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Combined Effects of Predator, Xylocoris Flavipes (Reuter) and Bacterium, Spinosad for Control of Stored Product Insect Pests

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COMBINED EFFECTS OF PREDATOR, *XYLOCORIS FLAVIPES* (REUTER) AND BACTERIUM, SPINOSAD FOR CONTROL OF STORED PRODUCT INSECT PESTS

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Entomology and Insect Biotechnology Laboratory
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DEDICATED

TO

MY PARENTS

AND

PARENTS-IN-LAW

DECLARATION

I hereby declare that the thesis entitled **Combined effects of predator, *Xylocoris flavipes* (Reuter) and bacterium, Spinosad for control of stored product insect pests** submitted in the Institute of Biological Sciences, University of Rajshahi, Bangladesh for the degree of **Doctor of Philosophy** is the result of my own investigation and was carried out under the supervisions of Professor Dr Md Wahedul Islam, Institute of Biological Sciences, University of Rajshahi and Professor Dr Selina Parween, Department of Zoology, University of Rajshahi, Bangladesh. This thesis has not been submitted elsewhere for any other degree or diploma.

ATUL CHANDRO SARKER
(candidate)

CERTIFICATE

This is to certify that the thesis entitled **Combined effects of predator, *Xylocoris flavipes* (Reuter) and bacterium, Spinosad for control of stored product insect pests** submitted by Atul Chandro Sarker for the degree of the **Doctor of Philosophy** is the record of bonafied research carried out at the Entomology and Insect Biotechnology laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh under our supervisions. All the data presented in the thesis are based on his own observations and no portion thereof has previously been published or submitted for any other degree or diploma.

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ABSTRACT

The flat grain beetle, *Cryptolestes pusillus* (Schon) (Coleoptera: Cucujidae) and the lesser grain borer, *Rhyzopertha dominica* (Fabricus) (Coleoptera: Bostrichidae) are the serious insect pest of stored commodities. *C. pusillus* is an internal and *R. dominica* is an internal feeder of whole wheat seed, flour, etc. The hemipteran predator, *Xylocoris flavipes* (Reuter) predates the eggs, larvae and pupae both of the pests in storage condition and checks their population in considerable level. A newly reduced risk broad-spectrum bacterium, Spinosad is effectively control the population of both *C. pusillus* and *R. dominica*. The effects of different life stages of both hosts on the biology of *X. flavipes* under laboratory condition were assessed. The influence of Spinosad on both hosts and predator and also the combined effects of *X. flavipes* and Spinosad on the population of *C. pusillus* and *R. dominica* were subsequently investigated.

The nymphs 1st up to 5th instar and adults of *X. flavipes* were found efficient to survive on eggs, larvae 1st up to 4th instar and pupae of the both insect pest *C. pusillus* and *R. dominica.* The mean duration of developmental period through five nymphal instars on eggs, larvae 1st up to 4th instars and pupae of were 15±2.00, 20±0.00, 22±0.58, 18±1.00, 14±1.15 and 12±1.15 days in *C. pusillus* and 18±1.00, 20±0.58, 16±2.00, 14±1.15, 12±1.15 and 13±0.58 days in *R. dominica* respectively. The adult female *X. flavipes* survived longer than the male. Average consumption rates of each nymph 1st up to 5th instar and adult stage of *X. flavipes* was found highest on eggs, 1st and 2nd instar larvae but lowest on 4th instar larvae and pupae of the both insect pests. The egg of *R. dominica* was more preferable than that of *C. pusillus*. Moreover, 1st, 2nd and 3rd instar larvae of the both host insect were more preferable to the predator than the other stages. The female predator always consumed more individuals of both the pests than the male. Average survivability rates of nymphs 1st up to 5th instar and adults were maximum on 1st and 2nd instar larvae and minimum on 4th instar larvae and pupae. The size of the female predator was found larger than the male at all stages studied. Based

on ratio 1:1, sex ratio was found the best (male and female almost equal in number) on 1st and 2nd instar larvae comparatively than that of other stages. Developmental period, adult longevity, consumption rates, survivability rates, size and sex ratio of *X. flavipes* always significant (P<0.001) in different life stages of both the insect pests.

The average percentage of egg hatchability (±SE) was the highest as 25.00±1.15 at 0.491µl/cm² and the lowest 5.00±1.02 at 7.863µl/cm² when *C. pusillus* was applied different concentrations of Spinosad and the results found highly significant (P<0.001). On the other hand, average (±SE) mortality of larvae, pupae and adults were found the highest as 14.00±2, 8.33±0.88 and 15.33±1.22 respectively at 7.883µl/cm² after 72h and the lowest 3.33±0.88, 1.67±0.33 and 5.00± 0.58 at 0.491µl/cm² Spinosad concentrations after 24h of exposure. The larvae (72h LC_{50} was 0.1755007µl/cm²) and adults (72h LC_{50} was 0.839572µl/cm²) were found more susceptible than pupae (72h LC_{50} was 35.94058µl/cm²).

Effects of separate concentrations of Spinosad on different stages of *R. dominica* were investigated and found that average percentage of egg hatchability (±SE) was the highest as 15.00±1.14 at 0.491µl/cm² and the lowest 0.33±1.03 at 7.863µl/cm². The effect of different concentrations on egg hatchability was highly significant (P<0.001). Average (±SE) mortality of larvae, pupae and adults were 13.33±0.88, 8.33±1.45 and 17.33±1.20 at 7.863µl/cm² after 72h and the lowest 4.67±0.33, 1.00±0.58 and 6.67±0.88 at 0.491µl/cm² after 24h of exposure periods. Larvae (72h LC_{50} was 0.5433412µl/cm²) and adults (72h LC_{50} was 0.466328µl/cm²) were found more susceptible than pupae (72h LC_{50} was 22.0538µl/cm²).

Different concentrations of Spinosad on different life stages of *X. flavipes* were investigated and recorded average percentage of egg hatchability (±SE) was highest 35.00±1.73 in control (untreated) and lowest 25.00±2.12 at 7.863µl/cm² concentrations. At 1.966, 0.983 and 0.491µl/cm² concentrations, the egg hatchability was almost similar like in control medium. Effect of different concentrations on egg hatchability was not significant (P<0.001) comparatively than that of control medium. Average (±SE) mortality of nymphs and adults was found highest as 6.67±1.76 and 5.00±0.45 at 7.863µl/cm² after 72h but lowest 1.00±0.58 and 1.67±0.67 at 0.491µl/cm² Spinosad

concentrations after 24h of exposure. A nymph (72h LC_{50} was 73.82966µl/cm²) was found more susceptible comparatively than that of the adult (72h LC_{50} was 331.5098µl/cm²). Moreover, at 0.491, 0.983 and 1.966µl/cm² concentrations after 24h to 72h of exposure, survivability of adults were found 94 to 84% respectively.

The adult population of *C. pusillus* and *R. dominica* was significantly ($P<0.001$) reduced by *X. flavipes* and different concentrations of Spinosad alone and their combinations after 3, 6, 9 and 12 months of storage than those of control medium. After 3 months, from Spinosad concentrations reduced the population from 57.69 to 66.12% compared to control. In combination this range was from 42.86 to 51.56%. After 6 months, this reduction percentage rate was increased in both concentrations and combinations. After 9 months, this reduction percentage rate was increased continuously and after 12 months the effect of both concentration and combination were found more effective compare to early storage period. In case of the predator alone, the population of the both insect pests was higher than those of concentrations and combinations. The population of both insect pests was found more susceptible to the combinations comparatively than that of concentrations. The population of *R. dominica* was found more susceptible to different concentrations and combinations than that of *C. pusillus* at all storage periods.




Chapter 1

General Introduction

INTRODUCTION

Insect pest of stored products and management scenario: Insects are the most diverse group of animals, including more than one million described species which are half of all known living organisms (Chapman 2006, Richard *et al*. 2007 and Wilson 2009). They appeared on the earth about 500 million years ago and are the oldest inhabitants of this planet (Saxena 1996). Some species of insects produce honey, silk and fibres, some of them pollinate flowering plants, and are now cultured primarily for pollination management in order to have sufficient pollinators in the field, orchard or greenhouse at bloom time (Smith *et al*. 1991). Some insects have also gained attention as potential sources of drugs and other medicinal substances (Aaron 2010). Fly larvae (maggots) were formerly used to treat wounds to prevent or stop gangrene, as they would only consume dead flesh. Some insects are also used as bio-control agents who are usually considered as beneficial because they can reduce the impact of pests and the use of pesticides in agriculture and food storage systems (Kenis and Branco 2010).

Although many insects attract the most attention positively but a large number of insects, alien insects and mite's species cause serious socio-economic hazards as pests of agriculture, horticulture, stored products, forestry and also may affect human or animal health (Kenis and Branco 2010). The struggle between men and insects began long before the dawn of civilization, has been continued without cessation to the present time and will continue, no doubt, as long as the human race endures. It is because of the fact that both men and certain insect species constantly want the same things at the same time (Metcalf and Flint 1962).

Men began to store foodstuffs right from the prehistoric days (Metcalf and Flint 1962, Retnakaran *et al.* 1985), to face the future demands and to ensure the supply of food but at times, insects cause so much damage to the crops that much of these get ruined. The inevitable world population growth placed increasing demands on the production of cereal and other food grains which comprise 67-80% of human food supply and diet (Kendall and Pimentel 1994, Dyson 1999). It is estimated

that losses of cereal grains in storage can range from 10 to 20% of overall production and a primary factor in these losses is the depredations of stored product insect pests (Phillips and Throne 2010). McEwen (1978) and Pimentel (1978) stated that world crop losses due to pests is approximately 35% of the total production in each year despite of best efforts.The crop loss is further increased at the post-harvest systems due to insects and other pests (Wright 1976). Globally only insect pests destroy approximately 14% of all potential food production (Pimentel 2007).

Pest problem have increased side by side with the increased amount of food stockpiled and the longer duration of storage (Khan and Mannan 1991). It is apparent that a number of insects act as enemies to the life of human, their pets and plants (Jha 1987). More than 2000 species of field and storage pests annually destroy approximately one third of world's food production, among which highest losses occur in developing Asian countries (Ahmed and Grainge 1986). A higher quality of the post harvested crops are damaged in the tropical countries by insects (Mondal and Port 1995, Mondal and Malek 1996) which are generally expressed in terms of direct weight and nutrient loss (Howe 1965a, Krishnamurthy 1975, Watters and Shuyler 1977). Moreover food losses due to insect infestation in the store is higher in tropical and sub tropical countries than in the temperate climatic zones (Girish *et al.* 1988). The annual costs arising from the two grain beetles *Oryzaephilus surinamensis* and *Rhyzopertha dominica* vary from 11.2 to 35.3 million € only in Germany (Reinhardt *et al*. 2003).

In some developed countries grain can be downgraded or rejected completely if even a single live insect is found (Pinniger *et al*. 1984, Anonymous 1990). In UK the Food Safety Act was amended recently; now all stores containing grain which might be destined for human consumption are treated as food premises and subject to inspection to ensure that food is not contaminated. The contamination of foods and animal feeds with mycotoxins is a worldwide problem (Kabak *et al*. 2006). The insect pests are detected in stored grain very soon after grain is brought in for storage (Hagstrum and Throne 1989, Dowdy and Mc Gauphey 1994) and they can

cause taint and contamination of grain with their excreta, cast skins and dead bodies (Scott 1991). Moreover, the presence of insects in stored foods directly affects both quantity and quality of the commodities (Hill 1978, Wilbur and Mills 1978, Burkholder and Faustini 1991, Khan and Mannan 1991).

A variety of pests are found in stored grain and cereal commodities and food processing facilities, depending upon geographic location, physical nature of the facility and the type of food being processed (Abd-El-Aziz 2011). Among the insect pests of stored commodities, the beetles (Coleoptera) are by far the most numerous group, followed by the moths (Lepidoptera) (Khan and Mannan 1991). Among these two groups other insects also share the stored habitat, and these are ants (Hymenoptera) (Smith 1965, Mallis 1982), cockroaches (Dictyoptera) (Cochran 1982, Mallis 1982), flies (Diptera) (Greenberg 1971, 1973, Mallis 1982), silverfish (Thysanura) (Mallis 1982) and springtails (Collembola) (Mallis 1982) psocids (Psocoptera) (Turner 1975, Mallis 1982). Globalization increased trade,, travel and transport and leading to an unprecedented homogenization of the world's biota by transport and subsequent establishment of organisms beyond their natural barriers (Wittenberg 2005). In Europe, 113 alien insect species are pests of stored products (Rees 2004) where as in Switzerland, about 800 alien species are established (Wittenberg 2005). In USA, a total of 61 species of insects have been reported at elevators or in flat storages (Hagstrum *et al*. 2010). The Canadian Grain Commission recognizes over 50 species of insects (including grain mites) as pests of stored grain. In Bangladesh 29 species of insect pests of stored products are listed by Alam (1971).

Among the insect pests the flat grain beetle, *Cryptolestes pusillus* (Schon.) is an external feeder of craked grains, a serious cosmopolitan pest of stored product commodities, and may subsequently build up huge population within very short time (Rahman *et al.* 2008). The genus has two other species *C. ferrugineus* (S) (Rusty grain beetle) and *C. turcicus* (Grourelle) (Flour mill beetle), having the same feeding habits. The lesser grain borer, *Rhyzopertha dominica* (F) is a primary pest of stored grains feeding the grain kernels, found in many regions of the world (Edde 2012).

The adults likely to fly back and forth between agriculture and non-agriculture land scapes (Edde *et al*. 2005, Mahroof and Phillips 2007, Jia *et al*. 2008 Mahroof *et al*. 2010) and is an internal feeder of sound grain. Hagstrum and Flinn (1994) reported that in farm bins *R. dominica* was present in 78.6% and *Cryptolestes* spp was present in 85.8%. Both *C. pusillus* and *R. dominica* are economically harmful and are available in stored cereal, cereal products and other commodities (Alam 1971).

Early attempts to control stored grain pests relied on methods such as mixing dry soil and wood ash with the grain, causing lethal dehydration of insects and the fumigant action of certain indigenous plant materials (Levinson and Levinson 1989). Control of stored-product pests is necessary to prevent contamination or adulteration of human foods. Persons involved in commodity or food storage, handling or processing, have the responsibility to prevent food adulteration (Abd-El-Aziz 2011). The middle decades of the twentieth century have been years of the revolution in the field of pest control triggered during the World War II by the discovery of DDT in 1939, and its successful utilization against a number of pests. It has been estimated that about 4.5 million metric tones of pesticides are used annually in the world agriculture (Smith and Van den Bosch 1967, Pimentel 1983). Pesticides sales in Bangladesh in 1954 was only 9 tons which up to 18902.5 tons of formulated products in the year 2000, went costing Tk 3001.56 million (Hasanuzzoha 2004) and the amounts of pesticides used in Bangladesh are being increased day by day.

Chemical pesticides are still indispensable in controlling insect pests both in field and storage due to their quick knockdown and killing properties. The efficacy of insecticides against storage pests varies greatly according to their chemical structures, insect species and the storage environment (Suchita *et al*. 1989). Indiscriminate and large-scale use of broad spectrum synthetic pesticides caused serious hazard including its persistence in the environment (Smith 1970, Wilkin and Fishwick 1981, Jolly *et al*. 1989, Bryne *et al*. 1994, Laliberte 1995, Bell *et al*. 1999, Rajapakse *et al*. 2000, Daglish and Wallbank 2002); toxicity to human beings (Anonymous 1981, Oudejans 1982, Hasanuzzoha 2004, Nayak *et al*.

2005), wild life including pollinator and economically beneficial insects (Munakata 1977, Pimentel 1981, 1983, Oudejans 1982, Daglish 2006); development of insect resistance to the insecticides (Georghiou and Mellon 1983, Champ 1986, Reichmuth 1992, Subramanyam 2006a); and finally, higher cost of crop production (Khan and Mannan 1991). Moreover, both multi-resistance and cross-resistance to pesticides has been reported in a large number of insects (Metcalf 1980, Georghiou and Mellon 1983). Sometimes, toxic residues of pesticides accumulate in the ecological food chain and become concentrated by bio-magnification (Metcalf and Luckman 1975). Concerns over pesticide operator safety and residues in our food and the environmental have led to a review of all organophosphorus pesticides by the Pesticide Safety Directorate in UK, their current advice is for farmers to avoid to use these pesticides altogether unless there is no alternative (Rooker 1999). Presently, their is a great concern in the post-harvest ecosystems throughout the world particularly in developing countries including Bangladesh (Champ 1979, Subramanyam and Hagstrum 1995). In spite of insecticides being the major means of defense against insect pests, the above mentioned problems have generated a sustained search for either alternative means of insects control methods for reducing the amount of insecticides required for the pest management (Mondal 1984a, Smet *et al*. 1990, Burkholder and Faustini 1991) or to search for eco-friendly new methods.Therefore, stored-product pest control strategies tend to emphasize the non-chemical aspects of pest control with the judicious use of pesticides (Abd-El-Aziz 2011).

In this respect, *Xylocoris flavipes* is a cosmoplitan predator of different preying on insect pests of stored commodities namely *Tribolium castaneum*, *T. confusum*, *Cryptolestes pusillus*, *Rhyzopertha dominica* and *Trogoderma granarium* (Ahmed *et al.*1991).Toews and Subramanyam (2004) found that Spinosad applied to stored wheat at 1 ppm was highly toxic to the parasitoids *Habrobracon hebetor* (Say), *Theolax elegans* (Westwood), and *Anisopteromalus calandrae* (Howard), yet not so to the warehouse pirate bug, *X. flavipes* (Reuter), which demonstrated 92% survival and was able to reproduce under these same conditions.

Spinosad will represent a valuable new addition to the limited arsenal of grain protectants and can positively impact global food security (Hertlein *et al.* 2011). Its combination of high efficacy, broad insect pest spectrum, low mammalian toxicity, and sound environmental profile is unique among existing products currently used for stored-grain protection (Thompson *et al.* 2000). In several countries, Spinosad are being widely used to protect stored products against insect pest including *C. pusillus* and *R. dominica* (Hertlein *et al*. 2011). Spinosad is minimally disruptive to beneficial insects and compatible with Integrated Pest Management (IPM) programs in many crops (Miles 2006 and Arthurs *et al*. 2007).

Unmanaged pest problems and unsafe pesticide use practices threaten human health and the environment. Full implementation of IPM is affordable and cost-effective and can reduce pesticide exposure, pesticide use and pest complaints. A coordinated global effort is critically needed to make safe and effective insect pest management the standard for all of stored products including paddy, rice, wheat, wheat flour, pulses, maize and sorghum (Thomas *et al.* 2009).

Keeping these views in mind and considering the merit of using natural pest control agents like predator, bacteria, etc., the research reported here was initiated to investigate the possible combined effects of the predator, *X. flavipes* and the bacterium, Spinosad for the control of two important stored product insect pests *C. pusillus* and *R. dominica*.

Test insects, their distribution and damage

Among the major insect pests causing serious damage to stored products *Cryptolestes pusillus* (Schon) and *Rhyzopertha dominica* (F.) were selected for the present research because they are of great threats to the stored grains and cereals of Bangladesh. These two insects have different feeding habit, the former one is an external feeder of broken grains and cereals, the later one is an internal feeder of whole grain. Both are consumed as prey by the warehouse pirate bug, *X. flavipes*.

***Cryptolestes pusillus*:** *C. pusillus*, flat grain beetle, is a member of the family Cucujidae, order Coleoptera. Granglbour in 1899 described this beetle under family named Laemophloeidae which consists of 500 species worldwide (Thomas and Leschen 2010). Nine pest species of *Cryptolestes* occurring in stored products are *C. capensis* (Waltl), *C. cornutus* (Thomas and Zimmermann), *C. divaricatus* (Grouvelle), *C. ferrugineus* (Stephens), *C. klapperichi* (Lefkovitch), *C. pusilloides* (Steel and Howe), *C. pusillus* (Schonherr), *C. turcicus* (Grouvelle) and *C. ugandae* (Steel and Howe) (Halstead 1993). In spite of Banks (1980) and Halstead (1993) publications, *Cryptolestes* specimens are usually identified to generic level only. *Cryptolestes pusillus* was first proposed by Schoenherr (1817). *C. pusillus* has two synonyms such as *Laemophloeus pusillus* and *Laemophloeus mintus*. The local name of *C. pusillus* is chapta beetle.

Distribution of *C. pusillus* : *C. pusillus* is most prevalent in warmer climates and occurs throughout the crop-growing regions of the world. It is particularly common in wet tropical and warmer temperate regions, but unable to survive in unheated premises in cooler temperate regions (CABI Crop Protection Compendium 2008, Halstead 1993). In Canada, *C. pusillus* has been recoded in grain elevators and flour mills feeding on damaged grain, preferably wheat (Bousquet 1990). So far, only cosmopolitan *C. ferrugineus*, *C. pusillus* and *C. pusilloides* have been detected in Australia (Halstead 1993). Adult beetles can fly. Although its origin is unknown, *C. pusillus* has been transported internationally in exported commodities.

Damage: The flat grain beetle, *C. pusillus* is one of the smallest and most common destructive major insect pests of stored grains (Davies 1949, Pajni and Bedi 1974, Barker 1976 and Hole *et al*. 1976). This beetle feeds on groundnuts, Coffee, barley, rice, sorghum, cocoa, wheat, flour, oilseeds, cassava root, dried fruits, chilies, maize and other dried stored food commodities (CABI Crop Protection Compendium 2008). In cooler and drier regions of the world, it is found mainly in cereal and cereal products, while in warmer and humid regions it infests a much wider range of products (Hole *et al*. 1976). The most favoured food of this

beetle is cracked cereal grains, which gives insect access to the germ, and provides better harborage and oviposition sites (LeCato 1974). Larvae feed preferentially on the germ of the whole kernels, but they also feed on the endosperm and sometimes hollow out the entire kernel. Growth of mold in the endosperm renders it more suitable as larval food. *Cryptolestes* species are apparently unable to feed on sound grain, but they can feed on kernels with very slight imperfections or injuries.

***Rhyzopertha dominica*:** The lesser grain borer *Rhyzopertha dominica* was first described by Fabricius in 1792 under the name *Synodendron dominicum* from specimens taken from nuts and roots imported from India (Chittenden 1911). Lesne (1896) later recorded the insect as *Rhyzopertha dominica* and published a full description of the insect under this name. *Rhyzopertha dominica* is a member of the family Bostrichidae under the order Coleoptera. There are about 550 bostrichid species in 99 genera of which 77 species in 26 genera occur in North America (Marske and Ivie 2003). Bostrichids are reddish-brown to dark-brown in colour of various in sizes, elongated, cylindrical in cross-section, and their head is invisible when viewed from above. The insects live mainly in dead and dried wood, and are pests of timber also (Potter 1935, Fisher 1950, Mathew 1987, Ivie 2002a, b).

Distribution: The origin of *R. dominica* is not known with certainty, but the consensus is that the Indian subcontinent is the native home of the insect because the region is the focus of many species of bostrichid (Chittenden 1911, Schwardt 1933 and Potter 1935). Nowadays, *R. dominica* is widely distributed around the world (Potter 1935 and Chujo 1958) and is a primary pest of stored grain in warmer regions lying in the belt between latitude 40° N and latitude 40° S of the equator (Potter 1935). *R. dominica* is largely distributed during the transport of grain (Doane 1919 and Chujo 1958) and was first noticed in the United States of America (USA) in specimens of wheat distributed from the Patent Office (Leconte 1862) and became established in the country in the early 1920s (Back and Cotton 1922). The establishment of *R. dominica* in the USA is believed to have been

augmented by importation of infested wheat from Australia (Doane 1919), and hence the insect sometimes referred as the "Australian wheat weevil" in early publications (Cotton and Good 1937). *R. dominica* is frequently captured in forest habitats and in grain storage environments, and the adults are likely to fly back and forth between agricultural and non-agricultural landscapes (Edde *et al.* 2005, Jia *et al.* 2008, Mahroof *et al*. 2010 and Mahroof and Phillips 2007).

R. dominica achieves its maximum reproductive success on dry grains, specially on wheat (Chittenden 1911, Schwardt 1933, Potter 1935, Bashir 2002 and Edde and Phillips 2006a,b). Published reports have shown that while *R. dominica* can tunnel in many woody plants, reproduction in most of them is generally poor (Wright *et al*. 1990, Edde and Phillips 2006a,b, Jia *et al*. 2008).

Damage: *R. dominica* is injurious to cereals; breeds in corn, rice, wheat, and in other substrates containing starch (Chittenden 1911). Grain infestations may result from residual insect populations inside storage structures and mixing of infested and uninfested grain or by *R. dominica* originating from outside sources (Sinclair 1982, Fields and Phillips 1994, Vela-Coiffier *et al*. 1997 and Hagstrum 2001). The flying adults generally enter a grain bin through the headspace, alight on the grain surface, and gradually moves through the grain mass in a slow downward progression (Sharangapani and Pingale 1957, Keever 1983, Vela-Coiffier *et al.* 1997 and Hagstrum 2001). *R. dominica* could move down into the grain mass to a depth of 12 m, which is deeper than observations for other grain beetles (Flinn *et al*. 2010). A wheat consignment containing more than 32 insect damaged kernels (IDK) per 100 g is designated as sample grade (Federal Grain Inspection Service 1997), which cannot be sold for human consumption (Flinn *et al*. 2004). There is a general trend toward a no tolerance for live insects and domestic flour mill contracts specify an upper rejection limit of 7 IDK per 100 g grain sample (Kenkel *et al*. 1993).

Grains infested by *R. dominica* have a characteristic sweetish odor, which is due to the male-produced aggregation pheromones (Khorramshahi and Burkholder 1981).

Adult feeding activities produce large amounts of frass, most of which consists of ovoid granules of apparently undigested endosperm mixed with a finer floury part (Breese 1960). The frass contain larvae exuvae, feces, fragments of immature insects, and other by-products, which could affect the end-use quality of the infested grain (Sanchez-Marinez *et al*. 1997, Seitz and Ram 2004 and Park *et al.* 2008). The larvae and adult *R. dominica* feed on both the germ and endosperm and are capable of reducing wheat kernels to the pericarp (Winterbottom 1922 and Campbell and Sinha 1976). The degree of feeding damage caused by adult *R. dominica* varies with beetle age, greater damage being caused by young adults (Rao and Wilbur 1972).

Several authors (Breese 1960, Campbell and Sinha 1976, Crombie 1944, Gay and Ratcliffe 1941, Golebiowska 1969, Howe 1965b, Rao and Wilbur 1972 and Wilbur and Halazon 1955) have investigated the amount of grain consumed by *R. dominica* under experimental conditions, but the estimate of losses varied among the authors. Campbell and Sinha (1976) reported 17% loss per kernel when *R. dominica* was allowed to develop from egg to pupa in wheat grains, whereas, Rao and Wilbur (1972) and Crombie (1944) reported 10 and 23% weight loss per wheat kernel, respectively.

Alternate insect control methods using at present

The pest management in stored commodities is facing many obstacles such as restrictions on the use of certain pesticides which caused resistance in pest population, pose possible health hazards and a risk of environmental contamination. Entomologists throughout the world spend a great deal of time and effort in attempt to determine the presence of insect pests in stored grains, check their infestations and design better and safer methods to bring them under control. Control measures of different nature are being adopted at farm, market and public sector storage that consist of use of native soar natural methods of control by plant materials or contact insecticides and fumigants (Maina and Lale 2004, Hussain *et al*. 2005).

Control of stored product insects is best achieved through an integration of physical, chemical and biological methods (Arthur 1996, Hagstrum *et al*. 1999,

Phillips and Throne 2010). However, in practice there is still a strong reliance on the use of chemicals applied to grains at the time of storage. To control an existing infestation, specially in grain that is not treated with a traditional chemical protectant, fumigants like phosphine is used. Promising techniques that have been developed and continues to be refined, is monitoring populations with insect pheromones and/or food attractants for detecting stored-product insects (Abd-El-Aziz 2011). Some chemical control products are under intense scrutiny due to concerns about human safety, insect resistance, environmental impacts and presence of chemical residues in raw and processed foods (Daglish and Wallbank 2002, Nayak *et al*. 2005, Daglish 2006). Alternative chemical control options to protect grain that do not suffer from the concerns outlined above are urgently needed and Spinosad is one such product that fills this void. Spinosad is currently registered in several countries as a grain protectant at a maximum labeled use rate of 1 ppm (1 mg/kg of grain) (Hertlein *et al*. 2011). Spinosad is effective against *R. dominica* (Subramanyam *et al*. 2003, Flinn *et al*. 2004 and Hertlein *et al*. 2011) and other economically important beetle including *C. pusillus* and moth pests associated with stored grain and is also effective against certain psocid species (Hertlein *et al.* 2011).

For a long time the biological control is an over-looked component of integrated pest management of stored product pest (Flinn 1998). Many species of natural enemies of insects occurs in stored product ecosystem (Brower *et al*. 1996). Among these the anthocorid bug, *X. flavipes* is a cosmopolitan predator of different prey (pests) of stored commodities namely *T. castaneum*, *T. confusum*, *C. pusillus*, *R. dominica* and *Trogoderma granarium* (Ahmed *et al*. 1991). Moreover, Computer-based decision support systems that use biological and environmental data to predict population trends and evaluate the need for insecticidal inputs have been developed for stored-product storage systems in several countries (Abd-El-Aziz 2011).

Environmental control

Insects in stored grain can be controlled by manipulating the physical environment or applying physical treatments to the grain and insects (Abd-El-Aziz 2011).

The factors of the physical environment are temperature, relative humidity or grain moisture content and relative composition of atmospheric gases in the intergranular air. Physical treatments include physical removal and physical barriers to prevent the entrance of insects, inert dusts, light and sound. Different types of physical control practices have been reviewed by a number of researchers (Shejhal 1980, Cline *et al*. 1985, Lapp 1986, Armitage 1987, Bell 1987, Lessard 1987, Navarro and Jay 1987, Wilkin and Nelson 1987, Cline and Press 1990, Banks and Field 1995, Golob 1997, Subramanyam and Hagstrum 2000).

In the developing countries of Asia and Africa the harvested grains, paddy, sorghum, millets, pulses or oil seeds are sun-dried before storage to eliminate insects if present (Rajendran and Chayakumari 2003). The eggs of the stored product insects could be eliminated by milling wheat to flour or by sieving flour through 210μm mesh (Yamanouchi and Takano 1980). Cooling of wheat by low volume aeration is used in modern Europe, Canada and United States (Arthur 1996) but cost of the cooling equipments and treatment is high (Longstaff 1999) which could not justify its economic use in developing countries. High temperature fluidized bed have reached a pilot scale for the disinfestations of grains and their products, and short exposures to temperature above 60ºC are generally effective for the disinfestations of grains (Evans 1981, 1987a, b). Insects coated in inert dusts dehydrate and die, so inert dusts have been used for centuries by aboriginal people in North America and Africa to control insects in their stored grain. According to Fields and Muir (1996) five types of inert dusts such as i) sands and other soil components are traditional insecticides used by aboriginal peoples as a protective layer on top of stored seed (Golob and Webley 1980); ii) Silica aerogel, produced by drying an aqueous solution of sodium silicate have been reported to control a number of insect species including *C. pusillus* and *R. dominica* (Le Patoural 1986, Desmarchelier and Dives 1987, White and Loschiavo 1989, Aldryhim 1991, 1993, Quarles, 1992); iii) non-silica dusts, such as rock phosphate, have been used in Egypt (Fam *et al*.1974) and lime (calcium oxide) provides insect control (Golob and Webley 1980); iv) Particle films (Kaolin and bentonite clays) also have potential

for use as a dry dust material in stored-product environments. The particle film M-96-018 was reported to be effective against the *Tribolium* species and appears to have a potential for use in management programs to control beetles within storage facilities (Arthur and Puterka 2002); v) Diatomaceous earth (DE) the fossilized remains of diatoms having low mammalian toxicity regarded as safe by the USA Environmental Protection Agency (Subramanyam and Hagstrum 1995) and (Anonymous 1991). DE also showed efficacy against *R. dominica* on stored wheat (Kavallierator *et al*. 2005, Athansassion and Kavallierator 2005), preventing economic damage of stored rice (Chanbang *et al*. 2008). However, it is difficult to control *R. dominica* within grain using DE alone (Arthur 2004b, Chanbang *et al*. 2007a, b).

Light may be of some use in luring flying insects into traps (Banks and Fields 1995). A 5-min exposure to 1 MHz sound at 14.5 Wcm^{-2}, killed all life stages of *S. granarius* at 26°C in wheat but commercial application is unexpected (Banks and Fields 1995). Controlled and modified atmosphere storage practices for protecting grains have also proved to be potential in stored insect pest control (Hyde *et al*. 1973, Anonymous 1984, Evans 1987b, Hulasare *et al*. 2005). Ozone is known as a sterilant of stored product insect pests at levels less than 45ppm (Abd-El-Aziz 2011). Packing materials very often produce barrier against the insect infestation in storage.

Sitophilus spp, *R. dominica*, *Plodia interpunctella*, *Lesioderma serricorne*, *Stegobius* are capable of penetrating food packaging but *Tribolium* spp, *C. pusillus*, *C. ferrugineus*, *Oryziphilu*s spp cannot penetrate intact packages and must enter through existing holes in the package (Highland 1991). Sabbaur and Abd-El-Aziz (2007) screened the most suitable packaging materials (muslin paper, cheesecloth, wax paper, gunny bags and polypropylene) for prevention of broad bean beetle infestation.

Control by radiation control

Various forms of electromagnetic energy have been considered potentially useful for insect control (Nelson 1967). These include ionizing radiation of extremely high frequency (X- rays and gamma rays) which may be used to cause mortality or to induce sterility. Potentially useful non-ionizing radiations are infrared, visible and

ultraviolet; infrared may be used to kill pests directly whereas visible and ultraviolet may be used to attract pests to traps (Crowdar 1986). Ionizing radiation damages organisms by causing the production of ions or free radicals-charged molecules that are highly reactive and chemical bonds can also be broken. Both types of ionizing irradiation have been used to control insects in grain stores (Fields and Muir 1996) To cause immediate death to stored product insects may require higher doses, sterilization of many species of insects can be accomplished at lower doses, but Rusty grain beetles are sterilized at only 0.6 kGy but saw-toothed grain beetles and red flour beetles require a 2.0 kGy dose (Banks and Fields 1995). The use of irradiation to suppress *T. castaneum* has been widely studied (Fontes *et al*. 1996, Hasan 1995, Khattak and Jilani 1984, Pajni and Virk 1978). To manage the pulse beetles (*Callosobruchus* spp) irradiation has become the major tool (Ghomomu 1989, 1991, Hussain and Imura 1989). A number of reports are present showing that the females coleopterans are more sensitive to gamma radiation than males (Ashraf and Brower 1974, Ahmed *et al.* 1976, Hasan 1995). Microwaves synersise with low temperature could provide an effective and friendly environmental treatment technique in IPM program (Valizadegan *et al*. 2009).

Chemical control

Chemical control of stored product insect pests includes some low risk insecticides, fumigants and the novel insect growth regulators. The developing countries of Asia and Africa still depend on the traditional chemical control method because of their quick positive results against the insect pests in the agriculture and storage system (Ferdous 2006).

Insecticides

Large number of insecticides have been used in the grain storages of the world since the time of World War II However, the after effects of the inorganic insecticides resulted in to develop organic insecticides. A large number of such organic insecticides are used in grain and cereals stores throughout the world. Many of the organic insecticides gave desired level of control against storage pests including *C. pusillus* and *R. domonica* in laboratory and in storages (Khan 1981, Rahman and Yadav 1985, Mondal 1986, Yadav 1987, Mondal 1988,

El-Sayed *et al*. 1988, Islam *et al*. 1989, Rajendran 1990, Ali *et al*. 1991, Collins and Cook 1998). New reduced-risk chemical insecticides are still developing to control the resistant starins of the storage insects.

Relative to other stored grain insect pests, *C. pusillus* and *R. dominica* are the most difficult insect pests to control with insecticidal grain protectants (Zettler and Cuperus 1990, Lorini and Galley 1999, Collins 2006); many of approved insecticides are either not effective against the insect pests including or the insects have developed resistance to them (Parween 1996). *R. dominica* has developed resistance to all approved organo-phosphorus insecticides (Navarro *et al*. 1987, Zettler and Cuperus 1990, Guedes *et al*. 1996, 1997, Lorini and Galley 1999, Collins 2006), and resistance of *R. dominica* to pyrethroid-based grain protectants is widespread (Lorini and Galley 1996, Collins 2006).

Fumigants: Vijayanna (2006) mentioned that fumigants are effective in pest control as these gaseous are suffocative or poisonous against the insects. Fumigation plays a major role in insect pest elimination in stored products. Phosphine and methyl bromide are the two common fumigants used for the management of stored-product insect pests worldwise (Rajendran and Sriranjini 2008). Phosphine once was the most important fumigant to control *R. dominica* (Collins 2006 and Daglish *et al*. 2010). However, some species including *C. pusillus* and *R. dominica* develop resistance to phosphine which became global issue (Herron 1990, Zettler and Cuperus 1990, Lorini and Collins 2006, Schlipalius *et al*. 2008, Newman 2010) and control failures have been reported in field situations in some countries (Taylor 1989, Collins *et al*. 2002). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance and therefore, is being phased out completely in 2015 (Taylor 1994, Fields and White 2002, Bartholomaeus and Haritos 2005). Chloropicrin and Candorsis have been reported from Italy as easiest alternatives to methyl bromide (Spotti 2004). Recently, the use of sulphuryl fluoride, a structural fumigant for termite and woodborer control, has been expanded to food commodities and food handling establishments (e.g., flourmills) in the USA, Canada and Europe (Prabhakaran 2006). New fumigants such as carbonyl sulphide and ethane dinitrile and the old

fumigant ethyl formate (alone and in mixture with CO_2) can be as alternatives for food and non-food commodities from the infestation of insect pests including *C. pusillus* and *R. dominica* (Damcevski *et al*. 2003).

Insect Growth Regulators (IGRs): The use of insect growth regulators (IGRs) in controlling of stored product insect pests was suggested by Thomas and Bhatnagar Thomas (1968). On the basis of mode of action, IGRs are divided into three categories such as (1) Juvenil hormones (JHs) and their analogues (JHAs) also called as juvenoids e.g. methoprene, hydroprene etc. which control physiological and behavioural activities (Arthir 2004, Collins 2006, Daglish *et al*. 1995, Daglish 2006), (2) ecdyson agonists which regulate the morphogenetic changes during metamorphosis and (3) chitin synthesis inhibitors (CSIs) or moult inhibitors (MIs) e.g. triflumuron, diflubenzuron etc. which interfere with chitin biosynthesis, prevent moulting and produce an imperfect cuticle and are effective suppressors of development for entire life cycle of insect pests (Post and Vincent 1973, Mulder and Gijswitj 1973, Post *et al.* 1974, Wills 1974, Mulder *et al*. 1975, Grosscurt 1978, Reynolds 1987, Wing and Aller 1990, Edwards and Menn 1981, Koolman 1989, Fox 1990, Heller *et al.* 1992, Oberlander *et al*. 1997). IGRs used in stored product systems in the United States and elsewhere include the insect juvenile hormone analogs (Arthur *et al*. 2006). Compared with the conventional insecticides, IGRs do not exhibit quick knock-down in insects or cause mortality, but the long- term exposure to these compounds largely stops the population growth as a result of the effects mentioned in both parents and progeny (Mondal and Parween 2000). Potentiality of IGRs against *Cryptolestes* spp. was studied by Kostyukovsky *et al*. (2000). IGRs affect development of immature insects includin *C. pusillus* and *R dominica*, methoprene affects the egg and larval stages of stored product insects and suppress progeny production of *R. dominica* (Mian and Mulla 1982a, b, Oberlander *et al*. 1997). The efficacy of IGRs against the stored product insects have been reviewed by Parween (1996), Mondal and Parween (2000).

IGRs represent low-risk, biologically based insecticides with potential for more adoption in the food industry in the future. The chemically synthetic nature of IGRs, however, are non-toxic to the environment and its biota (Fox 1990) and are also effective against the insecticides resistant strains of insect pests (Bengston 1987).

Botanicals as control measure

Botanicals are comparatively less expensive and are easy to prepare and use. Traditionally the farmers and stockers used plant materials or botanicals at early periods to protect stored grains against insect pests in many countries of Asia and Africa. Botanical Insecticides are locally available and are widely used to suppress stored product insect pests (Golob and Webley 1980). Specially plant may provide alternative potential to the currently used insect control agents as they constitute a rich source of bioactive molecules (Rajashekar *et al*. 2012), and are also safe for non-target animals, human being and ecofriendly (Srinivasa *et al.* 1993). Since last part of 20th Century number of plants have been screened for their insecticidal, repellent and antifeedant activities against the stored product insects, some of the latest reports are (Rajashekar *et al*. 2010, Mandal and Khalequzzaman 2010, Sabbur and Abd-El-Aziz (2007, 2010) Maia and Moore (2011), Khater (2012) and Dimetry (2012).

Plant oils are strong insecticides and effectively control the internal feeders of the grains at storage (Shukla *et al*. 1992, Xu *et al*. 1993, Yadav 1993, Obeng-Ofori 1995, Sanguanpang 1996, Shaaya *et al*. 1997, Liuv and Ho 1999, Keita *et al*. 2001, Haghtalab *et al*. 2009). Powders of different parts of a number of plants showed efficacy as repellent, antifeedant and growth inhibition along with toxicity against the stored product insects including *R. dominica* (Dakshinamurthy and Goel 1992, Tiwari 1994, El-Lakwah *et al*. 1994, Jembere *et al*. 1995, Mahal 2002). Besides that extracts of plant parts have been proved as potent insecticides and suggested to use as grain protectants (Shaaya *et al*. 1997, Sumita 2006, Mondal *et al*. 2012). In this context the neem-based compounds stand for unique position as insecticide in the stored and filled ecosystems (Ahmed and Grainge 1986). Compounds derived from plants continue to be assessed for their potential to

control storage pests (Allotey and Azalekor 2000). Botanical pesticides are always relatively safer for vertebrate animals and environments (Odderskaer *et al.* 2003). Although botanicals are environmentally friendly but they are uses in less quantity in Bangladesh due to lack of organized production and marketing (Ali 2004). Furthermore, interest has been shown in plant products ie, essential oils and their components for fumigants action since it is believed that natural compounds from plant sources may have the advantage over conventional fumigants in terms of low mammalian toxicity (not true all cases) rapid degradation and local availability (Rajendran and Sriranjini 2008).

Biological control

Biological control is an important component of integrated pest management of stored product pest (Flinn *et al*. 1998) that included various techniques such as use of resistant varieties, pheromones, sterile insects, pathogens, parasitoids, parasites, predators, etc. Biological control is a plausible alternative to chemical management of stored product pests, and a variety of examples of the use of natural enemies to protect stored raw commodities are provided by the literature viz., Brower (1990 and 1991), Brower and Press (1988), Brower and Mullen (1990), Flinn *et al.* (1994), Scholler *et al.* (1996), Flinn 1998, Flinn and Hagstrum 2001). Biological control was begun to be explored as a management strategy in food processing facilities since the last part of 20th Century. Many species of insect natural enemies occurs in stored product ecosystem (Brower *et al.* 1996) and these species represent potential biological control agents for the desired pests of food processing facilities (Hansen 1998, Prozell and Scholler 1994, 1998, Steidle *et al*. 2001), warehouses, and retail stores (Prozell *et al.* 1996). The anthocorid bug, *Xylocoris flavipes* (Reuter) is a cosmopolitan predator of different prey (pests) of stored commodities namely *T. castaneum*, *T. confusum*, *C. pusillus*, *R. dominica* and *T. granarium* (Ahmed *et al*. 1991, Rahman *et al*. 2009).

Biological control (Smith 1911) was first used in 1911 against the Mediterranean flour moth (Froggatt 1992) as alternative to conventional pesticides (Waage 1991). The theory of biological control was based on natural control which can be

observed in the balance of predator-prey and parasite-host population (Huffaker and Messenger 1976), thus reducing the commodity damage to tolerance levels (De Bach and Rosen 1991). Insect control program one aimed to avoidance, elimination or reduction of the factors which promote excessive multiplication of insects (Nayar *et al*. 1976). Haines (1989), Van Huis *et al*. (1991), Islam and Khan (2000) reviewed the status or research of the role of parasite and predators in the management of the stored pests.

Recently the predatory beetle *Teretriosoma nigrescens* (Lewis) has been introduce into West Africa to control *Prostephanus truncatus* (Horn) with moderate success (Markham *et al.* 1994). The impact of pesticides on natural enemies and the resulting outbreaks of secondary arthropod pests have been documented in many field agricultural systems (Croft 1990). Validation studies on the potential of natural enemies as biological controls agents as replacements for insecticidal protectants in storage system are in the focus of IPM programme throughout the world.

It is potential to select for insecticide resistance in certain parasitic and predatory species (Baker 1995, Baker and Arbogast 1995). Integrating the use of predatory/parasitic mites and insecticides for stored-grain pest management requires knowledge of the impact of the insecticides on the natural enemies or, in other words, their selectivity to natural enemies (Baker and Arbogast 1995, Goncalves *et al*. 2002). Goncalves *et al*. (2004) mentioned that the effect of insecticides on the mite species *Acarophenax lacunatus* (Cross and Krantz), an egg parasite of the stored grain pest *R. dominica* (F.). Deltamethrin was less selective in favor of the mite species. Nonetheless the parasitic mite was able to parasitize eggs of *R. dominica* on wheat treated with all the insecticides evaluated.

In some respects the artificial nature of man-made structures may serve to enhance biological control because augmentative natural enemies can be contained within the system and environmental conditions are relatively stable compared with those of natural systems. However, storage systems are also isolated from potential reservoirs of natural enemies, making inoculative or conservation biological control difficult. Thus, an inundative form of augmentative biological control may

be the most appropriate tactic for finished stored products (Scholler and Flinn 2000, Scholler *et al*. 1997). Releases of the warehouse pirate bug, *Xylocoris flavipes,* resulted in a 79-100% suppression of moth populations in small storages of peanuts, up to 99% reduction of sawtoothed grain beetle populations in 35-quart lots of corn and a 90-98% suppression of red flour beetles in a simulated peanut warehouse. Rice weevils in wheat spillage in small rooms were suppressed 96% by the parasitic wasp *Anisopteromalus calandrae.* When the egg parasitoid *Trichogramma pretiosum* and the larval parasitoid *Bracon hebetor* were released together in simulated peanut warehouses, they suppressed Indianmeal moth populations by 84% and almond moth (*Cadra cautella)* populations by 98%, *B. hebetor* alone supressed almond moth populations by 97.3%. A variety of natural enemies have been evaluated for use against stored product moths, and has also been evaluated as a potential biological control agent for stored product moths and beetles (Press *et al*. 1974, 1982, Keever *et al*. 1986, Krazpulski and Davis 1988). Furthermore, much of the literature concerning biological control of stored product moths involve releases of multiple species of natural enemies, including combinations of egg and larval parasitoids (Brower 1990), *H. hebetor* and *V. canescens* (Press *et al*. 1982), parasitoids and predators (Press *et al*. 1974, 1982, Keever *et al*. 1986, Krazpulski *et al.* 1988). Overall, *Trichogramma* species and *H. hebetor* have been the most frequently used natural enemies for stored product moths.

The advantages of biological control are free of side effects, safe to handle or use, occurs naturally, high degree of host specificity, cost effective, self perputation, searching ability and survive at low density. Research is continuing to determine the proper prescriptions for use of natural enemies in stored grain. Behavioral, ecological and physiological data are being collected that will facilitate effective deployment of parasitoids and predators. Storage situations other than grain bins, such as feed mills, food warehouses and food factories may be targeted areas for biological control in the future. As part of an IMP system for stored product management, biological control should help to reduce the use of pesticides on food and provide for high quality food products. Biological control in stored products is being regarded with increasing interest since they are nontoxic and do not damage human health or the environment.

