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Potentiality of Adenanthera pavonina (L.) (Leguminosae) and Dinarmus basalis (Rond.) (Pteromalidae) against Callosobruchus chinensis L. (Bruchidae)

Matin, Shah Md Abdul

University of Rajshahi

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THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
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RAJSHAHI UNIVERSITY, BANGLADESH

SUBMITTED

By
Shah Md Abdul Matin
B Sc (Honors), M Sc (Raj.)

Entomology and Insect Biotechnology Laboratory
Institute of Biological Sciences
University of Rajshahi
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DEDICATED

to

My Beloved Parents

DECLARATION

I hereby declare that the entire work submitted as a thesis entitled Potentiality of *Adenanthera pavonina* (L.) (Leguminosae) and *Dinarmus basalis* (Rond.) (Pteromalidae) against *Callosobruchus chinensis* L. (Bruchidae) 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 supervision of Dr Md Wahedul Islam, Professor, Institute of Biological Sciences, Rajshahi University, Rajshahi. The thesis contains no material which has been accepted for the award of any other degree or diploma elsewhere.

June 2014
Rajshahi University

(Shah Md Abdul Matin)
Candidate

CERTIFICATE

This is to certify that the thesis entitled Potentiality of *Adenanthera pavonia* (L.) (Leguminosae) and *Dinarmus basalis* (Rond.) (Pteromalidae) against *Callosobruchus chinensis* L. (Bruchidae) submitted for the degree of Doctor of Philosophy is bonafide original research work of Shah Md Abdul Matin, carried out at the Institute of Biological Sciences, University of Rajshahi under my supervision. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of the thesis for the award of the degree of Doctor of Philosophy.

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Shah Md Abdul Matin

CONTENTS

Abbreviation of the special words used in the text

#U	=	Number of insects used
% kill	=	Insects killed percent
+ve	=	Positive
µg	=	microgram
µl	=	micro liter
$CHCl_3$	=	Chloroform
cm^2	=	centimeter square
Cr %	=	corrected mortality percent
df	=	degree of freedom
E. Pr	=	Empirical Probit
et al.	=	and others (author)
EtOAc	=	Ethyl Acetate
Ex Pr	=	Expected Probit
F Pro	=	Final Probit
Fig.	=	Figure
fr	=	factor (s)
h	=	hour (s)
i.e.	=	that is
KI	=	Number of insects killed
LC_{50}	=	concentration required to kill 50% of test organisms
LD_{50}	=	dose required to kill 50% of test organisms
LDose	=	Log dose
MeOH	=	Methanol
mg	=	milligram (s)
ml	=	milliliter
mm	=	millimeter
mp	=	melting point
nm	=	nanometer
PDA	=	Potato Dextrose Agar
-ve	=	Negative
Weight	=	Weighting coefficient
Wk Pro	=	Working probit
χ^2	=	Chi-squared

A checklist of the tables provided

A checklist of the figures provided

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ABSTRACT

The petroleum ether, acetone, chloroform and methanol extracts of the leaves, seed and stem wood of *Adenanthera pavonina* (L.) were tested against *Callosobruchus chinensis* L. adults through residual film assay at doses of 2831, 2477, 2123, 1769 and 1415 µg/cm^2 on the surface of the Petri dishes, where the test insects were released to observe mortality or any sort of behavioral changes due to the action of the extracts compared to the controls. The seed extract was found to offer the highest mortality of the beetles than leaves and stems after 12h, 24h and 48 h of exposure respectively. According to the intensity of activity observed through mortality of the adult beetles the potentiality of the petroleum ether, acetone, chloroform and methanol extracts could be arranged in a descending order of chloroform > acetone > methanol >petroleum ether extracts.

In comparison to all other biological assays repellent activity of the *A. pavonina* extracts have been detected for seed, leaves and stem wood of petroleum ether, acetone, chloroform and methanol extracts respectively. All the test extracts offered repellency at 0.01% level of significance ($P<0.001$) except the stem (acetone) extract which was found 0.1% level of significance ($P<0.01$). According to the intensity of repellency the result could be arranged in a descending order: leaf (pet. ether extract) >leaf (chloroform extract) >seed (methanol extract > stem wood (acetone) extract and in all the cases significant differences were noticed.

The brine shrimp lethality effect of the above mentioned extracts also found promising and the seed extract was found to offer the highest mortality of the nauplii after 30 min, 24h and 48h of exposures respectively. According to the intensity of the activity it could be arranged in the following order: seed > leaf > stem wood extract.

The anitbacterial activity of *A. pavonina* extractives collected in petroleum ether, acetone, chloroform and methanol of leaves, seed and stem wood tested against 15 bacteria (6 Gram-positive bacteria, *S. aureus*, *B. cereus*, *B.*

megaterium, *B. Subtilis*, *S. lutea*, *S. –β-haemolyticus* and 9 Gram-negative bacteria, *S. typhi*, *S. dysenteriae*, *S. shiga*, *S. sonnei*, *S. boydii*, *E. coli*, *Klebsiella* sp., *P. aeruginosa*, *Proteus* sp.) at concentrations of 50 and 200µg/disc along with a standard antibiotic, Ciprofloxacin 30µg/disc. The *in vitro* screening for antimicrobial activity was carried out by using disc diffusion and micro broth dilution techniques. All the extracts showed significant inhibitory activity over the bacteria. The maximum diameter zone of inhibition 22 mm was recorded to chloroform fraction of the seed extract of *A. pavonia*. The susceptibility order of extracts were seed>leaf>stem.

The antifungal activity of the *A. pavonina* extractives collected on the above chemical solvents were tested against seven pathogenic fungi (*F. vasinfectum*, *A. fumigatus*, *A. niger*, *A. flavus*, *Mucor* sp., *C. albicans*, *P. notatum*) at concentrations of 50 and 200µg/disc along with a standard Nystatin (50µg/disc). Chloroform fraction of leaf extract showed higher activity 19 mm zone of inhibition than stem>seed as well as the fractions of chloroform>petroleum ether.

Among all the chloroform, methanol, petroleum ether and acetone extracts of the leaves, seed and stem wood of *A. pavonina*, only chloroform extracts of the seed and the stem wood were subjected to evaluate the minimum inhibition zones just depending on the intensity of activity. The MIC values of the chloroform extract of the seed was 256µg/ml against *B. cereus*; 128 µg/ml against *S.-β-haemolyticus*, *S. dysenteriae* and 64 µg/ml against *Klebsiella* sp. The MIC values of the chloroform extract of stem wood were 128 µg/ml against *S.-β-haemolyticus*; 64 µg/ml against *B. megaterium*, *S. sonnei* and 32 µg/ml against *S. typhi*.

Methanol and chloroform extracts of *A. pavonina* is toxic and repellent to *C. chinensis*. This study found that extracts of *A. pavonia* was not toxic to, and did not reduce the offspring of *Dinarmus basalis* (Rond.), a larval-pupal parasitoid of *C. chinensis*. Plant extracts was also not repellent to *D. basalis*.

In malathion, the higher percentage of suppression was 76.05% at 16 mg/cm^2 at the introduction level of 2 pairs but 34–52% suppression occurred at 8–4 mg/cm^2 at the introduction levels of 4, 8 and 12 pairs whereas 83.00% suppression was recorded at the introduction levels of 2 pairs at 2 mg/cm^2 in case of chloroform treated seeds of green gram. The highest parasitism 23.70% occurred at the introduction levels 12 pairs at 8 mg/cm^2 but lowest 8.30% at the introduction levels of 2 pairs at 16 mg/cm^2 in case of malathion The combinations of methanol and chloroform extracts of *A. pavonia* and parasitoid reduced populations of *C. chinensis*.

The overall assessment of toxicity of *A. pavonina* extracts individually and combinedly with parasitoid, *D. basalis* are very much promising and their efficacy on stored grain pests might have future to be used as a control agent or tool. It may open its possibility as a control agent for the insect pests as well.

Chapter 1

General Introduction

Chapter 1

General Introduction

1.1. Introduction

Pulses occupy an important place in the world food economy. It is a wonderful gift of nature as they nourish mankind with highly nutritive food, and keep the soil alive and productive. On account of these virtues, pulse crops remained an integral part of the sustainable agriculture production systems of the semi-arid tropics. The developing countries facing protein-calorie malnutrition and under-nutrition problems, pulses being the major source of dietary protein at cheaper cost playing important role to solve these problems. These are important constituents in the diet of a very large number of populations, of the developing countries, and are good source of protein which helps to supplement cereal diets, improving their protein nutritive value. The balanced amino-acid composition of cereal and pulse protein blend, effectively matches with milk. Pulses are also rich source of minerals like calcium, phosphorus, iron, etc. and certain vitamins. Although most legumes are consumed as dry grains, immature greed pods or green seeds.

Pulses are called as poor man's protein in the third world countries including Bangladesh. In Bangladesh per-capita consumption of animal protein is very low (Saehdeva and Sehgal 1985). Pulses are economically important crops of Bangladesh, because of their high protein content (20-30%) (Sharma 1984, Sulehrie *et al*. 1998) and can provide a comparatively cheaper alternative to animal proteins.

Different types of major pulses, e.g. Pea, *Pisum sativa* L., Gram, *Cicer arietinum* L., Lentil, *Lens culinaris* Medik, Black gram, *Vigna mungo* (L.), green gram, *Phaseolus auriux* Roxb. and cowpea, *Vigna unguiculata* (L.) are grown and consumed in Bangladesh. These pulse species contribute more

than 95% of total pulse production in the country (BARI 1999). In Bangladesh the total production of pulses 21290 metric tones in 70605 area (acres).

Present world production of pulses is estimated to be 50 million metric tones and, at an average price of $400 per metric ton, its total value would amount to $2 billion (Ali 1998). Pulses occupy 67.8 million hectares area and contribute 55.2 million tones to the world's food basket. Asia is the major pulse producing continent sharing 52.1% of the global hectarage. In Asia, India has lion share both in hectares and production. India has the distinction of being world's largest producer of pulses (13 million tones) but the average productivity is quite low (614 kg/ha) (Ali 1998). This is primarily due to abiotic and biotic stress and several management constraints under which these crops are grown.

The population of Bangladesh, as per the World Bank projection, will cross 173 million by 2020 (BARC 1995). The current production of pulses is 0.532 million tones (BBS 1997). If the present rate of per capita consumption of about 12 g day^{-1} is to be maintained in the year 2010, the demand for pulses is expected to be 0.672 million tones. This means the total production of pulses needs to be increased by about 28% by 2010 over the present production, with an average annual growth rate of 2.19% (Rahman *et al*. 2000).

The Government had given priority for pulse production policy. It launched the Crop Diversification Programme (CDP) with the assistance of the Canadian International Development Agency (CIDA) in 1990 to augment pulses, oilseeds, and tuber crops' production in the country. The themes of the CDP were : (1) increase area and production through utilization of fallow lands or periods; (2) introduction of new cropping patterns with increased cropping intensity, through introduction of new varieties and technologies; and (3) increase consumption and marketing. To achieve these goals the CDP has supported several components such as:

- Strengthening research for development of suitable high-yield technology packages.
- Seed production of improved varieties through the Bangladesh Agricultural Development Corporation (BADC).
- Strengthening of extension services through training and demonstration programs to familiarize farmers with the latest production technology (Rahman 2000).

Pest problems in stored pulse

Pest attack during storage reduces both quality and quantity of agricultural products. In tropical and subtropical countries the annual loss caused by insect pests ranges between 10-20% of the harvested products (Burkholder 1990). In Bangladesh, this loss ranges between 10-15% (Hussain 1996).Grain legumes are more difficult to store than cereals and they suffer greater damage from insects and microorganisms. This not only results in quantitative losses, but also in qualitative reduction of the nutritive value because of vitamin loss and deterioration of protein quality. The milling losses in insect-damaged grain are even higher as more breakage and powdering occur with such grain.

Bruchids and stored pulses

Bruchids, other wise referred to as 'Pulse beetles' are pests of pulses in both field as well as in storage. The bruchid beetels are prolific, breed rapidly, and cause serious deterioration in the nutritive value of the grain. Damage ranging 30 to 70% of the grain has been reported (Ali 1998). However, the loss caused to stored seeds far exceeds the damage caused to the crop in the field. In Bangladesh, three species of bruchids belonging to the common genus, *Callosobruchus* have been recorded. These are *C. chinensis* (L.), *C. analis* (F.) and *C. maculatus* (F.) are pests of legumes in field as well as storage. At 30^0C and 70% rh, same species of bruchids take only a few weeks to develop from egg to pupa (Ali 1998).

The bruchids attacked almost all the pulses but are serious pests of cowpea, mungbean, chickpea and lentil. The nature of damage due to pulse beetles is characterized by the circular holes in the infested pods and seeds, bored by the freshly hatched larva to feed the seeds and kernels.

In the warmer countries, especially where rainfall in abundant and the climate is warm and humid, it is extremely difficult to protect the stored grains from the infestations of the insect pests. Insects breed the year around with little interruption and hence extreme care is required to prevent heavy losses from their feeding activities. *C. chinensis* multiply rapidly in warm conditions and inflict serious damage within a short time. Six months after the initial infestation, 100% of the pulses may become infested and the weight loss may rise up to 48.9% in case of gram under the laboratory conditions (Singh 1965). One beetle could cause 3.5% weight loss to cowpea seeds (Booker 1967).

Biology of *Callosobruchus*

Pulse beetle, *Callosobruchus* sp. is the major pests of various stored pulses, causing extensive damage leading into huge economic loss. *C. chinensis* is a major pest of leguminous stored seeds. It is a pest of pulses, cowpea, soybean, gram, pigeon pea, lablab etc. The insect causes substantial loss to stored black gram *(Vigna mungo* (L.) and the infested seeds become unfit for either sowing or human consumption. The beetle exhibits a high degree of specificity for its growth and development towards various legumes. Earlier, the biology of the bruchid has been studied by many researchers on different pulses (Howe and Curie 1964, Raina 1970, Kumari *et al*. 1991, Pandey and Singh 1997, Singh and Kumari 2000, Singh and Borah 2001, Meghwal and Singh 2005). Laboratory studies on the biology of *C. chinensis* on the stored black gram revealed that the insect completed six generations from April to October. Total developmental period of *C. chinensis* was 31 d while it was 38.3 days during April – larval, pupal and total developmental period (egg to adult) was of 8.0, 18.39, 8.11 and 34.5 days respectively (Thakur and Pathania 2013).

Almost all the varieties of different pulses have been reported to be infested by this beetle (Singh et *al.* 1980). Its development in cowpea, red gram, green gram, bengal gram and lentil was within17.41 to 18.55 days (Singh et *al.* 1980). Pandey and Singh (1997) observed that entire development period from egg to adult stage on the seeds of chickpea *(Cicer arietinum)* and Urdu *(Vigna mungo)* was ranged from 20-28 days.

Adult beetle is 3-4 mm long, female being larger, brownish in colour, broader at shoulders and rounded posteriorly. There are dark patches on elytra and thorax. Adults show sexual dimorphism. Fecundity is about 100 eggs per female. Eggs are whitish, elongated and stuck on the grains or on pods and sometimes on the surface of the container. Incubation period is 3-6 days. Grubs are scarabeiform or cruciform, plump and with short legs and yellowish in colour. First in star larvae bear functional legs and a pair of thoracic plates to facilitate boring into the seeds. They feed on the inner contents of the grain and may damage several grains during development. Larval period may vary between 12 and 20 days. Pupation takes place inside the grain and pupa is dark brown in color. Completion of life cycle takes 4-5 weeks and there may be 6-7 overlapping generations in a year.

Both larvae and adults cause damage to the grains. They bite holes in the grains to enter inside and feed on kernel, damaging several grains in the process.

Plate 1: ***Callosobruchus chinensis***

Control strategy

Considering the economic benefit, methods of preventing losses in storage have gradually developed. Several protective measures like physical and mechanical, chemical, radiation, biological including the botanicals are recommended and practiced.

Physical and mechanical methods of control

The physical and mechanical methods of insect pests control are the oldest, and in some case most primitive. Control of insect pests by physical and mechanical techniques are taken to destroy them on to disrupt their normal physiological function, or to modify the environment to make it unsuitable for the pests to live. Physical and mechanical control methods often provide immediate and tangible results and are generally popular with, and convincing to, farmers (Saxena 1996). Different types of physical and mechanical control practices have been reviewed by a number of scientists (Watters 1972, Armitage 1987, Bell 1987, Lessard 1987, Navarro and Jay 1987, Wilkin and Nelson 1987, Nasiruddin 1988, Begum *et al*. 1991, Zdarkova 1996). However, these control measures are more or less costly and therefore not feasible for commercial storage agriculture (Saxena 1996) of developing countries.

Insect growth regulators (IGRs)

IGRs are known as biorationals or third generation insecticides, for their environmental safety and advanced development (Pedigo 1996). The use of IGRs as an alternative method of pest control and attracted much attention for stored product protection since 1970's. IGRs have been touted as long-term solution to the needs of insect pest management. Rup and Chopra (1984) reported that hydroprene treatment on unmated males and females of *C. maculatus* did not significantly decreased the fecundity with a dose of 7.5µg, whereas the fertility of eggs laid by treated females was affected on the first day of the oviposition even with a dose of 0.1µg. Male fertility was also affected. The F_1 adults showed morphological abnormalities and their developmental period was prolonged.

Five IGRs namely, GGA-29170, GGA-45128, MV-678, RO-20458 and fenoxycarb, that exhibited juvenile hormone (JH) activity, were evaluated for biological control and residual activity against *C. maculates*. The residual activity was evaluated at 2-week to 9-months post-treatment intervals. Most of these treatments showed high initial activity at 2-week interval resulting in a significant lower oviposition rate and showed a short residual life in this respect (Abo-El-Ghar 1992).

The main problem of IGR-treatment especially the juvenile hormone analogues (JHA) against any insect pest, is a very narrow window of susceptibility (Grooscurt 1978). However, most of the IGR-compounds have been found not to develop resistance in the pest organisms (Horn 1988), and active in storage ecosystem.

Biological control

Biological control has received increased attention in recent years and biological control agents are seen as essential and of first priority in building a pest control system (van Driesche and Bellows 1996). Biological control has been defined as the action of parasites, predators and pathogens in maintaining another organism's density at a lower average than world occur in their absence (DeBach 1964). van Driesche and Bellows (1996) refer to biological control as a population level process in which one species population lowers the numbers of another species by mechanism such as predation, parasitism, pathogenocity, or competition. There are various agents which are used for control insect pests i.e., parasites, parasitoids, predator, fungal, virus, bacterial, etc.

The importance of biological control

Utilization of biological agents to insect pests in storage situations is not a new concept, but it has only recently received serious attention. There are probably two reasons for this: first, because of the objection that introduction of parasitoids and predators would increase contamination of a product with

insect remains; and second, because of the observation that natural enemies appear in significant numbers only after a product has become heavily infested and serious damage has already occurred. Although the first criticism certainly has some validity, blanket rejection of biological control on the basis of sanitary requirements is unwarranted, because insect remains are of comparatively little concern in some products such as seed grain, animal feed and raw commodities that will be cleaned during processing. Also, since parasite and predaceous insects have essentially no ability to penetrate packages, they could be used to advantage in warehouses containing packaged commodities to reduce pest populations and thereby decrease the changes of commodity infestation. With regard to the second objection, it is true that when nature is allowed to make its course, populations of parasitoids and predators are slow to overtake and suppress pest populations.

Biological control of pests have received much attention due to the obvious hazards produced by chemical pesticides (Khan and Mannan 1991). Virtually, natural enemies affect all insect populations to some extent. For many species, natural enemies are the primary regulating force in the dynamics of their populations (Hill 1983). A natural enemy usually reduces the subject insect populations, the host or prey, by feeding on individuals, thereby promoting its own population at the expense of the population fed upon. Not only do this natural enemies help to prevent some insects from attaining pest status, but they also play a role in reducing the damage potential of significant pests (Hill 1983). Haines (1984) has reviewed the status of research on the role of parasitoids and predators in the management of storage pests.

Present status of biological control

Biological control, the use of living organisms to control pest population, dates from ancient times (Sweetman 1958, Waage and Greathhead 1988). However, 100 years have passed since Albert Koebele internationally introduced the Australian vedalia lady beetle via New Zealand (Caltagirone 1981) into California orange groves in 1889, where it spectacularly controlled

the cottony-cushion scale. This milestone marks the starts of modern classical biological control-the importation and release of an organism outside its natural range for the purpose of controlling a pest species.

A spectacular recent success in biological control has been the release of the specific parasitoid *Apoanygyrus lopezi* De Santis (= *Epidinocarsis lipezi* (De Santis) for the control of cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) in Africa. The parasitoid was introduced from South America into Nigeria in 1981, and by 1986 was established in 16 countries, covering an area of more than 750,000 km^2 (Winstanley 1995). Parasitoids, predators and pathogens have been employed in many cases to control insect pests in an efficient, cost effective and permanent manner (Hoy 1988). The success of biological control should be higher than the existing rate. The right biological control agent is more important that getting many agents and searching for the less common natural enemies might help to identify the right agent (Myers *et al*. 1989).

Future prospects of biological control

Despite the efforts of crop protection researchers over the past four decades, use of biological control against agricultural pests in limited relative to chemical and other methods (Frisbie and Adkisson 1985). The public wants safe pest control, but like most services and products this comes at a cost. The public will support only those biological systems that the effective and inexpensive, and that will replace the most harmful pesticides (Carlson 1988). Biological control is not being fully exploited as a method for the control of pest arthropods in forests, range lands and agriculture (Huffaker 1971, Tauber *et al*. 1985).

Additional and continuing efforts should be made in the traditional classical, augmentation and conservation approaches to biological control because the benefits associated with these approaches are already known to be substantial and can properly be improved.

Techniques of molecular biology are being employed to improve the efficacy of biological control in several ways. Some control agents act only very slowly, so there are opportunities to improve them either by increasing their natural virulence or by introducing new capability. Some agents have a very narrow host range; a better understanding of the molecular basis of specificity will suggest strategies for extending the range of species against which they are effective (Winstanley 1995).

Development of mass-rearing techniques is necessary for programmes with a short-term control effect. In glass hose only 15 natural enemies are presently reared on a large scale (van Lenteren and Woetes 1988). Although quality control methods are available, they are seldom applied (King and Leppla 1983). Innovations in long-term storage and release methods may lead to further reduction in costs of biological control, there by making it easier and economically more attractive to apply.

Advantages of biological control

The single biggest advantage of biological control is the high degree of specificity. This offers a means of controlling pest species without harming similar but beneficial species. For example, insect crops can be targeted without harm to bees and other important plant pollinators (Winstanley 1995).Biological control systems, by relying on the biochemistry and behaviour of naturally occurring organisms can, in some cases, be a cheaper alternative to the industrial manufacture of synthetic chemical agents. Cost-benefit analyses (DeBach 1974, Huffaker and Messenger 1976) show that the financial benefits are considerably higher in biological control than in chemical control. Biological control agents are self powered more stable and long lasting. Once they are established further investment in controlling the pest is not necessary. With the exception of a few cases there is no development of pest resistance to natural enemies as exists with chemical control (Coppel and Mertins 1977, Samways 1981).It does not cause environmental hazards, nor endanger humans or wildlife through pollution (DeBach 1974, van Emden

1974, Samways 1981).Biological control has no chemical residues in the plants and marketed products (Coppel and Mertins 1977).Biological control will not cause outbreaks of the pest population, or stimulate pest reproduction encouraging secondary pest outbreaks (Huffaker 1971, Samways 1981).

In stored product entomology, biological control was first used in 1911 against the Mediterranean flour moth (Froggatt 1912). Interest in biological control is increasing as consumers become more intolerant to pesticide residues. As availability of conventional pesticide decreases due to pest resistance, developmental cost and government registration and safety requirements (Waage 1991), search for other control measures have increased. The practically of the biological control is exemplified by the dominant role it plays in Integrated Pest Management in green house in Northern Europe (van Lenteren and Woets 1988).

The theory of biological control is based none “natural control” which can be observed in the balance of predator-prey and parasite-host populations (Huffaker and Messenger 1976). Natural enemies can be classified into several types, based on their life history, ecology, and population dynamics.

According to Nayar *et al*. (1976) the principal methods employed in biological control include the followings:

- Collecting parasites and predators from places of their origin and releasing them in places where they are absent.
- Collecting and storing the host insects in such a way as to kill them, but permitting the parasites to escape.
- Under favorable conditions large number of parasites and predators are reared and releasing them whenever needed.
- Importing parasites, predators or disease producing organisms from a foreign

The Parasitoid

Although the term parasitoid was introduced by Reuter in 1913. Doutt (1959) distinguished parasitoids from parasites on the basis that, parasitoids: a) at developing individual destroys its host, b) only the immature stages are parasitic, while the adults are free living, c) they are not heteroecious, d) they are generally similar in size to their host, e) the host is usually of the same taxonomic class, i.e. Insecta, and f) their action resembles that of predators more than parasites.

A parasitoid is defined by the feeding habit of its larva. The larva feeds exclusively on the body of its host, eventually killing it. Only a single host is required for the parasitoid to complete development, and often a number of parasitoids develop gregariously on the same host (God fray 1994). In all instances, the parasitoids kill their hosts, but in some circumstances, the host may live much of its full life before dying.
C. chinensis is parasitized by different parasitoids, *Dinarmus basalis* (Rond.) is one of them, belonging to the family Ptromalidae. This parasitoid is present in the natural storage condition and at certain time of the year, thus it considerably checks the pest population in the granaries.

Dinarmus basalis (Rond.)

Dinarmus basalis (Rondani) originally described by Rondani in 1877 as *Entedon basalis* parasitizing *Bruchus ciceri* in Egypt. Subsequently the name of the parasitoid was changed to *Bruchobius laticeps* by Ashmead (1904). The genus *Bruchobius* was erected by Ashmead in 1904 with *B. tateceps* from America as the type species. He described this species originally from America as a larval parasitoid of *B. quadrimaculatus*. Boucek (1974) redesigned the parasitoids as *Dinarmus basalis* (Rondani). This species has now been found to parasitism the larvae-pupae of different bruchids occurring in a number of warmer countries of the world including Bangladesh.

D. basalis is one of the dominant ectoparasitoid of *C. chinensis* in Bangladesh. It's body is elongated with metallic black colouration. The head appears black except the eyes and mandibles. Eyes are deep red. The scope, pedicel and the ring segments of the antennae, maxillary palpi, the labial palpi, and the tibia and tarsus are pale yellow. The head is hypognathous. The male insects are much smaller and less common compared to the females.

D. basalis lays egg on the body surface of the mature larvae, pre-pupae and pupae of *C. chinensis* which live within the seeds of *Vigna radiata* (L.) Wilczek. The female penetrates its sharp and pointed ovipositor through the surface of the seed coat and places an egg on the external surface of the host. After oviposition, the female withdraws the ovipositor from the seed and again inserts the ovipositor to form feeding tube extending from the surface of the host to outside the seed. She feeds on the host's fluid that oozes out through the feeding tube. It is believed that this food significantly increases the number of mature eggs in the ovaries of the parasitoid.

Eggs are Hymenopter form, dirty white in colour, smooth, elongate and rounded at both ends. There are three larval instars. The newly hatched larvae are smooth, translucent and dirty white. The larval body consists of 13 segments with different setae. One pre-pupa and pupa are recognized in the life history of *D. basalis*. The pupa is exarate. The fresh pupa appears white and become black in about 30 hours. The mean developmental time from oviposition to adult emergence at 30 ± 0.50C and 70% RH found to be 284.0 ± 1.41 whereas as 308.14 ± 1.02 h in case of female. The males of this species always emerged 24 h before the female. The detailed biology of this parasitoid described by Chatterji (1954), Islam (1995).

Chemical insecticides

With a greater awareness of the hazards associated with the use of synthetic organic insecticides, there has been an increased need to explore suitable

alternative methods of pest control. All insecticides are poisons, and the degree of toxicity varies greatly among them. Insecticide's mode of actions involve all the anatomical, physiological and biochemical responses to the chemical poison. Moreover, the fat present in the organism also undergoes reaction with the treated substance. It normally blocks metabolic processes in insects, but this is done in different ways by different compounds. According to their mode of action, the major groups of the most frequently used insecticides are i) nerve poison, ii) muscle poison and iii) physical toxicants (Pedigo 1996).

The synthetic insecticides which have widely been used all over the world during the past to control pests are chlorinated hydrocarbons, organophosphate compounds, carbamates, pyrethroids, etc. (Busvine 1971, WHO 1984, Lim and Visvalingam 1990, Setakana and Tan 1991, Curtis 1994; Kumar 1986). The most commonly used insecticides are DDT, dieldrin, lindane, chlordane, heptachlor, Malathion, fenitrothion, parathion, dichlovos and charbaril (WHO, 1984, 1992, 1995).

The application of synthetic pesticides to control agricultural pests has been a standard practice. However, with the growing evidence regarding detrimental effects of many of the conventional pesticides safer means of pest management has become very crucial. The loss of food grain during storage due to various insect pests is a very serious problem. More than 2000 species of field and storage pests annually destroy approximately one third of world's food production, valued at more than US $ 100 billion, among which highest losses (43% potential production) occur in developing Asian countries (Ahmed and Grainge 1986). Annual post-harvest losses resulting from insect damage, microbial deterioration and other factors are estimated to be 10-25% of worldwide production (Matthews 1993). Climate and storage conditions, especially in tropics, are often highly favorable for insect growth and development; control of these insects by chemical insecticides has then serious drawbacks (Sharaby 1988). The unsystematic use of chemical pesticides have given rise to many obvious serious problems, including

genetic resistance by pest species, toxic residues, increasing costs of application, environmental pollution, hazards from handling, etc. (Champ and Dyte 1976, Ahmed *et al.* 1981, Khanam *et al.* 1990, Pacheco *et al.* 1990, Sartori *et al.* 1990, Rajendran and Narasimhan 1994, Subramanyam and Hangstrum 1995, White and Leesch 1995, Jembere *et al.* 1995, Okonkwo and Okoye 1996).

Use of pesticides is often considered to be the most effective control technology for pests. But continuous of heavy use of some pesticides have created serious problems arising from factors, such as, direct toxicity to parasites, predators, pollinators, fish and man (Munakata 1977, Pimental 1981), pesticide resistance (Brown 1968, Georgiou and Taylor 1977, Schmutterer 1981, Waiss *et al.* 1981), and susceptibility of crop plants to insect pests (Pimental *et al.* 1980). Resistance to one or more pesticides have been reported in at least 477 species of insects and mites (Georghiou and Mellon 1983). Moreover, cross and multi-resistant strains in many important insect species have also been reported (Dyte 1970, Dyte and Holliday 1985, Irshad and Gillani 1990, Zettler 1991).

Plant products

Safe and inexpensive insecticides coupled with simple application methods are needed at the rural level (Periera and Wohlgemuth 1982). In many areas of the world locally available materials are widely used to protect stored products (Golob and Webley 1980). In the near past the search for naturally occurring antifeedants against pests of field crops and storage has been intensified (Islam 1983). A number of investigators isolated, identified and screened chemical compounds from different parts of many botanical families for insect feeding deterrence and growth inhibitor (Berney's and Chapman 1977, Doskotch *et al.* 1977, Jacobson 1977, Carpentier *et al.* 1979, Warthen 1979, Jurd and Manners 1980, Menn 1980).

Renewed interest in botanical pest control agents are motivated by three major objectives: i) to encourage traditional use of simple formulation of

locally available plant materials by farmers who can not afford commercial insecticides; ii) to identify sources of new botanical pesticides for commercial extraction; and iii) to elucidate the chemical structure of active principles. Botanical pest control agents extracted on large scale may also be used to replace for supplement the activity of existing synthetic pesticides against refractory pests. Structural elucidation of the active constituents may provide further inside into structure- activity relationships. Novel metabolites identified may serve as models for chemical synthesis of new pesticides with more desirable properties. The general pathway of the whole work is given in the Figs. 1 and 2; however the present work could be ahead of purification and pure compound bioassay. To trace the lead components for their further use to enhance the quality of human food it is necessary to go through several steps given in the following manner.

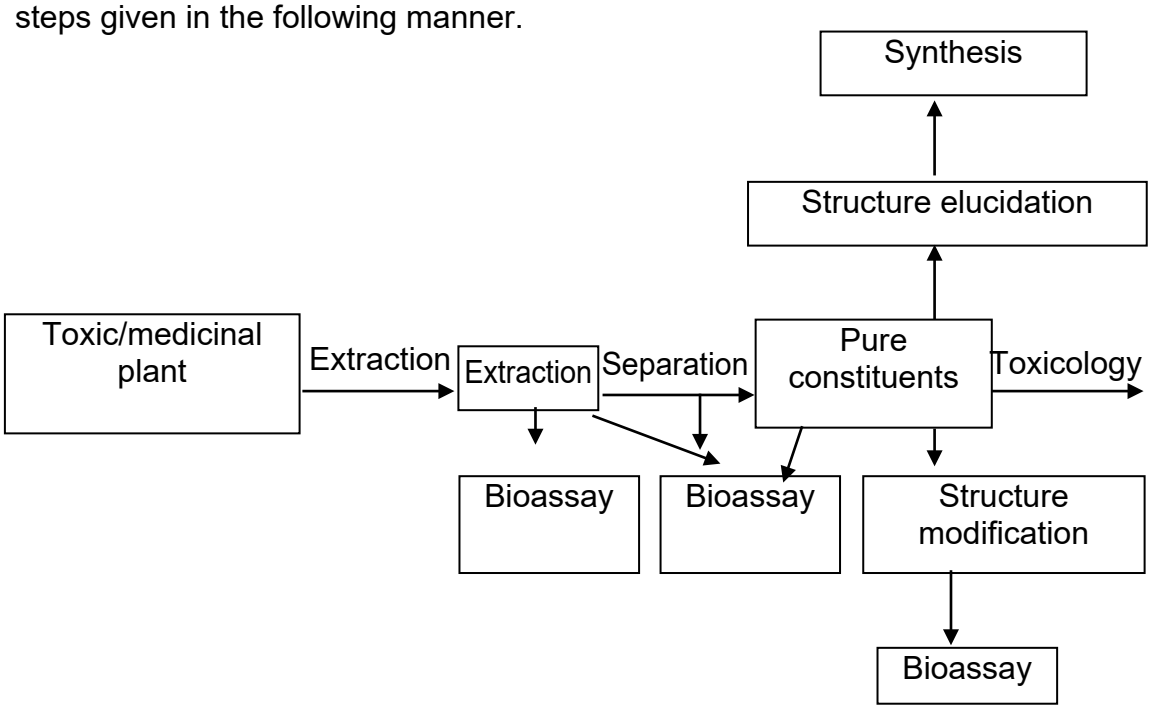


Fig. 1: The basic pathway from the plant to the bioactive constituents (Hostettmann 1995).

In recent years bioactive principles from natural origin have been subjected to investigation for pest control agents as well as for remedies of diseases without residual or side-effects. In fact, plant species is a vast repository of chemical substances that protect plants from attacks by phytophagous insects. Some of these chemicals may repel or kill the insects or deter them from feeding,

oviposition and reproduction. These properties of plants are of a great value in protecting stored commodities from insect infestation (Munakata 1977).

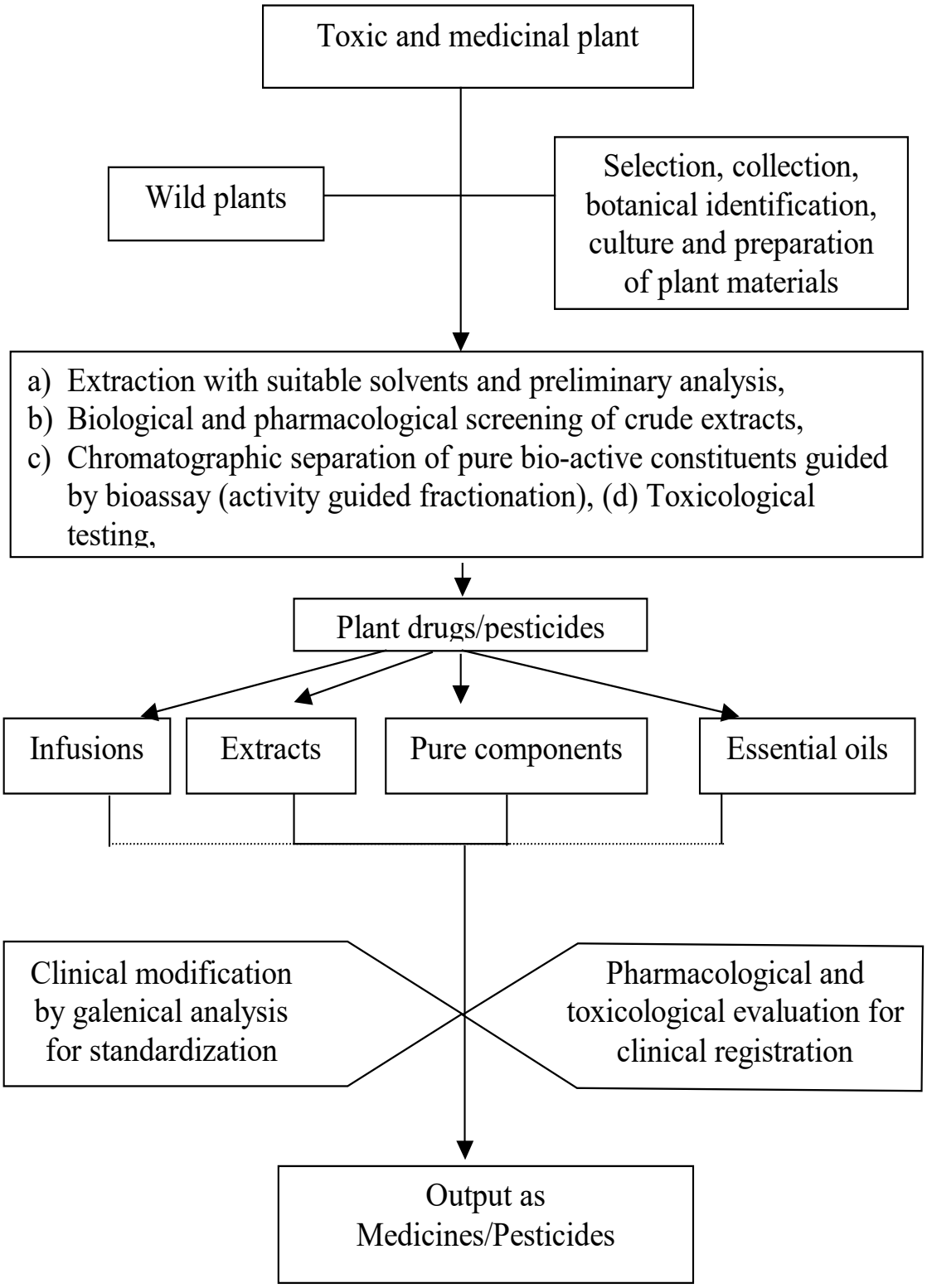


Fig. 2: Phytochemical investigation towards the outputs (Hostettmann *et al.* 1995).

Until now only a small part of the plant kingdom (estimated at 2,50,000 - 5,00,000 species around the globe) has been investigated phytochemically and the fraction subjected to biological and pharmacological screening is even lower. Amongst the most promising of the natural products investigated to date are metabolites. Although only about 10,000 secondary plant metabolites have been chemically identified, the total number of plant chemicals may exceed 4, 00,000. They are a vast commucopia of defense chemicals, comprising repellents, feeding and oviposition deterrents, growth inhibitors, sterilant, toxicant, etc. (Champagne *et al.* 1989).

