2002

E;ffect of gamma radiation Oil Tribolium castaneum (Herbst) and Tribolium co11fusum Duval

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EFFECT OF GAMMA RADIATION ON *TRIBOLIUM CASTANEUM* (HERBST) AND *TRIBOLIUM CONFUSUM* DUVAL

THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
RAJSHAHI UNIVERSITY, BANGLADESH

by

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June, 2002

Dedicated to my Parents

Declaration

I do hereby declare that the entire work submitted as the thesis entitled "**Effect of gamma radiation on *Tribolium castaneum* (Herbst) and *Tribolium confusum* Duval**" in the Institute of Biological Sciences, Rajshahi University, for the degree of **Doctor of Philosophy** is the result of my own investigation under the coordial supervision of Dr K A M S H Mondal, Professor, Institute of Biological Sciences and (Currently Pro-Vice Chancellor, Rajshahi University); Dr. Md. Mahbub Hasan, Professor, Department of Zoology and Dr. Selina Parween, Professor and Chairman, Department of Fisheries & Aquaculture, Rajshahi University.

This thesis has not been submitted or published to any other university for any other degree.

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Certificate

This is to certify that the contents reported in this thesis entitled **"Effect of gamma radiation on *Tribolium castaneum* (Herbst) and *Tribolium confusum* Duval"** are original work performed by Md. Hanif Mullick under our supervision for the degree of **Doctor of Philosophy.** There is no material in this thesis, previously published or submitted elsewhere for any other degrees.

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TABLE OF CONTENTS

ACKNOWLEDGMENTS

I express my most sincere gratitude to my supervisors Dr. K A M Shahadat Hossain Mondal, Professor Institute of Biological Sciences and Currently Pro-Vice-Chancellor, Rajshahi University; Dr. Md. Mahbub Hasan, Professor, Department of Zoology, Rajshahi University and Dr. Selina Parween, Professor & Chairman, Department of Fisheries and Aquaculture, Rajshahi University, for their valuable help, encouragement, suggestions, unceasing advise and guidance throughout the research and preparation of the manuscript.

I wish to express my indebtedness to Professor Md. Sohrab Ali, Director, Institute of Biological Science, University of Rajshahi, for his valuable suggestions and inspiration.

I am also grateful to the Ministry of Education, Government of the People's Republic of Bangladesh, for granting me deputation and the Institute of Biological Sciences, University of Rajshahi, for the financial support.

I would like to extend my gratitude to the Director, Institute of Food and Radiation Biology (IFRB), Atomic Energy Commission, Savar, Dhaka and Director General, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, for providing the gamma irradiation facilities.

My grateful thanks are due to Dr. S H Mullick and Dr. M Z Mullick of Jahangirnagar University,Savar Dhaka for their assistance and encouragement. Mr. Nashim Morshed, Computer Science Department, Khulna University, helped me in analyzing the data.

I also express my thanks to the all member of Institute of Biological Sciences, Rajshahi University, including the fellows for their help and inspiration.

Lastly, by no mean least, I owe a deep debt of gratitude to Foizunnesa Begum ,Mozaffar Hossain ,Naila and Master Hashib for their moral support and inspiration.

-The Author

PREFACE

Insects associated with raw grain and processed food cause both quantitative and qualitative losses. Insect infestations can occur just prior to harvest, during storage in a variety of structures such as cribs and metal or concrete bins, and in- transit in a variety of carriers. Stored-product insects often are found in warehouses, food-handling establishments, and retail grocery and pet stores. These insects can also breed in purchased food packages or food residues in a consumer's pantry, and may contaminate other food products stored in the pantry. Therefore, preventing economic losses caused by stored-product insects is important from the farmer's field to the consumer's table (Troller, 1993). Several tools (pesticides and alternatives) are available for managing insects associated with raw grain and processed food. Effective use of pesticides and alternatives requires a thorough understanding of pest ecology, the application of pesticides only when pest populations exceed acceptable levels, and an evaluation of risks, costs, and benefits. Scientific research during the last half of the 20th century has resulted in a better understanding of the biology, behaviour, and ecology of stored-product insect pests and their management. Stored-product protection towards the end of the 20th century has shifted from using only conventional pesticides to using a variety of pest management methods. Stored-product protection in the 21st century will involve developing and implementing integrated pest management (IPM) programmes with a greater emphasis on using alternatives to conventional pesticides. Although conventional pesticides will continue to play an important role in stored-product IPM, their use may be more limited. Pest

management programs, especially in developed countries, are changing to meet consumer's demand for food free of pesticide residues, address concerns about the safety of pesticides to humans, reduce the adverse effects of pesticides on the environment, delay pesticide resistance development in insects, and comply with tighter pesticide regulations. Many of the alternatives to conventional pesticides are more environmentally friendly and have low mammalian toxicity. However, unlike conventional pesticides, these alternative methods often do not provide effective or rapid suppression of pest populations, and may not be effective against all pest species. Furthermore, most alternative pest management methods often are more expensive than conventional pesticides, and have not been tested extensively under field conditions.

Both non-ionizing and ionizing radiation have been investigated for stored-product insect control. Two methods that show the promise for controlling insects without leaving harmful chemical residues are the use of gamma radiation or microwave radiation (Cornwell, 1966; Hamid *et al.*, 1968; Hasan, 1995; Hasan and Khan, 1998b; Halverson and Nablo, 2000). Of the two, irradiation of insects has attracted wide attention in various fields from development to genetics and through its possible application to insect pest eradication programme (Knipling, 1955). However, the control of insects in foodstuffs by irradiation depends on acquiring the necessary basic radiobiological knowledge, on advances in irradiation, and on both health and safety considerations.

This project is aimed study to the possible use of gamma irradiation in controlling stored product insect pests as an alternative to other conventional control methods.

ABSTRACT

The radiosensitivity of *T. castaneum* and *T. confusum* was determined with particular reference to male sterilisation. The younger stages were found to be more radisensitive than the older ones in both the species. Adults, male and female, showed the most radioresistance followed by the pupae > larvae > eggs as indicated by higher LD_{50} values. *T. castaneum* was more radioresistant throughout ontogeny compared to *T. confusum*.

The longevity studies showed that, mean survival time and dose rate were highly position correlated in both the species. They also showed that males were longer-lived than females in both the species. There was very little difference in LT50 at dose levels of 1-to 3-krad for both the sexes. The radiosensivity indices decreased as the both species. Studies evaluating mating performance with normal and irradiated individuals reveled that the fecundity was gradually suppressed with increased gamma doses irradiated either as pupae or adults for both *Tribolium* species.

The results of the reproductive potential showed that the maximum eggs per day per female was recorded for the cross schedule U♂X U♀ compared to crosses involving the irradiated individual for both the species and stages. Results show that, the lowest number eggs were observed in the cross schedules T♂X T♀ for both the species irradiated either pupae or adults. These results clearly indicate that egg-hatchability in *Tribolium* species was adversely affected by irradiating males and females. The present findings reveal that the patterns of hatchability followed the dose-dependent manner. It also indicates that the hatching was completely inhibited at a dose level of 3 krad.

The results of the tests illustrate that the maximum 25 percentage eggs were hatched at a dose level of 1 krad for both the species and stages, while it was only 10 percent at 2 krad. The results interprete that the sequence of order based on the hatching percentage was cross schedule $T♂ \times T♀ < T♂ \times U♀ < U♂ \times T♀$ for both the species and stages.

Chapter: One

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The genus *Tribolium* is commonly referred as "flour beetle" which includes a large number of species feeding on a variety of stored commodities throughout the world (Sokoloff, 1972). They cause losses of food intended for both human and animal consumption (Lal and Shrivastava, 1985, Kabir *et al.*, 1989). Pest problems have increased side by side with the increase in the amount of stockpiled and the longer duration of storage (Khan and Mannan, 1991). More than 20% losses may occur in tropical countries through insect attack after harvest (Alam, 1971 ; Mondal, 1994). It has been reported that nearly 2000 species of field and storage pests annually destroy approximately one third of world's food production valued about US $ 100 billion among which highest losses (43% of potential production) occur in developing Asian countries (Ahmed and Grainge, 1986). Storage techniques and climate conditions especially in the tropic countries are often highly favourable for insect growth and development (Khan and Mannan, 1991; Talukder and Howse, 1995). In Bangladesh huge amount of food grains are damaged annually by insect pests (Alam, 1971).

Among all insect pests of the stored products (Table1.1) the flour beetle, *Tribolium* species has long been recorded as serious pest. They are generally found in granaries, mills, warehouse, under the bark of trees, *etc.* Feeding on rice (Both husked and unhusked), wheat, flour, semolina, bleached and unbleached wheat flour, breakfast food, bran, cornmeal, barley flour and oat meal (Chittenden, 1897; Good, 1936; Husaain *et al.*, 1994). Good (1933) observed that the beetles living in chocolate, spices (red peper), various kinds of nuts and sometimes feeding on specimen in insect collection.