Parasitoids : Insects that parasitize or damage other insects and arthropods are most appropriately known as parasitoid. A parasitoid is parasitic in its immature stages but is free livng as adult. In all instances, parasitoids kill their host, but in some circumstances, the host may live much of its full time before dying. Hymenoptera and Diptera are the most important parasitoid groups of insects among six parasitoid including order (Pedigo 1996) which play an important role in the management of storage pests. Askew (1971) stated that there are over one lakh species of parasitic hymenoptera, but (Kerrich 1960) reported that there are five lakh hymenopteran parasitoids. Kapil and Chowdhury (1973) described nine hymenopteran parasitoids for *T. granarius.* Parasitoids that have been assessed for the biological control of stored product moths include multiple species of egg parasitoids within the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) (Brower 1990, Keever *et al*. 1986, Prozell and Scholler 1998, Steidle *et al*. 2001), the larval endoparasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichnuemonidae) (Press *et al*. 1982) and the larval ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) (Press *et al*. 1974, 1982, Krazpulski and Davis 1988, Brower 1990, Cline and Press 1990). The bethylid parasitoid *Plastanoxus westwoodi* (Kieffer) generally parasitized the larval and pupal stages of *C. pusillus* (Rahman 2006). The parasitoid *Theocolax elegans* (Westwood) reduced the population of *S. zaemais, T. castaneum* and *Cryptolestes* sp in a mixed infestation and synergistically produced better result with avidin maize powder in case of *T. castaneum* (Flinn *et al.* 2006). The parasitoid *Anisopteromalus calandrae* (How) attaks *Sitophilus* sp. *C. chinensis*, *O. surinamensis* and *R. dominica* (Mahal 2002), along with other species (Islam *et al*.1985). *R. dominica* is parasitized in the larval and pupal stages by the parasitoids *Lariophagus distinguendus* and *Chaetosphila elegans* (Durrant 1921). Pteromalid parasitoids such as *A. calendrae*, *L. distinguendus* and *T. elegans* are expected to suppress population of *Sitophilus* sp. and *R. dominica* (Flinn 1998, Imamura *et al*. 2004, Steidle *et al* 2000, Williams *et al.* 1971). Sixteen species of Hymenopteran parasitoids were collected from different localities in Greece (2002) on grain, tobacco and dried fruits; eight parasitoids attacked coleopteran hosts, six attacked

lepidopterans and two species attacked the both. According to Elipoulos *et al*. (2002) observed that *S. oryzae* and *R. domonica* were most frequently parasitized by *A. calandrae*, *H. sylvanidis*, *T. elegans*, *Venturia canescens*, *H. hebetor* and *C. tarsalis*; whereas, *Tribolium* spp., *Oryzaephilus* spp. and *Cryptolestes* spp. Were parasitized by *H. sylvanidis* and *C. tarsalis*. The authors also reported that *V. canescens* and *H. hevetor* preferred moth larvae the degree of dominant waps were in decreasing order as, *H. sylvanids*>, *A. calandrae*>, *Venturia canescens*> , *C. tarsalis*> *T. elegans*. Seven hymenopteran parasitoids belonging to families Pteromalidae, Braconidae, Bethylidae and Ichneumonidae were recorded from experimental culture of stored Bengal gram (*Cicer arietimum* L) and Masur (*Lens esculentus* L) in the BCSIR laboratories, Rajshahi, Bangladesh during 1982-1983. The parasitoids are *Dinarmus basalis* Rond., *A. calandrae*, *C. elegans*, *B. hebetor*, *Rhabdepysis* spp, *Holepyris* and *Diplazon* spp (Islam *et al*. 1985).

Predators

According to De Back (1964) the potential biocontrol agents either a predator or any other organisms must have the adaptability to the different physical conditions of the stored ecosystem, high host searching capacity, high reproductive rate, high degree of host specificity, good synchronization with the host, maximum host consumption rate, ability to survive in host-free period, changes in behaviour in relation to the density and dispersal of host or its own population. Predatory insects feed on eggs, larvae or nymphs, pupae and adults of host (Khan and Selman 1996). **P**redators that attack stored product insect pests are typically very small or larger and have a short life cycle with high reproductive capacity as long as hosts available with suitable environmental conditions (Scholer and Flinn 2000). Natural enemies can be released at a single location of the store and they will find and attack pests located deep inside crevices or with grain mass, unlike chemical pesticides (Tyler *et al.* 1983) and insect pathogens (Fuxa 1993). As pest insects have not yet developed resistance to parasitoids and predators (Hokkamen *et al*. 1995), continuous research on the prey-predator relationship of different predator species are going on.

X. flavipes is commonly known as the warehouse pirate bug (Hemiptera, Anthocoridae) and is cosmopolitan in distribution (Henry 1988, Gross 1954), commonly reported from storage habitats (Jay *et al*. 1968) in association with its prey, Lepidoptera and Coleoptera (Arbogast 1978). Release of *X. flavipes* resulted in a 79-100% suppression of moth populations in small storages of peanuts, up to 99% reduction of saw-toothed grain beetle populations and 90-98% suppression of red flour beetles in a simulated peanut warehouse. According to a number of entomologists, *X. flavipes* is one of the most efficient predators, which can kill and consume many stored product insect pests (Table 1).

Table 1 Stored product insects prey of *X. flavipes*

Order/Family	Species	References
Coleoptera Anobiidae	*Lasioderma serricorne* (F.)	Abdel-Rahman *et al*.1978-79
Bostrichidae	*Rhyzopertha dominica* (F.)	Abdel- Rahman *et al*.1978-79
	Prostophanus truncatus (Horn)	Helbig 1999
Bruchidae	*Acanthoscelides obtectus* (Say)	Sing *et al.* 2008a
	Callosobruchus maculatus (F.)	Sing *et al.* 2008a
Cucujidae	*Cathartus quadricollis* (Guerin)	Press *et al*. 1979
	Cryptolestes minitus	Wen and Deng 1988
	Cryptolestes pusillus (Schon)	Brower and Press 1992
Curculionidae	*Sitophilus orzae* (L.)	Abdel-Rahman *et al*.1978-79
	Sitophilus zeamais (Motsch.)	Wen and Deng 1988
Dermestidae	*Anttagenus unicolor* (Brahm)	Lecato 1976
	Trogoderma granarium Everts	Arbogast 1978
Mycetophogidae	*Typhaea stercorea* (L.)	Brower and Press 1992
Nitidulidae	*Carpophilus dimidiatus* (F.)	Brower and Press 1992
Silvanidae	*Oryzaephilus mercator*(Fauvel)	Press *et al.* 1979
	Oryzaephilus surinamensis (L.)	Abdel-Rahman *et al*.1978-79
	Ahasversus advena (Waitl)	Brower and Press 1992
Tenebrionidae	*Latheticus orzae* (Waterh)	Tawfik *et al*. 1982
	Tribolium castaneum (Herbst)	Lecato 1976
	Tribolium confusum (Duval)	Lecato 1976
	Stegobium paniceum (L.)	Awadallah *et al*. 1986
	Polorus ratzeburgi	Wen and Deng 1988
	Zabrotes subfasciatus (B.)	Sing *et al.* 2008a
Lepidoptera Gelechiidae	*Sitotroga cerealella* (Olivier)	Lecato and Arbogast 1979
Pyralidae	*Corcyra cephalonica* (Staint)	Tawfik *et al*. 1982
	Cadra cautella (Walker)	Press *et al*. 1974
	Ephestia kuehniella (Zeller)	Abdel-Rahman *et al*. 1978-79
	Plodia interpunctella (Hubner)	Abdel-Rahman *et al*. 1978-79
	Galleria mellonella (L.)	Arbogast 1978
Hymenoptera Braconidae	*Bracon hebetor* Say	Press *et al*. 1974

Bacteria : Microbial pesticides obtained from insect pathogens or their by-products are especially valuable biocontrol agent because their toxicity to non-target animals and human is extremely low compared to other commonly used insecticides, and are safe for both the pesticide user and consumers of the treated stored products or crops (Abd-El-Aziz 2011).

Currently, many entomopathogens were used for the control of invertebrate pests of agriculture, forestry and food storage (Subramanyam *et al.* 1999, 2002, Lacey *et al*. 2001, Fang *et al*. 2002a,b, Daglish *et al*. 2003, Khashaveh *et al*. 2008, Mahdneshin *et al*. 2009). Microbial agents are useful commercially only if they do not harm other natural organisms at recommended rate of use in the environment (Florence *et al*. 2003).

Among the entomopathogens bacteria is the most effective and dominant in microbial control of insects. *Bacillus thuringensis* (Bt) is a bacterium when sprayed on or eaten by an insect host, infects the host making it sick and then causing it to die. Bt variety is very host specific and works only on the caterpillar stages of moths and butterflies and to some extents to flies. Another bacterium *Beuveria bassiana* is also found as potent biocontrol aganet against the stored product insects. The effect of two microbial bacteria *(B. thuringiensis* and *B. bassiana*) and three plant extracts were studied on three stored product insects, *P. interpunctella, E. cautella* and *E. kuehniella*, the combined treatment with extracts *B. thuringiensis* caused significant enhancement of the pathogens, increasing the mortality in almost all insects (Sabbour 2003).

Recently discovered, Spinosad is a fermentation product of actinomycete bacteria, (*Saccharopolyspora spinosa* Mertz and Yao) which showed low mammalian, and avian toxicity, short environmental persistence and prescribed to use for control of stored product insect pests including *C. pusillus* and *R. dominica* (Subramanyam *et al.* 1999, 2002, Fang *et al*. 2002a,b, Daglish *et al*. 2003, Nayak *et al*. 2005, Bonjour *et al*. 2006, Hung et *al*. 2007, Athanassiou *et al*. 2008a, Vayias *et al*. 2010, Hertlein *et al*. 2011) in many integrated pest management programs, as, Spinosad is reported to kills insects relatively more quickly than other microbial pesticides (Bret *et al*. 1997).

Discovery of Spinosad: During the late 1950's, companies including The Dow Chemical Company and Eli Lilly and Company began to actively look for naturally occurring pest control products. As a result of these efforts, a scientist from the Natural Products division of Eli Lilly while vacationing in the Caribbean in 1982 visited abandoned rum still and collected several soil samples. These samples were taken to the laboratory to determine the presence of biological activity. Three years later the fermentation products from these samples were shown to have insecticidal activity, and by 1986 Eli Lilly's scientists identified a new actinomycete bacteria producing the biologically active substances, they named it *Saccharopolyspora spinosa.* Scientists identified the most highly active metabolites of S. *spinosa* in 1987. In 1995, because of its favorable environmental and toxicological profile, Spinosad was classified by the U.S. Environmental Protection Agency (EPA) as a reduced risk product and granted an accelerated registration review. During early 1997, the first Spinosad products, Tracer® and Conserve® were approved and launched in the U.S. for use on cotton, and on turf and ornamentals, respectively.

Now a day, *S. spinosa* colonies are grown using natural products such as soybean and cottonseed meal. Computers are used to control temperature, oxygen and nutrient levels to ensure maximum production of Spinosad. In Kenya, Spinosad was first registered for use as a grain protectant in 2003, registration in the United States was achieved in early 2005, with Spinosad's labeled use rate set at 1 ppm (1 mg ai/kg of grain), and its Maximum Residue Limit (MRL) or tolerance established at 1.5 ppm (Subramanyam 2006a,b, Hertlein *et al*. 2011). Spinosad is also currently registered for grain protection in a number of other countries, but widespread commercial launch has been deferred while awaiting final MRL or tolerance approvals in a few remaining key grain-importing countries (Hertlein *et al.* 2011).

Application of Spinosad: Spinosad's suitability as a stored grain protectant has been progressively highlighted in a series of scientific publications dating from 1999 (Subramanyam *et al*. 1999a, 2002, Fang *et al.* 2002a and Mutambuki *et al*. 2002).

Since then, Spinosad has been shown to provide highly effective and long-lasting control of important stored product pests on various grains (Toews and Subramanyam 2003, Nayak *et al*. 2005, Maier *et al*. 2006, Subramanyam 2006a, b, Huang and Subramanyam 2007, Huang *et al*. 2007, Subramanyam *et al*. 2007, Daglish *et al*. 2008, Athanassiou *et al.* 2008a,b, Chintzoglou *et al*. 2008a,b, Vayias *et al*. 2010a,b).

Spinosad effectively controls both the adults and immatures of same insect species, some other species are subjected to very high rate of mortality as larvae only. Spinosad also gradually reduce the overall population of many insect pests in storage and provide a satisfactory level of long control, and has already been proved as very effective against a range of stored-grain insect species, even at lower rates than the prescribed application rate, in both laboratories (Fang *et al*. 2002a, Toews and Subramanyam 2003, Nayak *et al*. 2005, Daglish and Nayak 2006) and field tests (Maier *et al*. 2006, Subramanyam *et al*. 2007, Daglish *et al*. 2008). A large number of stored product insect pests are controlled at 1 ppm concentration of Spinosad on various commodities which already were previously determined by many scientists are shown in Appendix table 1.

Toews and Subramanyam (2004) reported that Spinosad applied to stored wheat at 1 ppm was highly toxic to the parasitoids *H. hebetor*, *T. elegans*, and *A. calandre*, bit no toxicity was found against *X. flavipes*; survival rate was 92% and the live bugs were able to reproduce under the treated same conditions. Results obtained from this laboratory study were subsequently supported by field bin trials conducted by Parker *et al*. (2004a, b) in stored sorghum and by Parker and Falconer (2004, 2005, 2006 and 2007) in stored corn, where they showed no survival of the parasitoid *A. calandre* and only limited survival of the parasitoid *C. elegans*, over a storage period 10 to 24 months. Long lasting grain protecting potentialities against different specific life stages of different stored product insect pests make Spinosad the best than other synthetic insecticides.

Background of the study

Bangladesh is one of the major rice, wheat, maize and pulse growing country in the world, but large silos, granaries and warehouses are very limited in number. So, a large quantity of grains is stockpiled in small warehouses without following any scientific steps for keeping infestation away. In rural areas grain is still stored according to traditional indigenous methods and the same store or godown is used year after year without being properly cleaned. As a result, stores become quickly infested by insect pests. Warm and humid climate of the country provides more favourable environment for insect pests in storage. Moreover, food commodities are imported to this country almost throughout the year from many other countries, and new pests get the chance to enter the country. People have little knowledge about sanitation and contamination of insect pests in storage. For this cause, heavy loss of grain is occured in storage situation.

During the storage of grains and cereals or milled products use of insecticide is limited. Even within this limited application scientific methods or processes are not followed, due to lack of proper knowledge, training and skill (Ferdous 2006). Chemical control, in spite of its so many demerits, has been the primary method of pest control in the past and is still in use. As mentioned earlier, pesticide use in Bangladesh started from mid-fifties and gained momentum in late 1960 (Alam 1991). Until 2000, 17 companies (national and international) were involved in pesticide formulation, repacking, distribution and supply through their own sales and distribution network in Bangladesh (Hasanuzzoha 2004).

It is important that the proper knowledge should be present about the toxicological, effects of pesticidal threshold and care (Ali 2004). Prevention of food losses during postharvest storage is of paramount economic importance. Integrated pest management is now a widely accepted strategy in pest control in pre-and systems. Fumigants are used in the food silos, granaries and warehouses, but in the small storages usually the chemical insecticides are used. The ban on methylbromide and phosphine would affect the insect management in this system. Though IPM programmes are running on in both public and private grain stores, but more efficient, safe, eco-friendly and cost-effective agents are needed to be searched out. So, the present work will be add some new methods into the IPM programmes to make it more convenient, cost effective, and safe food to the consumers.

Objectives of the research

Entomologists, specially insect pest specialists are trying globally to solve the insect pest problems in storage. Many studies have been conducted in different parts of the world for mentioning the predatory abilities of various beneficial insects and for identifying the best bacterium to manage the store insect pests. Predacious bug, *X. flavipes* is a very potential biocontrol agent against the insect pests in storage (Arbogast 1976, 1978, Brower and Mullen 1990, Brower and Press 1992). The bacterium Spinosad obtained the registration for use in the grain stores of USA, Australia and Canada, and from the literature it was found that *X. flavipes* can survive and can produce offsprings in Spinosad treated medium (Towes and Subramanyum 2004). In this study *C. pusillus* and *R. dominica* as a model of external and internal feeder respectively were selected as they caused serious damage and infestation of important stored commodities like paddy, rice, whole wheat, wheat flour, maize, pulses, beans, maize, sorghum in Bangladesh (Alam 1971). Keeping these views in mind, this strategic plan was designed to accomplish this research by facilitating implementation of the potential of the predator, *X. flavipes* and bacterium, Spinosad in controlling the selected insect pests of stored product by treating separately and in combinedly. In this regard, the following specific observations were carried out.

- To detect the host-stage specific effects on biological parameters of *X. flavipes* on *C. pusillus* and *R. dominica*.
- To determine the effects of Spinosad on egg hatchability, 3rd instar larvae, pupae and adults of *C. pusillus*.
- To evaluate the effects of Spinosad on egg hatchability, 3rd instar larvae, pupae and adults of *R. dominica*
- To observe the effects of Spinosad on egg hatchability, 3rd instar nymphs, pupae and adults of *X. flavipes.*
- To find out the effects of *X. flavipes* and Spinosad on the population of *C. pusillus* and *R. dominica* separately and in combinedly.

Chapter 2

Review of Literature

Effects of environmental factors on insects

Environmental factors such as temperature, moisture, humidity, air, light, sound etc. are naturally not remain constant and equal in every parts of the world and throughout the year. These factors can change the development, survivability, progeny production, abundance and behaviour of insects of stored products. Birch (1945a) showed variation in duration of developmental stages of *R. dominica* at different temperature in wheat containing 14% moisture. *R. dominica* can be developed and reproduced even in grain with low moisture content, at a level of 8% (Golebiowska 1969). Light may be of some use in luring flying insects to entrap (Banks and Fields 1995). A 5-min exposure to 1 MHz sounds at 14.5 W cm^{-2} killed all stages of *S. granarius* at 26°C in wheat but commercial application is unexpected (Banks and Fields 1995). Arthur and Casada (2010) reported that suction aeration would be more beneficial than pressure aeration for controlling insect pest in wheat stored in the southern plains of the United States. Among the environmental factors temperature and humidity are usually the most influential abiotic factors affecting the population dynamics of insects in storage system (Flinn and Hagstrum 1990).

Temperature and humidity

Insects are poikilothermic and its rate of development varied with temperature and humidity (Andrewartha and Birch 1954, Kitching 1977, Flinn and Hagstrum 1990). The optimum conditions of temperature and humidity for development of stored product pests are between 25-35ºC and 50-70% (Howe 1965a, Haines 1991, Banks and Fields 1995, Fields and Muir 1996). A threshold for an adequate air temperature for aeration is 15ºC which is the lower temperature limit of development for most stored product insect pests (Howe 1965a, Fields 1992). Burrel (1967) reported that cooling prevented further development of the heavy infestation but since the insects were not killed, this control method should be used as a preventive rather than a cure. Lowering the temperature of commodities to prevent spoilage is an ancient technology. Part of the success of the underground storages used in ancient Egypt compared with above ground stores was due to

cooler temperatures of the underground stores (Banks and Fields 1995). The effectiveness of aeration can be increased in tropical climates or in the summer in temperate climates by cooling the air with refrigeration units. Low temperature and humidity reduce the rates of development, feeding, fecundity and survivability (Longstaff and Evans 1983). Burks *et al*. (1999) reported that low temperature prevents emergence of unparsitized larvae of the rice weevil *S. oryzae* while not affecting emergence of parasitoid *A. calandrae*.

Laboratory studies on the population of a bruchid, *C. maculatus* and its parasitoid *D. basalis* have demonstrated that the fecundity, duration of adult life and developmental time depended on humidity (Owdraogo *et al*. 1996). Okamoto (1972) observed maximum number of progeny of *A. calandrae* emerged from its host, *C. chinensis* at 70% rh and also mentioned that *A. calandrae* completed its life cycle within short period on different developmental stages of *C. chinensis* at 70% rh. Cave and Gaylor (1988) noted that parasitoid development and progeny emergence were higher at 75% rh and also noted that the mean developmental time of *Telenomus reynoldsi* (Hymenoptera:seclionidae) decreased with increasing humidity. *A calandrae* reared on *Sitophilus zeamais* at 75, 56 and 40% relative humidity and found that developmental time decreased slight but significantly when humidity increased (Smith 1993).

Temperature affected the suppression of *R. dominica* parasitized by *T. elegans*, the life span of the female adults of *R. dominica* decreased with increasing temperature, ranging between a mean value of 219 days at 20ºC and 75 days at 35ºC (Faroni and Garcia 1992). The developments of *R. dominica* was completed when relative humidity was 55% and above, but the development was inhibited when rh was less than 30% at any temperature (Longstaff 1999). At 30% rh life cycle of *R. dominica* required 30 to 40 days for completion (Potter 1935). Development of *R. dominica* was completed at temperature as low as 18ºC (Long staff 1999), the population of *R. dominica* was peaked in July to September and lowest during December to March, when the average temperature was <15ºC (Peng 1984). At 30ºC temperature the life cycle of *R. dominica* required

30-40 days for completion (Potter 1935). Birch (1945a) reported that *R. dominica* is a high temperature tolerant insect and it develops from egg to adult in shortest time at 34ºC. The larvae of *R. dominica* reared on whole meal flour required 29 days to complete their development at 26ºC (Howe 1950). Wheat variety also affects development and reproduction of internal grain feeders. Almeida and Amorim (1994) studied the reproduction of *R. diminica* reared on whole and broken wheat grains (Variety OC 854 and IAC 5) at 30ºC and 70% rh and found the fecundity was greater on variety OC 854 than on IAC 5 and longevity was greater on broken grain than on whole grain. Most of the larvae of *R. dominica* moult three times but a few larvae moulted four times before developing into pre pupa, pupa and adult, the total developmental time was 44 and 45 days at 26ºC and 30ºC temperature and 56% rh respectively Elek (1994). At both temperatures the first instar larval period was the longest and most variable and the least variable stages were none feeding egg, pre pupal and pupal stages.

Arbogast (1975) stated that the population growth of *X. flavipes* was greatly influenced by temperature and humidity. Development at 20ºC was protracted and characterized by a large variance in the duration of the developmental period, the time required to reach the adult stage ranged from 44 to 74 days, but at temperature increase from 30 to 35ºC produced no further change. Thus development was completed in 21-35 days at 25ºC, 14-24 days at 30ºC and 14-21 days at 35ºC. Mortality among the immature stages was highest at 35ºC, specially when the humidity was either very low or very high. In fact only 9% eggs were hatched at 35ºC and 32% rh though most developed to the point that the red compound eyes of the nymphs were visible through the chorion. Of the 9 nymphs that hatched, 6 died during the intermediate moult, and 3 died before they reached the 2nd stadium. Mortality was also relatively high at 20ºC when the rh was 35 or 65% and at 30ºC when the rh was 96%. The life span and oviposition period were longest at 20ºC, but the maximum rate of oviposition occurred at 25 and 30ºC. The life span of adult females and the duration of the oviposition period decreased as temperature increased. Life span and oviposition period was shortened at the

temperatures by high humidity (96-98% rh) and usually at a lesser extent by low humidity (33-35% rh). Fecundity was adversely affected by low and high temperature, thus the values of (GRR) were lower at 20 and 35ºC. Fecundity was also adversely affected by high humidity at all temperatures but low humidity apparently had an adverse affect only at 20ºC.

Abdel-Rahman *et al*. (1977) reported that the optimum temperature for oviposition of *X. flavipes* was 25ºC, the survivability rates of the eggs and the nymphs were increased at 35ºC. Hatching and survival of *X. flavipes* nymphs were optimum at 25ºC, the average number of eggs laid/female/days was found to increase by raising temperature. The eggs were hatched in between 2-7 days depending on temperature, (15 to 35ºC), increase of temperature enhanced development of the adult stage of *X. flavipe*s.

Faroni and Garcia (1992) observed the incubation period of *R. dominica* at 35, 32, 28, 24 and 18ºC temperature respectively and recorded the period to be 6.5, 6.7, 8.7, 11.2, 22.8 and 27.6 days. The incubation period was considerably shorter in summer than in winter in Bangladesh (Alam 1971). Parajulee *et al*. (1995) reported that in case of the predator *L. campestris* 43% rh slightly lowered eclosion rates (70-79%) than the higher rh 58 and 75% respectively. Sex differentiation of the predator was not affected by relative humidity at 30ºC (Parajulee and Phillips 1992, 1993).

Hashem (1989) reported the incubation period was 6.5 days at 30ºC temperature. Optimal temperature for development of *S. oryzae* is generally in the range of 22ºC to 27ºC (Howe 1965a, Field 1992) and in controlled laboratory studies; results indicate greater reproduction in *S. oryzae* at 27ºC versus 32ºC (Arthur and Throne 2003) however the reverse was true for *R. dominica* (Vardeman *et al.* 2006).

Russo *et al*. (2004) reported that the egg survival rate of *X. flavipes* predating on *T. castaneum* was high at 24 and 32ºC, and larvae survived longer than the first instar at 19ºC. At 21ºC the number of the larvae completing the immature stage was extremely low. Developmental time was significantly short at 32ºC. Female lived longer but fecundity was greatly reduced at 21ºC, and egg production was maximum at 32ºC.

The adult *X. flavipes* survived no more than 4-5 days at 19 to 32ºC. The developmental time from first instar to adult decreased with the increasing temperature, at 32ºC it was 13.7 days.

Khan *et al.* (2004) reported that high temperature (studied temperature 20, 25, 30 and 35ºC) shorten the developmental periods and increased the adults eclosion and progeny productions of the pulse beetle *C. chinensis.* The sex -ratio was distorted at higher temperature, but the reproductive potential and percent of fertility of the females were increased. Temperature range 25-30ºC has been proved to be the optimum for the development of pulse beetle.

Hasen *et al.* (2004) observed that in pest *S. cerealella* maximum fecundity (124 eggs per female) was obtained at 20ºC and 80% rh; and immature survivorship was highest at 80% rh and lowest at 44% rh; and minimum developmental time required at 80% rh at 32ºC.

Rees (2004) reported that shortest developmental period of *C. pusillus* is 22 days at 35ºC and 90% rh and breeding takes place at 17.5-37.5ºC and >50% rh where as the shortes developmental period of *C. pusillus* is 21 days at 35ºC and 90% rh breeding takes place at 20-42.5ºC and 40-90% rh. Some strains of *C. ferrugineus*, *C. capensis* and *C. turcicus* are highly cold tolerant and able to survive an extended period at or below 0ºC temperature. *C. ferrugineus* and *C. capensis* are able to breed under dried conditions than other species. In warmer regions of the world *C. ferrugineus* is frequently found with *C. pusillus*.

Rahman (2006) reported that the life history parameters of *X. flavipes* on *C. pusillus* showed optimum values at 25 and 30ºC and 70 and 90% rh. In another study, Rahman *et al*. (2007) observed the effect of temperature on the predator *X. flavipes* feeding on *C. pusillus* and recorded that highest number of eggs laid/female (27.27±2.52) and egg hatching rate (88.25±2.19%) at 30ºC, and the respective lowest value were 5.43±1.19 and 30.79±4.63% at 20ºC respectively, no eggs were laid at 15ºC. Mortality among immature stages was highest (51.71±1.48%) at 35ºC and were lowest (24.25±1.14) at 25ºC. Developmental

times decreased with the increased temperature. The sex rates (% female) of *X. flavipes* were 47.04, 56.68, 51.66 and 50.07 for 20, 25, 30 and 35ºC respectively, survivorship of oviposing females was highest at 25ºC but lowest at 35ºC. Rahman and Islam (2007) observed different parameters of life cycle of *C. pusillus* at 30±1ºC and found that oviposition rate, hatching rate, adult emergence and longevity of male and female were 4.89±0.35 egg/female/day, 91.22±1.02%, 88.44%, 156.11±1.37 day and 169.67±2.52 days respectively. *C. pusillus* like warmest areas within the grain bulk and move to the center during fall and winter (Flinn and Hagstrum 1998).

Ferdous *et al.* (2009) observed some biological parameters of *X. flavipes* feeding on *T. castaneum* at 30±1ºC temperatures in the laboratory and found the mean duration of developmental period through the five nymphal instars was 20.09 days. The mean incubation and nymphal periods were 3.5 and 16.57 days respectively. The percentage of adult emergence was 68.89 and the number of eggs laid per female per day ranged from 4.38 to 4.39. The mean life span was 6.6 days in males and 20.67 days in females.

Both temperature and relative humidity considerably affect the duration of nymphal stage, longevity and oviposition of *X. flavipes* (Abdel-Rahman *et al.* 1977 and Arbogast 1978). Arbogast *et al.* (1971) and Awadullah and Tawfik (1973) reported that the number of nymphal instars of *X. flavipes* varied from 2-6, but at 30ºC there was always five instars. Preying on *P. interpunctela* at 30ºC the life cycle of the predator was completed from 14-21 days (Arbogast 1975). At this temperature the incubation period was 4-5 days (Awadullah and Tawfik 1973, Arbogast 1975, 1978). Feeding on *T. castaneum* the longevity of the male and female predator was 5-43 and 8-37 days respectively, at 30ºC (Awadullah and Tawfik 1973).

Not only the prey species, but also the diet of the prey insect affected the biology of *X. flavipes*, which was coordinated with temperature and relative humidity (Saha 2007). Optimum developmental temperature was recorded as 30ºC and 70% rh when the prey species (*T. castaneum* and *T. confusum*) were reared on agar+flour; the oviposition period was also recorded the maximum at this temperature

rearing on the same prey species feeding the same food. Whereas, mortality (%) of immature stages was recorded at 25ºC and maximum mortality was observed at 35ºC and 30% rh when the prey were reared on yeast+flour.

Yousefnezhad-Irani and Asghar (2007) evaluated toxicity of Spinosad on adults *T. castaneum* and *S. oryzae* at different temperatures and exposure intervals. A commercial formulation of Spinosad, Tracer was used against 7-14d old adults of *T. castaneum* and *S. oryzae*, at 24, 28 and 32ºC and 65±5% RH. Mortality was recorded at 24, 48 and 72h post treatment. A dose dependent response was observed in both species. Similar trend was detected between mortality and time intervals. In both species an inverse relationship between LD_{50} values and temperature was detected. Based on LD_{50} values and none overlapping of 95% CL *S. oryzae* was more susceptible to Spinosad than *T. castaneum*.

Efficacy of Spinosad against *R. dominica* was not affected by temperature and moisture, during a maximum storage period of four months Fang and Subramanyam (2003).Two field studies provide data on residue levels and efficacy against *R. dominica* on wheat stored on farm in the Midwest of the USA. In one field study, efficacy and residues of Spinosad were found to be stable during 12 months of storage with a grain temperature range from -10 to 32ºC and a moisture range from 12.4 to 23% MC (Fang *et al*. 2002a). In the second field study grain temperature ranged from <10 to 35ºC and moisture ranged from 10.7 to 22.0% MC, but Spinosad efficacy did not change despite reduces falling by about 19% during 6 months of storage (Flinn *et al*. 2004).

Daglish *et al*. (2006) reported Spinosad applied at 0.5 and 1mg/kg against *R. dominica* was completely effective for 9 months at both 55 and 70% relative humidity with 100% adult mortality after 14 days of exposure and no live F_1 adults were produced. Athanassiou *et al*. (2008 a,b,c) tested the efficacy of Spinosad against adults *R. dominica, S. oryzae*, *T. confusum* on wheat and *P. truncatus* on maize at three temperatures 20, 25 and 30ºC and two relative humidity levels 55 and 75%. The author recorded mortality of *R. dominica* and *S. oryzae* was high even at 0.01 ppm of Spinosad, reaching 100% at 55% relative humidity and 30ºC

after 21 days of exposure. In several conditions tested Spinosad efficacy notably varied according to the temperature and humidity regimes. At 1 ppm, after 7 days of exposure mortality was low <14% but two weeks later >90% of the exposed adults were dead at 30ºC. At this exposure, the increase of temperature increased mortality, while no significant differences noted between the two relative humidity levels tested (Athanassiou *et al*. 2008 a,b,c).

Biology of *C. pusillus*

Mated female of *C. pusillus* deposits her eggs in crevices in the grain or in loose farinaceous materials. Eggs are small, slender and cylindrical, a typical egg is about 0.56 mm in length and 0.16 mm in width. The egg appears shiny white when first deposited but as development proceeds, it becomes pale yellowish. The incubation period lasts for 3-4 days.

The neonates are Cigar-shaped about 0.7 mm in length, yellowish-white in colour (Alam 1991). There are seven abdominal segments which are uniform in size, a longer eighth segment and the ninth segment which bears the anal hooks. The head and the spine like appendages are reddish brown; eyes are oval, each with three elongated bristles on the inner side. The 1st instar larva is active, restless and moves forward by contracting and expanding its body. The 2nd and 3rd instar larvae are same as the 1st instars larvae but the body becomes more elongate in shape. The head is wider than the rest of the body in the first instar, but then becomes narrower than the rest of the body in the later instars. After the first instar, there is a pair of lateral setae on the abdominal segments 1-7.

The mature 4th instar larva is about 3-4 mm in length and 0.33 mm in width, the body is milky white except the head and anal region which appear brownish. Two pale brownish longitudinal lines precede mid-dorsally starting from 1st thoracic segment ending at the 7th abdominal segment. Antennae three jointed, tip of each has an elongated hair and two short bristles. Mouth parts are distinct.

The mature 4th instar larva enters a pre-pupae stage, where the body becomes broader and silk glands forms on the prothorax. These glands are used to spin the silk cocoon in which pupation occurs (CABI Crop Protection Compendium 2008).

The pupal stage occurs within a silken cocoon. The cocoon remains attached to the larval food, and food particles may adhere to the surface of the cocoon. The pupa is of the exarate form, in which the legs and wings are free from the body and the abdomen is movable. The newly formed pupa is white, but becomes darker with age. The pupal cocoon is slightly longer than the mature larva (CABI Crop Protection Compendium 2008). Six elongated hairs at the frontal regions and brownish circular eyes are present. The male pupa is smaller in size (1.57 mm in length) and is devoid of the finger-like lobes but female pupa is larger (1.75 mm in length) having characteristic finger-like lobe, the gonapophyses. The pupal stage lasts for 4-5days. The freshly formed pupa is light yellowish which gradually turns to pale reddish before adult emergence.

Newly emerged adults are minute, flattened, oblong and light-brown in colour, but rapidly become reddish-brown. The body is about 1.5-2 mm long, depressed and elongate. Tarsi are five-segmented, except for the male hind tarsi which are four-segmented. However, as the basal segment is rather small, it may be only possible to count 4 segments on each tarsus or 3 on the male hind tarsus. The female antennae are about half the length of the body, while in the male they are about two thirds of the length of the body. Elytra have five parallel ridges. The biology of *C. pusillus* was observed at 30±1ºC temperature and 70±5 % relative humidity (Ahmed *et al*. 1994).

Sex differences: Male and female adults of *C. pusillus* can be identified by genitalia dissection under the microscope, the male genitalia is two banned accessory sclerites, curved shaped which middle is expand and both ends are gradually pin like and about 0.08 mm in size; whereas female genitalia is two closed banned accessory sclarites, long tube shaped which one end is spiracle and other end is open and about 0.125 mm in size with sclerotised part of burse (Figure -3) (Andras Szito 2011).

Biology of *R. dominica*

Mated female of *R. dominica* laid eggs singly or a raft or cluster among the frass or only on the food medium. The frass consists of half chewed or cutting food materials which are produced by *R. dominica.* The egg is opaque, whitish in colour with a waxy appearance when freshly laid, but after a little while takes on a pinkish colour (Kucerova and Stejskal 2008). The egg is about 0.5-0.6 mm in length and 0.2-0.25 mm in diameter (Thompson 1966, LeCato and Flaherty 1974 and Kucerova and Stejskal 2008). The average weight of 10 eggs was determined as 3×10 to 6×10 mg. The egg surface appears smooth, but scanning electron micrograph (SEM) magnification reveals a distinct granulated microstructure. The chorion, which has two layers, is about 2.7 µm in thickness (Kucerova and Stejskal 2008). The dark rusty tips of the mandibles and the abdominal thorn of the larva are visible through the chorion at the end of egg development (Kucerova and Stejskal 2008).

The mean daily oviposition rate is 10.4 eggs/day with a range of 4-15 eggs when the beetles are reared at 25°C and 70% rh (Howe 1950), the rate was somewhat lower, 6.5 eggs/day, for beetles reared at 29°C and 75% rh (Thompson 1966). The maximum number of eggs laid by female *R. dominica* in a day varies between 33 (Thompson 1966) and 45 eggs (Howe 1950). The pre-oviposition period was found to vary from 6 days (Thompson 1966) to 15 days (Schwardt 1933), and the oviposition period varies from 43 days at 25°C and 70% rh (Howe 1950) to 4 months at 34°C and 70% rh (Birch 1945 b,c).

The first instar larva is campodeiform, white or light yellowish in colour, with mandibulate mouthparts about 0.78 mm long and 0.13 mm wide across the head capsule (Potter 1935). The larva is very active, and moves rapidly about the grains (Winterbottom 1922). A terminal median spine is present in the first instar, (Potter 1935 and Howe 1950) has a short antennae beset with hairs. Thorax was three segmented and abdomen ten segmented. A number of long hairs were present dorsally and laterally on the abdominal segments. The larva often moved with a looping action. The duration of first instar larva was about 6 to 12 day. The 2nd instar larva losts the dorsal median spine at the posterior region of the body.

The second instar is similar in shape to the first instar, but larger in size (Winterbottom 1922), being about 1.1 mm long, and 0.17 mm across the head capsule (Potter 1935). The thoracic region is more differentiated from the abdominal region;. the last abdominal segment was horse shoe shaped, the two ends of the horse shoe slightly protuberated. The second instar larva lasts for 4-7days.

Third instar larva is recurved but they are able to straighten out. The thorax is somewhat larger in circumference than the abdomen, prothorax markedly enlarged. End of the abdominal segment showed a well-marked anal furrow. In its natural recurved state it has distinct lateral hypoplueral folds. Ventral surface of the body is flattened. The average body length and diameter of the head capsule of the third instar are 2.04 and 0.26 mm, respectively (Potter 1935).The third instars larva moults after 4-6days.

Head of the fourth instar larva is retracted into the thorax and longer than width, antennae are present at the sides of the head. The body is recurved with distinct pleural folds on the sides of the flattened ventral surface. Regularly arranged short hairs are present on body surface. Fifth to eighth segments of the body are much extended. The legs of pro-meso- and meta thoracic segments are equal in size; each leg is three segmented highly curved brown claw. Ventral region is whitish, head is light brown, and mandibles are dark-brown or nearly black (Chittenden 1911 and Winterbottom 1922). Length of the mature fourth instar is approximately 3.2 mm and the head is approximately 0.41 mm in diameter. The fourth instars larva lasts for 4-8days.

The pre-pupal stage does not involve moulting and can be distinguished from the mature larvae by their more elongated cylindrical shape and extended head as if forced out by the pressure of the fluid in the body (Potter 1935). It is a none feeding stage, relatively immobile, but is capable of limited wriggling. The average body length is 3.15 mm and diameter of the head capsule is 0.5 mm. The duration of the prepupal stage is very short, lasting about 1½ days. The pupae of *R. dominica* are exarate, i.e., the appendages are not fixed to the body. They are inactive, and body movement is limited to the abdominal segments. Young pupae are whitish in colour, later, brown pigment is laid down in the eye and mouthparts (Winterbottom 1922). Average lengths of the body and head capsule are 3.9 and 0.6 mm respectively. The rate of pupal development is proportional to temperature. At 70% rh, the pupal stage is completed in about 8 days at 25°C and 5-6 days at 28°C (Howe 1950). The optimum conditions for rapid development of the pupal stage are 34 °C and 70% rh in wheat kernels of 14% moisture content (Birch 1953, Birch 1945a,b,c); under these conditions, development of the pupa is completed in 4 days. The pupa exhibits the characteristic depressed head and enlarged thorax of the adult. The pupa lies in a cell excavated by the larva inside the grain. However, a pupal cell is not required for successful completion of the pupal stage (Schwardt 1933). If larvae are reared on flour, pupation occurs in an oval-shaped cavity hollowed out by the larva in the floury material (Winterbottom 1922).

During the pupal stage, it is possible to distinguish between the sexes. Sexual dimorphism is displayed at the tip of the abdomen. The genitalia of the females are divergent, three-segmented, and protuberant, whereas those of the males are convergent, two-segmented, and protuberant (Potter 1935 and Halstead 1963).

The newly formed adults emerge from the kernel by chewing through the outer grain layer and might go without food for about 3-5 days after emergence (Schwardt 1933). The adult beetle is 2-3 mm long and 0.8-1.0 mm in wide. Fresh body weight ranges from 0.99 to 1.38 mg, with a mean about 1.20 mg (Edde and Phillips 2006b). The insect is reddish-brown to dark brown in colour. The anterior margin of the dome-shaped thorax is crenulate, the surface of the thorax and the elytra are pitted. The pits on the elytra are arranged in 10-11 longitudinal punctures, giving them a striated appearance (Winterbottom 1922, Beiriger and Sites 1996). The pits become smaller toward the posterior of the abdomen. Each elytron has short setae that curve backward (Beiriger and Sites 1996). The elytra are slightly convex and there are no carinae or other protuberances to the declivity. The antenna is short, ten-segmented, and has a terminal three-jointed loose club (Chittenden 1911 and Chujo 1958).

Mean longevity of adult male and female *R. dominica* fed on wheat kernels at 28°C and 65% rh is 26 and 17 weeks respectively, about 4% of the male and 3% of female beetles tested in study lived for approximately 52 weeks (Edde and Phillips 2006b). Birch (1953) found that female *R. dominica* reared at 32.3°C and 70% rh on wheat kernels lived for 17.2 weeks on the average. However, mean longevity of male beetles in Birch's experiment was 20 weeks, which is 6 weeks shorter than observed by Edde and Phillips (2006b). This difference may relate to the fact that Birch kept males and females together while Edde and Phillips kept them separately. The extra resources spent in courtship may have shortened the lifespan of the male beetles in Birch (1953). Mean longevity values of starved adult male and female were 5.7 and 4.7 days, respectively (Edde and Phillips 2006b). Negative effects on reproduction and movement of adult *R. dominica* may occur

after 4 days of starvation (Daglish 2006 and Nguyen *et al.* 2008). The effects of starvation are more pronounced on female *R. dominica***,** presumably due to the greater energy demand on them for reproduction (Nguyen *et al.* 2008).

Sex differences: Adults of both sexes can be reliably separated by using the methods of Crombie (1941). Working under a dissecting microscope, the last three ventral abdominal segments of a live or freshly killed specimen can be gently squeezed until the tips of the genitalia appear. Often, squeezing may not be necessary for sexing female *R. dominica* because the tips of female genitalia are slightly extruded and easily discernible (Halstead 1963). In order to reduce mortality caused by squeezing, the insect can be sexed at the immature stage using the sexual dimorphism as described above for the pupal stage. A major setback to sexing the pupae is that it is found within whole grain kernels, making the collection for sexing difficult. This problem could be minimized by rearing the pupae on ground media of particle size between 1.4 and 2.0 mm diameter, which allows pupae to be removed from the medium easily, get sexed, and held for adult emergence (Cline 1973, Longstaff and Starick 1989). For males pupae, the genital papillae are convergent, 2- segmented and not protoberant while they are divergent, 3-segmented and protuberant while they are in females (Figure 4) (Potter 1933, Halstead 1963)

Biology of *X. flavipes*

Mated female of *X. flavipes* lays eggs on the food medium, scattered loosely throughout the habitat. Eggs are translucent, milky, elongate-oval and blackish with yellow spots. The egg ranged from 0.55 mm in length, the posterior end is broader than the anterior end. The anterior end is capped by a nearly circular operculum surrounded by an expanded rim of chorion. As eggs develop, they acquire a pale brownish colour. The egg hatchs in 4-6 days at 30±0.5ºC temperature and 70±0.5 % relative humidity.

Descriptions of the various life stages of *X. flavipes* is provided by Arbogast *et al* (1971) and Awadallah and Tawfik (1972). The nymph emerges by forcing open the operculum which usually remains attached to the shell by the embryonic cuticle. The 1st instar nymph looks pale brownish-yellow, lightly pigmented, with reddish eyes, antennae and legs. The nymph is about 0.60 mm in length and 0.09 mm in head capsule width. The nymph becomes large in size after the first moult and wings are visible. The developing ocelli appear after the second moult as small orange-red spots. Extensive black colouration first appears in the fourth instar, which is darker in the fifth. Fifth instar nymphs are dark brownish-yellow coloured with black tings on head and thorax. Black spots are present on the abdomen, wing pads are developed and extended up to metathorax. The 5th instar mature nymphs are about 1.50 mm in length and 0.25 mm in width. The mature nymphs are more predaceous than the immature nymphs.

The most sharply pronounced modifications are concentrated in the last moult of the 5th instars nymph to the adult. The adults are shining brownish black with irregularly distributed pale setae. The rostrum, antennae and legs appeared to be brownish yellow. Pronotum is slightly convex, sautellum is raised anteriorly and antennae are four segmented. Setae present on the third and fourth segments of the antennae.

Sex differences: Sexes of the adults are distinguished by the shape of the abdomen. The abdomen is bilaterally symmetrical in females, and in males it is notched on the left side of segments 8 and 9. The aedeagus of male arises within

the 9th segment and is directed to the left (Arbogast 1978). The piercing aedaegus of a male and abdomen of mated female of *X. flavipes* are shown in figure 5 (Backhouse *et al.* 2012). The females are larger than the males. Total developmental periods from egg to adult emergence of *X. flavipes* are 14-18 days. Both brachypterous and macropterous forms occur in *X. flavipes*, although the short-winged form was found to be most common in a sampled population (Arbogast 1978).

Managenemts techniques of *C. pusillus* and *R. dominica*

Insect identification programs can indicate the presence and abundance of particular species (Vela- Coffier *et al*. 1987, Reed *et al*. 1991 and Hagstrum 1998) and can reduce the illegal use of pesticides. In general, losses can be minimized when infestations are quickly identified and appropriate control measures implemented

(Abd-El-Aziz 2011). Many techniques are conducted from the beginning to still now for identification of insect pests in pre and post-harvest cereals and other commodities in storage and fields by many scientists viz., Burkholder and Ma 1985, Campbell *et al*. 2002, Loschiavo and Atkinson 1967, Loschiavo and Smith 1986, Lippert and Hagstrum 1987, Vela Coffier *et al*. 1997, Wakefield and Cogan 1999, Towes *et al*. 2005 Flinn *et al*. 2007, Schatzki and Fine 1988, Shumon *et al*. 1996, Panford 1987, Atui 2006, Magan and Evans 2000, Flinn *et al*. 2009 and recently, Computer based pest management (Abd El Aziz 2011) are used to identify the presence and manage the population of insect species in storages.

Cryptolestes pusillus

There have been several field trials (Reed and Harner 1998a, b, Casada *et al*. 2002, Arthur and Casada 2005) and modeling simulation studies (Flinn *et al*. 1997, Arthur and Flinn 2000) showing that an initial summer cooling cycle cooling to a level of about 24ºC, followed by second cooling to 15ºC in early autumn, severely limits population growth of stored product insects including *C. pusillus* in stored wheat.

Oliveira *et al*. (2003) reported that the biocontrol potential of the mite species *Acarophenax lacunatus* was able to parasitize eggs of *C. ferrugineus*. The mite caused significant reduction of 26% in the larval populations of *C. ferrugineus*. *A. lacunatus* can occur on colonies of *C. ferrugineus* (Cross and Krantz 1964, Faroni 1992).

Rahman (2006) observed bionomics of *Plastanoxus westwoodi* (Kieffer) on *C. pusillus* in the laboratory and reported that larval and pupal stages of *C. pusillus* are generally parasitized by beythylid parasitoid *P. westwoodi*. Fifty mated female of *P. westwoodi* suppressed the population of *C. pusillus* up to 81.31±4.54%. This parasitoid can be used as biocontrol agent of *C. pusillus* for easy handling, inexpensive culture techniques and their optimum progeny production. Female parasitoids are more active to suppress *C. pusillus* than male.

Effectiveness of aeration for controlling insect pest populations including *Cryptolestes* spp. in wheat during the summer months in the central and southern plains of the United States had reported (Reed and Harner 1998a, b, Arthur and

Casada 2005) and in rice stored in Arkansas (Ranali *et al*. 2002) and in eastern Texas (Arthur *et al.* 2008).

Arthur *et al.* (2010) conducted field trials in metal wheat storage bins to determine whether pressure aeration, pushing ambient air from the bottom or suction aeration, pulling air down from the top would be more effective at cooling the wheat mass and there by limiting insect population growth. Aeration was accomplished at an approximate air flow of 0.22 to 0. 31 m3/ min/t and was done by adjusting thermostatic controllers to operate the aeration fans when ambient temperatures fell below specified thresholds. Summer and autumn cooling cycles using suction aeration cooled the warmest part of the bin, the top of the grain mass always remained warmer than with suction aeration. This cooling effects was most pronounced in the upper surface of the grain mass and insect pest populations as measured by pith fall traps were consistently less in bins with suction versus pressure aeration. Results seem to indicate that suction aeration would be more beneficial than pressure aeration for controlling insect pests including *C. pusillus* in wheat stored in the southern plains of the United States.