However, farmers have been using plant extracts in pest control for centuries. Botanical compounds are a rich and promising source of new and alternative chemicals that may help replace synthetic insecticides for grain protection (Prakash and Rao 1997). Many plant-derived chemicals are insecticidal to stored-product pests (Jacobson 1989, Golob *et al*. 1999, Weaver and Subramanyam 2000). Azadirachtin from the Indian neem tree (*Azadirachta indica* A. Juss., Meliaceae) (Saxena *et al*. 1988, Jilani and Saxena 1990) and pyrethrum from chrysanthemums (Prakash and Rao 1997). This method of pest control provides an ideal source of low cost, safe and effective pesticides. Extracts of plant material rely on the solubility of the active components and it may cause repellent to insects (Sighamony *et al.* 1984), antifeedant or other type of bioactivities against insects (Jayasinghe and Fujimoto 1990, Morallo-Rejesus *et al.* 1990, Adalla *et al.* 1993, Facknath and Kawol 1993, Morallo-Rejesus *et al.* 1993, Kim *et al.* 1994, Naumann *et al.* 1994, Niber 1994, Rajuraman and Saxena 1994, Braver man and Chizov-Ginzburg 1997, Ho *et al.* 1997, El-Lakwah and Abdel-Latif 1998, Hermawan *et al.* 1998, Ndungu *et al.* 1999, Jannet *et al.* 2000).

Some plant families may accumulate a restricted number of anti-insect chemicals, so-called secondary metabolites, whilst other possesses a wide variety of different structural compounds. Secondary metabolites from plants include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other

minor chemicals can affect insects' life in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or an agent to modify oviposition. Essential oils and especially their main compounds monoterpenoids offer promising alternatives to classical fumigants (Papachristos and Stamopoulos 2003). These compounds act as fumigants against stored product insects (Liu and Ho 1999, Huang *et al*. 2000a, Kim and Ahn 2001, Papachristos and Stamopoulos 2002a), contact insecticides (Huang *et al*. 1997, 1999b, 2000b; Huang and Ho 1998, Chun *et al*. 2000, Tripathi *et al*. 2000, Tunç *et al*. 2000, Papachristos and Stamopoulos 2002a), antifeedant or repellent effects (Chiam *et al*. 1999, Kim *et al.* 2003a, b; Park *et al.* 2003a,b) and may also affect some biological parameters, such as growth rate, life span and reproduction (Regnault-Roger and Hamraoui 1995, Subramanian and Krishna 1996, Pascual-Villalobos 1996).

At the present time there are a number of botanical insecticides being marketed, which are extracted from neem, grapefruit seeds and garlic, among other plants. Besides, there are the synthetic copies of natural active ingredients like neo-nicotinoids where imidacloprid stands out. The use of plants as repellents is very old but has not received the necessary attention for proper development.

Bangladesh has a great treasure of promising plants. More than 500 plants are available in Bangladesh which have been reported to possess medicinal properties of some description or other and have been enumerated in the literature of indigenous drugs (Ghani 1998).

The various medicinal plant species included *Barleria* sp. (Acanthaceae), *rinum* sp. (Amaryllidaceae), *Annona squamosa* (Annonaceae), *Holarrhena antidysenterica* (Apocynaceae), *Bombax ceiba* (Bombacaceae), *Canna indica* (Cannaceae), *Wedelia chinensis* (Compositae), *Ipomoea mauritiana* (Convolvulaceae), *Cycas* sp. (Cycadaceae), *Ephedra* sp. Ephedraceae), *Adenanthera pavonina* (Leguminosae), *Cajanus cajan* (Leguminosae), *Mucuna pruriens* (Leguminosae), *Urena lobata* (Malvaceae), *Cocculus*

hirsutus (Menispermaceae), *Stephania japonica* (Menispermaceae), *Piper longum* (Piperaceae), *Glycosmis pentaphylla* (Rutaceae), *Murraya koenigii* (Rutaceae), *Tectona grandis* (Verbenaceae), and *Curcuma zedoaria* (Zingiberaceae).

Adenanthera pavonina L. is a woody Southeast Asian species of Leguminosae- Mimosoideae. It belongs to wild under exploited leguminous crop seeds and may be a potential source of feed for livestock and industrial uses (Balogun and Fetuga 1986). Workers dealt with this plant given emphasis mostly on chemical properties and their medicinal profile but a very few of them given touch on agricultural context. The present investigation has been designed to evaluate the efficacy of the plant parts as a possible source of biologically active secondary metabolites. Re-evaluation of potentiality of the extract of this plant in many projections in consideration of their versatile activity and for the possibility of their various uses in the environmentally friendly pest control sector of the contemporary Bangladesh. For this reason different parts of this plant, i.e. leaves, seed and stem wood of *A. pavonina* have been subjected to biological screening for their possible use in the modern pest control strategy.

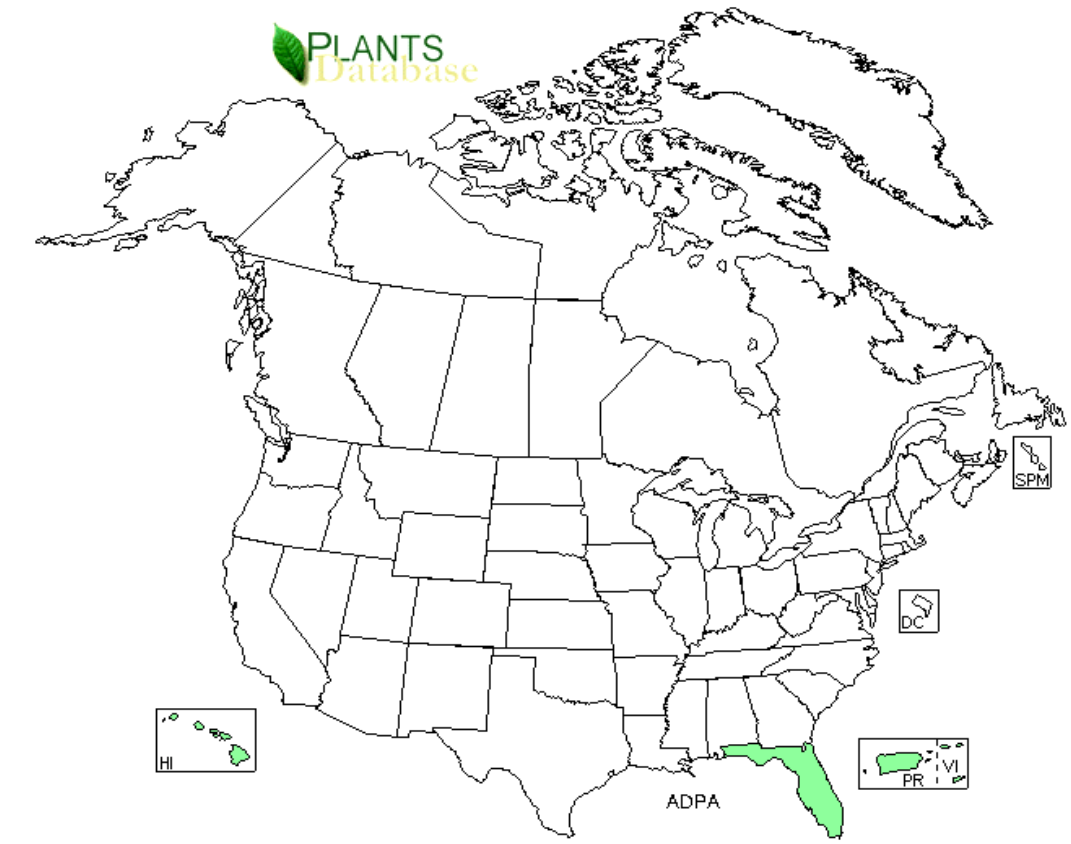
1.2. Back ground information on the test plant

1.2.1. Systemic position and distribution of *A. pavonina*

Adenanthera pavonina (L.), a genous of trees belonging to the (family Leguminosae, subfamily Mimosoideae) comprising some species, is an important tree in Southeast Asia and the Pacific islands. The scientific name is derived from a combination of the Greek *Aden*, "a gland," and *anthera*, "anther"; alluding to the anthers being tipped with a deciduous gland. Fabaceae or Leguminosae is a large and economically important family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. The name 'Fabaceae' comes from the defunct genus *Faba*, now included into *Vicia*. Leguminosae is an older name still considered valid, and refers to the typical fruit of these plants, which are called legumes. View over 100 Characteristics of 2,500 conservation plant

species and cultivars including appearance, use in conservation and restoration, growth requirements, and suitability for NRCS practices. It is the third largest family of flowering plants behind Orchidaceae and Asteraceae with 730 genera and over 19,400 species, according to the Royal Botanic Gardens 233 species are found in Bangladesh (Nasir and Ali 1977). *A. pavonina* is endemic to Southeast China and India, with first reports being recorded in India, Puerto Rico, Cuba, Jamaica, Trinidad, Tobago, Venezuela, Brazil, Costa Rica, Honduras, southern Florida, Southeastern Asia and Malaysia(Fig. 3). The red lens shaped Circassian seed are curiosities with travelers and are used as necklaces and ornamentals.The tree is sometimes called peacock flower, fence or sandalwood tree. The tree produced large quantities of red seed which are used as beads. According to Mabbery (Mabbery 1990), the plant is also called coral wood, red wood, coral pea, Barbados pride. *Adenanthera pavonina* L. (Family- Fabaceae) commonly known in Bangladesh as “Rakta Kombol”, is an important medicinal plant native to tropical Asia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions(Arzumand Ara *et al*. 2010).

The tree has been introduced throughout the humid tropics. It has been planted as a shade and ornamental tree in urban centers and gardens (Food and Agriculture Organization 1957). It has become naturalized in Chittagong, Chittagong Hill Tracts and Sylhet occasionally planted. It has several synonyms, i.e. *Adenanthera gersenii* Scheffer, *Adenanthera polita* Miq. The local names of the title species are Bengali: Raktakambal, Ranjan, Rakta Kanchan etc. The common English name of the tree is Red Sandalwood.



Region where *A. pavonina* grows in the world

Fig. 3. Geographical distribution of *A. pavonina*

1.2.2. **Morphological attributes and systemic position of *A. pavonina***

A. pavonina is a deciduous tree that reaches 60 m in height and up to 45 cm. The trunk is basically straight with smooth bark and many fissures. The spreading crown has relatively few leaves. The leaves are bipinnate and 30 to 60 cm long with numerous oblong leaflets that are rounded on both ends and have a small point at the apex.The tree blooms from the end of summer until winter. The flowers are pale yellow to orange and arranged in racemose inflorescences. The fruits (legumes) ripen in the fall or winter of the same year (Little and Wadsworth 1964). The seeds are ovate to elliptic-lenticular,

laterally flattened, biconvex in cross section and the seed coat is scarlet red to coral red, smooth, shiny, bony, and very hard and generally has no fracture lines.

Adenanthera pavonina L. is common throughout the lowland tropics up to 300-400 m. *A. pavonina* is a secondary forest tree favoring precipitation ranging between 3000-5000 mm for optimal growth. The tree is generally erect, having dark brown to grayish bark, and a spreading crown (Plate 2). Multiple stems are common, as are slightly buttressed trunks in older trees. The leaves are bipinnate with 2-6 opposite pairs of pinnate, oval-oblong with an asymmetric base and a blunt apex, being a dull green color on top and a blue-green beneath. The leaves yellow with age. Flowers are borne in narrow spike-like racemes. They are small, creamy-yellow in color, and fragrant. Each flower is star-shaped with five petals, connate at the base, and having 10 prominent stamens bearing anthers tipped with minute glands. The curved pods are long and narrow, 15-22 cm by 2 cm, with slight constrictions between seeds, and dark brown in color turning black upon ripening. Medium sized, deciduous tree; leaves with 2-6 pairs of pinnae; leaflets 6-12 pairs, 2-5 cm long, ovate, obtuse, glabrous, rather pale green, thin; flowers in slender, elongate racemes, borne in axils; flowers yellowish, sweet-fragrant; pods slender, linear, curved or contorted, dehiscent, thin, flattened, brown, smooth and shiny inside; seeds lenticular, suborbicular, hard, dark or bright red (Stone 1970).

Plate2: *Adenanthera pavonina*

The leathery pods curve and twist upon dehiscence to reveal the 8-12 showy seeds characteristic of this species. The hard-coated seeds, 7.5-9.0 mm in diameter, are lens-shaped, vivid scarlet in color, and adhere to the pods. The ripened pods remain on the tree for long periods and may persist until the following spring. There are reportedly 1600 seeds per pound (Little and Wadsworth 1964).

1.2.3. General components of *A. pavonina*

Investigation of bioactive compounds from plants for their possible use as ingredients of medicine and pesticides have been a history of decades, and a number of potential sources have also been traced so far. Previous phytochemical investigation of the plant reported the presence of robinetin, chalcone, butin and flavanol ampelopsin, stigmasterol glucosides, oleanolic acid, echinocystic acid, sapogenins and many other bioactive phyto-constituents (Yadev *et al*. 1976, Yeoh *et al*. 1984, Chandra *et al*. 1982, Mesbah *et al*. 2002, Shaiq *et al*. 2005, Enuo and Shishan 2007, Adedapo *et al*. 2009). The analgesic, anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxic and blood pressure reducing activities of the leaf and seed extracts and its isolated compounds have been reported (Nigam *et al*. 1973, Neerja *et al*. 1976 Adedapo *et al*. 2009, Rodrigo *et al*. 2007, Jayasinghe *et al*. 2006, Olajide *et al*. 2004, Mayuren *et al*. 2009). Phytochemical screening of *A. pavonina* was carried out by (Kothale and Rothe 2012) and they reported that the phytochemical screening of leaf and stem in various extracts i.e. petroleum ether, benzene, chloroform, acetone, ethanol, methanol, rectified spirit and water showed the presence of alkaloids, carbohydrate, proteins, tannins, saponin anthraquinone glycosides, cardiac glycosides, flavonoides and phenolic compounds, quinone, steroids. The majority of phytoconstituents are found in acetone, ethanol, methanol, rectified spirit and water extracts. Steroids present in the leaves and stem but not traced in petroleum ether, benzene, and chloroform. Coumarin test found weak presence only traced in the acetone in stem and leaf also. Saponin found positively in leaf in ethanol, methanol and water only. Stem extracts had

shown negative results. Quinone present in stem and leaf also in acetone, ethanol, methanol, rectified spirit extracts while, fixed oil and fats are totally absent in all extracts of stem and leaf. *β*- Resorcylic acid (2, 4-dihydroxybenzoic acid) is found in red sandalwood (*Pterocarpus santalinus*) and the related coral wood (*Adenanthera pavonina*). *β*- Resorcylic acid has thyroid peroxidase inhibitory effect (Haschek *et al*. 2010).

Bark contains saponins which on hydrolysis and methylation yield Meodeanolate and Me-echynocystate, stigmasterol, glucoside and fatty acids, oleanoic and echinocystic acids. Leaves contain alkaloid, octacosanol, dulcitol, ß-sitosterol glucoside and stigmasterol. Seeds contain HCN-glucoside, lignoceric acid, dulcitol, stigmasterol, stigmasterol glucoside and polysaccharide (Ghani 2003).

To our best knowledge, there have been very little studies published previously that examined chemicals accumulated in *A. pavonina* seeds. Those investigations led only to the isolation of a few protein trypsin inhibitors (Prabhu and Pattabiraman 1980, Richardson *et al.* 1986, Lam *et al.* 1999), flavonoids (Gennaro *et al.* 1972), fatty acids (FAs) (Kabele-Ngiefu *et al.* 1975, Balogun and Fetuga 1985, Sotheeswaran *et al.* 1994), triterpenoids (Yadav *et al.* 1976) and carbohydrates (Moreira *et al.* 1998).

1.2.4. Social utilities and folk medicinal use of the titled plant

Plant kingdom has always acted as a source of medication in all healing tradition of the world. Traditionally it had been used to treat many diseases such as asthma, boil, diarrhoea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic (Arzum and Ara *et al*. 2010, Burkil 1994, Ghani 2003). Several parts of the plant have been verified for its medicinal important hence, the bark and leaves are astringent, vulnerary, anthelmintic and aphrodisiac and are used in gonorrhea, ulcers, pharyngopathy, vitiated conditions of vata and gout and rheumatism. The seeds are bitter, astringent, sweet, cooling, aphrodisiac, anti-emetic and febrifuge. The roots are reported to be emetic in

nature (Hussain *et al*. 2010, Dash *et al*. 2010). The analgesic, anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxic and blood pressure reducing activities of the leaf and seed extracts and its isolated compounds have been reported (Adedapo *et al*. 2009, Rodrigo *et al.* 2007, Jayasinghe *et al*. 2006, Olajide *et al*. 2004, Mayuren and Ilavarasan 2009).

The seeds of *A. pavonina* mainly valued for the oil obtained from them, which has many industrial and medicinal uses. Seeds considered to have toxic properties; when cooked, edible and rich in fats and proteins. Raw seeds may be eaten. When cooked, it is easily digestible by humans and livestock, and is rich in fats and proteins. Seeds of *A. pavonina* contain appreciable amounts of proteins, crude fat and minerals comparable to commonly consumed staples. Seeds also represent a potential source of oil and protein that can alleviate shortages (Ezeagu *et al.* 2004).

Plate 3: Leaves and fruits with *A. pavonina*

1.2.5. Combined effect of plant extracts and parasitoid (*D. basalis*) against *C. chinensis* under laboratory conditions

Alternatives to traditional chemical insecticides such as predators, parasitoids, microbes and natural products have been gaining interest among researchers

concerned with developing Integrated Pest Management (IPM) approaches for insect control (Copping and Menn 2000). However, only a few of these alternative methods, such as pyrethrum- and *Bacillus thuringiensis* (Bt)-based products, have been commercially successful in the pesticide market. For several years, the entomologist has been investigating a variety of alternatives to conventional chemical pesticides in stored-product IPM. Controlling insect pests in stored grain and grain products can be very difficult because of the variety of species that can infest grain. Insect parasitoids have been shown to be effective in suppressing a limited number of pest species both in bulk grain storages and in food-processing facilities and warehouses (Scholler and Flinn 2000). One of the more effective parasitoids is *Dinarmus basalis* (Rond.), a small pteromalid wasp that attacks primary pulse pests, whose immature stages develop inside pulses, including *Callosobruchus* sp. (Islam *et al*. 1985, Islam *et al*. 2003). The present investigation identify a biopesticide that might be used to supplement the species-specific activity of the parasitoid, which, when used in combination with the parasitoid, would protect stored pulses from infested pests, and at the same time be non-toxic to the beneficial parasitoid species.

There are many indigenous plants that occur in nature. These plants play a major role in all the traditional system of medicine. Plants contain the rich source of natural products like vitamins, minerals and other immune modulators. Most of which have been used for human welfare specially to cure disease caused by pathogenic microorganisms. Some of them are extracted in different chemical solvents and are used for potential biopesticide for stored-product protection (Islam and Khalequzzaman 1992, Malek and Wilkins 1995, Talukder and Howse 1994, Huang *et al*. 2000, Kim *et al*. 2003a,b, Park *et al*. 2003a,b and Yeasmin *et al*. 2011). Some of the plant extracts could be used in combination with a parasitoid to control pulse pests (Haines 1984, van Huis *et al*. 1990a,b, Chun *et al*. 1992, Ouedraogo *et al*. 1996, Sanon *et al*. 1998, van Huis and de Rooy 1998, Islam and Khan 2000).

The release of insect parasitoids and predators into warehouse situations to suppress or eliminate residual or active populations of various stored-product insect has been studied by several workers (Le Cato *et al*. 1977, Press *et al*. 1975). Although many parasitoids and predators give excellent control in a small, confined environment, many questions need to be answered regarding warehouse releases (Cline *et al*. 1983).

Control of insects in storage facilities is essential for farmers, warehouse men, grain elevator operators and others. Many infestations of stored-grain originate from insects already present in the storage facilities. Elimination of these residual population, therefore, is a major objective in controlling stored-product insects (Press et al. 1984). The pulse beetle, *C. chinensis* is a major cosmopolitan pest of stored pulses often found in residual pulse debris and subsequently may build up huge populations.
D. basalis is a parasitoid of *C. chinensis* in larval, pre-pupal and pupal stages of the host. Very little information is, however, available for the suppressive effect of *D. basalis* on the population of *C. chinensis*, through combined with plant extracts. Monge and Huignard (1991) studied the population fluctuations of *C. maculatus* and its parasitoid *D. basalis* under storage in Niger, and Verma (1990) observed the host habitat location and host location by *D. basalis* of stored legumes in India. The purpose of the present study was to evaluate:

(i) The effect of plant extracts on parasitoid

(ii) The combined effect of plant extracts and parasitoid in reducing or eliminating *C. chinensis* infestations in laboratories.

1.3. Aim of this work

Preliminary tests showed that *A. pavonina* plant extracts did not affected *D. basalis*. Therefore, the present study hypothesized that the parasitoid could be used in combination with plant extracts, because the parasitoid attack the immature stages and the plant extracts is toxic to the adults, This hypothesis

was tested by exposing the parasitoid to plant extracts and evaluating the efficacy of the combination on the reduction of *C. chinensis* populations in a small-scale experiment with the following objectives to fulfill the need for safe stored pulses from infestation of insect pests.

1.4. Objectives of the present work

i. to screen the efficiency of *A. pavonina* plant extract for insecticidal potential against *C. chinensis* through dose-mortality bioassay;

ii. to determine the feeding deterrence and repellency as well as special efficacy against *C. chinensis* adults;

iii. to test the efficacy of the leaf, stem wood and seed extracts of *A. pavonina* as anti microbial assay and brine shrimp lethality assay and

iv. to assess the combined effect of plant extracts and parasitoid (*D. basalis*) against *C. chinensis* under storage condition.

Chapter 2

General Materials and Methods

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2.1. Selection of test organisms

Crucial to any investigation of plants with biological activities is the availability of suitable bioassays for monitoring the required effects. The test systems should ideally be simple, rapid, reproducible, and inexpensive. If active principles are only present at low concentration in the crude extract then bioassay should be high enough sensitive for their detection. Another factor of special relevance to plant extracts is the solubility of the sample, and finding a suitable system can pose problems.

The bioassays were carry on tests for insecticidal, larvicidal activities, and also for repellent potentials of the extractives of *A. pavonina, C. chinensis* (L.) was selected, because they are easily culture in the laboratory. For cytotoxicity test *Artemia salina* was selected, since it is being used in such cases as a model test agent. A number of bacteria and fungi were selected to carry out further efficiency tests of the extractives.

2.1.1. Collection and Culture of *C. chinensis*

Adult beetles of *C. chinensis* used were collected from the stock cultures in the Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh during April-October, 2012. The test insect was obtained from the separate culture raised from a single pair of *C. chinensis* on green gram seeds. For conducting studies on biology of the insect, three plastic containers (5 x 5 cm) containing 100 gm seeds were taken in with freshly emerged single pair of *C. chinensis* which was released in each of the three containers. Identification of the sexes was made by employing the method of Raina (1970). Mouth of the containers was

covered by muslin cloth and secured with rubber bands and later maintained in ambient laboratory conditions.

Adults were removed from these containers after death and total number of eggs laid by a single female on host grains was recorded. Eggs laid each day were kept in separate containers covered with muslin cloth and observations were recorded on incubation, developmental (larval and pupal) and total developmental period. Observations on incubation, larval and pupal period inside the grain were recorded by breaking the whole grain with the help of the needle and observing the stage of insect with the help of magnifying glass. The rearing of beetles was continued till the completion of sixth generation and adult longevity was recorded in respect of each generation. The culture of pulse beetle *C. chinensis* was reared in laboratory in pre-sterilized jars containing cowpea seeds, *Vigna radiata* L. Wilczek. This culture was kept at a temperature of 30± 0.5^0 C and a relative humidity of 70± 0.5 % (Plate 4 and 5).

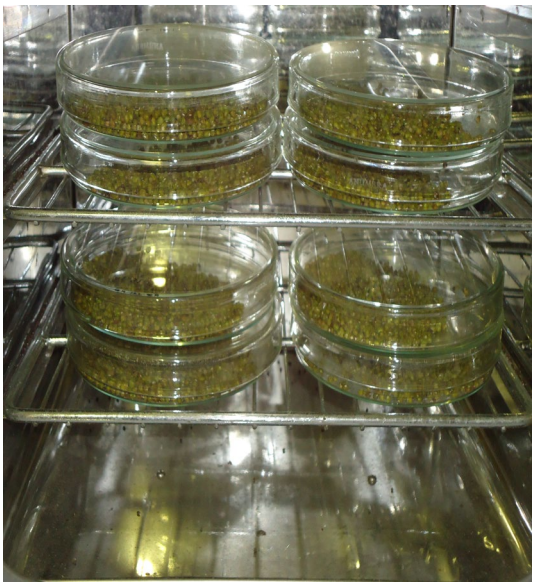

Plate4: Cultures of *C. chinensis* in an incubator.

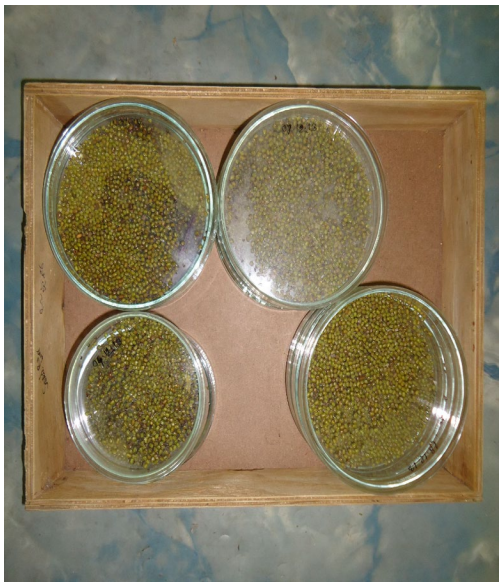

Plate5: *C. chinensis* under natural conditions.

2.1.1.1. Collection of adults

A huge number of beetles were reared to get a regular supply of the newly formed adults. When sufficient adults produced in the sub-cultures, they were collected from the food medium. For this purpose some pieces of filter paper were kept inside the beaker on the food. Adults crawled upon the paper and then the paper was taken out with a set forceps. Beetles were then collected in a small beaker (100 ml) with the help of a fine brush.

2.1.2. Collection and culture of brine shrimp nauplii for cytotoxicity test

There are many species within the genus of *Anostraca*, but the *A. salina* are very nice to grow, since the rate of successful hatches is very high. To conduct cytotoxicity test the brine shrimp nauplii were used because of its easy hatching and easy to use in the experiment. The eggs (cysts) were collected from aquarium shops. For their easy hatching and use the requirements were as follows:

- Salt water: 1.5 - 3 tablespoons of marine salt was added to 1 liter of pond water;
- Temperature: 26-28ºC (80-82ºF);
- Light: The beaker was placed near a window with sunlight;
- Aeration: Picking up some water carefully with a spoon and let it drop back to the beaker at least twice a day;
- Special attention: Brine shrimp eggs are sometimes very buoyant. Swirling of the water was done to knock down the eggs;

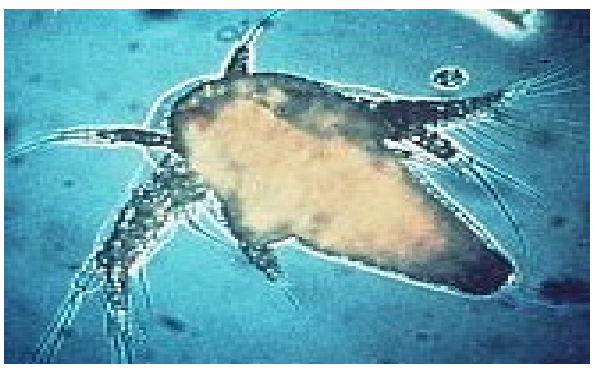

Plate 6: *Artemia salina* (Brine shrimp) nauplii

The cysts absorbed water and hatched after 24-48 hours, depending on their environment. Freshly hatched *A. salina* called nauplii and have a size of just 0.25mm (0.01inch). They molt like any other crawfish. When they grow to adult they molt about 17 times. Freshly hatched nauplii were used in this experiment.

2.1.3. Selection of microorganisms as test agents

The antimicrobial screening of a crude extract or a pure compound isolated from natural sources is essential to ascertain its activity against various types of microorganisms. It can be measured *in vitro* by a number of techniques among which the discs diffusion method (Bauer *et al*. 1966), is widely acceptable for the preliminary evaluation of antibacterial activity. Disc diffusion technique is essentially a qualitative or semi quantitative test indicating the sensitivity or resistance of microorganism to the test material. However, no distinction between bacteriostatic and bactericidal activity can be made by this method (Roland 1982).

2.1.3.1. Test agents for antibacterial activity

Antimicrobial activity of any plant or parts of a plant can be detected by observing the growth response of various microorganisms to the extracts of a plant or parts of a plant, which is placed in contact with them. Fifteen pathogenic bacterial isolates were selected for the test, 6 of which were gram positive and the remaining 9 were gram negative (Table 1).

Table 1: List of the pathogenic bacteria used in this investigation

Serial No.	Name of test organism	Strain #
Gram positive		
1.	*Staphylococcus aureus*	ATCC-259233
2.	*Bacillus cereus*	-
3.	*Bacillus megaterium*	QL-38
4.	*Bacillus subtilis*	QL-40
5.	*Sarcina lutea*	-
6.	*Streptococcus β-haemolyticus*	CRL

Gram negative		
7.	*Salmonella typhi*	-
8.	*Shigella dysenteriae*	AL-35587
9.	*Shigella shiga*	-
10.	*Shigella sonnei*	AJ-8992
11.	*Shigella boydii*	AL-17313
12.	*Escherichia coli*	FPFC-1407
13.	*Klebsiella* sp.	-
14.	*Pseudomonas aeruginosa*	-
15.	*Proteus* sp.	-

2.1.3.1.1. Collection and culture of test bacteria

These organisms of pure culture were primarily collected from the Department of Microbiology, University of Dhaka; Institute of Nutrition and Food Science (INFS), University of Dhaka and the Plant Pathology Laboratory of the Department of Botany, University of Rajshahi, and were further cultured at the Molecular Biology Laboratory, Institute of Biological Sciences, University of Rajshahi.

Culture media

A number of culture media are available to use in the demonstration of antibacterial activity of the test substances. These are:

i) Nutrient agar medium
ii) Nutrient broth medium
iii) Mueller-Hinton medium
iv) Tryptic Soy broth (TSB) medium
v) Trypticase Soy agar medium
vi) Staphylococcus defined medium
vii) Adams and Roe medium
viii) NTH agar or broth medium

While the nutrient agar medium was adopted to conduct experiments in this investigation.

The list of the composition of nutrient agar medium

Ingredient	Amount
Bactopeptone	0.5 gm
Sodium chloride	0.5 gm
Bactoyeast extract	1.0 gm
Bactoagar	2.0 gm
Distilled water	100 ml
pH	7.2 ± 0. 1 at 25°C

Preparation of the nutrient agar (DIFCO) medium

The instant nutrient agar (DIFCO) medium was weighed and then reconstituted with distilled water in a conical flask according to specification measurement (2.3% W/V). It was then heated in a water bath to dissolve the agar until a transparent solution was obtained.

Preparation of fresh culture of the pathogenic organisms

The nutrient agar medium was prepared and dispersed in a number of clean test tubes to prepare slants (5 ml in each test tube). The test tubes were plugged with cotton and sterilized in an autoclave at 121°C and 15 Ibs/sq. inch pressure for 15 minutes. After sterilization, the test tubes were kept in an inclined position for solidification. These were then incubated at 37.5°C to ensure sterilization. The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculating loop in an aseptic condition. The loop was burnt after each transfer of microorganisms to avoid contamination very carefully. The inoculated slants were then incubated at 37.5°C for 24 hours to assure the growth of test organisms. These fresh cultures were used for the sensitivity tests.

Selection of test method

The primary assay can be done in three ways, such as-

(a) Diffusion method;
(b) Dilution method; and
(c) Bioautographic method.

However, the diffusion method was used in this investigation.

Principles of the diffusion method

Diffusion assay (Barry 1976) is based on the ability of antibiotics to diffuse from a confined source through the nutrient agar gel and create a concentration gradient. If the agar is seeded or streaked with a sensitive organism, a zone of inhibition will result where the concentration exceeds the minimum inhibitory concentration (MIC) for the particular organism. In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions the known concentrations (µg/ml). The sterile (BBL, Cocksville, USA) filter paper (5mm diam.) discs were impregnated with known amounts of the test substances and dried. These test material discs were placed on plates containing nutrient agar medium seeded with the test organisms. These plates were kept at a low temperature (40^0C) for 24 hours to allow maximum diffusion. A number of events took place on the discs simultaneously that includes-

i) The dried discs absorb water from the agar medium and the material under test is dissolved.

ii) The test material diffuses from the discs to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel.

iii) There is a gradual change of test material concentration in the agar surrounding each disc.

To determine the most optimal concentration of extracts to be used in this study, sterile 7.5 mm filter paper discs were treated with 50 and 200 µl of the chloroform and methanol extracts (while the only solvents were used as control). The bacteria were inoculated on full–strength nutrient agar (Qualigens Fine Chemicals, Prod # 58673) by suspending loops in sterile de-ionized water. The bacterial suspension was then smeared on agar plates with a sterile glass-rod to ensure the entire surface of the agar had an even coating of the bacterial suspension. The test plates were divided into several areas and one filter paper disk was placed on each of the areas. The plates are then kept in an incubator (37ºC) for 12-18 h to allow the growth of the organisms. If any of the test materials has antimicrobial activity, it will inhibit

the growth of microorganisms just giving a clear distinct zone called 'zone of inhibition'. Biological activity of the *A. pavonia* components on bacterial growth was quantified in this way by measuring the diameter of zones of inhibition (in term of mm) deducing the size of the treated filter paper discs. The size of the inhibitory zone depends principally on the following factors-

i) Intrinsic antimicrobial sensitivity of the test sample
ii) Growth rate of the test microorganisms
iii) Diffusion rate of the freshly seeded test organisms
iv) Concentration of the freshly seeded test organisms
v) Amount of test sample on disc
vi) Thickness of the test medium in the Petri dishes
vii) Composition of the culture medium
viii) Size of inoculum
ix) Time of incubation
x) Temperature of incubation

Test materials used

I) Pet. ether, acetone, chloroform and methanol extracts of *A. pavonina* parts

II) Ciprofloxacin, (30µg/disc) as standard disc

Secondary assay

The simple assay quantities the relative potency, such as Minimum Inhibitory Concentration (MIC), of the lowest concentration of an antimicrobial agent required to inhibit the growth of the microorganisms *in vitro.*

Serial #	Apparatus and reagents to conduct antibacterial assay
1.	Crude extracts of Pet. ether, acetone, chloroform and methanol
2.	Standard disc (cyprofloxacin-30µg/disc).
3.	Pet. ether, Acetone, Chloroform and methanol
4.	Alcohol (95%)
5.	Filter paper discs (Sterilized)
6.	Petri dishes (120 mm diam.)
7.	Inoculating loop
8.	Sterile cotton

9. Test tubes
10. Sterile forceps
11. Micropipette (10 µl-100 µl)
12. Nose mask and hand gloves
13. Spirit burner & match box.
14. Rectified spirit
15. Nutrient agar media(DIFCO)
16. Laminar air flow unit (Bio-craft & Scientific Industries, India)
17. Incubator (Osk-9639A, Japan)
18. Refrigerator (Artston, Italy)
19. Autoclave (ALP Co. Ltd. KT-30L, Japan)

Sterilization procedure

The antibacterial screening was carried out in a laminar air flow unit and all types of precautions were highly maintained to avoid any type of contamination during the test. UV light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. Petri dishes and other glass-wares were sterilized in the autoclave at 121°C temperature and a pressure of 15 lbs/sq. inch for 15 minutes. Micropipette tips, culture media, cotton, forceps, blank discs etc. were also sterilized.

2.1.3.2. Test agents for antifungal activity

Plant derived compounds may offer potential leads for novel agents against systemic fungal diseases (Hufford and Clark, 1988) in man and plants. Chloroform and methanol extracts of *A. pavonia* samples (Leaves, stem wood and seeds) were used in this investigation for the detection of antifungal potentials (Table 2).

Table 2: List of the pathogenic fungi used in this investigation.

Serial No.	Name of test organisms
1.	*Fusarium vasinfectum*
2.	*Aspergillus fumigatus*
3.	*Aspergillus niger*
4.	*Aspergillus flavus*
5.	*Mucor* sp.
6.	*Candida albicans*
7.	*Penicillium notatum*

2.1.3.2.1. Collection and culture of test fungi

The fungal strains used in the sensitivity tests are given above. The pure cultures of the strains were collected from the Department of Pharmacy, University of Rajshahi and cultures were maintained in the Molecular Biology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Culture media

Potato dextrose agar (PDA) media were used to perform the antifungal activity tests and for the maintenance of the subcultures of the test organisms. The composition of the medium is given below:

Composition of the PDA medium

Ingredient	Amount
Potato	20.0 gm
Dextrose	2.0 gm
Agar	1.5 gm
Distilled water	100.0 ml

Preparation of the media

The constituents of the media were accurately weighed and dispersed in a conical flask with distilled water. It was heated in water bath to dissolve the ingredients until a transparent solution was obtained. The pH of the medium was adjusted to 5.6. The volume was adjusted by adding distilled water and sterilized in an autoclave at 121°C and 15 Lbs/sq. inch pressure for 15 minutes.

2.2. Collection of plant materials

In order to arrive at useful compounds in the shortest possible time, careful selection of plant material is obviously very important. Random collection is one method but it is more judicious to base the selection on certain criteria. By way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds (Huxtable, 1992). Similarly, folk used or popularly known very common toxic plants could be taken with desirable output, and one of the rotenone producing plant, *A. pavonina* has been selected for a thorough investigation.

Plate 7: Leaf

Plate 8: Seed

Plate 9: Stem Wood

Incase of very small plants, such as herbs, shrubs, grass, etc. normally the whole plant is subjected for extraction, because the distribution of constituents generally not vary too much. The presence of constituents in the heart-wood may disappear in the leaves; similarly constituents in the roots may not be the same that present in the fruits. Being a large timber plant, the distribution of compounds in different parts of this plant is obviously different and thus different parts of *A. pavonina* viz. Leaves, stem wood and seed have been collected from the Rajshahi University Campus.

2.2.1. Chemical extraction of the plant materials

The fresh plant materials were processed through the following way-

Leaves: Leaves were spread out to dry without heaping the material together. It was done under the shade avoiding direct sunshine.

Seeds: Peeling out the fruit shells the seeds were cut into small pieces and spread out to dry under a shade.

Stem wood: After peeling out the bark the stem-wood was processed by cutting them into small pieces as thin as possible and dried under a shade.

All the plant materials were individually powdered in a grinder machine. The powdered materials were weighed and placed in separate conical flasks to add sufficient amount of chloroform (500g × 1500ml × 3 times followed by filtration through What man filter paper at 24 h interval in the same collection flask) to yield the first extracts of the Leaves, seeds and stem-wood separately (Plate 10). The output extracts were poured in to glass vials and preserved in a refrigerator at 4ºC with proper labeling (Plate 11, 12, 13 and 14). For each of the samples four solvents have been used separately and successively.

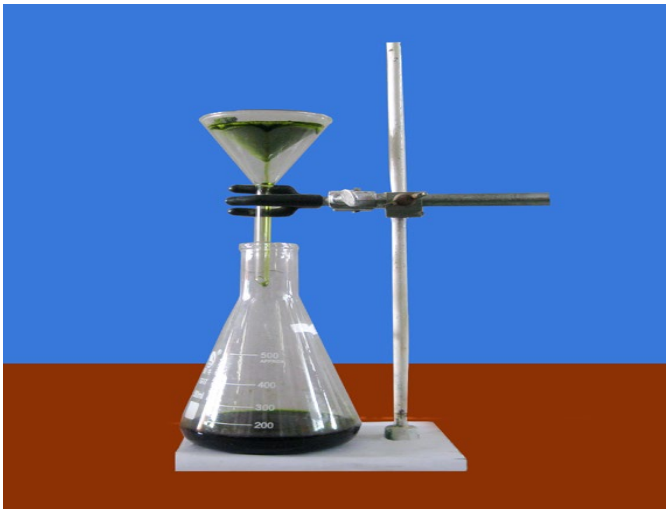

Plate10: Filtration of pet. Ether, acetone, chloroform and methanol extracts.

Plate11: Acetone extracts in vials

Plate12: Chloroform extracts in vials

Plate13: Pet. Ether extracts in vials

Plate14: Methanol extracts in vials

The pathway for the extraction, in detail, used in this investigation is given in Fig. 4.