Tribolium spp. are semi predators feeding on both living and dead materials.

Table 1.1. A list of insects associated with stored products (Metcalf and Flint, 1962; Alam, 1971; Wilbur and Mills, 1985; Kabir *et al.*, 1989; Gorham, 1990):

Species	Common names	Products infested
Coleoptera		
Anobiidae		
Lasioderma serricorne (F)	Cigarette beetle / tobacco beetle	Dried tobacco, food stuffs, turmeric.
L. testaceum L.	Cheeroot beetle	Dried tobacco, foodstuffs, turmeric
Stegobium paniceum (L)	Drug store beetle	Foodstuffs, stored turmeric, ginger, Chili, Coriander
Gastrulas indicus (L)	Book worm	Printed matter
Bostrichidae		
Rhyzopertha dominica (F.)	Lesser grain beetle	Rice, wheat maize, flour
Dinoderous ocellaris (F.)	Ghoon beetle	Dried bamboo, furniture, rice, wheat
Prostephanus truncatues (Horn)	Larger grain borer	Corn, soft wheat, dried cassava
Brenthidae		
Araeocerus fasciculatus (Deg.)	Coffee bean weevil	Coffee beans seeds
Bruchidae		
Callosobruchus chinensis (L.)	Pulse beetle	Oriental cowpea, pulses
C.maculatus (F.)	Spotted cowpea bruchid	Pulses
Bruchus pisorum (L.)	Pea weevil	Pulses
Carryon serratus (Oliv.)	Groundnut borer	Pulses, ground nuts
Acathoscelides obtectus (Say)	Bean bruchid	Pulses
Cerylonidae		
Mumidius ovalis (Beck)	-	Paddy, rice, corn, spices
Cleridae		
Necrobia rufipes (De Geer)	Red-legged ham beetle/ copra beetle	Copra, oil seeds, dried fish, rice, wheat, mixed feed
Cucujidae		
Cryptolestes ferrugineus (Stephen)	Rust–red grain beetle	Grains
C. pusillus (Schonherr)	Flat grain beetle	Grains

Table 1.1 (contd.)

Laemophloeus minutus (Oliv.)	Flat grain beetle	Rice, wheat, maize
Curculionidae		
Sitophilus oryzae (L.)	Rice weevil	Rice, maize, foodstuffs
S. zeamais Mostsch	Maize weevil	Maize, rice, sorghum, mung bean
Dermestidae		
Trogoderma granarium Everts	Khapra beetle	Grains, groundnut
Dermested lardarius L.	Larder beetle	Dried animal matter
Dermestes maculatus (Deg)	Hide beetle	Dried animal matter
Attagemus fasciatus L.	Carpet beetle	Dried animal matter
A. piceus Oliv.	Black carpet beetle	Grains, turmeric
Lophocateres pusillus (Klug)	Siamese grain beetle	Grains, turmeric
Mycetophagidae		
Typhaea stercorea (L.)	Hairy fungus beetle	Maize
Nitidulidae		
Carpophilus dimindiatus (F.)	Corn sap beetle	Rice, corn, flour
C. hemipterus (L.)	Dried fruit beetle	Dried fruits
Ptinidae		
Gibbium psylloides(Czenp)	-	Scavengers, bite holes in textiles
Ptinus tectus Boield	Australian spider beetle	Cereals, cereal products and species, often found as scavengers of miscellaneous debris
P. fur (L.)	White marked spider beetle	Cereals, cereal products and species, often found as scavengers of miscellaneous debris
Silvanidae		
Oryzaephilus surinamensis (L.)	Saw toothed beetle	Rice , wheat peas , flour wheat, mixed feed
O. mercator (Fauvel)	Merchant grain beetle	Wheat , mixed feed
Ahasverus advena (Walt)	Froeign grain beetle	Rice, maize
Tenebrionidae		
Tribolium castaneum (Herbst)	Red flour beetle	Grain, flour mixed feed
T. confusum Duval	Confused flour beetle	Grain, flour, mixed feed
Tenebrio molitor L.	Yellow mealworm	Flour, mixed feed
Latheticus oryzae (Waterh.)	Long-headed flour beetle	Flour, mixed feed

Table 1.1 (contd.)

Paloru subdepressus (Wall)	Depressed flour beetle	Grain, flour
P. ratzeburgii (Weissmann)	Small–eyed flour beetle	Cereal products
Alphitobius diaperinus (Panzer)	Lesser meal worm	Rice, wheat
A. laevigatus (F)	Black fungus beetle	Whole grains, wheat bran etc.
Gnathocerus cornutus(F.)	Broad horned flour beetle	Grain, flour
G. maxillosus (F.)	Slender horned flour beetle	Grain, flour
Trogositidae		
Tenebroides mauritanicus (L.)	Cadelle beetle	Grain, mixed feed
Lepidoptera **Galechiidae**		
Sitotroga cerealella (Oliv.)	Moth / Angoumois moth	Rice, wheat, maize flour
Hoffimannophila psedopretella (Straint)	Brown house moth	Grain and grain products; cause damage to carpets.
Endrosis sarcirtella	White–shouldered house moth	Grain and grain products; causing to carpets.
Phycitidae		
Cadra cautella (Walker)	Almond moth	Grains, dried fruits, almonds
Ephestia (Hub.) spp.	Tobacco moth	Tobacco, dried fruits, cocoa beans
E. (Anagasta) kuehniella (Zell)	Mediterranean flour moth	Flour
Plodia interpunctella (Hub.)	Indian meal moth	Dried fruits, meals, flour
Corcyra cephalonica Staint	Rice moth	Cereals, pulses, dried fruits and fishes
Hypospygia costalis (F.)	Clover Howard	Clover
Pyraustidae		
Doloessa viridis (Zeller)	Green rice moth	Milled rice, maize,sorghum
Tincidae		
Setomorpha tineoides Wallsingham	-	Dried tobacco
Tinea pellionella L.	Clothes moths	Woolen clothes, carpets , skin feathers
Psocoptera **Liposclelidae**		
Embidopsocus sp.	-	Rice, bean
Liposclelis entomophilus	-	Rice, maize mung bean
L. botrychophilus	-	Rice, maize, cassava .

T. castaneum and *T. confusum* are widely distributed throughout the world (Okumura and Strong, 1965; Sokoloff, 1972) including Bangladesh (Alam, 1971). They are largely disseminated in grains, flour, *etc*, transported for commercial purposes (Sokoloff, 1974). *Tribolium* spp. are unable to feed on whole cereal grains because their mouth parts are not suitable for attacking large and hard pieces of food grains and thus, they have the status of secondary pest. They damage the kernels (germ and endoplasm) of food grains. Although, the beetle are unable to eat sound grains yet they are found in grains in a large number and cause a great havoc and serious loss to the flours and grains that have previously been attacked by other pests e.g. weevils (Paddock and Reinhard, 1919). Sometimes severe infestation of flour or other materials may have a characteristic pungent odour as a result of gaseous secretion of the beetles (Payne, 1925; Englehardt *et al.*, 1965; Mondal, 1983, 1992). Viscosity and elasticity of the flour are markedly reduced. Payne (1925) reported that these affected food caused gastric disturbance if used. Park (1934b) remarked these types of food as "conditioned". Alam (1971) also reported that in the minor damage it imparts a nauseous smell and also changes the taste of the infested materials. The flour beetles contaminate more than they consume. A few beetles are enough to contaminate the flour adversely affected and create a pinkish taint making it unsuitable for human consumption (Mondal, 1992). This contamination results from the presence of living or dead insect or insect parts, cast exuviae, egg shells and pupal cases; noxious and presistent odours; webbing of food and the feces of the beetles (Mondal 1983 ; Khan and Mannan, 1991) and most importantly this contamination involves the accumulation of quinones (Roth, 1943; Mondal, 1985, 1992) given off by adult *Tribolium* and taken up by

the medium (Sokoloff, 1972). Many *Tribolium* spp. produce quinones which are mentioned below(Table 1.2)

Table1.2. Composition of the quinoid secretions of *T. confusum* (Loconti and Roth, 1953)

Compounds	Nature
2 – ethyl – 1, 4 – benzoquinone (ethylquinone)	***
2- methyl – 1, 4 – benzoquinone (methylquinone)	**
2 – methoxy – 1, 4- benzoquinone (methoxyquinone)	*
2 – ethylhydroquinone	*
Oil, Molecular weight 210 (– pentadecene)	**

*** Major components; ** Minor components; * Trace amount.

In *Tribolium* two pairs of well developed odoriferous glands are located one pair in the prothorax and the other in the abdomen of both sexes of adults. These are the source of these quinoid secretions (Good, 1936; Roth, 1943, 1945; Sokoloff, 1972, 1974).