Rhyzopertha dominica

The mite species *Acarophenax lacunatus* showed highest rate of parasitism on the egg of *R. dominica* and *T. castaneum*, leading to a significant decrease of populations of both species, and also reduced wheat weight loss (Cross and Krantz 1964) at 28±2ºC temperature and 65±5% relative humidity for 40 days (Oliveira *et al*. 2003). The mite caused a significant reduction of 61% in the larval population of *R. dominica*; Faroni (1992) and Faroni *et al.* (2000, 2001) stated that *A. lacunatus* is a potential biocontrol agent to regulate *R. dominica.*

Biocontrol agent used against stored grain insects will have to interact with insecticides used as grain protectants due to their heavy use especially in tropical areas. Therefore, the interaction between the pyrethroid insecticides bifenthrin and deltamethrin and *A. lacunatus* was assessed against *R. dominica* in a range of mite densities and insecticide doses for each compound. Despite the mite species presence on all insecticide doses, the lowest instantaneous rate of increase of

A. lacunatus was recorded with the highest doses of insecticides. The presence of the biological control agent at all doses of both insecticides suggests its tolerance to these compounds, which is a positive point for IPM programs. The biology of *A. lacunatus* and its potential as regulatory agent of population of *R. dominica* were reported by Faroni *et al*. (2000, 2001). There are reports of registance to organophosphates and the pyrethroid deltamethrin in Brazillian populations of *R. dominica* (Guedes *et al*. 1996, 1997, Lorini and Galley 1999, 2000).

Mahal *et al*. (2005) observed potentiality of *A. calandrae* in controlling residual populations of *R. dominica* in wheat grain in bulk at 46 m² room and found that 50 pairs of *A. calandrae* suppressed 83% of *R. dominica* in June to July and 74% in August- September.

The interaction between *A. lacunatus* and *A. calandrae* may be the promising tool for the integrated pest management of *R. dominica* (Goncalves *et al.* 2006) The use of *A. calandrae* alone demonstrated a low instantaneous rate of increase of *R. dominica* and a high protection of the wheat grains during 60 days storage. The association of *A. calandrae* with *A. lacunatus* led to the lowest number of immatures of *R. dominica*.

Chanbang *et al*. (2007) conducted laboratory trials to determine the effectiveness of diatomaceous earth (DE) against *R. dominica* on rough rice stored for eight weeks. DE products (Two) were equitoxic to pest insect. Mortality increased from 15.8 to 69.2% depending on the exposure interval. There was extensive progeny production in all treatments (including controls) and more progeny were produced at 32 than at 27ºC. Results showed that the two DE products did not completely suppress *R. dominica* on rough rice and combination treatments with another insecticide may be necessary to give complete control.

Efficacy of DE generally declines with increased relative humidity or grain moisture content and although mortality generally increases with temperature, mixed results have been reported for specific insect species and DE products (Arthur 2000, Fields and Korunic 2000, Vayias and Athanassiou 2004, Athanassiou *et al*. 2005). Other studies have repeatedly noted variation in

mortality of *R. dominica* and other stored grain insects exposed to commercial DE formulations, depending on grain type (Subramanyam *et al*. 1994, Arthur and Throne 2003, Arthur 2004, Athanassiou and Kavallieratos 2005).Tests with newer formulations of DE also show efficacy toward *R. dominica* on stored wheat (Kavallieratoes *et al*. 2005, Athanassiou and Kavallieratos 2005), and the research is still going on.

The natural enemy of the stored product insects, *C. elegans* was found to be very effective for suppressing *R. dominica* populations with augmentative release (Flinn *et al.* 1994, 1996). Flinn *et al.* (1996) observed 98 and 91% suppression of *R. dominica* compared to the control by *C. elagans* in 1993 and 1994 respectively.

Spinosad remained effective against house flies as well as against its cyclodiene resistant strain (Scott 1998, Scott *et al*. 2000), *R. dominica, S. oryzae* (Toews and Subramanyam 2003), *O surinamensis* (Fang *et al*. 2002). *R. dominica* is a leading pest species, which has developed resistance to several registered grain protectants over the years (Champ and Dyte 1976, Herron 1990, Collins *et al*. 1993, Zettler and Cuperus 1990, Collins 1998). Malathion resistant *R. dominica* strains are common in Australia and these insects are also cross-resistant to the organophosphorous compounds. Most of the results revealed that Spinosad was very effective *R. dominica* with an application rate 1mg/kg achieving complete control of all strains of this species (Fang *et al*. 2002a, b, Fang and Subramanyam 2003, Toews and Subramanyam 2003, Nayak *et al.* 2005).

Predatory abilities of *X. flavipes*

This predacious bug is considered to be a promising polyphagous candidate for biological control programmes as it has some advantageous characteristics. The minimum prey requirement for complete development is low. A high proportion of nymphae (33%) developed to adulthood with a food supply of only one larva per week (Lecato and Collins 1976). The predator is supposed to be easy to handle, because it uses cannibalism as its strategy for survival in times without prey. Arbogast (1978) found that the first nymphal stage is necessary prey for nymphae and adults in order to achieve complete development. However these

survival strategies are very important for this insect since it can only survive for a very short time without food (Arbogast *et al.* 1977). After a starvation period of 5 days, 95% of adult *X. flavipes* were dead. Brower and Press (1992) reported that the hemipteran predators suppressed 70-100% stored product pest population in the laboratory condition. Predatory hemipteran may invade prey both as immature and adults (Pedigo 1996). *X. flavipes* has shown promise as an effective biological control agent by suppressive growth of stored product insects in small (Jay *et al.* 1968) as well as moderate (Press *et al.* 1975, Arbogast 1976) volumes of commodities. Arbogast (1978) stated that *X. flavipes* has a high capacity to increase in number relative to its prey. Lecato and Collins (1976) mentioned that *X. flavipes* destroys large quantities of prey when prey is abundant. Awadallah *et al.* (1986) reported that *X. flavipes* when preyed only on the larvae of different pest insects, the predator fed on 105 larvae of *Corcyra cephalonica*, 112 larvae of *T. confusum*, 30 larvae of *Stegobium panicerum,* 148 larvae of *Lasioderma serricorni*,during 43 days of life span.

X. flavipes had been reported to suppress effectively the population of a number of direct grain feeders and also an efficient predator on different secondary feeders (Arbogast 1978 and Helbig 1999). *X. flavipes* efficiently predates on both coleopteran and lepidopteron pests (Arbogast 1975, Abdel Rahman *et al.* 1978-79, Brower and Mullen 1990, Jay *et al.* 1968, Lecato and Arbogast 1979, Lecato *et al.* 1977). *X. flavipes* developed faster, lived longer as an adult, survived better in the immature stages and laid more eggs when fed coleopteran larvae rather than lepidopteran larvae (Abdel Rahman *et al.* 1978-79). Ahmed *et al.* (2004) reported that a female *X. flavipes* killed 47.3±4.88 larvae of *C. pusillus* and laid 20.1±1.66 eggs during her life time (25.4±1.26 days).

Arbogast (1976) reported that the Sawtoothed grain beetle *O. surinamensis* population growth reduced 95% (after 16 weeks) when only 5 pairs of *X. flavipes* were introduced. Press *et al.* (1975) observed that *X. flavipes* effectively suppressed *T. castaneum* on 6-bu (ca, 227 liter) lots of farmers stock of peanuts contained in 64 cu-ft (ca 1812 liter) bins. *X. flavipes* suppressed about 97%

population of almond moth, *Cadra cautella* (Press 1989), and 90.4% population of *C. pusillus*, when only 50 pairs of *X. flavipes* were introduced (Brower and Press 1992).

Ishijima *et al.* (2005) studied the suppression effects of two predatory bugs, *X. flavipes* and *Joppeicus paradoxus* on the stored product insect *T. confusum* on whole wheat flour and found that the predators reduced 33% population of *T. confusum* after 25 days.

Lecato and Davis (1973) observed and reported that early and late instar nymphs and adult *X. flavipes* when exposed simultaneously to early or late instar larvae of the Indian meal moth, *P. interpunctella*, the red flour beetle, *T. castaneum*, the sawtoothed grain beetle, *O. surinamensi*s and the Cigarette beetle, *L. serricorne*. *X. flavipes* preyed on early and late instars of all species but the number killed depended partly on the size of the prey and sourse other factors. Press *et al.* (1973) demonstrated that *X. flavipes* can be reared on frozen or irradiated eggs of the indian meal moth *P. interpunctella*.

Lecato and Davis (1973 cit by press *et al.* 1974) found that *X. flavipes* preferred small larvae of large species and large larvae of small species.

Donnelly and Phillips (2001) compared the functional responses of *X. flavipes* with different densities of the prey species viz., *T. castaneum*, *O. surinamensis*, *P. interpunctella* and *R. dominica* in two different habitats, empty glass jars and glass jars filled with wheat kernels that were designed to stimulate more natural conditions in stored grain. Differences in the functional response of *X. flavipes* to all combinations of prey densities and grain conditions were compared with the predicted functional response curves from Holling's type 1 and type 11 models and to Hossell's type 111 model. The functional response of *X. flavipes* was best described by Holling's type 11 model, but a type 111 response occurred with prey that were more difficult to subdue, such as larvae. Numbers of prey attacked by females were greater than those *T. castaneum* attacked by males ($P<0.05$) in both habitats for some of the prey life stages and species. The maximum attack rates for

the different prey species in empty glass jars over 24h were as follows *T. castaneum* 27.3 small larvae, 1.6 large larvae *O. surinamensis* 24.3 small larvae, 17.4 large larvae, *P. interpunctella* 27.2 eggs, 23.7 small larvae, *R. dominica* 26.4 eggs and 16.6 internally feeding larvae. The maximum number killed for the different prey species in glass jars containing wheat over 48h were as follows *O. surinamensis* 13.7 small larvae, 12.8 large larvae, *P. interpunctella* 41.4 eggs, 14.7 small larvae, *R. dominica* 22.0 eggs and 12.1 internally feeding larvae. Experiments with wheat filled jars showed that single *X. flavipes* could locate and kill 27.1 out of 50 *P. interpunctella* eggs and 8.1 out of 10 *R. dominica* larvae inside kernel mixed into ≈ 18000 kernels of wheat in 48h.

Sing (1979) studied the functional response of *X. flavipes* to several different bruchid species infesting legumes. The authors reported that the true bug *X. flavipes* exhibited a type 11 functional response to the majority of cosmopolitan bruchid species evaluated when data were fit to Holling's disc equation. A negative correlation was detected between mean pest species body weight and rate of predation. The rate of attack on adult prey was quite low but fairly consistent with the large sized female predators generally more effective. The eggs and neonate larvae of *Acanthoscelides obtectus* were only accessible immature stages among all prey species examined. Predation on *A. obtectus* eggs and larvae was higher than on any adult bruchids. Mean predator kill of *A. obtectus* immature stages was 40 first instars or 10-20 eggs per 24h interval.

X. flavipes were successful at locating and killing *R. dominica* larvae that were feeding inside wheat kernels. In empty dish trials were observed and found that predators examined infested wheat kernels until the beetle larva's entrance hole was found, then the predator would insert its stylet through the entrance hole to bite and feed on the prey. Predators successfully found and killed nearly half of the *R. dominica* larvae feeding inside the small numbers of kernels dispersed among the 18000 kernels of wheat jar experiments (Donnelly and Phillips 2001).

Murata *et al.* (2007) evaluated the suppression of the confused flour beetle *T. confusum* by the anthocorid bug *X. flavipes* and the reduviid bug *Amphibolus venator.*

After 25 days *X. flavipes* alone showed 96.9% suppression of *T. confusum*, and *A. venator* alone showed 76.2% suppression, and both the predator bugs together showed 95.6% suppression of pest population. The rate of loss of whole wheat flour as an index of damage caused by *T. confusum* were 2.7, 6.4, 3.6 and 11.7% in *X flavipes* adults, *A. venator* adults, *A. venator* adults +*X. flavipes* adults, and control respectively. Furthermore *A. venator* attacked *X. flavipes* adults but not *X. flavipes* nymph. The author discussed on possibility of using both *X. flavipes* and *A. venator* against *T. confusum.*

X. flavipes preys on *eggs* of *C. cautella* (Brower and Mullen 1990), larvae of *T. castaneum* and *P. truncates* (Helbig 1999, Russo *et al.* 2004) and eggs and larvae of *R. dominica* and *P. interpunctella* (Brower and Press 1992). *X. flavipes* reduced population growth of *O. surinamensis* by 95% (Arbogast 1976), small population of *C. cautella* and *P. interpunctella* by more than 70% (Brower and Mullen 1990) and several species of small beetles including *T. castaneum*,*Typhaea stercorea* and *C. pusillus* by 100% (Brower and Press 1992). *X. flavipes* is one of the most dominant predators of many stored product insect pests including *C. pusillus* (Rahman *et al.* 2007). Although *X. flavipes* is the most successful against small sized externally developing prey particularly accessible eggs and early larval stages those are neither heavily selerotized nor overly hirsute (Lecato and Davis 1973).

Stimulated *X. flavipes* direct a scent gland exudates thought to be defensive in nature over a wide area (Remold 1963). The consistent rapid liquefaction of large adult bruchid prey after attack by *X. flavipes* suggests that the predator utilizes enzymatic salivary venom for extra oral digestion a common strategy of predaceous arthropods preying on large prey with intractable cuticles (Cohen 1995). The potential of a knockdown or disorientation effect from the scent gland secretion (Phillips *et al.* 1995) combined with the catastrophic disruption of neuromuscular function (Blum 1981) caused by the injection of salivary venom could account for the predators ability to kill the comparatively more difficult adult bruchid prey.

Imamura *et al.* (2008) observed biological aspects and predatory abilities of predatory bugs that prey on stored product insects. Biological controls in stored products are being regarded with increasing interest since they are nontoxic and do not damage human health or the environment. Several species of predatory bugs have been studied as biological control agents. Specifically *X. flavipes* is advantageous because it has high population increase capacity and wide distribution. *X. flavipes* has been reported to suppress population of small insects but it can not predate large insects and internal grain feeding insects. As *A. venator*, *P. biannulipes* and *J. paradoxus* can attack large insects.

Ishijima *et al.* (2005) conducted laboratory experiment to test the suppression effects of *X. flavipes* and another predatory bug *J. paradoxus*, whose adults can attack large prey. The reduction rates of *T. confusum* populations with the release of *X. flavipes* and *J. paradoxus* were 97% and 67% respectively. In the *J. paradoxus* treated groups, *T. confusum* adults were completely eliminated by *J. paradoxus* adults. However, when *X. flavipes* and *J. paradoxus* were released simultaneously the reduction rate was only 35%. Intraguiled predation has also been reported between *X. flavipes* and *Bracon hebetor* (Say) which is a parasitoid of pyralid moth larvae (Press *et al.* 1974). The reduction rates of *P. interpunctella* with the release of *B. hebetor* and *X. flavipes* were 74% and 22% respectively. When *B. hebetor* and *X. flavipes* were released simultaneously, the reduction rate was 52.6%. The number of *B. hebetor* was also reduced when *X. flavipes* was present indicating that *X. flavipes* had fed on *B. hebetor* as well.

The biocontrol efficacy of the anthocorids *Lyctocoris campestris* (Parajulee *et al.* 1994) and *X. flavipes* (Lecato 1976, Lecato *et al.* 1977, Brower and Press 1992, Brower *et al.* 1996, Donnelly and Phillips 2001) against stored product pests under a variety of environmental conditions is well documented although evaluations with bruchid prey have been comparatively limited.

Sing (1979) and Sing and Arbogast (2008a,b) evaluated *X. flavipes* predation under highly simplified conditions that excluded assessing the potential for the stored commodity, predator density and the lag between infestation and predator introduction to confound host finding and attack success. Intra specific competition

and prey predator population oscillation as influenced by predator density and the timing of predator introduction to the stored commodity would also play an important role in the development of an effective treatment protocol for any operational use of *X. flavipes* to manage bruchid infestations of stored legumes.

Predative association with either one species feeding on another or both species feeding on each other may occur in grain silos and predation among stored grain beetles on the trophic level has been described extensive in laboratory experiments (Lefkovitch 1968, Sokoloff 1974, Ciesielska 1975, Lecato 1975, Suresh *et al.* 2001, Hulasare *et al.* 2005).

Ferdous *et al.* (2009) studied some biological parameter of *X. flavipes* feeding on *T. castaneum* in the laboratory. A single *X. flavipes* consumed 45 larvae of *T. castaneum* in its 1st, 2nd and 3rd nymphal instars. One predator can consume 135 larvae and 56 pupae on *T. castaneum* in its total life time. A mass culture technique of *X. flavipes* on *T. castaneum* was studied under laboratory conditions. The predator separately produced 1974.67 F_1 progeny from a single release of 50 mated females. The percentage of female predator in the culture was 48.89. A single predator in its five nymphal stages consumed 48-64 prey larvae of *C. pusillus* (Rahman 2006). Saha (2007) reported that the highest fecundity of *X. flavipes* was found to be 39.6 and 39.4 when *T. castaneum* and *T. confusum* fed on agar +flour respectively. Rahman (2006) reported an average of 677.0 adults of *X. flavipes* was mass produced after 45 days feeding on *C. pusillus*. Islam and Kabir (1992) described the mass culture technique of another pteromalid parasitoid *Dinarmus basalis* on *C. chinensis* in the laboratory.

An ideal bacterium: Spinosad is a reduced risk broad-spectrum bacterium, commercially available product from Dow Agro Sciences (Indianapolis, Indiana, USA). Spinosad is derived by fermentation from a naturally-occurring soil actinomycete, *Saccharopolyspora spinosa* (Mertz and Yao 1990) (Bacteria: Actinobacteridae) (Boek *et al*. 1994), belongs to a new class of insecticides called the NATURALYTES. As these products are created by biosynthesis of *S. spinosa*, so Spinosad has been classified as a bioinsecticide (Copping and Menn 2000).

Spinosad is active after contact (Spark *et al.* 1999), It affects nicotinic acetylcholine and gamma amino butyric acid (GABA) receptor sites of the insects nervous system and so far has proved non-cross-resistant to any other known insecticide (Salgado and Sparks 2005). In addition, Spinosad exhibits low mammalian toxicity and a high favorable environmental profile (Thompson *et al*. 2000, Cleveland *et al.* 2001), and no adverse effects on predatory insects such as ladybirds, lacewings, big eyed bugs or minute pirate bugs (Dow Elanco 1994, Williams *et al*. 2003, Stark *et al.* 2004). Spinosad's impact on beneficial insect populations under field conditions are typically short lived and followed by rapid recovery (Williams *et al*. 2003, Miles and Eelen 2006). In the environment, Spinosad degrades when exposed to sunlight and is quickly metabolized when washed into soil (Saunders and Bret 1997, Liu *et al*. 1999). Moreover, Spinosad residues are highly stable on grains stored in bins, with a length of protection ranging from 6 months to 2 years (Hertlein *et al*. 2011). All these attributes make Spinosad an ideal bacterium for use in the stored product integrated pest management and insecticide resistance management programs.

Chemical description of Spinosad

The chemical name of Spinosad is Spinosyn A and Spinosyn D. Chemical structures of Spinosad and naturally occurring mixture of Spinosyns A and D are shown below (Figure 4 and 5). When the bacterium *S. spinosa* is allowed to grow aerobically in an aqueous growth medium, it produces a number of biologically active metabolites called Spinosyn, which are large complex molecules containing mostly carbon, hydrogen and oxygen arranged in a unique 4-ringed system, one ring of which is a macrocyclic lactone. The 4-ringed system has two sugar molecules attached. About 24 Spinosyn are produced in the fermentation and there are only minor structural differences, such as the presence or absence of a methyl group in various locations (Crouse *et al*. 1999), Spinosyn D has one more methyl group than Spinosyn A. Extraction of the medium and subsequent recrystallization gives Spinosad, which contains about 90% Spinosyns and 10% impurities from the

growth medium, and the Spinosyn fraction contains about 85% Spinosyn A and 15% Spinosyn D in the final products (Mertz and Yao 1990, Kirst *et al*. 1992 and Sparks *et al*. 1999).

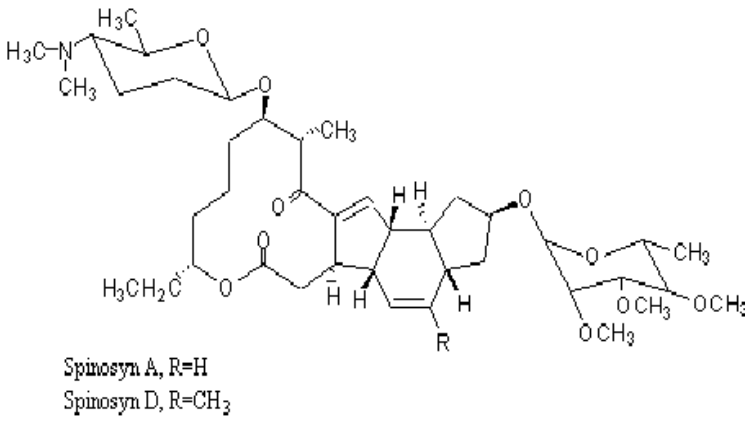


Figure 4 Chemical structure of Spinosad.

Spinosyn A is 2-[(6-deoxy-2,3,4-tri-O-methyl-*alpha*-L-mannopyranosyl)oxy)-13-[(5- dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethy l2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a,16b-tetradecahydro-14-methyl-1H-as-indaceno(3,2-d) oxacyclododecin-7,15-dione.

Spinosyn D is 2-((6- deoxy-2,3,4-tri-o-methyl-*alpha*-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a,16b-tetradecahydro-4,14-dimethyl-1H-as-indaceno (3,2- d)oxacyclododecin-7,15-dione (Dow 1997, Jachetta 2001).

Molecular formula of Spinosyn A and D are $C_{41}H_{65}NO_{16}$ and $C_{42}H_{67}NO_{16}$ respectively; molecular weight are 731.98 and 745.99 respectively. The trade names of Spinosad are Success® Naturalyte®, Tracer®, Spintor, Spinoace, Boomerang, Laser and Extinosad

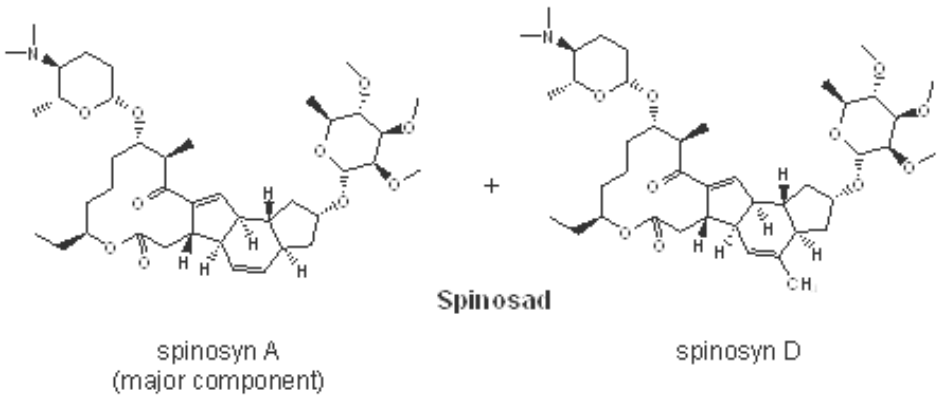


Figure 5 Spinosad: A naturally occurring mixture of spinosyns A and D.

Properties of Spinosad: Tan or white low melting crystals (m.p.84-99.5°C Spinosyn A, m.p. 161-170°C) Spinosyn D, which have low volatility and an earthy odor. Crystals are soluble in a number of organic solvents, Solubility is higher in polar solvents (acetone, dichloromethane, acetontrile, and methanol) than in non-polar solvents (hexane). Crystals have low solubility in water, though spinosyn A is more soluble than spinosyn D. Water solubility increases as solutions become more acidic. The aqueous solutions are basic with pH about 8, and the Spinosyn react with acids to form salts that have higher water solubility (Thompson *et al*. 2000). A major degradative pathway for Spinosad is photolysis (Saunders and Bret 1997) and this limits its residual efficacy in crop markets to about 7-14 days. However, in farm bins and other enclosed grain storage environments where sunlight is lacking, Spinosad can be highly stable. A total of eight studies evaluated the stability of Spinosad residues in corn and wheat by using analytical techniques. Six of these were field studies (Fang *et al*. 2002b, Flinn *et al*. 2004, Daglish and Nayak 2006, Maier *et al*. 2006, Subramanyam *et al*. 2007 and Daglish *et al*. 2008), and two were laboratory studies (Blanc *et al*. 2004 and Chintzoglou *et al*. 2008b). In general, Spinosad residues on treated wheat and corn appear to be relatively stable over storage periods ranging from 6 to12 months, even when subjected to wide fluctuations in temperature (−10–32 °C) and humidity (35–85%). Szabela (2005) indicated that analytical measures of Spinosad residues through time support the conclusion that in some cases Spinosad is capable of adequately protecting grains

in storage for up to two years. Where used, linked bioassays confirmed no loss in biological efficacy over these same time periods. Total Spinosad residue declines in these studies ranged from 39% loss over seven and half months to virtually nil loss over 12 months, with other reported values in-between. Chintzoglou *et al*. (2008b) reported unusually rapid degradation of Spinosad residues on corn – much higher than those observed on wheat or rice in the same study; this is the only study showing degradation of a dry Spinosad formulation.

Efficacy of Spinosad

Spinosad is currently targeted only at Lepidoptera and thrips, spinosyns and spinosoids are inherently broad spectrum, showing activity against insects in the orders coleoptera, diptera, homoptera, hymenoptera, isopteran, orthoptera, Lepidoptera, siphonaptera and thysanoptera as well as mites (Thompson *et al.* 1995).

The efficacy of Spinosad on the insect nervous system is unique and different from that of other insect control products (Salgado 1997). Spinosad is practically non toxic to predator insects and insect pathogen (Bret *et al.* 1997), though it is toxic to insect parasitoids and honey bees but the toxicity is significantly much less than many synthetic insecticides (Schoonover and Larson 1995, Bret *et al.* 1997). Insects that are resistant to known synthetic insecticides do not cross resistant to Spinosad. Spinosad is rapidly absorbed and extensively metabolized in a rat. Within 48h of dosing, 60-80% of Spinosad or its metabolites are excreted through urine or feces (EPA 1997, Dow 1997).

Subramanyam *et al.* (1999b) evaluated the effectiveness of Spinosad applied to 14% moisture wheat grain against adults of *R. dominica*, *S. oryzae*, *O. surinamensis*, *T. castaneum* and larvae of *P. interpunctella.* Spinosad at 1 mg/kg provided complete control of *R. dominica* adults within 8 days, and completely suppressed emergence of *P. interpunctella* adults. No F_1 adults of *R. dominica* were produced after 14 days of exposure. *O. surinamensis* and *T. castaneum* were moderately and least susceptible to Spinosad, respectively,

based on adult mortality. However, significant of reductions in the production of F_1 adults of *S. oryzae*, *O. surinamensis* and *T. castsneum* occurred at rates ≥ 3mg/kg.

Toews *et al.* (2003) evaluated contact toxicity of Spinosad to adults of *R. dominica*, *S. oryzae* and *T. castaneum* after 24 or 48h of exposure. Spinosad at a dose >0.05 mg/cm² affected significantly *R. dominica* at 24 and 48h exposures followed by *S. oryzae* and *T. castaneum*. The 24h LD_{50} values were 0.0004, 0.077 and 0.189 mg/cm² for *R. dominica, S. oryzae* and *T. castaneum* respectively. All *R. dominica* adults were dead following 48h exposure Spinosad where as mortality of *S. oryzae* and *T. castaneum* ranged from 10 to 85% and 12 to 48% respectively.

Sanon *et al*. (2010) reported that a dry Spinosad formulation applied at 0.94 ppm to cowpeas provided up to six months of continuous protection when these treated seeds were bagged in plastic and then stored under typical warehouse conditions in Burkina Faso. Control of *C. maculatus* by Spinosad was better than that provided by the commercial standard pyrethroid, deltamethrin. Ghelani *et al*. (2009) demonstrated that Spinosad controlled key stored grain pests of pearl millet in India (*R. dominica*, *T. castaneum*, and *C. cephalonica*) for up to 12 months of storage. However, this study was conducted under laboratory conditions, and Spinosad was applied at a rate of 2 ppm instead of its anticipated global-wide registered use rate of 1 ppm. Thus, these results cannot be considered directly predictive of future commercial performance. In a laboratory study, Vayias *et al*. (2010a) showed that a Spinosad liquid SC formulation applied to barley at 1 ppm provided nearly complete control of *R. dominica* and *S. oryzae* for six months and adequate control of *C. ferrugineus* for four to six months. Control of *T. confusum* was only moderate in this study.

Spinosad applied to wheat at 0.1 and 1.0 mg/kg resulted in adult mortality and preventing population growth of *R. dominica* (Fang *et al.* 2002a,b, Subramanyam *et al.* 1999), and of 1 mg/kg was necessary for complete control and progeny suppression of *C. ferrugineus*, *C. pusillus* and *T. castaneum* (Subramanyam *et al.* 1999).

Huang *et al.* (2007) also reported more or less similar results from Spinosad treated stored corns. More or less each of the Spinosad formula showed potentialites against almost all the insect pest of stored grains. Number of such successful reports have been published from different countries of the world, specially from USA and Australia. The works of Thompson *et al*. (1997), Nayak *et al.* (2005), Daglish and Nayak (2006), Getchell (2006), Yousefnezhad Irani *et al*. (2007) Athanassiou *et al*. (2008a,b,c), Daglish *et al*. (2008), Hussain *et al*. (2009), Mollaie *et al*. (2011), Mutambuki *et al*. (2012), Subramanyam *et al*. (2012) should be reviewed before introducing Spinosad in any IPM programme.

Toews *et al.* (2003) reported contact toxicity of Spinosad to adults of *R. dominica*, *S. oryzae*, *T. castaneum*, *T. confusum*, *C. ferrugineus* and *O. mercator* on four different surfaces. Aqueous Spinosad suspension was sprayed to concrete, galvanized steel, unwaxed floor tile and waxed floor tile to obtain deposits of 0.05 or 0.1mg/cm². Approximately 24h after application, 30 adult beetles were confined by species to each untreated and Spinosad treated surface, and knowckdown for 24h was assessed. Mortality of all other species exposed to Spinosad was 99-100%. *Tribolium* spp. were highly susceptible to Spinosad on concrete (99-100% mortality), however on unwaxed floor tile, steel and waxed floor tile recovery on food after knockdown resulted in only 72-92% mortality. Results suggest that Spinosad has excellent contact activity against adults of stored product insects, especially on concrete and has potential for use as a general surface, spot or crack/crevice spray to control insects in empty bins, warehouses, food processing facilities and retail stores.

More field evaluations of Spinosad are necessary to establish its future performance in commercial stored product environments. Field studies are particularly valuable as predictive tools, because they tend to reflect the combined impact of Spinosad on adults, immatures, and progeny production under conditions of continuous exposure-aspects of which are difficult to simulate under laboratory conditions (Toews and Subramanyam, 2004). Published field studies with Spinosad are comparatively less (Fang *et al*. 2002a, Flinn *et al*. 2004a,b and

Subramanyam *et al*. 2007), and in Bangladesh context published literature on Spinosad efficacy against the stored product insects are very scare.

Toews *et al.* (2005) investigated the survival of stored product insect natural enemies in wheat treated with Spinosad in laboratory and pilot scale experiments. The predator *X. flavipes* and the parasitoids *H. hebetor*, *T. elegans* and *A. calandrae* when exposed to wheat treated Spinosad at 0.05-1 mg/kg, *X. flavipes* was the only species that survived (92% survival). However,when combined treatments with Spinosad and *X. flavipes* was tried against *T. castaneum*, about 90% inhibition of immature population was achieved, both in laboratory and field trials. There is substantial evidence to show that Spinosad at 1 mg/kg is not effective in killing all exposed adults but this rate is effective in suppressing progeny of *T. castaneum* (Fang *et al.* 2002a,b) thereby limiting any population growth to contamination from external sources or other forms of immigration.

A general lack of survival among hymenopterans exposed to Spinosad was also reported in the literature (Tillman and Mulrooney 2000, Mason *et al.* 2002, Michaud 2003). There is limited evidence to suggest that *X. flavipes* is more pesticide tolerant than parasitoids and pest insects (Baker and Arbogast 1995, Press *et al.* 1978). This a good sign for storage managers to use both thesetwo natural enemies to control the stotred product insects

Yousefnezhad Irani *et al*. (2007) observed toxicity of Spinosad on adults *T. castaneum* and *S. oryzae* at different temperatures and exposure intervals and reported based on LD_{50} values and more overlapping of 95% CL, *S. oryzae* was more susceptible to Spinosad than *T. castaneum*. The trend of species susceptibility to Spinosad was similar to those reported by (Subramanyam *et al*. 2004, Hung *et al*. 2004 and Flinn *et al*. 2004a, b).

Spinosad is primarily a stomach poison with some contact activity and it represents a new class of insecticides acting by a neurotoxin with a novel mode of action and act as against at the post synaptic cholinergic ion channels and GABA gated ion channels (Salgado 1998, Salgado *et al*. 1998, Thompson *et al*. 2000).

Spinosad has an excellent environmental and mammalian toxicological profile (Crouse and Sparks 1998, Sparks *et al*. 1999, Thompson *et al*. 2000). Spinosad exhibits wide margin of safety to many beneficial insects and related organisms (Schoonover and Larson 1995, Elzen *et al*. 2000) and is therefore considered a selective insecticide (Miles and Dutton 2000).

Suppression of the subsequent generation is one of the basic characteristics of a successful grain protectant (Arthur 1996). Spinosad is capable to giving long term protection without a loss in efficacy (Fang *et al*. 2002a, Maier *et al*. 2006), through contact, ingestion and through the nervous system.

Chapter 3

General Methodology

Selection of the test insects: pests and predator

Pests: The flat grain beetle, *Cryptolestes pusillus* (Schon) is one of the common major pests of stored grains, it is particularly common in wet tropical and warmer temperate regions (Halstead 1993, CABI Crop Protection Compendium 2008). It is an external feeder of the stored cereal and other commodities. Incubation, larval, pre pupal and pupal period of *C. pusillus* are 3-4d, 19-21d, 3-4d and 4-5d respectively and the total developmental period is 30-35d. The eggs, larvae, pre-pupae and pupae are normally visual. Culture of *C. pusillus* in the laboratory is simple and subsequently produces huge population within short time. Both larvae and adults of *C. pusillus* damage a number of stored commodities (Cotton 1963).

The lesser grain borer, *Rhyzopertha dominica* (Fabricus) is another serious damaging pest of stored grains it is an internal feeder and cosmopolitan in distribution. The incubation, larval, pre pupal and pupal periods are 4-6d, 18-33d, 1d and 2-4d respectively. Total developmental time required as 30-41d. Eggs, larvae, pre pupae, pupae and adults are normally visual. Culture of *R. dominica* in the laboratory is easy. By feeding and making circular holes into the cereals *R. dominica* causes heavy weight loss and also affects the nutritional and baking quality as well as germination capacity of the grain (Patel and Valand 1994). So for easy culture techniques, requisite quantity of both the pest species were available within a short time under the laboratory conditions.

Predator: Under the laboratory conditions hemipteran predators can suppress about 70-100% stored product insect pest populations under the laboratory conditions (Brower and Press 1992). Among the hemipteran predators, the warehouse pirate bug, *Xylocoris flavipes* (Reuter) is a potent candidate to suppress the coleopteran and lepidopteran stored-product insect pests. It is cosmopolitan in distribution (Gross 1954, Arbogast 1979). The incubation and nymphal periods are 4-5d and 10-16d respectively. The developmental period is required 20d which may vary 14-15d depending on food, temperature and humidity. Adult male and female longevity are 6d and 20d respectively. Culture of *X. flavipes* is easily established in laboratory. *X. flavipes* is commercially available in USA to manage

the stored products insect pests (Mason *et al.* 2001). They are commonly found in grain storage facilities and their presence usually indicates an established infestation of grain pests. *X. flavipes* lives on eggs, larvae and pupae of insect pests.

Selection of bacterium

Spinosad is a reduced-risk commercial bacterial insecticide which belongs to a new class of insecticides called the NATURALYTES. Spinosad provides highly effective and long-lasting (up to two years) control of more or less all species of stored insect pests on various grains (Hertlein *et al*. 2011) and so far has proved non-cross-resistant to other insecticides (Salgado and sparks 2005). In addition, spinosad exhibits low mammalian toxicity and a highly environmental profile (Cleveland *et al*. 2001). Spinosad shows wide margins of safety to many beneficial insects and related organisms (Schoonover and Larson 1995, Elzen *et al*. 2000). It degrades quickly when exposed to sunlight (UV light) (Liu *et al.* 1999), therefore it is considered as a selective insecticide (Miles and Dutton 2000). Spinosad is already registered as a grain protectant in Kenya, USA, South Africa, Canada, European Union, Australia, Japan etc. at the maximum use rate of 1ppm (1mg/kg of grain) and its Maximum Residue Limit (MRL) established at 1.5ppm (Hertlein *et al*. 2011). Moreover, Spinosad's unique and non-cross-resistant mode of action will make it a valuable new tool in stored grain resistance management.

Food medium used

Food for host: White wheat, whole wheat flour and Brewer's yeast were collected from the granaries of Shaheb Bazer, Rajshahi, Bangladesh. Wheat grains were cleaned by sieving through 500 micrometer aperture sieve (Wire cloth company, Newark, New Jersey 07104, USA) and sterilized in an oven at 100 ºC for 8h. Whole wheat flour and powdered Brewer's yeast ratio was 19:1 (Park and Frank 1948, Park 1962, Zyromska-Rudzka 1996) were mixed. Both wheat flour and yeast were passed previously through 125 micrometer aperture sieve (Wire cloth company, Newark, New Jersey 07104, USA) and were sterilized at 120 ºC for 6h in an oven. The sterilized wheat and standard food mixture were kept in separate plastic containers for 15 days to equilibrate its moisture content with that of the

laboratory with the minimum of 13% (Khan 1981, Mondal 1984a) and were used throughout the experiments. New foods wheat and standard food were prepared wherever necessary.

Food for predator: Continuous supply of eggs, larvae up to 4th instars, pupae of *C. pusillus* and *R. dominica* were provided as food for *X. flavipes* during maintained culture in the laboratory. Culture of beetles was maintained according to Mondal and Parween (1997) in beakers containing food medium.

Culture of *C. pusillus*

Collection of beetle: Adults of *C. pusillus* were collected from the rearing culture maintained in the Integrated Pest Management Laboratory (IPM), Institute of Biological Sciences, University of Rajshahi since ten years.

Collection of eggs: Six hundred unsexed adults of *C. pusillus* were collected and divided into three groups, each having 200 adults and were introduced to separate glass petri dishes (9cm diame) containing 50g food (whole wheat flour and Brewer's yeast, ratio19:1) and allowed to oviposit. After 24h, the adults were sieved and eggs were collected sieving by 125 micrometer aperture sieve. The collected eggs were cleaned by gently tapering the paper on a piece of black paper and by separating flour particles. Cleaned eggs were identified under compound microscope and transferred to petri dishes with the help of fine camel hair brush. Eggs were kept 3-4d for hatching.

Collection of larvae, pre-pupae, pupae and adults: After hatching, 1st, 2nd, 3rd and 4th instars larvae were obtained from 4-5d, 5-6d, 4-5d and 6-7d respectively. Pre pupae and pupae were seen into the cocoon at 3-4d and 4-5d. The larvae and pupae were confirmed by random examining under a magnifying glass. Pupae emerged as adults at 4-5d. All the cultures were conducted in the Control Temperature (CT) room at 30±0.5ºC temperature and 70±0.5% relative humidity to ensure constant and regular supply of different life stages of *C. pusillus* of known age throughout the study period.

Determination of sex: The male-female sexes were determined by the following characters

i) Antennae was about two thirds length of the body in male and half length of the body in female.

ii) Male hind tarsi were 4 segmented but female hind tarsi were 5 segmented

iii) Males were normally small in shape-size than females.

Culture of *R. dominica*

Collection of beetle: Adults of *R. dominica* were collected from the stock culture maintained in Integrated Pest Management Laboratory, Institute of Biological Sciences, University of Rajshahi since ten years.

Collection of eggs: Six hundred unsexed adults of *R. dominica* were collected and divided into 3 groups, each having 200 adults and were kept in separate petri dishes (15 cm daime) containing 100g of white wheat and allowed to oviposit. After 24 h, the adults were sieved out and eggs were separated using by 500 and 125 micrometer aperture sieves for adults and eggs respectively. Eggs were examined under compound microscope and transferred to glass petri dishes (15 cm daime) with the help of fine camel hair brush. The collected eggs were kept 4-6d for hatching.

Collection of larvae, pre pupae, pupae and adults: After hatching, the 1st, 2nd, 3rd and 4th instars larvae, pre pupae and pupae were obtained from 6-12d, 4-7d, 4-6d, 4-8d, 1d and 2-4d respectively and were confirmed by random examining under a magnifying glass. Adults were emerged directly from pupae. All cultures were conducted in the Control Temperature room at 30±0.5ºC temperature and 70±0.5 % relative humidity to ensure constant and regular supply of various kinds of life stages of *R. dominica* of known ages.

Determination of sex: It is difficult to identify the sex of *R. dominica* in their larval and adult stages. Sex of *R. dominica* was separated in the pupal stage by the microscopic test of exogenital papillae by removed of attached exuvae (Porter 1935). The sexed pupae were then placed in separate petri dishes for the emergence of adults. Survivorship of pupae was reduced by sexing procedure and the degree of reduction varied considerably with the culture media used (Long staff and Starick 1989).

Culture of *X. flavipes*

Collection of the adult bug: Adults of *X. flavipes* were collected from the stock culture maintained in the Integrated Pest Management laboratory, Institute of Biological Sciences and University of Rajshahi for six years.

Collection of eggs: One hundred adults of *X. flavipes* were placed in a beaker (500 ml) containing sufficient food (1st and 2nd instars larvae and pupae of *C. pusillus* and *R. dominica*). After 24 h, adults were replaced with the help of a fine camel brush. Eggs were found at the bottom of the beaker and examined them under compound microscope. The collected eggs were kept at 4-5d for hatching.

Collection of nymphs and adults: The newly hatched nymphs were determined using by magnifying glass and transferred very carefully with the help of fine camel hair brush to a beaker (500 ml) containing 1st and 2nd instars larvae of *C. pusillus* and *R. dominica* as food. The 2nd, 3rd, 4th and 5th instars nymphs were obtained from the culture on the 3rd, 5th, 8th and 12th d from hatching respectively (Arbogast 1971). The nymphal instars were estimated by counting the exuviae deposited in the petri dish. The 5th instars nymphs transferred into adults.

The culture of *X. flavipes* was maintained in beakers (500 ml) containing food medium (wheat flour and whole wheat infested by either *C. pusillus* or *R. dominica*). The mouth of the beaker was covered with fine cloth and tied with rubber band. Beakers were kept in the Control Temperature room at 30±0.5ºC and 70±5 % rh.

Determination of sex: Sex of *X. flavipes* was separated at the adult stage. Shape of the adult female's abdomen is bilaterally symmetrical and in male it is notched on the left side of the segments 8 and 9.

Source of Spinosad

Spinosad is light gray to white in colour with slight odor stale water. About 500ml of Spinosad (PRN- MAPP-12054, cafno 20012- 019, Lot No–3068404) was obtained from Dow Agro Sciences, UK. Concentration of spinosad was 120g spinosad/Litre.

Preparation of concentration

Using by 3 ml syringe 0.5 ml (equal to 32 drops) of Spinosad were taken in glass vial (5 ml) and using a micropipette 2 ml distilled water were added. The vial was shaken vigorously for equal mixing of Spinosad and water. Filter papers (9 cm diameter) were soaked in the prepared solution and allowed to evaporate at room temperature overnight hanging by strings. This 0.5 ml solution had the concentration equal to 7.863 µl/cm^2, determined using the formula given by Athanassiou *et al.* 2008. Other concentrations of Spinosad as 3.932, 1.966, 0.983 and 0.491 µl/cm^2 were than prepared by serial dilution, by taking half drops of Spinosad in each step and adding 2 ml distilled water.

Precautions

All experiments were conducted under same laboratory conditions. Frass, spoiled substance, faecal materials, dead beetles, cocoon, etc. gradually accumulate in the culture media and make it dirty, unhealthy and damped. To avoid such unhealthy condition, the original cultures of both predator and prey insects were sieved after every 10-12d and fresh food was added to the culture. All glass wares, equipments and sieves were sterilized at 180ºC for 6h in an oven just before use. Washing detergents were used to clean all the materials. The experimental desks were cleaned everyday.

Statistical analysis

Data were analyzed by factorial ANOVA, Tukey's test, Probit analysis using some statistical software like Minitab (version 14), MS Excel 2003 and Bio stat 2009.

Chapter 4

Host-stage specific effects on biological parameters of *X. flavipes*

Introduction

Stored commodities are attacked by Lepidoptera, Coleoptera and Acari (Sing and Watters 1985, Khan and Mannan 1991). They are consist of many external and internal feeders such as *C. pusillus* and *R. dominica* and are found occasionally infesting grain (Subramanyam 2006a,b). Insect pest management in stored commodities with chemicals is facing many challenges due to concerns about human safety, insect resistance, environmental impacts and presence of residues in raw and processed foods (Hagstrum *et al*. 1999, Phillips *et al*. 2000, Daglish and Wallbank 2002, Nayak *et al*. 2005, Daglish and Nayak 2006). Therefore, there is an urgent need to develop alternative methods for their control. Biological control has been studied as an alternative option (Arbogast 1985, Haines 1984, 1991, Brower *et al.* 1996, Scholler and Flinn 2000) and it is also depended on the potentiality of biocontrol agents. Among the predatory bugs, the warehouse pirate bug, *X. flavipes* is one of the biological control agents of many stored product insect pests (Brower *et al*. 1996, Scholler *et al*. 1997, Visarathanonth *et al*. 1990, 1994, Imamura *et al*. 2008). Actually, *X. flavipes* showed promise in suppressing populations of stored product insect pests (Jay *et al*. 1968) and preyed on early and late instar of many insect species but the number depending partly on the size of the prey and possibly other factors (Lecato and Davids 1973). Age of the host insect can have a profound effect on development and oviposition of parasitoids/predators (Vinson and Iwantsch 1980) because nutritional status and accessibility of the host may change with age.

Several workers have described the effects of temperature, humidity and host species as foods on the biology of *X. flavipes* (Birch 1945a,b,c, Howe 1953 Arbogast 1975, Press *et al*. 1976, Russo *et al*. 2004, Herra *et al.* 2005, Ferdous 2006, Saha 2007, Rahman *et al.* 2009). Before releasing the predator in the store to control insect pests, a thorough knowledge is needed about their biology. Many parasitoids/predators lay more eggs in certain host instar than the other. However, no specific information has been available concerning host *C. pusillus* and *R. dominica* stage specific effects on the biology of *X. flavipes*. Moreover, it was

important to measure the developmental period, adult longevity, consumption rate, survivability, size and sex ratio of *X. flavipes* on different life stages of host *C. pusillus* and *R. dominica*. This led to the conduct of the test.

Materials and Methods

Host: Two hundreds newly emerged healthy and mated females of *C. pusillus* were collected from previous established culture (Chapter 3). They were introduced in a Petri dish (15cm diameter) containing 10g fresh food (wheat flour and yeast ratio, 19:1 in weight). After 24 h, adults were sieved out from Petri dish and kept them in another Petri dish (15cm diameter) with same fresh food. The eggs along with foods were placed in a Petri dish (9cm diameter). The Petri dish was kept within wooden folder for the save from other organisms. The wooden folder was sifted to CT room at 30±0.5ºC temperature and 70±0.5% relative humidity. A series of culture were maintained for regular supply of eggs, larvae up to 4^{th} instar and pupae of *C. pusillus*. Through the same procedure as described above eggs, larvae up to 4^{th} instar and pupae of *R. dominica* were reared for regular supply. Different life stages of both hosts were used as food in the present tests.

Predators: Fifty newly emerged healthy and mated females of *X. flavipes* were collected from earlier established culture (Chapter 3) and introduced in a glass beaker (500 ml) with 1^{st} and 2^{nd} instar larvae of *C. pusillus* and *R. dominica* as food and allowed to oviposite. Two filter papers were placed at the bottom of the beaker. Mouth of the beaker was covered with the fine cloth and rubber band to prevent moving out of the insects. After 24 h, the adults were removed to another beaker (500 ml) using by a fine camel brush and the deposited eggs were collected in a Petri dish (9 cm diameter) tapping by the filter papers for hatching. The newly hatched nymphs were carefully transferred to Petri dish (9 cm diameter) one by one using by fine camel brush.