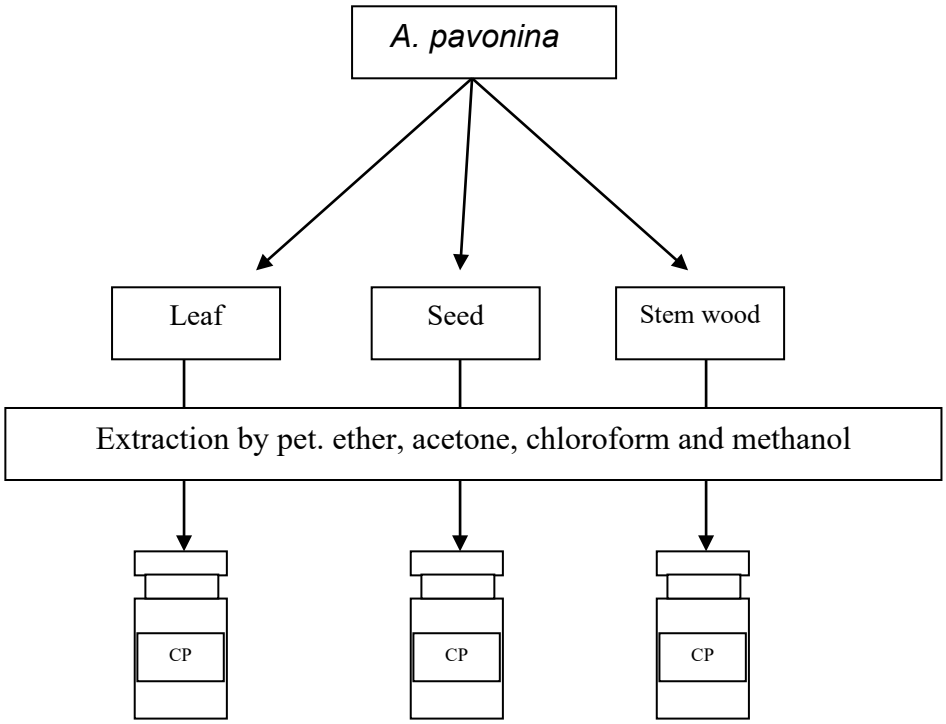

Fig. 4: Collection of extracts from different parts of *A. pavonia*

2.3. Crude extract bioassay

For the selection of bioassays to employ in research on plant constituents, the first step is to choose suitable target organisms. The complexity of the bioassay has to be designed as a function of the facilities and resources available. A list of bioassays taken in this investigation is shown in below:

Types of tests	**Test agents**
Insecticidal	*C chinensis* L. adults
Repellent activity test	*C. chinensis* L. adults
Cytotoxicity test	*A. salina*
Antimicrobial activity test:	
1. Antibacterial	Fifteen pathogenic bacteria
2. Antifungal	Seven pathogenic fungi

2.3.1. Preparation of doses for insecticidal assay

This is also one basic application method for doses of toxic substances to any insect population. The test material has been dissolved in an organic solvent with a certain concentration to apply to a Petri dish of known surface area. After application being volatile the solvent evaporates out immediately simply with the atmospheric temperature. Thus, the ingredient goes to make film on the surface of the Petri dish. Released insects within this captivity might have contact with the substance distributed evenly on the floor. However, being covered with the upper lid of the Petri dish there could have a captive environment with the extract distributed even in the air inside and may cause mortality by suffocation. Mortality suffocation may cause promptly if there is any volatile bioactive principles in the test material.

All extracts were diluted with the solvents in which they were extracted and the actual amount of extracted matter in a dose was recorded (Plate 15). The application of dose was carried out by residual film method (Busvine 1971). All the petroleum ether, acetone, chloroform and methanol extracts of the leave, seed and stem wood of *A. pavonina* were tested against *C. chinensis* adults through residual film assay at doses of 2831, 2477, 2123, 1769 and 1415 µg/cm^2 concentrations.

Plate 15: Preparation of doses for surface film test

2.3.1.1. Application of doses on insects

To conduct surface film activity test 60 mm Petri dishes were taken for all the doses and for their replications. One ml of each of the doses were poured into the lower part of the Petri dish and allowed them to dry out. Being volatile the solvent was evaporated out within a few minutes. Ten insects were released in each of the treated Petri dish. A control experiment by applying the only solvent into the Petri dish was also set at the same time under the same conditions (Plate 16).

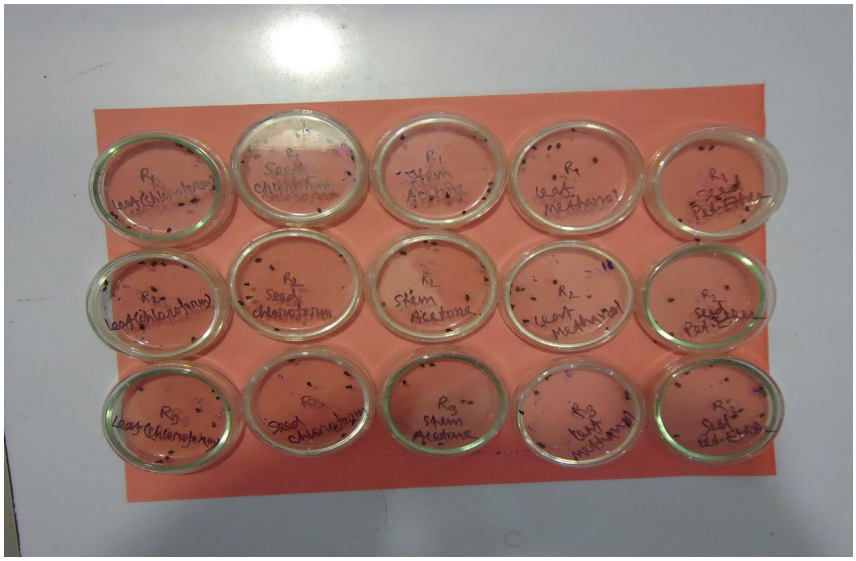

Plate 16: Bioassay using the plant extracts against *C. chinensis* adults by surface film method.

2.3.1.1.1. Reading and analysis of data for insecticidal activity

The experimental Petri dishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and the mortality was counted after every 24h, 48h and 72h and the data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recovery of the insects if occurred.

The mortality recorded was corrected by the Abbott's (1925) formula in the following manner:

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

Where,

P_r = Corrected mortality (%)

P_o = Observed mortality (%)

P_c = Control mortality (%), sometimes called natural mortality (%).

Then mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using software developed in the Department of Agricultural Environmental Science, University of Newcastle upon Tyne, U.K. The dose-mortality relationship was expressed as a median lethal dose (LD_{50}).

2.3.1.2. Preparation of doses for the repellency test

All the test extracts of leaves, seed and stem wood of *A. pavonina* collected in petroleum ether, acetone, chloroform and methanol at dose levels 471.50, 235.75, 117.88, 58.94, and 29.47 µg/cm^2 on filter paper for conduct the repellent activity test against adult beetles of *C. chinensis* .

2.3.1.2.1. Application of doses for repellency of insects

The repellency test used was adopted from the method (No. 3) of McDonald *et al*., (1970) with some modifications by Talukder and Howse (1993, 1994). Half filter paper discs (What man No. 40, 9 cm diam.) were prepared and selected doses of all the petroleum ether, acetone, chloroform and methanol extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 10-15 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (9 cm diam.), the inner surface of which was smeared with flu on to prevent insects escaping. The orientation of the same

was changed in the replica to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each filter-paper circle (Plate17). Each concentration was tested five times. Insects that settled on each half of the filter paper discs were counted after 1 h and then at hourly intervals for 5 h. No significant difference was detected between the repellency of only solvent impregnated and untreated filter papers in tests designed to check for any possible influence of solvents. The average of the counts was converted to percentage repellency (*PR*) using the formula of Talukder and Howse (1993, 1995):

$$PR = 2(C - 50),$$

Where, C is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values for attractant activity.

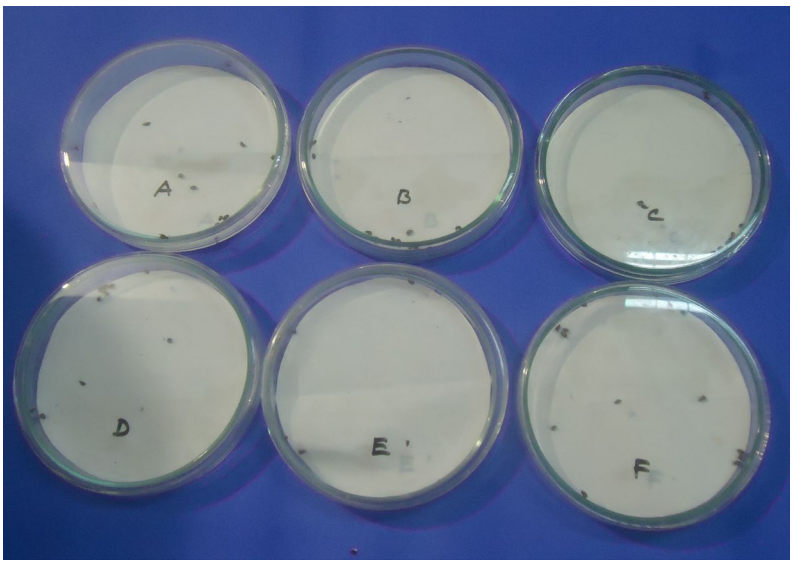

Plate17 -: Experiment for repellency test

2.3.1.2.2. Reading and analysis of data for repellency test

Repellency was observed for one-hour interval and up to five successive hours of exposure, just by counting the number if insects in the treated and non-treated part of the filter paper spread on the floor of the 90 mm Petri dish. The values in the recorded data were then calculated for percent repellency, which was again developed by arcsin transformation for the calculation of ANOVA.

2.3.1.3. Culture of *A. salina* for cytotoxicity test

Test materials:

(i) *A. salina* Leach (brine shrimp eggs or cycts)
(ii) Sea salt (non-ionized NaCl)
(iii) Small tank with perforated dividing dam to hatch the shrimp
(iv) Lamp to attract the nauplii
(v) Pipette (1 ml and 5 ml)
(vi) Micropipette (10-200µl adjustable)
(vii) Test tubes (5 ml)
(viii) Magnifying glass

Since the lethality test involves the culture of brine shrimp nauplii, i.e., the nauplii should be grown in the seawater, while the seawater contains 3.8% of sodium chloride. Accordingly 3.8% sodium chloride solution was made by dissolving sodium chloride (38 gm) in distilled water (1000 ml) and was filtered off. The P^H of the brine water thus prepared was maintained between 8 and 9 using $NaHCO_3$.

Brine water was taken in a small tank and shrimp eggs (1.5 gm/L) were added to one side of the perforated divided tank with constant oxygen supply. Constant temperature (37ºC) and sufficient light were maintained to give the sufficient aeration. After 48 hours, matured shrimp as nauplii (larvae) was collected and used for the experiment.

2.3.1.3.1. Preparation and application of doses on *A. salina*

Pet. ether, acetone, Chloroform and methanol extracts of the *A. pavonina* samples were applied against the brine shrimp nauplii. For leaves and stem wood samples 4 mg were initially dissolved in 200µl of pure dimethylsulfoxide (DMSO) to make them hydrophilic before adding 19.98 ml of water to get a concentration of 200 ppm for each of the samples separately which were used as stock solutions for all the extracts and from these concentrations other successive doses were prepared separately for each of the extracts through serial dilution method. Then a series of following concentrations made from the stock solutions were 200, 100, 50, 25, 12.5 and 6.25 ppm for all the extracts separately. However, for the seed extract 2 mg was initially

dissolved in 100µl of pure dimethylsulfoxide (DMSO) to make it hydrophilic before adding 19.98 ml of water to get a concentration of 100 ppm which was used as the stock solution for the seed extract. Then a series of following concentrations was made from the stock solution as 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ppm for the seed extract.

Brine shrimp eggs were hatched in simulated seawater to get nauplii. Test samples were prepared by the addition of calculated amount of DMSO (dimethylsulfoxide) for obtaining desired concentration of the test sample. The nauplii were counted by visual inspection and were taken in vials containing 5 ml of seawater. Then samples of different concentrations were added to the pre-marked vials with the help of a micropipette. The vials were left for 24 hours and then the nauplii were counted again to find out the cytotoxicity of the test agents and compared to the results with positive control.

2.3.1.3.2. Reading and analysis of data for cytotoxicity

The test tubes containing the nauplii along with the treated brine water were kept on a rack near the window in the laboratory. The recorded mortality was analyzed according to Finney (1947) and Busvine (1971) as it was done in the previous phase of experiments with both adults and larvae.

Preparation and application of doses for antimicrobial assays

Preparation and application of doses on bacteria

Preparation of the test plates

The test plates were prepared according to the following procedure:

i) The nutrient agar medium prepared previously was poured in 15 ml quantity in each of the clean test tubes and plugged with cotton pads.

ii) The test tubes and the Petri dishes were sterilized in an autoclave at 121°C and 15 lbs/sq. inch pressure for 15 minutes and were transferred into a laminar air flow unit and then allowed to cool down to 45°C to 50°C.

iii) The test organisms were transferred from the fresh subculture to the test tubes containing 15 ml autoclaved medium with the help of an

incubating loop in an aseptic condition. Then the test tubes were shaken by rotation to get a uniformed suspension of the organism.

iv) The bacterial suspensions were immediately transferred to the sterile Petri dishes in an aseptic area. The Petri dishes were rotated several times, first clock wise and then anticlockwise to assure homogenous distribution of the test organisms. The media were poured into Petri dishes in such a way that it could give a uniform depth of approximately 4 mm.

v) Finally, when the medium was cooled down to room temperature in a laminar air flow unit, it was stored in a refrigerator (at 4°C).

Preparation of the discs treated with the test samples

For the preparation of the discs containing chloroform and methanol extracts the following procedures were utilized. Three types of discs were prepared for antimicrobial screening. These are as follows:

(a) Sample discs- Sterilized filter paper discs having 5 mm in diameter (BBL, Cocksville USA) were prepared with the help of a punch machine and were taken in a blank Petri dish. Sample solution of desired concentration (10 μg/disc) was applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for a few minutes for complete removal of the solvent.

(b) Standard discs- These are used to compare the antibacterial activity of the test materials. In the present study, discs containing (30 μg/disc) of the antibiotic Ciprofloxacin were used as standard discs for the comparison with the extract treated ones.

(c) Control/blank discs- These were used as negative controls to ensure that the residual solvents on the filter paper were not active themselves. These were prepared in the previous manner applying only solvent to the discs and were used to examine the effect of the solvents used.

Preparation of test samples

In both cases, the doses were prepared 50 and 200 µg/discs separately of chloroform and methanol extracts and the standard Ciprofloxacin was used 30 µg/disc.

Placement of the discs and incubation

i) By means of a pair of sterile forceps, the dried crude extract discs and standard disc were placed gently on the solidified agar plates seeded with the test organisms to ensure contact with the medium.

ii) The plates were then kept in a refrigerator at 4°C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium.

iii) Finally, the plates were incubated at 37.5°C for 24 hours in an incubator.

Precaution

The discs were placed in such a way that they were not closer than 15 mm to the edge of the plate and were placed apart enough to prevent overlapping of the zones of inhibition.

Measurement of the zones of inhibition

After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones in term of mm with a transparent scale.

Preparation and application of doses on fungi

Preparation of the test plates

The test plates were prepared according to the following procedure:

i) About 10 ml of distilled water was poured in several clean test tubes and plugged with cotton pads.

ii) The test tubes, Petri dishes, glass rods, cotton pads and the medium were sterilized by autoclave and then transferred to the laminar air flow cabinet.

iii) About 6 ml of the medium was poured carefully into the medium sized Petri dishes separately and were rotated several times, first

clockwise and then anticlockwise to assure homogenous thickness of the medium and allowed to cool down and solidity at about 30°C.

iv) The test tubes containing distilled water were inoculated with fresh culture of the test fungi and were shaken gently to form a uniformed suspension of the organism because of their high prevalence of sporulation process.

v) Separate piece of cotton were immerged in the test tubes with the help of individual glass rods and gently rubbed the medium. The pieces of cotton were then discarded.

vi) Finally, the plates were stored in a refrigerator (4°C) overnight.

Preparation of the discs containing test samples

(a) **Sample discs-** Sterilized filter paper discs (5 mm diam.) were taken by the forceps in the plates. Crude extracts of chloroform (50µg and 200µg) were applied on separate discs with the help of micropipettes in an aseptic condition. These discs were left for a few minutes for complete removal of the solvent.

(b) **Standard discs-** These were used to compare the antibacterial activity of the test material. In the present study, ready-made Nystatin 50µg/disc was used as standard disc for comparison with the efficacy of the test extracts.

(c) **Control/blank discs-** These were used as negative controls to ensure that the residual solvent on the filter paper were not active themselves. These were prepared in the previous manner applying only solvent to the discs and were used to examine the effect of the solvents used.

Note:

Preparation of the discs, test samples, standard sample and placement of the discs, diffusion, incubation and measurement of zones of inhibition were almost same with the antibacterial screening. Here, only the incubation period was replaced by 48-72 hours at room temperature.

Determination of Minimum Inhibitory Concentrations (MIC) for the antibacterial agents

Minimum Inhibitory Concentration (MIC) may be defined as the lowest concentration of an antimicrobial drug to inhibit the growth of the test organism. There are two methods of experiments to determine the MIC values are as follows:

i) Serial tube dilution technique or turbidimetric assay (Reiner, 1982).
ii) Paper disc plate technique or agar diffusion assay (Bauer *et. al.* 1966).

Here 'Serial tube dilution technique' was followed using nutrient broth medium to determine the MIC values of chloroform extracts against the following 3 gram positive and 4 gram negative pathogenic bacteria.

Gram positive bacteria

(a) *Streptococcus-β-haemolyticus*
(b) *Bacillus cereus*
(c) *Bacillus subtilis*

Gram negative bacteria

(d) *Shigella dysenteriae*
(e) *Shigella typhi*
(c) *Shigella shiga*
(d) *Shigella sonnei*

Preparation of inoculum

Fresh cultures of the test organisms were grown at 37.5°C for two days on the nutrient agar medium. Bacterial suspensions were then prepared in sterile nutrient broth medium in such a manner that the suspension contains 107 CFU/ml. These suspensions were used as inocula.

Preparation of the sample solution

The stock solution was prepared by dissolving 1.024 mg of crude extracts in 2 ml of DMSO. Thus the solution with a concentration of 1.024 mg/ml was obtained.

Procedure of serial tube dilution technique

i) Twelve (12) autoclaved test tubes were taken, nine of which marked as 1, 2, 3, 4, 5, 6, 7, 8 & 9 and the rest were assigned as Cm = (medium), Cs = (medium + compound) and Ci = (medium + inoculum).

ii) One ml of sterile nutrient broth medium was added to each of the 12 test tubes.

iii) One ml of the sample solution was added to the first test tube and mixed well.

iv) One ml content from the 1st test tube was transferred by the sterile pipette to the 2nd test tube and mixed uniformly. Then 1 ml of this mixture was transferred to the 3rd test tube. This process of serial dilution was continued up to the 9th test tube.

v) One drop (10µl) of properly diluted inoculum was added to each of the 9 test tubes and mixed well.

vi) For the control test tube, 1 ml of the sample solution was added and mixed well, while 1 ml of this mixed content was discarded. This was to check the clarity of the medium in presence of diluted solution of the compound.

vii) 10 µl of the inoculum was added to the control test tube Ci to observe the growth of the organism in the medium used.

viii) The control test tube Cm, containing medium only was used to confirm the sterility of the medium.

ix) All the test tubes were incubated at 37.5°C for 18-24 hours. The MIC is the lowest drug concentration at which there is no growth of the organism.

Combined effect of plant extracts and parasitoid (*D. basalis*) against *C. chinensis* under laboratory conditions

C. chinensis was reared in one-pint glass jars on green gram seeds. Possible insect contaminations were eliminated by heating the seeds at a temperature of 60^0C for 15 h in an incubator. The seeds were then thoroughly washed, carefully dried under the sun, tempered to 13-14% moisture content and stored in air-light containers.

Stock cultures were set up by releasing about 200 adults in the jars, each containing 1 kg green gram seeds and the mouth of the jars were covered with muslin and tied with rubber bands.

The adults were allowed to ovipositors on the seeds for 24 h to obtain sufficient number of eggs. Then the adults were removed to another glass jars for further host eggs. Thus a series of stock cultures were maintained in the laboratory for constant supply of these insects to conduct the experiments.

D. basalis was maintained in ½ pint mason jars. Thirty mated females were introduced into the glass jars containing 12-15 days-old infested seeds of *C. chinensis*. After 12-14 days, progeny emerged from the parasitized seeds. The newly emerged parasitoids were again supplied with 12-15 days-old infested seeds of green gram. The process was repeated every 12-15 days for getting continuous supply of adult progeny for different experiments.

Two separate tests were run and each test was replicated three times.

Effect of plant extracts on parasitoid

Different doses of plant (*A. pavonina*) extracts of methanol (16, 8, 4 mg/ml) and chloroform (2, 1, 0.5 mg/ml) were used in this study. The procedure for getting extracts is described in previous Chapter. Studies were conducted at 30 ± 0.5^0C and 70 ± 0.5% RH.

Direct Contact Toxicity

The bottom of the Petri dishes (11 cm diam) was socked with 16 mg of methanol. After proper drying 20 1-d-old *D. basalis* adults were placed in a Petri dishes containing 20 mg green gram seed, 3 g green gram seeds infested with 12-15 days-old infested seeds, or as a control, uninfested green gram seeds. The number of dead *D. basalis* was recorded each day until all adult *D. basalis* died. In experiments conducted at the same time, honey was provided as food for *D. basalis* with a filter paper streaked with honey. The similar process was continued in other doses 8, 4 mg/ml. In chloroform, the doses were 2, 1 and 0.5 mg/ml and the process was repeated as described in case of methanol.

Choice Test

One section of a divided Petri dish was filled with 5 g of untreated green gram, and the other section was filled with treated 16 mg/ml methanol green gram seeds. Green gram in both sections contained 12-15 days-old *C. chinensis* infested seeds. A single 1-d-old *D. basalis* female was placed in each Petri dish and allowed to oviposit for 0, 24, 48, or 96 h. Untreated and treated green gram from the two dish sections were collected separately and placed in vials (29 by 80 mm) with screen lids, and the number of emerged *D. basalis* adults in each was recorded after 3 weeks. The process was continued in case of 8 and 4 mg/ml of methanol and different doses of chloroform.

No-Choice Test

Twenty 1-d-old *D. basalis* females were placed in vials with 10 g of green gram, which contained 12-15 days-old *C. chinensis*, and treated at 4, 8 and 16 mg/ml green gram seed. The parasitoids were removed after 0, 1, 3, 5, or 7 d. The numbers of emerged *D. basalis* and *C. chinensis* were counted after 3 weeks. Similar process was applied in case of different doses of chloroform.

Combined effect of plant extracts and parasitoid

The Petri dishes (11 cm diam) were soaked with 4, 8 and 16 mg/ml of methanol extracts of seed of *A. pavonina* separately. After proper drying clean green gram seeds were placed. Mated females of *C. chinensis* were released in the Petri dish for egg laying up to three days and then the females were removed. The mated females of *D. basalis* were released in the Petri dishes after the initial infestation of the seeds were 12-15 days-old. The release rates were 2, 4, 8 and 12. The Petri dishes were kept in CT (Controlled temperature) room at 30±0.5ºC temperature and 70±0.5% relative humidity. The grain was sifted, and the number of live and dead adults of *D. basalis* and *C. chinensis* was counted. Three replications were used for each dose and control. The same procedure was applied incase of chloroform using 2, 1 and 0.5 mg/ml.

Analysis of variance (ANOVA) followed by Tukey's multiple range test was used to determine if there were significant differences among treatments (Mian and Mulla (1982a).

Chapter 3

Results

Chapter 3

Results

3.1. Bioactivity of the crude extracts

3.1.1. Insecticidal activity against *C. chinensis* adults

All the petroleum ether, acetone, chloroform and methanol extracts of the leaves, seed and stem wood of *A. pavonina* were tested against *C. chinensis* adults through residual film assay at doses of 2831, 2477, 2123, 1769 and 1415 µg/cm^2 on the surface of the Petri dishes, where the test insects were released to observe mortality or any sort of behavioral changes due to the action of the extracts compared to their controls. The results have been presented in Appendix Tables (1- 36) and Figures (5-16) for the mortality recorded. To trace acute toxicity (if exists) an observation of mortality was made after 12h after application of the doses, however usual observations were made after 24h and 48h of exposures.

The data was subjected to probit analysis and the LD_{50} values were shown in Tables 3, 4and 5. The seed extract was found to offer the highest mortality of the beetles, with the LD_{50} values were 4646.414, 3106.171 and 2018.911 µg/cm^2 for the chloroform extracts; 4231.617, 4218.390 and 2161.584 µg/cm^2 for the acetone extracts; 4537.363, 3758.050 and 2394.528 µg/cm^2 for the methanol extracts and 4029.546 , 4104.532 and 2432.645 µg/cm^2 for the pet. ether extracts for 12h, 24h and 48h of exposures respectively. Observation after 12h assured acute toxicity positively, however, the LD_{50} value was simply larger. Depending on toxicity the leaf extract gives LD_{50} values 9993.172, 8552.961 and 7167.948 µg/cm^2 for the acetone extract; 13957.44, 11211.07 and 7445.673 µg/cm^2 for the pet. ether extract; 8769.089, 9181.241 and 7763.914 µg/cm^2 for the chloroform extract and 9320.703, 9239.611 and 8818.821 µg/cm^2 for the methanol extract after 12, 24 and 48 h of exposure respectively; which was just followed by the stem wood extract to give the

LD_{50} values 12800.32, 11376.43 and 8823.608 µg/cm^2 for the pet. ether extract; 12099.02, 9799.158 and 7249.375 µg/cm^2 for the acetone extract; 12053.19, 9064.269 and 6306.757 µg/cm^2 for the chloroform extract and 11224.51, 8066.323 and 5592.335 µg/cm^2 for the methanol extract for 12, 24 and 48 h of exposure respectively.

According to the intensity of activity observed through mortality of the adult *C. chinensis* the potentiality of the petroleum ether, acetone, chloroform and methanol extracts could be arranged in a descending order of chloroform > acetone > methanol >petroleum ether extracts.

Table 3: Dose mortality effects of *A. pavonina* seed extracts (pet. ether, acetone, chloroform and methanol) against *C. chinensis* adults.

Test extract	Time exposed (h)	LD_{50} value µg/cm^2	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	12	4029.546	3040.105	5341.016	Y = -13.724+5.194X	1.537(3)
	24	4104.532	2807.799	6000.136	Y =-6.013+3.048X	0.224(3)
	48	2432.645	2230.889	2652.638	Y =-10.635+4.617X	0.330(3)
Acetone	12	4231.617	3044.489	5881.634	Y = -11.829+4.640X	1.578(3)
	24	4218.390	2748.362	6474.701	Y =-4.746+2.689X	3.626E-02(3)
	48	2161.584	2009.114	2325.627	Y = -10.737+4.719X	1.272(3)
Chloroform	12	4646.414	2960.347	7292.786	Y=-7.636+3.446X	0.232(3)
	24	3106.171	2531.771	3810.892	Y=-6.454+3.280X	0.190(3)
	48	2018.911	1879.221	2168.983	Y=-10.904+4.812X	1.355(3)
Methanol	12	4537.363	3052.271	6745.017	Y=-11.325+4.464X	1.385(3)
	24	3758.050	2889. 551	4887.597	Y = -9.463+4.046X	1.024(3)
	48	2394.528	2254.381	2543.387	Y=-17.041+6.522X	5.321(3)

The overall assessment of toxicity of *A. pavonina* extracts are very much promising and their efficacy on stored grain pests might have future to be used as a control agent or tool. It may open its possibility as a control agent for the insect pests as well.

Table 4: Dose mortality effects of *A. pavonina* leaf extracts (pet. ether, acetone, chloroform and methanol) against *C. chinensis* adults.

Test extract	Time exposed (h)	LD_{50} value µg/cm^2	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	12	13957.44	6817.663	28574.33	Y = -11.557+3.995X	0.262(3)
	24	11211.07	7210.572	17431.06	Y = -10.775+3.895X	2.964E-02(3)
	48	7445.673	6641.745	8346.892	Y = -17.412+5.789X	0.668(3)
Acetone	12	9993.172	7385.677	13521.24	Y = -14.964+4.991X	0.117(3)
	24	8552.961	6938.465	10543.12	Y = -12.948+4.564X	0.444(3)
	48	7167.948	6446.421	7970.223	Y = -16.149+5.486X	1.545(3)
Chloroform	12	8769.089	7338.51	10478.54	Y = -22.007+6.850X	2.152(3)
	24	9181.241	7158.734	11775.15	Y = -13.474+4.662X	0.294(3)
	48	7763.914	6701.33	8994.983	Y = -14.480+5.008X	0.645(3)
Methanol	12	9320.703	7452.133	11657.8	Y =-22.827+7.010X	1.018(3)
	24	9239.611	7261.293	11756.92	Y = -15.426+5.151X	0.279(3)
	48	8818.821	7116.806	10927.88	Y = -14.363+4.908X	0.530(3)

Table 5: Dose mortality effects of *A. pavonina* stem wood extracts (pet. ether, acetone, chloroform and methanol) against *C. chinensis* adults.

Test extrac	Time exposed (h)	LD_{50} value µg/cm^2	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	12	12800.32	7069.525	23176.730	Y= -11.191 + 3.942X	0.031
	24	11376.43	6906.73	18738.66	Y =-8.375 +3.298X	3.198E-02
	48	8823.608	6647.416	11712.22	Y =-8.623 +3.453 X	5.685E-02
Acetone	12	12099.02	7033.855	20811.70	Y = -9.743 +3.611x	2.387E-02
	24	9799.158	6772.172	14179.13	Y=-7.985+3.253X	1.332E-03
	48	7249.375	6207.77	8465.757	Y = -9.703+3.809X	0.258
Chloroform	12	12053.19	7112.16	20426.90	Y = -11.171+3.962X	0.175
	24	9064.269	7002.897	11732.43	Y =-11.787+4.242X	0.054
	48	6306.757	5929.698	6707.792	Y = -19.769+6.519X	1.268
Methanol	12	11224.51	7237.74	17407.33	Y = -10.897 +3.925X	9.541E-03
	24	8066.323	6681.735	9737.825	Y = -11.594+ 4.248X	0.192
	48	5592.335	5305.335	5894.861	Y =-19.277+ 6.478 X	0.849

4.1.3. Cytotoxicity against *A. salina* nauplii

The dose-mortality assay of *A. pavonina* extracts against the brine shrimp (*A. salina*) nauplii has been done through test tube treatment method for all the petroleum ether, acetone, chloroform and methanol extracts of leaves, seed and stem wood. Most of the test extracts showed remarkable dose-mortality effects against the 1 day nauplii of *A. salina* and the result has been presented in Appendix Tables (37-72) and Figures (17-28). The seed extract was found to offer the highest mortality of the nauplii, with the LC_{50} values were 444.648, 14.709 and 5.678 ppm for the chloroform extracts; 846.206, 32.924 and 11.762 ppm for the methanol extracts; 6601.437, 99.821 and

13.470 ppm for the pet. ether extracts and 3181.012, 47.757 and 14.425 ppm for the acetone extracts for 30 min, 24h and 48h of exposures respectively. The LC_{50} values for the stem extract were18255.69, 99.179 and 39.897 ppm for the chloroform extracts; 8575.589, 139.213 and 56.989 ppm for the methanol extracts; 7266.57, 697.739 and 68.312ppm for the pet. ether extracts and 745099, 192.026 and 39.527 ppm for the acetone extracts after 30 min, 24h and 48h of exposures respectively. The LC_{50} values for the leaf extract were 4256.71, 49.081 and 25.539 ppm for the chloroform extracts; 13202.60, 83.859 and 44.246 ppm for the methanol extracts; 15469.24, 549.935 and 63.791ppm for the pet. ether extracts and 43235.51, 105.242 and 46.046 ppm for the acetone extracts for 30 min, 24h and 48h of exposures respectively (Tables 6-8). To consider acute toxicity (if exists) of the extracts a reading of data is made after 30 min. of exposure, and in this case the result was positive, while the LC_{50} values were comparatively larger. According to the intensity of activity the results of the extracts against the brine shrimp nauplii could be arranged in the following order: seed > leaf > stem extract.

Table 6: Cytotoxicity through dose-mortality of *A. pavonina* leaf extracts (pet. ether, acetone, chloroform and methanol) against *A. salina* nauplii.

Test extract	Time exposed	LC_{50} value (ppm)	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	30 min	15469.24	137.4437	1741058	Y = 2.339 +0.635X	0.389 (4)
	24 h	549.935	179.709	1682.885	Y = 2.821+0.795X	1.137(4)
	48 h	63.791	39.563	102.857	Y = 3.337+0.921X	1.228(4)
Acetone	30 min	43235.51	31.943	5.852E+07	Y = 3.073+0.416X	0.101(4)
	24 h	105.242	60.326	183.602	Y=3.348+0.817X	0.105(4)
	48 h	46.046	29.479	71.922	Y = 3.264+1.044X	0.743(4)
Chloroform	30 min	4256.71	219.277	82633.13	Y =2.691+0.636X	0.348(4)
	24 h	49.081	26.259	91.736	Y =3.778+ 0.723X	0.249(4)
	48 h	25.539	14.159	46.063	Y =3.646+0.962X	0.344(4)
Methanol	30 min	13202.60	115.401	1510458	Y = 2.864+ 0.518X	0.162(4)
	24 h	83.859	46.035	152.759	Y = 3.603 + 0.726X	0.401(4)
	48 h	44.246	26.783	73.094	Y =3.458+0.937X	1.682(4)

Table 7: Cytotoxicity through dose-mortality of *A. pavonina* seed extracts (pet. ether, acetone, chloroform and methanol) against *A. salina* nauplii.

Test extract	Time exposed	LC_{50} value (ppm)	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	30 min	6601.437	57.700	755264.70	Y =3.020+0.518X	0.162 (4)
	24 h	99.821	50.501	197.308	Y =3.337+0.832X	6.689E-02(4)
	48 h	13.470	7.182	25.264	Y =4.004+0.882X	0.234 (4)
Acetone	30 min	3181.012	67.055	150904.20	Y =3.177+0.520X	0.435 (4)
	24 h	47.757	28.254	80.722	Y = 3.565+0.854X	0.300 (4)
	48 h	14.425	8.743	23.800	Y =3.751+1.078X	0.150 (4)
Chloroform	30 min	444.648	93.614	2111.985	Y =3.175+0.690X	9.481E-02 (4)
	24 h	14.709	6.693	32.322	Y =4.214+0.673X	0.195 (4)
	48 h	5.678	2.591	12.443	Y = 4.218+1.037X	0.702 (4)
Methanol	30 min	846.206	107.583	6655.92	Y =3.027+0.674X	0.168 (4)
	24 h	32.924	18.637	58.163	Y = 3.840+0.765X	0.189 (4)
	48 h	11.762	6.659	20.775	Y =3.898+1.029X	0.594 (4)

Table 8: Cytotoxicity through dose-mortality of *A. pavonina* stem extracts (pet. ether, acetone, chloroform and methanol) against *A. salina* nauplii.

Test extract	Time exposed	LC_{50} value (ppm)	95% Conf. Limits		Regression equation	χ^2 Value (df)
			Lower limit	Upper limit		
Pet. Ether	30 min	7266.57	248.445	212533.80	Y =1.755+0.840X	0.647 (4)
	24 h	697.739	208.878	2330.734	Y =2.645+0.828X	0.954 (4)
	48 h	68.312	44.174	105.639	Y =3.127+1.021X	0.176 (4)
Acetone	30 min	745099	2903859	1.912E+12	Y =3.238+0.300X	0.123 (4)
	24 h	192.026	88.802	415.237	Y =3.351+0.722X	0.155 (4)
	48 h	39.527	24.212	64.528	Y =3.429+0.984X	0.574 (4)
Chloroform	30 min	18255.69	66.731	4994224	Y =3.082+0.450X	0.166 (4)
	24 h	99.179	52.125	188.710	Y =3.610+0.696X	4.875E-02 (4)
	48 h	39.897	24.167	65.864	Y =3.460+0.962X	0.398 (4)
Methanol	30 min	8575.589	172.861	425433.50	Y =2.638+0.601X	0.297 (4)
	24 h	139.213	71.126	272.478	Y =3.428+0.733X	0.290 (4)
	48 h	56.989	35.272	92.077	Y =3.360+0.934X	0.802 (4)

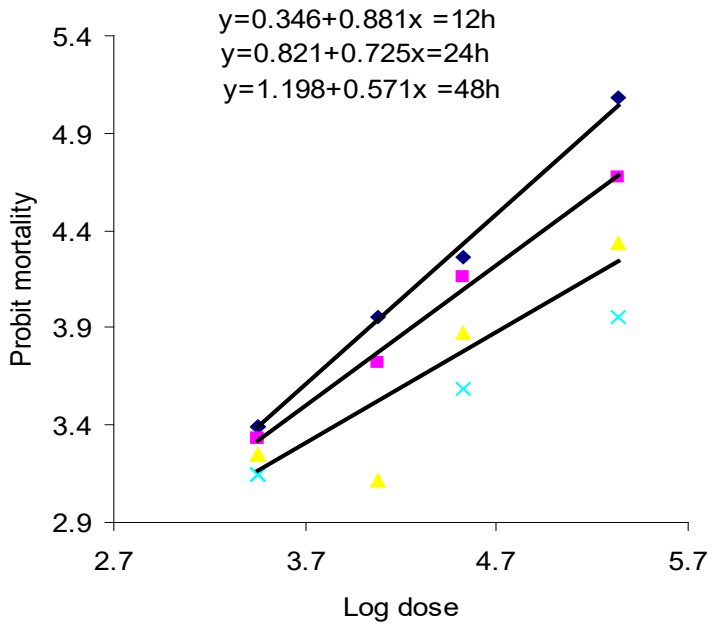

Figure 5: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonina* seed extract in petroleum ether after 12, 24 and 48 h of exposure.

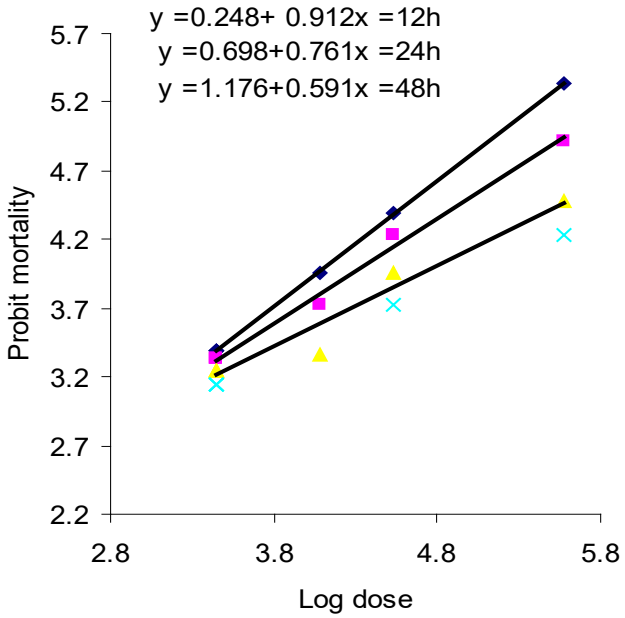

Figure 6: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonina* seed extract in acetone after 12, 24 and 48 h of exposure.