The problem of the continued widespread use of pesticides, and particularly the incorporation of the words 'environmental pollution' into common vocabulary have caused scientists to look seriously at any ideas for pest control which do not involves traditional insecticides. In this connection, gamma irradiation appears to be a potential alternative to chemicals for insect control in stored products (Laudani, *et al.*, 1965; Golumbic and Davis, 1966; Tilton, 1974; Khalequzzaman and Hasan, 1989; Cheng *et al.*, 1991; Hasan and Khan, 1998; Halverson and Nablo, 2000). Radiation has also been used successfully to control several insect species and may prove effective or applicable in controlling many other pests

(Hussain and Imura, 1989). Highland (1991) reported that ionizing radiation has a distinct advantage over fumigation with chemicals, because there is no possibility of harmful chemical residues.

Tilton (1974) proposed that gamma radiation also appears to be in considerable use in some circumstances because it is thought that insect resistance is unlikely occur. Various forms of electromagnetic energy have been considered potentially useful for insect control (Nelson, 1967). These include ionizing radiation of extremely high frequency (X rays and γ rays) which might be used to cause mortality or to induce sterility. Potentially useful non-ionizing radiation are infra red, visible and ultraviolet ray. Gamma ray might be lethaly introduced to any form or stages of the insect pest. So, efficacy of gamma irradiation is more applicable than other conventional method.

Infra-red may be used to kill pests directly; whereas visible and ultraviolet ray may be used to attract pests to traps (Crowder, 1986). Nelson *et al.* (1966) concluded that the most important factors influencing the effectiveness of gamma rays for stored grain insect control were:

a. Frequency and field intensity.
b. Pulse modulation in 5 – 20 ms pulse widths.
c. Particle size of the host medium.
d. Moisture content of the medium.
e. Species and developmental stage of the pest.
f. Age of the insect.

Of these factors the first and last two appear to be the most important in physical control (Crowder, 1986).

There are two kinds of practical application in physical control *i.e.* gamma radiation. One is the direct killing and the other is the sterilisation by irradiation of the whole pest population (O' Brien and Wolfe, 1963).

There are several factors in ^{60}Co gamma rays which include:

- Gamma rays have a short length of electro magnetic waves.
- It has no mass
- It has no charge
- Wave length of gamma ray is more shorter than a visible wave length of light ray
- It's velocity is 3×10^{10} cm/sec
- It does not disperse by the electric field
- It does not disperse by the magnetic field
- It reacts in photographic plates
- It creates fluorescence when it reflected on any matters
- Gamma rays have an ionisation power. This power is less effective than α-ray or β-ray
- It has a power of penetrating.

This power is much more than α-ray or β-ray. It has a reflectory and refractory power. It has also a power of interference and diffraction as like as light beam.

Ionizing radiation in the application is now a recognised method of treating food and seeds. The essential differences between the irradiation process for grain and the use of chemical methods of insect control are given below in Table 1.3:

Table 1.3. The differences between the irradiation process and use of chemical methods of insect control (Cornwell and Bull 1960):

Irradiation	Chemical treatment
a. The product is conveyed to the treatment	The insecticide / fumigant is brought to be product.
b. Design of the irradiation plan is compatible with 100% control. Homogenous treatment.	b. Efficiency is limited by physical factors of distribution and penetration. Failure frequency results from inefficient application.
c.The process is automatic. No human error or operator prejudice.	c. Application is subject to human error.
d. Design completely hazard proof.	d. Operators may be subject to human error.
e. Nothing is added in the product.	e. Toxic residues may remain.
f. Treatment is instantaneous.	f. Delay may be encountered in holding grain under gas during airing off.

Cornwell (1960) expressed considerable scepticism about the promises of lowered prices of gamma emitters and put his faith in electron accelerators with high out put *e.g.* 200 tons/ hr. Although, Horne and Brownwell (1962) were concerned about the mechanical problems of such machines and believed that the deep penetrating power of gamma rays provided the answer. Most of the equipment designs published are for gamma irradiation commonly by ^{60}Co which have been used more frequently than any other source for irradiating insects.

Sokoloff (1977) have reported that gamma rays and X rays (100 kv and higher) are equal in their biological effectiveness. The advantages of using gamma rays from Cobalt sources are that gamma emitters are more economical to operate than X ray sources and large quantities of voluminous materials such as grain or flour can be irradiated at one time. Much of the research with gamma rays has been directed to determining the effectiveness of these rays in pest control.

Horne and Brownwell (1962) suggested that mobile irradiation could be installed in railcars or ships. It was hoped to extend the use of radiation facilities to those who would not need year round irradiation and to spread the large initial and running costs among many users.

Total cost of irradiative disinfestation facilities seems to be higher than traditional methods of preservation but it could be reduced by using minimal effective doses and by extending the facilities for multipurpose utilisation of the source (Highland, 1991). Moreover, retention of the quality and nutritive value of the food products would be additional benefits of this radiation technology (Bhuiya *et al.*, 1991).

Impact of radiation disinfestation are greatly affected by intrinsic factors such as the age, sex, stage and strain of the insect and many extrinsic factors, including the temperature, infestation site, food, dose rate and type of radiation (Highland, 1991). Because of the numerous variable factors, each application of radiation technology must be studied and planned thoroughly so that the final procedure is effective, economical, safe and acceptable to the consuming public. An operator of the gamma plant must be well trained and also be a radiobiologist or concerned person.

Radiosensitivity may possesses either lethal or sterile or substerile effects on the different stages of the flour beetles. Sometimes behavioural effects are determined, such as a ceasation of feeding or loss of movement by insects.

Hasan *et al.*(1989) observed that lethality is gauged by determining the emergence of adults from the irradiated pre-adults stages or by determining the survival of metamorphic stages from previous stages that were irradiated.

In fact, the lethal effects are not always necessary for the control of the insects. Sterilizing effect would be the important criterion with the radiation. The level of irradiation will fulfil the requirements for disinfestation. Sterilizing doses are lower and would therefore be more economical than lethal doses. Highland (1991) also proposed that the presence of live insects even sterile ones may be unacceptable and selection of sterilizing versus lethal doses must be based on an array of factors.

According to Brower (1973) if radiation is to be used as a means of controlling insects in grain and other store commodities the dose must be sufficient to irreversibly sterilize or to kill within a predetermined time period all species present at the time of treatment. Therefore, the comparative radiation sensitivity of all species commonly found infesting these products must be determined.

Numerous researchs have been conducted on the effect of gamma radiation on many aspects of stored product pests including *Tribolium* spp. (Cork, 1957; Sokoloff, 1961; Tilton *et al.*, 1966a; Yang *et al.*, 1970; Hasan, 1995; Hasan and Khan, 1998; Halverson and Nablo, 2000). There was no conflict in their reports about radiosensitivity on *Tribolium* species.

Most of the researchers have reported that the females of Coleoptera are more sensitive to gamma irradiation than the males (Rieman and Flint, 1967; Grosch, 1971;

Ahmed *et al.*, 1976c). The reasons for this difference are unclear (Ashraf and Brower, 1974). However, irradiation effects on the testis of Coleoptera are more radiosensitive than ovary.

In the present research project the effectiveness (*i.e.* radiosensitivity or radioresistant) of gamma radiation on both *T. castaneum* (Herbst) and *T. confusum* Duval were studied with special reference to the sterile insect technique (SIT).

Chapter: Two

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The early work on gamma rays led to understanding the biological effects of ionizing radiation on stored-product insects (Hasan and Khan, 1998b). Besides mortality effects, insects exposed to radiation had arrested development, sterility, and reduction in fecundity. Storage pest species vary widely in their sensitivity to ionising irradiation (Ducoff, 1972; Hasan 1995), the underlying causes of these differences were not known (Vardell *et al.* 1978). No satisfactory predictor for insect radio sensitivity has been established though there have been numerous attempts (Cole *et al.* 1959; Menhinick and Crossley 1969; Willard and Cherry, 1975; Nakakita *et al.* 1985; Mehta *et al.* 1990a).

2.1. Radio sensitivity in insects

Review deals the effect of radiation on various aspects of storage insects and also the possible use of radiation as a physical control measure in pest control management.