Bioassays: Newly hatched healthy 70 nymphs of *X. flavipes* were kept in 7 Petri dishes (9 cm diameter) separately (per Petri dish with 10 nymphs) containing with 200 eggs, 25 larvae of each instar and 10 pupae of *C. pusillus* as food. After every 24 h, consumed or killed life stages of *C. pusillus* by *X. flavipes* were observed

and counted. Food were balanced adding by same life stage and were cleaned discarding by dead insects daily. Same procedure was followed as described above for *R. dominica*. The nymphs were regularly observed for ecdysis, number of nymphal ecdysis was recorded alone with the duration for each instar. Regular supply of eggs, larvae up to 4th instar and pupae of *C. pusillus* and *R. dominica* was maintained until the death of *X. flavipes*. The numbers of adult's emergence as well as total developmental time and longevity of adults were observed. Prey consumption rate and no. of survivability of *X. flavipes* were observed and counted. The length (mm) of male and female adults was measured using by an ocular micrometer and the sexes were differentiated by compound microscope. All the experiments were replicated three times and conducted in CT room at 30 ± 0.5ºC and 70 ± 0.5% relative humidity.

Data analysis: The developmental period, adult longevity, prey consumption rate, survivability, size and sex ratio of *X. flavipes* were determined statistically compared to control using by factorial ANOVA. The comparison of mean was done by Tukey's test (1953).

Results and Observation

***C. pusillus* as prey of *X. flavipes*:** *C. pusillus* were reared on standard food. The mean developmental period from egg hatching to adult emergence of *X. flavipes* on different life stages of *C. pusillus* have shown in Figures 10A and Appendix table 2. Developmental period and its variance depended upon the different life stages of host (Figure 10B). *X. flavipes* was found able to complete development on eggs, larvae up to 4th instar and pupae of host but not on adults. On eggs, larvae up to 4th instar and pupae the mean developmental durations were recorded as 15±2.00, 20±0.00, 22±0.58, 18±1.00, 14±1.15 and 12±1.15 days respectively (Appendix Table 2).The age specific distribution of each nymph up to 5th instar varied on different life stages of prey (Figure 10A). The developmental period was maximum on 2nd instar larvae and was minimum on the pupae Appendix table 2). The effects of different life stages of host on the developmental period was highly significant ($P<0.001$) (Appendix tables 3-7).

Life span of the predator varied with the different life stages of host. Adult of *X. flavipes* were found to be very active on the 2nd to 4th instar larvae Figure 10A,B). The highest longevity of the females was 31±1.15 days on 2nd instar larvae and lowest was 14±1.15 days on pupae (Appendix table 2). The effect of different life stages of prey on adult longevity was highly significant (P<0.001) (Appendix tables 8 and 9).

Average (%) number of daily consumed or killed eggs, larvae up to 4th instar and pupae of *C. pusillus* by nymphs up to 5th instar and adults of *X. flavipes* were presented in Figure 12A and Appendix table 18. Consumption rate of *X. flavipes* was found to be varied on stages of host (Figure 12B). Effect of different life stages of *C. pusillus* on nymphs and adults of *X. flavipes* was found highly significant (P<0.001) (Appendix tables 19-25). The female predator always consumed more prey than the male. 2nd instar larvae of the beetle were found more preferable to the predator compared to other stages. A single *X. flavipes* can consume 3.33±0.33, 4.33±0.33, 5.67±0.33, 6.33±0.33, 8.67±0.33, 12.33±0.33 and 14.33±0.33 2nd instar larvae of *C. pusillus* per day in its 1st, 2nd, 3rd, 4th, 5th, adult male and female stages respectively (Appendix table 18). So an individual *X. flavipes* needs total 164.97, 129.01, 88.33, 66.01, 25.01 and 31 numbers of eggs, larvae up to 4th instar and pupae of *C. pusillus* to become adult. Number of eggs, larvae up to 4th instar and pupae of prey were consumed by *X. flavipes* as 130.64, 114.64, 98.64, 77.3, 69.36 and 34.64 respectively in adult male and as 413.40, 326.60, 286.66, 226.60, 186.60 and 113.40 respectively in adult female.

Average survivability (%) of *X. flavipes* (nymphs up to 5th instar and adults) was recorded as maximum when fed on eggs, larvae up to 2nd instar 3rd, 4th instar larvae and pupae (Figure 14A, Appendix table 34). Survivability of *X. flavipes* was depended upon different life stages of *C. pusillus* (Figure 14B). The effect of host stages on survivability of nymphs up to 5th instar and adults of *X. flavipes* was found highly significant (P<0.001) (Appendix tables 35-41).

Normally females are larger in size than males. Size of the adult *X. flavipes* was more when they fed on the larvae up to 4th instar of *C. pusillus* than when they fed

on eggs and pupae (Figures 16A, Appendix table 50). The largest the males and females were measured as 1.80±0.01 and 2.10±0.01 mm respectively feeding on 3rd instar larvae and shortest sizes were 1.50±0.06 and 1.70±0.06 mm respectively on eggs (Appendix table 50). The effect of different life stages of host on adult size was highly significant (P<0.001) (Figure 16B, Appendix tables 51 and 52).

Sex ratio of the emerged predators differed while feeding different life stages of *C. pusillus* (Figures 18A, Appendix table 56). Sex differentiation of the emerged adults of *X. flavipes* was affected by the life stages of *C. pusillus* which were preyed by the predator. The sex-ratio of *X. flavipes* was found to fluctuated depending on the larval instar and other stages of prey. In all cases more number of female bugs were produced when the immature predators fed in 1st instar host larvae, the sex ratio was obtained closer to the normal one, the resulted sex ratio (male:female) was 45:55. Effect of different life stages on sex ratio was highly significant ($\chi^2 = 13.67$, df = 5, P<0.001) (Figure 18B, Appendix tables 57 and 58).

***R. dominica* as prey of *X. flavipes*:** *X. flavipes* were successful at locating and killing *R. dominica* larvae and pupae that were feeding inside wheat kernels. The mean developmental period from egg hatching to adult emergence of *X. flavipes* on different life stages of *R. dominica* have been shown in Figures 11A and Appendix table 10. Developmental period and its variance depended upon the different life stages of the prey (Figure 11B). *X. flavipes* was able to complete development on eggs, larvae up to 4th instar and pupae of the prey. On eggs, larvae up to 4th instar and pupae the mean developmental durations were 18±1.00, 20±0.58, 16±2.00, 14±1.15, 12±1.15 and 13±0.58 days respectively (Appendix table 10). The age specific distribution of each nymph up to 5th instar varied on different life stages of prey (Figure 11A). The developmental period was maximum on 2nd instar larvae but minimum on 4th instar larvae (Appendix table 10). The effects of different life stages of prey on the developmental period was highly significant (P<0.001) (Appendix tables 11-15).

Life span of the predator varied with the different life stages of prey. Adult of *X. flavipes* were found to be very active on the 2nd to 4th instar larvae. The highest

longevity of the females was 34±2.31days on 2nd instar larvae and lowest was 15±1.15 days on pupae of the host (Appendix table 10). The effect of different life stages of prey on adult longevity was highly significant (P<0.001) (Appendix tables 16 and 17).

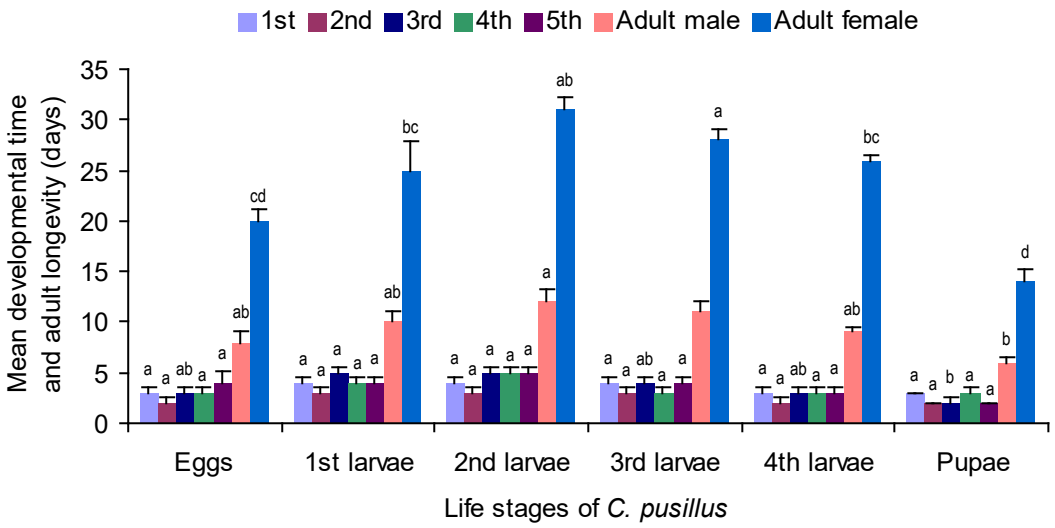
Mean number of daily consumed eggs, larvae up to 4th instar and pupae of *R. dominica* by nymphs up to 5th instar and adults of *X. flavipes* were presented in Figure 13A and Appendix table 26. Consumption rate of *X. flavipes* was found to very on life stages of prey (Figure 13B). Effect of different life stages of *R. dominica* on nymphs and adults of *X. flavipes* was found highly significant (P<0.001) (Appendix tables 27-33). The female predator always consumed more prey than the male. 1st instar larvae of prey were found more preferable to the predator comparatively than other stages. One *X. flavipes* consumed 3.33±0.88, 4.67±1.20, 5.00±0.58, 6.33±0.88, 8.67±1.20, 10.00±1.15 and 14.00±1.15.33 1st instar larvae of *R. dominica* per day in its 1st, 2nd, 3rd, 4th, 5th, adult male and female stages respectively (Appendix table 26). So an individual *X. flavipes* needs total 260, 104, 86.32, 78.67, 52.30, and 30.99 numbers of eggs, larvae up to 4th instar and pupae of *R. dominica* to became adult. Number of eggs, larvae up to 4th instar and pupae of prey were consumed by *X. flavipes* as 213.30, 100, 93.30, 86.70, 63.30 and 30 respectively in adult male and 572.74, 528, 271.26, 249.26, 183.26 and 88 respectively in adult female.

Average survivability (%) of *X. flavipes* (nymphs up to 5th instar and adults) on eggs, larvae up to 2nd instar were found maximum comparatively than that of 3rd, 4th instar larvae and pupae (Figure 15A, Appendix table 42). Survivability of *X. flavipes* was depended upon different life stages of *R. dominica* (Figure 15B). The effect of host stages on survivability of nymphs up to 5th instar and adults of *X. flavipes* was found highly significant (P<0.001) (Appendix tables 43-49).

Normally females are larger than males. Adult male and female size of *X. flavipes* was when the larvae upto 4th instar of *R. dominica* was fed by the predator, whereas the adult size was less when it fed on 4th instar larvae and pupae (Figures 17A, Appendix table 53). The highest size of the males and females was

1.85±0.01 and 2.15±0.02 mm feeding on 2nd instar larvae, and lowest was 1.65±0.04 and 1.90±0.03 mm feeding on pupae (Appendix table 53). The effect of different life stages of prey on adult size was highly significant (P<0.001) (Figure 17B, Appendix tables 54 and 55).

Sex ratio of the emerged predators differed while feeding on different life stages of *R. dominica* (Figures 19A, Appendix table 59). The sex ratio of *X. flavipes* reared on different life stages of *R. dominica* always showed preference to the females, but fluctuation of the male-female ratio was recorded among the stage of the prey insect. Based on ratio 1:1, sex ratio of *X. flavipes* was found the best on 1st and 2nd instar larvae comparatively than other stages. Effect of different life stages on sex ratio was highly significant ($\chi^2 = 12.76$, df = 5, P<0.001) (Figure 19B, Appendix tables 60 and 61).



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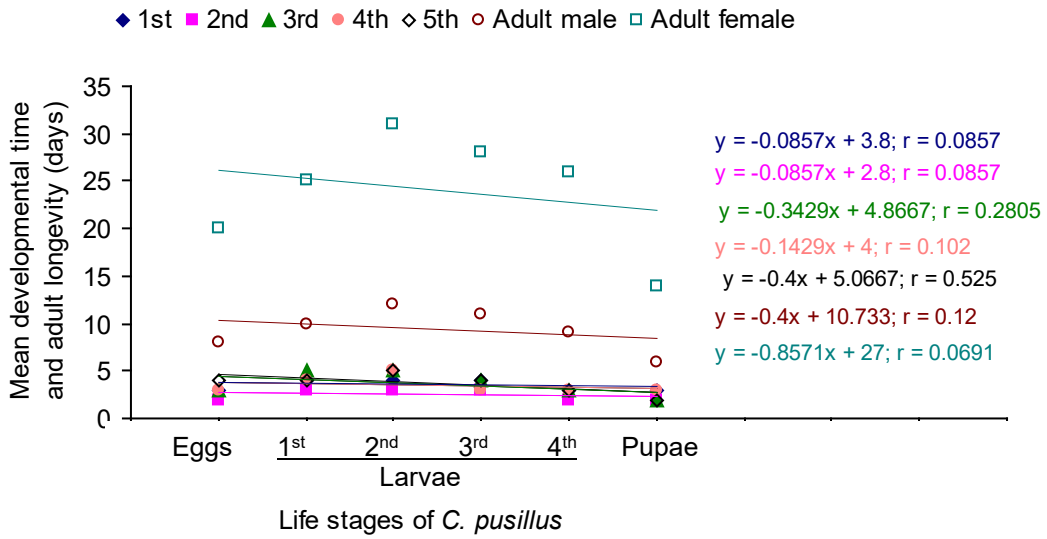
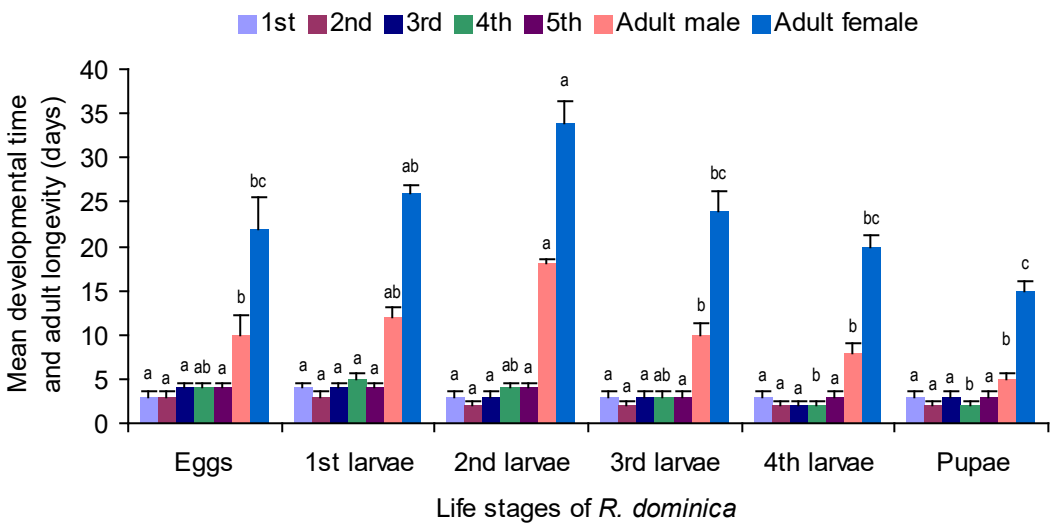


B

Figure 10A Mean developmental time and adult longevity of *X. flavipes* on different life stages of *C. pusillus*.

10B Relationship between mean developmental time and adult longevity of *X. flavipes* and different life stages of *C. pusillus*.



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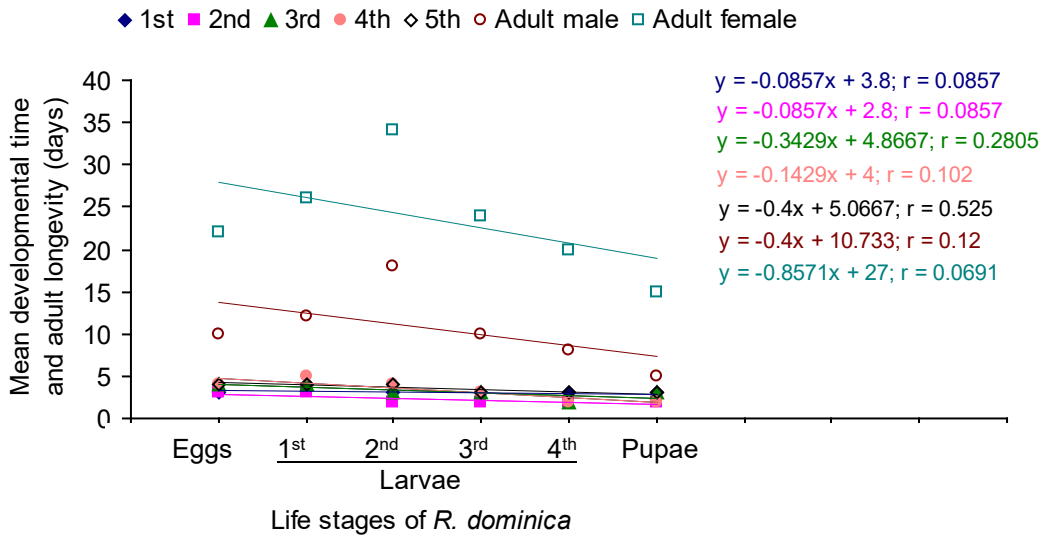
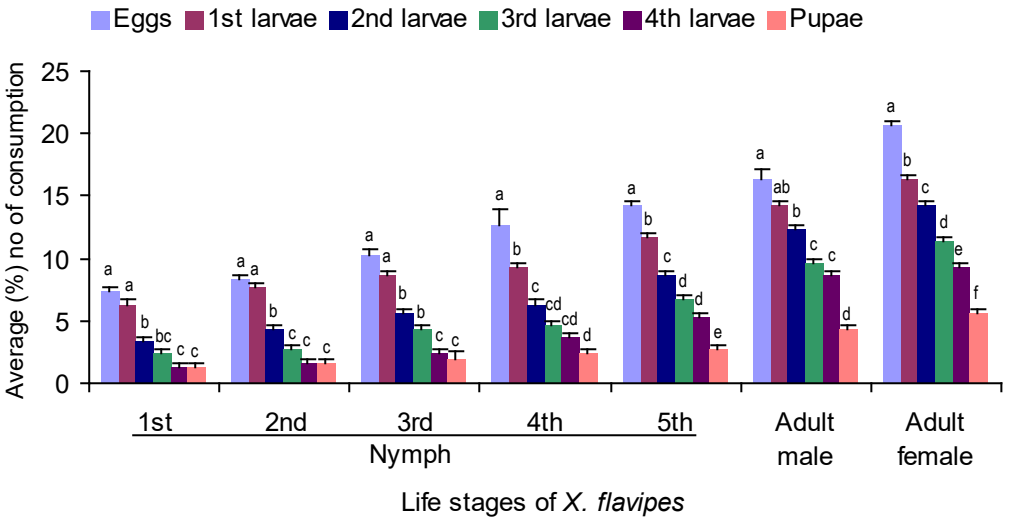


B

Figure 11A Mean developmental time and adult longevity of *X. flavipes* on different life stages of *R. dominica*

11B Relationship between mean developmental time and adult longevity of *X. flavipes* and different life stages of *R. dominica*



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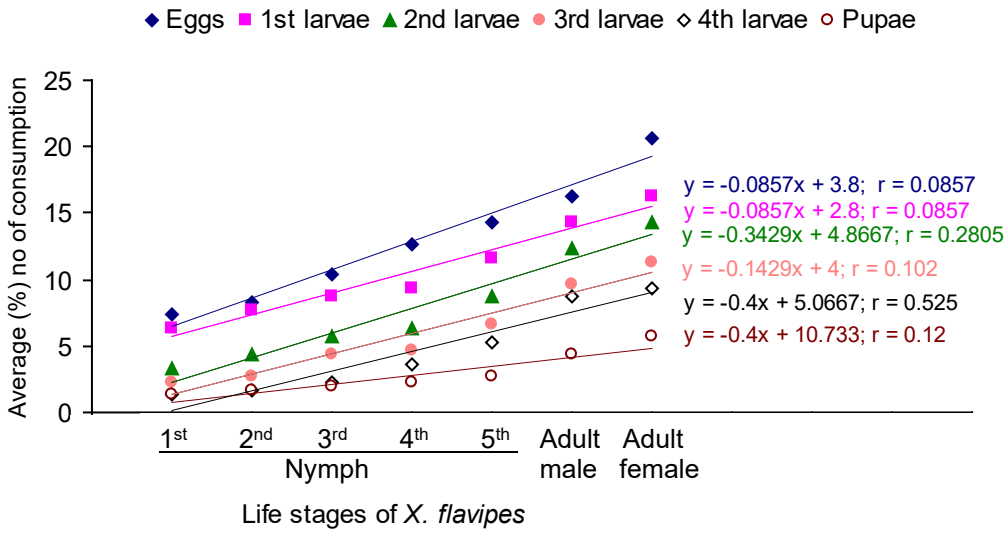
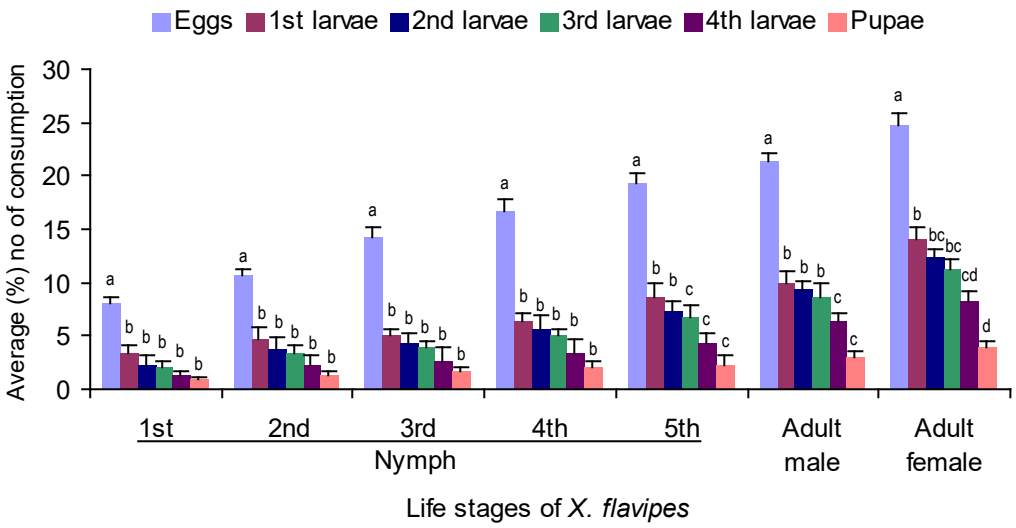


B

Figure 12A Average (%) no of *C. pusillus* (eggs, larvae up to 4th instar and pupae) consumed per day by different life stages of *X. flavipes*

12B Relationship between consumption rate of *X. flavipes* and different life stages of *C. pusillus* per day



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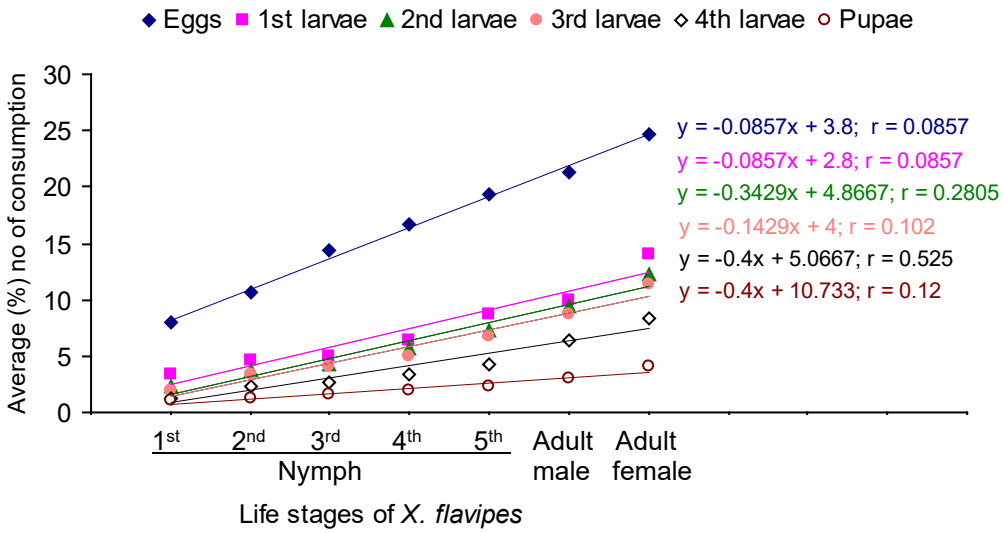
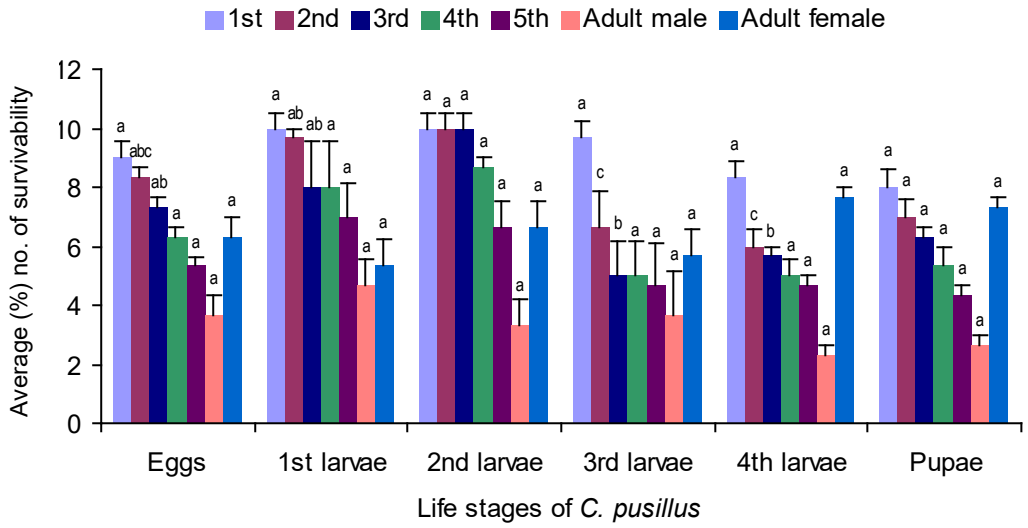


B

Figure 13A Average (%) no of *R. dominica* (eggs, larvae up to 4th instar and pupae) consumed per day by different life stages of *X. flavipes*

13B Relationship between consumption rate of *X. flavipes* and different life stages of *R. dominica* per day



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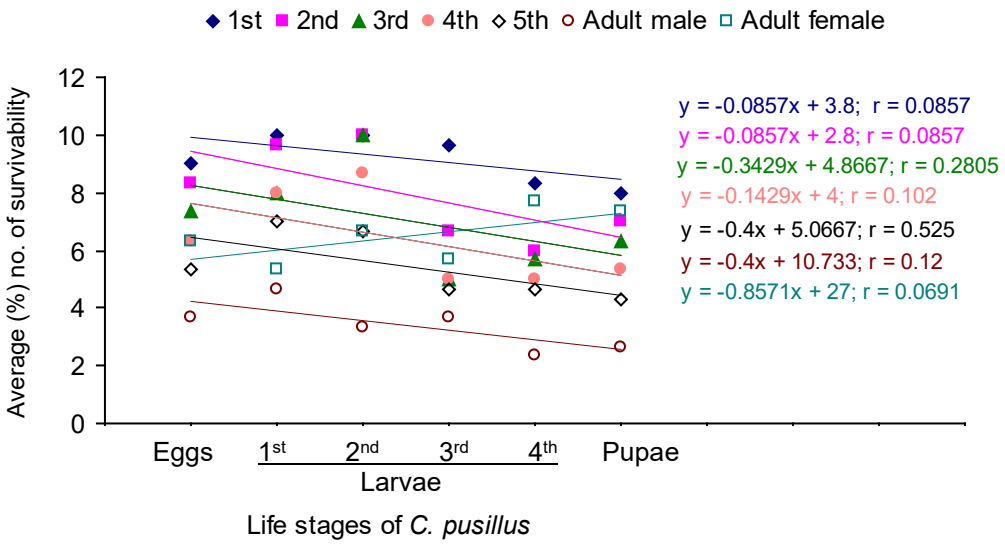
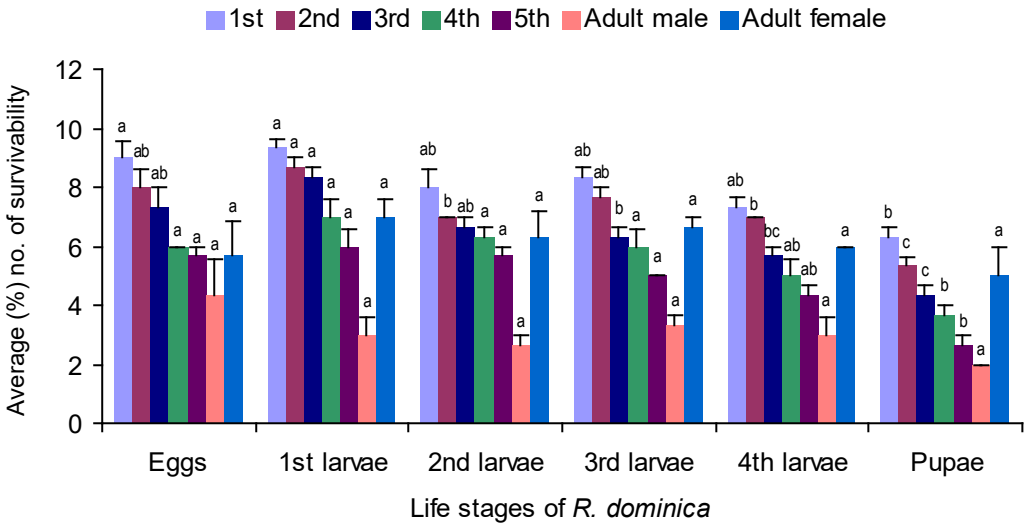


B

Figure 14A Average (%) no. of survivability of different life stages of *X. flavipes* on different life stages of *C. pusillus*

14B Relationship between average no. of survivability of different life stages of *X. flavipes* and different life stages of *C. pusillus*



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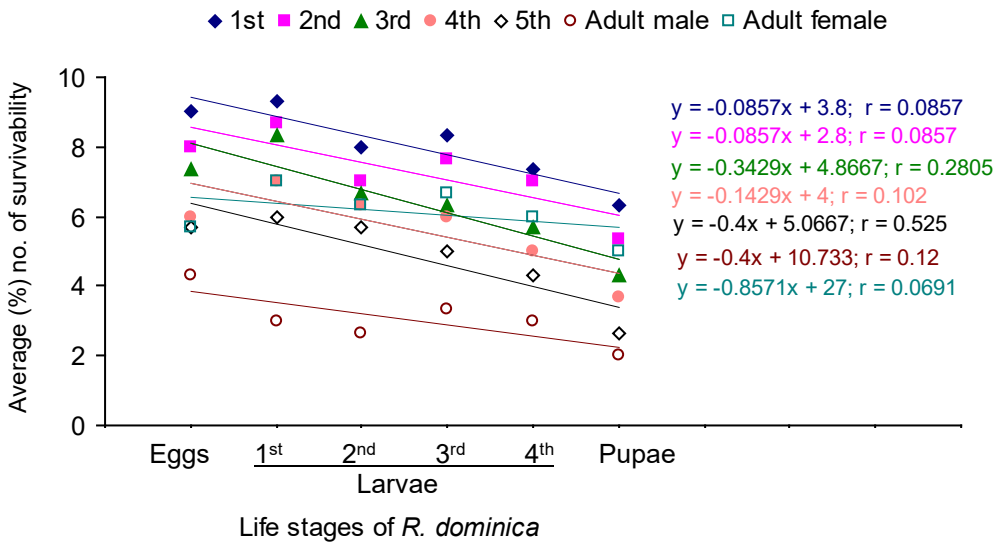
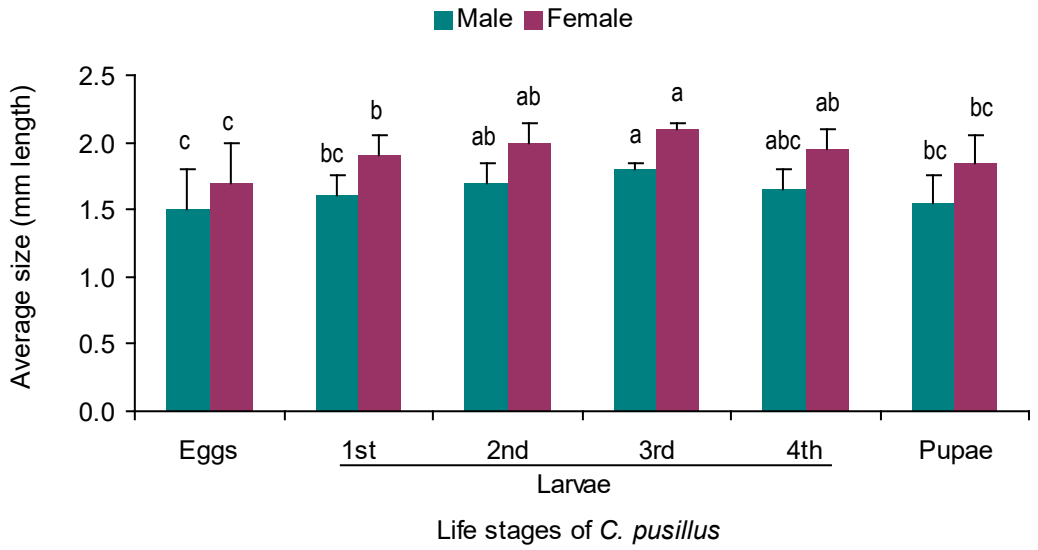


B

Figure 15A Average (%) no. of survivability of different life stages of *X. flavipes* on different life stages of *R. dominica*

15B Relationship between average no. of survivability of different life stages of *X. flavipes* and different life stages of *R. dominica*



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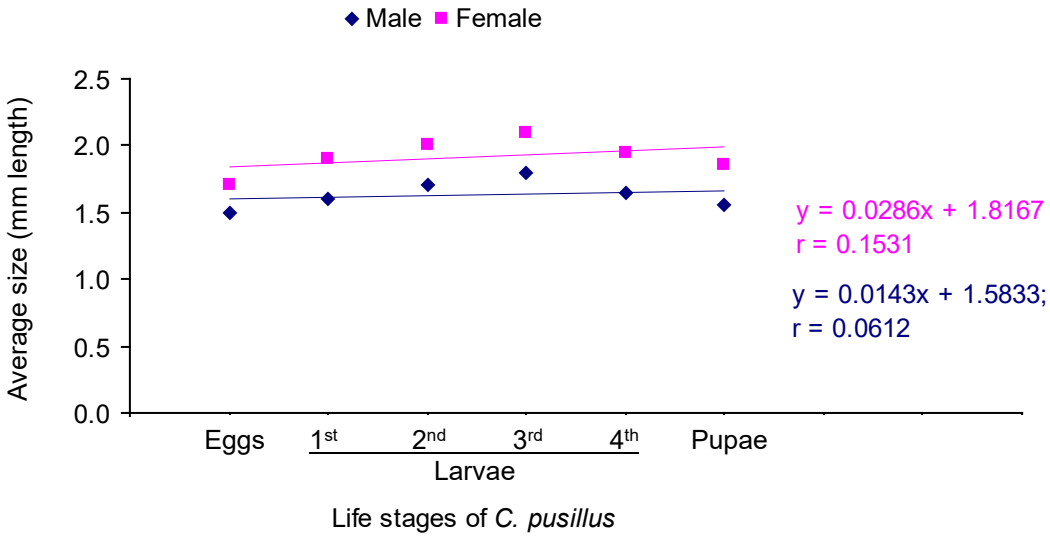
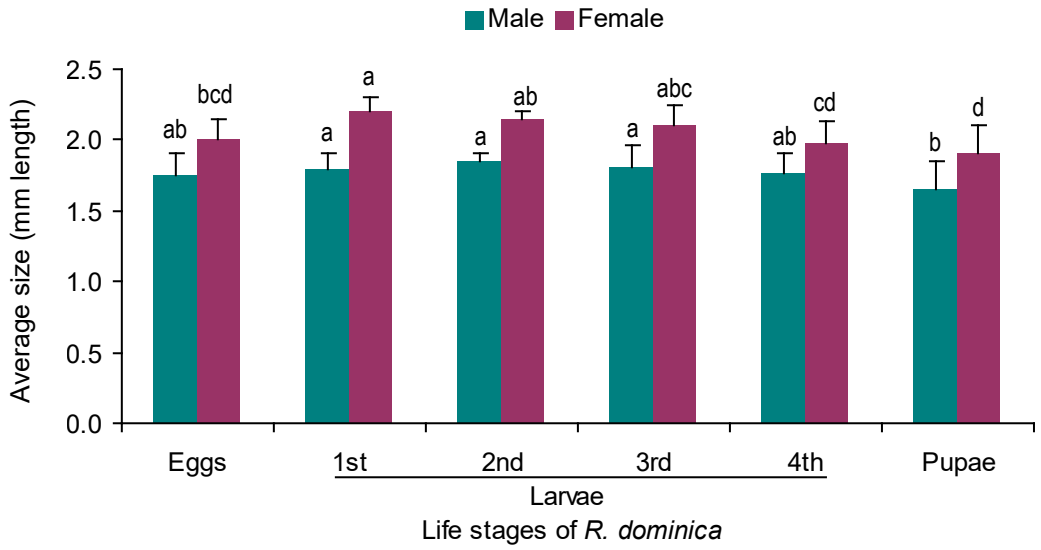


B

Figure 16A Average size (mm in length) of adult *X. flavipes* fed on different life stages of *C. pusillus*

16B Relationship between average size (mm in length) of adult *X. flavipes* and different life stages of *C. pusillus*



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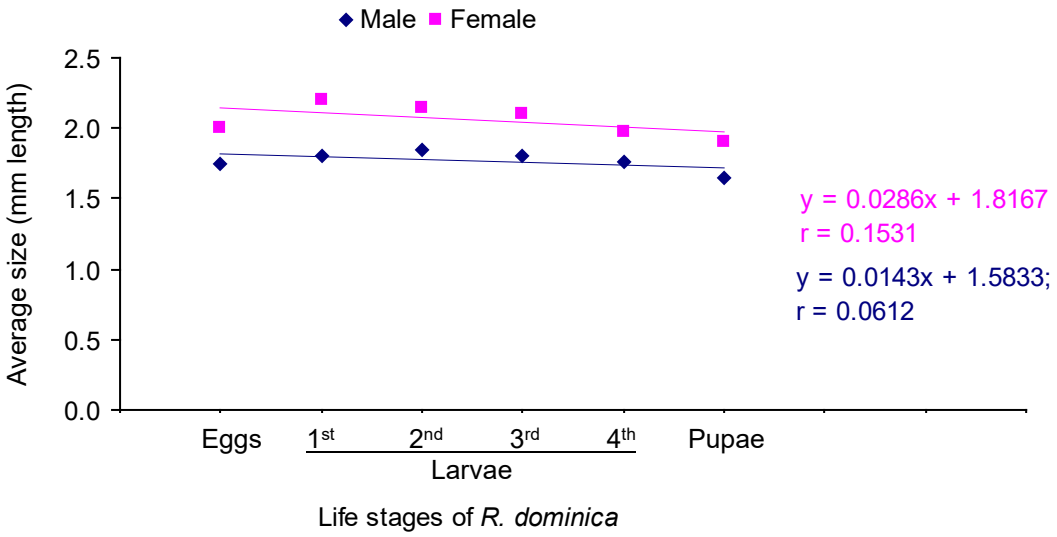
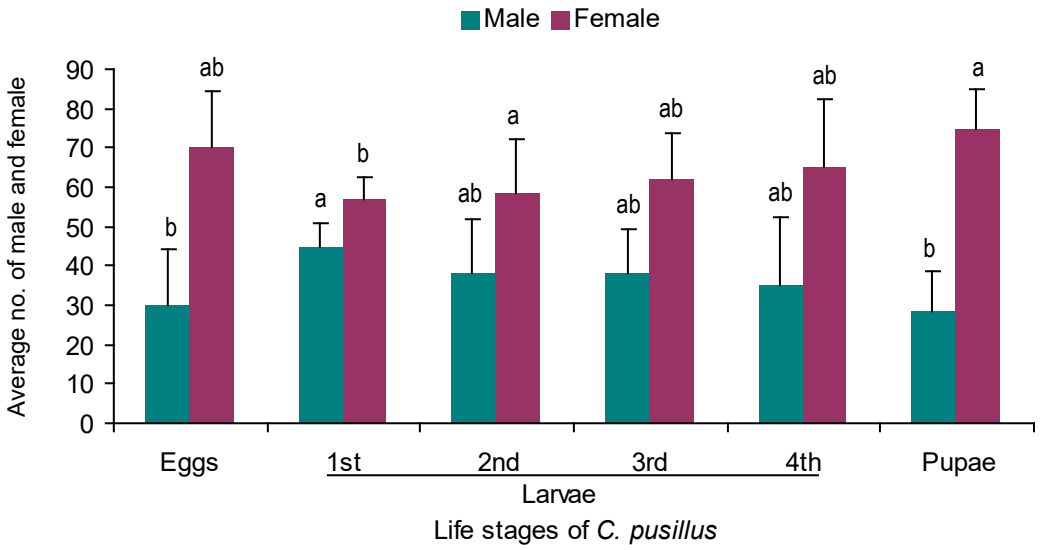


B

Figure 17A Average size (mm in length) of adult *X. flavipes* fed on different life stages of *R. dominica*

17B Relationship between average size (mm in length) of adult *X. flavipes* and different life stages of *R. dominica*



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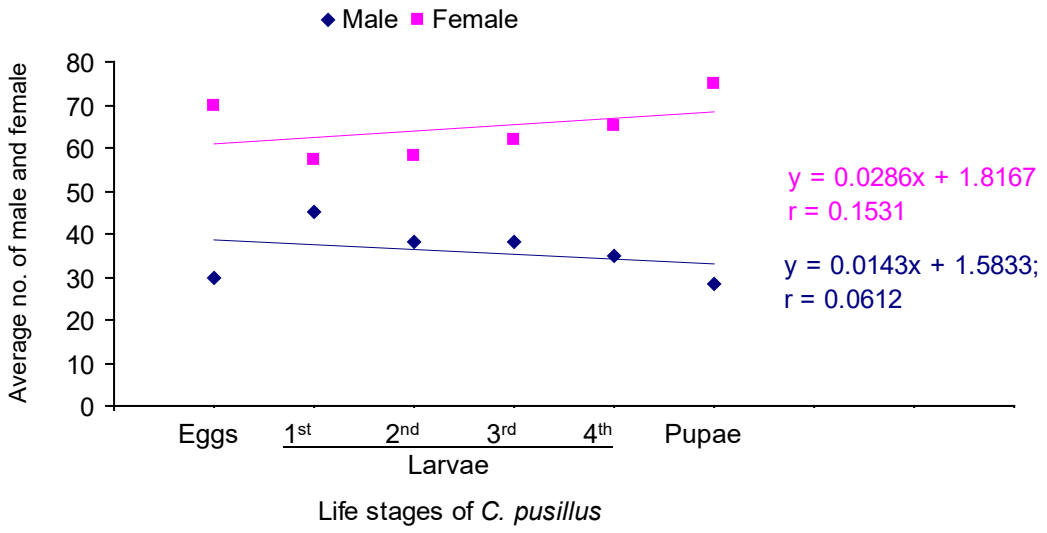
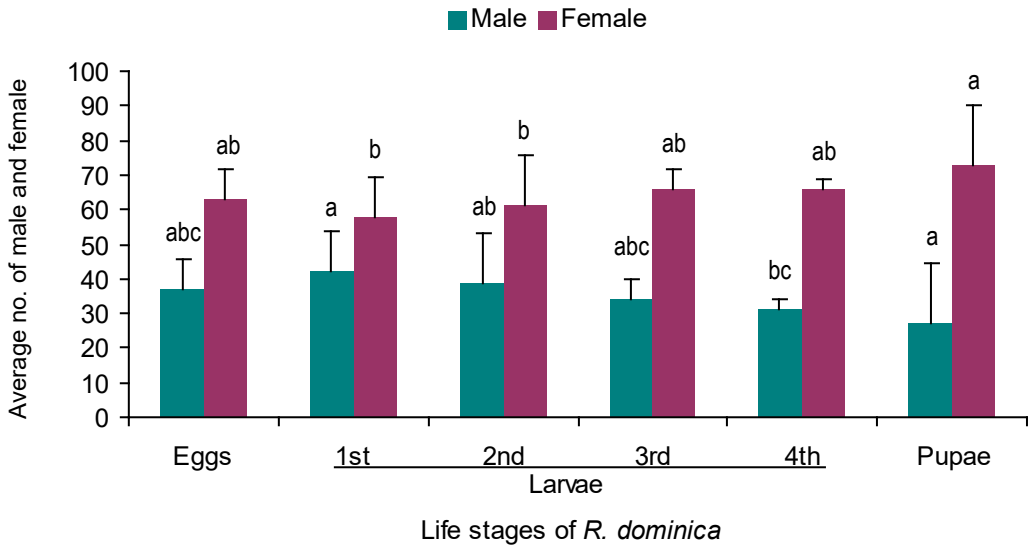


B

Figure 18A Average no. of *X. flavipes* male and female fed on different life stages of *C. pusillus*

18B Relationship between average sex ratio of *X. flavipes* and different life stages of *C. pusillus*



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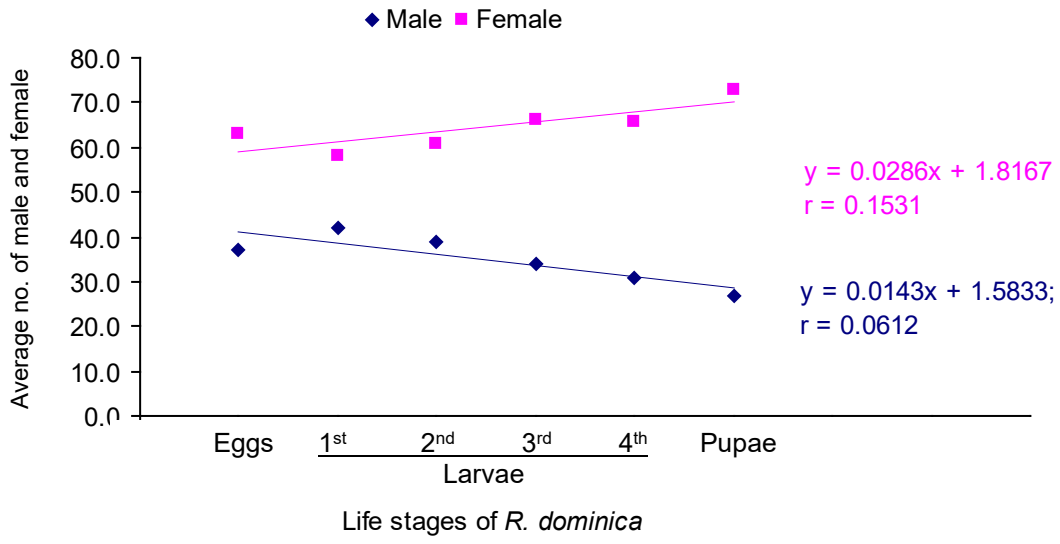


B

Figure 19A Average no. of *X. flavipes* male and female fed on different life stages of *R. dominica*

19B Relationship between average sex ratio of *X. flavipes* and different life stages of *R. dominica*

Discussion

Different life stages of hosts *C. pusillus* and *R. dominica* which used as food played an important role in controlling developmental periods, adult longevity, survivability, size and sex ratio of the predator *X. flavipes*. The consumption rate of individual predator varied widely depending on the stage of the prey. The predator developed faster, lived longer as an adult, survived better in the immature stage and laid more eggs when fed on coleopteran larvae rather than lepidopteran larvae (Abdel Rahman *et al*. 1978-79). *C. pusillus* and *R. dominica* were the most suitable prey of *X. flavipes* (Brower and Press 1992, Abdel Rahman *et al*. 1978-79). As intrinsic factors both temperature and relative humidity considerably affect the duration of nymphal and adult stage of *X. flavipes* (Abdel Rahman *et al*. 1977 and Arbogast 1978), similar results was found on different life stages of both *C. pusillus* and *R. dominica* respectively.