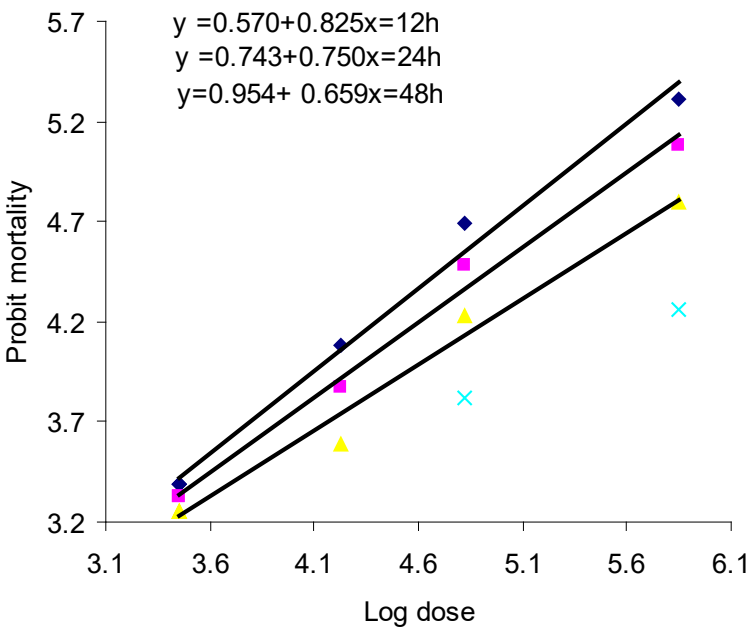

Figure 7: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonina* seed extract in chloroform after 12, 24 and 48 h of exposure.

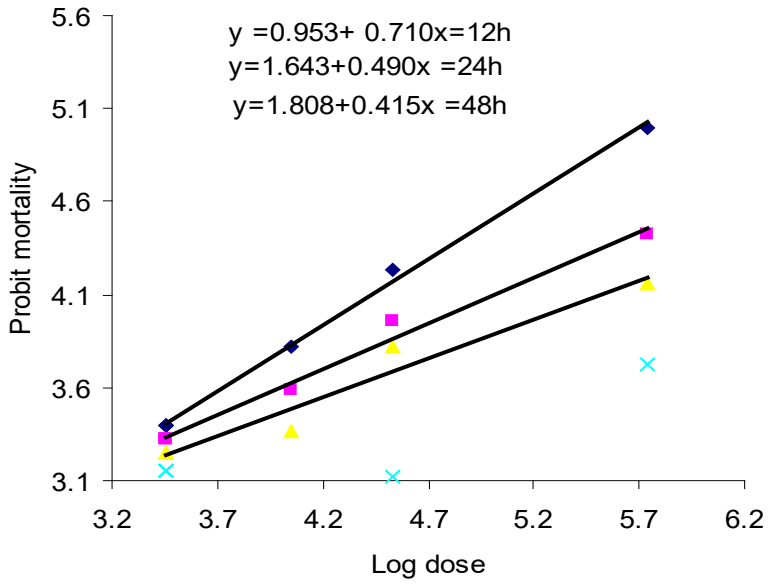

Figure 8: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonina* seed extract in methanol after 12, 24 and 48 h of exposure.

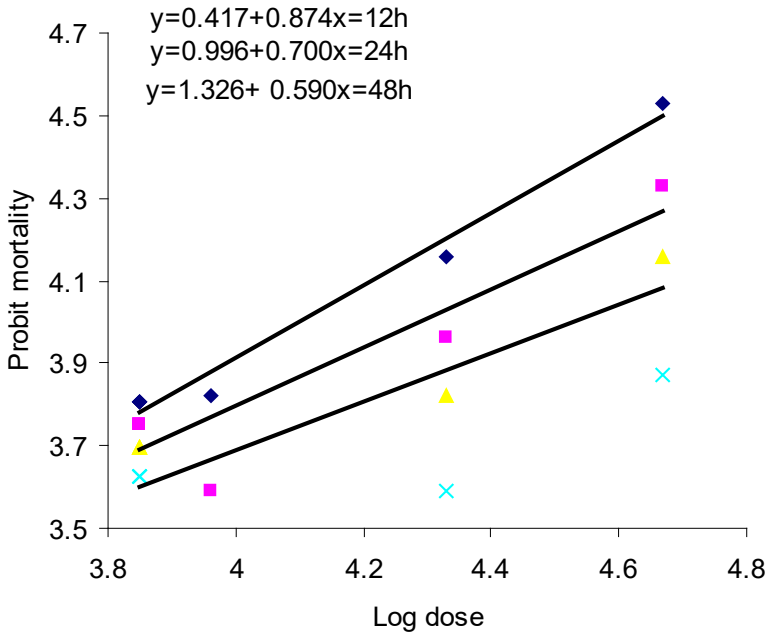

Figure 9: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* stem extract in petroleum ether after 12, 24 and 48 h of exposure.

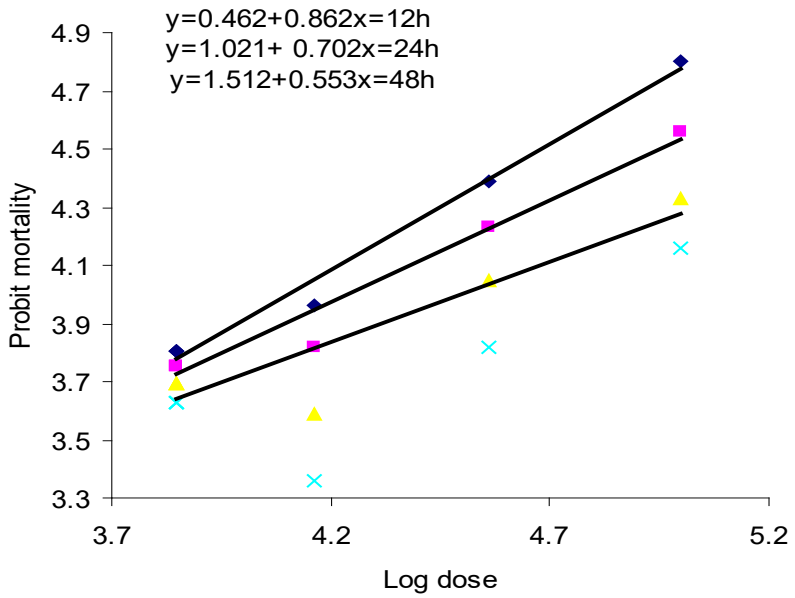

Figure 10: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* stem extract in acetone after 12, 24 and 48 h of exposure.

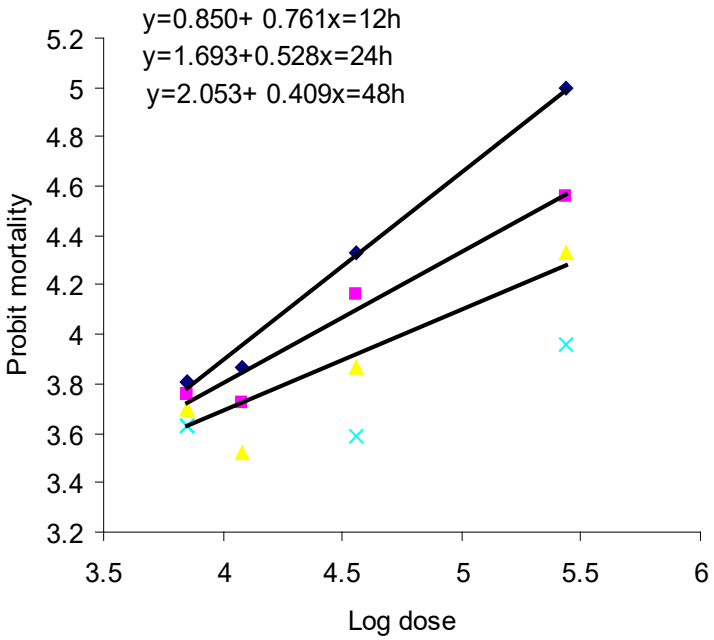

Figure 11: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* stem extract in chloroform after 12, 24 and 48 h of exposure.

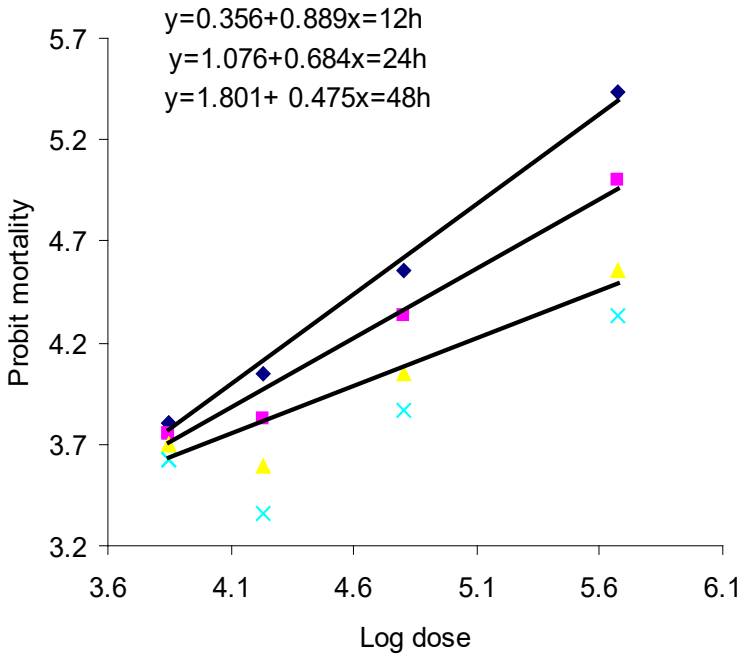

Figure 12: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* stem extract in methanol after 12, 24 and 48 h of exposure.

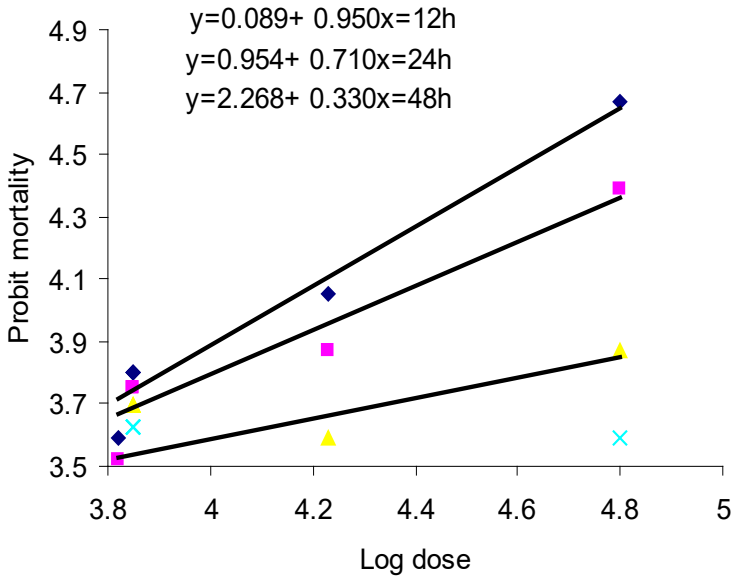

Figure 13: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* leaf extract in petroleum ether after 12, 24 and 48 h of exposure.

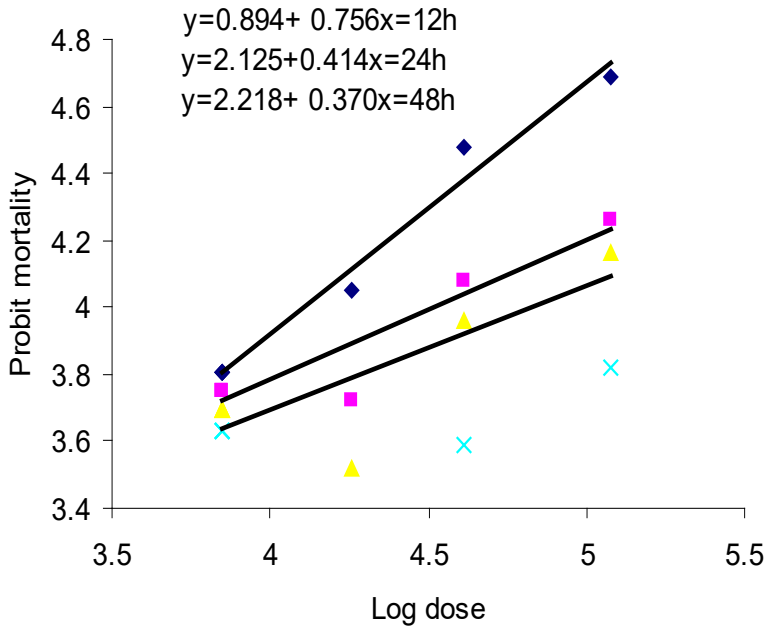

Figure 14: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* leaf extract in acetone after 12, 24 and 48 h of exposure.

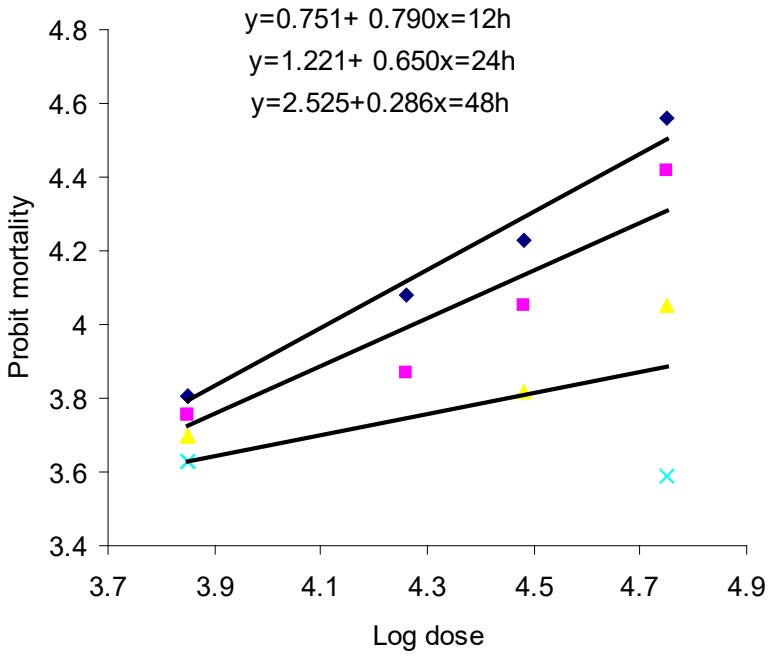

Figure 15: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* leaf extract in chloroform after 12, 24 and 48 h of exposure.

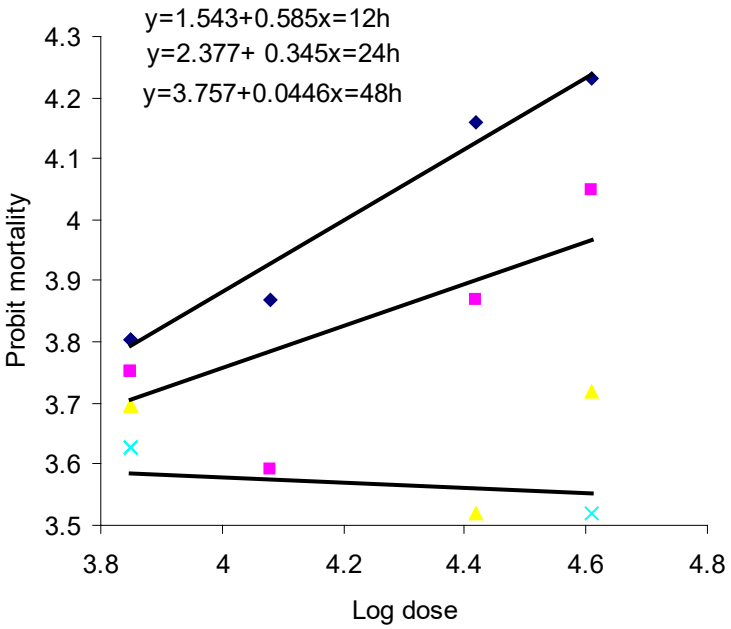

Figure 16: Regression line of probit mortality of adult *C. chinensis* and log dose of *A. pavonia* leaf extract in methanol after 12, 24 and 48 h of exposure.

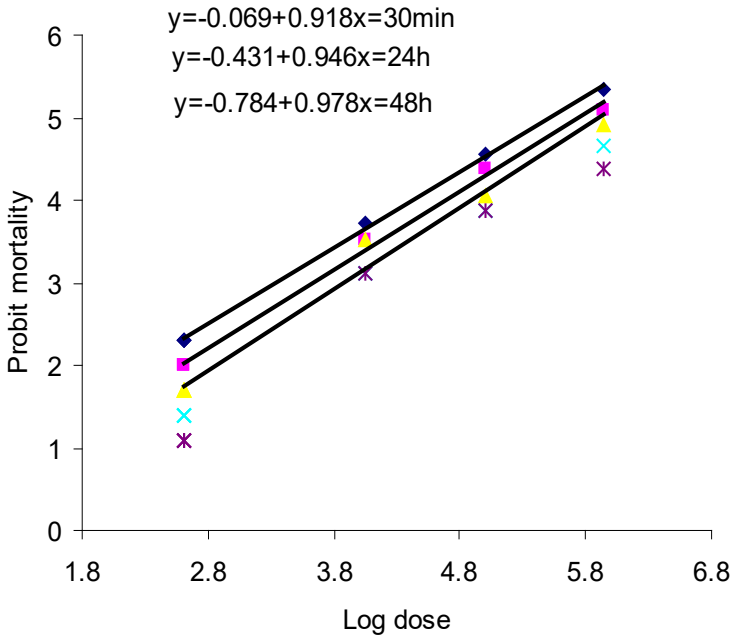

Figure 17: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* leaf extract in petroleum ether after 30min, 24 and 48 h of exposure

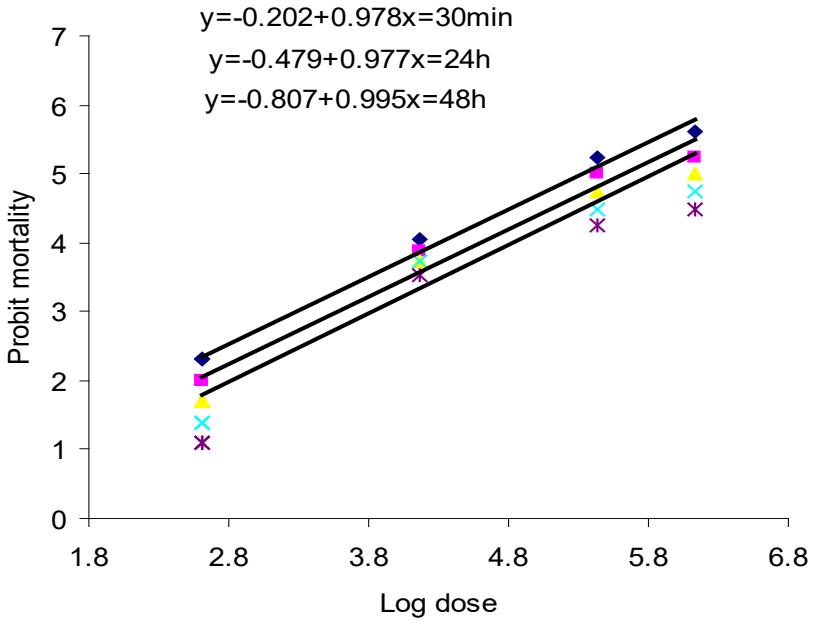

Figure 18: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* leaf extract in acetone after 30min, 24 and 48 h of exposure.

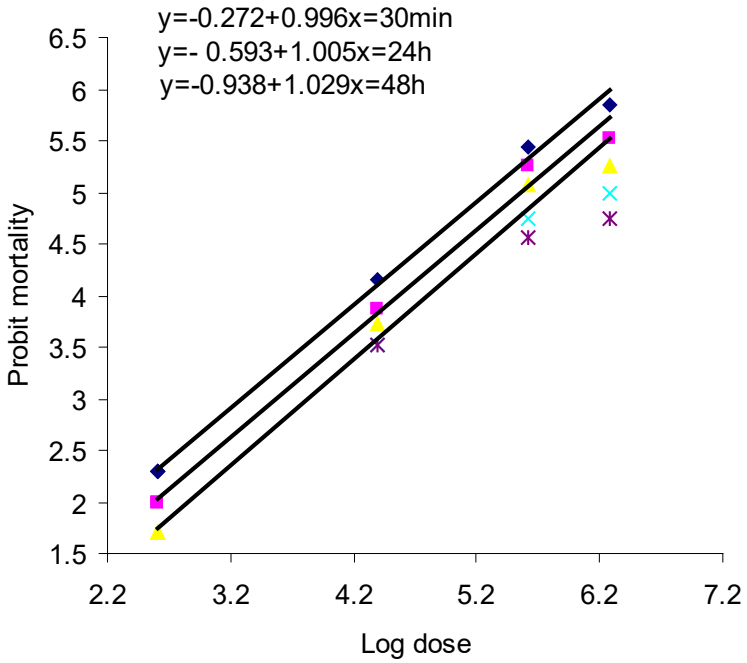

Figure 19: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* leaf extract in chloroform after 30min, 24 and 48 h of exposure.

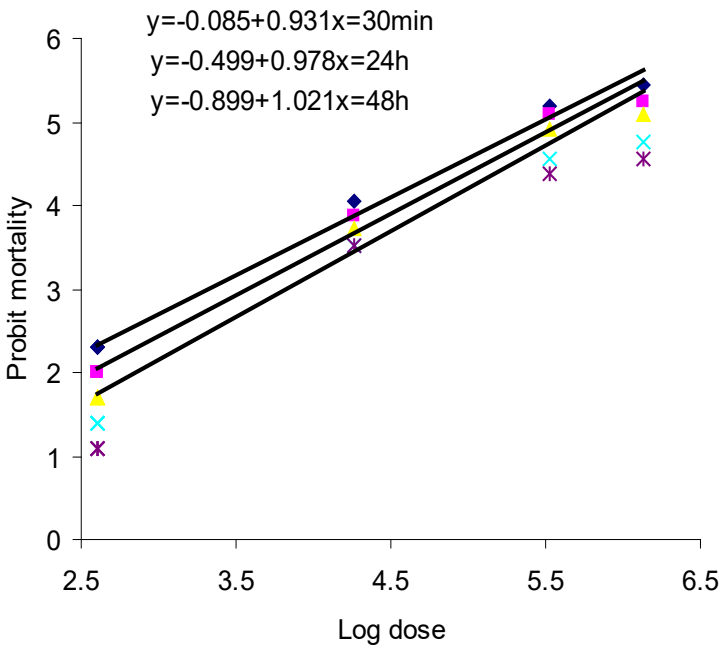

Figure 20: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* leaf extract in methanol after 30min, 24 and 48 h of exposure

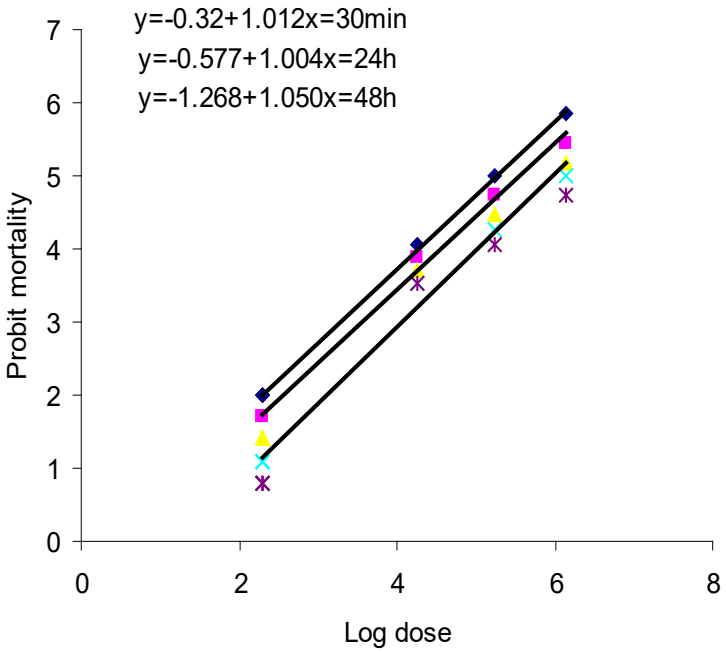

Figure 21: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* seed extract in petroleum ether after 30min, 24 and 48 h of exposure.

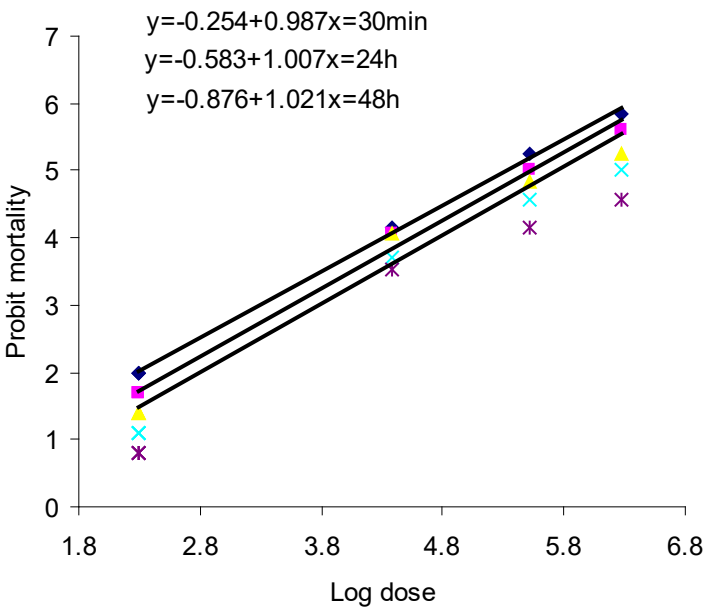

Figure 22: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* seed extract in acetone after 30min, 24 and 48 h of exposure.

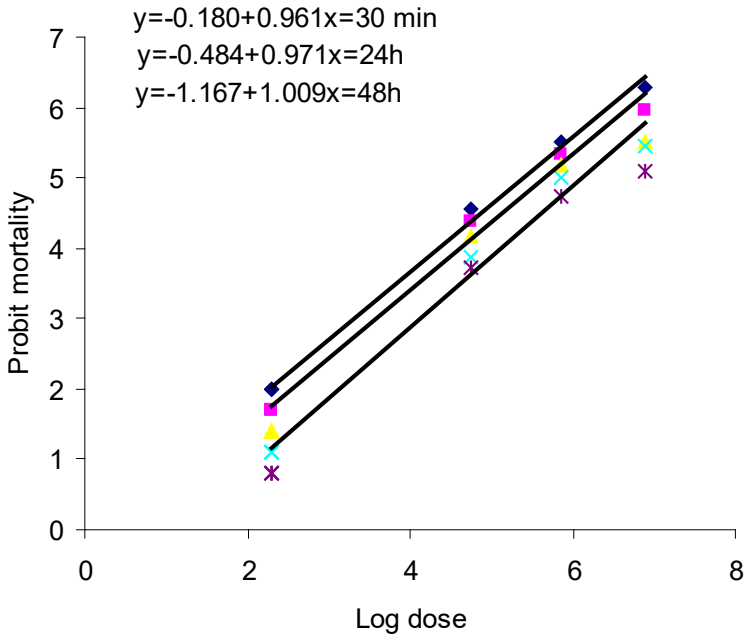

Figure 23: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* seed extract in chloroform after 30min, 24 and 48 h of exposure

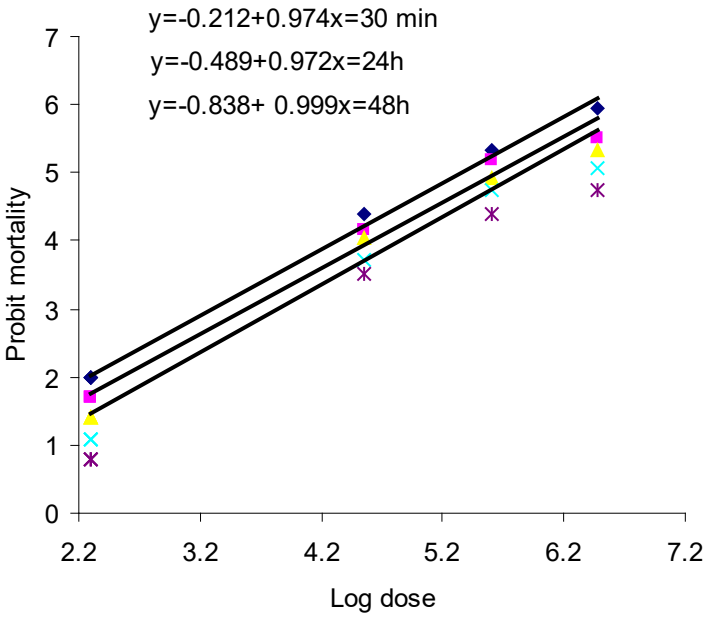

Figure 24: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* seed extract in methanol after 30min, 24 and 48 h of exposure.

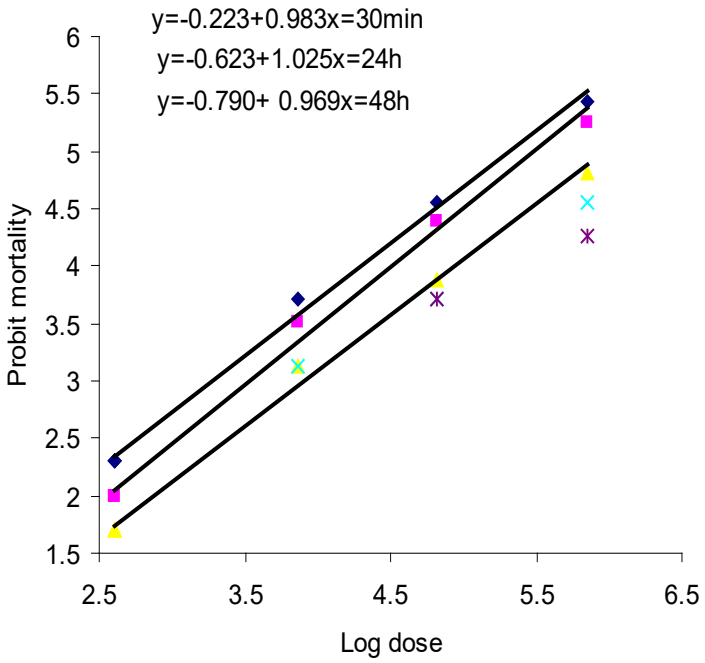

Figure 25: Regression line of probit mortality of *Artemia salina* larva and log dose of *Adenanthera pavonia* stem extract in petroleum ether after 30min, 24 and 48 hours of exposure.

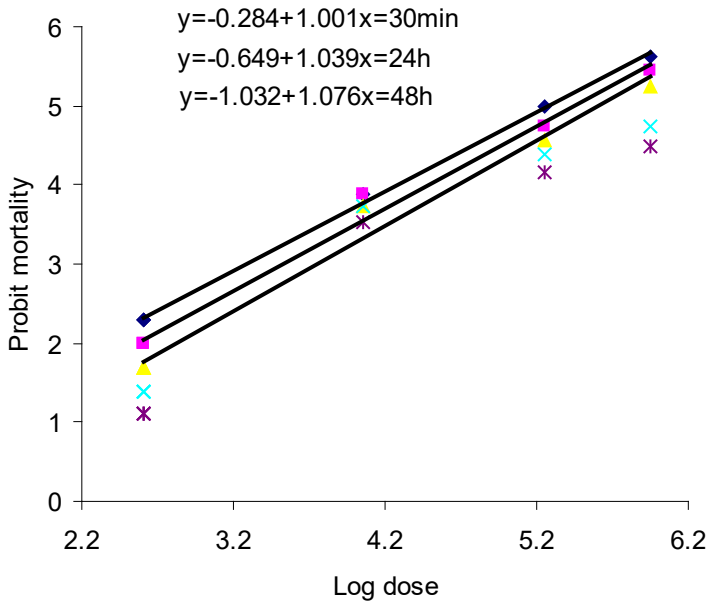

Figure 26: Regression line of probit mortality of *Artemia salina* larva and log dose of *Adenanthera pavonia* stem extract in acetone after 30min, 24 and 48 hours of exposure.

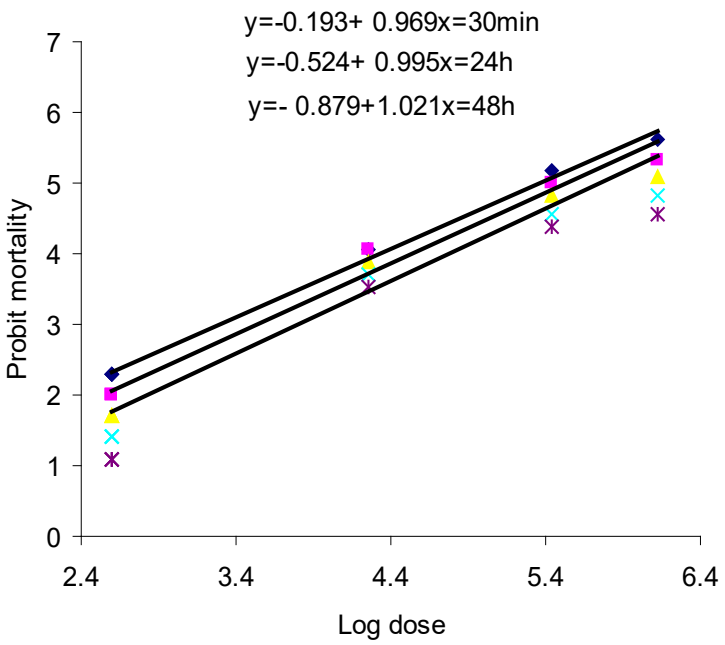

Figure 27: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* stem extract in chloroform after 30min, 24 and 48 h of exposure

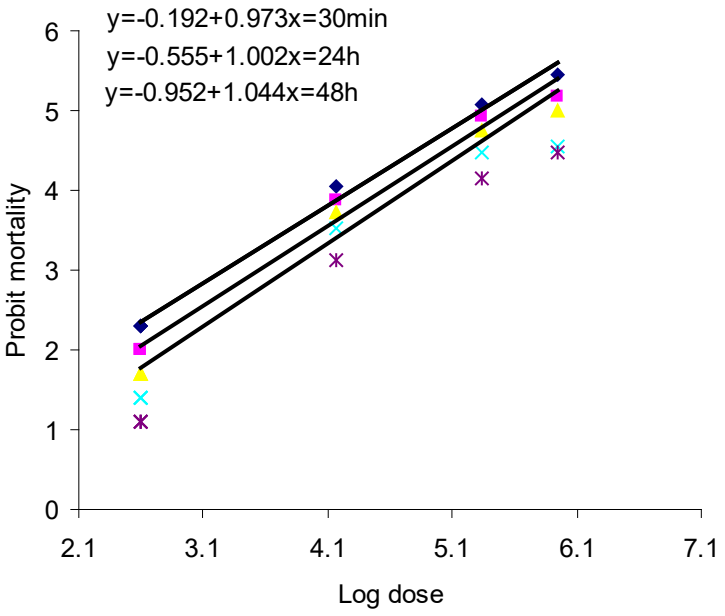

Figure 28: Regression line of probit mortality of *A. salina* larva and log dose of *A. pavonia* stem extract in methanol after 30min, 24 and 48 h of exposure

4.1.4. Repellency against *C. chinensis* adults

All the test extracts of leaves, seed and stem wood of *A. pavonina* collected in petroleum ether, acetone, chloroform and methanol showed repellent activity against adult beetles of *C. chinensis* at dose levels 471.50, 235.75, 117.88, 58.94, and 29.47 µg/cm^2 on filter paper. The data was recorded with 1 hour interval for up to 5 hours of exposure and the percent repulsion data was then subjected to ANOVA after transforming into arcsin percentage values Appendix Tables (116 - 122); and the result has been presented in Tables (9-11). The F values have been established were 11.155, 10.771, 11.481, 36.693, 39.862, 32.042, 38.787, 5.993, 3.622, 0.439, 5.191 and 3.514 for the analysis between doses and 0.260, 3.867, 0.772, 0.970, 1.176, 1.241, 0.134, 0.229, 0.939, 0.720, 0.458 and 1.514 for the analysis between time interval for seed, leaves and stem wood of petroleum ether, acetone, chloroform and methanol extracts respectively.

Among the tested $CHCl_3$ extracts all the rest offered repellency at 0.01% level of significance ($P<0.001$) except the stem (acetone) extract which was found active at 0.1% level of significance ($P<0.01$). According to the intensity of repellency the result could be arranged in a descending order: leaf (pet. ether extract) >leaf (chloroform extract) >seed (methanol extract) > stem wood (acetone) extract and in all the cases significant differences.

Table 9: ANOVA results of repellency by *A. pavonina* seed extracts against *C. chinensis* adult.

Test material	Extract	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Seed	Petro. Ether	Dose effect	1469.839	4	367.4598	11.15526**	0.000162
		Time effect	34.31488	4	8.57872	0.260431	0.898986
		Error	527.0479	16	32.9405		
		Total	2031.202	24			
Seed	Acetone	Dose effect	1133.241	4	283.3103	10.77072**	0.000198
		Time effect	406.8754	4	101.7188	3.867084	0.022031
		Error	420.8602	16	26.30376		
		Total	1960.977	24			
Seed	Chloroform	Dose effect	710.1249	4	177.5312	11.48068**	0.000138
		Time effect	47.72102	4	11.93025	0.771512	0.559418
		Error	247.4155	16	15.46347		
		Total	1005.261	24			
Seed	Methanol	Dose effect	2403.7	4	600.925	36.69287***	7.16E-08
		Time effect	63.5225	4	15.88063	0.969681	0.45108
		Error	262.0346	16	16.37716		
		Total	2729.257	24			

(* * * =Highly significant, * * = Significant, * = Insignificant).

Table 10: ANOVA results of repellency by *A. pavonina* leaf extracts against *C. chinensis* adult.

Test material	Extract	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Leaf	Petro. Ether	Dose effect	4327.371	4	1081.843	39.86151***	3.96E-08
		Time effect	127.625	4	31.90624	1.175615	0.358588
		Error	434.2405	16	27.14003		
		Total	4889.237	24			
Leaf	Acetone	Dose effect	4332.852	4	1083.213	32.04243***	1.87E-07
		Time effect	167.7981	4	41.94952	1.240905	0.333239
		Error	540.8893	16	33.80558		
		Total	5041.539	24			
Leaf	Chloroform	Dose effect	3000.608	4	750.1519	38.78669***	4.81E-08
		Time effect	10.39778	4	2.599446	0.134405	0.967322
		Error	309.4472	16	19.34045		
		Total	3320.453	24			
Leaf	Methanol	Dose effect	876.2613	4	219.0653	5.993308**	0.00382
		Time effect	33.54518	4	8.386294	0.229437	0.917832
		Error	584.8265	16	36.55166		
		Total	1494.633	24			

(* * * =Highly significant, * * = Significant, * = Insignificant).

Table 11: ANOVA results of repellency by *A. pavonina* stem extracts against *C. chinensis* adult.

Test material	Extract	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Stem	Petro. Ether	Dose effect	3734.096	4	933.524	3.621934**	0.027628
		Time effect	968.5539	4	242.1385	0.939461	0.466351
		Error	4123.87	16	257.7419		
		Total	8826.519	24			
Stem	Acetone	Dose effect	185.7219	4	46.43046	0.43863*	0.778896
		Time effect	304.8857	4	76.22142	0.720066	0.590654
		Error	1693.655	16	105.8534		
		Total	2184.262	24			
Stem	Chloroform	Dose effect	1333.825	4	333.4564	5.190596**	0.007104
		Time effect	117.7617	4	29.44043	0.458271	0.7652
		Error	1027.879	16	64.24241		
		Total	2479.466	24			
Stem	Methanol	Dose effect	1511.371	4	377.8427	3.513904**	0.030581
		Time effect	651.1877	4	162.7969	1.513997	0.245151
		Error	1720.446	16	107.5279		
		Total	3883.005	24			

(* * * =Highly significant, * * = Significant, * = Insignificant).

4.1.4. Antimicrobial activities of the test extracts

4.1.4.1. Antibacterial activity

The anitbacterial activity of *A. pavonina* extractives collected in petroleum ether, acetone, chloroform and methanol of leaves, seed and stem wood were tested against 15 bacteria (6 Gram-positive bacteria *S. aureus, B. cereus, B. megaterium, B. subtilis, S. lutea, S.-β -haemolyticus* and 9 Gram-negative bacteria *S. typhi, S. dysenteriae, S. shiga, S. sonnei, S. boydii, E. coli, Klebsiella* sp., *P. aeruginosa, Proteus* sp. at concentrations of 50 and 200µg/disc along with a standard antibiotic, Ciprofloxacin 30µg/disc. The results obtained are shown in Tables (12-17) and Plates (18-20).