Stored product insects differ markedly in their tolerance to gamma radiation (Tilton *et.al.* 1966a ; Hasan and Khan, 1998). The tolerance vary considerably even within a single genus (Cornwell *et.al.* 1957). Since the mechanism of this differential tolerance is not well understood, the only method that can be used to develop an effective dose level to control stored product insects is to obtain experimental evidence for each species. The developmental stages of insects are continuously renewing their cells and tissues hence the particular stage of the insect may determine its sensitively to ionising radiation and once injured, particular stages may be capable of continuing development, depending on the level of radio sensitivity.

Irradiation at appropriate dose assures complete lethality to all the stages of insects by penetrating through the grains. So, immature stages growing inside the grains can not escape. Moreover, radiation treatment does not leave any residual effect. Considering these positive effects of radiation, number of scientists tried to control stored grain pests by using this technique. But surprisingly very little attention has been given to protect stored grains from insect damage by radiation (Haque, 1963; Quaraishi and Metin, 1963; Srisan,1963; Kumagai, 1969; Podany, 1977).

Gamma radiation appears to be a potential alternative to chemicals for insect control in stored products (Laudani *et al.*, 1965; Golumbic and Davis, 1966; Tilton, 1974). Comparative studies on the radiation sensitivity among stored product insect pests are important for radiation control because many species may be present in infested grains and the dosage must be high enough to control the most resistant species present. Furthermore, sensitivity varies depending on stages and strains (Cornwell, 1966; Vereecke and Pelerents, 1969; Brower, 1972a.b. Ionising radiation has been successfully used to control several insect species and may prove effective or applicable in controlling many others.

Tilton and Brower (1973) studied on 27 species of grain infesting beetles, showed that the small species in the families cuculionidae and cucujidae were more radiosensitive than the generally larger species in the family Tenebriondae. The problem is that size, developmental time and many other intrinsic factors can not be separated so that a definitive test can be conducted of course, large and small strains of species can be selected that have 2 or 3 fold differences in weight. However, these differences were polygenic and

in species of *Tribolium* may be produced by as many as 280 different genes (Enfield, 1972). As a result differences in radio sensitivity between strains may be caused by either genetic differences or size differences, and an exact determination of cause is difficult (Vardell *et al.,* 1978).

The potential commercial use of gamma radiation for insect disinfestations of bark grain (Cornwell, 1966) has made it imperative that the possible development of radiation resistance by stored product insects be investigated. Accidental exposure of some insects to substerilizing doses of radiation procedures may occur. Minimum radiation levels would probably be used for reason of economy and dosage would probably not be completely uniform in a large scale irradiator (Tilton *et al.,* 1971). Therefore, it is important to know whether insects receiving substerilizing doses for several successive generation can produce radiation resistant populations. Increased radio resistance has been reported for some insects but not for stored product insects (Cornwell and Morris, 1959).

Cogburn *et al.* (1971) reported that the treatment with gamma and infra-red radiation potentiated each other when used for the control of Angoumois grain moth, *Sitotroga cerealella* (Oliver). Tilton *et al.* (1966a) also obtained similar results with gamma and microwave treatment whilst working on some stored grain insect pests. Early research by Bughio (1977) showed that the susceptibility of *Chilo partellus* eggs to gamma radiation was correlated with the development age of the eggs as well as the dose of radiation applied. Reichlle (1969) reported that the eggs of the bagworm *Thyridopteryx ephemeraeformis*, during early embryonic development appeared to be about ten times more radiosensitive than the eggs immediately preceding hatching. Insects may become

more sensitive to other types of stress after exposure to ionising radiation.

Earlier embryonic development stages are more vulnerable to gamma radiation. An order of susceptibility among the stages of *Callosobruchus chinensis* (L) is as egg >> 7 day old larvae > 10 day larvae > mature pupae > new adults (Hussain and Imura, 1989). The LD_{50} of *Tribolium* was much higher than Hussain and Imura's findings, except for the pupae. These contradictions could be attributable to differences in the strains examined, dose rates and pre and post irradiation conditions (Pendelbury, 1966; Tilton and Brower, 1985).

Sensitivity to irradiation decreased as development progressed from the egg to the adult of *Oryzaephilus surinamensis* (L.) (Younes and Ahmed, 1977). The authors found that the duration of the irradiated stages was prolonged by irradiation and short retardation in development was generally increased by increament of the dose. This result agrees with that found by many other investigators (Howden 1957, Davich and Lindquist, 1962, Younes, 1970; Hasan and Saleh Reza, 1993).

Bhuiya *et.al.* (1991) noted a dose dependent reduction in adult emergence from irradiated eggs, larvae and pupae of the Angoumois grain moths, *S. cerealella* and the saw toothed grain beetle, *Oryzaephilus surinamensis*. They also found that adult development from irradiated eggs ceased at a dose of 0.15 - 0.20 KGy (1 Krad=1.00 x 10^2 Gy) while emergence from treated larvae ceased at 0.25 - 0.40 Gy.

Mehta and Sethi (1990) observed that the percentage of adult emergence from different age groups of irradiated eggs decreased with increasing doses of radiation.

Hussain *et.al.* (1994)

reported that *C. chinensis* can effectively be controlled by direct irradiation of beans containing immature stages at lower doses.

O'Brien and Wolfe (1963) reported that adult insects are at least 100 times less sensitive to the effects of radiation than the vertebrates. This phenomenon was explained in two ways: (1) a generalised law was formulated in 1906 as the Bergonic-Tribondeau law: the sensitivity of cells to irradiation is in direct proportion to their reproductive activity and inversely proportional to their degree of differentiation; (2) a special feature of many insects after they hatch from the eggs in that very little cell division occurs during larval life. Cell division and differentiation of tissues occur during embryonic development in the egg, so that in larval life, growth occurs primarily by enlargement of cell volume without an increase in cell number. There are other short bursts of mitotic activity, just before moulting and (where a pupal form occurs) in the later stages of pupation. There is also evidence that the amount of irradiation needed to cause death depends on the species involved.

Cornwell *et al.* (1957) observed that different species of *Laemophloeus* vary considerably in response to irradiation. Erdman (1962), Park *et al.* (1958), Van den Bruel and Bollaerts (1960) also obtained similar results in different species of *Sitophilus* and *Tribolium*.

2.2. Lethal doses and other effects on insects

Tilton *et al.* (1966a) found that 51.3% of the eggs of *Tribolium confusum*, hatched after treatment with 0.13 Kgy, but all the insects were dead at day 21. Only 30% of the larvae treated with 0.13 KGy pupated but 94.6% of the pupae treated with the same doses emerged as adults. Similar results were also found in other tests to determine the lethality of irradiated stored pests (Hasan *et al.*, 1989; Mehta *et al.*, 1991; Saxena *et al*,. 1992).

Bartlett and Bell (1962) using two strains of *T. castaneum* subjected to X- radiation found that the females of the heavier strain were more resistant to somatic damage than the males and that genetic damage was not so great in the heavy strain as in the light strain, specially when both percents were irradiated. Differences in radiosensitivity resulting solely from age and metamorphic stage have been shown by many workers (Peredel'skii, 1956). In general it has been shown that the older insects are more resistant to radiation than younger ones and this resistance increase slightly through ontogeny (Cornwell and Bull, 1960).

Fractionated treatments were applied in *Calandra granaria* at intervals of more than a few minutes were less effective than continuing radiation of the same total dose (Jefferies and Cornwell, 1958). Subsequently, Jefferies (1962) reported that while fractionated doses were less effective in reducing the amount of reproduction from insects, treated as eggs, larvae or pupae this effect was not noted with treated adults. He also added that a doses sufficient to cause complete sterility in adults would also sterilise all other stages whether as single or fractionated doses.

A dose of 16 Krad was adequate to control stored product colcoptera in bulk grain (Cornwell, 1966). Watters (1968) noted that stored product moths were more resistant than beetles. If young adult flour beetles are X-irradiated, the time course of mortality decreased after doses which kill only a small proportion, doses which kill most and 5 times greater than the dose to kill almost all exposed beetles. This has been noted explicitly in some previous works (Rogers and Hilchey, 1960; Ducoff and Bosma, 1967). Infra-red radiation caused similar effects on *T. confusum* (Cork, 1957)

Tenebrio molitor (Menhinick and Crossley, 1969) and is also apparent on *Rhodnius prolixus*.

Larval sensitivity is depended on age at treatment, e.g. older larvae are less susceptible than younger ones (Qureshi *et al.*, 1970). Ismail *et al.* (1987) (Oliver) also found that the pupae of *S. cerealella* are the most resistant metamorphic stage to radiation. Brower and Tilton (1973) found that adults of *T. madens* and *T. castaneum* emerged from 100 Krad treated pupae were with elytral and occasionally other deformities. Whereas, Halberstaedter *et al.* (1943), Donnelly (1960) and others reported that in many species of insects, the young eggs, larvae and pupae are more radiosensitive than their later stages.