W Islam 1993 found that the developmental time of *Anisopteromalus calandrae* How from egg to adult emergence is 237.6±1.83 h for males and 206.57±0.78 h for females at 30±0.5°C and 70±0.05 rh. *Dinarmus basalis* Rond was found to attack and successfully complete its development within the larvae, pre-pupae and pupae of *Callosobruchus chinensis* L. and adult parasitoid emerged after complete life cycle within 12-14 days (Islam *et al.* 1985). At 30ºC temperature there are five nymphal instar of *X. flavipes* and the instar number may vary from 2-6 (Arbogast *et al*. 1971, Awadullah and Tawfik 1973). On *P. interpunctella* at 30ºC temperature, life of the predator was found to complete in 14-21 days (Arbogast 1975). In the present study mean developmental periods of nymphal instar was obtained as 12±1.15 - 22±0.58 days and 12±1.15 - 20±0.58 days feeding on different life stages of *C. pusillus* and *R. dominica* respectively.

Awadallah and Tawfik (1973) reported that adult males and females of *X. flavipes* when provided with *T. castaneum,* lived for 5-43 and 4-37 days respectively in average. However, the present study revealed that the adult males lived for 8±1.15, 10±1.15, 12±1.15, 11±1.15, 9±0.58, 6±0.58 days and 10±2.31, 12±1.15, 18±0.58, 10±1.33, 8±1.15, 5±0.58 days and females lived for 20±1.15, 25±2.89, 31±1.15, 28±1.15, 26±0.58, 14±1.15 days and 22±3.46, 26±0.88, 34±2.31, 24±2.31, 20±.15, 15±1.15 days in average on eggs, 1st, 2nd, 3rd, 4th instar larvae and pupae

respectively of *C. pusillus* and *R. dominica*. Abdel Rahman *et al*. (1977) found that raising temperature 15-35ºC enhanced development of the eggs and the nymphal stages and shortened the life span of the adult stage of *X. flavipes*.

The female *Dinarmus basalis* Rond fed on body fluids of *Callosobruchus chinensis* L. through the feeding tubes on the way of oviposition like many other Pteromalids and continuously deposited their eggs throughout their adult life (Islam 1991). *X flavipes* consumption on adult insect pests explained not only by the greater challenge to subdue large prey, but it also correlated with daily ingestion rate and gut capacity, the nutritional resources of large prey generally exceed the daily food requirements of small predators (Peters 1983). *X. flavipes* killed significantly more 'stimulating' larval prey than 'easy' egg prey (Lecato and Arbogast 1979, Russo *et al*. 2004). Arbogast (1978) stated that *X. flavipes* has a high capacity to increase in number relative to its prey. Lecato and Collins (1976) mentioned that *X. flavipes* destroys large quantities of prey when prey is abundant. It was observed in the present study that when an excess of eggs, 1st, 2nd, 3rd, 4th instar larvae and pupae of *C. pusillus* and *R. dominica* were provided, each predator killed an average of 300 eggs, 49 larvae and 25 pupae of *C. pusillus* and 400 eggs, 49 larvae and 10 pupae of *R. domonica* during their life time. But when the different life stages of host were provided separately, each predator destroyed an average of 400 eggs, 60 larvae and 28 pupae of *C. pusillus* and 500 eggs, 50 larvae and 20 pupae. The feeding intensity of *X. flavipes* differs depending on the species of the prey and their life stages. Awadallah *et al*. (1986) reported that the predator when preyed only on the larvae of different pest insects, the predator fed on 105 larvae of *Cocyra cephalonica*, 112 larvae of *T. confusum*, 30 larvae of *Stegobium panicerum*, 148 larvae of *Lasioderma serriocorni* during 43 days of life span. The previous reports showed that *X. flvipes* reduced population growth of *Oryzaephilus surinamensis* by 95% (Arbogast 1976), small population of *C. cautella* and *P. interpunctella* by more 70% (Brower and Mullen 1990) and several species of small beetles including *T. castaneum*, *Typhaea sterocorea*, *C. pusillus*, *R. dominica* 70-100% (Brower and Press 1992). Hymenopteran

parasitoid, life time reproductive success is strongly correlated with the number and quality of parasitized hosts (Potting *et al*. 1997). However, during this feeding period, the predator devoured 63 eggs only why such differences occur in feeding activity in relation to the prey species and life stages, the answer lies in the optimal diet theory which assumes that predators are able to rank prey in order of profitability (Charnov 1976). It is an evident from the present and previous studies on the biology of *X. flavipes* for growth and reproduction potentiality the predator needs energy rich food, Hence they feed on less number of pupae than the number of eggs or larvae.

Active feeding period regulates not only oviposition but also duration of egg laying and total life span. A female *X. flavipes* killed 47.3±4.88 larvae of *C. pusillus* and laid 20.1±1.66 eggs during her life time (25±1.26) days on *C. pusillus* larvae (Ahmed *et al*. 2004). It has been reported that an adult *X. flavipes* killed only 1 or 3 late instar larvae of *T. castaneum* in 24 h (Donnelly *et al*. 2001). When can prey on adult *Tribolium*. (Nishi *et al*. 2004) *X. flavipes* does not easily prey on adults. The present study showed that *X. flavipes* can't prey on adults of *C. pusillus* and *R. dominica*. Imamura *et al*. (2008) observed that the population of internal grain feeding insects such as *Sitophilus* and moth such as *C. cautella* and *P. interpunctella* were less affected by the predator compared with small external feeder such as *O. surinamensis* by the predator. The present study indicated the similar results of the above findings.

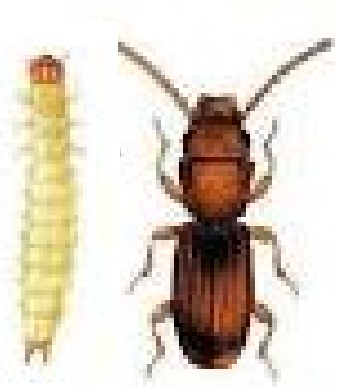
Survivability of immature stages of predator was maximum on 2nd instar larva and 3rd instar larvae and was minimum on pupae of *C. pusillus*. But in case of *R. dominica* it was maximum on 1st and 2nd instar larvae and was minimum on 4th instar larvae and pupae. Arbogast (1975) reported that survivability among immature stages of *X. flavipes* was lowest at 35ºC temperature.

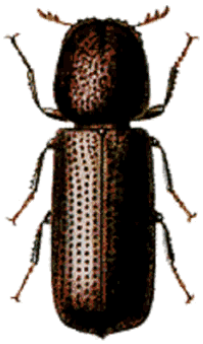
Lecato and Davis (1973) reported that the early instar nymph, late instar nymph and adult *X. flavipes* length (mean±SE) was 1.09± 0.01, 1.88± 0.02 and 2.22± 0.05 mm

respectively. In thepresent study, it was found that adult male size (length) was 1.50±0.06, 1.60±0.03, 1.75±0.03, 1.80±0.01, 1.65±0.03, 1.55±0.04 mm and 1.75±0.03, 1.80±0.02, 1.85±0.01, 1.81±0.03, 1.76±0.03, 1.65±0.04 mm and adult female size (length) was 1.70±0.06, 1.90±0.03, 2.00±0.03, 2.10±0.01, 1.95±0.03, 1.85±0.04 mm and 2.00±0.03, 2.20±0.06, 2.15±0.02, 2.10±0.01, 1.98±0.05, 1.90±0.03 mm in average on eggs, 1st, 2nd, 3rd, 4th instar larvae and pupae respectively of *C. pusillus* and *R. dominica*.

The sex ratio (%) of emerging adults of *X. flavipes* ranged from 0.98 to 0.76 at different host stage specific, but it was not significant from 1:1 for any of the life stages. Russo *et al*. (2004) found similar result for *X. flavipes*. Parajulee and Phillips (1993) reported a similar rate of *Lyctocoris carpestrist* at 30ºC and 60-70% relative humidity.

Taking into account the time of development, adult longevity, consumption rate, survivability, size and sex ratio the most suitable stage of the prey were 2nd instar larvae of *C. pusillus* and *R. dominica,* which produced the largest predator individuals in the shortest period of exposure. Overall *X. flavipes* proved itself as an effective predator of insect pest in grain storage and a potential controlling agent against *C. pusillus* and *R. dominica*.






Chapter 5

Effects of Spinosad on *C. pusillus* and *R. dominica*

Introduction

One of the challenges for 21st Century is to grow more food for the growing population of the world, and for food security (Pimental *et al.* 1994). For the protection of stored grains and other food commodities needs sustainable insect pest management of stored products, considering the safety of the consumers and the environment, within a cost effective way. *C. pusillus* is one of the serious external feeder and common major pests (Halstead 1993, Ahmed and Khatun 1994) and *R. dominica* is one of the most injurious internal feeder and a major pest (Potter 1935, Crombie 1941), occurring in all areas of the world where grain is produced and stored (Chittenden 1911, Chanbang *et al.* 2007, Jia *et al.* 2008, Edde 2012).

Both pests virtually feed on all kinds of stored grain and milled cereal products and causes immense damage to the tropical and subtropical countries throughout the world including Bangladesh (Dhaliwal 1976, Kirkpatrick and Cagle 1978, Hossain *et al.* 1986). The damage is caused by both the larval and adults stages of *C. pusillus* and *R. donimica* (Cotton 1963, Campbell and Sinha 1976, Arbogast 1991, Jia *et al*. 2008). Due to their high fecundity, polyphagous nature, quick adaptation against insecticides, control of these pests for a long time is quiet difficult and rather impossible.

Chemical control has been the most efficient and effective means for protection of stored product insect pests, but indiscriminate use of the pesticides has led to widespread resistance in insects and other arthropod pests. Continuous and enormous use of same or similar groups of synthetic pesticides causes problem of pesticide residues in foodstuff and other environmental contamination. Moreover, synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use (Keita *et al.* 2001). This has promoted the necessity for the development of new, safe, biodegradable alternate insecticides that could be feasible and effective for insect pest management in the stored ecosystem.

Spinosad appears to be one of the most promising new grain protectant (Thompson *et al*. 1997) and derived from soil bacteria, *S. spinosa*. Spinosad has rapid contact and ingestion activity in insects of fields and stores (Sparks *et al*. 1995, Bret *et al.* 1997, Toews and Subramanyam 2003), causing excitation of the nervous system, leading to cessation of feeding and paralysis (Ghosh *et al.* 2010). Because of its low mammalian toxicity and highly favourable environmental profile (Cleveland *et al*. 2001) Spinosad was screened against the insect pests to determine its effectiveness as a grain protectant (Bret *et al*. 1997, Subramanyam *et al.* 1999, 2002, Fang *et al*. 2002a, Mutambuki *et al*. 2002). Spinosad was found to be highly effective and provide long lasting (6 months to 2 years) control of stored product insect pests on various grains (Toews and Subramanyam 2003, Nayak *et al*. 2005, Maier *et al.* 2006, Subramanyam 2006 a, b, Huang and Subramanyam 2007, Huang *et al*. 2007, Subramanyam *et al*. 2007, 2012, Daglish *et al*. 2008, Chintzoglou *et al.* 2008a, b, Vayias *et al*. 2010a, b, Ghosh *et al*. 2010, Athanassiou *et al*. 2008a, b, 2009a,b, 2010a,b 2011, Hertlein *et al*. 2011). Although Spinosad is registered in the USA at a level rate of 1ppm and its maximum residue limit (MRL) rate of 1.5ppm on stored grains, it is not yet being marketed in the USA (Athanassiou *et al*. 2010, Hertlien *et al*. 2011). Laboratory and field tests on stored wheat showed that Spinosad at 1 mg/kg of grain were effective against several insect pests including the *R. dominica*, *C. ferrugineus*, and *P. interpunctella* (Fang *et al*. 2002a,b, Flinn *et al*. 2004 Huang *et al.* 2004). Spinosad cross resistance to organophosphate pyrethroids and methoprene had not been observed against a number of stored product insects, and it was found highly effective against *R. dominica* adults, killing them within 7 days and completely suppressing the progeny production of the beetle (Subramanyam *et al.* 2012). Previous studies have shown that *R. dominica* is highly susceptible to liquid or dry Spinosad even at low rates (Huang and Subramanyam 2007, Getchell and Subramanyam 2008).

So far the effect of Spinosad on *C. pusillus* and *R. dominica* has been conducted in a very few levels in Bangladesh. The aim of this study was to evaluate the effect of Spinosad on different life stages of *C. pusillus* and *R. dominica* at different exposure periods under laboratory conditions.

Materials and Methods

Insects: 2-d old eggs, 14-19d old larvae, pupae and 2d old adults of *C. pusillus* and 2d old eggs, 26-31d old larvae, pupae and 2d old adults of *R. dominica* were collected from previous established culture (Chapter 3) and were used in different tests of the present study.

Concentrations: The concentrations of Spinosad as 0.491, 0.983, 1.966, 3.932 and 7.863 µl/cm² were prepared using the method described in Chapter 3 and were used in this experiment.

Bioassays: Prepared concentrations treated filter papers were kept in Petri dishes (9 cm diameter) which were cleaned using by cotton with ethyl alcohol and dried immediately.

Eggs: Nine hundred 2d old eggs of *C. pusillus* and *R. dominica* were collected separately by sieving the food medium using the methods of Khan and Selmon (1981). Eggs were placed in Petri dishes containing filter paper either treated separately with 0.491, 0.983, 1.966, 3.932 and 7.863 µl/cm² concentrations of Spinosad and distilled water only, and all the Petri dishes were covered with lid. These Petri dishes were kept in CT (Controlled Temperature room at 30±0.5ºC temperature and 70±0.5% relative humidity, without controlling light. Three replications were used for each concentration and control also. Fifty eggs were used in each replicate (N=150). The eggs were observed daily under compound microscope for the hatching of larvae. The numbers of hatched and unhatched eggs were counted every 24h up to 10 days. Egg hatching was confirmed by counting the number of 1st instar larvae. The mortality of the eggs was assessed by counting the unhatched eggs after 10 days.

Larvae: Three hundred sixty 14-19 d old larvae of *C. pusillus* and 26 – 31d old larvae of *R. dominica* were placed in the Petri dishes containing filter paper either treated separately with the above mentioned concentrations of Spinosad and distilled water only and the Petri dishes were covered with lid. All the Petri dishes kept in CT room at 30±0.5ºC temperature and 70±0.5% relative humidity.

Three replications were used in each concentration and control. Twenty larvae were used in each replicate (N=60). Mortality was recorded after 24-, 48- and 72h-, after treatment (HAT). Those larvae that did not move when probed or shaken in the light and mild heat considered to be dead (Yousefnezhad-Irani and Asghar 2007).

Pupae: The experiment was set with the pupae of C. pusillus and *R. dominica* in similar way as set for the egg and larval stages. Three replications were used in each concentration and control. Twenty pupae were used separately for each concentration of Spinosad (as mentioned) and control. Mortality was recorded after 24-, 48- and 72- HAT. Pupae were considered dead when they did not move by any proved and shaken in the light and mild heat (Yousefnezhad-Irani and Asghar 2007).

Adults: The experiments were conducted with the same concentrations of Spinosad with a control batch using 2-d old adults of *C. pusillus* and *R. dominica*. Three replications for each of the concentrations and the control were continued. The experiments were conducted in Petri dishes similarly like the eggs, larvae of pupal stages. Mortality was counted after 24-, 48- and 72- HAT. Adults were considered to be dead when probing with a hot needle failed to produce a response (Yousefnezhad-Irani and Asghar 2007).

Data analysis: The mortality data were corrected by Abbott's (1925) formula wherever needed, the formula is

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where, P_r = corrected mortality (%)

P_o = observed mortality (%)

P_c = control mortality (%)

PRC value was calculated according to Mian and Mulla (1982) by the following formula

$$PRC = \frac{C - T}{C} \times 100$$

Where, C = No. of population in control

T = No. of population in treated media

All data were analyzed by Factorial ANOVA to compare mortality percentage as the response variables with concentrations, life stages, exposure periods. For comparison of the means Tukey's test (1953) was used. Lethal concentrations and the associated 95% limit of confidence were calculated by Probit analyses.

Results and Observation

Effect on egg hatchability: Spinosad showed a concentration related effects on the average percentage of egg hatchability of *C. pusillus* and *R. dominica* (Figure 20 and 21, Appendix table 62 and 77).

Average percentage of egg hatchability (±SE) was the lowest (5.00±1.02) at 7.863 µl/cm² concentration and the highest (25.00±1.15) at 0.491µl/cm² concentration in *C. pusillus*. At 7.863, 3.932, 1.966, 0.983 and 0.491 µl/cm² concentrations, the average percent of egg hatchability of *C. pusillus* was less than that of the control and PRC value was the highest 88.10% at 7.863µl/cm² and the lowest was 40.48% at 0.491 µl/cm²(Appendix Table 62). The effect of different concentrations on egg hatchability was found highly significant (P<0.001) (Appendix table 63).

In case of *R. dominica,* average percentage of egg hatchability (±SE) was lowest 0.33±1.03 at 7.863 µl/cm² concentrations and highest 15.00±1.14 at 0.491 µl/cm² concentrations. At 7.863, 3.932, 1.966, 0.983 and 0.491 µl/cm² concentrations, the average percent of egg hatchability was significantly less than that of the control and PRC value was the highest 99.13% at 7.863 µl/cm² concentrations whereas lowest 60.53% at 0.491 µl/cm² concentrations(Appendix table 77). The effect of different concentrations on egg hatchability was found highly significant (P<0.001) (Appendix table 78).

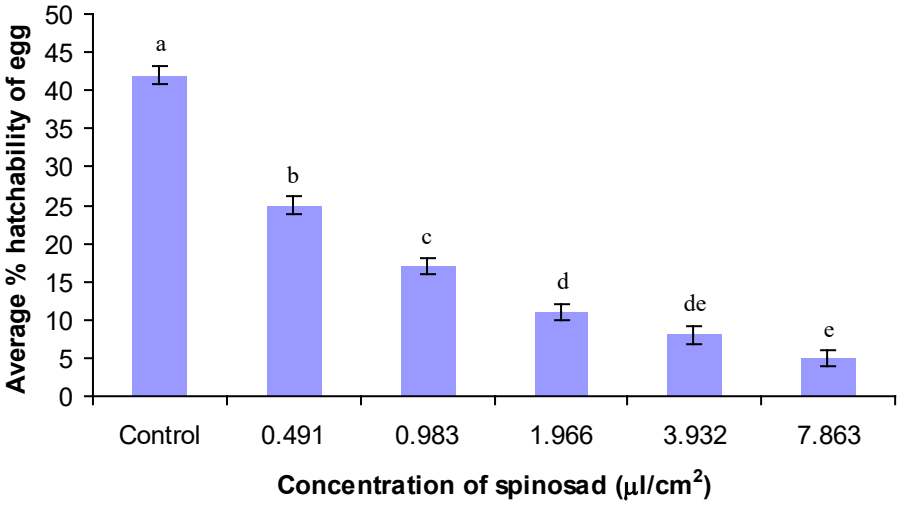


Figure 20 Average (%) hatchability of *C. pusillus* on different concentrations of Spinosad.

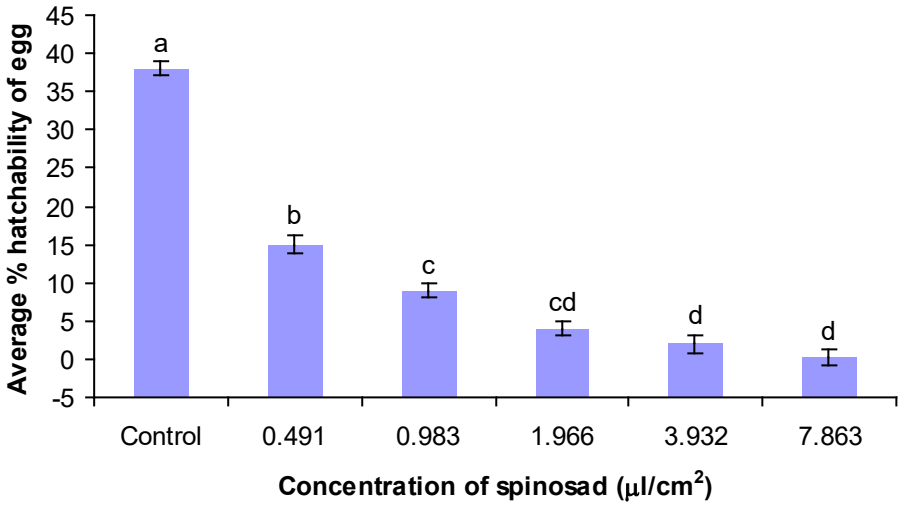


Figure 21 Average (%) hatchability of *R. dominica* on different concentrations of Spinosad.

Toxicity on larvae: The larval mortality of *C. pusillus* was observed on prepared concentrations of Spinosad after 24-, 48- and 72 hours after treatment (HAT) and the results of the experiments along with statistical analyses are shown in Figure 22, Table 2 and Appendix table 64-66, 74. All the Spinosad concentrations by contact were found to be toxic to the larvae compare to control. Toxicity of Spinosad was increased with the increase of concentration and exposure time. There were significant differences in the mean mortality of larvae between exposure periods ($F=58.038$, $df=2$, $P<0.0001$) and between concentrations ($F=38$, df-5, $P<0.001$). In addition, the interaction between exposure periods and concentration was significant (Appendix table 74). LC_{50} at 24-h was 18.208, 48-h was 5.912 and 72-h was 0.175 µl/cm^2 Figure 15 (Figure 28, Appendix Table 73).

In case of *R. dominica*, the larval mortality was observed on prepared concentrations of Spinosad after 24, 48 and 72 h of exposure and the results statistical analyses are shown in Figure 23, Table 3 and Appendix tables 79-81, 89. All the concentrations acted as larvicide by contact. Average mortality (±SE) was highest 13.33±0.88 at 7.863 µl/cm² concentration after 72 h and lowest 4.67±0.33 at 0.491 µl/cm² concentrations after 24 h of exposure (Table 3). There were significant differences in the mean mortality of larvae between exposure times ($F=57.026$, $df=2$, $P<0.001$) and between concentrations ($F=56.006$, df-5, $P<0.001$). In addition, the interaction between exposure time and concentration was significant ($F=7.795$, df-10, $P<0.001$) (Appendix table 89). LC_{50} at 24-h was 9.22978, 48 h was 2.835366 and 72 h was 0.5433412µl/cm² (Figure 28, Appendix table 88).

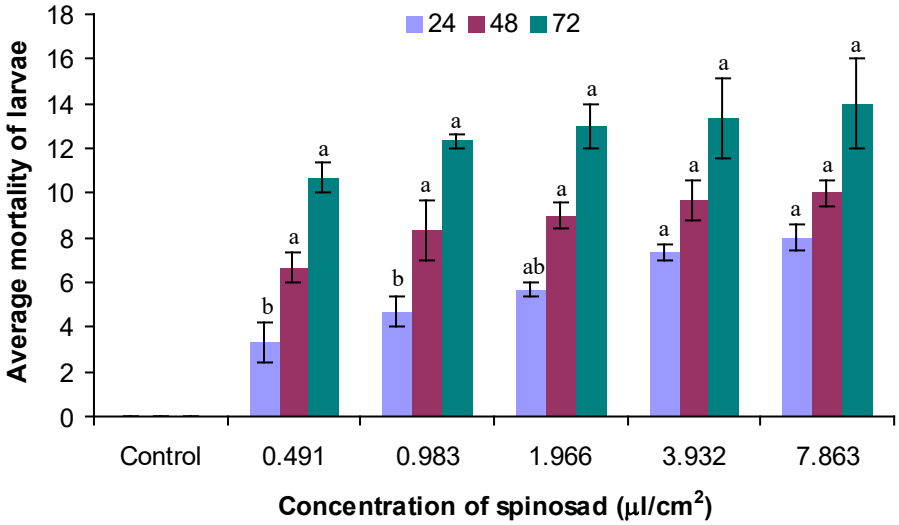


Figure 22 Average mortality of *C. pusillus* larvae on different concentrations of Spinosad after 24, 48 and 72 h period of exposure

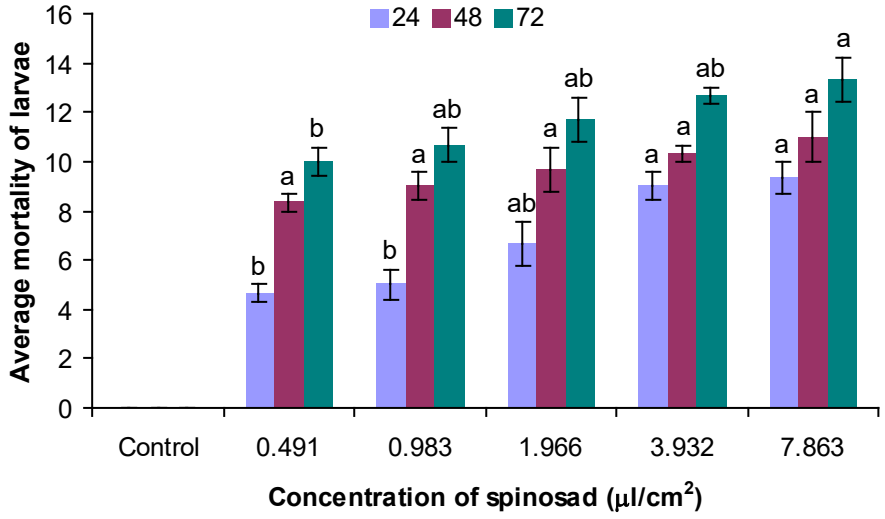


Figure 23 Average mortality of *R. dominica* larvae on different concentrations of Spinosad after 24, 48 and 72 h period of exposure

Toxicity on pupae: Pupal mortality of *C. pusillus* at different concentrations of Spinosad 24-, 48- and 72 HAT was observed and the results were shown in Figure 24, Table 2 and Appendix tables 67-69, 75. The concentrations were found to effective causing pupal mortality by contact action. compare to control. Average mortality (±SE) was highest 8.33±0.88 at 7.863 µl/cm² concentration after 72 h and lowest was 1.67±0.33 at 0.491 µl/cm² concentration after 24 h of exposure (Table 2). There were significant differences in the mean mortality between exposure periods (F=30.79, df=2, P<0.001) and between concentrations (F=61.524, df-5, P<0.001). In addition, the interaction between exposure period and concentration was significant (F=2.558, df-10, P<0.001) (Appendix table 75). LC_{50} at 24-h was 568.5706 , 48 h was 1841.139 and 72 h was 35.94058 µl/cm² (Figure 28, Appendix Table 73).

Pupal mortality of *R. dominica* at different concentrations of Spinosad after 24, 48 and 72 h of exposure was observed on and the results were shown in Figure 25, Table 3 and Appendix table 82-84,90. The tested concentrations were found to be potential causing pupal mortality by contact compared to control. Average mortality (±SE) was highest 8.33±1.45 at 7.863 µl/cm² concentration after 72 h and lowest 1.00±0.58 at 0.491 µl/cm² concentration after 24 h of exposure (Table 3). There were significant differences in the mean mortality between exposure periods (F=32.986, df=2, P<0.001) and as well as between concentrations (F=23.986, df-5, P<0.001). In addition, the interaction between exposure periods and concentration was significant (F=4.622, df-10, P<0.001) (Appendix table 90). LC_{50} at 24-h was 1138.777, 48 h was 231.1335 and 72 h was 22.0538 µl/cm² (Figure 28, Appendix table 88).

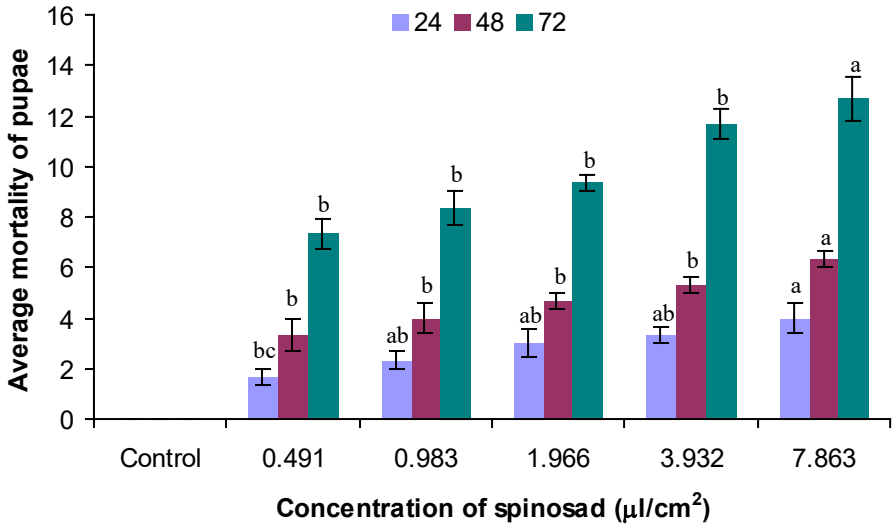


Figure 24 Average mortality of *C. pusillus* pupae on different concentrations of Spinosad after 24, 48 and 72 h period of exposure

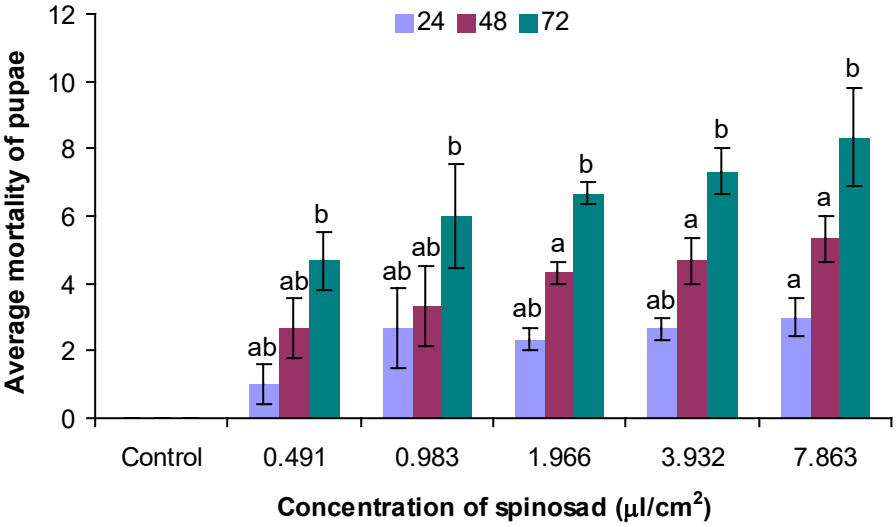


Figure 25 Average mortality of *R. dominica* pupae on different concentrations of Spinosad after 24, 48 and 72 h period of exposure

Toxicity on adults: At all tested concentrations of Spinosad, adult mortality of *C. pusillus* were higher than that of larval and pupal stages (Table 2). Adult mortality of *C. pusillus* on different concentrations of Spinosad after 24-, 48- and 72 HAT was observed and the results with their statistical analyses are presented in Figures 26, Table 2 and Appendix tables 70-72, and 76. The concentrations were found to be potential causing adult mortality based on the contact compare to control. Average mortality (±SE) was highest 15.33±1.22 at 7.863µl/cm² after 72 h and lowest was 5.00±0.58 at 0.491µl/cm² after 24 h of exposure (Table 2). There were significant differences in the mean mortality of adults between exposure periods ($F=202.970$, $df=2$, $P<0.001$) and between concentrations ($F=42.617$, $df=5$, $P<0.001$). In addition, the interaction between exposure period and concentration was significant ($F=8.350$, $df=10$, $P<0.001$) (Appendix table 76). The LC_{50} values after 24- h was 7.995, 48- h was 2.145 and 72- h was 0.839 µl/cm² (Figure 28, Appendix table 73).

The tested concentrations of Spinosad, 2d old adult mortality of *R. dominica* were higher than that of larval and pupal stages (Table 3). Adult mortality of *R. dominica* on different concentrations of Spinosad after 24, 48 and 72 h of exposure was observed and the results with statistical analyses were shown in Figure 27, Table 3 and Appendix table 85-87, 91. Different concentrations were found to be potential causing adult mortality by contact compare to control. Average mortality (±SE) was highest 17.33±1.20 at 7.863 µl/cm² concentrations after 72 h and lowest 6.67±0.88 at 0.491 µl/cm² concentrations after 24 h of exposure (Table 3). There were significant differences in the mean mortality of adults between exposure periods ($F=36.122$, $df=2$, $P<0.001$) and between concentrations ($F=83.468$, $df=5$, $P<0.001$). In addition, the interaction between exposure time and concentration was significant ($F=2.017$, $df=10$, $P<0.001$) (Appendix table 91). LC_{50} at 24-h was 6.951654, 48 h was 0.9590641 and 72 h was 0.466328 µl/cm² (Figure 28, Appendix table 88). Spinosad showed a concentration related effects on adult mortality. The potentiality was higher in all treatments with the increase of concentrations and exposure periods.

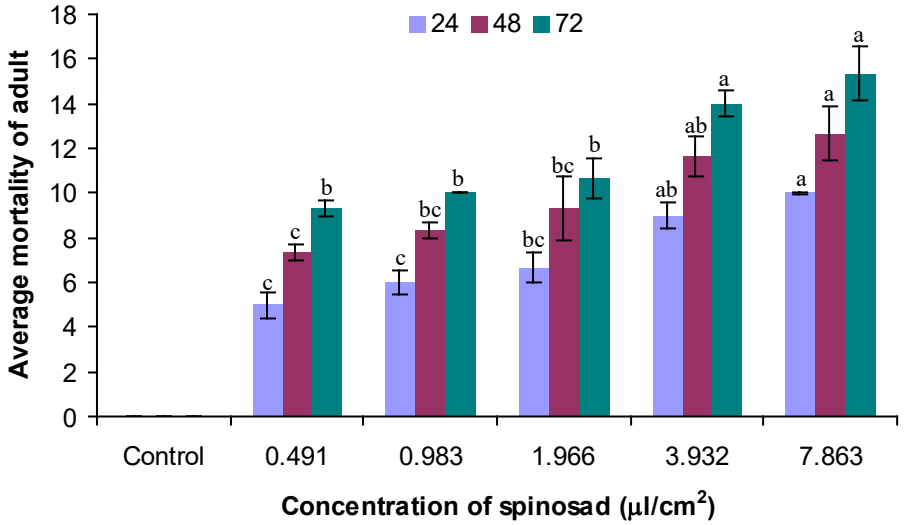


Figure 26 Average mortality of *C. pusillus* adults on different concentrations of Spinosad after 24, 48 and 72 h period of exposure.

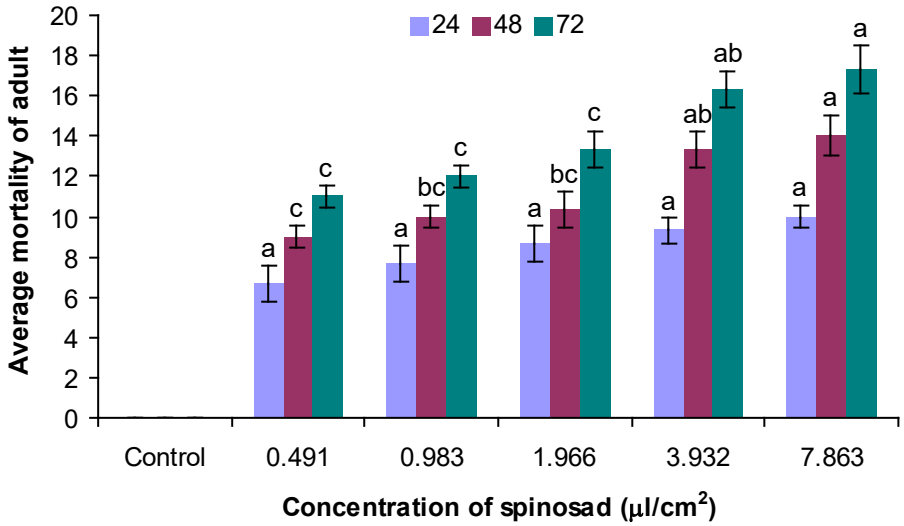


Figure 27 Average mortality of *R. dominica* adult on different concentrations of Spinosad after 24, 48 and 72 h period of exposure

Table 2 Mortality (mean±SE) of 14-19d larvae, pupae and 2d adults of *C. pusillus* at different concentrations of Spinosad after different exposure periods.

Exposure periods (h)	Concentrations (µl/cm^2)	Mortality (mean ± SE)					
		Larvae	PRC	Pupae	PRC	Adults	PRC
24	Control	0.00±0.00c	-	0.00±0.00c	-	0.00±0.00d	-
	0.491	3.33±0.88b	16.65	1.67±0.33bc	8.35	5.00±0.58c	25.00
	0.983	4.67±0.67b	23.35	2.33±0.33ab	11.65	6.00±0.58c	30.00
	1.966	5.67±0.33ab	28.35	3.00±0.58ab	15.00	6.67±0.67bc	33.35
	3.932	7.33±0.33a	36.65	3.33±0.33ab	16.65	9.00±0.58ab	45.00
	7.863	8.00±0.58a	40.00	4.00±0.58a	20.00	10.00±0.058a	50.00
48	Control	0.00±0.00b	-	0.00±0.00c	-	0.00±0.00d	-
	0.491	6.67±0.67a	33.35	3.33±0.67b	16.65	7.33±0.33c	36.65
	0.983	8.33±1.33a	41.65	4.00±0.58b	20.00	8.33±0.33bc	41.65
	1.966	9.00±0.58a	45.00	4.67±0.33ab	23.35	9.33±1.45abc	46.65
	3.932	9.67±0.88a	48.35	5.33±0.33ab	26.65	11.67±0.88ab	58.35
	7.863	10.00±0.58a	50.00	6.33±0.33a	31.65	12.67±1.20a	63.35
72	Control	0.00±0.00b	-	0.00±0.00c	-	0.00±0.00c	-
	0.491	10.67±0.67a	53.35	5.00±0.58b	25.00	9.33±0.33b	46.65
	0.983	12.33±0.33a	61.65	5.67±0.67ab	28.35	10.00±0.00b	50.00
	1.966	13.00±1.00a	65.00	6.33±0.33ab	31.65	10.67±0.88b	53.35
	3.932	13.33±1.76a	66.65	7.00±0.58ab	35.00	14.00±0.58a	70.00
	7.863	14.00±2.00a	70.00	8.33±0.88a	41.65	15.33±1.22a	76.65

Note: Means with same letter do not significantly differed from each other (Tukey's Test)

Table 3 Mortality (mean±SE) of 26-31d larvae, pupae and 2d adults of *R. dominica* at different concentrations of Spinosad after different exposure periods

Exposure periods (h)	Concentrations (µl/cm²)	Mortality (mean ± SE)					
		Larvae	PRC	Pupae	PRC	Adults	PRC
24	Control	0.00±0.00c	-	0.00±0.00a	-	0.00±0.00b	-
	0.491	4.67±0.33b	23.35	1.00±0.58ab	5.00	6.67±0.88a	33.35
	0.983	5.00±0.58b	25.00	2.67±1.20ab	13.35	7.67±0.88a	38.35
	1.966	6.67±0.88ab	33.35	2.33±0.33ab	11.65	8.67±0.88a	43.35
	3.932	9.00±0.58a	45.00	2.67±0.33ab	13.35	9.33±0.67a	46.65
	7.863	9.33±0.67a	46.65	3.00±0.58a	15.00	10.00±0.58a	50.00
48	Control	0.00±0.00b	-	0.00±0.00b	-	0.00±0.00d	-
	0.491	8.33±0.33a	41.65	2.67±0.88ab	13.35	9.00±0.58c	45.00
	0.983	9.00±0.58a	45.00	3.33±1.20ab	16.65	10.00±0.58bc	50.00
	1.966	9.67±0.88a	48.35	4.33±0.33a	21.65	10.33±0.88bc	51.65
	3.932	10.33±0.33a	51.65	4.67±0.67a	23.35	13.33±0.88ab	66.65
	7.863	11.00±1.00a	55.00	5.33±0.67a	26.65	14.00±1.00a	70.00
72	Control	0.00±0.00c	-	0.00±0.00a	-	0.00±0.00d	-
	0.491	10.00±0.58b	50.00	4.67±0.88b	23.35	11.00±0.58c	55.00
	0.983	10.67±0.67ab	53.35	6.00±1.55b	30.00	12.00±0.58c	60.00
	1.966	11.67±0.88ab	58.35	6.67±0.33b	33.35	13.33±0.88bc	66.65
	3.932	12.67±0.33ab	63.35	7.33±0.67b	36.65	16.33±0.88ab	81.65
	7.863	13.33±0.88a	66.65	8.33±1.45b	41.65	17.33±1.20a	86.65

Note: Means with same letter do not significantly differed from each other (Tukey's Test)

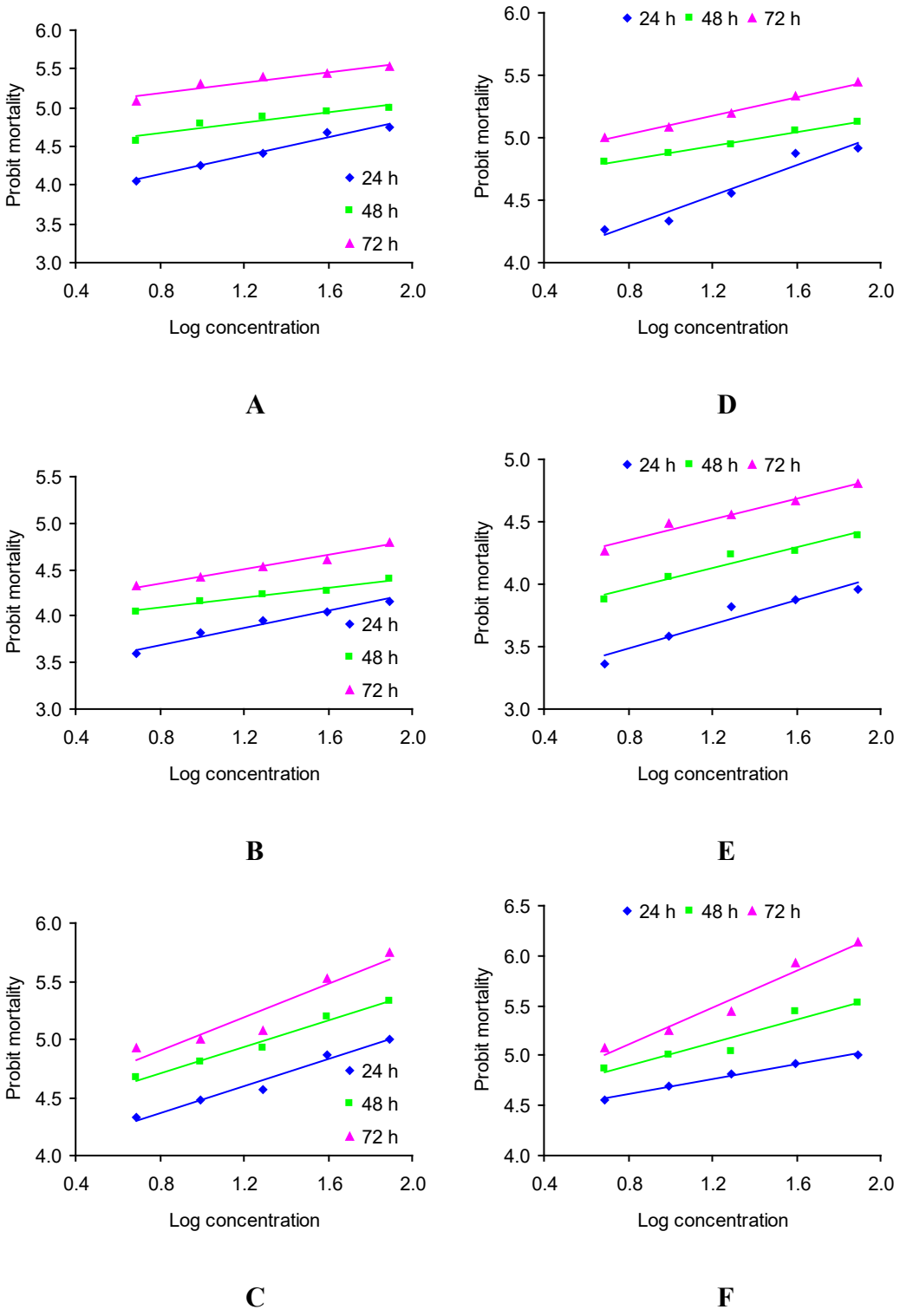


Figure 28 Regression lines of probit mortality on log concentration of Spinosad on *C. pusillus* (A = larvae, B = pupae and C = adults) and *R. dominica* (D = larvae, E = pupae and F = adult).

Discussion

Results of the present experiments revealed that there were significant impacts of the contact activity different concentrations of Spinosad and exposure periods against egg hatchability of *C. pusillus* and *R. dominica*. Spinosad concentrations were found to be toxic against 14-19 d old larvae, pupae and 2 d old adults of *C. pusillus* and 2d old eggs, 26-31d old larvae, pupae and 2d old adults of *R. dominica*. Adults were the most susceptible in comparison with that of larvae and pupae at concentration and longest exposure period. Mortality of pupae was found the least number followed by larvae and adults. Egg hatchability was found to be dependent on the concentration. Similar effects of Spinosad have been reported on other stored-product insects by Mutambuki *et al*. (2003), Chintzoglou *et al*. (2008a, b), Aarthi and Murugan (2010), Athanassiou *et al*. (2010a,b), Mollaie *et al*. (2011).

Naturally, eggs of *R. dominica* are laid singly or in groups/clusters on the exterior of the kernel (Potter 1935). Eggs hatch and the active neonate bores inside the kernel, where it completes development up to the adult stage (Howe 1950, Arbogast 1991). Upon reaching the adult stage, *R. dominica* emerges from the kernel and creates a large exit hole (Potter 1935, Rees 1995). Triflumuron caused 100% egg mortality of *O. surinamensis*, *R. dominica* and *T. castaneum* when they were exposed for four weeks (Mian and Mulla 1982a). Similar results were reported by Mazid *et al*. (2006), Eisa *et al*. (1986), Saxena and Mathur (1981 a, b) and Saxena and Kumar (1989).

LC_{50} values of Spinosad against the larvae of *C. pusillus and R. dominica* 24-, 48-, and 72 HAT were 18.208, 5.912 and 0.1755 and 9.230, 2.835 and 0.543µl/cm^2 respectively, revealed that this bacterial insecticide is quite potent to kill the larvae at very low concentration, but will require a longer exposure (72-h). Mortality of 50 and 70% were achieved after 48- and 72 h. The toxic effect of Spinosad begun during 48-h of exposure. As the treatments were given by contact, probably an oral treatment with Spinosad would be able to produce higher rate of mortality in the larvae at more lower concentrations and shorter exposure periods.

It was reported that *E. kuehniella* larvae were more susceptible to Spinosad compared to *P. interpunctella* larvae. Spinosad at the concentrations of 0.1, 0.25, 0.5, 0.75 and 1 mg/kg completely suppressed larval survival of *E. kuehniella* (Mollaie *et al.* 2011). Spinosad at (1 mg/kg) resulted 95% suppression of larval survival and adult emergence of *P. interpunctella* was achieved. On the contrary, Spinosad at 0.1 mg/kg resulted 100% larval mortality of *E. kuehniella.* Differences in toxicity of Spinosad was recorded on the might be due different commodity used as pest food (Fang *et al*. 2002a, Chintzoglou *et al*. 2008a,b). Fang *et al*. (2002a), Huang *et al*. (2007), Huang and Subramanyam (2007), reported that susceptibility of *P. interpunctella* larvae to Spinosad was dose dependent. Toews and Subramanyam (2003) reported that mortality of *T. castaneum* was increased with increasing the concentrations of Spinosad by contact treatment. In addition, the mortality of adults in all concentrations was dependent to exposure time, and 72 HAT caused the highest mortality. The application rates of 0.1 and 0.5 ppm Spinosad gave 83 and 100% mortality respectively of *Cryptolestes* spp on Spinosad- treated maize (Huang and Subramanyam 2007). Bond *et al*. (2004) reported that the naturally derived insecticide Spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Cetin *et al*. (2005) worked on the evaluation of the naturally derived insecticide Spinosad against *Culex pipiens* L. (Diptera: Culicidae) larvae in septic tank water in Antalya. Additional studies have reported the larvicidal properties of Spinosad in this and other mosquito species (Liu *et al*. 2004a, b, Cetin *et al*. 2005 b, Darriet *et al*. 2005, Darriet and Corbel 2006 and Romi *et al*. 2006).