4.1.4.1.1. Antibacterial activity of the seed extracts

4.1.4.1.1.2. Antibacterial activity of the seed (chloroform and methanol) extracts

For the seed ($CHCl_3$) extract only *B. cereus*, *B. megaterium*, *S .- β -haemolyticus*, *S. dysenteriae* and *E. coli* were responsive with inhibition zones 14,15, 25,18, 22 mm and 12, 10 mm for 200 and 50 µg/disc application, and for the MeOH extract *B. cereus*, *B. megaterium, B. subtilis, S .-β-haemolyticus* and *Klebsiella* sp. were responsive with inhibition zones 18, 16, 20, 21, 16 mm and 10, 10, 09 mm respectively for the same doses (Table 12); while the inhibition zones for the standard Ciprofloxacin 30µg/disc were 28, 30, 32, 32, 29, 29, and 31mm for the above mentioned test agents respectively.

Table 12: Antibacterial activity of the seed (chloroform and methanol) extracts of *A. pavonina* and the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
Gram positive bacteria					
S. aureus		-		-	26
B. cereus	-	14	-	18	28
B. megaterium	-	15	-	16	30
B. subtilis	-	-	10	20	32
S. lutea	-	-	-	-	26
S.-β -haemolyticus	12	25	10	21	32
Gram negative bacteria					
S. typhi	-	-	-	-	33
S. dysenteriae	10	18	-	-	29
S. shiga	-	-	-	-	30
S. sonnei	-	-	-	-	30
S. boydii	-	-	-	-	29
E. coli	-	22	-	-	29
Klebsiella sp.			09	16	31
P. aeruginosa	-	-	-	-	31
Proteus sp.	-	-	-	-	33

4.1.4.1.1.3. Antibacterial activity of the seed (Pet. ether and Acetone) extracts

For the seed (Pet. ether) extract only *B. cereus*, *B. megaterium, S. lutea* *S .- β -haemolyticus*, *S. typhi*, *S. dysenteriae, S. boydii* and *Klebsiella* sp. were responsive with inhibition zones 21, 20, 22, 18, 20, 21, 16, 20 mm and 11, 10, 12, 09, 10, 11, 09, 12 mm for 200 and 50 µg/disc application, and for the acetone extract *B. cereus*, *B. megaterium, S. lutea, S. dysenteriae, S. boydii* and *Klebsiella* sp. were responsive with inhibition zones 16,15,21,16,18,19 mm and 07, 06, 10, 08,11,10 mm respectively for the same doses (Table 13); while the inhibition zones for the standard Ciprofloxacin 30 µg/disc were 28, 30, 26, 32, 33, 29, 29, and 31 mm for the above mentioned test agents respectively.

Table 13: Antibacterial activity of the seed (pet. ether and acetone) extracts of *A. pavonina* and the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Pet. ether extract		Acetone extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
Gram positive bacteria					
S. aureus	-	-	-	-	26
B. cereus	11	21	07	16	28
B. megaterium	10	20	06	15	30
B. subtilis	-	-	-	-	32
S. lutea	12	22	10	21	26
S.-β -haemolyticus	09	18	-	-	32
Gram negative bacteria					
S. typhi	10	20	-	-	33
S. dysenteriae	11	21	08	16	29
S. shiga	-	-	-	-	30
S. sonnei	-	-	-	-	30
S. boydii	09	16	11	18	29
E. coli	-	-	-	-	29
Klebsiella sp.	12	20	10	19	31
P. aeruginosa	-	-	-	-	31
Proteus sp.	-	-	-	-	33

4.1.4.1.2. Antibacterial activity of the leaf (chloroform and methanol) extracts

The leaf (chloroform) extracts were responsive to *S. aureus, B. megaterium, S.-β -haemolyticus , S. typhi, S. dysenteriae* and *Klebsiella* sp. with inhibition zones 16, 15, 20,15, 17,18 mm and 08, 09,10, 08, 06 and 08 mm for 200 and 50µg/disc application, and the MeOH extract was responsive to *S. aureus, B. megaterium, S.-β -haemolyticus , S. typhi* and *Klebsiella* sp. with inhibition zones 18, 22, 17, 16,14 mm and 09,10, 08, 07 and 06 mm respectively for the same doses (Table 14); while the inhibition zones for the standard Ciprofloxacin 30µg/disc were 33, 31, 30, 31, 30 and 28 mm for the above mentioned test agents respectively.

Table 14: Antibacterial activity of the leaf (chloroform and methanol) extracts of *A. pavonina* and the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
Gram positive bacteria					
S. aureus	08	16	09	18	33
B. cereus	-	-	-	-	28
B. megaterium	09	15	10	22	31
B. subtilis	-	-	-	-	30
S. lutea	-	-	-	-	31
S.- β -haemolyticus	10	20	08	17	30
Gram negative bacteria					
S. typhi	08	15	07	16	31
S. dysenteriae	06	17	-	-	30
S. shiga	-	-	-	-	31
S. sonnei	-	-	-	-	30
S. boydii	-	-	-	-	30
E. coli	-	-	-	-	31
Klebsiella sp.	08	18	06	14	28
P. aeruginosa	-	-	-	-	30
Proteus sp.	-	-	-	-	31

4.1.4.1.2.1 Antibacterial activity of the leaf (Pet. ether and Acetone) extracts

The leaf extract (pet. ether and acetone) was responsive to *B. cereus*, *B. megaterium, S.- β –haemolyticus, S. dysenteriae, S. shiga, S. boydii* and *Klebsiella* sp. with inhibition zones 19, 21, 23, 17, 18, 24, 19 mm and 09,10,11,09,08,12 and 09 mm for 200 and 50µg/disc application, and the acetone extract was responsive to *B. cereus, B. megaterium, S. dysenteriae, S. shiga* and *S. boydii* with inhibition zones 20, 24, 18, 20, 21 mm and 10,12, 08, 09 and 10mm respectively for the same doses (Table 15); while the inhibition zones for the standard Ciprofloxacin 30µg/disc were 30, 31, 30, 30, 31, 30 and 28 mm for the above mentioned test agents respectively.

Table 15: Antibacterial activity of the leaf (Pet. ether and acetone) extracts of *A. pavonina* and the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Pet. Ether extract		Acetone extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
Gram positive bacteria					
S. aureus	-	-	-	-	33
B. cereus	09	19	10	20	30
B. megaterium	10	21	12	24	31
B. subtilis	-	-	-	-	30
S. lutea	-	-	-	-	31
S.- β -haemolyticus	11	23	-	-	30
Gram negative bacteria					
S. typhi	-	--	-	-	31
S. dysenteriae	09	17	08	18	30
S. shiga	08	18	09	20	31
S. sonnei	-	-	-	-	30
S. boydii	12	24	10	21	30
E. coli	-	-	-	-	31
Klebsiella sp.	09	19	-	-	28
P. aeruginosa	-	-	-	-	30
Proteus sp.	-	-	-	-	31

4.1.4.1.3. Antibacterial activity of the stem wood (chloroform and methanol) extracts

The stem wood (chloroform) extracts were responsive to *B. cereus*, *B. megaterium, B. subtilis, S.-β-haemolyticus*, *S. typhi*, *S. dysenteriae*, *S. sonnei,* and *Klebsiella sp*. with inhibition zones 20, 20, 18, 22, 21, 20, 21 and 20 mm and 9, 8, 7, 10, 9, 8, 9 and 8 mm for 200 and 50 µg/disc application, and the MeOH extract was responsive to *B. cereus*, *B. subtilis, S.-β-haemolyticus*, *S. typhi* and *S. boydii* with inhibition zones 15, 14, 17, 18, 16 mm and 6, 6, 7, 8 and 7 mm for the same doses (Table 16); while the inhibition zones for the standard Ciprofloxacin 30 µg/disc were 28, 30, 30, 33, 33, 30, 31, 31 and 28 mm for the above mentioned test agents respectively.

Table 16: Antibacterial activity of stem wood (chloroform and methanol) Of *A. pavonina* and standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/dic	
Gram positive bacteria					
S. aureus	-	-	-	-	28
B. cereus	09	20	06	15	28
B. megaterium	08	20	-	-	30
B. subtilis	07	18	05	14	30
S. lutea	-	-	-	-	31
S.- β -haemolyticus	10	22	07	17	33
Gram negative bacteria					
S. typhi	09	21	08	18	33
S. dysenteriae	08	20	-	-	30
S. shiga	-	-	-	-	30
S. sonnei	09	21	-	-	31
S. boydii	-	-	07	16	31
E. coli	-	-	-	-	30
Klebsiella sp.	08	20	-	-	28
P. aeruginosa	-	-	-	-	30
Proteus sp.	-	-	-	-	28

4.1.4.1.3.1 Antibacterial activity of the stem wood (Pet. ether and acetone) extracts

In case of the stem wood extract (Pet. ether) *B. cereus*, *B. megaterium, S.-β-haemolyticus*, *S. dysenteriae, S. sonnei* and *Klebsiella* sp. were responsive with inhibition zones 20, 20, 23, 20, 20, 16mm and 09, 08, 10, 10, 08, 08 mm for 200 and 50 µg/disc application, and for the acetone extract *S. aureus, B. cereus*, *S.-β-haemolyticus*, *S. dysenteriae S. sonnei* and *E. coli* were responsive with inhibition zones 17, 15, 16, 15, 18, 14, 16mm and 07, 06, 07, ,09, 06, 07mm for the same doses (Table 17); while the inhibition zones for the standard Ciprofloxacin 30 µg/disc were 30, 28, 30, 30, 30, 31 and 30mm for the above mentioned test agents respectively.

Table 17: Antibacterial activity of the stem wood (pet. ether and acetone) extracts of *A. pavonina* and the standard Ciprofloxacin.

Test organisms	Diameter of zone of inhibition (in mm)				
	Pet. ether extract		Acetone extract		Ciprofloxacin 30µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
Gram positive bacteria.					
S. aureus	-	-	07	17	30
B. cereus	09	20	06	15	28
B. megaterium	08	20	-	-	30
B. subtilis	-	-	-	-	31
S. lutea	-	-	-	-	31
S.- β -haemolyticus	10	23	07	16	30
Gram negative bacteria					
S. typhi	-	-	-	-	33
S. dysenteriae	10	20	09	18	30
S. shiga	-	-	-	-	31
S. sonnei	08	20	06	14	30
S. boydii	-	-	-	-	33
E. coli	-	-	07	16	31
Klebsiella sp.	08	16	-	-	30
P. aeruginosa	-	-	-	-	33
Proteus sp.	-	-	-	-	31

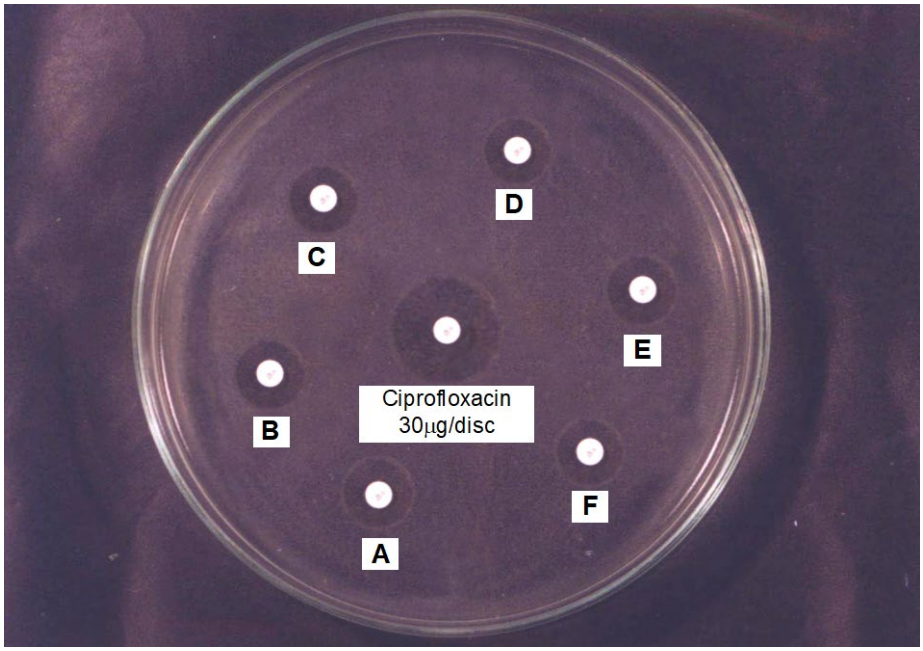

Plate 18: Effect of chloroform, methanol, pet. ether and acetone extracts from seed of *A. pavonina* and the standard Ciprofloxacin (30µg/disc) against A: *S.-β –haemolyticus*; B: *S. dysenteriae;* C: *E. coli*; D: *Klebsiella* sp.; E: *B. cereus*; F: *B. megaterium*

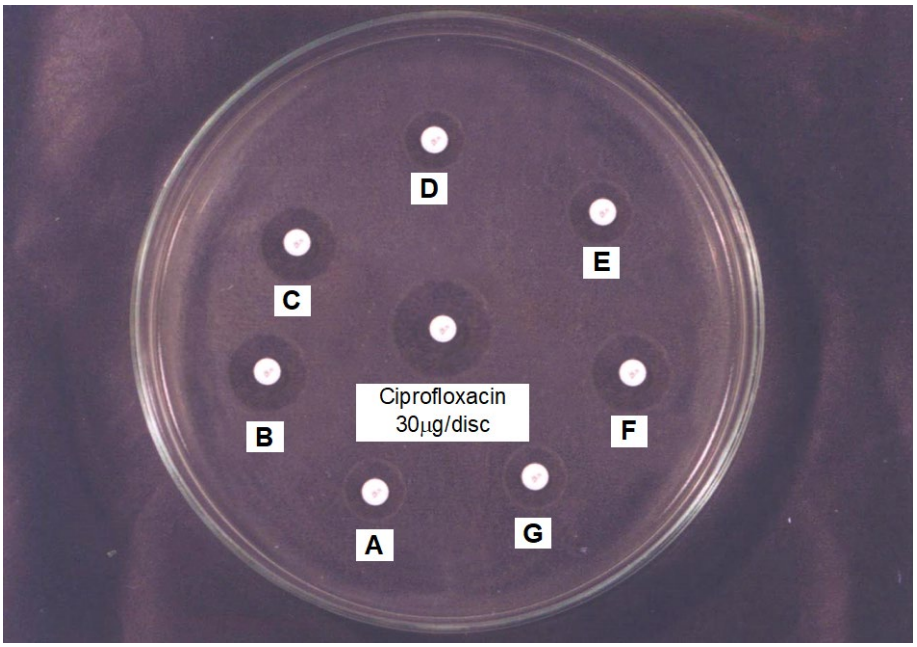

Plate 19: Effect of chloroform and methanol, pet. ether and acetone extracts from leaf of *A. pavonina* and the standard Ciprofloxacin (30µg/disc) against A: *S. aureus*; B: *B. megaterium*; C: *S.-β -haemolyticus*; D: *S. dysenteriae*; E: *S. shiga*; F: *S. boydii*; G: *Klebsiella* sp.

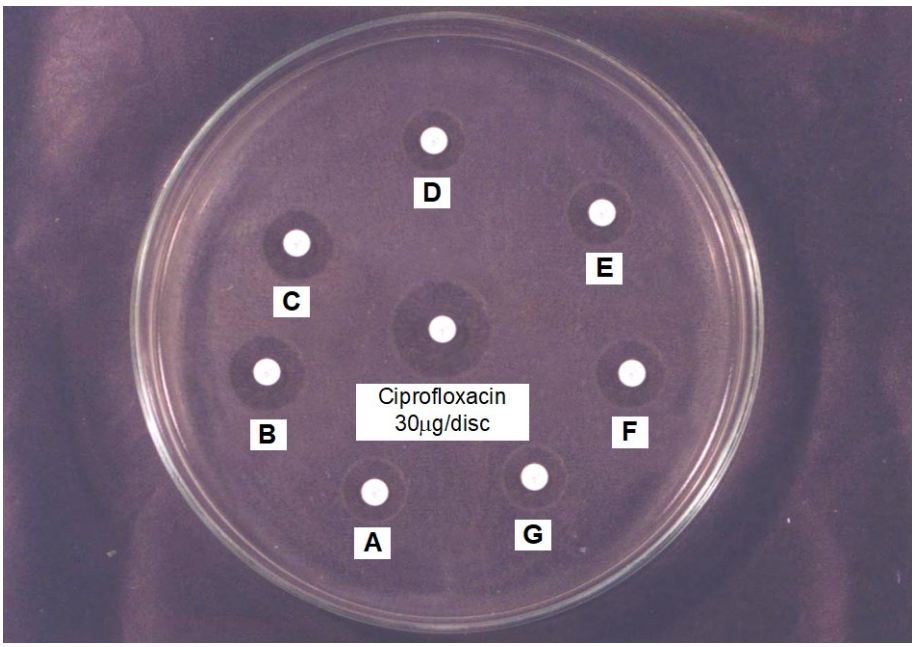

Plate 20: Effect of chloroform and methanol, pet. ether and acetone extracts from stem wood of *A. pavonina* and the standard Ciprofloxacin (30µg/disc) against A: *B. megaterium*; B: *S.-β -haemolyticus;* C: *S. typhi;* D: *S. dysenteriae;* E: *S. sonnei;* F: *Klebsiella* sp.; G: *B. cereus*

Minimum inhibitory concentrations (MICs) against test bacteria

Among all the chloroform, methanol, pet. ether and acetone extracts of the leaves, seed and stem wood of *A. pavonina* only chloroform extracts of the seed and the stem wood were subjected to evaluate the minimum inhibition zones just depending on the intensity of activity. This was done due to lack of adequate laboratory supports. The results of the MIC values have been presented in Tables (18 to 19). The MIC value of the chloroform extract of the seed was 256µg/ml against *B. cereus,* and 128µg/ml against *S.- β – haemolyticus, S. dysenteriae* and 64µg/ml against *Klebsiella* sp.

Table: 18 Minimum inhibitory concentrations (MICs) of the chloroform extract of seed against four pathogenic bacteria.

Test tube No.	Nutrient broth medium added (ml)	Seed extract (µg/ml)	Inoculum added (µl)	*S. -β- haemolyticus*	*S. dysenteriae*	*Klebsiella sp.*	*B. cereus*
1	1	512	10	-	-	-	-
2	1	256	10	-	-	-	-
3	1	128	10	-	-	-	+
4	1	64	10	+	-	+	+
5	1	32	10	+	+	+	+
6	1	16	10	+	+	+	+
7	1	8	10	+	+	+	+
8	1	4	10	+	+	+	+
9	1	2	10	+	+	+	+
10	1	1	10	+	+	+	+
Cm	1	0	0	-	-	-	-
Cs	1	512	0	-	-	-	-
Ci	1	0	10	+	+	+	+
Results of MIC values in (µg/ml)				128	128	64	256

+ = Growth - = No growth

The MIC values of the chloroform extract of seed were 128µg/ml against *S.- β –haemolyticus, S. dysenteriae;* 64µg/ml against *Klebsiella* sp. and 256µg/ml against *B. cereus*.

Table: 19. Minimum inhibitory concentrations (MICs) of the chloroform extract from the stem wood against four pathogenic bacteria.

Test tube No.	Nutrient broth medium added (ml)	Stem wood extract (µg/ml)	Inoculum added (µl)	*S. - β - haemolyticus*	*S. sonnei*	*S. typhi*	*B. megaterium*
1	1	512	10	-	-	-	-
2	1	256	10	-	-	-	-
3	1	128	10	+	-	-	-
4	1	64	10	+	+	-	+
5	1	32	10	+	+	+	+
6	1	16	10	+	+	+	+
7	1	8	10	+	+	+	+
8	1	4	10	+	+	+	+
9	1	2	10	+	+	+	+
10	1	1	10	+	+	+	+
Cm	1	0	0	-	-	-	-
Cs	1	512	0	-	-	-	-
Ci	1	0	10	+	+	+	+
Results of MIC values in (µg/ml)				128	64	32	64

+ = Growth - = No growth.

The MIC values of the chloroform extract of stem wood were 128µg/ml against *S.- β –haemolyticus;* 64µg/ml against *B. megaterium, S. sonnei* and 32µg/ml against *S. typhi*.

Antifungal activity

Antifungal activity of the *A. pavonina* extractives collected in petroleum ether, acetone, chloroform and methanol of leaves, seed and stem wood were tested against seven pathogenic fungi *F. vasinfectum*, *A. fumigatus*, *A. niger*,

A. flavus, Mucor sp., *C. albicans*, *P. notatum* at concentrations of 50 and 200µg/disc along with a standard Nystatin (50µg/disc). The results obtained are shown in Tables (20-25) and Plates (21-23).

Antifungal activity of the leaf (chloroform and methanol) extracts

In case of the leaf extracts (chloroform) *A. fumigatus* and *C. albicans* were responsive with inhibition zones 16, 19mm; and 09, 10mm for 200 and 50µg/disc application, and for the methanol extract *A. fumigatus, Mucor* sp. and *C. albicans* were responsive with inhibition zones 12, 13, 17mm; and 06, 07, 09mm for the same doses (Table 20), while the inhibition zones for the standard Nystatin 50µg/disc were 30, 30, 28mm for the above mentioned test agents respectively.

Table 20: Antifungal activity of leaf (chloroform and methanol) extracts of *A. pavonina* and standard Nystatin.

Test Fungi	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	-	-	-	-	28
A. fumigatus	09	16	06	12	30
A. niger	-	-	-	-	28
A. flavus	-	-	-	-	26
Mucor sp.	-	-	07	13	30
C. albicans	10	19	09	17	28
P. notatum	-	-	-	-	30

Antifungal activity of leaf (pet. ether and acetone) extracts

For the leaf extract (Pet. ether) *F. vasinfectum*, *A. flavus* and *C. albicans* were responsive with inhibition zones 18, 16, 19 mm and 09, 07,10mm for 200 and 50 µg/disc application, and for the acetone extract *F. vasinfectum* and *C. albicans* were responsive with inhibition zones 16, 15mm and 08, 07mm for the same doses (Table 21) while the inhibition zones for the standard Nystatin 50 µg/disc were 28, 31 and 28mm for the above mentioned test agents respectively.

Table: 21 **Antifungal activity of leaf (pet. ether and acetone) extracts of *A. pavonina* and standard Nystatin.**

Test Fungi	Diameter of zone of inhibition (in mm)				
	Pet. Ether extract		Acetone extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	09	18	08	16	28
A. fumigatus	-	-	-	-	30
A. niger	-	-	-		28
A. flavus	07	16	-	-	31
Mucor sp.	-		-	-	30
C. albicans	10	19	07	15	28
P. notatum	-	-	-	-	30

Antifungal activity of stem wood (chloroform and methanol) extracts

In case of the stem wood extract (chloroform) *F. vasinfectum*, *A. fumigatus and C. albicans* were responsive with inhibition zones 15, 16, 16mm and 07, 08, 09mm for 200 and 50µg/disc application, and for the methanol extract *F. vasinfectum*, *A. fumigatus, Mucor* sp. and *C. albicans* were responsive with inhibition zones 14, 11, 13, 15 mm and 07, 06, 07, 08mm for the same doses (Table 22); while the inhibition zones for the standard Nystatin 50µg/disc were 29, 32, 31and 30 mm for the above mentioned test agents respectively.

Table: 22 Antifungal activity of stem wood (chloroform and methanol) extracts of *A. pavonina* and standard Nystatin.

Test Fungi	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	07	15	07	14	29
A. fumigatus	08	16	06	11	32
A. niger	-	-	-	-	30
A. flavus	-	-	-	-	28
Mucor sp.	-	-	07	13	31
C. albicans	09	16	08	15	30
P. notatum	-	-	-	-	28

Antifungal activity of stem wood (pet. ether and acetone) extracts

For the stem wood extract (Pet. ether) *F. vasinfectum, Mucor* sp. and *P. notatum* were responsive with inhibition zones 16,17, 14mm and 08 ,07, 07 mm for 200 and 50 µg/disc application, and for the acetone extract *A. fumigatus, Mucor* sp. and *P. notatum* were responsive with inhibition zones 12, 13, 11mm and 06, 07, 06mm for the same doses (Table 23); while the inhibition zones for the standard Nystatin 50 µg/disc were 30, 31 , 28 and 30 mm for the above mentioned test fungi.

Table 23: Antifungal activity of stem wood (pet. ether and acetone) extracts of *A. pavonina* and the standard Nystatin.

Test Fungi	Diameter of zone of inhibition (in mm)				
	Pet. Ether extract		Acetone extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	08	16	-	-	30
A. fumigatus	-	-	06	12	31
A. niger	-	-	-	-	27
A. flavus	-	-	-	-	28
Mucor sp.	07	17	07	13	28
C. albicans	-	-	-	-	30
P. notatum	07	14	06	11	30

Antifungal activity of seed (chloroform and methanol) extracts

For the seed extract (chloroform) *F. vasinfectum*, *A. fumigatus, A. flavus* and *C. albicans* were responsive with inhibition zones 19, 17, 14,15mm and 10, 09, 07, 07mm for 200 and 50 µg/disc application, and for the methanol extract *A. fumigatus, A. flavus* and *C. albicans* were responsive with inhibition zones 12,10, 12 mm and 07, 05, 06 mm for the same doses (Table 24); while the inhibition zones for the standard Nystatin 50 µg/disc were 30, 33, 30, 32mm for the above mentioned test agents respectively.

Table 24: Antifungal activity of seed (chloroform and methanol) extracts of *A. pavonina* and the standard Nystatin.

Test Fungi	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	10	19	-	-	30
A. fumigatus	09	17	07	12	33
A. niger	-	-	-	-	33
A. flavus	07	14	05	10	30
Mucor sp.	-	-	-	-	30
C. albicans	07	15	06	12	32
P. notatum	-	-	-	-	28

Antifungal activity of seed (pet. ether and acetone) extracts

In case of the seed extract (pet. ether) only *A. fumigatus* and *P. notatum* were responsive with inhibition zones 18, 16mm and 09, 08mm for 200 and 50 µg/disc application, and for the acetone extract *A. fumigatus, Mucor* sp. and *P. notatum* were responsive with inhibition zones 15, 16, 12mm and 07,08,06 mm for the same doses (Table 25); while the inhibition zones for the standard Nystatin 50 µg/disc were 30, 30 and 28 mm for the above mentioned test fungi respectively.

Table 25: Antifungal activity of seed (pet. ether and acetone) extracts of *A. pavonina* and the standard Nystatin.

Test Fungi	Diameter of zone of inhibition (in mm)				
	Pet. Ether extract		Acetone extract		Nystatin 50µg/disc
	50µg/disc	200µg/disc	50µg/disc	200µg/disc	
F. vasinfectum	-	-	-	-	30
A. fumigatus	09	18	07	15	30
A. niger	-	-	-	-	28
A. flavus	-	-	-	-	30
Mucor sp.	-	-	08	16	30
C. albicans	-	-	-	-	28
P. notatum	08	16	06	12	28

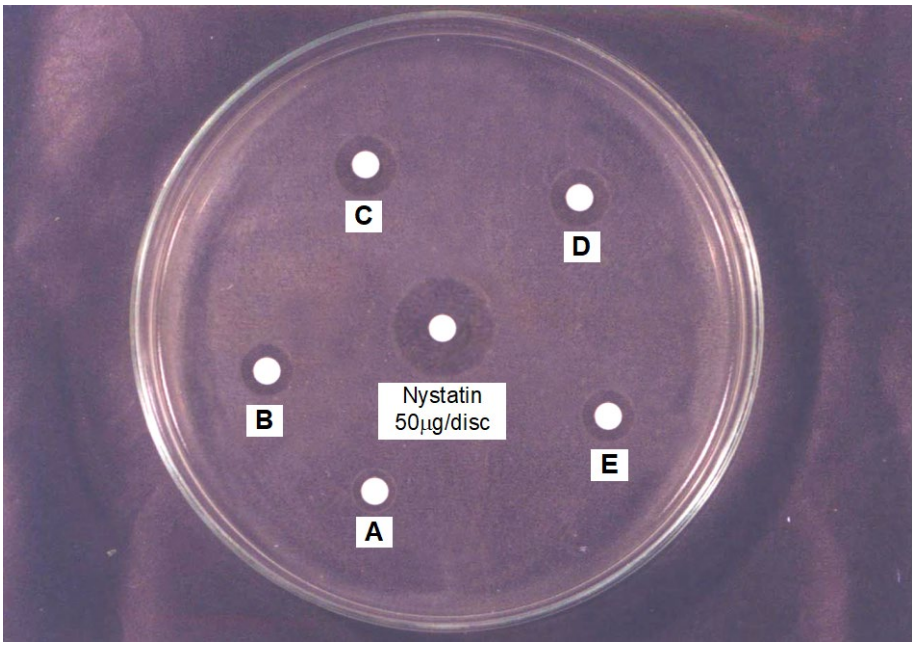

Plate 21: Effect of chloroform, methanol, pet. ether and acetone extracts from leaf of *A. pavonina* and the standard Nystatin (50µg/disc) against A: *Mucor* sp.; B: *A. fumigatus*; C: *C. albicans*; D: *A. flavus*; E: *F. vasinfectum*

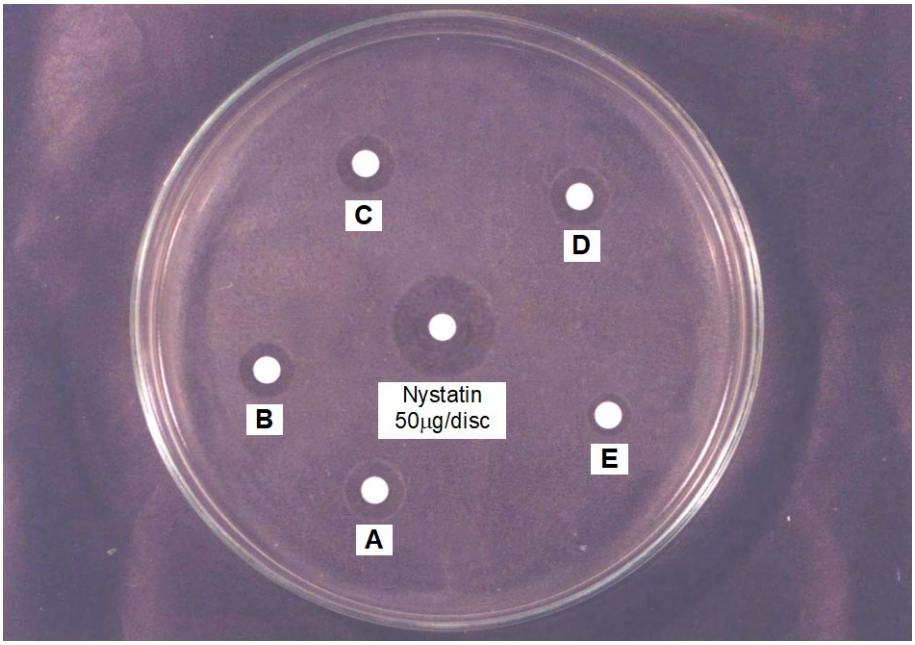

Plate 22: Effect of chloroform, methanol, pet. ether and acetone extracts from stem wood of *A. pavonina* and standard Nystatin (50µg/disc) against A: *A. fumigatus*; B: *Mucor* sp.; C: *C. albicans*; D: *F. vasinfectum*; E: *P. notatum*.

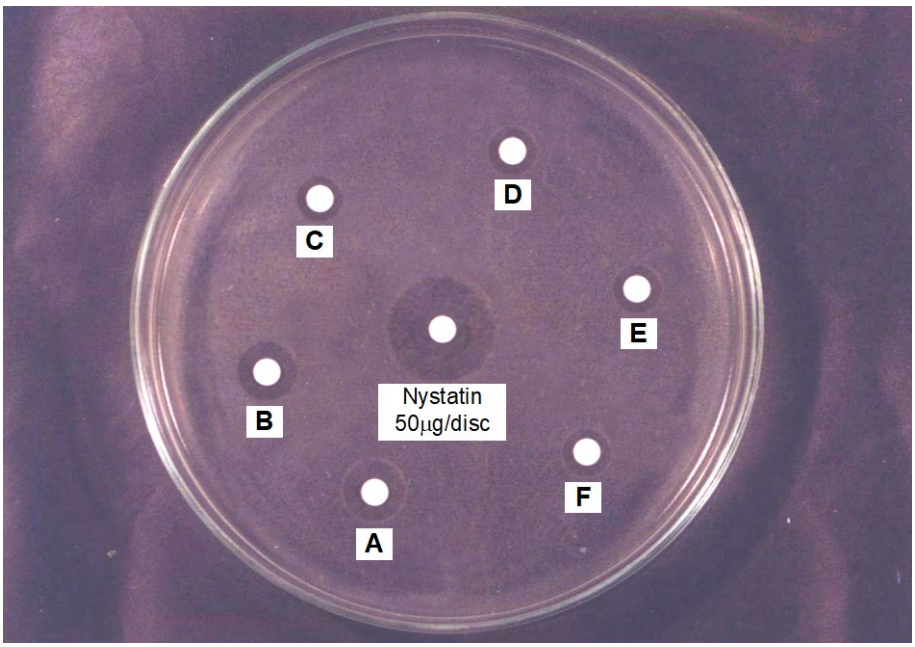

Plate 23: Effect of chloroform, methanol, pet. ether and acetone extracts from seed of *A. pavonina* and standard Nystatin (50µg/disc) against A: *F. vasinfectum*; B: *A. fumigatus*; C: *A. flavus*; D: *Mucor* sp.; E: *P. notatum*; F: *C. albicans*

Table 26: Summary of biological activity of the pet. ether, acetone, chloroform and methanol extracts of *A. pavonina* at a glance.

Test types	Activity traced	Leaves	Stem wood	Seed
Insecticidal activity	*C. chinensis*	√	√	√
Repellency	*C. chinensis*	√	√	√
Cytotoxicity	*A. salina*	√	√	√
Antimicrobial	Antibacterial activity	√*	√	√
	Antifungal activity	√	√	√

√= activity found, √*= weak activity found

Combined effect of plant extracts and parasitoid (*D. basalis*) against *C. chinensis* under laboratory conditions

Direct Contact Toxicity

Studies to determine the effect of contact toxicity of *D. basalis* on different doses of methanol and chloroform extracts of *A. pavonia* are presented in Figure 29.The data indicated that survivability of *D. basalis* on the above chemicals are varied. The percentage of survival was recorded 20% when *D. basalis* was 4th day of age but it was 7th day (20%) when *D. basalis* was supplied host containing seeds daily in case of methanol. Significant differences of survival of *D. basalis* between doses were noticed (P<0.000) (Appendix Table…..). The maximum longevity was observed on 6th day (30%) when *D. basalis* was provided no hosts at16 µg/cm^2. On the other hand, when *D. basalis* was supplied with host containing seeds the highest longevity was recorded 35% when the age of *D. basalis* 9 or 10 days at 4 µg/cm^2 but it was 10 days at 16 µg/cm^2 doses.

The data further showed that 60% mortality of *D. basalis* was recorded on the first day when offered 2 µg/cm^2 chloroform treated extracts but it was 50% on the second day at 0.5 µg/cm^2 when chloroform was supplied with hosts. It was 55% at 1 µg/cm^2 on second day. Significant differences of longevity of *D. basalis* was recorded between when *D. basalis* was supplied without host and host containing seeds (12-15-day old *C. chinensis*) (P<0.0001).

Maximum longevity was observed when *D. basalis* treated extracts of *A. pavonia* with methanol but it was minimum on chloroform.

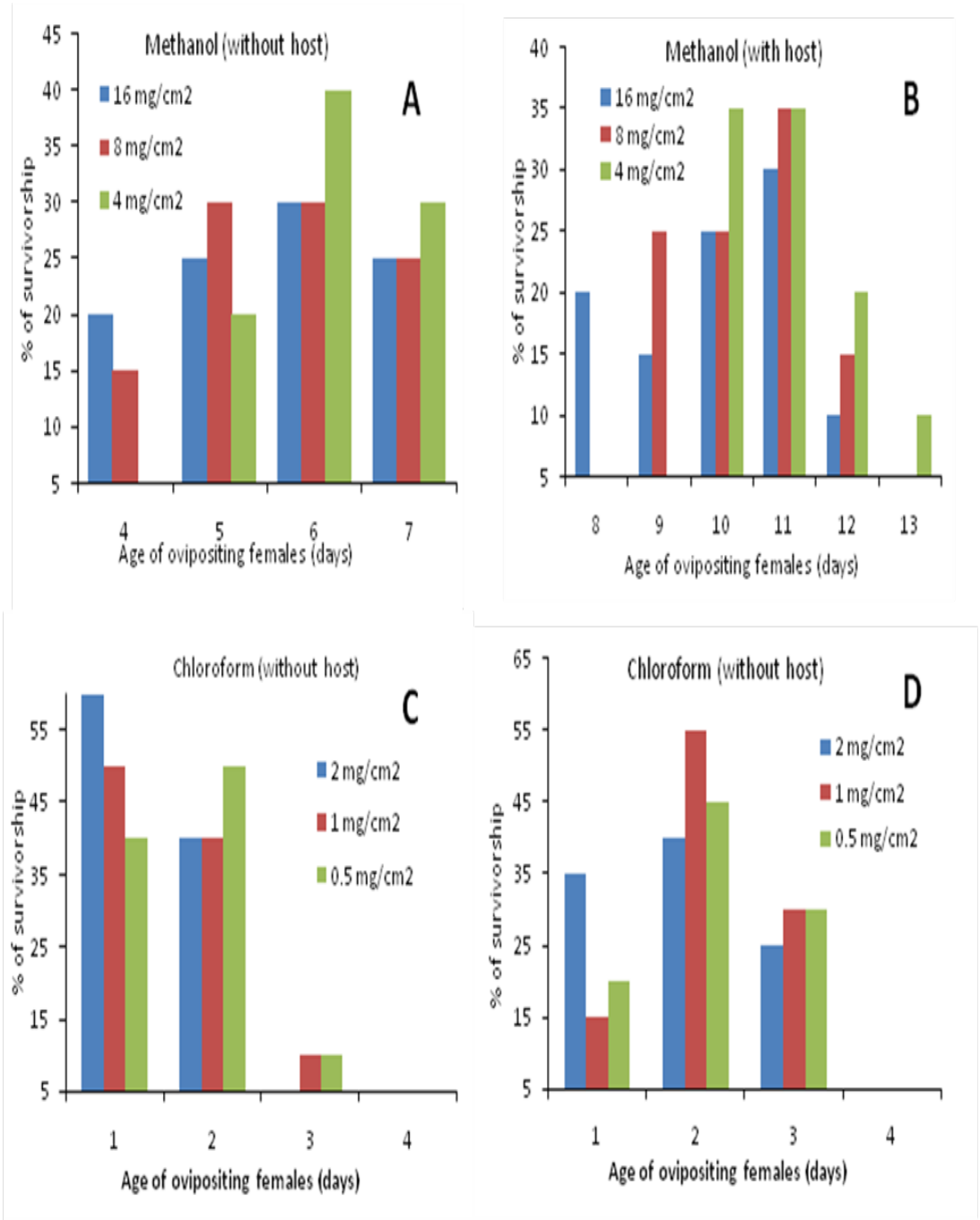

Figure 29: Percent survivorship of *D. basalis* in chloroform, methanol extracts of *A. pavonia* (without host – A and with host - B) and (without host - C and with host - D) infested seeds of green gram.

Choice Test

The F_1 progeny of *D. basalis* emerging from the treated and untreated methanol and chloroform extracts of *A. pavonia* are summarized in Tables (27 and 28) in separate doses. It was noted that methanol was most preferred by *D. basalis* than chloroform. Significant differences was noticed in progeny production ($P<0.0001$).

In methanol, maximum number of F_1 progeny were emerged after 96 h of oviposition. The minimum number was after 24 h. There was significant differences of F_1 progeny production among the tested doses ($P<0.0001$). Highest number of progeny (33.67) were emerged when the seeds were treated with dose 8 µg/cm^2.