In general, *T. confusum* and *T. destructor* were equally sensitive and are more sensitive than *T. castaneum* and *T. madens*, which likewise were equally sensitive (Brower, 1975c). These similarities are reflected in table 2.1.

To determine the doses necessary for the commercial application of gamma radiation, it is necessary to determine the minimum effective dose required to sterilise the most resistant age and metamorphic stage of those species likely to be found in the various stored products (Tilton *et al.*, 1966a). It is also necessary to determine the minimum effective dose needed to kill those stages of insects likely to be found in packaging operation (Highland, 1991).

Probit dose for sterilization of 50% of the males treated was more correlated with the phylogenetic relationship and chromosome number than with the weight or length of the developmental period (Brower, 1975). *T. confusum* and *T. destructor* are smallest and the

largest species respectively belong to a single species group with 18 chromosomes. While, *T. castaneum* and *T. madens* are small and larger species in the same group having 20 chrosomes (Table 2.1). The larger species develop more slowly than the smaller species. Thus, phylogenetic relationship appears to be more important than physical size or rate of development in determining radio sensitivity. This correlation may be due to difference in number of the chromosomes possessed by the two groups as proposed by Sparrow *et al.* (1963) or on other undiscovered differences.

Table 2.1. Comparison of *Tribolium* species radiosensitivity with various biological characteristics of the species

Species	Sterilising dose(rad)[d] or 50% (Males's)	Phylogenetic group[a]	Number of chromosome[b]	Weight (mg)[c] (Male's)	Developmental time (days)[c]
T.confusum	4350[c]	*confusum*	18	2.43	24.3[a]
T.destructor	4400[c]	*confusum*	18	5.44	45.5[cd]
T.madens	7600[c]	*castaneum*	20	3.50	25.3[d]
T.castaneum	8300[c]	*castaneum*	20	2.08	19.4[d]

a. Hinton (1948), b. Smith and Brower (1974), c. Brower (1975), d. Sokoloff (1972).

2.3 Life span of irradiated insects

Davey (1919) first observed the longevity of *T. confusum* after exposure to X-radiation. Later Cork (1957) noted a similar effect with gamma radiation. Ducoff (1975) observed the longevity was prolonged at low dose levels of radiation, whereas after exposure to high or moderate doses, it was shortened. The sex of the beetles play an important role in response to radiation. Davey or Cork did not separate their results by sex. This was an important

omission because in the same species, including *Drosophila subobscura* (Lamb 1964), *Trogoderma glabrum* , *Attagenus piceus* and *Acheta domesticus* (Hunter and Krithayakiern, 1971) the effects were more marked in the females. In several other species, including *Culicoides variipennis* (Jones, 1967) and *Laspeyresia pomonella* (White and Hutt, 1970) the effect was approximately equal in the two sexes. Benz (1970) noted that the increase in lifespan appears limited to the males in the larch bud moth. *Zeiraphera diniana*. Bhatnagar *et al.* (1965) observed in *Musca domestica* that isolation of individual house flies also extends the lifespan of males more than that of females. Ragland and Sohal (1973) also reported some results.

Irradiated males of screw worm flies, *Cochliomyia homonivorax* (Coquerel) having reduced longevity, may be an indication that they were sexually more aggressive than untreated ones (Baumhover, 1965). If this hypothesis is correct, a reduction in the longevity of irradiated male *Stomoxys nigra* is compensated by an increase in their sexual efficiency (Ramsamy, 1977). Banmhover (1965) added that an increase of sexual aggressiveness in males caused a corresponding decrease in the longevity of females to which they were mated.

Cork (1957) found that the life span may be increased by suitable doses of radiation. Doubtless, in some insects the relationship between fertility and longevity would be influenced by the developmental stage of the insects at the time of

irradiation (Proverbs and Newton, 1962). Grosch (1956) noted that irradiated adults live longer than normal insects that are not fed, but do not live as long as control insects that receive food. Adult feeding does not seems to be important in the codling moth. In preliminary experiments in which moths were fed a dilute aqueous solution, no difference was found between the life span of irradiated (as pupae) and normal adults.

After exposure to certain doses of radiation, the nutritional state of adults of *T.castaneum* affected their longevity (Roger and Hilchey, 1960). The authors also added that at least two dose dependent modes of mortality response to irradiation with high energy electrons were exhibited by the beetle and starvation was not the principal reason for death. Although the longevity of an irradiated insect may be influenced by the subsequent nutrition of the insect, the relationship between longevity and nutrition as affected by ionising radiation is wide open for further investigation.

Reduction in adult longevity by X-irradiation of the embryos of *Habrobracon juglandis* (Ashmead) was more sensitive criterion of induced sterility (Erdman, 1960). Insects those are exposed to ionising radiation, frequently become temporarily lethargic (Proverbs and Newton, 1962). Irradiation of larvae may cause a permanent cessation of development even though the insect remains alive for an abnormally long period (Whiting, 1950; Bletchy and Fisher, 1957; Cornwell *et al.*, 1957).Irradiation of larvae may cause a permanent cessation of development even though the insect remains alive for an abnormally long period.

Cessation of development is similar to true diapause and at least one author has reported that true diapause was induced when mature last instar larval of *Pectinophora malvella* Hb. were irradiated. When mature fifth instar larvae of the codling moth were subjected to

23.25 Krad many of the larvae failed to pupate and remained alive for about 2 weeks. If true diapause had been induced these larvae should have lived for several months (Proverbs and Newton, 1962).

Ducoff (1986) mentioned that insects which have been exposed to ionising radiation as young adults frequently exhibit greater mean longevity than do controls. This increased longevity has been observed in several genera of Diptera, Lepidoptera, Coleoptera and Orthoptera. Within these genera the extent of radiation enhanced longevity may or may not vary between sexes or be affected by the ploidy of the genome. These observation led many authors to suggest the reduction of sexual activities or gonodal functions by radiation as one possible cause of radiation induced life lengthening (Lamb, 1964; Tilton *et al.*, 1966a; Ducoff, 1975; Allen and Sohal, 1982; Ducoff, 1986; Hasan, 1997, 1998). Effect of radiation on the mean longevity of some stored products insect species has been shown in Table 2.2.

Table 2.2. Radiosensitivity of some selected stored product insect pests were treated at 15,000 rads.

Species	Sex	Mean Logevity (days)	LT_{50}	LT_{10o}	RI*	References
Coleoptera						
Lasioderma serricone	♀ & ♂	4.7	2.8	12.0	0.60	Hasset & Jenkins 1952
Sitophilus oryzae	♀ & ♂	7.6	3.6	12.0	0.47	,,
Dermestes ater	♀ & ♂	8.4	7.0	-	0.83	,,
Attagenus piceus	♀ & ♂	9.3	6.8	-	0.73	,,

Species	Sex	Mean Logevity (days)	LT_{50}	LT_{10o}	RI*	References
Herpalus pennsylvanicus	♀ & ♂	224.4	6.0	-	0.03	Menhinick & Crossley 1969
Philonthus sp.	♀ & ♂	70.0	40.0	-	0.57	Edwards 1969
Tenebrio molitor	♀ & ♂	29.2	7.7	-	0.26	Menhinick & Crossley 1968
Conotrachelus nenuphar	♂	90.5	10.3	15.7	0.11	Willard 1970
C. nenuphar	♀	57.0	9.3	13.3	0.16	Willard 1970
Lepidoptera						
Plodia interpunctella	♀ & ♂	4.8	3.8	5.3	0.80	-
Anagasta kuchniella	♀ & ♂	5.4	3.7	7.8	0.69	-
Ephestia clutella	♀ & ♂	3.8	2.8	4.3	0.74	-
Cadra cautella	♀ & ♂	2.0	1.2	2.3	0.60	-
Cadra figulilella	♀ & ♂	2.8	1.8	3.5	0.64	-
Sitotroga cerealella	♀ & ♂	4.2	3.8	6.0	0.91	-
Ostrinia nubibai	♂	8.0	7.3	12.5	0.91	-

* RI = Radiosensitivity Index; LT = Longevity Times [from Willard (1970)]

Eposures to high temperature greatly increased longevity in *Drosophila* females and caused great damage to the ovaries and radiation also produced ovarian damage. Decreased egg laying would enhanced female longevity by conserving protein and energy reserves for the somatic tissues. Tilton *et al.* (1966a) reported similarly that the female longevity was after increased irradiation of the beetles *Trogoderma* and *Attagemus*. The physical activity may

also be increased (Ragland and Sohal, 1975) which can markedly affect longevity (Sohal and Buchanon, 1981). Allen and Sohal (1982) argued that enhanced longevity of moderately irradiated male houseflies could be attributed to reduced metabolic rate in otherwise highly active flies. Females also showed some reduction in metabolic activity but their longevity was reduced of the some doses.