As pupa is the non-feeding stage of *C. pusillus* and *R. dominica only* contact treatment is possible to give. As a whole the pupal stage was found to be comparatively tolerant (PRC value was <50% at maximum concentration and longer exposure) to Spinosad treatment. To achive more toxic effect against the pupae of *C. pusillus* and *R. dominica* more higher concentrations of Spinosad have to be applied. There is a lack of literature reporting the Spinosad

toxicity at the pupal stages of stored product insects, so the present result is quite difficult to compare.

Bonjur *et al*. (2011) conducted field experiments with 0, 25, 50 and 70 ppm concentrations of ozone in steel bins containing wheat against seven stored grain pests after and found that adult pupae of *P. interpunctella* were more susceptible to those concentrations after 1, 2, 3 and 4 d of exposure. Kovendan *et al*. (2012) found that pupal mortality of *Aedes aegypti* was 12% by the treatment of Spinosad at 20 ppm and it has been increased to 64% at 100 ppm after 24 h and the LC_{50} value of pupae was 93.44 ppm. The above results are more or less similar to the present findings.

Athanassiou *et al*. (2010a, b) evaluated adult mortality of *Cryptolestes* spp on 0.1 and 0.5 ppm Spinosad treated wheat and recorded 86.7±3.3 and 97.80±2.2 % mortal effect respectively after 14 days, however, the mortality of control adults was 26.70±5.8 %. In the present study the PRC values were 50, 63.35 and 76.65 at the highest concentration of Spinosad (7.863 µl/cm^2) exposed 72-h. The higher concentrations needed in the present study might be due to the contact treatement and lower exposure (72 h compared to 14d).

Subramanyam *et al*. (1999) carried out an experiment to establish the efficacy of 1, 3, 6, 10, 15 and 20 mg/kg concentrations of Spinosad on wheat after 8 and 14 d of exposure and found that the mortality of *R. dominica* adults was 100%. Fang *et al*. (2002a) found 100% mortality of *R. dominica* adults on wheat treated with 1 ppm of Spinosad after 7 days of exposure. For instance, Toews and Subramanyam (2003) reported that *T. castaneum* was by far more tolerant to Spinosad than *R. dominica* and *S. oryzae.* Similar results have also been reported by other researchers (Huang *et al*. 2004, Nayak *et al*. 2005, Getchell 2006). Daglish and Nayak (2006) reported that Spinosad residues were stable for 9 months on wheat, without loss of insecticidal activity against *R. dominica.* In another study, Daglish *et al*. (2006) found that Spinosad applied at 0.5 or 1mg/kg was completely effective for 9months at both 55 and 70% RH, with 100% adult mortality of *R. dominica* after 14 days of exposure and no live f_1 adults produced.

Arthur (2004a, b) reported mortality of *R. dominica* adults exposed to dust and liquid formulations of methoprene. Athanassiou *et al*. (2008a) found that at 0.01ppm of Spinosad at 30°C temperature and 75% relative humidity after 7, 14 and 21 d of exposure average percent mortality of *R. dominica* (± SE) was achieved 55.6 ± 7.0, 84.5 ± 8.1 and 94.4 ± 2.2 respectively. An another experiment, Athanassiou *et al.* (2010a) evaluated the efficacy of 0.1 and 0.5 ppm concentrations of Spinosad against adults of *R. dominica* on wheat after 14 d of exposure and found that average percent mortality of *R. dominica* (± SE) were 100.0 ± 0.0 and 100.0 ± 0.0 respectively whereas 3.3 ± 1.7 mortality was in control. *C. maculatus* appears to be highly susceptible to Spinosad at a low rate of 0.3 ppm (Sanon *et al*. 2010). The present findings are similar to the above results.

The present results indicate that Spinosad is an effective tool to control all the life stages of *C. pusillus* and *R. dominica* the degree of toxicity of Spinosad can be ranked as eggs>adults > larvae> pupae. So, it can be concluded that low concentrations of Spinosad would be potential to control *C. pusillus* and *R. dominica* in storage system.

Chapter 6

Effects of Spinosad on *X. flavipes*

Introduction

Among the predatory bugs, *X. flavipes* is more stout bodied cosmopolitan insect and one of the most challenging efficient predator of many insect pests in storage commodities (Gross 1954, Arbogast 1979, Brower *et al*. 1996 and Imamura *et al.* 2008). This predacious bug successfully controls the population of small insect pests of storage particularly by sucking fluids of eggs and early instar larvae that are neither heavily sclerotized nor overly hirsute (Lecato and Davids 1973, Sing and Arbogast 2008a,b). This bug has already been commercialized in North-America and is marketed for biocontrol of stored product insects (Mason and Hubner 2001).

There is limited evidence to suggest that *X. flavipes* is more pesticide-tolerant than parasitoids and pest insects. Baker and Arbogast (1995) reported *X. flavipes* to be 4-fold and 10-fold more tolerant to malathion than *A. calandrae* and *H. hebetor,* respectively. Press *et al*. (1978) reported that *X. flavipes* generally exhibited greater tolerance to the insecticides permethrin, fenitrothion, pirimiphos-methyl, pyrethrins_piperonyl butoxide, and malathion than three prey species, including *T. castaneum*, *L. serricorne* and *P. interpunctella.*

Use of synthetic pesticides causes some unfortunate consequences such as environmental pollution, pests/hosts resistance and toxicity to other non-target organisms including human beings, biological pesticides from microbial origin are environmentally safe pesticides. Among microbial insecticides, Spinosad is specially valuable because its non-toxicity to non-target animals and human beings (Aarthi and Murugan 2010). In field crop markets, where it has been sold since 1997, Spinosad is minimally disruptive to beneficial insects and compatible with Integrated Pest Management (IPM) programs in many crops (Miles 2006 and Arthur *et al*. 2007). A portion of Spinosad's selectivity in crop markets derives from its intrinsic toxicity profile relative to beneficial insects, which can vary by taxa, but with Spinosad generally being less toxic to predators than to parasitoids (Michaud 2003 and Williams *et al.* 2003). A second important factor contributing to

Spinosad's favorable selectivity is its relatively transient presence in the environment due to photolytic breakdown. Spinosad's impact on beneficial insect populations under field conditions are typically short lived and followed by rapid recovery (Williams *et al*. 2003 and Miles and Eelen 2006). However, in grain storage environments where sunlight is not present, Spinosad's residual efficacy has been shown to be much longer, on the order of six months to two years. Therefore, Spinosad's impact on beneficial insects can be quite different in grain versus crop environments. In a labroatory study, Toews and Subramanyam (2004) found that Spinosad applied to stored wheat at 1 ppm was highly toxic to the parasitoids *H. hebetor*, *T. elegans* and *A. calandrae* yet not so to *X. flavipes*, which demonstrated 92% survival and was able to reproduce under these same conditions. The findings from this laboratory study were subsequently supported by field bin trials conducted by Parker *et al.* (2004a and 2004b) in stored sorghum and Parker and Falconer (2004, 2005, 2006 and 2007) in stored corn, where they showed no survival of the parasitoid *A. calandrae* and only limited survival of the parasitoid *C. elegans* when grain was treated with Spinosad at 1 ppm and parasitoid populations were monitored over storage periods ranging from 10 to 24 months. However, populations of *X. flavipes* remained relatively unaffected by Spinosad in the similar sorghum bin study. These results suggest *X. flavipes* can survive and reproduce in grains treated with Spinosad at rates up to 1 ppm, whereas parasitoid populations will be markedly reduced due either to the direct toxicity of Spinosad or indirectly through Spinosad's impact on their host species. Spinosad is likely to be no worse than many other grain protectants with respect to its impact on beneficial species.

Spinosad appears to be very compatible with many predatory insects of the crop field like green lacewing (*Chrysoperla carnea*), ladybird beetle (*Hippodamia convergens*), minute pirate bug (*Orius laevigatus*), big-eyed bug (*Geocoris punctipes*), and damsel bug (*Nabis* spp.) (Thompson *et al.* 2000). Moreover, Spinosad is safe to nymphs and adults of many natural enemies including

X. flavipes (Ghosh *et al*. 2010). Previous research has focused the effects of Spinosad on many natural enemies or beneficial insects (Schoonover and Larson 1995, Bret *et al.* 1997, Boucher 1999, Tillman and Mulrooney 2000, Thompson *et al.* 2000, Consoli *et al.* 2001, Mason *et al*. 2002, Williams *et al*. 2003, Michaud 2003, Towes and Subramanyam 2004, Wang *et al*. 2005, Subramanyum *et al*. 2007, Daglish *et al*. 2008, Ghosh *et al*. 2010) whereas, very few experiments have yet been conducted to explore the effects of Spinosad on *X. flavipes*. Therefore, the present investigation was undertaken to evaluate the stage specific susceptibility of X. *flavipes* to several concentrations of Spinosad at different exposure periods in the laboratory condition which are likely to be compatible with stored product ecosystems that make the powerful and well Integrated Pest Management strategies.

Materials and Methods:

Insects: 2d old eggs, 4d old nymphs and 2d old adults of *X. flavipes* were collected from the stock culture (Chapter 3) and used in the present experiments.

Concentrations used: The concentrations of Spinosad applied to the pests *C. pusillus* and *R. dominica*, were also used in this experiment to observe that the applicable dosages for the pest control is either affect the predator or not.

Bioassays: Concentration treated filter papers were kept in Petri dishes (9 cm diameter) which were cleaned by cotton with ethyl alcohol and dried immediately.

Eggs: Nine hundred 2d old eggs of *X. flavipes* were collected by sieving the food medium using according to Khan and Selmon (1981). Eggs were placed in Petri dish containing filter paper either treated separately with 0.491, 0.983, 1.966, 3.932 and 7.863µl/cm² concentrations of Spinosad and distilled water only, and Petri dishes were covered with lid. The petri dishes were kept in CT (Controlled tempareture) room at 30±0.5ºC temperature and 70±0.5% relative humidity, Three replications were used for each concentration and control. Fifty eggs were used in each replicate (N=150). The egg was observed daily under compound microscope until hatching. The numbers of hatched and unhatched eggs were

counted every 24h up to 10 days. Eggs hatching were confirmed by counting the number of 1st instar nymphs. The mortality of eggs was assessed by counting the unhatched eggs after 10 days.

Nymphs: Three hundred sixty 4d old nymphs of *X. flavipes* were placed in the Petri dishes containing filter paper either treated separately with prepared concentrations of Spinosad or distilled water only and the Petri dishes were covered with lid. All Petri dishes kept in CT room at 30±0.5ºC temperature and 70±0.5% relative humidity. Three replications were carried out in each concentration and control. Twenty nymphs were used in each replicate (N=60). Mortality was recorded after 24,- 48 and 72h after post treatment. Those nymphs that did not move when probed or shaken in the light and mild heat considered to be dead (Yousefnezhad-Irani and Asghar 2007).

Adults: The experiment was conducted with the same concentrations of Spinosad, with a control batch using 2d old adults in three raplication for eacg of the concentrations and the control. The experiment was conducted in Petri dishes similarly like the egg and nymphal stages. Mortality was counted after 24, 48 and 72 HAT. Adults were considered to be dead when probing with a hot needle failed to produce a response (Yousefnezhad-Irani and Asghar 2007).

Data analysis: The mortality data were corrected by Abbott's (1925) formula. The data were analyzed by Factorial ANOVA to compare mortality percentage as the response variable and concentrations, life stages and exposure periods. For the comparison of means the Tukey's test (1953) was used. Lethal concentrations and the associated 95% limit of confidence were calculated by Probit analyses. PRC values were calculated according to the formula of Mian and Mulla (1982a).

Results and Observation

Effects on egg hatchability: Spinosad inhibited egg hatchability of *X. flavipes* at all concentrations (Figure 29, Appendix table 92). In average 25-35% eggs o0f the predator were hatched. The hatchability was higher in all treatments with the increase of concentrations. Egg hatchability was decreased with the increased concentrations, and the PRC values ranged from 2.86-28.57 (Appendix table 92).

The effect of different concentrations on egg hatchability was found significant (P<0.001) (Appendix table 93).

Toxicity on nymphs: 4d old nymphs of *X. flavipes* were used and mortality recorded on different concentrations of Spinosad after 24, 48 and 72 h of exposure. The findings with statistical analyses are shown in Figure 30, Table 4, and Appendix tables 94-96 and 101. Different concentrations were found to be potential causing very few nymphal mortality based on the contact treatment compare to control (Table 4). Average percent mortality (±SE) was highest 6.67±1.76 at 7.863µl/cm² after 72 h and lowest was 1.00±0.58 at 0.491µl/cm² after 24 h of exposure (Table 4). At 95% confidence limit lower to upper 24 h LC_{50} was 432.6535 (2.922949-237196.1), 48 h LC_{50} was 137.838 (3.740582-5079.244) and 72 h LC_{50} was 73.82966 µl/cm² (3.22157-1691.976) respectively (Figure 32, Appendix table 100). There were significant differences in the mean mortality of larvae between exposure periods (F=37.587, df=2, P<0.001) and between concentrations (F=12.611, df-5, P<0.001). In addition, the interaction between exposure periods and concentration was significant (F=5.460, df-10, P<0.001) (Appendix table 101). Moreover, Nymphal mortality was found higher than that of adults (Table 4).

Toxicity on adults: Two day old adult mortality of *X. flavipes* were found less than that of nymphal stages at all tested concentrations of Spinosad (Table 4). Adult mortality of *X. flavipes* on different concentrations of Spinosad after 24, 48 and 72 h of exposure and the statistical analyses were shown in Figure 31, Table 4 and Appendix table 97-99 and 102. The tested concentrations were found to be effective causing adult mortality based on the ingestion and contact compare to control. Average percent mortality (±SE) was highest 5.00±0.45 at 7.863µl/cm² concentrations after 72 h and lowest 1.67±0.67 at 0.491µl/cm² concentrations after 24 h of exposure (Table 4). At 95% confidence limit lower to upper 24 h LC_{50} was 492.8509 (3.859698-62932.93), 48 h LC_{50} was 348.9742 (4.223628-28833.73) and 72 h LC_{50} was 331.5098µl/cm² (2.651994-41440.03) respectively (Figure 32, Appendix table 100). There were significant differences in the mean mortality of adults between exposure periods ($F=7.386$, $df=2$, $P<0.001$) and between concentrations ($F=5.219$, $df=5$, $P<0.001$). In addition, the interaction between exposure period and concentration was significant ($F=2.432$, $df=10$, $P<0.001$)

(Appendix table 102). Spinosad showed a concentration related effects on adult mortality. The potentiality was higher in all treatments with the increase of concentrations and exposure periods. Moreover, at 0.491, 0.983 and 1.966µl/cm² concentrations of Spinosad, after 24 h to 72 h of exposure survivability of *X. flavipes* adults were found range 94 to 84% respectively (Table 4).

Table 4 Effects of different concentrations of Spinosad on 4d nymphs and 2d adults of *X. flavipes* at different exposure periods.

Exposure periods (h)	Concentrations (µl/cm^2)	Mortality (mean ± SE)			
		Nymphs	PRC	Adults	PRC
24	Control	0.00±0.00a	-	0.00±0.00b	-
	0.491	1.00±0.58ab	5.00	1.67±0.67a	8.35
	0.983	1.33±0.67ab	6.65	1.33±0.33a	6.65
	1.966	1.67±0.33ab	8.35	2.00±0.05a	10.00
	3.932	2.33±0.33a	11.65	2.67±0.33a	13.35
	7.863	3.00±0.58a	15.00	3.33±0.33a	16.65
48	Control	0.00±0.00c	-	0.00±0.00d	-
	0.491	2.33±0.33b	11.65	1.33±0.33cd	6.65
	0.983	3.33±0.67ab	16.65	2.00±0.03bc	10.00
	1.966	4.00±0.08ab	20.00	2.33±0.33bc	11.65
	3.932	5.00±0.03a	25.00	3.00±0.03ab	15.00
	7.863	5.33±0.88a	26.65	4.00±0.58a	20.00
72	Control	0.00±0.00b	-	0.00±0.00d	-
	0.491	3.33±0.33ab	16.65	2.00±0.05c	10.00
	0.983	4.00±0.58a	20.00	3.00±0.58bc	15.00
	1.966	5.00±0.58a	25.00	3.33±0.33bc	16.65
	3.932	6.00±0.04a	30.00	3.67±0.33ab	18.35
	7.863	6.67±1.76a	33.35	5.00±0.45a	25.00

Note: Means with same letter do not significantly differed from each other (Tukey's Test)

Figure 32 Regression lines of probit mortality on log concentration of Spinosad on *X. flavipes* (A = nymphs and B = adults).

Discussion

Due to the very low mammalian toxicity (Breslin *et al*. 2000) and rapid breakdown in the environment (Cleveland *et al.* 2002, Thompson *et al*. 2002), there can be little doubt that Spinosad represents an important improvement over conventional synthetic pesticides in terms of safety to farm workers, traders and the consumers of pesticide-treated agricultural post harvest cereal, grain products and other stored commodities. In total, there were 228 observations on the impact of Spinosad on 52 species of natural enemies of which 162 involved predators (27 species) and 66 involved parasitoids (25 species) where as 71% (42/59) of laboratory studies and 79% (81/103) of field-type studies were conducted on predators in which Spinosad was not harmful to predators (Williams *et al*. 2003). As Spinosad appears to have low toxicity to many beneficial insects (Elzen *et al*.1998), it has potential for use in Integrated Pest Management (IPM) systems. In the present study, it is clear that there is no impact of 0.491, 0.983 and 1.966µl/cm² concentrations of Spinosad on egg hatchability, survivability of nymphs and adults of the predator, *X flavipes* in comparison with the control. The results showed that egg hatchability of *X. flavipes* was almost similar the control at lower three concentrations of Spinosad after 10 d of exposure. Previous studies suggesting the safety of Spinosad to eggs of Chrysopids employed topical applications and did not assay consumption by active life stages.

Schoonover and Larson (1995) found that LC_{50} of the predator, *Orius insidiosus* nymph was 200 ppm, Torres *et al.* (1999) found that LC_{50} of the predator, *Podisus nigrispinus* nymph was 45 ppm and Vinuela *et al.* (1998) found that LC_{50} of *P. maculiventris* nymph was 33 ppm respectively to moderate concentrations (30-200 ppm) of Spinosad. Toews and Subramanyam (2004) estimated that the mean number of *X. flavipes* nymphs ($F=4.07$, df= 1, 11, $P=0.069$) and adults ($F=1.88$, df= 1, 11, $P=0.197$) recovered was similar between the control and treatments. Effects of fungus *Beauveria bassiana* strain Bb-RSB on *Orius sauteri* (Hemiptera: Anthocoridae) were examined under laboratory conditions and found that *O. sauteri* nymphs treated with either low or high *B. bassiana* concentrations

were able to complete their life cycle as well as control nymphs, mortality was very low and adult longevity was not affected (Gao *et al*. 2012). These results also supported the present findings.

The lack of lethal infections indicates that *O. sauteri* is not within the physiological host range of this strain of *B. bassiana* (Zimmermann 2007).

Schoonover and Larson (1995) found that LC_{50} of *Phytoseiulus persimilis* adult was 200 ppm and Torres *et al.* (1999) found that LC_{50} of *Podisus nigrispinus* adult was 53 ppm respectively to moderate concentrations (30-200 ppm) of Spinosad. Ludwing and Oetting (2001) obtained ambiguous results with their two tests of Spinosad for compatibility with *O. insidiosus* for control of thrips on greenhouse chrysanthemums. Miles and Dutton (2000) reported Spinosad as highly toxic to parasitic Hymenoptera in greenhouses, but concluded it was compatible with *O. insidiosus*, *C. rufilabris* and the coccinellids *Hippodamia convergens* and *Coccinella septempunctata*. Tillman and Mulroney (2000) observed toxicity of Spinosad to three parasitoid species in cotton, *Bracon mellitor*, *Cardiochiles nigriceps* and *Cotesia marginiventris* although the coccinellids *Coleomegilla maculata* and *H. convergens* were unaffected. Similarly, Mason *et al.* (2002) demonstrated toxicity of Spinosad to *Trichogramma inyoense*, an egg parasitoid of *Mamestra configurata*. Toews and Subramanyam (2004) observed that absolute densities derived from sieving all the grain at the end of the study showed differences among treatments in the number of *X. flavipes* adults ($F=12.73$, $df=1,4$, $P=0.023$) but not nymphs ($F=2.92$, $df=1,4$, $P=0.163$). These results are similar to the present study because the adult survivability was ranged 84 to 94% at lower three concentrations of Spinosad after 24 to 72 h of expusure.

Tillman and Mulrooney (2000) found that counts of a hemipteran predator *Geocoris punctipes* (Say) (Hemiptera: Lygaidae), were not affected by Spinosad in cotton fields. *X. flavipes* is a more stout-bodied insect, and was perhaps, sucking behabiour of body fluids of live soft bodied small insects and also less affected by the sieving. A general lack of survival among hymenopterans exposed to Spinosad was also reported in the literature (Tillman and Mulrooney 2000, Mason *et al*. 2002, Michaud 2003).

A recent review of predator and parasitoid susceptibility to Spinosad concluded that this product represented one of the most judicious insecticides available for the conservation of predator populations (Williams *et al.* 2003). However, the majority of laboratory and field studies in natural and semi-natural conditions report moderately harmful or harmful effects on populations of hymenopteran parasitoids (Bernardo and Viggiani 2000, Hill and Foster 2000, Tillman and Mulrooney 2000, Mason *et al*. 2002).

Assessment of the impact of Spinosad on non-target insects is particularly relevant now that synthetic Spinosyn analogues (Spinosoids) are being developed for increased environmental stability and an altered spectrum of insecticidal activity (Crouse *et al*. 2001, Sparks *et al*. 2001).

Laboratory dose-mortality studies are of limited use in predicting the impact of a toxicant on non-target invertebrate populations in the field (Stark *et al*. 1995, Wright and Verkerk 1995, Longley and Jepson 1997). Species or stage-related differences in biology and behaviour can significantly influence the susceptibility of natural enemies to pesticides (Longley and Jepson 1997, Verkerk *et al*.1998). This was particularly evident when considering the impact of Spinosad on immature parasitoids developing in *S. frugiperda* eggs and larvae. Similar effects, including an inability to spin a cocoon or pupation or Spinosad-induced mortality at the moment of adult emergence have been observed in parasitoids of other taxa (Suh *et al*. 2000, Gahbiche 2001, Mason *et al*. 2002).

Predators generally suffer insignificant sub-lethal effects following exposure to Spinosad, whereas parasitoids often show sub-lethal effects including loss of reproductive capacity, reduced longevity, etc. All studies agree that Spinosad residues degrade quickly in the field, with little residual toxicity at 3-7 days post application (Williams *et al*. 2003).

Spinosad shows no effects on predatory insects such as ladybirds, lacewings, big-eyed bugs or minute pirate bugs (Copping 2001). Spinosad is slow acting compared to conventional synthetic insecticides, but is more rapid than most entomopathogens (Bret *et al*. 1997).

Clearly, caution is required when making assumptions about pesticide impact on beneficial organisms based exclusively on toxicity data generated in laboratory studies (Stark *et al*. 1995). It is becoming increasingly clear that species or stage-related differences in biology and behaviour and even crop type can significantly influence the susceptibility of non-target invertebrates to pesticides (Longley and Jepson 1997, Verkerk *et al.* 1998). Moreover, the fact that a natural enemy survives exposure to a poison does not necessarily mean that it will perform as well as a non-intoxicated con specific; many of the indirect sub-lethal effects on natural enemy function (foraging, predation, etc.) and/or reproduction cannot be detected by laboratory dose/mortality assays (Wright and Verkerk 1995, Longley and Jepson 1996). In addition, natural enemies subjected to multiple routes of exposure to pesticides may respond in unexpected ways that would be impossible to predict based on single route laboratory toxicity tests (Banken and Stark 1998, Kunkel *et al*. 2001).

The need for accurate assessment of the environmental impact of agrochemicals is an issue of international concern (Croft 1990, Levitan *et al*. 1995, Reus *et al*. 2002). This information is specially relevant now that large areas are being treated with Spinosad, for example to control fruit flies (Peck and McQuate 2000, Prokopy *et al*. 2000, Vargas *et al*. 2001). Toews and Subramanyam (2004) found that the survival of natural enemies, the predator *X. flavipes* was 87.6±2.7% in untreated wheat (control) and was >90% in wheat treated with 1mg/kg Spinosad.

It can be concluded that there is little impact of 0.491, 0.983 and 1.966µl/cm² concentrations of Spinosad on egg hatchability, survivability of nymphs and adults of *X flavipes* which is negligible. These concentrations of Spinosad may be used with the predator, *X. flavipes* for the well management of many stored product insect pests.




Chapter 7

Effects of *X. flavipes* and Spinosad on pest population after different storage periods

Introduction

Control of stored product insects is considered the best achieved through an integration of physical, chemical, and biological methods (Arthur 1996, Hagstrum *et al*. 1999, Phillips and Throne 2010). In this context in storage facilities, where light is absent the bacterial insecticide Spinosad could be a potential agent; and was found to be remain stable for a long period (up to two years), thus it can provide long-term protection for stored grains (Fang *et al*. 2002b, Fang and Subramanyam 2003, Arthur *et al.* 2006, Hertlein *et al.* 2011). Spinosad has already been proved very effective against a range of stored-grain insect species, even at lower rates than the application rate, in both laboratory (Fang *et al.* 2002a, Toews and Subramanyam 2003, Nayak *et al*. 2005, Daglish and Nayak 2006) and field tests (Maier *et al*. 2006, Subramanyam *et al.* 2007, Daglish *et al.* 2008) and there by The US Environmental Protection Agency approved for its use as a grain protectant in 2005, at the application rate of 1ppm (mg/kg of grain) (Subramanyam 2006a,b).

Bret *et al*. (1997) reported that Spinosad was much less toxic to beneficial insects in field crops than synthetic pesticides. Schoonover and Larson (1995) reported that Spinosad was practically nontoxic to the insidious flower bug, lady beetle, phytoseid mite, and common green lacewing. Boucher (1999) reported that Spinosad applied to bell peppers effectively controlled the pepper maggot but it did not reduce populations of beneficial arthropods, including unspecified species of Coccinellidae, Chrysopidae, Cecidomyiidae, Syrphidae, Nabidae, and hymenopteran-parasitized Aphididae. Mason *et al.* (2002) found that Spinosad was toxic to the parasitoids *Trichogramma inyoense* Pinto and Oatman (Hymenoptera: Trichogrammatidae) and *Microplitis mediator* Haliday (Hymenoptera: Braconidae). Surprisingly, the predacious bug, *X. flavipes* can survive 92% and reproduce at 1ppm Spinosad (Towes and Subramanyam 2004).

Stored grain insects can coexist with other arthropods, which may act as natural enemies, within storage and hence a binary combination of insecticide and predator may give more complete control than the application of a single

insecticide or predator. Many combinations of Spinosad with beneficial insects such as predator, parasitoids and with insecticides as a stored grain protectant have been progressively highlighted in a series of scientific publications (Towes and Subramanyam 2004, Parker *et al*. 2004a, 2004b, Szabela 2005, Williams *et al.* 2003, Miles and Eelen 2006, Miles 2006, Arthurs *et al*. 2007, Bonjour *et al*. 2006, Parker and Falconer 2006, 2007, Nayak and Daglish 2007, Subramanyam *et al.* 2007, Daglish 2008, Daglish *et al*. 2008, Chintzoglou *et al*. 2008a, Huang *et al*.2009, Ghosh *et al.* 2010, Vayias *et al.* 2010b, Athanassiou *et al.* 2010a,b). Combination is needed, specially when mortality from Spinosad is not complete or progeny production occurs, even when parental adults are killed on different grains (Fang *et al*. 2002a, Athanassiou *et al*. 2008c, Chintzoglou *et al*. 2008). Conversely, *X. flavipes* affects progeny production or immature stages but generally adults are not affected (Lecato and Davids 1973, Arbogast 1979, Brower and Mullen 1990, Brower and Press 1992, Helbig 1999, Donnely and Phillips 2001, Russo *et al*. 2004, Sing and Arbogast 2007). Hence, a combination of *X. flavipes* and Spinosad may be more effective than the application of each alone. Lower rates of Spinosad were used to determine if even small amounts of Spinosad combined with *X. flavipes* would improve the control of different stored grain species, including those life stages of insect pests that are not controlled by either *X. flavipes* or Spinosad. Considering these, in the present investigation, the potentiality of predator *X. flavipes* and Spinosad (contact treatment) alone and in combination were evaluated to control *C. pusillus* and *R. domonica* supplied with standard food at different storage periods.

Materials and Methods

Insects: Unsexed 2d old adults of predator *X. flavipes* and 5d old adults of *C. pusillus* and *R. dominica* were collected from the respective stock culture (Chapter 3) using by aspirator and were used in this experiment.

Standard food: White wheat, wheat flour and brewer's yeast were cleaned and sterilized using the methods as described in chapter 3. Firstly, wheat flour and brewer's yeast were mixed (ratio 19:1 in weight) and prepared mixture food.

Secondly, the standard food were prepared with white wheat and mixture food where the ratio was 3:1 between wheat and mixture food. Finally, the standard food 10.5kg was made for the experiments.

Bioassays: Thirty six pieces of filter papers (9cm diameter) were soaked in 0.491, 0.983 and 1.966 µl/cm² concentrations of Spinosad and 12 pieces of filter papers (9cm diameter) treated with only 2ml distilled water and were dried them at room temperature using the methods as described in chapter 5. 200g of standard food were taken in a cylindrical plastic container (12 cm diameter, 20 cm in height), such 24 containers were prepared for *C. pusillus* and another 24 containers were prepared for *R. dominica*. One hundred adults of *C. pusillus* in 1:1 ratio of male and female were introduced in each of 24 containers. Similarly 100 adults 1:1 male and female *R. dominica* were introduced in another 24 set of containers. The mouths of all containers were covered by the fine clothes with the help of rubber bands and were kept in the CT room at 30±0.5ºC temperature and 70±0.5% relative humidity without controlling light. After 25 days, each filter paper either treated with distilled water only (control) or treated with different concentrations of Spinosad was divided into 4 pieces and were inserted into standard food of each container at different layers separately. Then 30 unsexed adults of *X. flavipes* were released in the container either treated with distilled water or treated with different concentrations of Spinosad for both species. The mouths of all the containers were covered similarly and were placed in the CT room at same environmental conditions.

After 3, 6, 9 and 12 months the containers were opened, adults were removed sieving by 125 and 500 micrometer aperture sieve. The total numbers of live and dead adults were counted separately, the dead adults were discarded. In case of *C. pusillus,* adult predator were not found after every exposure period, so after each three months 30 unsexed adults of *X. flavipes* were added to the container. Few larvae of *C. pusillus* were found, their numbers were added to the adults. In case of *R. dominica,* always there found adult *X. flavipes,* so there was no need to add new predators in the experiment. Only adults were counted for *R. dominica* as

this species complete larval and pupal development within the wheat kernels. After counting an additional 5g of standard food was added to each container to avoid the conditioning of food medium due to overcrowding or shortage (Mondal 1985).

Another set of experiment was conducted with 30 unsexed adult predators released separately in containers containing either *C. pusillus* or *R. dominica* adults rearing on same quality of standard food and similar experimental conditions, and stored for same periods. For control batch similar numbers of *C. pusillus* and *R. dominica* adults were released in standard food without providing any predator or Spinosad treated filter paper. All the experiments were replicated three times. With each concentrations all the experiment were done under same laboratory conditions.

Data Analyses: All data were analyzed by Factorial ANOVA to compare mortality percentage as the response variable and concentrations, life stages, exposure periods main effects. For the comparison of means the Tukey's test (1953) was used. The percent reduction of population to control (PRC) was calculated according to Mian and Mulla (1982a) by the following formula.

Percentage reduction in population

$$(\text{PRC}) = \frac{\text{No. of population in control - No. of population in treated media}}{\text{No. of population in control}} \times 100$$

or, $$\text{PRC} = \frac{\text{C - T}}{\text{C}} \times 100$$

Where, C = No. of population in control

T = No. of population in treated media

Results and observations

Effects on adult population of *C. pusillus*: The data of the present experiment revealed that the combined effects of different concentrations of Spinosad and *X. flavipes* reduced the population growth of *C. pusillus* after different storage periods in comparison with that of control (untreated) and control (treated with 30 unsexed adults *X. flavipes* only) (Figure 33, Table 5). In plastic container within standard food after 3, 6, 9 and 12 months of exposure in control batch (untreated) the initial 100 adults of *C. pusillus* were increased up to 400±4.62, 600±5.77,

825±2.89 and 1020±5.77 adult individuals respectively where as, the highest number of host population were reduced (PRC 55.25, 48.33, 37.09 and 29.90) in 30 *X. flavipes* +1.966 µl/cm² combination, among the different used combinations. The lowest number of host population were suppressed (PRC 85.50, 79.83, 74.79 and 70.20) by only 30 *X. flavipes* at the same periods and conditions. Different used concentrations of Spinosad were found potential to reduce maximum number of *C. pusillus* populations in comparison with control (Table 5). From 3 to 12 months different used concentrations of Spinosad singly or in combination with predator, *X. flavipes* were found effective to suppress *C. pusillus* population (Table 5). Each level of concentrations and combinations were found effective to suppress the population of *C. pusillus* significantly (P<0.001) (Appendix tables 103-106).

Table 5 Effects of 2d unsexed adults of *X. flavipes* with different concentrations of Spinosad separately and in combination against the adult population of *C. pusillus* at different storage periods. [Initial prey population 100 adults, 1:1]

Predator/ Concentration	No/Rates (µl/cm^2)	Exposure period (months)							
		3		6		9		12	
		Total population	PRC	Total population	PRC	Total population	PRC	Total population	PRC
Control (untreated)		400±4.62a		600±5.77a		825±2.89a		1020±5.77a	
Control (Adult *X. flavipes* treated only)	30	58±4.62g	85.50	121±5.77g	79.83	208±4.62h	74.79	304±2.31h	70.20
Spinosad	0.491	83±1.73f	79.25	170±2.89f	71.67	305±2.89g	63.30	429±5.20g	57.90
	0.983	97±4.04ef	75.75	208±4.62e	65.33	343±1.73f	58.42	470±2.89f	53.00
	1.966	115±2.89de	71.25	239±5.20d	60.17	377±4.04e	54.30	516±3.46e	49.40
Adults *X. flavipes* + Spinosad	30 + 0.491	121±3.46d	69.75	241±3.46d	59.83	398±4.62d	51.76	564±2.31d	44.70
	30 + 0.983	146±3.46c	63.50	272±1.15c	54.67	444±2.31c	46.18	645±2.89c	36.70
	30 + 1.966	179±5.20b	55.25	310±2.89b	98.33	519±5.20b	37.09	715±5.77b	29.9

Note: Means with same letter do not significantly differed from each other (Tukey's Test)

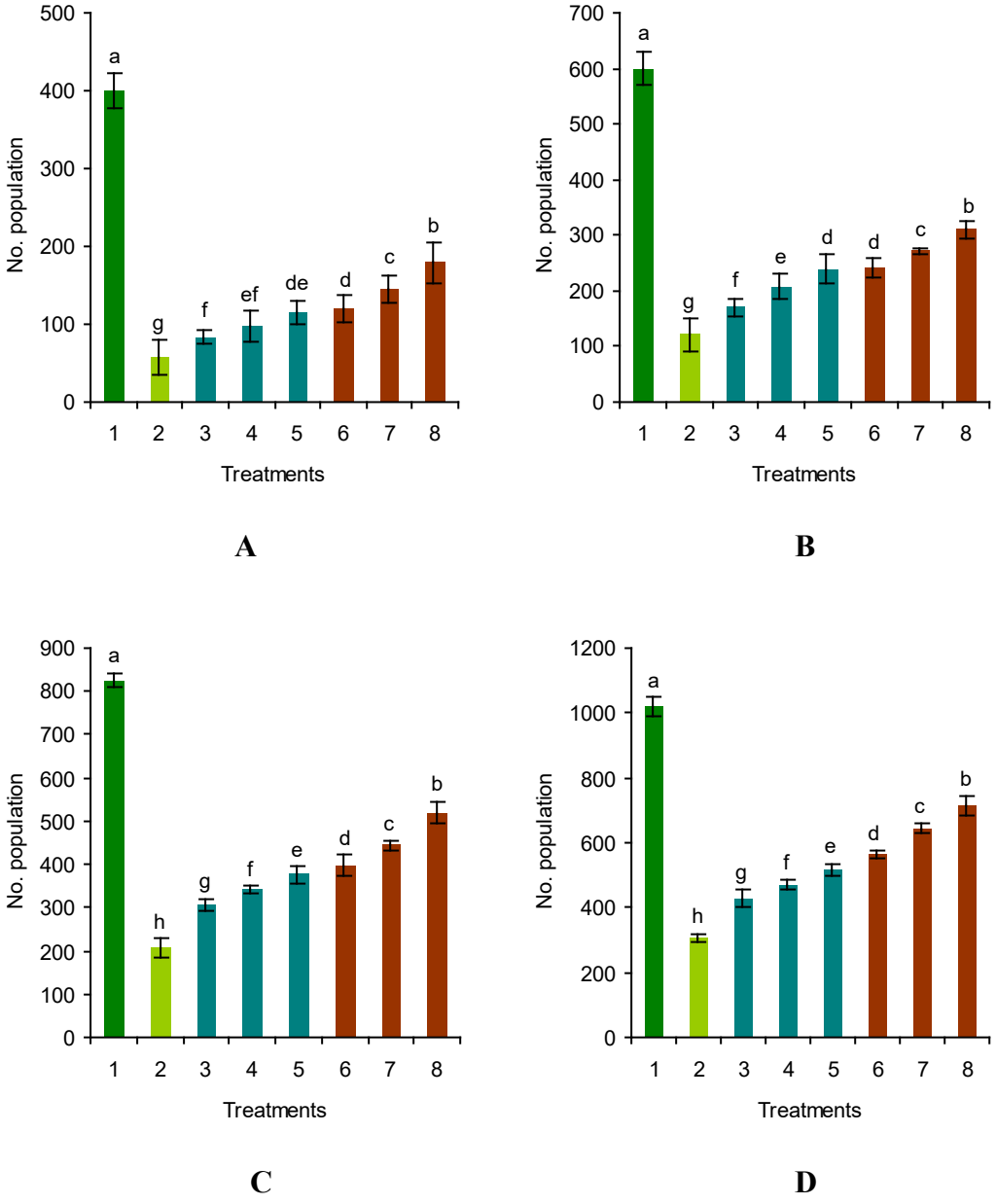


Figure 33 A, B, C and D showing the effects of unsexed adults *X. flavipes* with Spinosad on the adult population of *C. pusillus* after 3, 6, 9 and 12 months storage periods (Treatment 1 = Control (Untreated), 2 = Control (treated with 30 adult *X. flavipes*), 3 = 0.491 µl/cm^2 Spinosad, 4 = 0.983 µl/cm^2 Spinosad, 5 = 1.9966 µl/cm^2 Spinosad, 6 = 0.491 µl/cm^2 Spinosad + 30 adults *X. flavipes*, 7 = 0.983 µl/cm^2 Spinosad + 30 adults *X. flavipes*, 8 = 1.966 µl/cm^2 Spinosad + 30 adults *X. flavipes*,

Effects on adult populations of *R. dominica*: The data of the present experiment revealed that the combined effects of different used concentrations of Spinosad and *X. flavipes* reduced the population growth of *R. dominica* after different storage periods in comparison with that of control (untreated) and control (treated with 30 unsexed adult *X. flavipes*) (Figure 34, Table 6). In plastic container within standard food, after 3, 6, 9 and 12 months of exposure in control batch (untreated) the initial 100 adults of *R. dominica* were increased up to 735±2.89, 1220±11.55, 1790±11.55 and 2150±5.77 adult individuals respectively where as, the highest number of host population were reduced (PRC 42.86, 27.86, 11.28 and 1.10) in 30 *X. flavipes* +1.966 µl/cm² combination, among the different used combinations. The lowest number of host population were suppressed (PRC 81.77, 74.02, 67.49 and 61.21) by only 30 *X. flavipes* at the same periods and conditions. Different concentrations of Spinosad were found effective to reduce more number of host populations in comparison with control (Table 6). From 3 to 12 months different used concentrations of Spinosad singly or in combination with *X. flavipes* were found effective to suppress the host population (Table 6). Each level of concentrations and combinations were found effective to suppress the population of *R. dominica* significantly ($P<0.001$) (Appendix tables 107-110).

Predator, *X. flavipes* were not found susceptible to used concentrations. But in all used concentrations and combinations *R.* d*ominica* was found more susceptible than *C. pusillus* at the same time and conditions. Used concentrations of Spinosad were found potential during long time singly and in combination with *X. flavipes* to suppress the populations of both hosts (Tables 5 and 6). In case of population suppression of *C. pusillus* and *R. dominica* limited benefits were achieved at all combinations in comparison with singly concentrations of Spinosad or only with *X. flavipes* alone (Tables 5 and 6).

Table 6 Effects of 2d unsexed adults of *X. flavipes* with different concentrations of Spinosad separately and in combination against the adults populations of *R. dominica* at different storage periods. [Initial prey population 100 adults, 1:1]

Predator/ Concentrations	No/Rates (µl/cm^2)	Exposure period (months)							
		3		6		9		12	
		Total population	PRC	Total population	PRC	Total population	PRC	Total population	PRC
Control (untreated)		735±2.89a		1220±11.55a		1790±11.55a		2150±5.77a	
Control (Adult *X. flavipes* treated only)	30	134±2.31h	81.77	317±4.04h	74.02	582±1.15h	67.49	834±2.31g	61.21
Spinosad	0.491	249±5.20g	66.12	621±2.89g	49.10	1032±6.93g	42.35	1411±6.35f	34.37
	0.983	279±6.81f	60.68	691±3.46f	43.36	1134±2.31f	36.65	1598±4.62e	25.67
	1.966	311±5.77e	57.69	723±1.73e	40.74	1208±4.62e	32.51	1780±5.77d	17.20
Adults *X. flavipes* + Spinosad	30 + 0.491	356±3.46d	51.56	781±3.61d	36.15	1257±4.04d	29.78	1978±4.62c	8.00
	30 + 0.983	389±4.62c	47.07	835±2.89c	31.56	1354±2.31c	24.36	2054±2.31b	4.47
	30 + 1.966	420±2.89b	42.86	880±5.77b	27.86	1588±2.89b	11.28	2127±4.04a	1.10

Note: Means with same letter do not significantly differed from each other (Tukey's Test)

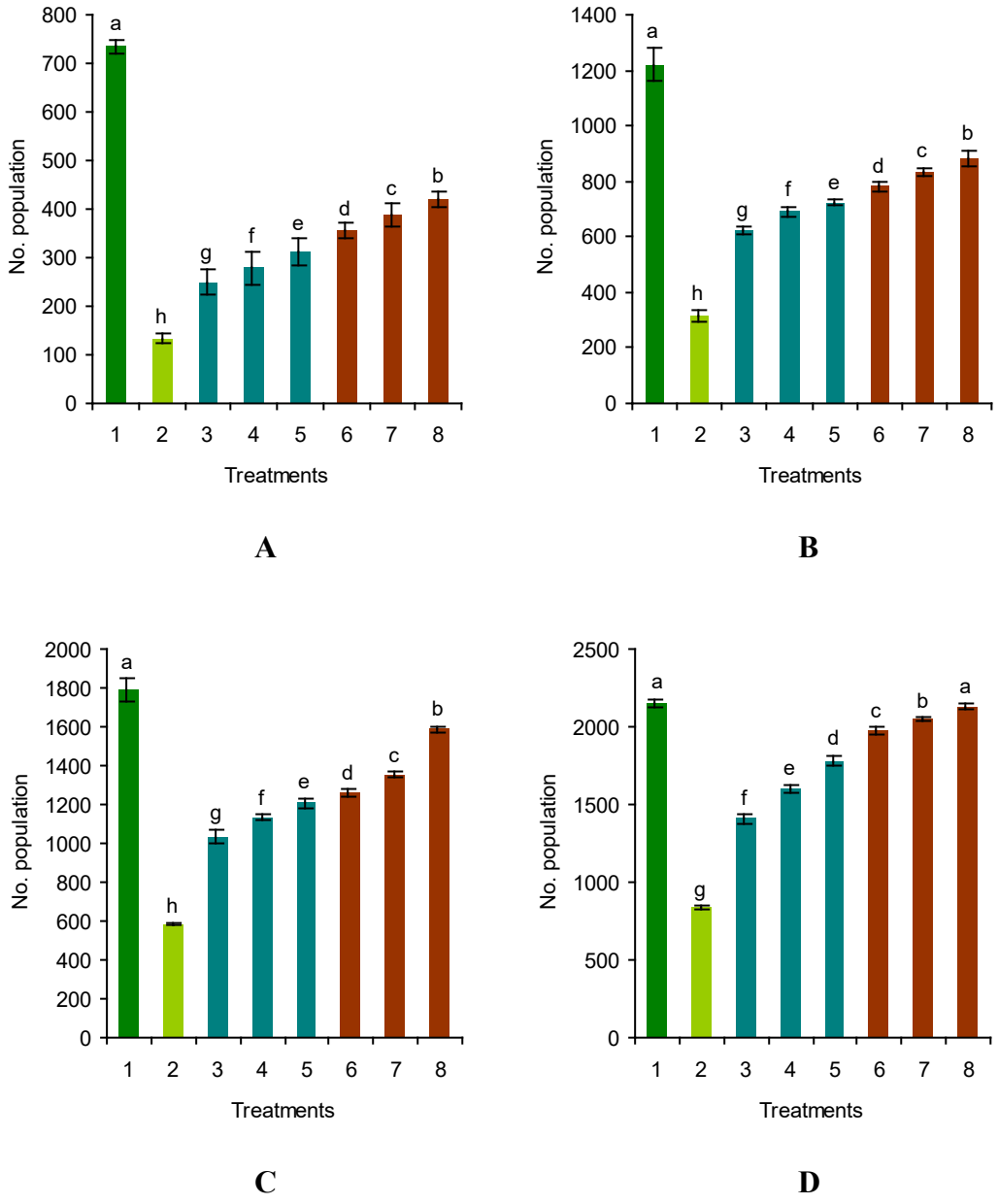


Figure 34 A, B, C and D showing the effects of unsexed adults *X. flavipes* with Spinosad on the adult population of *R. dominica* after 3, 6, 9 and 12 months storage periods (Treatment 1 = Control (Untreated), 2 = Control (treated with 30 adult *X. flavipes*), 3 = 0.491 µl/cm^2 Spinosad, 4 = 0.983 µl/cm^2 Spinosad, 5 = 1.9966 µl/cm^2 Spinosad, 6 = 0.491 µl/cm^2 Spinosad + 30 adults *X. flavipes*, 7 = 0.983 µl/cm^2 Spinosad + 30 adults *X. flavipes*, 8 = 1.966 µl/cm^2 Spinosad + 30 adults *X. flavipes*,

Discussion

Combined effects of *X. flavipes* with different concentrations of Spinosad inhibited the population build up of *C. pusillus* and *R. dominica*. The published reports showed that different concentrations of Spinosad (Fang *et al*. 2002a, Toews and Subramanyam 2003, Nayak *et al*. 2005, Daglish and Nayak 2006, Maier *et al*. 2006, Subramanyam *et al*. 2007, Daglish *et al*. 2008) and *X. flavipes* (Arbogast 1984, Haines 1984, Brower 1990, 1991, Brower *et al*. 1996, Nilakhe and Parker 1990, Scholler *et al*. 1997, Adler and Scholler 1998, Scholler and Flinn 2000, Lecato and Collins 1976) separately as potential agents which suppressed *C. pusillus* and *R. dominica* populations effectively.

Toews and Subramanyam (2004) reported that suppression of *T. castaneum* population was achieved in Spinosad or Spinosad +*X. flavipes* treatments and *X. flavipes* survived (92%) and reproduced in Spinosad treated wheat normally which was in agreement with the findings of the present result.

Arbogast (1976) reported that *O. surinamensis* population growth reduced 95% (after 16 wk) when only 5 pairs of *X. flavipes* were introduced. Brower and Press (1992) reported that *X. flavipes* suppressed 90.4% populations of *C. pusillus* when only 50 pairs of *X. flavipes* were introduced.

A single release of 50 pairs of *A. calandrae* suppressed 95.3% of residual populations of *S. oryzae* in wheat (Press *et al*. 1984) but 32.8 and 34.3% of *S. oryzae* and *R. dominica* respectively (Ahmed and Kabir 1995). *C. elegans* was very effective for suppressing *R. dominica* population with augmentative release (Flinn *et al*. 1994, 1996). Flinn *et al*. (1996) observed 98 and 91% suppression of *R. dominica* compared to the control by *C. elegans* in 1993 and 1994 respectively.