Table 27. Number of emerged *D. basalis* adults from methanol treated infested seeds in different exposure periods.

Days of oviposition of parasitoids	Doses (mg/cm^2)	Range	Numbers of F_1 progeny of *D. basalis* (Mean ±)	Total
0			0	
	16	10-12	11.00 ± 0.58	
24	8	14-16	15.00 ± 0.58	
	4	14-16	15.00 ± 0.58	
	16	16-18	17.33 ± 0.67	
48	8	20-28	24.00 ± 2.31	
	4	20-23	21.67 ± 0.88	
	16	18-28	22.00 ± 3.06	
96	8	30-36	33.67 ± 1.86	
	4	28-34	31.33 ± 1.76	

ANOVA

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	6414.688	3	2138.229	5.517912	0.019917	3.862548
Columns	6747.188	3	2249.063	5.803928	0.01728	3.862548
Error	3487.563	9	387.5069			
Total	16649.44	15				

On the other hand, 24 h was optimum for progeny production D. basalis than 48 and 96 h when A. pavonia extracts was treated with choloroform. Significant differences were noticed among doses of extracts of *A. pavonia* (P<0.0001). The maximum progeny were produced when the seeds were treated with 1.5 µg/cm^2 (7.0).

Table 28. Number of emerged *D. basalis* adults from chloroform treated infested seeds in different exposure periods.

Days of oviposition of parasitoids	Doses (mg/cm^2)	Range	Numbers of F_1 progeny of *D. basalis* (Mean ±)	Total
0			0	40
	2	4-8	5.3 ± 1.33	
24	1	5-8	6.67 ± 0.88	
	1.5	6-8	7.00 ± 0.58	
	2	1-2	1.67 ± 0.33	
48	1	1-3	2.00 ± 0.58	
	1.5	1-4	1.67 ± 1.20	
	2			
96	1			
	1.5			

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	115.1875	3	38.39583	1.803915	0.216492	3.862548
Columns	543.1875	3	181.0625	8.506688	0.005411	3.862548
Error	191.5625	9	21.28472			
Total	849.9375	15				

No-choice Test

Table 29 shows both *C. chinensis* and *D. basalis* emergence un different release rates of *D. basalis* in no-choice test. In all the doses malathion treated seeds of *A. pavonia*, hosts and parasitoids were emerged successfully. The highest number of *C. chinensis* (28.00) was emerged at 4 mg/cm^2 after 1 day of release of *D. basalis* and lowest number was 207.33 at the same doses after 7 day of release in malathion treated seeds. There was highly significant

differences noticed among doses (P<0.0001) and as well as release rates (P<0.0001) of *C. chinensis*.

The maximum number of *D. basalis* (87.67) was emerged at 4 mg/cm^2 at the release rates of 7 day where as minimum number at 16 mg/cm^2 13.67 after 1 day release of *D. basalis*.

The highest emergence of *C. chinensis* (296.0) was recorded at 1.5 and 2.0 doses after 3 and 5 days of release when *A. pavonia* seeds were treated with different doses of chloroform (Table 30). The emergence of *C. chinensis* in different doses was well as different release rates were highly significant (P<0.0001). On the other hand, emergence of *D. basalis* was highest (14.33) at 1.5 dose of *A. pavonia* after 1 day release but minimum (1.0) at 2 mg/cm^2 doses. No parasitoid was emerged after 7 day of release of *D. basalis*. Both the doses and release rates were highly significant (P<0.0001).

Table 29 Number of emerged *C. chinensis* and *D. basalis* emerged from infested seeds treated with methanol in different durations.

Days after release of parasitoids	Doses (mg/cm^2)	Number of *C. chinensis* * (Mean ± SE)	Number of *D. basalis* * (Mean ± SE)	Total
0		291.0 (287-296)	0	291.0
	16	276.33 ± 0.88 (275-278)	13.67 ± 0.88 (12-15)	290.00 ± 0.0
1	8	279.67 ± 0.88 (278-281)	15.00 ± 0.58 (14-16)	294.67 ± 1.33
	4	280.00 ± 1.0 (279-282)	16.00 ± 1.0 (15-18)	296.00 ± 2.0
	16	257.67 ± 3.84 (250-262)	28.33 ± 1.76 (25-31)	286.33 ± 4.3
3	8	246.67 ± 1.76 (244-250)	33.00 ± 1.15 (31-35)	279.67 ± 0.67
	4	248.67 ± 5.2 (240-258)	34.00 ± 2.0 (30-36)	282.67 ± 3.52
	16	240.67 ± 1.45 (238-243)	46.67 ± 1.76 (44-50)	287.33 ± 2.85
5	8	235.33 ± 2.9 (230-240)	58.33 ± 0.88 (57-60)	293.67 ± 3.38
	4	222.33 ± 1.45 (220-225)	69.00 ± 2.08 (66-73)	291.33 ± 0.88
	16	217.67 ± 1.45 (215-220)	76.33 ± 0.88 (69-72)	288.00 ± 2.0
7	8	212.33 ± 1.45 (210-215)	81.33 ± 2.9 (76-86)	293.67 ± 2.18
	4	207.33 ± 3.71 (200-212)	87.67 ± 4.63 (80-96)	295.00 ± 2.65

* Range in parenthesis

ANOVA

Number of *C. chinensis* emerged from infested seeds treated with methanol in different durations.

Source of Variation	SS	DF	MS	F	P
Subject	546.847	8			
A	4406.36	3	1468.787	162.2485	<0.0001
Subj x A	217.2639	24	9.0527		
B	1538286.75	3	512762.25	13706.0062	<0.0001
Subj x B	897.875	24	37.4115		
A x B	17123.0833	9	1902.5648	210.1655	<0.0001
Subj x A x B	651.7917	72	9.0527		
Total	1562129.9722	143			

Number of *D. basalis* emerged from infested seeds treated with methanol in different durations.

Source of Variation	SS	DF	MS	F	P
Subject	107.5556	8			
A	45	3	15	Infinity	<0.0001
Subj x A	0	24	0		
B	3616	3	1205.3333	32.2857	<0.0001
Subj x B	896	24	37.3333		
A x B	135	9	15	Infinity	<0.0001
Subj x A x B	0	72	0		
Total	4799.5556	143			

Table 30. Number of C*. chinensis* and *D. basalis* emerged from infested seeds treated with chloroform in different durations.

Days after release of parasitoids	Doses (mg/cm^2)	Number of *C. chinensis* *(Mean ± SE)	Number of *D. basalis* *(Mean ± SE)	Total
0		294.0 (287-296)	0	291.0 ±0.0
	2	284.33 ± 2.19 (280-287)	8.0 ± 0.58 (7-9)	292.33 ± 1.67
1	1	284.33 ± 1.2 (282-286)	11.00 ± 0.58 (10-12)	295.33 ± 1.76
	1.5	272.00 ± 2.5 (269-277)	14.33 ± 0.33 (14-15)	286.33 ± 2.33
	2	289.00 ± 1.53 (286-298)	8.33 ± 0.88 (7-10)	297.33 ± 0.67
3	1	289.67 ± 0.88 (288-291)	9.00 ± 0.0 (9-9)	298.67 ± 2.96
	1.5	282.00 ± 3.05 (278-288)	10.33 ± 0.33 (10-11)	292.33 ± 3.05
	2	296.00 ± 1.15 (294-298)	1.00 ± 0.58 (1-2)	297.00 ± 1.33
5	1	292.67 ± 1.33 (290-294)	2.00 ± 0.58 (1-2)	294.67 ± 0.88
	1.5	296.00 ± 0.58 (295-297)	0.33 ± 0.33 (0-1)	246.33 ± 0.58
	2	295.00 ± 0.58 (294-296)	0.0 ± 0.0 (0-0)	295.00 ± 0.58
7	1	286.67 ± 2.40 (282-290)	0.0 ± 0.0 (0-0)	286.67 ± 2.40
	1.5	287.33 ± 1.67 (284-289)	0.0 ± 0.0 (80-96)	287.33 ± 1.67

* Range in parenthesis

ANOVA

Number of *C. chinensis* emerged from infested seeds treated with chloroform in different durations.

Source of Variation	SS	DF	MS	F	P
Subject	71.8472	8			
A	422.5764	3	140.8588	27.6562	<0.0001
Subj x A	122.2361	24	5.0932		
B	2197076.85	3	732358.9514	60357.4302	<0.0001
Subj x B	291.2083	24	12.1337		
A x B	633.7292	9	70.4144	13.8252	<0.0001
Subj x A x B	366.7084	72	5.0932		
Total	2198985.1597	143			

Number of *D. basalis* emerged from infested seeds treated with chloroform in different durations.

Source of Variation	SS	DF	MS	F	P
Subject	12.8472	8			
A	64.5556	3	21.5185	13.4777	<0.0001
Subj x A	38.3194	24	1.5966		
B	462.75	3	154.25	57.956	<0.0001
Subj x B	63.875	24	2.6615		
A x B	929.6667	9	103.2963	64.6977	<0.0001
Subj x A x B	114.9584	72	1.5966		
Total	1686.9722	143			

Combined effect of plant extracts and parasitoid

The combined effect of *C. chinensis* at different doses of malathion and chloroform of *A. pavonia* extracts by *D. basalis* are presented in Tables (31 and 32) *D. basalis* exhibited a significant suppression of *C. chinensis* at all the doses tested (P<0.0001). Highest population was recorded in malathion but lowest in chloroform.

In malathion, 307.33 total population of *C. chinensis* and *D. basalis* were recorded at an introduction levels of 4 h of the parasitoids at 16 µg/cm^2. The population was 314.67 at the introduction levels of 4 h at 8 µg/cm^2. The lowest population was recorded after 12 h of introduction at 4 µg/cm^2. The release of parasitoid was also significant (P<0.0001). *D. basalis* was emerged 75.0 at 4 mg/cm^2 doses after 8 day. It was 24.67 at 16 mg/cm^2 dose after 2 days release. Significant differences were noticed within doses and release rates (P<0.0001). The interaction among doses and release rates were also significant (P<0.0001).

Table 31. Number of C*. chinensis* and *D. basalis* population from different release rates in malathion infested seeds of green gram.

Days after release of parasitoids	Doses (mg/cm^2)	Number of *C. chinensis* *(Mean ± SE)	Number of *D. basalis* *(Mean ± SE)	Total
0			0	291.0
	16	272.67 ± 2.67 (270-278)	24.67 ± 0.88 (23-26	297.33 ± 3.38
2	8	280.00 ± 1.15 (278-280)	27.00 ± 0.58 (26-28)	307.00 ± 1.53
	4	278.00 ± 1.15 (276-278)	29.33 ± 0.67 (28-30)	307.33 ± 1.33
	16	262.00 ± 2.31 (258-262)	50.00 ± 1.15 (48-52)	312.00 ± 3.06
4	8	262.67 ± 6.36 (250-270)	52.00 ± 1.53 (50-55)	314.67 ± 4.84
	4	258.00 ± 8.11 (244-272)	53.00 ± 1.53 (50-56)	311.67 ± 6.94
	16	243.67 ± 1.45 (241-246)	65.00 ± 0.58 (64-66)	308.67 ± 1.76
8	8	235.00 ± 1.73 (232-238)	71.00 ± 0.58 (70-72)	306.00 ± 2.31
	4	230.67 ± 0.67 (230-232)	75.33 ± 1.76 (72-78)	306.00 ± 2.0
	16	233.33 ± 3.53 (228-240)	69.33 ± 1.76 (66-72)	302.67 ± 1.76
12	8	225.33 ± 1.33 (224-228)	70.0 ± 1.15 (68-72)	295.33 ± 2.40
	4	221.33 ± 1.33 (220-224)	68.0 ± 1.15 (66-70)	289.33 ± 0.67

* Range in parenthesis

ANOVA

Number of C*. chinensis* population from different release rates in malathion infested seeds of green gram.

Source of Variation	SS	DF	MS	F	P
Subject	445.125	8			
A	2428.2222	3	809.4074	56.6923	<0.0001
Subj x A	342.6528	24	14.2772		
B	1620190	3	540063.3333	14840.7373	<0.0001
Subj x B	873.375	24	36.3906		
A x B	12738.6667	9	1415.4074	99.1376	<0.0001
Subj x A x B	1027.9583	72	14.2772		
Total	1638046	143			

Number of D*. basalis* population from different release rates in malathion infested seeds of green gram.

Source of Variation	SS	DF	MS	F	P
Subject	121.9306	8			
A	4020.6319	3	1340.2106	262.1849	<0.0001
Subj x A	122.6806	24	5.1117		
B	62403.1875	3	20801.0625	450.9207	<0.0001
Subj x B	1107.125	24	46.1302		
A x B	7708.8958	9	856.544	167.5654	<0.0001
Subj x A x B	368.0416	72	5.1117		
Total	75852.4931	143			

On the other hand, in chloroform the highest population (320.0) of both *C. chinensis* and *D. basalis* were recorded after 4 day of release at the dose of 1 µg/cm^2. The population were more or less similar 301.0, 301.67 and 303.67 at 2, 1 0.5 µg/cm^2 doses after 2 h of release. Statistical significance were noticed of different doses (P<0.0001) and release rates (P<0.0001) in case of *C. chinensis*.

Emergence of *D. basalis* at different doses were significant (P<0.0001) and release rates also significant (P<0.0001). Interaction between doses and release rates were also significant (P<0.0001).

The data of the experiment reveal that the combined effects of the extracts of methanol and chloroform of *A. pavonia* and parasitoid, *D. basalis* reduced the population of *C. chinensis* in different release rates at each doses of solvents (Table 32).

Table 32. Number of C*. chinensis* and *D. basalis* population from different release rates in chloroform infested seeds of green gram.

Days after release of parasitoids	Doses (mg/cm^2)	Number of *C. chinensis* *(Mean ± SE)	Numbers of *D. basalis* *(Mean ± SE)	Total
0			0	291.0
	2	284.00 ± 3.05 (280-290)*	17.00 ± 0.58 (16-18	301.00 ± 3.51
2	1	284.00 ± 3.05 (278-288	17.67 ± 1.45 (15-20)	301.67 ± 4.33
	1.5	284.67 ± 2.9 (284-290)	19.00 ± 1.0 (17-20)	303.67 ± 3.76
	2	292.00 ± 1.15 (290-294)	26.00 ± 1.15 (24-28)	318.00 ± 1.15
4	1	291.33 ± 1.76 (288-294)	28.67 ± 0.67 (28-30)	320.00 ± 1.15
	1.5	286.67 ± 2.4 (282-290)	28.00 ± 1.15 (26-30)	314.67 ± 2.40
	2	251.33 ± 2.4 (248-256)	33.33 ± 2.4 (30-38)	284.67 ± 1.33
8	1	238.00 ± 1.15 (236-240)	39.67 ± 0.88 (38-41)	277.67 ± 1.67
	1.5	244.67 ± 2.33 (241-249)	40.33 ± 0.88 (39-42)	285.00 ± 3.21
	2	248.33 ± 0.88 (247-250)	36.00 ± 0.57 (35-37)	284.33 ± 0.33
12	1	253.67 ± 3.18 (250-260)	36.67 ± 0.88 (35-38)	290.33 ± 3.38
	1.5	255.33 ± 5.46 (248-266)	42.00 ± 1.15 (40-44)	297.33 ± 4.37

* Range in parenthesis

ANOVA

Number of C*. chinensis* population from different release rates in chloroform infested seeds of green gram.

Source of Variation	SS	DF	MS	F	P
Subject	69321.875	8			
A	2218.1	3	739.3667	42.665	<0.0001
Subj x A	467.9	24	17.3296		
B	1701996.075	3	567332.025	80.9667	<0.0001
Subj x B	189188.425	24	7006.9787		
A x B	10680.9	9	1186.7667	51.9302	<0.0001
Subj x A x B	1851.1	72	22.8531		
Total	1975724.375	143			

Number of *D. basalis* population from different release rates in chloroform infested seeds of green gram.

Source of Variation	SS	DF	MS	F	P
Subject	1423.475	8			
A	1002.825	3	334.275	16.8842	<0.0001
Subj x A	534.55	24	19.7981		
B	16541.7	3	5531.9	69.9134	<0.0001
Subj x B	2129.425	24	78.8676		
A x B	1248.075	9	138.675	7.9775	<0.0001
Subj x A x B	1408.05	72	17.3833		
Total	24288.1	143			

The percentage suppression of *C. chinensis* by *D. basalis* at different levels of introduction in two different solvents are presented in Table 33. *D. basalis* suppressed *C. chinensis* populations at all levels of introduction. In malathion, the percentage suppression was 76.05 % at 16 mg/cm^2 at the introduction level of 2 pairs but 34 – 52% suppression occurred at 8 – 4 mg/cm^2 at the introduction levels of 4, 8 and 12 pairs.

On the other hand, 83.00 % suppression was recorded at the introduction levels of 2 pairs at 2 mg/cm^2 in case of chloroform treated seeds of green gram but 60.0 – 75.0 % suppression occurred at the introduction levels of 4 – 12 pairs at 1 and 1.5 mg/cm^2 doses.

Parasitism occurred in all the release rates at separate doses. The highest parasitism 23.70% occurred at the introduction levels 12 pairs at 8 mg/cm^2 but lowest 8.30% at the introduction levels of 2 pairs at 16 mg/cm^2 in case of malathion.. Similarly, 14.13% parasitism occurred at the introduction levels of 12 pairs at 1.5 doses whereas only 5.65% parasitism occurred at the introduction levels of 2 pairs at 2 mg/cm^2 doses.

Table 33. Percent suppression and parasitism of *C. chinensis* by *A. pavonia* extracts and *D. basalis* in different release rates at different concentrations.

Malathion	Release rates	Doses (mg/cm^2)	% Suppression	% Parasitism
		16	76.05	8.30
	2	8	73.87	8.79
		4	71.70	9.54
		16	52.83	16.03
	4	8	51.55	15.89
		4	51.23	17.01
		16	39.06	21.06
	8	8	33.85	23.20
		4	30.25	24.27
		16	34.80	22.91
	12	8	34.60	23.70
		4	37.61	23.00
Chloroform		2	83.00	5.65
	2	1	82.21	5.86
		1.5	81.31	6.26
		2	75.78	8.18
	4	1	73.54	8.96
		1.5	74.07	8.90
		2	68.94	11.71
	8	1	62.58	14.29
		1.5	60.46	14.15
		2	66.14	12.66
	12	1	66.97	12.63
		1.5	60.38	14.13

Chapter 4

Discussion

Chapter 4

Discussion

The use of phytochemicals as well as plant products such as powder, oil and extracts for the control of stored-product insect pests has agricultural importance and recently has received much more attention because these insecticidal compounds are safer than the synthetic pesticides and can be easily obtained from plants with less sophisticated methods. Being situated in the Oriental Region (Sub Tropical) Bangladesh has a huge diversity of species with a plenty of promising plants of which *Adenanthera pavonina* was taken into consideration, because this plant has been appeared into human notice as a source of phytochemical screening of leaf and stem in various extracts i.e. petroleum ether, benzene, chloroform, acetone, methanol, rectified spirit and water shows that there is presence of alkaloids, carbohydrate, proteins, tannins, saponin anthraquinone glycosides, cardiac glycosides, flavonoides and phenolic compounds, quinone, steroids. As a native plant of Bangladesh much attention was not paid to work out its potentiality or for its further possibilities of contribution to the overall development of our country. Thus, the present experiments have been carried out to investigate the extractives of different parts of *Adenanthera pavonina* viz. leaves, seeds and stem wood for their insecticidal, insect repellent and feeding activities against *C. chinensis,* cytotoxic activity against *A. salina* and antimicrobial activity against a number of pathogenic bacteria and fungi and finally the combined effects of plants extracts and parasitoid, *D. basalis*.

The petroleum ether, acetone, chloroform and methanol extracts of the leaves, seeds and the stem wood of *A. pavonina* showed insecticidal potentials and their LD_{50} values have been established as well. The seed extracts (of all the solvents) offered highest mortality of the *C. chinensis* beetles, however the comparative

higher doses of the leaf and stem wood extracts indicate weaker action all the three test extracts offered mortality within 24 hours of application just to prove their acute toxicity. Both the leaf and stem wood were found to possess bioactive potential(s) with comparatively higher insecticidal activity after the seed extract, but the leaf extract is comparatively mild in activity followed by the activity of the stem wood extract against *C. chinensis* adults through surface film assay.

The present results support the previous works (Olajide *et al*. 2004) on the same material collected in methanol that gave LD_{50} value1.36g/kg. These findings also receive supports from the report of Zaoui *et al.* (2002), as they have mentioned the insecticidal activity of the methanol extract of *A. pavonina.* Mortality rates in the treated mice were recorded 24 hours after the treatment and any behavioral changes, locomotion, convulsions and mortality were observed for next 14 days. In this case, acute toxicity is involved in estimation of LD_{50} which is usually an initial screening step in the assessment and evaluation of the toxic characteristics of a substance. Oral consumption of methanol extract produce 80%, 60%, 40% and 20% mortality at the doses of 1800 mg/kg, 1575 mg/kg, 1350 mg/kg and 1125 mg/kg body weight respectively. Treated animals those were alive, showed some symptoms associated with toxicity like slow movement and drowsiness at these doses level. The LD_{50} value of the test substances was found 1453.44 mg/kg body weight in mice.

Olajide *et al*. (2004) reported the presence of cardiac glycosides in the methanol extract of *A. pavonina* seeds although there is no known study on the cardiovascular effect of the extract. Methanol extract of seeds of *A. pavonina* was also evaluated for pharmacological effects in animal models. The study demonstrated the anti-inflammatory and analgesic effect of *A. pavonina* extract (Olajide *et al*. 2004). Blood pressure lowering effect of *A. pavonina* seed extract on normotensive rats was reported by (Aduragbenro *et al*. 2009). Anti-diarrheal activity and acute toxicity of methanolic bark extract of *A. pavonina* Linn was

done by (Ara *et al*. 2013) which revealed good results of activity of the extractives of the experimental plant.

The present results also support the previous works (Ahmed *et al.* 2012) where explained the acute systemic toxicity of four mimosaceous plants leaves in mice. They noticed after the intraperitoneal administration of 100 mg/kg dose of *A. pavonia., Peltophorum roxburghii* Degener., *Prosopis cineraria* Druce, and *Prosopis juliflora* DC., no adverse reactions were seen for 48 hours. So, all studied extracts at the same doses considered to be safe as they give normal activity. Where as 150 mg/kg i.p. considered to be safe only for the extract of *A. pavonia* and remaining three plant extracts are being considered toxic as they cause 100% mortality of mice till 48 hours. The 200 mg/kg i.p. of all studied extracts considers being toxic. The final median lethal dose (LD_{50}) value was calculated by recorded 0 and 100% survival as the square root of the product of the lowest lethal doses and highest non-lethal doses (Kalu *et al*. 2011). The calculated LD_{50} for *A. pavonia* was to be 141.42 mg/kg body weight where as for *P. roxburghii*, *P. cineraria* and *P. juliflora* was 122.47 mg/kg body weight.

These results are in agreement with those reported on the significant in the acute toxicity test revealed that LD_{50} of the total ethanol extract of the leaves of the target plant was found to be 5.8 g/kg body wt. From this result, the plant is considered to be safe, this is confirmed by what was reported about the seeds, young leaves and fruits that could be cooked and eaten (Corner 1997, Benthall, 1946 and Clark and Thaman 1993).

The *Adenanthera* extracts also screened for nematicidal activity by the previous workers. The seeds are bitter, astringent, sweet, cooling, aphrodisiac, anti-emetic and febrifuge. They are useful in gout, burning sensation, hyperdipsia, vomiting, fever and giddiness. Powder of the seed is applied as a poultice to abscess to promote suppuration. Seeds contain HCN-glucoside, lignoceric acid, dulcitol, stigmasterol, stigmasterol glucoside and polysaccharide Ghani (2003). The

seeds have been found to be effective in cardiovascular diseases in pregnancy. The ground seeds are used to treat boils and inflammatory reactions and also useful for external application; it hastens suppuration Kirtikar and Basu (1933). Decoction of leaves is used to treat gout and rheumatism Burkill (1994). Ahmed *et al*. (2010) demonstrated the effect of local tree seeds in the control of root knot nematode *Meloidogyne javanica* (Treub) chitwood and growth promotion of chickpea (*Cicer arietinum* L.) and mung bean (*Vigna radiata* L.).Their results indicate that, with increasing extract concentrations, hatching of eggs were gradually decreased. They suggested that 100% extract concentration was best for reducing the hatching of eggs, followed by 50% concentration. The best nematicidal activity was shown by *L. leucocephala* which showed 0% hatching, followed by *A. pavonina* that showed more promising control of *M. javanica* egg hatching than *Eucalyptus* spp., and *A. indica.* Mortality was gradually increased with exposure time. *A. indica* 100% concentration extracts showed 100% mortality, followed by 50% concentration; then, *A. pavonina* and *L. leucocephala* showed 85% and 89% mortality, respectively, in 100% concentration extracts, followed by *Eucalyptus* spp*.* Various neem products, including neem cake, its oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts have been found to be nematicidal against several species of parasitic nematodes (Alam 1991), attacking vegetables and legumes (Haseeb *et al*. 2005). They concluded that the use of *A*. *indica, A. pavonina, L. leucocephala* and *Eucalyptus* spp., seed extracts as seed treatment and soil drenching has the potential to reduce severity of galls in roots and enhance the plant growth. However, further research including a more quantitative approach is needed to explore the control of damage caused by nematodes. Ntalli *et al.* (2010) reported the Phytochemistry and nematicidal activity of the essential oils from 8 Greek lamiaceae aromatic plants and 13 terpene components.

Cytotoxicity test was also carried out which revealed good results of activity of the extracts of the experimental plant. The test materials were found cytotoxic,

while the test was carried out through dose-mortality assay it was possible to established the LC_{50} values as part of this experimentation. According to the intensity of activity the results of the extracts against the brine shrimp nauplii could be arranged in the following order: seed > leaf > stem wood and the toxicity offered by the extracts were very much promising. In support of these findings screening results for cytotoxicity by many previous researchers done on an allied species of *Adenanthera* were available. Cytotoxicity of crude extracts of *A. pedunculata* and *A. pavonina* was determined using the brine shrimp assay Ajaiyeoba *et al*. (2006).

Some earlier workers reported various parts of the target plants against the analgesic, anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxicity and blood pressure reducing activities of the leaf and seed extracts and its isolated compounds have been reported by Nigam *et al.* (1973), Neerja *et al.* (1976), Adedapo *et al.* (2009), Rodrigo *et al.* (2007), Jayasinghe *et al.* (2006), Olajide *et al.* (2004), Mayuren and Ilavarasan (2009). Ali *et al.* (2013) reported the acute toxicity, brine shrimp cytotoxicity, anathematic and relaxant potentials of fruits of Rubus fruticosus Agg.

These results also support of the acute anti-inflammatory test of two doses of total ethanol extract (50 and 100 mg/ kg body wt.) and 100 mg/ kg body wt. dose of the successive extracts of the leaves revealed that significant anti-inflammatory effects are exhibited by oral administration of the doses of the different extracts. They significantly inhibited the rat paw oedema weight induced by carrageenan. This effect was found to be dose dependant in the total ethanol extract. The most potent extract was the total ethanol extract, 100 mg/kg b. wt. (91.27 %) followed by aqueous ethanol extract (89.15 %), chloroform extract (79.89 %), ethyl acetate extract (70.10 %), petroleum ether extract (65.34 %) in comparison with indomethacin (100 % potency).

The present results are in agreement with those reported on the significant *in vivo* anti-inflammatory activities exhibited by the extracts of *A. pavonina* in the carrageenan-induced rat paw oedema model providing scientific evidence to the traditional uses of this plant for treatment of various inflammatory conditions, e.g. gout, rheumatism and tumours Arzumand *et al.* (2010). These results are also supported by the significant anti-inflammatory activity in rats exhibited by the ethanol leaf extract of *A. pavonina,* which may be related to the presence of active constituents such as flavonoids, *β*-sitosterol and stigmasterol. A possible mechanism may also be due to the inhibition of prostaglandin synthesis Mayuren and Ilavarasan (2009)**.**

The present results also support the ethanolic extracts of *Derris scandens* (Roxb.) Benth, along with other test extracts showed cytotoxicity (IC_{50}<30 µg/ml) against lung and prostate cancer cell lines (Acharya and Thomas 2007). Another similar work was also available done on cytotoxicity. These tests showed LC_{50} of petroleum ether, chloroform and methanol extracts on *A. salina* Leach as 1.14, 1.1, and 54.9mg/l respectively. Chemical analysis revealed the presence of fatty acids, steroids, triterpenoids, alkaloids, phenols, and phenyl propanoids, tannin, and mucilage in the extracts (Uyub *et al.* 2010).

So, insecticidal potentials of *A. pavonina* or of its related species needs no further proof, while certain other experimental results carried out by different researchers worldwide mentioned here, were tested against different other multicellular organisms, viz. nematodes, mites and brine shrimp nauplii.

These findings also receive supports from the report of Burkil (1994), Kirtikar and Basu (1981), Watt *et al*. (1962), Ghani (2003), as they have mentioned flavonoids, saponins, and alkaloids, etc. are present in the seeds and bark of the test plant. Various parts of this plant have also been used for the treatment of asthma, boil, diarrhea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic. However, the methanol extract of the seeds of *A. pavonia* was evaluated for pharmacological effects in animal models Olajide *et al.* (2004).The

row seeds are toxic and may cause intoxication. The seeds to be rich in oil and proteins, and easily digested by both human and livestock. The bark extracts of *A. pavonina* were effective in inhibiting the growth of both gram positive as well as gram negative bacteria (Arshad *et al*. 2011). A methanol extract of the seeds of *A. pavonia* was evaluated for pharmacological effects in animal models (Olajide *et al*. 2004). The antifungal activity of peptides extracted from *A. Pavonia* seeds were assessed by Ribeiro *et al.* (2012).

Some earlier workers reported various plant materials including essential oils in checking the multiplication of pests in stores (Krishnamurti and Rao 1944, Su *et al.* 1972, Sangapa 1977). Antiproliferative activity of *A. pavonina* was traced by Sun *et al*. (2010). Tannins were present in the target plant parts which have astringent and detergent properties were also present and can be used against diarrhea (Trease and Evnas 2002, Bruneton 1999). There has been an assertion by Trease and Evans (2002) that naturally cardiac glycosides are used for treatment of various diseases associated with the heart such as in controlling supraventicular (atrial) cardiac arrythmias, it also exert a slowing and strengthening effect on failing heart (Essiett *et al*. 2010). The presence of this compound in *A. pavonia* could be useful in the treatment of diseases associated with the heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery.

No such previous works have been traced that reveals repellent activity of the *A. pavonina* extracts through experiments directly on *C. chinensis* adults, while it was done in the present investigation to establish high degree of repellent potentials of the leaf, seed, and stem wood extracts of the experimental plant. Repellency by the petroleum ether, acetone, chloroform, and methanol extracts of *A. pavonina* against *C. chinensis* adults was very much promising, while all the extracts found to repel at 0.01% level of significance ($P<0.001$) except the stem (acetone) extract which was found active at 0.1% level of significance ($P<0.01$). The repellency record triggers a hope for the use of *A. pavonina* extracts as repellents since most of the extracts repelled the beetles significantly. These results are in agreement with similar works of McDonald *et al.* (1970),

Khan (1981), Talukder and Howse (1995) and Hebbalkar *et al*. (1992). Abdullah *et al.* (2011) assessed the mortality and repellency of the chloroform extracts of different parts of *Urena sinuate* on *Tribolium castaneum* adults. The root and stem extracts showed significant repellent effects on the beetles but the fruit and leaf extracts produced no repellency at all. Singh *et al.* (2001) carried out antifeedant activity tests of some famous (of bioactive potentials) plants that help understanding repellent potentiality of some medicinal plant extracts. Mondal *et al*. (2011) assessed the repellent activity of *Derris indica* extracts against *T. castaneum* adults. The fruit shell, leaves, seed and stem bark extracts showed repellent activity but the stem wood, root bark and root wood extracts produced no repellency at all.

The antioxidant activity of herbal extract is due to various biochemicals, which act as inhibitors of the process of oxidation, and thus have diverse physiological role in the body. Antioxidant activities of crude extracts were determined using DPPH radical Spectrophotometer assay using α-tocopherol (Roberta *et al*. 2006). Antifungal, Antioxidant and Cytotoxic activity of *Acronychia pedunculata* and *A. pavonina* was traced by Rodrigo *et al.* (2007).The free radicals are in those molecules/atoms that possess an unpaired electron in their outermost orbit, which is capable of inducing chain reaction and thereby damaging different types of cells, resulting into accelerated aging, various diseases, stress, and also hampering the defense mechanisms of the body. Various Indian medicinal herbs of common use are known to have remarkable antioxidant properties; while 25 Indian medicinal plants including *A. pavonina*, *D. indica* and *D. robusta* were tested to show antioxidant potentials. Different parts of the selected plants were tested individually for their antioxidant activity. The scientific names, Thai names, part used, and percentage yield of plant extracts were reported by (Tunsaringkarn *et al.* 2012). They observed the inhibitory activity of Heinz body induction *in vitro* antioxidant model and tannin concentration of Thai mimosaceous plant extracts. The results showed that *Xylia xylocarpa* (Roxb.) Taub.bark has the highest yield, followed by *A. pavonina* leaves, *X. xylocarpa*

(Roxb.) Taub. leaves, *Samanea saman* (Jacq.) Merr. Leaves and *Acacia catechu* (L.f.) Wild leaves at 22.69, 20.72, 19.44, 17.88, and 16.38 %, respectively.

Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect, etc. (van Wyk and Gericke 2000). It is logical to expect biologically active compounds to be produced by plants as a chemical defense measure against their enemies. Natural products have been a consistently successful source in drug discovery and offer more opportunities to find antimicrobial drugs or lead compounds (Wang *et al*. 2006).

The leaves, seed and stem wood of *A. pavonina* petrolium ether, acetone, chloroform and methanol extracts were subjected to evaluate the antimicrobial activity (on some selected pathogenic bacteria and fungi) and the minimum inhibitory action just depending on the intensity of activity against the selected Gram positive and Gram negative bacteria. The activities found were very much promising. The seed extracts offered the MIC values 256 µg/ml against *B. cereus*. and 128 µg/ml against *S.- β –haemolyticus, S. dysenteriae* and 64 µg/ml against *Klebsiella* sp. and of the stem wood were 128µg/ml against *S.- β – haemolyticus,* 64 µg/ml against *B. megaterium, S. sonnei* and 32 µg/ml against *S. typhi*. This result is also supported by the previous findings. Alcoholic and aqueous extracts of the fresh bark are reported to exhibit marked antibacterial activity against *Micrococcus pyogenes* var. *aureus* (Anon 1969).

Findings on antimicrobial activity by previous workers support the recent outcome of this investigation. Antibacterial screening of the bark of *A. pavonina* was demonstrated by Hussain *et al*. (2011). Ethanolic and aqueous extracts showed the highest activity against all the tested bacteria. These results were compared with the Zones of inhibition produced by commercially available standard antibiotics. Extracts of *A. pavonina* and *A. pedunculata* exhibit antibacterial activity against methicillin-sensitive and resistant strains of

Staphylococcus aureus, *Enterococcus faecalis* and *Pseudomonas aeruginosa* Jayasinghe *et al*. (2006).

The present work concluded that the plant extract inhibited bacterial growth but their effectiveness varied. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The Millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases (Girish and Satish 2008, Toama *et al*. 1974).These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources (Livermore and Nikaido 1994, Eruteya and Odunfa 2009). In general, the inhibitory activity of bark extracts was seen on both gram positive as well as gram negative strains. In most cases the antibacterial activity of bark extracts was more than the many of the antibiotics used. All the extracts are very effective antibacterial agents, except petroleum ether which was least effective against all the bacterial strains. The results of diameter of zones of inhibition showed that the bark extracts were effective in inhibiting the growth of both gram positive as well as gram negative bacteria up to varying extents, which may be due to the presence of alkaloids, glycosides, flavonoids, tannins, saponins, and triterpenoids. Antibacterial activity of three medicinal Thai plants against *Campylobacter jejuni* and other food borne pathogens were reported by Dholvitayakhun *et al.* (2012). They demonstrated that leaves of *A. pavonina, M. olifera* and *A. squamosa* are used in traditional Thai medicine to treat dysentery and other diseases. *A. pavonina* contained flavonoids, terpines and tannins, and was the most active extract against *C. jejuni*, inhibiting growth at 62.5-125 μgmL^{-1}. The result show that *A. pavonina* and *A. squamosa* could potentiality is used in modern applications aimed at the treatment of food borne diseases. Antifungal activity of crude extracts was determined against *Cladosporium*

cladosporioides by thin layer chromatography (TLC) autobiography method (Adikaram and Bandara 1998).

The present results also support of four non-traditional galactomannans were isolated from seeds of *A. pavonia*, *C. pulcherrima, G. triacanthos* and *S. japonica* and a simple methodology using only ethanol and water as solvents was developed for their extraction in view of their use e.g. in the demanding area of food industry. All the galactomannans from those plants were obtained by aqueous extraction followed by precipitation with ethanol, but the extraction procedure of galactomannan from the seeds of *S. japonica* required an acidic pretreatment in order to effectively separate the hull from the endosperm. Other works used solvents as chloroform (Mirzaeva *et al*. 1998) and petroleum ether (Amin *et al*. 2007, Uner and Altınkurt 2004) to extract galactomannans, which are not authorized in the food industry (List of Codex Specifications for Food Additives 2008). The extraction yield of each of the galactomannans was determined, as well as their monosaccharide composition, M/G ratio, purity, intrinsic viscosity and viscosity average molecular mass, thus providing a convenient measure of the hydrodynamic volume of individual polymer coils. This finding also support of the antibacterial activity against *E. coli*, *B. subtilis, P. aeruginosa and S. aureus* and inhibitory effect on glucoamylase of ethanolic extracts isolated at different temperatures from seeds of *Syzygium cumini* was investigated *in vitro* (Gangadhar *et al.* 2011).

The methanol extract of seeds of *A. pavonina* showed dose-dependent and significant analgesic and anti-inflammatory activity (Olajide *et al*. 2004). Study showed *A. pavonina* seed extract has the potential to have a blood pressure lowering effect. Serum biochemistry changes suggest the extract has a tonic effect on kidneys and liver and may play a central role in drug metabolism. Absence of kidney, liver and testes lesions suggest the plant is safe for medicinal use (Aduragbenro *et al.* 2009). Study showed the seed oil to be rich in neutral lipids (86.2%) and low in polar lipids (13.8%). Unsaturated fatty acids were as

high as 71%, the predominant FA showed to be linoleic, oleic and lignocerotic acids. Stigmasterol was the major steroid. Results indicate the possible applications of the oil in the pharmaceutical and medical fields as drug and cosmetic active ingredient carriers (Robert Zarnowski *et al.* 2004). The stem-bark of *A. pavonia* showed significant antioxidant activity, presumably with more activity than a-tocopherol (Rodrigo *et al.* 2007). Study isolated a trypsin inhibitor (ApTI) from *A. pavonina* seed and was tested for insect growth regulatory effect. Chronic ingestion resulted in significant reduction in larval survival. Results showed (ApTI) have a potential anti metabolic effect when ingested by *A. kuehniella* (Macedo *et al.* 2010). Bark extracts were tested for anthelmintic activity against *Phretima posthuma* and *Ascardia galli*. Phytochemical screening of bark yielded alkaloids, carbohydrates, glycosides, saponin, phytosterols, fixed oil and fats, phenolics and flavonoids. Results showed significant anthelmintic activity (Dash *et al.* 2010). Study of alcoholic and aqueous seed extracts in alloxan-induced diabetic rats produced favorable changes in the lipid profile of diabetic rats along with better glycemic control (Krishnaveni *et al*. 2011). Study developed very stable formulations of submicron oil-in-water emulsions from *A. pavonia.* Study of seed extract in neuropathic pain in streptozotocin-induced diabetic rats showed attenuation of development of diabetic neuropathy when compared to pregabalin. Results suggest a possible benefit in preventing progression of diabetic nephropathy (Ramdas *et al.* 2012). Study evaluated a stem bark extract for antitumor activity on Dalton's ascitic lymphoma in male Swiss albino mice. Results demonstrated tumor inhibitory activity with increase in life span accompanied by reduction in WBC in treated mice (Arihara Siva Kumar G *et al*., Indian Journal of Research in Pharmacy and Biotechnology). Study evaluated an aqueous seed extract in streptozotocin-induced diabetic rats. Results showed reduced development of diabetic nephropathy in STZ-induced diabetic rats and can have a beneficial effect in reducing progression of diabetic nephropathy (Ramdas *et al*. 2012).