Lamb (1966) reported that the long term life shortening effects of ionising radiation occurs because radiation either accelerates the natural ageing process or causes precocious ageing. Accelerated ageing would occur if radiation caused an increase in the rate of the ageing processes throughout life; precocious ageing would occur if the animal aged rapidly in the period immediately after radiation but thereafter the time course of the ageing process was unaltered.

The induced repair theory of radiation enhanced insect longevity leads to several predictions that are amendable to experimental testing (Ducoff, 1976):

1. Irradiation should lead to greater radiation resistance in those insects (*e.g.* adult diptera) lacking somatic cell renewal.
2. Irradiated insects should exhibit increased resistance to environmental stresses; particularly stresses affecting postmitotic tissues in which resistance declines with age.
3. Other DNA damaging factors (e.g. UV, alkylating agents) when used on adults should also produce increase in longevity and in stress resistance.
4. Irradiation should exert similar increase of longevity to radiation resistance or to stress resistance in other organisms lacking somatic cell renewal.

Ageing and radiation were inter related i.e. T/E (Here, T = Survival Time and E = life expectancy of the controls). On the contrary, if ageing had no effect upon intrinsic radiation so one would find that a given dose would have the same effect at all ages; *e.g.* a certain dose might half the life span of an insect (O' Brien and Wolfe 1963) and life span should decrease with age (Sacher, 1957; Wharton and Wharton, 1959).

2.4 Responsible factors for death of irradiated insects

According to Ducoff (1972), irradiation of insect larvae may lead to one or more number of responses in addition to simple lethality. These include delay in pupation (Bourgin *et al.*, 1956), developmental abnormalities in the adult (Ducoff & Bosma, 1966), death during the pupal stage or failure of emergence (Vinson *et al.*, 1969) and imaginal death soon after eclosion (Yang & Ducoff, 1969). The relationships between these responses and larval death are not clear and differ among the various orders of Insecta.

Furthermore, few investigations have furnished data on all of these responses. As the waves of mitotic activity after each moult and the drastic recognisation during metamorphosis represent physiological crises for the irradiated insect, it is unlikely that survival studies on irradiated larvae can provide any direct evidence on mechanisms of death (Ducoff, 1972). The author added that if groups of adults of a given insect species exposed to a graded series of doses, exhibit the same time course of mortality then it is reasonable to postulate apartienlar mode or hierarchy of modes of death as in irradiated mammals. These situation may be complicated by a number of factors including the relatively short adult life spans of many insects, the variations between insect orders in their dependence on cell renewal and

by the profound influence of temperature and other environmental factors on metabolic rate and on duration of life.

Mortality curves from infra-red were that acute mortality caused by high doses of radiation was the result of damage to the central nervous system (Nöthel, 1968). Direct evidence of nervous damage by ionising radiation in insects is scarce. Ultra structural changes in the nucleus and cytoplasm of brain cells of Odonata larvae (*Calopteryx splendens*) exposed to high doses (100 Krad).

Responsible factor of death in irradiated adult insects is not well understood as in mammals which exhibit three specific modes of radiation induced death, namely, the haemopoietic mode the gastrointestinal mode (Quastler *et al.*, 1951) and the central nervous system mode (Langham *et al.*, 1956). Many adult insect species succumb to moderate (lethal-mid lethal) doses of radiation from several Gy in *Anthonomous* to 10 Gy in *Tribolium* (Ducoff *et al.*, 1971, Glenn and Ducoff, 1976). While the wasps and the Dipterns (Allen and Sohal, 1982) require high doses like 100 Gy. Besides the differences in the magnitude of the lethal dose, the patterns of radiation induced mortality for the two groups are distinctly dissimilar.

In the more sensitive group death occurs within a restricted post irradiation time period, the on set and duration of which are independent of dose, so that there is a characteristic acute LD_{50} associated with each particular insect species or strain. In the high dose group, radiation induced death does not occur in any specific post irradiation time period and increasing the dose cause progressively earlier death, which indicates the two different

patterns of mode of radiation death. There is substantial evidence from partial body irradiation (Lee, 1964), from histological studies (Riemann and Flint, 1967) and from dose fractionation studies (Lai and Ducoff, 1978) that the dose independent pattern represents a mode of death which is the consequence of damage to the proliferative cells in the mid gut epithelium.

The insect groups in which the adult stage is of fairly long duration and in which cell renewal takes place, radiation mortality is confined to a fairly circumscribed period. The time to development of this period of mortality and its duration are affected only slightly if at all by the magnitude of the dose over a range from mid lethal to at least several times that required to kill all exposed animals. These facts constitute a strong argument for the operation of a single specific mode of death (Ducoff, 1972). This possibility is supported by the progressive decrease in survival time with increasing dose in the high dose range in *Tribolium* (Rogers and Hilchey, 1960, Tilton *et al.*, 1966a; Ducoff *et al.*, 1969) and in many of the other species cited.

2.5 Sterility technique in irradiated insects

In applied entomology, the use of sterile insects to control insects is a more popular and evolutionary technique now a days. The origin of the idea and development of the technique are intimately related to research on the screw worm fly, *Cochliomyia hominivorax* (Coquerel). Knipling (1955) first developed an idea of sterile male release technique (SMRT) in insects. Today it is the most utilised genetic control method for the insect. Whitten and Pal (1974) with other entomologist involved in SMRT research are departing from the concept of 100% induced sterility.

There are two distinct procedures in SMRT. The first procedure involves the rearing, sterilisation and release of the sterile insects to mix with and compete for mates with those of the natural population (Knipling, 1959).

Following nine requirements for the feasibility of utilising the sterility method for control of insect population, these include:

1. Availability of a method of inducing sterility without serious adverse effects on mating behaviour and competitiveness.

2. A method of mass rearing of the insect involved.

3. Quantitative information regarding population densities at a low level in the population cycle.

4. A practical way of reducing natural populations to levels manageable with sterile insects.

5. Information on rate of population increase as a guide for determining the number of sterile insects required to over flood the natural population.

6. Cost of current method of control plus losses incurred by the insect pest must be higher than the combined cost of initial reduction of the natural population and rearing, treating and releasing the required number of sterile insects.

7. If complete population control can not be maintained because of reinfestation by migrating insects or new introductions then the cost of maintaining complete control by continual sterile insect releases must be favourable in relation to the costs for current methods of control plus additional losses caused by the insects.

8. There would be justification for employing the sterile insect release method even it is more costly than current measures for control, provided the method is advantageous in overcoming hazard to man or his environment.

9. The sterile insects to be release must not cause undue losses to foods and must not create hazards for man which might out weigh the benefits obtained by achieving and maintaining population control.

O' Brien and Wolfe (1963) reported that sterility may be caused by (i) infecundity in females, (ii) aspermia or sperm inactivation in males, (iii) inability to mate, (iv) dominant lethal mutation in the reproductive cells of either the male or the female. All these conditions led to sterility and can be produced in insects by ionising radiation.

A lose of fecundity which is equivalent to a depression in egg production has been repeatedly observed after treatment of female insects with ionising radiation (Grosch, 1962; LaChance and Leverich, 1965) and with ingested radioisotopes (Grosch *et al.*, 1956; Grosch, 1959).

The doses of radiation induce a dominant lethal mutations in sperm inactivation were mainly available for species that reproduce parthenogenetically (O'Brien and Wolfe, 1963). Most investigatons on sperm inactivation in parthenogenetic insects agree that inactivation of sperm by irradiation did not occur until complete doninant lethality was attained (Stancati, 1932; Whiting, 1938; Clark *et al.*, 1957l; Lee, 1964).

Adult male *D. melanogaster* were almost sterilised by doses of 5 -10 Krad, but there was no evidence of sperm inactivation because the sperm fertilised the eggs and participated in the zygote formation (Demerce and Kaufimum, 1941). Some workers suggested that some

sperms can be inactivated by fairly low doses of radiation (Yanders, 1959; Lefevre and Jonsson, 1962). Yander (1964) also concluded that some aspect of sperm behaviour, possibly motality was affected by X-rays at doses in the range commonly used in genetic and sterility studies.

Makee (1989) suggested that successful application of SMRT can be made, there were several essential pieces of information required on the economic importance of the insect as well as on sterilisation, mass rearing and release operations and their cost. This involves assessment of the population size of the insects, details of the life history of the insect and determination of sterilising dose and its effects on the behaviour and competitiveness of the treated insect.