Athanassiou *et al*. (2010a,b) evaluated adults of *Cryptolestes* spp. and *R. dominica* on wheat treated with 0.1 and 0.5 ppm concentrations of Spinosad and 1 and 5ppm concentrations of methoprene applied alone or in combination after 14 d of exposure and found that average percent mortality (± SE) were 86.7 ± 3.3, 97.8 ± 2.2 in Spinosad alone and 37.8 ± 9.7, 37.8 ± 9.7 in methoprene alone, 80.0 ± 5.8 in Spinosad 0.1 ppm + methoprene 1 ppm,

90.0 ± 2.9 in Spinosad 0.1 ppm + methoprene 5 ppm, 98.9 ± 1.1 in Spinosad 0.5 ppm + methoprene 1 ppm and 98.9 ± 1.1a Spinosad 0.5 ppm + methoprene 5 ppm where as 26.7 ± 5.8 was in control for *Cryptolestes* and were 100.0 ± 0.0, 100.0 ± 0.0 in Spinosad alone and 2.2 ± 1.5b, 5.6 ± 3.0 in methoprene alone, 100.0 ± 0.0 in Spinosad 0.1 ppm + methoprene 1 ppm, 100.0 ± 0.0 in Spinosad 0.1 ppm + methoprene 5 ppm, 100.0 ± 0.0 in Spinosad 0.5 ppm + methoprene 1 ppm and 100.0 ± 0.0 Spinosad 0.5 ppm + methoprene 5 ppm where as 3.3 ± 1.7 was in control for *R. dominica.*

Daglish and Nayak (2006) reported that Spinosad residues were stable for 9 months on wheat, without loss of insecticidal activity against *R. dominica* a devastating pest of stored wheat worldwide. Spinosad applied at 0.5 or 1mg/kg was completely effective for 9 months at both 55 and 70% RH, with 100% adult mortality of *R. dominica* after 14 days of exposure and no live f_1 adults produced reported (Daglish *et al*. 2006) in their another study. As Spinosad appears to have low toxicity to many beneficial insects (Elzen *et al*.1998), it has potential for use in Integrated Pest Management (IPM) systems.

Sharififard *et al.* (2011) observed interactions between *Metarhizium anisopliae* and sub lethal doses of Spinosad for control of *M. domestica* and found that average percent (±SE) mortality of adults was 44±4, and 72.4±1.79 for fungus alone, 21±1.24, 32±1.7 and 39±1.7 for Spinosad alone but ranged from 66–87% and 89–95% in combination treatments of 105 and 107 spore/g fungus with 0.5, 1 and 1.5µg/gm doses of Spinosad respectively after 9 d of exposure and concluded that the interaction between *M. anispliae* and Spinosad indicated a synergetic effect that increased the house fly mortality as well as reduced the lethal time.

Kovendan *et al*. (2012) observed bioefficacy of larvicdial and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, Spinosad against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae) and found that after 24 h of exposure methanolic leaf extract of *C. papaya* against the first- to fourth-instar larvae and pupae of values LC_{50}=I instar was 51.76 ppm, II instar was 61.87 ppm, III instar was 74.07 ppm, and IV instar was 82.18 ppm, and pupae was 440.65 ppm, respectively, and bacterial insecticide, Spinosad against the first to

fourth instar larvae and pupae of values LC_{50}=I instar was 51.76 ppm, II instar was 61.87 ppm, III instar was 74.07 ppm, and IV instar was 82.18 ppm, and pupae was 93.44 ppm, respectively. Moreover, combined treatment of values of LC_{50}=I instar was 55.77 ppm, II instar was 65.77 ppm, III instar was 76.36 ppm, and IV instar was 92.78 ppm, and pupae was 107.62 ppm, respectively

In a laboratory study, Vayias *et al*. (2010a) showed that a Spinosad liquid SC formulation applied to barley at 1 ppm provided nearly complete control of *R. dominica* and *S. oryzae* for six months and adequate control of *C. ferrugineus* for four to six months. Control of *T. confusum* was only moderate in this study. They repoted potential use of *Beauveria bassiana* against the western flower thrips *Frankliniella occidentalis* without reducing the effectiveness of its natural predator *Orius sauteri* (Hemiptera: Anthocoridae) were observed (Gao *et al*. 2012). They found that total nymphal developmental time of *O. sauteri* increased 3-7% and the adult longevity decreased 9-13%. One explanation is that *F. occidentalis* larvae contaminated by *B. bassiana* may be poor quality prey for *O. sauteri* because infection makes the larvae deficient in certain essential nutrients (Simelane, Steinkraus and Kring 2008) or creates a buildup of fungal toxins or metabolites, which may slow development and shorten adult longevity of *O. sauteri* (Leckie *et al*. 2008). Although development of immature *O. sauteri* was slowed by feeding on *B. bassiana*-infected prey, the experiment revealed no significant differences in mortality rates among nymphs given different prey types. This result indicates that *O. sauteri* did not acquire lethal amounts of the pathogen through transmission from the prey (Gao *et al*. 2012). Consequently, the results presented here suggest that the slight negative effects on *O. sauteri* when provided *F. occidentalis* larvae contaminated by *B. bassiana* formulation are most probably prey-quality mediated rather than direct effects of the *B. bassiana* formulation (Sobhy *et al*. 2010). Other strains of *B. bassiana* have been shown to infect Anthocoridae species but with relatively low rates of successful infection (Ludwig and Oetting 2001, Dunkel and Jaronski 2003).

Overall, the findings of the present investigation revealed that *X. flavipes* and Spinosad has good potential properties against *C. pusillus* and *R. dominica* as target species of insect pest control programs.

Chapter 8
Summary and Conclusion

Summary

The present study was carried out to observe the effects of two stored product insect pests, *C. pusillus* and *R. dominica* as the host on the biology of the predator, *X. flavipes* and also to find out the effects of bacterium, Spinosad on the predator and the host. Moreover, the predator and bacterium separately and in combination were used to suppress the population of the both insect pests.

Host (*C. pusillus* and *R. dominica*) stage-specific effects on biology of *X. flavipes*

The eggs, larvae 1st up to 4th instar and pupae of *C. pusillus* and *R. dominica* played significant (P<0.001) role on the biology of *X. flavipes*.

In case of *C. pusillus*, the developmental time from egg hatching to adult emergence of *X. flavipes* were found as 15±2.00, 20±0.00, 22±0.58, 18±1.00, 14±1.15 and 12±1.15 days on eggs, larvae 1st up to 4th instar and pupae. Duration of each nymph 1st up to 5th instar was fluctuated on different life stages. The maximum developmental period occurred in 2nd instar larvae but it was the minimum on pupae. The developmental period on the different life stages of host was highly significant (P<0.001) which indicated that the developmental periods of *X. flavipes* depended upon different life stages of *C. pusillus*. The highest longevity of the female adult was 38±1.15 days on 3rd instar larvae and male adult 12±1.15 days on 2nd instar larvae and the lowest 14±1.15 days in female, 6±0.58 days in male on pupae. Life span of the predator varied inversely with host stages. The effect of different life stages of host on adult longevity was highly significant (P<0.001).

Nymphs 1st up to 5th instar and adults of *X. flavipes* consumed more eggs, 1st and 2nd instar larvae than those of 3nd, 4th instar larvae and pupae of *C. pusillus*. One 1st instar nymph consumed a few number 4th instar larvae (1.33±0.33) and large number eggs (7.33±0.33) per day. The 2nd, 3rd, 4th and 5th instar nymphs consumed more eggs, larvae 1st up to 4th instar and pupae comparatively than 1st instar nymphs. The adult female consumed more number of 1st instar larvae (16.33±0.33) than the male (14.33±0.33). The adult was found to consume average

number pupae 4.33±0.33 in male and 5.67±0.33 in female daily. No adult of *C. pusillus* was killed or consumed by any life stages of *X. flavipes*.

Average number of survivability of *X. flavipes* (nymphs 1st up to 5th instar and adult) on eggs, larvae up to 2nd instar were found maximum comparatively than that of 3rd, 4th instar larvae and pupae. Survivability of immature and adult stages of predator was maximum on 1st and 2nd instar larvae and minimum on pupae of *C. pusillus*. The effect of host stages was found highly significant ($P<0.001$).

Normally, females were found large size in length than males on eggs, larvae 1st up to 4th instar and pupae. Larvae 1st up to 4th instar of *C. pusillus* when fed on, adult male and female size (mm length) of *X. flavipes* were found prolonged than eggs and pupae. The highest size in length of the male and females were as1.80±0.01 mm and 2.10±0.01mm on 3rd instar larvae and the lowest was 1.50±1.50 mm and 1.70±0.06 mm on eggs. Adult size was highly significant ($P<0.001$) on different life stages of host.

Sex ratio of the emerged predators differed on life stages of *C. pusillus*. Different life stages of *C. pusillus* reared on standard food (Wheat and Yeast; ratio 19:1), when fed on *X. flavipes*, sex of the emergence adults showed preference for more number of female production than the male. The immature stages of *X. flavipes* feeding on eggs, larvae up to 4th instar and pupae on the same food, become adult and the ratio between male and female were 1:2.33, 1.12:1.42, 1.27:1.94, 1.27:2.07, 1.17:2.17 and 1.41:3.75 respectively. Based on ratio 1:1, sex ratio was found the best on 1st and 2nd instar larvae comparatively than other stages.

In case of *R. dominica*, the developmental time from egg hatching to adult emergence of *X. flavipes* were 18±1.00, 20±0.58, 16±2.00, 14±1.15, 12±1.15 and 13±0.58 days respectively on eggs, larvae up to 4th instar and pupae. Duration of each nymph up to 5th instar was found dissimilar on used stages. The maximum developmental period occurred in 2nd instar larvae but it was minimum on 4th instar larvae. The effects of different life stages of host on the developmental period was highly significant ($P<0.001$) which indicated that the developmental

periods of *X. flavipes* depended upon different life stages of *R. dominica*. The highest longevity of the adult was 34±2.31 days in female, 18± 0.58 days in male on 2nd instar larvae and the lowest was 15±1.15 days in female, 5±0.58 days in male on pupae. Life span of the predator varied inversely with host stages. The effect of different life stages of *R. dominica* on adult longevity was highly significant (P<0.001).

Nymphs 1st up to 5th instar and adults of *X. flavipes* consumed more eggs and 1st instar larvae than those of 2nd to 4th instar larvae and pupae of *R. dominica*. One 1st instar nymph consumed a few number 4th instar larvae (1.33±0.33) and large number eggs (8.00±0.58) per day. 2nd, 3rd, 4th and 5th instar nymphs consumed more eggs, larvae up to 4th instar and pupae comparatively than 1st instar nymphs. The adult female consumed more 1st instar larvae (14.00±1.15) than the male (10.00±1.15). The adult was found to consume average number pupae 3.00±0.58 in male and 4.00±0.58 in female daily. The results noticed that adult *R. dominica* was not killed or consumed by any stage of *X. flavipes*.

Average number of survivability of *X. flavipes* (nymphs up to 5th instar and adult) on eggs, larvae up to 2nd instar were found maximum comparatively than that of 3rd, 4th instar larvae and pupae. Survivability of immature and adult stages of predator was maximum on eggs, 1st and 2nd instar larvae and minimum on pupae of *R. dominica*. The effects of host stages was found highly significant (P<0.001).

Normally, females were found large size in length than males on eggs, larvae up to 4th instar and pupae. Larvae up to 4th instar of *R. dominica* when fed on, adult male and female size (mm length) of *X. flavipes* was larger comparatively than eggs and pupae. The highest size in length of the male and females was as 1.85±0.01 mm and 2.15±0.02 mm on 2nd instar larvae and lowest was 1.75±0.03 mm on egg and 1.90±0.03 mm on pupae. The effect of different life stages of host on adult size was highly significant (P<0.001).

Sex ratio of the emerged predators differed in life stages of *R. dominica*. Different life stages of host reared on standard food (Wheat and Yeast; ratio 19:1), when

were fed on *X. flavipes*, sex of the emergence adults showed preference for more female production than the male. The immature stages of *X. flavipes* feeding on eggs, larvae up to 4th instar and pupae on the same food, become adult and the ratio between males and females were found as 1.23:2.10, 1.05:1.45, 1.30:2.03, 1.13:2.20, 1.03:2.18 and 1.35:3.65 respectively. Based on ratio 1:1, sex ratio was found the best on 1st and 2nd instar larvae comparatively than other stages.

Overall, *X. flavipes* proved itself as an effective predator of insect pests in stored commodities and a potential controlling agent against *C. pusillus* and *R. dominica*.

Effects of Spinosad on host and predator

In case of *C. pusillus,* Spinosad showed a concentration related effects on the percentage of average egg hatchability. Average percentage of egg hatchability (±SE) was lowest 5.00±1.02 at 7.863µl/cm² but highest 25.00±1.15 at 0.491µl/cm² concentrations. At 7.863, 3.932, 1.966, 0.983 and 0.491µl/cm² concentrations, the average percent of egg hatchability was less than that of the control medium. PRC value was the highest 88.10% at 7.863µl/cm² where as the lowest was 40.48% at 0.491µl/cm² concentrations. The effect of different concentrations on egg hatchability was found significant ($P<0.001$).

C. pusillus 14-19d old larvae were used and the mortality was observed on different concentrations of Spinosad after 24, 48 and 72h of exposure. The concentrations of o.491, 0.983, 1.966, 3.982 and 7.863µl/cm² were found to be potential causing larval mortality based on the ingestion and contact compare to control. Average mortality (±SE) was the highest 14.00±2.00 at 7.863µl/cm² after 72h and lowest 3.33±0.88 at 0.491µl/cm² concentrations after 24h of exposure. At 95% confidence limit lower to upper 24h LC_{50} was 18.208 (4.379743-75.69753), 48h LC_{50} was 5.912 (1.421441-24.59105) and 72h LC_{50} was 0.176µl/cm² (0.01161457-2.651883) respectively. There were significant differences in the mean mortality of larvae between exposure periods ($F=58.038$, $df=2$, $P<0.001$) and between concentrations ($F=38.389$, $df=5$, $P<0.001$). In addition, the interaction between exposure periods and concentration rates was significant ($F=12.411$, $df=10$, $P<0.001$).

Moreover, Spinosad showed concentration rate and exposure period related effects on larval mortality.

The concentrations were o.491, 0.983, 1.966, 3.982 and 7.863µl/cm² were found to be potential causing pupal mortality based on the ingestion and contact compare to control. Average mortality (±SE) was highest 8.33±0.88 at 7.863µl/cm² after 72h and lowest 1.67±0.33 at 0.491µl/cm² Spinosad concentrations after 24h of exposure. At 95% confidence limit lower to upper 24h LC_{50} was 568.571 (2.246793-143881.9), 48h LC_{50} was 1841.139 (0.09236452-36700190) and 72h LC_{50} was 35.941µl/cm² (2.061289-626.6588) respectively. There were significant differences in the mean mortality between exposure periods ($F=30.791$, $df=2$, $P<0.001$) and between concentrations ($F=61.524$, $df=5$, $P<0.001$). In addition, the interaction between exposure period and concentration rates was significant ($F=2.558$, $df=10$, $P<0.001$). Moreover, Spinosad showed concentration rate and exposure period related effects on pupal mortality.

The above concentrations were found to be potential causing adult mortality based on the ingestion and contact compare to control. Average mortality (±SE) was highest 15.33±1.22 at 7.863µl/cm² after 72h and lowest 5.00±0.58 at 0.491µl/cm² Spinosad concentrations after 24h of exposure. At 95% confidence limit lower to upper 24h LC_{50} was 7.995 (2.947408-21.68888), 48h LC_{50} was 2.145 (1.191995-3.860422) and 72h LC_{50} was 0.840µl/cm² (0.446121-1.580054) respectively. There were significant differences in the mean mortality of adults between exposure periods ($F=202.970$, $df=2$, $P<0.001$) and between concentrations ($F=42.617$, $df=5$, $P<0.001$). In addition, the interaction between exposure period and concentration was significant ($F=8.350$, $df=10$, $P<0.001$). Spinosad showed a concentration related effects on adult mortality. The potentiality was higher in all treatments with the increase of concentration rate and exposure periods.

In case of *R. dominica,* Spinosad showed a concentration related effects on the percentage of average egg hatchability. Average percentage of egg hatchability (±SE) was lowest 0.33±1.03 at 7.863µl/cm² and highest 15.00±1.14 at 0.491µl/cm² concentrations. At 7.863, 3.932, 1.966, 0.983 and 0.491µl/cm² concentrations, the

average percent of egg hatchability was significantly lower than that of the control. The PRC value was the highest 99.13% at 7.863µl/cm² concentrations where as lowest 60.53% at 0.491µl/cm² concentrations. The effect of different concentrations on egg hatchability was found highly significant (P<0.001).

R. dominica 26-31d old larvae were used and mortality was observed on different concentrations of Spinosad after 24, 48 and 72h of exposure. The observed concentrations o.491, 0.983, 1.966, 3.982 and 7.863µl/cm² were found to be potential causing larval mortality based on the ingestion and contact compare to control. Average mortality (±SE) was highest 13.33±0.88 at 7.863µl/cm² after 72h and lowest 4.67±0.33 at 0.491µl/cm² concentrations after 24h of exposure. At 95% confidence limit lower to upper 24h LC_{50} was 9.230 (3.402471-25.03735), 48h LC_{50} was 2.835 (0.0.683601-10.46294) and 72h LC_{50} was 0.543µl/cm² (0.1273662-2.317881) respectively. There were significant differences in the mean mortality of larvae between exposure times (F=57.026, df=2, P<0.001) and between concentrations (F=56.006, df=5, P<0.001). In addition, the interaction between exposure time and concentration rates were significant (F=7.795, df=10, P<0.001).

The pupal mortality of *R. dominica* on different concentrations of Spinosad after 24, 48 and 72h of exposure were observed at o.491, 0.983, 1.966, 3.982 and 7.863µl/cm² concentrations and found to be potential causing pupal mortality based on the ingestion and contact compare to control. The average mortality (±SE) was highest 8.33±1.45 at 7.863µl/cm² after 72h and lowest 1.00±0.58 at 0.491µl/cm² concentrations after 24h of exposure. At 95% confidence limit lower to upper 24h LC_{50} was 1138.777 (1.990441-651521.7), 48h LC_{50} was 231.134 (2.478723-21552.51) and 72h LC_{50} was 22.054 µl/cm² (2.711695-179.3602) respectively. There were significant differences in the mean mortality between exposure periods (F=32.986, df=2, P<0.001) and between concentrations (F=23.986, df=5, P<0.001). In addition, the interaction between exposure period and concentration rate was significant (F=4.622, df=10, P<0.001).

The observed concentrations were found to be potential causing adult mortality based on the ingestion and contact compare to control. Average mortality (±SE) was highest 17.33±1.20 at 7.863µl/cm² after 72h and lowest 6.67±0.88 at 0.491µl/cm² concentrations after 24h of exposure. At 95%, confidence limit lower to upper 24h LC_{50} was 6.952 (1.68851-28.62021), 48h LC_{50} was 0.959 (0.4546266-2.02328) and 72h LC_{50} was 0.466µl/cm² (0.2381866-0.912989) respectively. There were significant differences in the mean mortality of adults between exposure periods (F=36.112, df=2, P<0.001) and between concentrations (F=83.468, df=5, P<0.001). In addition, the interaction between exposure time and concentration was highly significant (F=2.017, df=10, P<0.001). Spinosad showed a concentration related effects on adult mortality. The potentiality was higher in all treatments with the increase of concentration rate and exposure period.

In case of *X. flavipes* the average percentage of egg hatchability (±SE) was lowest 25.00±2.12 at 7.863µl/cm² and highest 35±1.73 at 0.491µl/cm² concentrations. At 1.966, 0.983 and 0.491µl/cm² concentrations, the average percent of egg hatchability of *X. flavipes* was almost similar as in the control (untreated). PRC value was highest 28.57 at 7.863µl/cm² where as lowest 2.86% at 0.491µl/cm² concentrations. The effect of different concentrations of Spinosad on egg hatchability noted highly significant (P<0.001).

Mortality was observed on different concentrations of Spinosad after 24, 48 and 72h of exposure of 4d old nymphs of *X. flavipes*. The noted concentrations were found to be potential causing very few nymphal mortality based on the ingestion and contact compare to control. Average mortality (±SE) was highest 6.67±1.76 at 7.863µl/cm² after 72h and lowest 1.00±0.58 at 0.491µl/cm² concentrations after 24h of exposure. At 95%, confidence limit lower to upper 24h LC_{50} was 432.654 (2.922949-237196.1), 48h LC_{50} was 137.838 (3.740582-5079.244) and 72h LC_{50} was 73.830µl/cm² (3.22157-1691.976) respectively. There were significant differences in the mean mortality of larvae between exposure periods (F=15.068, df=2, P<0.001) and between concentrations (F=13.562, df=5, P<0.001). In addition, the interaction between exposure periods and concentration rates was significant (F=3.083, df=10, P<0.001). Moreover, nymphal mortality was found slightly higher than that of adults.

The 2d old adult mortality of *X. flavipes* on different concentrations o.491, 0.983, 1.966, 3.982 and 7.863µl/cm² of Spinosad after 24, 48 and 72h of exposure was observed. The average mortality (±SE) was the highest 5.00±0.45 at 7.863µl/cm² after 72h and the lowest was 1.67±0.67 at 0.491µl/cm² concentrations after 24h of exposure. At 95%, confidence limit lower to upper 24h LC_{50} was 492.851 (3.859698-62932.93), 48h LC_{50} was 348.974 (4.223628-28833.73) and 72h LC_{50} was 331.510µl/cm² (2.651994-41440.03) respectively. There were significant differences in the mean mortality of adults between exposure periods ($F=7.386$, $df=2$, $P<0.001$) and between concentrations ($F=5.219$, $df=5$, $P<0.001$). In addition, the interaction between exposure period and concentration was significant ($F=2.432$, $df=10$, $P<0.001$). The potentiality was higher in all treatments with the increase of concentrations and exposure periods. Moreover, at o.491, 0.983 and 1.966µl/cm² concentrations of Spinosad, after 24h to 72h of exposure survivability of *X. flavipes* adults were found ranging 94 to 84% respectively.

Combined effects of *X. flavipes* and Spinosad on the population of *C. pusillus* and *R. dominica*

The combined effects of different concentrations of Spinosad and *X. flavipes* reduced the population growth of *C. pusillus* after different storage periods in comparison with that of control (untreated) and control (treated with 30 adults *X. flavipes* only). In plastic container within standard food, after 3, 6, 9 and 12 months of exposure in control batch (untreated) the initial 100 adults of *C. pusillus* were increased up to 400±4.62, 600±5.77, 825±2.89 and 1020±5.77 adult individuals respectively where as, the highest number of host population were reduced (PRC 55.25, 48.33, 37.09 and 29.90) in 30 *X. flavipes* +1.966µl/cm² combination, among the different combinations. The lowest number of host population were suppressed (PRC 85.50, 79.83, 74.79 and 70.20) by only 30 *X. flavipes* at the same periods and conditions. Different concentrations of Spinosad were found potential to reduce more number of *C. pusillus* populations in comparison with control. From 3 to 12 months different concentrations of

Spinosad alone or in combination with *X. flavipes* were found effective to suppress the *C. pusillus* population. Each level of concentrations and combinations were found potential to suppress the population of *C. pusillus* significantly (P<0.001).

The combined effects of different concentrations of Spinosad and the predator, *X. flavipes* reduced the population growth of host *R. dominica* after different storage periods in comparison with that of control (untreated) and control (treated with 30 unsexed adult *X. flavipes*). In plastic container within standard food, after 3, 6, 9 and 12 months of exposure in control batch (untreated) the initial 100 adults of *R. dominica* were increased up to 735±2.89, 1220±11.55, 1790±11.55 and 2150±5.77 adult individuals respectively where as, the highest number of host population were reduced (PRC 42.86, 27.86, 11.28 and 1.10) in 30 *X. flavipes* +1.966µl/cm² combination, among the different combinations. The lowest number of host population were suppressed (PRC 81.77, 74.02, 67.49 and 61.21) by only 30 *X. flavipes* at the same periods and conditions. Different concentrations of Spinosad were found potential to reduce more number of host populations in comparison with control. From 3 to 12 months different used concentrations of Spinosad alone or in combination with *X. flavipes* were found effective to suppress the host population. Each level of concentrations and combinations were found potential to suppress the population of *R. dominica* significantly (P<0.001).

X. flavipes were not found susceptible to used concentrations. But in all used concentrations and combinations, host *R.* d*ominica* was found more susceptible than the host *C. pusillus* at the same time and conditions. Used concentrations of Spinosad were found potential during long time alone and in combination with predator, *X. flavipes* to suppress the populations of both hosts. In case of population suppression of both hosts *C. pusillus* and *R. dominica*, limited benefits were achieved at all combinations in comparison with alone concentrations of Spinosad or only with predator, *X. flavipes*.

Conclusion

X. flavipes a well known challenging predator of many stored products insect pests is already now a day commercially available in the USA as a biological control agent. On the other hand, bacterium, Spinosad provides long term grain protection through the control of adult and immature stages of insect pests in storage. Spinosad is minimally disrupted to beneficial insects and compatible with Integrated Pest Management (IPM) program.

The present investigation revealed nymph up to 5th instar and adult *X. flavipes* can kill and consume eggs, larvae up to 4th instar and pupae of *C. pusillus* and *R. dominica*. Eggs, larvae up to 4th instar and pupae of the both insect pests fluctuated duration of developmental periods of each nymph, adult longevity, consumption rate, survivability rate, size and sex ratio of the predator. *X. flavipes* preferred 1st, 2nd and 3rd instar larvae followed by the 4th instar larvae and pupae. The female predator always consumed more prey than the male.

Moreover, the effects of different concentration of Spinosad on egg hatchability and on mortality of larvae, pupae and adult of the both insect pests were found more potential. The concentration rate and exposure period were highly significant in all stages of the both pests. In case of the predator, egg hatchability and mortality rate of nymph and adults showed results almost same as in the control medium. Lower concentrations (0.491, 0.983 and 1.966µl/cm^2) were found not significant on egg, nymph and adult of the predator.

Overall, it was found that the effects of *X. flavipes* and different concentration of Spinosad separately and in combination were highly significant ($P<0.001$) comparatively than in control to reduce the adult population of *C. pusillus* and *R. dominica* after 3, 6, 9 and 12 months of exposure in storage.

Therefore, the predator, *X. flavipes* and bacterium, Spinosad can be used effectively in the management of *C. pusillus* and *R. dominica* in storage, which is very important from environmental as well as Integrated Pest Management (IPM) and Global Protection Point (GPP) of views.

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Appendices

Appendix table 1 Effect of 1ppm of Spinosad against the immature and adult stages of different stored product insects

Insect pests	Life stage	Commodities	References
Plodia interpunctella	Immature	Wheat, corn, sunflower	Subramanyam *et al*. (1999), Fang *et al*. (2002a) and Huang *et al*. (2007)
Corcyra cephalonica	Immature	Corn	Huang and Subramanyam (2003), Shrama and Michaelraj (2006)
Cadra cautella	Immature	Corn	Subramanyam (2004)
Sitotroga cerealella	Immature	Wheat, corn	Huang *et al*. (2007)
Rhyzopertha dominica	Immature Adult	Wheat, corn, rice, sorghum, barley	Subramanyam *et al*. (1999), Fang *et al*. (2002a), Daglish *et al*. (2003), Nayak *et al*. (2005), Bonjour *et al*. (2006), Huang *et al*. (2007), Athanassiou *et al*. (2008c), Vayias *et al*. (2009b, 2010b)
Prostephanus truncatus	Adult	Wheat	Athanassiou *et al*. (2008c)
Cryptolestes ferrugineus	Immature Adult	Wheat, corn, sorghum, barley	Daglish *et al*.(2003), Huang *et al*. (2007) and Vayias *et al*. (2010b)
Cryptolestes pusillus	Immature Adult	Wheat	Subramanyam *et al*. (2002)
Sitophilus zeamais	Immature Adult	Wheat, corn	Huang *et al*. (2007)
Sitophilus oryzae	Immature Adult	Wheat, corn, sorghum, barley, rice	Subramanyam *et al*. (1999), Daglish and Wallbank (2002), Fang *et al*. (2002a), Nayak *et al*. (2005), Bonjour *et al*. (2006), Huang *et al*. (2007), Athanassiou *et al*. (2008c, 2009a), Vayias *et al*. (2009b, 2010b)
Tribolium confusum	Immature Adult	Wheat, barley, rice	Huang *et al*. (2007) and Athanassiou *et al*. (2008c)
Tribolium castaneum	Immature Adult	Wheat, corn, sorghum, sunflower	Subramanyam *et al*. (1999), Fang *et al*. (2002a), Daglish *et al*. (2003), Nayak *et al*. (2005), Bonjour *et al*. (2006), Huang *et al*. (2007)
Oryzaephilus surinamensis	Immature Adult	Wheat, corn, sorghum	Subramanyam *et al*. (1999), Fang *et al*. (2002a), Bonjour *et al*. (2006)
Lepinotus reticulatus	Immature Adult	Wheat, corn. rice	Athanassiou *et al*. (2009a)
Liposcelis entomophila	Immature Adult	Wheat, corn, rice	Daglish *et al*. (2003), Nayak *et al*. (2005), Nayak and Daglish (2007), Athanassiou *et al*. (2009a)
Liposcelis decolor	Immature Adult	Wheat	Daglish *et al*. (2003), Nayak *et al*. (2005), Nayak and Daglish (2007), Huang *et al*. (2009)
Liposcelis bostrychophila	Immature Adult	Wheat, corn, rice	Daglish *et al*. (2003), Nayak *et al*. (2005), Nayak and Daglish (2007), Huang *et al*. (2009), Athanassiou *et al*. (2009a)
Liposcelis paeta	Immature Adult	Wheat, corn, rice	Daglish *et al*. (2003), Nayak *et al*. (2005), Nayak and Daglish (2007), Athanassiou *et al*. (2009a)

Appendix table 2 Developmental periods and adult longevity of *X. flavipes* fed on different life stages of *C. pusillus* under laboratory condition at 30±0.5°C temperature and 70±0.5% relative humidity

Life stages of *C. pusillus*	Diet (g)	Mean Developmental periods (day) of nymphal instar					Total duration (day) of nymphsal stages	Adult longevity (day) of *X. flavipes*		Total duration (day)	
		1st	2nd	3rd	4th	5th		Male	Female	Male	Female
Eggs	2	3±0.58a	2±0.58a	3±0.58ab	3±0.58a	4±1.15a	15±2.00bcd	8±1.15ab	20±1.15cd	23	35
1st larvae	3	4±0.58a	3±0.58a	5±0.58a	4±0.58a	4±0.58a	20±0.00ab	10±1.15ab	25±2.89bc	30	45
2nd larvae	3	4±0.58a	3±0.58a	5±0.58a	5±0.58a	5±0.58a	22±0.58a	12±1.15a	31±1.15ab	39	53
3rd larvae	3	4±0.58a	3±0.58a	4±0.58ab	3±0.58a	4±0.58a	18±1.00abc	11±1.15a	28±1.15a	29	56
4th larvae	3	3±0.58a	2±0.58a	3±0.58ab	3±0.58a	3±0.58a	14±1.15de	9±0.58ab	26±0.58bc	23	40
Pupae	2	3±0.0a	2±0.0a	2±0.58b	3±0.58a	2±0.0a	12±1.15d	6±0.58b	14±1.15d	18	26

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 3 Factorial ANOVA showing the effects of different life stages of. *C. pusillus* on longevity of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	1671	1	1671	5.305	0.254
Host	18420	5	3684	11.695	0.054
Rep	4710	2	2355	7.476	0.157
Host*Diet	4521	3	1507	4.784	0.820
Host*Rep	908	1	908	2.883	0.528
Rep*Diet	1321	1	1321	4.194	0.362
Error	1575	5	315		
Total	33126	18			

Appendix table 4 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	1674	1	1674	7.440	0.245
Host	18125	5	3625	16.111	0.054
Rep	4980	2	2490	11.067	0.125
Host*Diet	4950	3	1650	7.333	0.820
Diet*Rep	607	1	607	2.698	0.528
Rep*Host	998	1	998	4.436	0.321
Error	1125	5	225		
Total	32459	18			

Appendix table 5 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	1765	1	1765	6.418	0.242
Host	22590	5	4518	16.429	0.039
Rep	4682	2	2341	8.513	0.142
Host*Diet	4503	3	1501	5.458	0.811
Diet*Rep	936	1	936	3.404	0.415
Rep*Host	1328	1	1328	4.829	0.326
Error	1375	5	275		
Total	37179	18			

Appendix table 6 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	1682	1	1682	4.806	0.251
Host	18080	5	3616	10.331	0.061
Rep	4430	2	2215	6.329	0.125
Host*Diet	4509	3	1503	4.294	0.845
Diet*Rep	925	1	925	2.643	0.625
Rep*Host	1319	1	1319	3.469	0.148
Error	1750	5	350		
Total	32695	18			

Appendix table 7 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	1648	1	1648	4.395	0.245
Host	18095	5	3619	9.650	0.071
Rep	4452	2	2226	5.936	0.136
Host*Diet	4410	3	1470	3.920	0.645
Diet*Rep	939	1	939	2.504	0.528
Rep*Host	1313	1	1313	3.501	0.138
Error	1875	5	375		
Total	32732	18			

Appendix table 8 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of adult male

Source	SS	DF	MS	F	P value
Diet	1653	1	1653	3.983	0.325
Host	18095	5	3619	8.720	0.072
Rep	4472	2	2236	5.388	0.145
Host*Diet	4284	3	1428	3.440	0.645
Diet*Rep	932	1	932	2.246	0.529
Rep*Host	1331	1	1331	3.207	0.134
Error	2075	5	415		
Total	32842	18			

Appendix table 9 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on longevity of adult female

Source	SS	DF	MS	F	P value
Diet	1621	1	1621	3.413	0.245
Host	17630	5	3526	7.423	0.015
Rep	4450	2	2225	4.684	0.132
Host*Diet	4593	3	1531	3.223	0.412
Diet*Rep	945	1	945	1.989	0.558
Rep*Host	1363	1	1363	2.869	0.164
Error	2375	5	475		
Total	32977	18			

Appendix table10 Developmental periods and adult longevity of *X. flavipes* fed on different life stages of *R. dominica* under laboratory condition at 30±0.5°C temperature and 70±0.5% relative humidity

Life stages of *R. dominica*	Diet (g)	Mean Developmental periods (day) of nymphal instar					Total duration (day) of nymphsal stages	Adult longevity (day) of *X. flavipes*		Total duration (day)	
		1st	2nd	3rd	4th	5th		Male	Female	Male	Female
Eggs	2	3±0.58a	3±0.58a	4±0.58a	4±0.58ab	4±0.58a	18±1.00ab	10±2.31b	22±3.46bc	28	40
1st larvae	3	4±0.58a	3±0.58a	4±0.58a	5±0.58a	4±0.58a	20±0.58a	12±1.15ab	26±0.88ab	32	46
2nd larvae	3	3±0.58a	2±0.58a	3±0.58a	4±0.58ab	4±0.58a	16±2.00abc	18±0.58a	34±2.31a	34	50
3rd larvae	3	3±0.58a	2±0.58a	3±0.58a	3±0.58ab	3±0.58a	14±1.15bc	10±1.33b	24±2.31bc	24	38
4th larvae	3	3±0.58a	2±0.58a	2±0.58a	2±0.58b	3±0.58a	12±1.15c	8±1.15b	20±1.15bc	20	32
Pupae	2	3±0.58a	2±0.58a	3±0.58a	2±0.58b	3±0.58a	13±0.58bc	5±0.58b	15±1.15c	18	28

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 11 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	1771	1	1771	6.440	0.254
Host	18920	5	3784	13.760	0.054
Rep	4910	2	2455	8.927	0.157
Host*Diet	4821	3	1607	5.844	0.820
Diet*Rep	1008	1	1008	3.665	0.528
Rep*Host	1421	1	1421	5.167	0.362
Error	1375	5	275		
Total	34226	18			

Appendix table 12 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	1774	1	1774	9.337	0.245
Host	18625	5	3725	19.605	0.054
Rep	4910	2	2455	12.921	0.125
Host*Diet	5121	3	1707	8.982	0.820
Diet*Rep	998	1	998	5.253	0.528
Rep*Host	1098	1	1098	5.779	0.321
Error	950	5	190		
Total	33476	18			

Appendix table 13 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	1765	1	1765	8.209	0.242
Host	18590	5	3718	17.293	0.039
Rep	4882	2	2441	11.353	0.142
Host*Diet	4953	3	1651	7.679	0.811
Diet*Rep	1036	1	1036	4.819	0.415
Rep*Host	1428	1	1428	6.642	0.326
Error	1075	5	215		
Total	33729	18			

Appendix table 14 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	1782	1	1782	5.940	0.251
Host	18580	5	3716	12.387	0.061
Rep	4630	2	2315	7.717	0.125
Host*Diet	4779	3	1593	0.310	0.845
Diet*Rep	1025	1	1025	3.417	0.625
Rep*Host	1419	1	1419	4.730	0.148
Error	1500	5	300		
Total	33715	18			

Appendix table 15 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	1748	1	1748	4.540	0.245
Host	19095	5	3819	9.919	0.071
Rep	4652	2	2326	6.042	0.136
Host*Diet	4536	3	1512	3.927	0.645
Diet*Rep	1039	1	1039	2.699	0.528
Rep*Host	1413	1	1413	3.670	0.138
Error	1925	5	385		
Total	34408	18			

Appendix table 16 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of adult male

Source	SS	DF	MS	F	P value
Diet	1753	1	1753	5.009	0.325
Host	19095	5	3819	10.911	0.072
Rep	4872	2	2436	6.960	0.145
Host*Diet	4884	3	1628	4.651	0.645
Diet*Rep	998	1	998	2.851	0.529
Rep*Host	1531	1	1531	4.374	0.134
Error	1750	5	350		
Total	34883	18			

Appendix table 17 Factorial ANOVA showing the effects of different life stages of *R. dominica* on longevity of adult female

Source	SS	DF	MS	F	P value
Source	SS	DF	MS	F	P value
Diet	1721	1	1721	4.049	0.245
Host	18630	5	3726	8.767	0.015
Rep	4850	2	2425	5.706	0.132
Host*Diet	5193	3	1731	4.073	0.412
Diet*Rep	1045	1	1045	2.459	0.558
Rep*Host	1563	1	1563	3.678	0.164
Error	2125	5	425		

Appendix table 18 Average (%) consumption rate by different life stages of *X. flavipes* per day on different life stages of *C. pusillus* under laboratory condition

Life stages of *C. pusillus*	Diet (g)	Average (%) consumption rate of *X. flavipes*						
		Nymphs					Adults	
		1st	2nd	3rd	4th	5th	Male	Female
Eggs	2	7.33±0.33a	8.33±0.33a	10.33±0.33a	12.67±1.20a	14.33±0.33a	16.33±0.88a	20.67±0.33a
1st larvae	3	6.33±0.33a	7.67±0.33a	8.67±0.33a	9.33±0.33b	11.67±0.33b	14.33±0.33ab	16.33±0.33b
2nd larvae	3	3.33±0.33b	4.33±0.33b	5.67±0.33b	6.33±0.33c	8.67±0.33c	12.33±0.33b	14.33±0.33c
3rd larvae	3	2.33±0.33bc	2.67±0.33c	4.33±0.33b	4.67±0.33cd	6.67±0.33d	9.67±0.33c	11.33±0.33d
4th larvae	3	1.33±0.33c	1.67±0.33c	2.33±0.33c	3.67±0.33cd	5.33±0.33d	8.67±0.33c	9.33±0.33e
Pupae	2	1.33±0.33c	1.67±0.33c	2.00±0.58c	2.33±0.33d	2.67±0.33e	4.33±0.33d	5.67±0.33f

Note: Means with same letter do not significantly differed from each other Tukey's Test, $P<0.001$

Appendix table 19 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	70982	1	70982	4.693	0.258
Host	15281800	5	3056360	202.073	0.001
Rep	26152	2	13076	0.865	0.765
Host*Diet	2033490	3	677830	44.815	0.425
Diet*Rep	43775	1	43775	2.894	0.502
Rep*Host	28569	1	28569	1.889	0.762
Error	75625	5	15125		
Total	17560393	18			

Appendix table 20 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	70859	1	70859	4.973	0.248
Host	15283175	5	3056635	214.500	0.003
Rep	26030	2	13015	0.913	0.745
Host*Diet	2032290	3	677430	47.534	0.158
Diet*Rep	43625	1	43625	3.0614	0.425
Rep*Host	28236	1	28236	1.981	0.623
Error	71250	5	14250		
Total	17555465	18			

Appendix table 21 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	70458	1	70458	3.866	0.246
Host	1581290	5	3056258	167.696	0.004
Rep	26724	2	13362	0.733	0.369
Host*Diet	2014440	3	671480	36.844	0.145
Diet*Rep	43425	1	43425	2.383	0.369
Rep*Host	28632	1	28632	1.571	0.758
Error	91125	5	18225		
Total	3856094	18			

Appendix table 22 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	70441	1	70441	4.116	0.252
Host	15281620	5	3056324	178.576	0.005
Rep	26916	2	13458	0.786	0.335
Host*Diet	2017080	3	672360	39.285	0.248
Diet*Rep	43145	1	43145	2.521	0.365
Rep*Host	28635	1	28635	1.673	0.762
Error	85575	5	17115		
Total	17553412	18			

Appendix table 23 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	70448	1	70448	4.369	0.215
Host	15281810	5	3056362	189.542	0.002
Rep	26938	2	13469	0.835	0.333
Host*Diet	2017410	3	672470	41.704	0.263
Diet*Rep	43126	1	43126	2.674	0.275
Rep*Host	28621	1	28621	1.775	0.632
Error	80625	5	16125		
Total	17548978	18			

Appendix table 24 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of adult male

Source	SS	DF	MS	F	P value
Diet	70451	1	70451	7.473	0.248
Host	15283195	5	3056639	194.072	0.021
Rep	26884	2	13442	0.853	0.312
Host*Diet	2014680	3	671560	42.639	0.148
Diet*Rep	43121	1	43121	2.738	0.639
Rep*Host	28632	1	28632	1.818	0.425
Error	78750	5	15750		
Total	17545713	18			

Appendix table 25 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on consumption rate of adult female

Source	SS	DF	MS	F	P value
Diet	60362	1	60362	3.939	0.251
Host	15282105	5	3056421	199.440	0.002
Rep	30882	2	15441	1.008	0.452
Host*Diet	1864740	3	621580	40.560	0.458
Diet*Rep	40136	1	40136	2.629	0.741
Rep*Host	28131	1	28131	1.836	0.458
Error	76625	5	15325		
Total	17381981	18			

Appendix table 26 Average (%) consumption rate by different life stages of *X. flavipes* per day on different life stages of *R. dominica* under laboratory condition

Life stages of *R. dominica*	Diet (g)	Average (%) consumption rate of *X. flavipes*						
		Nymphs					Adults	
		1st	2nd	3rd	4th	5th	Male	Female
Eggs	2	8.00±0.58a	10.67±0.67a	14.33±0.88a	16.67±1.20a	19.33±0.88a	21.33±0.88a	24.67±1.20a
1st larvae	3	3.33±0.88b	4.67±1.20b	5.00±0.58b	6.33±0.88b	8.67±1.20b	10.00±1.15b	14.00±1.15b
2nd larvae	3	2.33±0.88b	3.67±1.20b	4.33±0.88b	5.67±1.20b	7.33±0.88b	9.33±0.88b	12.33±0.88bc
3rd larvae	3	2.00±0.58b	3.33±0.88b	4.00±0.58b	5.00±0.58b	6.67±1.20bc	8.67±1.20b	11.33±0.88bc
4th larvae	3	1.33±0.33b	2.33±0.88b	2.67±1.20b	3.33±1.45b	4.33±0.88bc	6.33±0.88c	8.33±0.88cd
Pupae	2	1.00±0.00b	1.33±0.33b	1.67±0.33b	2.00±0.58b	2.33±0.88c	3.00±0.58c	4.00±0.58d

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 27 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	40093	1	40093	5.627	0.480
Host	2071065	5	414213	58.135	0.015
Rep	86706	2	43353	6.085	0.001
Host*Diet	154470	3	51490	7.227	0.999
Diet*Rep	13449	1	13449	1.888	0.067
Rep*Host	41535	1	41535	5.829	0.003
Error	35625	5	7125		
Total	2442943	18			

Appendix table 28 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	40091	1	40091	6.440	0.481
Host	2071075	5	414215	66.540	0.012
Rep	126738	2	63369	10.170	0.001
Host*Diet	154350	3	51450	8.265	0.985
Diet*Rep	13445	1	13445	2.150	0.062
Rep*Host	41525	1	41525	6.670	0.001
Error	31125	5	6225		
Total	2478349	18			

Appendix table 29 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	40085	1	40085	4.859	0.382
Host	2070625	5	414125	50.197	0.011
Rep	127250	2	63625	7.712	0.005
Host*Diet	153750	3	51250	6.212	0.915
Diet*Rep	13351	1	13351	1.618	0.025
Rep*Host	41245	1	41245	4.999	0.006
Error	41250	5	8250		
Total	2487556	18			

Appendix table 30 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	40152	1	40152	3.287	0.582
Host	2070710	5	414142	33.904	0.024
Rep	127272	2	63636	5.210	0.002
Host*Diet	153150	3	51050	4.179	0.812
Diet*Rep	13314	1	13314	1.080	0.022
Rep*Host	41225	1	41225	3.375	0.012
Error	61075	5	12215		
Total	2506898	18			

Appendix table 31 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	40521	1	40521	2.952	0.125
Host	2070560	5	414112	30.172	0.032
Rep	127348	2	63674	4.639	0.005
Host*Diet	153300	3	51100	3.723	0.425
Diet*Rep	13145	1	13145	0.958	0.021
Rep*Host	41215	1	41215	3.002	0.041
Error	68625	5	13725		
Total	2514714	18			

Appendix table 32 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of adult male

Source	SS	DF	MS	F	P value
Diet	40314	1	40314	8.860	0.110
Host	2072125	5	414425	91.082	0.032
Rep	26204	2	13102	2.880	0.005
Host*Diet	153360	3	51120	11.235	0.125
Diet*Rep	13143	1	13143	2.888	0.021
Rep*Host	41269	1	41269	9.070	0.041
Error	22750	5	4550		
Total	2369165	18			

Appendix table 33 Factorial ANOVA showing the effects of different life stages of *R. dominica* on consumption rate of adult female

Source	SS	DF	MS	F	P value
Diet	40316	1	40316	7.642	0.120
Host	2072375	5	414475	78.573	0.014
Rep	26202	2	13101	2.484	0.006
Host*Diet	153450	3	51150	9.697	0.131
Diet*Rep	13140	1	13140	2.491	0.041
Rep*Host	41251	1	41251	7.820	0.012
Error	26375	5	5275		
Total	2373109	18			

Appendix table 34 Average percent (±SE) no. of survivability of different life stages of *X. flavipes* on different life stages of *C. pusillus* under laboratory condition

Life stages of *C. pusillus*	Diet (gm)	Average (%) no. of survivability of *X. flavipes*						
		Nymphs					Adults	
		1st	2nd	3rd	4th	5th	Male	Female
Eggs	2	9.00±0.58a	8.33±0.33abc	7.33±0.33ab	6.33±0.33a	5.33±0.33a	3.67±0.67a	6.33±0.67a
1st larvae	3	10.00±0.00a	9.67±0.33ab	8.00±1.53ab	8.00±1.53a	7.00±1.15a	4.67±0.88a	5.33±0.88a
2nd larvae	3	10.00±0.00a	10.00±0.00a	10.00±0.00a	8.67±0.33a	6.67±0.88a	3.33±0.88a	6.67±0.88a
3rd larvae	3	9.67±0.58a	6.67±1.20c	5.00±1.15b	5.00±1.15a	4.67±1.45a	3.67±1.45a	5.67±0.88a
4th larvae	3	8.33±0.58a	6.00±0.58c	5.67±0.33b	5.00±0.58a	4.67±0.33a	2.33±0.33a	7.67±0.33a
Pupae	2	8.00±0.58a	7.00±0.58bc	6.33±0.33ab	5.33±0.67a	4.33±0.33a	2.67±0.33a	7.33±0.33a

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 35 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	299.39	1	299.39	14.022	0.000
Host	3212.20	5	642.44	30.090	0.000
Rep	463.50	2	231.75	10.85	0.922
Host*Diet	268.38	3	89.46	4.190	0.005
Diet*Rep	70.64	1	70.64	3.308	0.801
Rep*Host	98.62	1	98.61	4.618	0.330
Error	106.75	5	21.35		
Total	4519.48	18			