To our best knowledge, there have been very little studies published previously that examined chemicals accumulated in *A. pavonina* seeds. Those investigations led only to the isolation of a few protein trypsin inhibitors (Prabhu and Pattabiraman 1980, Richardson *et al.* 1986, Lam *et al.* 1999), flavonoids (Gennaro *et al.* 1972), fatty acids (Kabele-Ngiefu *et al.*1975, Balogun and Fetuga 1985, Sotheeswaran *et al.* 1994), triterpenoids (Yadav *et al.* 1976) and Carbohydrates (Moreira *et al.* 1998). In the present work, we show detailed compositions of lipids present in the oil of *A. pavonina* seeds. Moreover, a possible use of this oil for a submicron oil-in water (o/w) lipid emulsion formulation is also described by (Miguel *et al.* 2009).

So far the results of the present investigation concern the biological activity of *A. pavonia* is of course promising, which was further authenticated by the outcomes of the previous researches around the world discussed in this chapter. Bangladesh being the homeland of this famous plant might have a bright future of earning foreign currency by exporting different products or preparations of this plant. The seeds offered the highest toxicity to the majority of the test agents, and 100% highest activity to the multicellular test organisms; followed by the leaf extracts. So, seeds could be an export item, as well as the leaf products (for what there is an international market), and easy formulation of the products is necessary while the folk use of the *Adenanthera* products given hints in this regard. To achieve the goal with much success in proper utilization of this promising plant and for the marketing of its products it is necessary to launch a huge investigation for further substantial empirical assessments.

Combined effect of plant extracts and parasitoids

Parasitoids and insecticides are generally incompatible because beneficial insects are often more susceptible to insecticides materials than their hosts (Croft 1990, Scholler and Flinn 2000). For example, diatomaceous earth is more toxic to *A. calandrae* than to *S. oryzae* (Perez-Mendoza *et al*. 1999). By direct contact, diatomaceous earth takes > 1 h to kill 50% of *A. calandrae*, but it takes 24 h to kill 50% of its host, *S. oryzae* (Perez-Mendoza *et al*. 1999). I contrast, the present

study demonstrated that plant extracts of methanol and chloroform and parasitoid, *D. basalis* were compatible for reducing populations of *C. chinensis*. It had no direct contact toxicity, and it did not affect parasitism by *D. basalis*. Plant extracts had no effect on the emergence of *D. basalis* and its host. *D. basalis* always survive highest when supplied host containing seeds (12-15 day old) because age of the host may have a profound effect on oviposition and development of parasitoids (Vinson and Iwantsch 1980). The female *D. basalis* penetrates its sharp and pointed ovipositior through the surface of the seed-coat and places an egg on the external surface of the seed coat and places an egg. After ovipositing the female withdraws the ovipositior from the seed and again inserts it to form a feeding tube extending from the surface of the body of the host to out side the seed and feeds on the host fluids that oozes out through feeding tube. It is believed that this food significantly increases the number of mature eggs in the ovaries (Edwards 1954, DeBach 1979). Various investigations (Wylie 1963, Hegazi *et al*. 1977) showed that such food differences profoundly affected parasitoid longevity and fecundity.

The present study found that *D. basalis* suppressed populations of *C. chinensis* in different release rates. Islam *et al*. (2003) reported the ability of *D. basalis* to suppress residual population of *C. maculatus* in black gram debris and found lowest density 5 and 10 pairs suppressed *C. maculatus* population 52-62% and 71.78% respectively; 20-30 pairs 80-89% and 85-95% respectively. Press et al. (1984) found that *Anisopteromalus calandrae* (How.) suppressed the population of 90% of *Sitophilus oryzae* (L.) in wheat debris at introduction levels of 30, 40 and 50 pairs.

Flinn *et al*. (2006) studied the protection of stored maize from insect pests using a two-component biological control method consisting of a hymenopteran parasitoid, *Theocolax elegans*, and transgenic avidin maize powder. Small-scale tests were conducted in plastic jars containing 3 kg of non-transgenic maize. They tested treatments of 0.3% powdered avidin maize, the parasitoid, and the

combination of the parasitoid plus 0.3% powdered avidin maize in plastic jars. One pair each of *S. zeamais*, *T. castaneum* and *C. ferrugineus* were added to each jar. After 8 weeks, the entire contents of each jar were examined for adult insects. They found control and avidin maize powders had no detrimental effects on *T. elegans*. The parasitoid suppressed populations of *S. zeamais*. The parasitoid-avidin combination treatment produced the greatest percentage reduction for all three tested insects and resulted in 78%, 94% and 70% reductions in populations of *S. zeamais*, *T. castaneum* and *C. ferrugineus*, respectively, when compared to the control treatment. The percentage reductions for the parasitoid treatment were 70%, 8% and 20% for *S. zeamais*, *T. castaneum* and *C. ferrugineus* were reduced by 10%, 85% and 40%, respectively. They recommended that the combination treatment of avidin maize powder plus the release of parasitoid was superior to either treatment alone when applied to mixed populations of internal and external feeders.

Hou *et al*. (2004) investigated the control of stored-product beetles with combinations of protein-rich pea flour and parasitoids and found that protein-rich pea flour is toxic and repellent to three major stored-grain pests: the rice weevil, *Sitophilus oryzae* L.; the red flour beetle, *Tribolium castaneum* (Herbst); and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). They mentioned that protein-rich pea flour was not toxic to, and did not reduced the offspring of *Anisopteromalus calandrae* (Howard), a parasitoid of *S. oryzae*, nor did it reduce offspring of *Cephalonomia waterstoni* (Gahan), a parasitoid of *C. ferrugineus*. Protein-rich pea flour was also not repellent to *A. calandrae* they recorded. The combinations of protein-rich pea flour and parasitoid reduced populations of *S. oryzae* they found. In the present investigations similar results were found in methanol and chloroform extracts of *A. pavonina* and parasitoid, *D. basalis*.

Protein-rich pea flour and its extract are toxic to stored-product beetles and, at a concentration of 0.1%, can control these insects in a granary studied by Hou *et al*. (2004). To reduce the concentration of protein-rich pea flour needed to control

stored-product beetles, natural products or currently used grain protectants (diatomaceous earth, neem, *Bacillus thuringiensis* (Berliner), malathion, and pyrethrum) were mixed with protein-rich pea flour in wheat. They tested mixtures against *S. oryzae*, *T. castaneum* and *C. ferrugineus* and found neem and protein-rich pea flour acted sysnergistically against *T. castaneum* and malathion and protein-rich pea flour acted synergistically against *S. oryzae*. Protein-rich pea flour combined with diatomaceous earth or pyrethrum acted additively against *S. oryzae*. Our study also reported combined effect of two insecticides and parasitoids.

Islam and Kabir (1995) studied biological control potential of *D. basalis*, a larval-pupal ectoparasitoid of the pulse beetle, *C. chinensis* and found that *D. basalis* was introduced at six densities to suppress residual populations of *ca* 600 pulse beetle, *C. chinesis*, breeding in red lentil debris in a 46.24 m^2 room. The release rates were 5, 10, 20, 30, 40 and 50 pairs of parasitoids. The highest density, 50 pairs, suppressed the *C. chinensis* population 100% in the April-May period, but in the July-August period, 30 pairs could achieve this result. More progeny per female parasitoid were produced at the lower parasitoid densities than at the higher levels in both periods they recorded. In another test, *ca* 500 pulse beetles per 275 g of lentils, kept separately in each of three types of bags (amemian, jute and polypropylene) were exposed in the room to 50 pairs of parasitoids. Highest percentage of suppression and parasitism occurred in amemian bags and lowest in polypropylene bags when a single release of 50 pairs of *D. basalis* were made during both periods they concluded.

Finally it can be concluded that *A. pavonina* plant extracts and parasitoid, *D. basalis* combinedly may be an excellent protection against *C. chinensis* infestation in warehouse. *D. basalis* is a generalist parasitoid and parasitizes a number of stored-product Coleoptera (Islam *et al*. 1985). Because *D. basalis* is not adversely affected by the methanol and chloroform plant extracts, the combination of this parasitoid and plant extract may help suppress populations of stored-product insects. More research on importance of pharmaceutical of reported plant is needed in this respect.

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Appendices

AppendixTable1: Dose-mortality effect of Seed extract (pet. ether) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
2831	3.452	60	11	18.333	18	4.08	4.189	4.094	28.26	4.204
2477	3.394	60	9	15.000	15	3.96	3.906	3.970	24.30	3.902
2123	3.327	60	6	10.000	10	3.72	3.578	3.750	16.14	3.554
1769	3.248	60	2	3.333	3	3.12	3.191	3.116	9.24	3.143
1415	3.151	60	0	0.000	0	0.00	2.717	2.320	4.56	2.639

Results:

Y = -13.724+5.194X
Chi-squared is 1.537 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.605
LD_{50} is 4029.546µg/cm^2
95% confidence limits are 3040.105 to 5341.016µg/cm^2

AppendixTable2: Dose-mortality effect of Seed extract (pet. ether) of *Adenenthera pavonina* against *C. chinensis* adults after 24 h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
2831	3.452	60	19	31.667	32	4.53	4.499	4.540	33.48	4.508
2477	3.394	60	14	23.333	23	4.26	4.323	4.266	31.92	4.331
2123	3.327	60	12	20.000	20	4.16	4.120	4.170	28.26	4.127
1769	3.248	60	8	13.333	13	3.87	3.881	3.873	22.20	3.886
1415	3.151	60	5	8.333	8	3.59	3.587	3.596	16.14	3.590

Results:

Y =-6.013+3.048X
Chi-squared is 0.224 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.613
LD_{50} is 4104.532µg/cm^2
95% confidence limits are 2807.799 to 6000.136µg/cm^2

AppendixTable3: Dose-mortality effect of Seed extract (pet. ether) of *Adenenthera pavonina* against *C. chinensis* adults after 48 h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
2831	3.452	60	38	63.333	63	5.33	5.312	5.318	36.96	5.304
2477	3.394	60	32	53.333	53	5.08	5.043	5.075	38.22	5.036
2123	3.327	60	22	36.667	37	4.67	4.733	4.662	36.96	4.727
1769	3.248	60	15	25.000	25	4.33	4.366	4.330	31.92	4.361
1415	3.151	60	9	15.000	15	3.96	3.917	3.970	24.30	3.913

Results:

Y =-10.635+4.617X

Chi-squared is 0.330 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.386
LD_{50} is 2432.645µg/cm^2
95% confidence limits are 2230.889 to 2652.638µg/cm^2

AppendixTable4: Dose-mortality effect of Seed extract (Acetone) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
2831	3.452	60	11	18.333	18	4.08	4.183	4.094	28.26	4.190
2477	3.394	60	9	15.000	15	3.96	3.923	3.970	24.30	3.921
2123	3.327	60	6	10.000	10	3.72	3.622	3.730	18.12	3.610
1769	3.248	60	3	5.000	5	3.36	3.265	3.375	10.80	3.242
1415	3.151	60	0	0.000	0	0.00	2.830	2.410	5.52	2.792

Results:

Y = -11.829+4.640X
Chi-squared is 1.578 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.627
LD_{50} is 4231.617µg/cm^2
95% confidence limits are 3044.489 to 5881.634µg/cm^2

AppendixTable5: Dose-mortality effect of Seed extract (Acetone) of *Adenenthera pavonina* against *C. chinensis* adults after24 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	19	31.667	32	4.53	4.543	4.516	34.86	4.534
2477	3.394	60	16	26.667	27	4.39	4.385	4.394	31.92	4.378
2123	3.327	60	13	21.667	22	4.23	4.201	4.218	30.18	4.198
1769	3.248	60	9	15.000	15	3.96	3.983	3.970	24.30	3.985
1415	3.151	60	6	10.000	10	3.72	3.717	3.720	20.16	3.725

Results:

Y =-4.746+2.689X
Chi-squared is 3.626E-02 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.625
LD_{50} is 4218.390µg/cm^2
95% confidence limits are 2748.362 to 6474.701µg/cm^2

AppendixTable6: Dose-mortality effect of Seed extract (Acetone) of *Adenenthera pavonina* against *C. chinensis* adults after 48 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	43	71.667	72	5.58	5.554	5.556	34.86	5.553
2477	3.394	60	38	63.333	63	5.33	5.282	5.358	37.62	5.279
2123	3.327	60	28	46.667	47	4.92	4.968	4.915	38.04	4.963
1769	3.248	60	18	30.000	30	4.48	4.596	4.460	34.86	4.589
1415	3.151	60	13	21.667	22	4.23	4.141	4.246	28.26	4.131

Results:

Y = -10.737+4.719X
Chi-squared is 1.272 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.335
LD_{50} is 2161.584µg/cm^2
95% confidence limits are 2009.114 to 2325.627µg/cm^2

AppendixTable7: Dose-mortality effect of Seed extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	13	21.667	22	4.23	4.283	4.218	30.18	4.258
2477	3.394	60	11	18.333	18	4.08	4.070	4.078	26.34	4.058
2123	3.327	60	8	13.333	13	3.87	3.825	3.873	22.20	3.828
1769	3.248	60	5	8.333	8	3.59	3.534	3.596	16.14	3.555
1415	3.151	60	2	3.333	3	3.12	3.179	3.116	9.24	3.221

Results:

Y=-7.636+3.446X
Chi-squared is 0.232 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.667
LD_{50} is 4646.414µg/cm^2
95% confidence limits 2960.347 to 7292.786µg/cm^2

AppendixTable8: Dose-mortality effect of Seed extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	26	43.333	43	4.82	4.865	4.838	37.62	4.868
2477	3.394	60	23	38.333	38	4.69	4.673	4.686	36.06	4.678
2123	3.327	60	18	30.000	30	4.48	4.450	4.480	33.48	4.458
1769	3.248	60	13	21.667	22	4.23	4.187	4.246	28.26	4.198
1415	3.151	60	7	11.667	12	3.82	3.865	3.822	22.20	3.880

Results:

Y=-6.454+3.280X
Chi-squared is 0.190 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.492
LD_{50} is 3106.171µg/cm^2
95% confidence limits 2531.771 to 3810.892µg/cm^2

AppendixTable9: Dose-mortality effect of Seed extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	48	80.000	80	5.85	5.734	5.830	31.92	5.706
2477	3.394	60	37	61.667	62	5.31	5.450	5.294	36.06	5.427
2123	3.327	60	32	53.333	53	5.08	5.122	5.065	38.04	5.105
1769	3.248	60	25	41.667	42	4.80	4.734	4.792	36.96	4.724
1415	3.151	60	14	23.333	23	4.26	4.260	4.252	30.18	4.257

Results:

Y=-10.904+4.812X
Chi-squared is 1.355 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.305
LD_{50} is 2018.911 µg /cm^2
95% confidence limits 1879.221 to 2168.983µg/cm^2

AppendixTable 10: Dose-mortality effect of Seed extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
2831	3.452	60	10	16.667	17	4.05	4.077	4.037	26.34	4.086
2477	3.394	60	7	11.667	12	3.82	3.829	3.822	22.20	3.826
2123	3.327	60	5	8.333	8	3.59	3.544	3.596	16.14	3.527
1769	3.248	60	3	5.000	5	3.36	3.206	3.375	10.80	3.174
1415	3.151	60	0	0.000	0	0.00	2.792	2.320	4.56	2.741

Results:

Y = -11.325+4.464 X
Chi-squared is 1.385 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.657
LD_{50} is 4537.363 µg /cm^2
95% confidence limits 3052.271 to 6745.017µg/cm^2

AppendixTable 11: Dose-mortality effect of Seed extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
2831	3.452	60	19	31.667	32	4.53	4.537	4.516	34.86	4.502
2477	3.394	60	13	21.667	22	4.23	4.279	4.218	30.18	4.267
2123	3.327	60	9	15.000	15	3.96	3.987	3.970	24.30	3.996
1769	3.248	60	7	11.667	12	3.82	3.642	3.864	18.12	3.676
1415	3.151	60	2	3.333	3	3.12	3.220	3.121	10.80	3.284

Results:

Y = -9.463+4.046X
Chi-squared is 1.024 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.575
LD_{50} is 3758.050 µg /cm^2
95% confidence limits 2889. 551to 4887.597µg/cm^2

AppendixTable 12: Dose-mortality effect of Seed extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
2831	3.452	60	46	76.667	77	5.74	5.483	5.699	36.06	5.474
2477	3.394	60	30	50.000	50	5.00	5.114	4.990	38.04	5.096
2123	3.327	60	17	28.333	28	4.42	4.689	4.416	36.06	4.659
1769	3.248	60	12	20.000	20	4.16	4.185	4.170	28.26	4.142
1415	3.151	60	6	10.000	10	3.72	3.569	3.750	16.14	3.510

Results:

Y=-17.041+6.522X
Chi-squared is 5.321 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.379
LD_{50} is 2394.528µg /cm^2
95% confidence limits 2254.381 to 2543.387µg/cm^2

AppendixTable 13: Dose-mortality effect of leaf extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	14	23.333	23	4.26	4.353	4.266	31.92	4.362
6369.43	3.804	60	11	18.333	18	4.08	4.050	4.078	26.34	4.049
5661.71	3.753	60	8	13.333	13	3.87	3.712	3.894	20.16	3.698
4954.00	3.695	60	3	5.000	5	3.36	3.328	3.360	12.48	3.301
4246.28	3.628	60	0	0.000	0	0.00	2.884	2.410	5.52	2.843

Results:

Y = -22.007+6.850X
Chi-squared is 2.152 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.943
LD_{50} is 8769.089µg /cm^2
95% confidence limits 7338.51 to 10478.54µg/cm^2

AppendixTable 14: Dose-mortality effect of leaf extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	18	30	30	4.48	4.489	4.48	33.48	4.473
6369.43	3.804	60	13	21.667	22	4.23	4.268	4.218	30.18	4.260
5661.71	3.753	60	10	16.667	17	4.05	4.022	4.037	26.34	4.021
4954.00	3.695	60	7	11.667	12	3.82	3.742	3.836	20.16	3.751
4246.28	3.628	60	3	5	5	3.36	3.419	3.36	14.28	3.439

Results:

Y = -13.474+4.662X
Chi-squared is 0.294 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.963
LD_{50} is 9181.241µg /cm^2
95% confidence limits 7158.734 to 11775.15µg/cm^2

AppendixTable 15: Dose-mortality effect of leaf extract (chloroform) of *Adenenthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	24	40.000	40	4.75	4.812	4.76	37.62	4.798
6369.43	3.804	60	20	33.333	33	4.56	4.575	4.544	34.86	4.569
5661.71	3.753	60	17	28.333	28	4.42	4.310	4.426	31.92	4.313
4954.00	3.695	60	10	16.667	17	4.05	4.010	4.037	26.34	4.023
4246.28	3.628	60	5	8.333	8	3.59	3.663	3.596	18.12	3.687

Results:

Y = -14.480+5.008X
Chi-squared is 0.645 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.890
LD_{50} is 7763.914µg /cm^2
95% confidence limits 6701.33 to 8994.983µg/cm^2

AppendixTable 16: Dose-mortality effect of leaf extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	11	18.333	18	4.08	4.158	4.094	28.26	4.161
6369.43	3.804	60	8	13.333	13	3.87	3.837	3.873	22.20	3.841
5661.71	3.753	60	5	8.333	8	3.59	3.478	3.630	14.28	3.482
4954.00	3.695	60	2	3.333	3	3.12	3.071	3.135	7.86	3.075
4246.28	3.628	60	0	0.000	0	0.00	2.601	2.230	3.72	2.606

Results:

Y =-22.827+7.010X
Chi-squared is 1.018 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.969
LD_{50} is 9320.703µg /cm^2
95% confidence limits 7452.133 to 11657.8 µg/cm^2

AppendixTable 17: Dose-mortality effect of leaf extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 24 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	17	28.333	28	4.42	4.380	4.426	31.92	4.403
6369.43	3.804	60	12	20	20	4.16	4.154	4.17	28.26	4.168
5661.71	3.753	60	8	13.333	13	3.87	3.900	3.878	24.30	3.904
4954.00	3.695	60	4	6.667	7	3.52	3.614	3.529	18.12	3.606
4246.28	3.628	60	3	5	5	3.36	3.283	3.375	10.80	3.261

Results:

Y = -15.426+5.151X
Chi-squared is 0.279 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.966
LD_{50} is 9239.611µg /cm^2
95% confidence limits 7261.293 to 11756.92 µg/cm^2

AppendixTable 18: Dose-mortality effect of leaf extract (methanol) of *Adenenthera pavonina* against *C. chinensis* adults after 48 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	21	35.000	35	4.61	4.526	4.60	34.86	4.531
6369.43	3.804	60	13	21.667	22	4.23	4.306	4.234	31.92	4.306
5661.71	3.753	60	10	16.667	17	4.05	4.060	4.037	26.34	4.055
4954.00	3.695	60	6	10.000	10	3.72	3.780	3.72	20.16	3.771
4246.28	3.628	60	4	6.667	7	3.52	3.458	3.54	14.28	3.442

Results:

Y = -14.363+4.908X
Chi-squared is 0.530 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.945
LD_{50} is 8818.821µg /cm^2
95% confidence limits 7116.806 to 10927.88 µg/cm^2

AppendixTable 19: Dose-mortality effect of leaf extract (pet.ether) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	7	11.667	12	3.82	3.815	3.822	22.20	3.822
6369.43	3.804	60	5	8.333	8	3.59	3.632	3.596	18.12	3.639
5661.71	3.753	60	4	6.667	7	3.52	3.428	3.54	14.28	3.434
4954.00	3.695	60	2	3.333	3	3.12	3.196	3.116	9.24	3.203
4246.28	3.628	60	1	1.667	2	2.95	2.928	2.945	6.60	2.935

Results:

Y = -11.557+3.995X
Chi-squared is 0.262 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.145
LD_{50} is 13957.44µg /cm^2
95% confidence limits 6817.663 to 28574.33 µg/cm^2

AppendixTable 20: Dose-mortality effect of leaf extract (pet.ether) of *Adenenthera pavonina* against *C. chinensis* adults after 24 h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	13	21.667	22	4.23	4.233	4.218	30.18	4.222
6369.43	3.804	60	10	16.667	17	4.05	4.051	4.037	26.34	4.043
5661.71	3.753	60	8	13.333	13	3.87	3.848	3.873	22.20	3.844
4954.00	3.695	60	5	8.333	8	3.59	3.617	3.596	18.12	3.618
4246.28	3.628	60	3	5	5	3.36	3.351	3.36	12.48	3.357

Results:

Y = -10.775+3.895X

Chi-squared is 2.964E-02 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.050
LD_{50} is 11211.07µg /cm^2
95% confidence limits 7210.572 to 17431.06 µg/cm^2

AppendixTable 21: Dose-mortality effect of leaf extract (pet.ether) of *Adenenthera pavonina* against *C. chinensis* adults after 48 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	25	41.667	42	4.80	4.872	4.812	37.62	4.872
6369.43	3.804	60	22	36.667	37	4.67	4.604	4.659	36.06	4.607
5661.71	3.753	60	16	26.667	27	4.39	4.305	4.394	31.92	4.311
4954.00	3.695	60	8	13.333	13	3.87	3.966	3.878	24.30	3.976
4246.28	3.628	60	5	8.333	8	3.59	3.574	3.596	16.14	3.588

Results:

Y = -17.412+5.789X
Chi-squared is 0.668 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.872
LD_{50} is 7445.673µg /cm^2
95% confidence limits 6641.745 to 8346.892 µg/cm^2

AppendixTable 22: Dose-mortality effect of leaf extract (acetone) of *Adenenthera pavonina* against *C. chinensis* adults after 12 h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	14	23.333	23	4.26	4.262	4.252	30.18	4.252
6369.43	3.804	60	10	16.667	17	4.05	4.030	4.037	26.64	4.023
5661.71	3.753	60	6	10	10	3.72	3.769	3.72	20.16	3.768
4954.00	3.695	60	4	6.667	7	3.52	3.475	3.54	14.28	3.479
4246.28	3.628	60	2	3.333	3	3.12	3.134	3.116	9.24	3.145

Results:

Y = -14.964+4.991X
Chi-squared is 0.117 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.100
LD_{50} is 9993.172µg /cm^2
95% confidence limits 7385.677 to 13521.24 µg/cm^2

AppendixTable 23: Dose-mortality effect of leaf extract (acetone) of *Adenenthera pavonina* against *C. chinensis* adults after 24 h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	21	35	35	4.61	4.625	4.605	36.06	4.624
6369.43	3.804	60	18	30	30	4.48	4.413	4.48	33.48	4.416
5661.71	3.753	60	11	18.333	18	4.08	4.176	4.094	28.26	4.182
4954.00	3.695	60	9	15	15	3.96	3.908	3.97	24.30	3.917
4246.28	3.628	60	5	8.333	8	3.59	3.598	3.596	16.14	3.612

Results:

Y = -12.948+4.564X
Chi-squared is 0.444with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.932
LD_{50} is 8552.961µg /cm^2
95% confidence limits 6938.465 to 10543.12 µg/cm^2

AppendixTable 24: Dose-mortality effect of leaf extract (acetone) of *Adenenthera pavonina* against *C. chinensis* adults after 48 h of exposure.

Dose µg/cm²	*Log dose*	*# used*	*# Kill*	*% Kill*	*Corr. %*	*Emp. probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
7077.14	3.850	60	32	53.333	53	5.08	4.967	5.065	38.04	4.969
6369.43	3.804	60	23	38.333	38	4.69	4.718	4.688	36.96	4.718
5661.71	3.753	60	14	23.333	23	4.26	4.440	4.27	33.48	4.438
4954.00	3.695	60	12	20	20	4.16	4.124	4.17	28.26	4.120
4246.28	3.628	60	7	11.667	12	3.82	3.760	3.836	20.16	3.752

Results:

Y = -16.149+5.486X
Chi-squared is 1.545 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.855
LD_{50} is 7167.948µg /cm^2
95% confidence limits 6446.421 to 7970.223 µg/cm^2

Appendix Table 25: Dose-mortality effect of stem extract (pet. ether) of *Adenanthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	9	15	15	3.96	3.972	3.97	24.3	3.985
6369.43	3.804	60	7	11.667	12	3.82	3.795	3.836	20.16	3.805
5661.71	3.753	60	5	8.333	8	3.59	3.597	3.596	16.14	3.603
4954.00	3.695	60	3	5	5	3.36	3.372	3.36	12.48	3.375
4246.28	3.628	60	2	3.333	3	3.12	3.113	3.116	9.24	3.111

Results:

Y = -11.191 + 3.942X
Chi-squared is 0.031 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.107
LD_{50} is 12800.32µg /cm^2
95% confidence limits 7069.525 to 23176.730µg/cm^2

Appendix Table 26: Dose-mortality effect of stem extract (pet. ether) of *Adenanthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	15	25	25	4.33	4.313	4.33	31.92	4.320
6369.43	3.804	60	12	20	20	4.16	4.163	4.17	28.26	4.169
5661.71	3.753	60	9	15	15	3.96	3.995	3.97	24.30	4.001
4954.00	3.695	60	7	11.667	12	3.82	3.805	3.822	22.20	3.809
4246.28	3.628	60	5	8.333	8	3.59	3.585	3.596	16.14	3.589

Results:

Y =-8.375 +3.298X

Chi-squared is 3.198E-02 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.056
LD_{50} is 11376.43µg /cm^2
95% confidence limits 6906.73 to 18738.66µg/cm^2

Appendix Table 27: Dose-mortality effect of stem extract (pet. ether) of *Adenanthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	22	36.667	37	4.67	4.683	4.659	36.06	4.669
6369.43	3.804	60	19	31.667	32	4.53	4.520	4.516	34.86	4.511
5661.71	3.753	60	15	25	25	4.33	4.337	4.33	31.92	4.334
4954.00	3.695	60	12	20	20	4.16	4.130	4.17	28.26	4.134
4246.28	3.628	60	8	13.333	13	3.87	3.890	3.873	22.20	3.903

Results:

Y = -8.623 + 3.453 X
Chi-squared is 5.685E-02 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.946
LD_{50} is 8823.608µg /cm^2
95% confidence limits 6647.416 to 11712.22µg/cm^2

Appendix Table 28: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	12	20	20	4.16	4.148	4.17	28.26	4.159
6369.43	3.804	60	9	15	15	3.96	3.985	3.97	24.30	3.994
5661.71	3.753	60	7	11.667	12	3.82	3.803	3.822	22.20	3.809
4954.00	3.695	60	5	8.333	8	3.59	3.596	3.596	16.14	3.600
4246.28	3.628	60	3	5	5	3.36	3.358	3.36	12.48	3.358

Results:

Y = -9.743 +3.611x
Chi-squared is 2.387E-02 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.083
LD_{50} is 12099.02 µg /cm^2
95% confidence limits are 7033.855 to 20811.70µg /cm^2

Appendix Table 29: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr . %***	***Emp probit***	***Expt probit***	***Work probit***	***Weig ht***	***Final probit***
7077.14	3.850	60	20	33.333	33	4.56	4.552	4.544	34.86	4.540
6369.43	3.804	60	16	26.667	27	4.39	4.401	4.39	33.48	4.391
5661.71	3.753	60	13	21.667	22	4.23	4.233	4.218	30.18	4.225
4954.00	3.695	60	10	16.667	17	4.05	4.042	4.037	26.34	4.036
4246.28	3.628	60	7	11.667	12	3.82	3.822	3.822	22.20	3.818

Results:

Y = -7.985 + 3.253X
Chi-squared is 1.332E-03 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.991
LD_{50} is 9799.158µg /cm^2
95% confidence limits are 6772.172 to 14179.13µg /cm^2

Appendix Table 30: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	30	50	50	5.0	4.971	4.99	38.04	4.960
6369.43	3.804	60	25	41.667	42	4.8	4.794	4.792	36.96	4.786
5661.71	3.753	60	20	33.333	33	4.56	4.597	4.544	34.86	4.591
4954.00	3.695	60	15	25	25	4.33	4.373	4.33	31.92	4.370
4246.28	3.628	60	12	20	20	4.16	4.115	4.17	28..26	4.115

Results:

Y = -9.703+3.809X
Chi-squared is 0.258 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.860
LD_{50} is 7249.375 µg /cm^2
95% confidence limits are 6207.77 to 8465.757 µg /cm^2

Appendix Table 31: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	11	18.333	18	4.08	4.091	4.078	26.34	4.084
6369.43	3.804	60	8	13.333	13	3.87	3.902	3.878	24.30	3.902
5661.71	3.753	60	6	10	10	3.72	3.691	3.73	18.12	3.700
4954.00	3.695	60	4	6.667	7	3.52	3.451	3.54	14.28	3.470
4246.28	3.628	60	2	3.333	3	3.12	3.174	3.116	9.24	3.205

Results:

Y = -11.171+3.962X
Chi-squared is 0.175 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.081
LD_{50} is 12053.19 µg /cm^2
95% confidence limits are 7112.16 to 20426.90 µg /cm^2

Appendix Table 32: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonina* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	***Log dose***	***# used***	***# Kill***	***% Kill***	***Corr. %***	***Emp. probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
7077.14	3.850	60	20	33.333	33	4.56	4.553	4.544	34.86	4.544
6369.43	3.804	60	15	25	25	4.33	4.354	4.33	31.92	4.350
5661.71	3.753	60	12	20	20	4.16	4.132	4.17	28..26	4.133
4954.00	3.695	60	8	13.333	13	3.87	3.880	3.873	22. 20	3.887
4246.28	3.628	60	5	8.333	8	3.59	3.589	3.596	16.14	3.603

Results:

Y =-11.787+4.242X
Chi-squared is 0.054 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.957
LD_{50} is 9064.269µg /cm^2
95% confidence limits are 7002.897 to 11732.43µg /cm^2

Appendix Table 33: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonina* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	40	66.667	67	5.44	5.332	5.422	36.96	5.326
6369.43	3.804	60	30	50	50	5.00	5.035	5.000	38.22	5.028
5661.71	3.753	60	20	33.333	33	4.56	4.703	4.558	36.96	4.694
4954.00	3.695	60	15	25	25	4.33	4.327	4.330	31.92	4.316
4246.28	3.628	60	9	15	15	3.96	3.892	3.975	22.20	3.878

Results:

Y = -19.769+6.519X
Chi-squared is 1.268with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.800
LD_{50} is 6306.757µg /cm^2
95% confidence limits are 5929.698 to 6707.792µg /cm^2

Appendix Table 34: Dose-mortality effect of stem extract (Methanol) of *Adenanthera pavonina* against *C. chinensis* adults after 12h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	13	21.667	22	4.23	4.222	4.218	30.18	4.214
6369.43	3.804	60	10	16.667	17	4.05	4.041	4.037	26.34	4.034
5661.71	3.753	60	7	11.667	12	3.82	3.838	3.822	22.20	3.833
4954.00	3.695	60	5	8.333	8	3.59	3.608	3.596	18.12	3.606
4246.28	3.628	60	3	5	5	3.36	3.342	3.360	12.48	3.343

Results:

Y = -10.897 + 3.925X
Chi-squared is 9.541E-03 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 4.050
LD_{50} is 11224.51µg /cm^2
95% confidence limits are 7237.74 to 17407.33µg /cm^2

Appendix Table 35: Dose-mortality effect of stem extract (Methanol) of *Adenanthera pavonia* against *C. chinensis* adults after 24h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	25	41.667	42	4.80	4.764	4.792	36.96	4.758
6369.43	3.804	60	20	33.333	33	4.56	4.569	4.544	34.86	4.564
5661.71	3.753	60	15	25	25	4.33	4.352	4.330	31.92	4.347
4954.00	3.695	60	10	16.667	17	4.05	4.105	4.056	28.26	4.101
4246.28	3.628	60	8	13.333	13	3.87	3.820	3.873	22.20	3.816

Results:

Y = -11.594+ 4.248X
Chi-squared is 0.192 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.907
LD_{50} is 8066.323µg /cm^2
95% confidence limits are 6681.735 to 9737.825µg /cm^2

Appendix Table 36: Dose-mortality effect of stem extract (Methanol) of *Adenanthera pavonia* against *C. chinensis* adults after 48h of exposure.