Pradham *et al.* (1971) noted that the number of sterile males has to be higher than of the native males; the sterile males must be released when the native population was very low. Otherwise the native population should be reduced by applying other control methods. The author also added that to enhance the effectiveness of SMRT the control area should be naturally isolated to prevent reinfestation. Other artificial barriers should be constructed by using insecticides, herbicides or cultural practise.

One of the most suitable areas for applying SMRT is against the stored product insects (Tilton and Brower, 1983). This is because unlike field insect populations, stored product insect population are limited in number.

Therefore, relatively small number of sterile males are required for release. In other words it would be easy to achieve a high ratio of sterile to native males. Secondly, the population size can be easily controlled using insecticides to kill as many individuals as possible before

releasing the sterile males. Thirdly, stored product insect populations can be easily detected using pheromone traps (Brady *et al.*, 1971) light traps (Kirkpatrick *et al.*, 1970). Therefore the release of sterile males can be timed to be during the adult stage. Fourthly, the rearing methods for stored product insects are not difficult, that can be carried out at any time. Large numbers of insects for irradiation can therefore be obtained whenever needed.

Pradham *et.al.* (1971) noted that when SMRT was to be applied in storage, several requirements should be considered Firstly, the storage structure has to be insect proof and must be disinfested by fumigation or spraying. Also the food stuff has to be without any insect infestations and should therefore be fresh or fumigated. Finally, to prevent the build up of the insect population a suitable number of sterile males should be released before closing the store.

Makee (1989) proposed that SMRT can solve the insecticide problems though it has some disadvantages. This technique is species specific, whereas several species of stored product insects can infest a commodity at the same time. However, this problem can be solved by releasing sterile male against the most destructive species. An example of controlling primary insects in store leading to the reduction in the secondary insect infestations. As a result of treatment the ability of sterilised males to compete with native males to mate may be impaired. North and Hold (1968) suggested that this can be overcome by releasing a high number of sterile males to increase the sterile to normal male ratio by using a low dose of irradiation which does not affect the competitiveness of treated males. These doses were termed sub sterilising doses and these were very effective.

Ahmed *et al.* (1976a) cited that in classical SMRT, only males would be released but in reality both sexes were released to avoid problems with mass sexing. However, it was not until pointed out that both sexes released simultaneously were theoretically as effective as males alone, that this modification was generally accepted (Ailam and Galun, 1967). Guerra *et al.* (1972, 1974) proposed that test with the tobacco budworm, *Heliothis frescens* (F.) confirmed the degree of control that was greater when both sexes were released than when a single sex was released.

Over a range of relatively low doses, a linear relationship exists between the dose and level of sterility induced in irradiated insects. There were many findings that pointed towards a correlation between dose rate and certain types of damage to the insect. A general type of dose rate dependent damage has been demonstrated for the bruchid beetle *C. maculates* (Naharin *et al.*, 1971). A reduction of the LD_{50} value with higher does rates had been demonstrated for three other species of stored product insects (Jefferies and Banham, 1966).

The level of radiation sterilisation of Cowpea Weevil depends upon the physiological age of the insect. The dose required to achieve complete sterility increased with increasing age but susceptibility to substerilising doses varied. The longer duration to overcome the induced effects may account for the greater resistance of the eggs and first instar larvae compared to the older stages (Ghomomu, 1989).

The fertility of irradiated adults were observed to reduce with increasing doses of radiation (Cornwell, 1966; Tilton and Brower, 1971; Chand and Sehgal, 1979; Abdel Salam, 1989; Hasan and Saleh Reza, 1993; Hasan 1998, 1999).

The variability in the susceptibility to radiation induced sterilisation of the developmental stages of stored insects were reported by other workers (Soliman, 1972; Gonen, 1975; Brower, 1978, Ghomomu, 1991) observed that the eggs, larvae, pupae and adults of *T. castaneum* were sterilised with 8.6, 8.1, 6.2 and 8.6 krad respectively, while these stages of *T. confusum* were sterilised with 3.4, 6.4, 4.7 and 7.1 krad respectively. Jefferies (1962) achieved 99.9% sterility of eggs, larvae, early and late pupae and adults of *O. surinamensis* at 8, 6.5, 7.7, 12 and 15.3 krad respectively. Cornwell (1966) in *Sitophilus* spp. and in *Zabrotes subfasciatus* reported variability in the susceptibility of developmental stages. The susceptibility of stored-product insect species exposed to 160 Gy is reflected in Fig. 2.1.

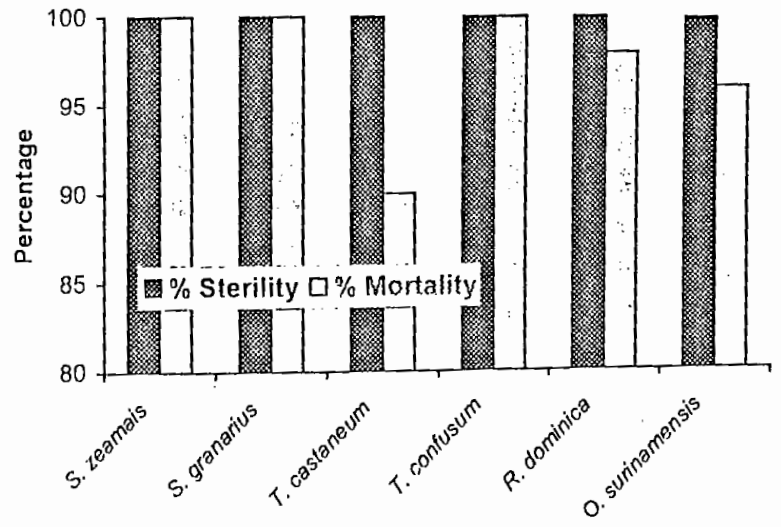


Fig.2.1. Susceptibility of stored product insect species exposed to 160 Gy

Abdel-Salam (1989) noted that for the success of SMRT the sterilised males should compete favourably with the non sterilised males. This requirement necessitates that the sterilising dose of radiation does not significantly impair the sexual vigour or the longevity of the treated insects (Tilton *et al.*, 1966a; Hasan, 1998). From a lower radiation dose more vigour and competitive sterile males might be obtained. Brower (1979b) showed that it appears that sub sterilised males have the greatest probability of success. The same findings were also quoted by Abdel-Salam (1977) whilst working *on T. castaneum*.

Chapter: Three

GENERAL MATERIAL AND METHODS

GENERAL MATERIAL AND METHODS

3.1. Methods of culturing *Tribolium* species

Both *T. castaneum* and *T. confusum* used in the present experiments were obtained from Integrated Pest Management Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi.

The following food media consisting wholemeal flour and yeast at a ratio of 19:1 were used for the culture of these species (Hasan and Selman, 1993):

3. 2. Determination of larval instars

Newly hatched one hundred larvae were put into petridishes containing approximately 30g of food medium and were reared in incubator at 30±1^{0}C. The 3rd, 6th, 9th, 12th and 16th days (plate 3.1, 3.2 and 3.3) from hatching for as second, third, fourth, fifth and sixth instars larvae whilst the and newly hatched larvae were used as first instar (Mondal, 1984b). The food medium was replaced by a fresh one on every fourth day to avoid conditioning by the larvae (Park, 1935; Mondal, 1984a). Most of the larvae had six instars (Good, 1936; Mondal, 1984a).

All the cultures were maintained in an incubator at 30+1^0 C without controlling the light or relative humidity.