Appendix table 36 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	297.45	1	297.45	14.524	0.005
Host	3711.55	5	742.31	36.245	0.001
Rep	622.90	2	311.45	15.208	0.922
Host*Diet	298.74	3	99.58	4.862	0.005
Diet*Rep	60.21	1	60.21	2.940	0.801
Rep*Host	86.32	1	86.32	4.215	0.330
Error	102.40	5	20.48		
Total	5179.57	18			

Appendix table 37 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	399.15	1	399.15	21.992	0.005
Host	3491.25	5	698.25	38.471	0.005
Rep	462.30	2	231.15	12.736	0.985
Host*Diet	264.69	3	88.23	4.861	0.005
Diet*Rep	65.02	1	65.02	3.582	0.785
Rep*Host	96.32	1	96.32	5.307	0.521
Error	90.75	5	18.15		
Total	4869.48	18			

Appendix table 38 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	345.31	1	345.31	16.779	0.005
Host	3710.75	5	742.15	36.061	0.005
Rep	223.16	2	111.58	5.422	0.995
Host*Diet	240.54	3	80.18	3.896	0.852
Diet*Rep	75.42	1	75.42	3.665	0.925
Rep*Host	95.10	1	95.10	4.620	0.936
Error	102.90	5	20.58		
Total	4793.18	18			

Appendix table 39 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	295.58	1	295.58	11.614	0.006
Host	3445.60	5	689.12	27.077	0.005
Rep	181.16	2	90.58	3.559	0.725
Host*Diet	267.54	3	89.18	3.504	0.852
Diet*Rep	55.42	1	55.42	2.178	0.925
Rep*Host	90.10	1	90.10	3.540	0.859
Error	127.25	5	25.45		
Total	4462.65	18			

Appendix table 40 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of adult male

Source	SS	DF	MS	F	P value
Diet	298.12	1	298.12	13.399	0.009
Host	3126.80	5	625.36	28.106	0.005
Rep	186.50	2	93.25	4.191	0.814
Host*Diet	256.86	3	85.62	3.848	0.485
Diet*Rep	40.43	1	40.43	1.817	0.425
Rep*Host	92.56	1	92.56	4.160	0.693
Error	111.25	5	22.25		
Total	4112.52	18			

Appendix table 41 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on no. of survivability of adult female

Source	SS	DF	MS	F	P value
Diet	345.42	1	345.42	29.243	0.008
Host	2628.10	5	525.62	29.282	0.006
Rep	199.24	2	99.62	5.545	0.858
Host*Diet	585.45	3	195.15	10.872	0.425
Diet*Rep	90.42	1	90.42	5.037	0.431
Rep*Host	102.28	1	102.28	5.698	0.574
Error	89.75	5	17.95		
Total	4040.66	18			

Appendix table 42 Average percent (±SE) no. of survivability of different life stages of *X. flavipes* on different life stages of *R. dominica* under laboratory condition

Life stages of *R. dominica*	Diet (gm)	Average (%)survivability of *X. flavipes*						
		Nymphs					Adults	
		1st	2nd	3rd	4th	5th	Male	Female
Eggs	2	9.00±0.58a	8.00±0.58ab	7.33±0.67ab	6.00±0.00a	5.67±0.33a	4.33±1.20a	5.67±1.20a
1st larvae	3	9.33±0.33a	8.67±0.33a	8.33±0.33a	7.00±0.58a	6.00±0.58a	3.00±0.58a	7.00±0.58a
2nd larvae	3	8.00±0.58ab	7.00±0.00b	6.67±0.33ab	6.33±0.33a	5.67±0.33a	2.67±0.33a	6.33±0.88a
3rd larvae	3	8.33±0.33ab	7.67±0.33ab	6.33±0.33b	6.00±0.58a	5.00±0.00a	3.33±0.33a	6.67±0.33a
4th larvae	3	7.33±0.33ab	7.00±0.00b	5.67±0.33bc	5.00±0.58ab	4.33±0.33ab	3.00±0.58a	6.00±0.00a
Pupae	2	6.33±0.33b	5.33±0.33c	4.33±0.33c	3.67±0.33b	2.67±0.33b	2.00±0.00a	5.00±1.00a

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 43 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of 1st instar nymphs

Source	SS	DF	MS	F	P value
Diet	623.10	1	623.10	4.965	0.016
Host	23472.00	5	4694.40	37.406	0.000
Rep	1257.80	2	628.90	5.011	0.025
Host*Diet	279.30	3	93.10	0.741	0.720
Diet*Rep	222.50	1	222.50	1.773	0.199
Rep*Host	154.60	1	154.60	1.232	0.387
Error	627.50	5	125.50		
Total	26636.80	18			

Appendix table 44 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of 2nd instar nymphs

Source	SS	DF	MS	F	P value
Diet	623.25	1	623.25	4.888	0.015
Host	23472.10	5	4694.42	36.819	0.001
Rep	1257.92	2	628.96	4.933	0.022
Host*Diet	279.30	3	93.10	0.730	0.625
Diet*Rep	222.50	1	222.50	1.745	0.215
Rep*Host	154.60	1	154.60	1.213	0.147
Error	637.50	5	127.50		
Total	26647.27	18			

Appendix table 45 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of 3rd instar nymphs

Source	SS	DF	MS	F	P value
Diet	623.35	1	623.35	4.788	0.016
Host	23474.25	5	4694.85	36.064	0.001
Rep	1256.50	2	628.25	4.826	0.022
Host*Diet	2410.56	3	803.52	6.172	0.625
Diet*Rep	302.48	1	302.48	2.323	0.214
Rep*Host	154.62	1	154.62	1.188	0.147
Error	650.90	5	130.18		
Total	28872.66	18			

Appendix table 46 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of 4th instar nymphs

Source	SS	DF	MS	F	P value
Diet	723.40	1	723.40	5.466	0.018
Host	27974.25	5	5594.85	42.273	0.001
Rep	1200.50	2	600.25	4.535	0.022
Host*Diet	2680.56	3	893.52	6.751	0.622
Diet*Rep	322.48	1	322.48	2.437	0.214
Rep*Host	254.63	1	254.63	1.923	0.149
Error	661.75	5			
Total	33817.57	18			

Appendix table 47 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of 5th instar nymphs

Source	SS	DF	MS	F	P value
Diet	822.38	1	822.38	5.843	0.016
Host	33471.95	5	6694.39	47.562	0.002
Rep	1224.48	2	612.24	4.350	0.021
Host*Diet	2380.26	3	793.42	5.637	0.582
Diet*Rep	292.82	1	292.82	2.080	0.245
Rep*Host	354.25	1	354.25	2.517	0.485
Error	703.75	5	140.75		
Total	39249.89	18			

Appendix table 48 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of adult male

Source	SS	DF	MS	F	P value
Diet	712.85	1	712.85	5.186	0.020
Host	33473.10	5	6694.62	38.595	0.002
Rep	1256.84	2	628.42	4.572	0.022
Host*Diet	2679.54	3	893.18	6.497	0.592
Diet*Rep	272.36	1	272.36	1.981	0.250
Rep*Host	254.47	1	254.47	1.851	0.471
Error	687.30	5	137.46		
Total	39336.46	18			

Appendix table 49 Factorial ANOVA showing the effects of different life stages of *R. dominica* on no. of survivability of adult female

Source	SS	DF	MS	F	P value
Diet	622.47	1	622.47	4.585	0.021
Host	28471.60	5	5694.32	41.947	0.002
Rep	1396.84	2	698.42	5.145	0.022
Host*Diet	2380.56	3	793.52	5.845	0.592
Diet*Rep	252.14	1	252.14	1.857	0.250
Rep*Host	454.28	1	454.28	3.346	0.471
Error	678.75	5	135.75		
Total	34256.64	18			

Appendix table 50 Average (±SE) adult size (mm in length) of *X. flavipes* on different life stages of *C. pusillus* under laboratory condition.

Adult size (mm in length) of *X. flavipes*	Different life stages of *C. pusillus*					
	Eggs	1st	2nd	3rd	4th	Pupae
Male	1.50±0.06c	1.60±0.03bc	1.70±0.03ab	1.80±0.01a	1.65±0.03abc	1.55±0.04bc
Female	1.70±0.06c	1.90±0.03b	2.00±0.03ab	2.10±0.01a	1.95±0.03ab	1.85±0.04bc

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 51 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on size of adult male

Source	SS	DF	MS	F	P value
Diet	219.74	1	219.74	4.867	0.282
Host	3250.95	5	650.19	14.401	0.002
Rep	896.30	2	448.15	9.926	0.017
Host*Diet	265.44	3	88.48	1.960	0.989
Diet*Rep	75.87	1	75.87	1.680	0.978
Rep*Host	470.98	1	470.98	10.351	0.002
Error	225.75	5	45.15		
Total	5405.03	18			

Appendix table 52 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on size of adult female

Source	SS	DF	MS	F	P value
Diet	199.48	1	199.48	5.659	0.251
Host	3241.10	5	648.22	18.389	0.003
Rep	850.30	2	425.15	12.061	0.021
Host*Diet	298.44	3	99.48	2.822	0.852
Diet*Rep	96.69	1	96.69	2.743	0.852
Rep*Host	470.82	1	470.82	13.357	0.003
Error	176.25	5	35.25		
Total	5333.08	18			

Appendix table 53 Average (±SE) adult size (mm in length) of *X. flavipes* male and female on different life stages of *R. dominica* under laboratory condition

Adult size (mm in length) of *X. flavipes*	Different life stages of *R. dominica*					
	Eggs	1st	2nd	3rd	4th	Pupae
Male	1.75±0.03ab	1.80±0.02a	1.85±0.01a	1.81±0.03a	1.76±0.03ab	1.65±0.04b
Female	2.00±0.03bcd	2.20±0.06a	2.15±0.02ab	2.10±0.01abc	1.98±0.05cd	1.90±0.03d

Note: Means with same letter do not significantly differed from each other Tukey's Test, $P<0.001$

Appendix table 54 Factorial ANOVA showing the effects of different life stages of *R. dominica* on size of adult male

Source	SS	DF	MS	F	P value
Diet	120.47	1	120.47	1.717	0.248
Host	3371.10	5	674.22	9.611	0.005
Rep	578.30	2	289.15	4.122	0.025
Host*Diet	57.75	3	19.25	0.274	0.874
Diet*Rep	16.74	1	16.74	0.239	0.874
Rep*Host	474.82	1	474.82	6.769	0.006
Error	350.75	5	70.15		
Total	4969.93	18			

Appendix table 55 Factorial ANOVA showing the effects of different life stages of *R. dominica* on size of adult female

Source	SS	DF	MS	F	P value
Diet	121.47	1	121.47	1.681	0.251
Host	3371.25	5	674.25	9.332	0.007
Rep	578.62	2	289.31	4.004	0.026
Host*Diet	58.08	3	19.36	0.268	0.876
Diet*Rep	16.25	1	16.25	0.2245	0.858
Rep*Host	474.74	1	474.74	6.570	0.009
Error	361.25	5	72.25		
Total	4981.66	18			

Appendix table 56 Average (±SE) number (%) of *X. flavipes* adult male and female on different life stages of *C. pusillus* under laboratory condition.

Adults of *X. flavipes*	Different life stages of *C. pusillus*					
	Eggs	1st	2nd	3rd	4th	Pupae
No. of Male	30.00±2.89b	45.00±1.15a	38.33±2.73ab	38.00±2.31ab	35.00±3.46ab	28.33±2.03b
No. of Female	70.00±2.89ab	55.00±1.15b	61.67±2.73b	62.00±2.31ab	65.00±3.46ab	71.67±4.04a

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 57 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on number of adult male

Source	SS	DF	MS	F	P value
Diet	17416	1	17416	2.130	0.148
Host	348375	5	69675	8.523	0.001
Rep	24266	2	12133	1.484	0.271
Host*Diet	17325	3	5775	0.706	0.726
Diet*Rep	11904	1	11904	1.456	0.276
Rep*Host	38846	1	38846	4.752	0.006
Error	40875	5	8175		
Total	499007	18			

Appendix table 58 Factorial ANOVA showing the effects of different life stages of *C. pusillus* on number of adult female

Source	SS	DF	MS	F	P value
Diet	17258	1	17258	2.297	0.145
Host	346295	5	69259	9.217	0.002
Rep	24464	2	12232	2.141	0.262
Host*Diet	17295	3	5765	0.767	0.459
Diet*Rep	11941	1	11941	1.589	0.251
Rep*Host	38936	1	38936	5.182	0.007
Error	37570	5	7514		
Total	483759	18			

Appendix table 59 Average (±SE) number(%) of *X. flavipes* adult male and female on different life stages of *R. dominica* under laboratory condition.

Adults of *X. flavipes*	Different life stages of *R.. dominica*					
	Eggs	1st	2nd	3rd	4th	Pupae
No. of Male	37.00±1.73abc	42.00±2.31a	39.00±2.89ab	34.00±1.15abc	31.00±0.58bc	27.00±3.46a
No. of Female	63.00±1.73ab	58.00±2.31b	61.00±2.89b	66.00±1.15ab	69.00±2.85ab	73.00±3.46a

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 60 Factorial ANOVA showing the effects of different life stages of *R. dominica* on number of adult male

Source	SS	DF	MS	F	P value
Diet	110.74	1	110.74	1.463	0.281
Host	2950.95	5	590.19	7.850	0.002
Rep	800.30	2	400.15	5.325	0.017
Host*Diet	2176.44	3	725.48	9.654	0.945
Diet*Rep	95.21	1	95.21	1.267	0.962
Rep*Host	470.98	1	470.98	6.267	0.004
Error	375.75	5	75.15		
Total	6980.37	18			

Appendix table 61 Factorial ANOVA showing the effects of different life stages of *R. dominica* on number of adult female

Source	SS	DF	MS	F	P value
Diet	118.62	1	118.62	1.438	0.325
Host	3251.25	5	650.25	7.885	0.005
Rep	896.64	2	448.32	5.437	0.019
Host*Diet	2250.45	3	750.15	9.096	0.814
Diet*Rep	93.21	1	93.21	1.130	0.478
Rep*Host	825.23	1	825.23	10.006	0.005
Error	412.35	5	82.47		
Total	7847.75	18			

Appendix table 62 Effects of different concentrations of Spinosad on hatchability of eggs of *C. pusillus*

Concentrations	Dose rates (µl/cm^2)	Total no of eggs	Total eggs hatched	Average % of egg hatched (±SE)	PRC value
Control	Untreated	150	126	42.00±1.12a	-
Spinosad	0.491	150	75	25.00±1.15b	40.48
	0.983	150	51	17.00±1.05c	59.52
	1.966	150	33	11.00±1.10d	73.81
	3.932	150	24	8.00±1.11de	80.95
	7.863	150	15	5.00±1.02e	88.10

Note: Means with same letter do not significantly differed from each other Tukey's Test , P<0.001

Appendix table 63 Factorial ANOVA showing the effects of different concentrations of Spinosad on hatchability eggs of *C. pusillus*

Source	SS	DF	MS	F	P value
Concentration	550.770	5	110.154	31.240	0.001
Exposure period	420.780	2	210.390	59.668	0.002
REP	14.364	2	7.182	2.037	0.135
Concentration * REP	34.150	10	3.415	0.969	0.017
Exposure period * Concentration	63.890	10	6.389	1.812	0.011
Exposure period * REP	8.044	4	2.011	0.570	0.199
Error	74.046	21	3.526		
Total	1166.044	54			

Appendix table 64 Dose mortality data of 14-19d old *C. pusillus* larvae treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	10	16.667	17	4.05	4.068	4.037	26.34	4.063
0.983	0.9925536	60	14	23.333	23	4.26	4.249	4.252	30.18	4.243
1.966	1.29358	60	17	28.333	28	4.42	4.430	4.420	33.48	4.422
3.932	1.594607	60	22	36.667	37	4.67	4.611	4.659	36.06	4.602
7.863	1.895579	60	24	40	40	4.75	4.792	4.740	36.96	4.782

$Y = 3.649872 + 0.5973316 X$ $\chi^2 = 0.2013416$ (3 df)

$LC_{50} = 18.20812$ µl/cm^2 95% Conf. limits are 4.379743 to 75.69753 µl/cm^2

Appendix table 65 Dose mortality data of 14-19d old *C. pusillus* larvae treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	20	33.333	33	4.56	4.630	4.551	36.06	4.627
0.983	0.9925536	60	25	41.667	42	4.80	4.733	4.792	36.96	4.731
1.966	1.29358	60	27	45	45	4.87	4.836	4.890	37.62	4.835
3.932	1.594607	60	29	48.333	48	4.95	4.939	4.940	38.04	4.939
7.863	1.895579	60	30	50	50	5.00	5.042	5.000	38.22	5.043
Y = 4.388323 + 0.3452384 X					χ^2 = 0.5288744 (3 df)					
LC_{50} = 5.912253 µl/cm^2					95% Conf. limits are 1.421441 to 24.59105 µl/cm^2					

Appendix table 66 Dose mortality data of 14-19d old *C. pusillus* larvae treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	32	53.333	53	5.08	5.14591	5.065	38.04	3.14457
0.983	0.9925536	60	37	61.667	62	5.31	5.24705	5.332	37.62	5.24212
1.966	1.29358	60	39	65.000	65	5.39	5.34803	5.37	36.96	5.33952
3.932	1.594607	60	40	66.667	67	5.44	5.44902	5.429	36.06	5.43692
7.863	1.895579	60	42	70.000	70	5.52	5.55000	5.5	34.86	5.53430
Y = 4.92096 + 0.3235642 X					χ^2 = 0.6214318 (3 df)					
LC_{50} = 0.1755007 µl/cm^2					95% Conf. limits are 0.01161457 to 2.651883 µl/cm^2					

Appendix table 67 Dose mortality data of *C. pusillus* pupae treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	5	8.333	8	3.59	3.642	3.596	18.12	3.660
0.983	0.9925536	60	7	11.667	12	3.82	3.780	3.836	20.16	3.791
1.966	1.29358	60	9	15	15	3.96	3.916	3.970	24.30	3.923
3.932	1.594607	60	10	16.667	17	4.05	4.053	4.037	26.34	4.054
7.863	1.895579	60	12	20	20	4.16	4.190	4.170	28.26	4.186
Y = 3.356467 + 0.4377169 X					χ^2 = 0.1827011 (3 df)					
LC_{50} = 568.5706 µl/cm^2					95% Conf. limits are 2.246793 to 143881.9 µl/cm^2					

Appendix table 68 Dose mortality data of *C. pusillus* pupae treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	10	16.667	17	4.05	4.062	4.037	26.34	4.055
0.983	0.9925536	60	12	20	20	4.16	4.140	4.170	28.26	4.135
1.966	1.29358	60	13	21.667	22	4.23	4.218	4.218	30.18	4.214
3.932	1.594607	60	14	23.333	23	4.26	4.296	4.252	30.18	4.294
7.863	1.895579	60	16	26.667	27	4.39	4.374	4.394	31.92	4.374
Y = 3.872518 + 0.2643514 X					χ^2 = 0.1104122 (3 df)					
LC_{50} = 1841.139 µl/cm^2					95% Conf. limits are 0.09236452 to 36700190 µl/cm^2					

Appendix table 69 Dose mortality data of *C. pusillus* pupae treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	15	25	25	4.33	4.312	4.330	31.92	4.309
0.983	0.9925536	60	17	28.333	28	4.42	4.425	4.420	33.48	4.421
1.966	1.29358	60	19	31.667	32	4.53	4.538	4.516	34.86	4.532
3.932	1.594607	60	21	35	35	4.61	4.651	4.605	36.06	4.644
7.863	1.895579	60	25	41.667	42	4.80	4.764	4.792	36.93	4.755
Y = 4.053233 + 0.3704698 X					χ^2 = 0.1278024 (3 df)					
LC_{50} = 35.94058 µl/cm^2					95% Conf. limits are 2.061289 to 626.6588 µl/cm^2					

Appendix table 70 Dose mortality data of 2d *C. pusillus* adult treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	15	25	25	4.33	4.3019	4.330	31.92	4.300
0.983	0.9925536	60	18	30	30	4.48	4.475	4.480	33.48	4.474
1.966	1.29358	60	20	33.333	33	4.56	4.648	4.551	36.06	4.648
3.932	1.594607	60	27	45	45	4.87	4.821	4.890	37.62	4.822
7.863	1.895579	60	30	50	50	5.00	4.994	4.990	38.04	4.996
Y = 3.900657 + 0.5777386 X					χ^2 = 0.5446673 (3 df)					
LC_{50} = 7.995372 µl/cm^2					95% Conf. limits are 2.947408 to 21.68888 µl/cm^2					

Appendix table 71 Dose mortality data of 2d *C. pusillus* adult treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	22	36.667	37	4.67	4.640	4.659	36.06	4.639
0.983	0.9925536	60	25	41.667	42	4.80	4.812	4.812	37.62	4.809
1.966	1.29358	60	28	46.667	47	4.92	4.984	4.915	38.04	4.979
3.932	1.594607	60	35	58.333	58	5.20	5.156	5.190	38.04	5.148
7.863	1.895579	60	38	63.333	63	5.33	5.328	5.318	36.96	5.318
Y = 24.249114 + 0.563959 X					χ^2 = 0.2347469 (3 df)					
LC_{50} = 2.145135 µl/cm^2					95% Conf. limits are 1.191995 to 3.860422 µl/cm^2					

Appendix table 72 Dose mortality data of 2d *C. pusillus* adult treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	28	46.667	47	4.92	4.820	4.942	37.62	4.840
0.983	0.9925536	60	30	50	50	5.00	5.036	5.000	38.22	5.047
1.966	1.29358	60	32	53.33.	53	5.08	5.252	5.098	37.62	5.254
3.932	1.594607	60	42	70	70	5.52	5.468	5.510	36.06	5.461
7.863	1.895579	60	46	76.667	77	5.74	5.684	5.730	33.48	5.668
Y = 4.364444 + 0.6877889 X					χ^2 = 1.608753 (3 df)					
LC_{50} = 0.839572 µl/cm^2					95% Conf. limits are 0.446121 to 1.580054 µl/cm^2					

Appendix table 73 Regression equations, χ^2 values, LC_{50} values and 95% confidence limits for Spinosad against 14-19 d larvae, pupae and 2 d adults of *C. pusillus* after 24, 48 and 72 h of exposure.

Concentration of Spinosad (µl/cm^2)	Life stage of *C. pusillus*	Exposure period (h)	Regression equation	χ^2 for heterogeneity	LC_{50} (µl/ cm^2)	95% confidence limits	
						Lower	Upper
Control 0.491 0.983 1.966 3.932 7.863	Larvae	24	Y =3.649872+0.5973316 X	0.2013416	18.20812	4.379743	75.69753
		48	Y =4.388323+0.3452384 X	0.5288744	5.912253	1.421441	24.59105
		72	Y =4.92096+0.3235642 X	0.6214318	0.1755007	0.01161457	2.651883
Control 0.491 0.983 1.966 3.932 7.863	Pupae	24	Y =3.356467+0.4377169 X	0.1827011	568.5706	2.246793	143881.9
		48	Y =3.872518+0.2643514 X	0.1104122	1841.139	0.09236452	36700190
		72	Y =4.053233+0.3704698 X	0.1278024	35.94058	2.061289	626.6588
Control 0.491 0.983 1.966 3.932 7.863	Adults	24	Y =3.900657+0.5777386 X	0.5446673	7.995372	2.947408	21.68888
		48	Y =24.249114+0.563959 X	0.2347469	2.145135	1.191995	3.860422
		72	Y =4.364444+0.6877889 X	1.608753	0.839572	0.446121	1.580054

Appendix table 74 Factorial ANOVA showing the effects of different concentrations of Spinosad on the 14-19d old larval mortality of *C. pusillus* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	695.780	5	139.156	38.389	0.000
Exposure period	420.778	2	210.389	58.038	0.001
REP	14.778	2	7.389	2.038	0.245
Concentration * REP	35.440	10	3.544	0.978	0.027
Exposure period * Concentration	124.110	10	12.411	3.424	0.001
Exposure period * REP	15.948	4	3.987	1.100	0.207
Error	76.125	21	.3.625		
Total	1382.959	54			

Appendix table 75 Factorial ANOVA showing the effects of different concentrations of Spinosad on pupal mortality of *C. pusillus* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	804.425	5	160.885	61.524	0.000
Exposure period	161.038	2	80.519	30.791	0.002
REP	15.026	2	7.513	2.873	0.140
Concentration * REP	28.570	10	2.857	1.093	0.389
Exposure period * Concentration	66.890	10	6.689	2.558	0.006
Exposure period * REP	6.964	4	1.741	0.666	0.154
Error	54.915	21	2.615		
Total	1137.828	54			

Appendix table 76 Factorial ANOVA showing the effects of different concentrations of Spinosad on the adult mortality of *C. pusillus* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	898.150	5	179.630	42.617	0.000
Exposure period	1711.038	2	855.519	202.970	0.000
REP	7.948	2	3.974	0.943	0.610
Concentration * REP	402.800	10	40.280	9.556	0.000
Exposure period * Concentration	351.960	10	35.196	8.350	0.000
Exposure period * REP	19.948	4	4.987	1.183	0.571
Error	88.515	21	4.215		
Total	3480.359	54			

Appendix table 77 Effects of different concentrations of Spinosad on hatchability of eggs of *R. dominica*

Concentrations	Dose rates (µl/cm^2)	Total no of eggs	Total eggs hatched	Average % of egg hatched (±SE)	PRC value
Control	Untreated	150	114	38.00±0.95a	-
Spinosad	0.491	150	45	15.00±1.14b	60.53
	0.983	150	27	9.00±1.01c	76.32
	1.966	150	12	4.00±0.84cd	89.47
	3.932	150	6	2.00±1.14d	94.74
	7.863	150	1	0.33±1.03d	99.13

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 78 Factorial ANOVA showing the effects of different concentrations of Spinosad on hatchability eggs of *R. dominica*

Source	SS	DF	MS	F	P value
Concentration	354.925	5	70.985	13.851	0.001
Exposure period	400.956	2	200.478	39.118	0.008
REP	31.050	2	15.525	3.029	0.053
Concentration * REP	402.910	10	40.251	7.854	0.011
Exposure period * Concentration	407.660	10	40.766	7.954	0.123
Exposure period * REP	81.412	4	20.353	3.971	0.015
Error	107.625	21	5.125		
Total	1786.538	54			

Appendix table 79 Dose mortality data of 26-31d *R. dominica* larvae treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	14	23.333	23	4.26	4.216	4.252	30.18	4.207
0.983	0.9925536	60	15	25	25	4.33	4.402	4.330	33.48	4.395
1.966	1.29358	60	20	33.333	33	4.56	4.588	4.544	34.86	4.582
3.932	1.594607	60	27	45	45	4.87	4.774	4.870	36.96	4.769
7.863	1.895579	60	28	46.667	47	4.92	4.960	4.915	38.04	4.957
Y = 3.776951 + 0.6223563 X					χ^2 = 0.6917343 (3 df)					
LC_{50} is 9.22978 µl/cm^2					95% Conf. limits are 3.402471 TO 25.03735 µl/cm^2					

Appendix table 80 Dose mortality data of 26-31d *R. dominica* larvae treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration ($\mu l/cm^2$)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	25	41.667	42	4.80	4.792	4.792	36.96	4.796
0.983	0.9925536	60	27	45	45	4.87	4.876	4.890	37.62	4.877
1.966	1.29358	60	29	48.333	48	4.95	4.960	4.940	38.04	4.957
3.932	1.594607	60	31	51.667	52	5.05	5.044	5.050	38.22	5.038
7.863	1.895579	60	33	55	55	5.13	5.128	5.115	38.04	5.118
Y = 4.611174 + 0.2676746 X					χ^2 = 0.0251174 (3 df)					
LC_{50} is 2.835366 $\mu l/cm^2$					95% Conf. limits are 0.7683601 to 10.46294 $\mu l/cm^2$					

Appendix table 81 Dose mortality data of 26-31d *R. dominica* larvae treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration ($\mu l/cm^2$)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	30	50	50	5.00	4.984	4.990	38.04	4.984
0.983	0.9925536	60	32	53.333	53	5.08	5.097	5.075	38.22	5.096
1.966	1.29358	60	35	58.333	58	5.20	5.210	5.228	37.62	5.208
3.932	1.594607	60	38	63.333	63	5.33	5.323	5.318	36.96	5.320
7.863	1.895579	60	40	66.667	67	5.44	5.436	5.429	36.06	5.432
Y = 4.726218 + 0.372455 X					χ^2 = 0.03347778 (3 df)					
LC_{50} is 0.5433412 $\mu l/cm^2$					95% Conf. limits are 0.1273662 to 2.317881 $\mu l/cm^2$					

Appendix table 82 Dose mortality data of *R. dominica* pupae treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration ($\mu l/cm^2$)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	3	5	5	3.36	3.424	3.360	14.28	3.448
0.983	0.9925536	60	5	8.333	8	3.59	3.572	3.596	16.14	3.587
1.966	1.29358	60	7	11.667	12	3.82	3.720	3.836	20.16	3.726
3.932	1.594607	60	8	13.333	13	3.87	3.868	3.873	22.20	3.865
7.863	1.895579	60	9	15	15	3.96	4.016	3.955	26.34	4.004
Y = 3.129547 + 0.4611072 X					χ^2 = 0.4201939 (3 df)					
LC_{50} is 1138.777 $\mu l/cm^2$					95% Conf. limits are 1.990441 to 651521.7 $\mu l/cm^2$					

Appendix table 83 Dose mortality data of *R. dominica* pupae treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration ($\mu l/cm^2$)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	8	13.333	13	3.87	3.910	3.878	24.30	3.916
0.983	0.9925536	60	10	16.667	17	4.05	4.035	4.037	26.34	4.039
1.966	1.29358	60	13	21.667	22	4.23	4.160	4.246	28.26	4.161
3.932	1.594607	60	14	23.333	23	4.26	4.285	4.252	30.18	4.283
7.863	1.895579	60	16	26.667	27	4.39	4.410	4.390	33.48	4.405
Y = 3.636407 + 0.4053654 X					χ^2 = 0.2772174 (3 df)					
LC_{50} is 231.1335 $\mu l/cm^2$					95% Conf. limits are 2.478723 to 21552.51 $\mu l/cm^2$					

Appendix table 84 Dose mortality data of *R. dominica* pupae treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	14	23.333	23	4.26	4.300	4.252	30.18	4.292
0.983	0.9925536	60	18	30	30	4.48	4.427	4.480	33.48	4.421
1.966	1.29358	60	20	33.333	33	4.56	4.554	4.544	34.86	4.550
3.932	1.594607	60	22	36.667	37	4.67	4.681	4.659	36.06	4.679
7.863	1.895579	60	25	41.667	42	4.80	4.808	4.812	37.62	4.808
Y = 3.995484 + 0.4286425 X					χ^2 = 0.1803207 (3 df)					
LC_{50} is 22.0538 µl/cm^2					95% Conf. limits are 2.711695 to 179.3602 µl/cm^2					

Appendix table 85 Dose mortality data of 2d *R. dominica* adult treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	20	33.333	33	4.56	4.576	4.554	34.86	4.565
0.983	0.9925536	60	23	38.333	38	4.69	4.687	4.686	36.06	4.679
1.966	1.29358	60	26	43.333	43	4.82	4.798	4.818	36.96	4.793
3.932	1.594607	60	28	46.667	47	4.92	4.909	4.915	38.04	4.906
7.863	1.895579	60	30	50	50	5	5.020	5	38.22	5.020
Y = 4.303998 + 0.3778334 X					χ^2 = 0.05872917 (3 df)					
LC_{50} is 6.951654 µl/cm^2					95% Conf. limits are 1.68851 to 28.62021 µl/cm^2					

Appendix table 86 Dose mortality data of 2d *R. dominica* adult treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	27	45	45	4.87	4.828	4.890	37.62	4.842
0.983	0.9925536	60	30	50	50	5.00	5.002	5.000	38.22	5.006
1.966	1.29358	60	31	51.667	52	5.05	5.176	5.050	38.04	5.170
3.932	1.594607	60	40	66.667	67	5.44	5.350	5.422	36.96	5.334
7.863	1.895579	60	42	70	70	5.52	5.524	5.500	34.86	5.500
Y = 4.465182 + 0.544706 X					χ^2 = 1.017773 (3 df)					
LC_{50} is 0.9590641 µl/cm^2					95% Conf. limits are 0.4546266 to 2.02328 µl/cm^2					

Appendix table 87 Dose mortality data of 2d *R. dominica* adult treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	32	53.333	53	5.08	5.010	5.075	38.22	5.020
0.983	0.9925536	60	36	60.000	60	5.25	5.287	5.280	37.62	5.285
1.966	1.29358	60	40	66.667	67	5.44	5.564	5.416	34.86	5.551
3.932	1.594607	60	49	81.667	82	5.92	5.841	5.868	30.18	5.816
7.863	1.895579	60	52	86.667	87	6.13	6.118	6.132	24.30	6.081
Y = 4.410579 + 0.8814548 X					χ^2 = 0.8944054 (3 df)					
LC_{50} is 0.466328 µl/cm^2					95% Conf. limits are 0.2381866 to 0.912989 µl/cm^2					

Appendix table 88 Regression equations, χ^2 values, LC_{50} values and 95% confidence limits for Spinosad against 26-31 d larvae, pupae and 2 d adults of *R. dominica* after 24, 48 and 72 h of exposure.

Concentration of Spinosad (µl/cm^2)	Life stage of *R. dominica*	Exposure period (h)	Regression equation	χ^2 for heterogeneity	LC_{50} (µl /cm^2)	95% confidence limits	
						Lower	Upper
Control 0.491 0.983 1.966 3.932 7.863	Larvae	24	Y =3.776951+0.6223563 X	0.6917343	9.22978	3.402471	25.03735
		48	Y =4.611174+0.2676746 X	0.0251174	2.835366	0.7683601	10.46294
		72	Y =4.726218+0.372455 X	0.03347778	0.5433412	0.1273662	2.317881
Control 0.491 0.983 1.966 3.932 7.863	Pupae	24	Y =3.129547+0.4611072 X	0.4201939	1138.777	1.990441	651521.7
		48	Y =3.636407+0.4053654 X	0.2772174	231.1335	2.478723	21552.51
		72	Y =3.995484+0.4286425 X	0.1803207	22.0538	2.711695	179.3602
Control 0.491 0.983 1.966 3.932 7.863	Adults	24	Y =4.303998+0.3778334 X	0.05872917	6.951654	1.68851	28.62021
		48	Y =4.465182+0.544706 X	1.017773	0.9590641	0.4546266	2.02328
		72	Y =4.410579+0.8814548 X	0.8944054	0.466328	0.2381866	0.912989

Appendix table 89 Factorial ANOVA showing the effects of different concentrations of Spinosad on the 14-19d old larval mortality of *R. dominica* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	5749.705	5	1149.941	56.006	0.000
Exposure period	2341.148	2	1170.574	57.026	0.000
REP	80.704	2	40.352	1.966	0.128
Concentration * REP	200.410	10	20.041	0.976	0.001
Exposure period * Concentration	1605.670	10	160.567	7.795	0.000
Exposure period * REP	79.992	4	19.998	0.974	0.417
Error	431.967	21	20.527.		
Total	10101.596	54			

Appendix table 90 Factorial ANOVA showing the effects of different concentrations of Spinosad on the pupal mortality of *R. dominica* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	179.945	5	35.989	11.516	0.000
Exposure period	151.112	2	75.556	23.986	0.002
REP	13.334	2	6.667	2.133	0.063
Concentration * REP	164.890	10	16.489	5.276	0.000
Exposure period * Concentration	144.530	10	14.453	4.622	0.000
Exposure period * REP	11.588	4	2.897	0.927	0.010
Error	65.625	21	3.125		
Total	731.024	54			

Appendix table 91 Factorial ANOVA showing the effects of different concentrations of Spinosad on the adult mortality of *R. dominica* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	1105.945	5	221.189	83.468	0.000
Exposure period	191.444	2	95.722	36.122	0.003
REP	4.334	2	2.167	1.635	0.615
Concentration * REP	25.890	10	2.589	0.977	0.031
Exposure period * Concentration	53.440	10	5.344	2.017	0.001
Exposure period * REP	10.224	4	2.556	0.965	0.066
Error	55.650	21	.2.650		
Total	1446.927	54			

Appendix table 92 Effects of different concentrations of Spinosad on hatchability of eggs of *X. flavipes*

Concentrations	Dose rates (µl/cm^2)	Total no of eggs	Total eggs hatched	Average % of egg hatched (±SE)	PRC value
Control	Untreated	150	105	35.00±1.73a	-
Spinosad	0.491	150	102	34.00±1.15ab	2.86
	0.983	150	99	33.00±1.21ab	5.71
	1.966	150	96	32.00±1.36ab	8.57
	3.932	150	90	30.00±2.89ab	14.29
	7.863	150	75	25.00±2.12b	28.57

Note: Means with same letter do not significantly differed from each other Tukey's Test, P<0.001

Appendix table 93 Factorial ANOVA showing the effects of different concentrations of Spinosad on hatchability eggs of *X. flavipes*

Source	SS	DF	MS	F	P value
Concentration	144.090	5	28.769	12.599	0.001
Exposure period	64.032	2	32.020	37.489	0.002
REP	4.702	2	2.231	0.876	0.389
Concentration * REP	22.452	10	2.278	5.540	0.003
Exposure period * Concentration	15.539	10	1.499	3.608	0.005
Exposure period * REP	3.305	4	0.849	2.011	0.123
Error	0.000	0	.		
Total	754.000	54			

Appendix table 94 Dose mortality data of 4d *X. flavipes* nymphs treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	3	5	5	3.36	3.350	3.360	12.48	3.344
0.983	0.9925536	60	4	6.667	7	3.52	3.500	3.519	16.14	3.500
1.966	1.29358	60	5	8.333	8	3.59	3.650	3.596	18.12	3.653
3.932	1.594607	60	7	11.667	12	3.82	3.800	3.822	22.20	3.807
7.863	1.895579	60	9	15	15	3.96	3.950	3.970	24.30	3.962
Y = 2.990045 + 0.5126829 X					χ^2 = 0.07527256 (3 df)					
LC_{50} is 832.6535 µl/cm^2					95% Conf. limits are 2.922949 to 237196.1 µl/cm^2					

Appendix table 95 Dose mortality data of 4d *X. flavipes* nymphs treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	7	11.667	12	3.82	3.866	3.822	22.20	3.871
0.983	0.9925536	60	10	16.667	17	4.05	4.008	4.037	26.34	4.010
1.966	1.29358	60	12	20	20	4.16	4.150	4.170	28.26	4.149
3.932	1.594607	60	15	25	25	4.33	4.292	4.320	30.18	4.287
7.863	1.895579	60	16	26.667	27	4.39	4.434	4.390	33.48	4.426
Y = 3.55203 + 0.4612296 X					χ^2 = 0.16153 (3 df)					
LC_{50} is 137.838 µl/cm^2					95% Conf. limits are 3.740582 to 5079.244 µl/cm^2					

Appendix table 96 Dose mortality data of 4d *X. flavipes* nymphs treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	10	16.667	17	4.05	4.048	4.037	26.34	4.050
0.983	0.9925536	60	12	20	20	4.16	4.182	4.170	28.26	4.181
1.966	1.29358	60	15	25	25	4.33	4.316	4.330	31.92	4.313
3.932	1.594607	60	18	30	30	4.48	4.450	4.480	33.48	4.444
7.863	1.895579	60	20	33.333	33	4.56	4.584	4.544	34.86	4.575
Y = 3.748117 + 004364652 X					χ^2 = 0.09504318 (3 df)					
LC_{50} is 73.82966 µl/cm^2					95% Conf. limits are 3.22157 to 1691.976 µl/cm^2					

Appendix table 97 Dose mortality data of 2d *X. flavipes* adult treated with different concentrations of Spinosad after 24 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	3	5	5	3.36	3.386	3.360	12.48	3.396
0.983	0.9925536	60	5	8.333	8	3.59	3.552	3.596	16.14	3.557
1.966	1.29358	60	6	10	10	3.72	3.718	3.720	20.16	3.718
3.932	1.594607	60	8	13.333	13	3.87	3.884	3.873	22.20	3.879
7.863	1.895579	60	10	16.667	17	4.05	4.050	4.037	26.34	4.040
Y = 3.027043 + 0.5342836 X					χ^2 = 0.04168511 (3 df)					
LC_{50} is 492.8509 µl/cm^2					95% Conf. limits are 3.859698 to 62932.93 µl/cm^2					

Appendix table 98 Dose mortality data of 2d *X. flavipes* adult treated with different concentrations of Spinosad after 48 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	4	6.667	7	3.52	3.532	3.519	16.14	3.537
0.983	0.9925536	60	6	10	10	3.72	3.684	3.730	18.12	3.687
1.966	1.29358	60	7	11.667	12	3.82	3.836	3.822	22.20	3.842
3.932	1.594607	60	9	15	15	3.96	3.988	3.970	24.30	3.997
7.863	1.895579	60	12	20	20	4.16	4.140	4.170	28.26	4.152
Y = 3.175748 + 0.514919 X				χ^2 = 0.07186127 (3 df)						
LC_{50} is 348.9742 µl/cm^2				95% Conf. limits are 4.223628 to 28833.73 µl/cm^2						

Appendix table 99 Dose mortality data of 2d *X. flavipes* adult treated with different concentrations of Spinosad after 72 hours of treatment.

Concentration (µl/cm^2)	Log concentration	No of insects	Kill	% kill	Corr. %	Emp. probit	Expt. probit	Work probit	Weight	Final probit
0.491	0.6910847	60	6	10	10	3.72	3.760	3.720	20.16	3.766
0.983	0.9925536	60	9	15	15	3.96	3.894	3.975	22.20	3.897
1.966	1.29358	60	10	16.667	17	4.05	4.028	4.037	26.34	4.029
3.932	1.594607	60	11	18.333	18	4.08	0.162	4.094	28.26	4.160
7.863	1.895579	60	15	25	25	4.33	4.296	4.320	30.18	4.291
Y = 3.464713 + 0.4360996 X				χ^2 = 0.3258066 (3 df)						
LC_{50} is 331.5098 µl/cm^2				95% Conf. limits are 2.651994 to 41440.03 µl/cm^2						

Appendix table 100 Regression equations, χ^2 values, LC_{50} values and 95% confidence limits for Spinosad against 4 d nymphss and 2 d adults of *X. flavipes* after 24, 48 and 72 h of exposure.

Concentration of Spinosad (µl/cm^2)	Life stage of *X. flavipes*	Exposure period (h)	Regression equation	χ^2 for heterogeneity	LC_{50} (µl/cm^2)	95% confidence limits	
						Lower	Upper
Control 0.491 0.983 1.966 3.932 7.863	Nymphss	24	Y =2.990045+0.5126829 X	0.07527256	832.6535	2.922949	237196.1
		48	Y =3.55203+0.4612296 X	0.16153	137.838	3.740582	5079.244
		72	Y =3.748117+004364652 X	0.09504318	73.82966	3.22157	1691.976
Control 0.491 0.983 1.966 3.932 7.863	Adults	24	Y =3.027043+0.5342836 X	0.04168511	492.8509	3.859698	62932.93
		48	Y =3.175748+0.514919 X	0.07186127	348.9742	4.223628	28833.73
		72	Y =3.464713+0.4360996 X	0.3258066	331.5098	2.651994	41440.03

Appendix table 101 Factorial ANOVA showing the effects of different concentrations of Spinosad on the nymphsal mortality of *X. flavipes* after different exposure periods.

Source	SS	DF	MS	F	P value
Concentration	144.095	5	28.819	13.562	0.000
Exposure period	64.038	2	32.019	15.068	0.003
REP	4.704	2	2.352	1.107	0.449
Concentration * REP	102.850	10	10.285	4.840	0.001
Exposure period * Concentration	65.520	10	6.552	3.083	0.006
Exposure period * REP	19.408	4	4.852	2.283	0.128
Error	44.625	21	2.125.		
Total	445.240	54			

Appendix table 102 Factorial ANOVA showing the effects of different concentrations of Spinosad on the adult mortality of *X. flavipes* after different exposure periods

Source	SS	DF	MS	F	P value
Concentration	109.990	5	21.998	5.219	0.000
Exposure period	61.592	2	30.796	7.306	0.000
REP	5.098	2	2.549	0.605	0.685
Concentration * REP	65.870	10	6.587	1.563	0.108
Exposure period * Concentration	102.150	10	10.215	2.423	0.243
Exposure period * REP	35.664	4	8.916	2.115	0.953
Error	88.515	21	4.215		
Total	468.879	54			

Appendix table 103 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *C. pusillus* after 3 months of exposure

Source	SS	DF	MS	F	P value
Treatment	180975	3	60325	65.929	0.281
Concentration	180531	1	180513	197.303	0.003
Rep	780033	3	260011	284.165	0.019
Treatment * Concentration	30555	3	10185	11.131	0.974
Treatment * Rep	13500	1	13500	14.754	0.962
Concentration* Rep	170624	2	85312	93.237	0.002
Error	10065	11	915		
Total	1366283	24			

Appendix table 104 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *C. pusillus* after 6 months of exposure

Source	SS	DF	MS	F	P value
Treatment	174039	3	58013	53.966	0.145
Concentration	245528	1	245528	228.398	0.001
Rep	982503	3	327501	304.652	0.262
Treatment * Concentration	32556	3	10852	10.095	0.731
Treatment * Rep	14225	1	14225	13.233	0.274
Concentration* Rep	168026	2	84013	78.152	0.004
Error	11825	11	1075		
Total	1628702	24			

Appendix table 105 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *C. pusillus* after 9 months of exposure

Source	SS	DF	MS	F	P value
Treatment	927675	3	309225	235.152	0.172
Concentration	652220	1	652220	495.985	0.001
Rep	45756	3	15252	11.598	0.125
Treatment * Concentration	271500	3	90500	68.821	0.761
Treatment * Rep	12740	1	12740	9.688	0.317
Concentration* Rep	57220	2	28610	21.757	0.003
Error	14465	11	1315		
Total	1981976	24			

Appendix table 106 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *C. pusillus* after 12 months of exposure

Source	SS	DF	MS	F	P value
Treatment	123599	3	41193	24.965	0.016
Concentration	215000	1	215000	130.303	0.000
Rep	1450710	3	483570	293.073	0.025
Treatment * Concentration	1664688	3	554896	336.301	0.720
Treatment * Rep	143228	1	143228	86.805	0.199
Concentration* Rep	525000	2	262500	159.091	0.387
Error	18150	11	1650		
Total	4140355	24			

Appendix table 107 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *R. dominica* after 3 months of exposure

Source	SS	DF	MS	F	P value
Treatment	165036	3	55012	66.681	0.084
Concentration	172517	1	172517	209.112	0.002
Rep	840198	3	280066	339.474	0.000
Treatment * Concentration	24555	3	8185	9.921	0.925
Treatment * Rep	12519	1	12519	15.175	0.754
Concentration* Rep	166732	2	83366	101.050	0.013
Error	9075	11	825		
Total	1390632	24			

Appendix table 108 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *R. dominica* after 6 months of exposure

Source	SS	DF	MS	F	P value
Treatment	159084	3	53028	57.328	0.129
Concentration	233548	1	233548	252.484	0.001
Rep	921006	3	307002	331.894	0.000
Treatment * Concentration	24786	3	8262	8.932	0.951
Treatment * Rep	12230	1	12230	13.222	0.802
Concentration* Rep	166124	2	83062	89.797	0.021
Error	10175	11	925		
Total	1526953	24			

Appendix table 109 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *R. dominica* after 9 months of exposure

Source	SS	DF	MS	F	P value
Treatment	898173	3	299391	244.401	0.000
Concentration	642440	1	642440	524.441	0.000
Rep	52500	3	17500	14.286	0.922
Treatment * Concentration	268383	3	89461	73.029	0.005
Treatment * Rep	10640	1	10640	8.686	0.801
Concentration* Rep	53223	2	26611	21.223	0.330
Error	13475	11	1225		
Total	1938834	24			

Appendix table 110 Factorial ANOVA showing the effects of adult *X. flavipes* and different concentrations of Spinosad on the adult population of *R. dominica* after 12 months of exposure

Source	SS	DF	MS	F	P value
Treatment	120279	3	40093	25.456	0.480
Concentration	214213	1	214213	136.008	0.015
Rep	1390059	3	463353	294.192	0.001
Treatment * Concentration	1544700	3	514900	326.921	0.999
Treatment * Rep	113449	1	113449	72.029	0.067
Concentration* Rep	483096	2	241548	153.364	0.003
Error	17325	11	1575		
Total	3883121	24			