Dose µg/cm²	Log dose	# used	# Kill	% Kill	Corr. %	Emp. probit	Expt probit	Work probit	Weight	Final probit
7077.14	3.850	60	45	75	75	5.67	5.665	5.67	33.48	5.662
6369.43	3.804	60	40	66.667	67	5.44	5.372	5.422	36.96	5.366
5661.71	3.753	60	30	50	50	5.00	5.045	5.00	38.22	5.034
4954.00	3.695	60	20	33.333	33	4.56	4.673	4.551	36.06	4.659
4246.28	3.628	60	15	25	25	4.33	4.245	4.32	30.18	4.225

Results:

Y = -19.277 + 6.478 X
Chi-squared is 0.849 with 3 degrees of freedom
No significant heterogeneity
Log LD_{50} is 3.748
LD_{50} is 5592.335µg /cm^2
95% confidence limits are 5305.335 to 5894.861µg /cm^2

Appendix Table 37: Dose-mortality effect of leaf extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 30 min. of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	5	16.667	17	4.05	3.969	4.062	12.15	3.992
200	2.301	30	3	10	10	3.72	3.785	3.72	10.08	3.801
100	1.999	30	2	6.667	7	3.52	3.600	3.529	9.060	3.609
50	1.699	30	2	6.667	7	3.52	3.416	3.54	7.140	3.418
25	1.398	30	1	3.333	3	3.12	3.232	3.121	5.40	3.227
12.5	1.097	30	1	3.333	3	3.12	3.048	3.135	3.93	3.036

Results:

Y = 2.339 +0.635X
Chi-squared is 0.389 with 4 degrees of freedom
No significant heterogeneity
Log LC_{50} is 4.189
LC_{50} is 15469.24
95% confidence limits are 137.4437 to 1741058

Appendix Table 38: Dose-mortality effect of leaf extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	15	50	50	5	4.866	5.02	18.81	4.890
200	2.301	30	10	33.333	33	4.56	4.635	4.551	18.03	4.651
100	1.999	30	8	26.667	27	4.39	4.405	4.39	16.74	4.411
50	1.699	30	5	16.667	17	4.05	4.175	4.056	14.13	4.172
25	1.398	30	4	13.333	13	3.87	3.945	3.878	12.15	3.933
12.5	1.097	30	4	13.333	13	3.87	3.714	3.894	10.08	3.694

Results:

Y = 2.821+0.795X
Chi-squared is 1.137 with 4 degrees of freedom
No significant heterogeneity
Log LC_{50} is 2.740
LC_{50} is 50 IS 549.935
95% confidence limits are 179.709 to 1682.885

Appendix Table 39: Dose-mortality effect of leaf extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	25	83.333	83	5.95	5.767	5.926	15.96	5.734
200	2.301	30	19	63.333	63	5.33	5.483	5.321	18.03	5.457
100	1.999	30	16	53.333	53	5.08	5.199	5.065	19.02	5.180
50	1.699	30	14	46.667	47	4.92	4.915	4.915	19.02	4.903
25	1.398	30	11	36.667	37	4.67	4.631	4.659	18.03	4.625
12.5	1.097	30	8	26.667	27	4.39	4.347	4.394	15.96	4.348

Results:

Y = 3.337+0.921X

Chi-squared is 1.228 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.805

LC_{50} is 63.791

95% confidence limits are 39.563 to 102.857

Appendix Table 40: Dose-mortality effect of leaf extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	6	20	20	4.16	4.150	4.17	14.13	4.155
200	2.301	30	5	16.667	17	4.05	4.026	4.037	13.17	4.030
100	1.999	30	4	13.333	13	3.87	3.902	3.878	12.15	3.904
50	1.699	30	3	10	10	3.72	3.778	3.72	10.08	3.779
25	1.398	30	3	10	10	3.72	3.654	3.73	9.060	3.654
12.5	1.097	30	2	6.667	7	3.52	3.530	3.519	8.070	3.529

Results:

Y = 3.073+0.416X

Chi-squared is 0.101 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 4.636

LC_{50} is 43235.51

95% confidence limits are 31.943 to 5.852E+07

Appendix Table 41: Dose-mortality effect of leaf extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	20	66.667	67	5.44	5.468	5.429	18.03	5.474
200	2.301	30	18	60	60	5.25	5.226	5.28	18.81	5.228
100	1.999	30	15	50	50	5.00	4.984	4.99	19.02	4.982
50	1.699	30	12	40	40	4.75	4.742	4.74	18.48	4.736
25	1.398	30	9	30	30	4.48	4.501	4.46	17.43	4.490
12.5	1.097	30	7	23.333	23	4.26	4.259	4.252	15.09	4.244

Results:

Y=3.348+0.817X

Chi-squared is 0.105 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.022

LC_{50} is 105.242

95% confidence limits are 60.326 to 183.602

Appendix Table 42: Dose-mortality effect of leaf extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	26	86.667	87	6.13	5.995	6.136	14.13	5.980
200	2.301	30	22	73.333	73	5.61	5.678	5.610	16.74	5.666
100	1.999	30	18	60	60	5.25	5.362	5.24	18.48	5.352
50	1.699	30	15	50	50	5.00	5.045	5.00	19.11	5.037
25	1.398	30	12	40	40	4.75	4.728	4.74	18.48	4.723
12.5	1.097	30	9	30	30	4.48	4.412	4.48	16.74	4.409

Results:

Y = 3.264+1.044X

Chi-squared is 0.743 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.663

LC_{50} is 46.046

95% confidence limits are 29.479 to 71.922

Appendix Table 43: Dose-mortality effect of leaf extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	8	26.667	27	4.39	4.322	4.394	15.96	4.346
200	2.301	30	6	20.000	20	4.16	4.138	4.170	14.13	4.155
100	1.999	30	4	13.333	13	3.87	3.955	3.878	12.15	3.963
50	1.699	30	3	10.000	10	3.72	3.772	3.720	10.08	3.772
25	1.398	30	2	6.667	7	3.52	3.588	3.519	8.07	3.580
12.5	1.097	30	2	6.667	7	3.52	3.405	3.540	7.14	3.389

Results:

Y =2.691+0.636X

Chi-squared is 0.348 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 3.629

LC_{50} is 4256.71

95% confidence limits are 219.277 to 82633.13

Appendix Table 44: Dose-mortality effect of leaf extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	22	73.333	73	5.61	5.650	5.61	16.74	5.659
200	2.301	30	20	66.667	67	5.44	5.436	5.429	18.03	5.441
100	1.999	30	18	60	60	5.25	5.222	5.28	18.81	5.223
50	1.699	30	16	53.333	53	5.08	5.008	5.075	19.11	5.006
25	1.398	30	12	40	40	4.75	4.794	4.74	18.48	4.788
12.5	1.097	30	10	33.333	33	4.56	4.580	4.544	17.43	4.571

Results:

Y =3.778+ 0.723X

Chi-squared is 0.249 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.691

LC_{50} is 49.081

95% confidence limits are 26.259 to 91.736

Appendix Table 45: Dose-mortality effect of leaf extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	27	90	90	6.28	6.190	6.27	12.15	6.149
200	2.301	30	24	80	80	5.85	5.890	5.80	15.09	5.860
100	1.999	30	21	70	70	5.52	5.591	5.50	17.43	5.570
50	1.699	30	18	60	60	5.25	5.292	5.28	18.81	5.281
25	1.398	30	15	50	50	5.00	4.993	4.99	19.02	4.991
12.5	1.097	30	12	40	40	4.75	4.694	4.74	18.03	4.702

Results:

Y =3.646+0.962X

Chi-squared is 0.344 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.407

LC_{50} is 25.539

95% confidence limits are 14.159 to 46.063

Appendix Table 46: Dose-mortality effect of leaf extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	7	23.333	23	4.26	4.212	4.252	15.09	4.213
200	2.301	30	5	16.667	17	4.05	4.056	4.037	13.17	4.057
100	1.999	30	4	13.333	13	3.87	3.901	3.878	12.15	3.901
50	1.699	30	3	10	10	3.72	3.746	3.72	10.08	3.745
25	1.398	30	2	6.667	7	3.52	3.590	3.519	8.07	3.589
12.5	1.097	30	2	6.667	7	3.52	3.435	3.54	7.140	3.433

Results:

Y = 2.864+ 0.518X

Chi-squared is 0.162 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 4.121

LC_{50} is 13202.60

95% confidence limits are 115.401 to 1510458

Appendix Table 47: Dose-mortality effect of leaf extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	21	70	70	5.52	5.490	5.51	18.03	5.493
200	2.301	30	17	56.667	57	5.18	5.270	5.202	18.81	5.274
100	1.999	30	16	53.333	53	5.08	5.051	5.075	19.11	5.055
50	1.699	30	14	46.667	47	4.92	4.832	4.942	18.81	4.837
25	1.398	30	10	33.333	33	4.56	4.613	4.551	18.03	4.618
12.5	1.097	30	8	26.667	27	4.39	4.394	4.394	15.96	4.400

Results:

Y = 3.603 + 0.726X

Chi-squared is 0.401 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.924

LC_{50} is 83.859

95% confidence limits are 46.035 to 152.759

Appendix Table 48: Dose-mortality effect of leaf extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	26	86.667	87	6.13	5.922	6.136	14.13	5.896
200	2.301	30	20	66.667	67	5.44	5.634	5.430	16.74	5.614
100	1.999	30	18	60	60	5.25	5.346	5.240	18.48	5.332
50	1.699	30	16	53.333	53	5.08	5.058	5.075	19.11	5.050
25	1.398	30	12	40	40	4.75	4.769	4.74	18.48	4.768
12.5	1.097	30	10	33.333	33	4.56	4.481	4.57	16.74	4.486

Results:

Y =3.458+0.937X

Chi-squared is 1.682 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.646

LC_{50} is 44.246

95% confidence limits are 26.783 to 73.094

Appendix Table 49: Dose-mortality effect of seed extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	7	23.333	23	4.26	4.212	4.252	15.09	4.213
100	1.999	30	5	16.667	17	4.05	4.056	4.037	13.17	4.057
50	1.699	30	4	13.333	13	3.87	3.901	3.878	12.15	3.901
25	1.398	30	3	10	10	3.72	3.746	3.72	10.08	3.745
12.5	1.097	30	2	6.667	7	3.52	3.590	3.519	8.07	3.589
6.25	0.796	30	2	6.667	7	3.52	3.435	3.54	7.140	3.433

Results:

Y =3.020+0.518X

Chi-squared is 0.162 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 3.820

LC_{50} is 6601.437

95% confidence limits are 57.700 to 755264.70

Appendix Table 50: Dose-mortality effect of seed extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	18	60	60	5.25	5.238	5.28	18.81	5.251
100	1.999	30	15	50	50	5.00	4.996	4.99	19.02	5.000
50	1.699	30	12	40	40	4.75	4.753	4.74	18.48	4.750
25	1.398	30	9	30	30	4.48	4.510	4.46	17.43	4.500
12.5	1.097	30	7	23.333	23	4.26	4.268	4.252	15.09	4.249
6.25	0.796	30	5	16.667	17	4.05	4.025	4.037	13.17	3.999

Results:

Y =3.337+0.832X

Chi-squared is 6.689E-02 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.999

LC_{50} is 99.821

95% confidence limits are 50.501 to 197.308

Appendix Table 51: Dose-mortality effect of seed extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
200	2.301	30	26	86.667	87	6.13	6.085	6.087	13.17	6.033
100	1.999	30	24	80	80	5.85	5.808	5.80	15.09	5.768
50	1.699	30	20	66.667	67	5.44	5.530	5.416	17.43	5.502
25	1.398	30	17	56.667	57	5.18	5.253	5.202	18.81	5.237
12.5	1.097	30	15	50	50	5.00	4.976	4.99	19.02	4.971
6.25	0.796	30	12	40	40	4.75	4.698	4.74	18.03	4.706

Results:

Y =4.004+0.882X

Chi-squared is 0.234 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.129

LC_{50} is 13.470

95% confidence limits are 7.182 to 25.264

Appendix Table 52: Dose-mortality effect of seed extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
200	2.301	30	8	26.667	27	4.39	4.387	4.394	15.96	4.375
100	1.999	30	6	20	20	4.16	4.225	4.15	15.09	4.218
50	1.699	30	5	16.667	17	4.05	4.063	4.037	13.17	4.061
25	1.398	30	5	16.667	17	4.05	3.901	4.062	12.15	3.905
12.5	1.097	30	3	10	10	3.72	3.739	3.72	10.08	3.748
6.25	0.796	30	2	6.667	7	3.52	3.577	3.519	8.07	3.591

Results:

Y =3.177+0.520X

Chi-squared is 0.435 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 3.503

LC_{50} is 3181.012

95% confidence limits are 67.055 to 150904.20

Appendix Table 53: Dose-mortality effect of seed extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	21	70	70	5.52	5.531	5.50	17.43	5.531
100	1.999	30	18	60	60	5.25	5.273	5.28	18.81	5.274
50	1.699	30	15	50	50	5.00	5.014	5.00	19.11	5.017
25	1.398	30	13	43.333	43	4.82	4.756	4.82	18.48	4.760
12.5	1.097	30	10	33.333	33	4.56	4.497	4.57	16.74	4.503
6.25	0.796	30	6	20	20	4.16	4.239	4.15	15.09	4.245

Results:

Y = 3.565+0.854X

Chi-squared is 0.300 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.680

LC_{50} is 47.757

95% confidence limits are 28.254 to 80.722

Appendix Table 54: Dose-mortality effect of seed extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	27	90	90	6.28	6.247	6.23	11.10	6.230
100	1.999	30	24	80	80	5.85	5.918	5.87	14.13	5.906
50	1.699	30	22	73.333	73	5.61	5.589	5.584	17.43	5.582
25	1.398	30	18	60	60	5.25	5.261	5.28	18.81	5.257
12.5	1.097	30	15	50	50	5.00	4.932	4.99	19.02	4.933
6.25	0.796	30	10	33.333	33	4.56	4.603	4.551	18.03	4.609

Results:

Y =3.751+1.078X

Chi-squared is 0.150 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.159

LC_{50} is 14.425

95% confidence limits are 8.743 to 23.800

Appendix Table 55: Dose-mortality effect of seed extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	***Log dose***	***# used***	***# Kill***	***%Kill***	***Corr %***	***Emp probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
200	2.301	30	12	40	40	4.75	4.774	4.74	18.48	4.761
100	1.999	30	10	33.333	33	4.56	4.561	4.544	17.43	4.553
50	1.699	30	8	26.667	27	4.39	4.348	4.394	15.96	4.346
25	1.398	30	6	20	20	4.16	4.135	4.17	14.13	4.138
12.5	1.097	30	4	13.333	13	3.87	3.922	3.878	12.15	3.931
6.25	0.796	30	3	10	10	3.72	3.710	3.72	10.08	3.723

Results:

Y =3.175+0.690X

Chi-squared is 9.481E-02 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.648

LC_{50} is 444.648

95% confidence limits are 93.614 to 2111.985

Appendix Table 56: Dose-mortality effect of seed extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	***Log dose***	***# used***	***# Kill***	***%Kill***	***Corr %***	***Emp probit***	***Expt probit***	***Work probit***	***Weight***	***Final probit***
200	2.301	30	24	80	80	5.85	5.787	5.83	15.96	5.763
100	1.999	30	21	70	70	5.52	5.581	5.50	17.43	5.560
50	1.699	30	19	63.333	63	5.33	5.375	5.318	18.48	5.358
25	1.398	30	17	56.667	57	5.18	5.169	5.165	19.02	5.155
12.5	1.097	30	15	50	50	5.00	4.963	4.99	19.02	4.952
6.25	0.796	30	12	40	40	4.75	4.757	4.74	18.48	4.750

Results:

Y =4.214+0.673X

Chi-squared is 0.195 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.168

LC_{50} is 14.709

95% confidence limits are 6.693 to 32.322

Appendix Table 57: Dose-mortality effect of seed extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
200	2.301	30	29	96.667	97	6.88	6.599	6.759	8.07	6.604
100	1.999	30	27	90	90	6.28	6.287	6.23	11.10	6.292
50	1.699	30	25	83.333	83	5.95	5.975	5.984	14.13	5.980
25	1.398	30	21	70	70	5.52	5.662	5.52	16.74	5.668
12.5	1.097	30	20	66.667	67	5.44	5.350	5.422	18.48	5.355
6.25	0.796	30	16	53.333	53	5.08	5.038	5.075	19.11	5.043

Results:

Y = 4.218+1.037X

Chi-squared is 0.702 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 0.754

LC_{50} is 5.678

95% confidence limits are 2.591 to 12.443

Appendix Table 58: Dose-mortality effect of seed extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
200	2.301	30	10	33.333	33	4.56	4.590	4.544	17.43	4.578
100	1.999	30	8	26.667	27	4.39	4.380	4.394	15.96	4.375
50	1.699	30	6	20	20	4.16	4.171	4.170	14.13	4.172
25	1.398	30	5	16.667	17	4.05	3.962	4.062	12.15	3.970
12.5	1.097	30	3	10	10	3.72	3.753	3.72	10.08	3.766
6.25	0.796	30	2	6.667	7	3.52	3.544	3.519	8.07	3.563

Results:

Y =3.027+0.674X

Chi-squared is 0.168 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.927

LC_{50} is 846.206

95% confidence limits are107.583 to 6655.92

Appendix Table 59: Dose-mortality effect of seed extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	22	73.333	73	5.61	5.609	5.61	16.74	5.599
100	1.999	30	19	63.333	63	5.33	5.377	5.318	18.48	5.369
50	1.699	30	17	56.667	57	5.18	5.146	5.165	19.02	5.139
25	1.398	30	14	46.667	47	4.92	4.914	4.915	19.02	4.909
12.5	1.097	30	12	40	40	4.75	4.683	4.74	18.03	4.678
6.25	0.796	30	8	26.667	27	4.39	4.451	4.39	16.74	4.448

Results:

Y = 3.840+0.765X

Chi-squared is 0.189 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.518

LC_{50} is 32.924

95% confidence limits are18.637 to 58.163

Appendix Table 60: Dose-mortality effect of seed extract (Methanol) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
200	2.301	30	28	93.333	93	6.48	6.336	6.424	10.08	6.267
100	1.999	30	25	83.333	83	5.95	6.009	5.923	13.17	5.957
50	1.699	30	21	70	70	5.52	5.682	5.52	16.74	5.647
25	1.398	30	19	63.333	63	5.33	5.355	5.318	18.48	5.337
12.5	1.097	30	16	53.333	53	5.08	5.028	5.075	19.11	5.027
6.25	0.796	30	12	40	40	4.75	4.700	4.74	18.48	4.717

Results:

Y =3.898+1.029X

Chi-squared is 0.594 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.070

LC_{50} is 11.762

95% confidence limits are 6.659 to 20.775

Appendix Table 61: Dose-mortality effect of stem extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	4	13.333	13	3.87	3.924	3.878	12.15	3.942
200	2.301	30	3	10	10	3.72	3.688	3.73	9.060	3.689
100	1.999	30	2	6.667	7	3.52	3.452	3.54	7.140	3.436
50	1.699	30	1	3.333	3	3.12	3.216	3.121	5.40	3.183
25	1.398	30	1	3.333	3	3.12	2.979	3.172	3.30	2.930
12.5	1.097	30	0	0	0	0	2.743	2.32	2.28	2.677

Results:

Y =1.755+0.840X

Chi-squared is 0.647 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 3.861

LC_{50} is 7266.57

95% confidence limits are 248.445 to 212533.80

Appendix Table 62: Dose-mortality effect of stem extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	13	43.333	43	4.82	4.790	4.818	18.48	4.800
200	2.301	30	10	33.333	33	4.56	4.546	4.544	17.43	4.551
100	1.999	30	8	26.667	27	4.39	4.302	4.394	15.96	4.301
50	1.699	30	4	13.333	13	3.87	4.058	3.873	13.17	4.052
25	1.398	30	3	10	10	3.72	3.814	3.72	11.10	3.803
12.5	1.097	30	3	10	10	3.72	3.570	3.75	8.07	3.554

Results:

Y =2.645+0.828X

Chi-squared is 0.954 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.844

LC_{50} is 697.739

95% confidence limits are 208.878 to 2330.734

Appendix Table 63: Dose-mortality effect of stem extract (Pet. ether) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	24	80	80	5.85	5.817	5.80	15.09	5.784
200	2.301	30	20	66.667	67	5.44	5.502	5.416	17.43	5.476
100	1.999	30	18	60	60	5.25	5.187	5.24	19.02	5.169
50	1.699	30	13	43.333	43	4.82	4.873	4.838	18.81	4.862
25	1.398	30	10	33.333	33	4.56	4.558	4.544	17.43	4.554
12.5	1.097	30	7	23.333	23	4.26	4.243	4.252	15.09	4.247

Results:

Y =3.127+1.021X

Chi-squared is 0.176 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.834

LC_{50} is 68.312

95% confidence limits are 44.174 to 105.639

Appendix Table 64: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	5	16.667	17	4.05	4.024	4.037	13.17	4.019
200	2.301	30	4	13.333	13	3.87	3.931	3.878	12.15	3.928
100	1.999	30	4	13.333	13	3.87	3.838	3.873	11.10	3.838
50	1.699	30	3	10	10	3.72	3.745	3.72	10.08	3.748
25	1.398	30	3	10	10	3.72	3.652	3.73	9.060	3.658
12.5	1.097	30	2	6.667	7	3.52	3.560	3.519	8.07	3.567

Results:

Y =3.238+0.300X

Chi-squared is 0.123 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 5.872

LC_{50} is 745099

95% confidence limits are 2903859 to 1.912E+12

Appendix Table 65: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	18	60	60	5.25	5.219	5.28	18.81	5.230
200	2.301	30	15	50	50	5.00	5.005	5.00	19.11	5.013
100	1.999	30	12	40	40	4.75	4.792	4.74	18.48	4.795
50	1.699	30	10	33.333	33	4.56	4.578	4.544	17.43	4.578
25	1.398	30	8	26.667	27	4.39	4.365	4.394	15.96	4.360
12.5	1.097	30	6	20	20	4.16	4.151	4.17	14.13	4.143

Results:

Y =3.351+0.722X

Chi-squared is 0.155 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.283

LC_{50} is 192.026

95% confidence limits are 88.802 to 415.237

Appendix Table 66: Dose-mortality effect of stem extract (Acetone) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	25	83.333	83	5.95	5.970	5.984	14.13	5.989
200	2.301	30	22	73.333	73	5.61	5.680	5.61	16.74	5.693
100	1.999	30	20	66.667	67	5.44	5.391	5.422	18.48	5.397
50	1.699	30	18	60	60	5.25	5.102	5.24	19.02	5.100
25	1.398	30	12	40	40	4.75	4.813	4.76	18.81	4.804
12.5	1.097	30	9	30	30	4.48	4.524	4.46	17.43	4.508

Results:

Y =3.429+0.984X

Chi-squared is 0.574 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.597

LC_{50} is 39.527

95% confidence limits are 24.212 to 64.528

Appendix Table 67: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	7	23.333	23	4.26	4.260	4.252	15.09	4.253
200	2.301	30	5	16.667	17	4.05	4.120	4.056	14.13	4.118
100	1.999	30	5	16.667	17	4.05	3.981	4.062	12.15	3.982
50	1.699	30	4	13.333	13	3.87	3.842	3.873	11.10	3.847
25	1.398	30	3	10	10	3.72	3.703	3.72	10.08	3.711
12.5	1.097	30	2	6.667	7	3.52	3.564	3.519	8.07	3.576

Results:

Y =3.082+0.450X

Chi-squared is 0.166 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 4.261

LC_{50} is 18255.69

95% confidence limits are 66.731 to 4994224

Appendix Table 68: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	20	66.667	67	5.44	5.419	5.429	18.03	5.422
200	2.301	30	17	56.667	57	5.18	5.211	5.202	18.81	5.212
100	1.999	30	15	50	50	5.00	5.002	5.00	19.11	5.002
50	1.699	30	13	43.333	43	4.82	4.794	4.818	18.48	4.793
25	1.398	30	10	33.333	33	4.56	4.586	4.544	17.43	4.583
12.5	1.097	30	8	26.667	27	4.39	4.378	4.394	15.96	4.374

Results:

Y =3.610+0.696X

Chi-squared is 4.875E-02 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.996

LC_{50} is 99.179

95% confidence limits are 52.125 to 188.710

Appendix Table 69: Dose-mortality effect of stem extract (Chloroform) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	26	86.667	87	6.13	6.003	6.087	13.17	5.963
200	2.301	30	22	73.333	73	5.61	5.704	5.606	15.96	5.673
100	1.999	30	19	63.333	63	5.33	5.405	5.321	18.03	5.384
50	1.699	30	16	53.333	53	5.08	5.105	5.065	19.02	5.094
25	1.398	30	13	43.333	43	4.82	4.806	4.838	18.81	4.805
12.5	1.097	30	10	33.333	33	4.56	4.507	4.544	17.43	4.515

Results:

Y =3.460+0.962X

Chi-squared is 0.398 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.601

LC_{50} is 39.897

95% confidence limits are 24.167 to 65.864

Appendix Table 70: Dose-mortality effect of stem extract (methanol) of *Adenanthera pavonia* against *Artemia salina* after 30 min of exposure.

Dose ppm	*Log dose*	*# used*	*# Kill*	*%Kill*	*Corr %*	*Emp probit*	*Expt probit*	*Work probit*	*Weight*	*Final probit*
400	2.602	30	6	20	20	4.16	4.236	4.15	15.09	4.200
200	2.301	30	5	16.667	17	4.05	4.037	4.037	13.17	4.020
100	1.999	30	4	13.333	13	3.87	3.839	3.873	11.10	3.839
50	1.699	30	3	10	10	3.72	3.641	3.73	9.060	3.658
25	1.398	30	2	6.667	7	3.52	3.443	3.54	7.140	3.477
12.5	1.097	30	1	3.333	3	3.12	3.244	3.121	5.400	3.296

Results:

Y =2.638+0.601X

Chi-squared is 0.297 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 3.933

LC_{50} is 8575.589

95% confidence limits are 172.861 to 425433.500

Appendix Table 71: Dose-mortality effect of stem extract (methanol) of *Adenanthera pavonia* against *Artemia salina* after 24h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	19	63.333	63	5.33	5.345	5.318	18.48	5.336
200	2.301	30	16	53.333	53	5.08	5.122	5.065	19.02	5.115
100	1.999	30	14	46.667	47	4.92	4.898	4.942	18.81	4.895
50	1.699	30	12	40	40	4.75	4.675	4.74	18.03	4.674
25	1.398	30	9	30	30	4.48	4.452	4.48	16.74	4.453
12.5	1.097	30	6	20	20	4.16	4.228	4.15	15.09	4.232

Results:

Y =3.428+0.733X

Chi-squared is 0.290 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 2.144

LC_{50} is 139.213

95% confidence limits are 71.126 to 272.478

Appendix Table 72: Dose-mortality effect of stem extract (methanol) of *Adenanthera pavonia* against *Artemia salina* after 48h of exposure.

Dose ppm	Log dose	# used	# Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
400	2.602	30	25	83.333	83	5.95	5.828	5.902	15.09	5.791
200	2.301	30	20	66.667	67	5.44	5.538	5.416	17.43	5.509
100	1.999	30	17	56.667	57	5.18	5.247	5.202	18.81	5.228
50	1.699	30	15	50	50	5.00	4.956	4.99	19.02	4.947
25	1.398	30	10	33.333	33	4.56	4.666	4.551	18.03	4.666
12.5	1.097	30	9	30	30	4.48	4.375	4.49	15.96	4.384

Results:

Y =3.360+0.934X

Chi-squared is 0.802 with 4 degrees of freedom

No significant heterogeneity

Log LC_{50} is 1.756

LC_{50} is 56.989

95% confidence limits are 35.272 to 92.077

Appendix Table73: Repellency of *C. chinensis* by seed (pet.ether) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm²)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	10	9	7	9	7	9.00	8.00	8.00	8.00	7.666	80.00	60.00	60.00	60.00	53.32	63.44	50.77	50.77	50.77	46.89
		R2	8	8	9	8	7															
		R3	9	7	8	7	9															
236	10	R1	6	9	7	9	7	8.00	7.666	7.666	8.00	7.333	60.00	53.32	53.32	60.00	46.66	50.77	46.89	46.89	50.77	43.05
		R2	8	6	9	7	7															
		R3	10	8	7	8	8															
118	10	R1	7	9	6	7	8	7.333	7.000	6.666	7.000	7.000	46.66	40.00	33.32	40.00	40.00	43.05	39.23	35.24	39.23	39.23
		R2	8	6	9	5	5															
		R3	7	6	5	5	8															
59	10	R1	5	7	8	5	8	6.333	6.000	7.333	7.333	7.333	26.66	20.00	46.66	46.66	46.66	31.05	26.56	43.05	43.05	43.05
		R2	8	6	8	7	7															
		R3	6	5	6	10	7															
30	10	R1	7	6	5	4	6	6.000	6.666	6.666	6.000	6.333	20.00	33.32	33.32	20.00	26.66	20.56	35.24	35.24	26.56	31.05
		R2	7	9	7	6	8															
		R3	4	5	8	8	5															

Appendix Table 74: Repellency of *C. chinensis* by seed (acetone) of *A. pavconina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	9	10	9	8	10	9.666	9.333	9.000	8.333	9.000	93.32	86.66	80.00	66.66	80.00	75.00	68.53	63.44	54.70	63.44
		R2	10	9	10	9	8															
		R3	10	9	8	8	9															
236	10	R1	10	10	8	9	9	9.666	8.333	9.000	8.000	8.666	93.32	66.66	80.00	60.00	73.32	75.00	54.70	63.44	50.77	58.89
		R2	10	6	9	7	10															
		R3	9	9	10	8	7															
118	10	R1	6	8	10	7	8	8.333	9.000	9.000	8.666	8.333	66.66	80.00	80.00	73.32	66.66	54.70	63.44	63.44	58.89	54.70
		R2	9	10	8	9	9															
		R3	10	9	9	10	8															
59	10	R1	9	8	8	9	7	8.666	7.666	8.000	8.000	8.000	73.32	53.32	60.00	60.00	60.00	58.89	46.89	50.77	50.77	50.77
		R2	7	6	7	8	9															
		R3	10	9	9	7	8															
30	10	R1	8	6	5	5	7	8.000	7.666	8.000	7.000	7.333	60.00	53.32	60.00	40.00	46.66	50.77	46.89	50.77	39.23	43.05
		R2	7	9	10	9	8															
		R3	9	8	9	7	7															

Appendix Table 75: Repellency of *C. chinensis* by seed (chloroform) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose ($\mu g/cm^2$)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	8	9	10	9	7	7.000	7.666	8.000	8.000	7.333	40.00	53.32	60.00	60.00	46.66	39.23	46.89	50.77	50.77	43.05
		R2	6	8	7	8	6															
		R3	7	6	7	7	9															
236	10	R1	6	8	5	8	7	6.666	6.666	7.333	6.333	7.000	33.32	33.32	46.66	26.66	40.00	35.24	35.24	43.05	31.05	39.23
		R2	9	6	8	5	6															
		R3	5	6	9	6	8															
118	10	R1	7	5	6	4	7	6.000	6.666	6.666	6.333	6.666	20.00	33.32	33.32	26.66	33.32	26.56	35.24	35.24	31.05	35.24
		R2	6	8	8	7	5															
		R3	5	7	6	8	8															
59	10	R1	6	9	8	7	5	6.666	6.666	6.666	6.333	6.666	33.32	33.32	33.32	26.56	33.32	35.24	35.24	35.24	31.05	35.24
		R2	8	6	5	6	8															
		R3	6	5	7	6	7															
30	10	R1	8	6	5	4	7	6.666	6.333	6.000	6.333	6.333	33.32	26.66	20.00	26.66	26.66	35.24	31.05	26.56	31.05	31.05
		R2	7	9	4	9	8															
		R3	5	4	9	6	4															

Appendix Table 76: Repellency of *C. chinensis* by seed (methanol) of *Apavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	10	10	9	9	9	9.333	9.000	9.333	9.000	9.000	86.66	80.00	86.66	80.00	80.00	68.53	63.44	68.53	63.44	63.44
		R2	9	9	9	10	8															
		R3	9	8	10	8	10															
236	10	R1	8	6	9	8	7	9.000	8.000	8.666	8.333	8.666	80.00	60.00	73.32	66.66	73.32	63.44	50.77	58.89	54.70	58.89
		R2	9	10	8	10	9															
		R3	10	8	9	7	10															
118	10	R1	8	9	8	7	10	8.000	7.666	7.000	8.000	8.000	60.00	53.32	40.00	60.00	60.00	50.77	46.89	39.23	50.77	50.77
		R2	7	6	5	8	6															
		R3	9	8	8	9	8															
59	10	R1	4	8	9	10	7	7.333	7.666	7.000	7.666	7.000	46.66	53.32	40.00	53.32	40.00	43.05	46.89	39.23	46.89	39.23
		R2	8	7	6	6	8															
		R3	10	8	6	7	6															
30	10	R1	6	7	8	9	8	7.000	7.333	6.666	7.000	6.666	40.00	46.66	33.32	40.00	33.32	39.23	43.05	35.24	39.23	35.24
		R2	9	8	5	4	9															
		R3	6	7	7	8	3															

Appendix Table 77: Repellency of *C. chinensis* by leaf (pet. ether) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	9	10	10	10	10	9.666	10.00	9.666	9.333	9.666	93.32	100.0	93.32	86.66	93.32	75.00	90.00	75.00	68.53	75.00
		R2	10	10	9	9	10															
		R3	10	10	10	9	9															
236	10	R1	9	10	9	10	9	9.000	9.333	9.333	9.000	9.000	80.00	86.66	86.66	80.00	80.00	63.44	68.53	68.53	63.44	63.44
		R2	8	9	9	9	10															
		R3	10	9	10	8	8															
118	10	R1	8	7	10	8	8	8.000	8.333	9.000	7.666	7.666	60.00	66.66	80.00	53.32	53.32	50.77	54.70	63.44	46.89	46.89
		R2	7	8	8	6	7															
		R3	9	10	9	9	8															
59	10	R1	6	3	8	7	6	7.333	7.000	7.666	7.666	7.000	46.66	40.00	53.32	53.32	40.00	43.05	39.23	46.89	46.89	39.23
		R2	10	8	7	9	9															
		R3	6	10	8	7	6															
30	10	R1	5	7	6	8	8	7.333	7.333	7.000	7.666	7.333	46.66	46.66	40.00	53.32	46.66	43.05	43.05	39.23	46.89	43.05
		R2	8	9	7	7	6															
		R3	9	6	8	8	8															

Appendix Table 78: Repellency of *C. chinensis* by leaf (acetone) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	8	9	10	9	8	9.000	9.000	9.000	8.666	9.000	80.00	80.00	80.00	73.32	80.00	63.44	63.44	63.44	58.89	63.44
		R2	10	8	9	7	10															
		R3	9	10	8	10	9															
236	10	R1	7	8	9	7	10	8.666	7.333	8.000	8.000	8.333	73.32	46.66	60.00	60.00	66.66	58.89	43.05	50.77	50.77	54.70
		R2	9	6	8	9	9															
		R3	10	8	7	8	6															
118	10	R1	5	9	8	7	8	7.000	7.666	7.666	6.000	6.666	40.00	53.32	53.32	20.00	33.32	39.23	46.89	46.89	26.56	35.24
		R2	7	6	9	5	7															
		R3	9	8	6	6	5															
59	10	R1	6	7	5	8	6	7.000	6.000	6.333	6.666	5.666	40.00	20.00	26.66	33.32	13.32	39.23	26.56	31.05	35.24	21.39
		R2	8	6	8	5	4															
		R3	7	5	6	7	7															
30	10	R1	5	8	6	4	5	6.000	6.333	6.333	5.666	6.000	20.00	26.66	26.66	13.32	20.00	26.56	31.05	31.05	21.39	26.56
		R2	6	7	8	6	7															
		R3	7	4	5	7	6															

Appendix Table 79: Repellency of *C. chinensis* by leaf (chloroform) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	9	7	8	9	8	8.000	7.333	7.666	8.000	7.333	60.00	46.66	53.32	60.00	46.66	50.77	43.05	46.89	50.77	43.05
		R2	7	8	9	7	7															
		R3	8	7	6	8	7															
236	10	R1	8	6	7	6	5	6.666	6.666	6.666	6.333	6.666	33.32	33.32	33.32	26.66	33.32	35.24	35.24	35.24	31.05	35.24
		R2	7	8	6	7	7															
		R3	5	6	7	6	8															
118	10	R1	6	5	6	7	6	6.000	5.666	5.666	6.000	5.666	20.00	13.32	13.32	20.00	13.32	26.56	21.39	21.39	26.56	21.39
		R2	7	8	5	6	7															
		R3	5	4	6	5	4															
59	10	R1	7	5	6	7	5	5.333	6.000	5.333	5.333	6.000	6.66	20.00	6.66	6.66	20.00	14.89	26.56	14.89	14.89	26.56
		R2	5	6	5	5	7															
		R3	4	7	5	4	6															
30	10	R1	6	5	4	6	4	5.333	5.666	5.666	5.333	5.333	6.66	13.32	13.32	6.66	6.66	14.89	21.39	21.39	14.89	14.89
		R2	3	7	6	4	7															
		R3	7	5	7	6	5															

Appendix Table80: Repellency of *C. chinensis* by leaf (methanol) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	8	7	6	7	6	7.000	6.666	7.000	6.000	6.000	40.00	33.32	40.00	20.00	20.00	39.23	35.24	39.23	26.56	26.56
		R2	7	5	8	6	5															
		R3	6	8	7	5	7															
236	10	R1	5	6	5	8	6	5.333	5.666	6.000	7.000	6.000	6.66	13.32	20.00	40.00	20.00	14.89	21.39	26.56	39.23	26.56
		R2	7	5	6	7	5															
		R3	4	6	7	6	7															
118	10	R1	6	5	6	7	6	6.000	6.000	5.666	6.000	5.666	20.00	20.00	13.32	20.00	13.32	26.56	26.56	21.39	26.56	21.39
		R2	7	6	5	6	5															
		R3	5	7	6	5	6															
59	10	R1	5	6	5	6	5	5.333	5.666	5.333	5.333	5.666	6.66	13.32	6.66	6.66	13.32	14.89	21.39	14.89	14.89	21.39
		R2	6	5	6	4	7															
		R3	5	6	5	6	5															
30	10	R1	7	5	7	5	7	5.666	5.333	5.333	5.666	5.333	13.32	6.66	6.66	13.32	6.66	21.39	14.89	14.89	21.39	14.89
		R2	3	5	5	7	5															
		R3	7	6	4	5	4															

Appendix Table 81: Repellency of *C. chinensis* by stem (pet. ether) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	6	5	6	7	5	5.666	6.000	6.000	6.333	6.666	13.32	20.00	20.00	26.66	33.32	21.39	26.56	26.56	31.05	35.24
		R2	5	7	5	6	7															
		R3	6	6	7	8	8															
236	10	R1	5	7	6	7	5	5.000	6.000	6.000	6.333	6.000	00.00	20.00	20.00	26.66	20.00	00.00	26.56	26.56	31.05	26.56
		R2	4	6	5	6	7															
		R3	6	5	7	6	6															
118	10	R1	4	6	4	5	7	5.333	5.666	4.333	5.333	6.000	6.66	13.32	-13.34	6.66	20.00	14.89	21.39	-21.39	14.89	26.56
		R2	7	4	5	6	5															
		R3	5	7	4	5	6															
59	10	R1	4	5	6	5	5	5.000	4.666	5.666	5.333	4.000	00.00	-6.66	13.32	6.66	-20.00	00.00	-14.89	21.39	14.89	-26.56
		R2	6	3	5	6	4															
		R3	5	6	6	5	3															
30	10	R1	6	5	3	6	4	4.333	5.333	5.000	5.333	4.333	-13.34	6.66	00.00	6.66	-13.34	-21.39	14.89	00.00	14.89	-21.39
		R2	4	6	5	4	5															
		R3	3	5	7	6	4															

Appendix Table 82: Repellency of *C. chinensis* by stem (acetone) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	7	8	5	6	7	6.000	5.333	5.333	6.000	5.333	20.00	6.66	6.66	20.00	6.66	26.56	14.89	14.89	26.56	14.89
		R2	6	5	7	5	3															
		R3	5	3	4	7	6															
236	10	R1	6	7	5	4	6	5.333	5.666	6.666	5.333	5.666	6.66	13.32	33.32	6.66	13.32	14.89	21.39	35.24	14.89	21.39
		R2	4	4	7	5	7															
		R3	6	6	8	7	4															
118	10	R1	4	5	3	6	7	5.666	5.333	4.666	5.666	6.000	13.32	6.66	-6.68	13.32	20.00	21.39	14.89	14.89	21.39	26.56
		R2	7	6	5	4	5															
		R3	6	5	6	7	6															
59	10	R1	6	7	5	4	3	5.333	5.666	5.333	6.000	5.333	6.66	13.32	6.66	20.00	6.66	14.89	21.39	14.89	26.56	14.89
		R2	5	4	7	8	7															
		R3	5	6	4	6	6															
30	10	R1	3	4	7	5	7	5.666	5.000	5.333	5.666	5.666	13.32	00.00	6.66	13.32	13.32	21.39	00.00	14.89	21.39	21.39
		R2	7	5	5	6	4															
		R3	7	6	4	6	6															

Appendix Table 83: Repellency of *C. chinensis* by stem (chloroform) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	9	7	8	6	7	8.000	6.000	7.000	7.000	6.333	60.00	20.00	40.00	40.00	26.66	50.77	26.56	39.23	39.23	31.05
		R2	8	6	9	8	6															
		R3	7	5	4	7	6															
236	10	R1	8	6	5	4	5	6.333	6.000	6.333	5.333	6.000	26.66	20.00	26.66	6.66	20.00	31.05	26.56	31.05	14.89	26.56
		R2	6	7	6	7	6															
		R3	5	5	8	5	7															
118	10	R1	6	5	4	3	5	5.333	6.000	5.666	5.333	5.000	6.66	20.00	13.32	6.66	00.00	14.89	26.56	21.39	14.89	00.00
		R2	4	6	7	8	3															
		R3	6	7	6	5	7															
59	10	R1	5	6	7	5	6	6.000	5.666	5.333	6.000	5.666	20.00	13.32	6.66	20.00	13.32	26.56	21.39	14.89	26.56	21.39
		R2	6	7	5	6	7															
		R3	7	4	4	7	4															
30	10	R1	4	5	6	4	7	5.333	6.000	5.333	5.666	6.000	6.66	20.00	6.66	13.32	20.00	14.89	26.56	14.89	21.39	26.56
		R2	5	7	5	7	6															
		R3	7	6	5	6	5															

Appendix Table 84: Repellency of *C. chinensis* by stem (methanol) of *A. pavonina* with percent repulsion and arcsin transformation data.

Dose (µg/cm^2)	Insects used	Replication	Hourly observation					Average of hourly observation (Nc)					Percent repulsion (PR) PR = (Nc – 5) × 20%					Arcsin transformation data				
			1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
472	10	R1	7	8	5	7	6	6.666	6.000	5.333	6.333	5.666	33.32	20.00	6.66	26.66	13.32	35.24	26.56	14.89	31.05	21.39
		R2	6	5	7	5	8															
		R3	7	5	4	7	3															
236	10	R1	6	7	5	4	5	5.666	6.000	5.333	5.666	5.333	13.32	20.00	6.66	13.32	6.66	21.39	26.56	14.89	21.39	14.89
		R2	5	4	6	5	7															
		R3	6	7	5	8	4															
118	10	R1	5	8	6	3	5	5.333	6.333	5.666	5.333	5.666	6.66	26.66	13.32	6.66	13.32	14.89	31.05	21.39	14.89	21.39
		R2	4	5	4	8	6															
		R3	7	6	7	5	6															
59	10	R1	6	7	5	3	4	5.666	5.333	5.666	5.333	5.000	13.32	6.66	13.32	6.66	00.00	21.39	14.89	21.39	14.89	00.00
		R2	4	5	6	8	5															
		R3	7	4	6	5	6															
30	10	R1	5	6	4	5	6	5.333	5.666	4.666	4.333	5.333	6.66	13.32	-6.68	-13.34	6.66	14.89	21.39	-14.89	-21.39	14.89
		R2	6	4	5	3	5															
		R3	5	7	5	5	5															

Appendix Table 85: Survival of *D. basalis* of different doses of methanol treated seeds of *A. pavonia* without host

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.380952	2	0.190476	0.167832	0.847446	3.885294
Columns	149.8095	6	24.96825	22	8.09E-06	2.99612
Error	13.61905	12	1.134921			
Total	163.8095	20				

Appendix table 86: Survival of *D. basalis* of different doses of chloroform treated seeds of *A. pavonia* without host

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0	2	0	0	1	4.102821
Columns	330.6667	5	66.13333	49.6	9.77E-07	3.325835
Error	13.33333	10	1.333333			
Total	344	17				

Appendix Table 87: Survival of *D. basalis* of different doses of methanol treated seeds of *A. pavonia* with host

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0	2	0	65535	#NUM!	4.102821
Columns	0	5	0	65535	#NUM!	3.325835
Error	0	10	0			
Total	0	17				

Appendix Table 88: Survival of *D. basalis* of different doses of chloroform treated seeds of *A. pavonia* with host

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0	2	0	65535	#NUM!	4.102821
Columns	0	5	0	65535	#NUM!	3.325835
Error	0	10	0			
Total	0	17				