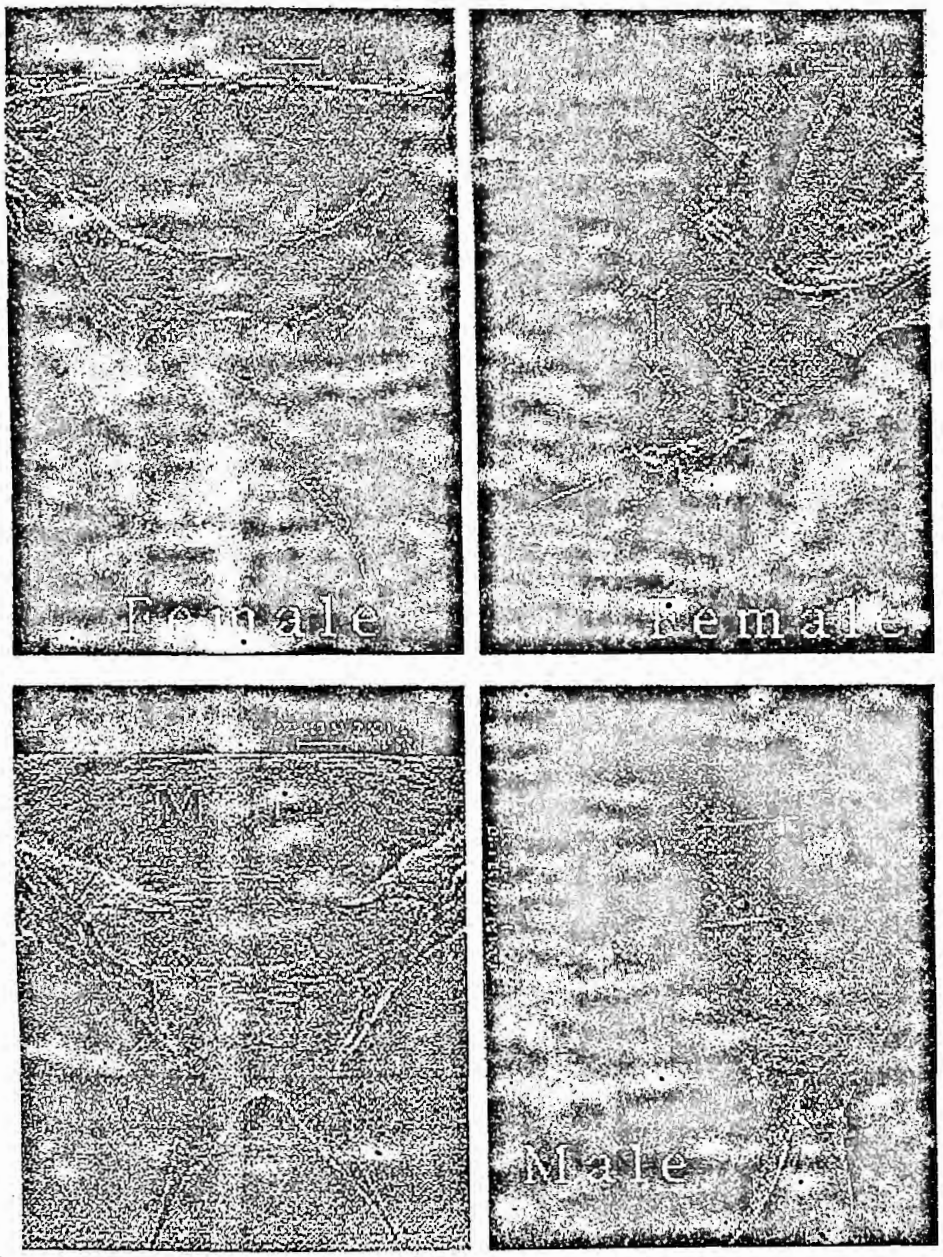


Plate. 3.1. Exogenital processes of the female pupae of *T. castaneum* and *T. confusum*

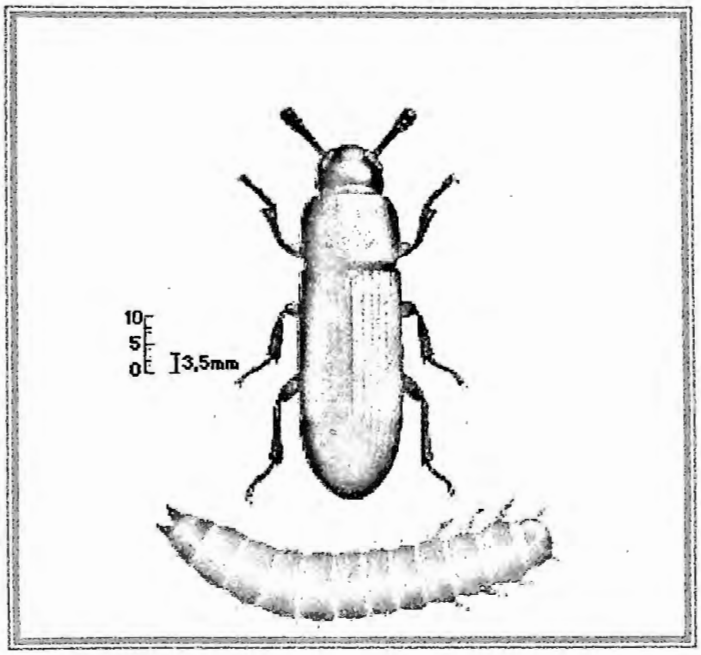


Plate 3.2: Adult and larvae of *T. castaneum*

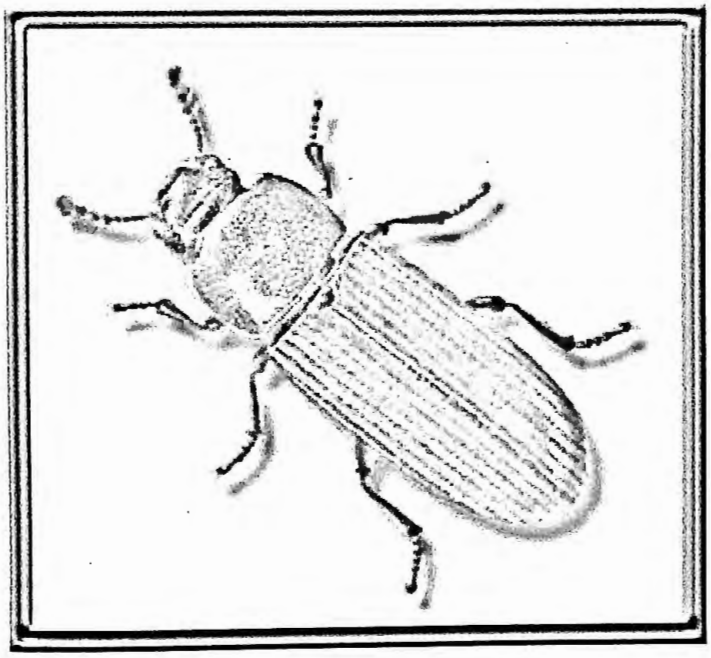


Plate 3.3. Adult of *T. confusum*

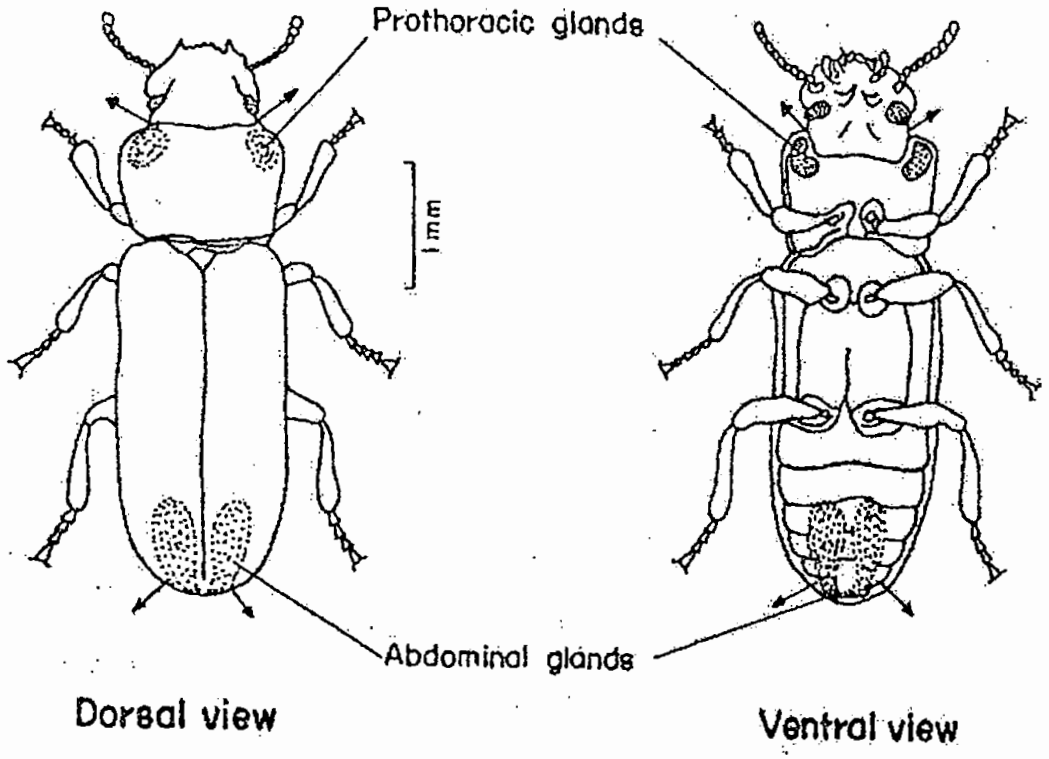


Plate 3.4. Drawing of adult *Tribolium* showing the position of prothoracic and abdominal odoriferous gland reservoirs. The arrows indicate the regions from which the secretion is emitted (After Roth, 1943)

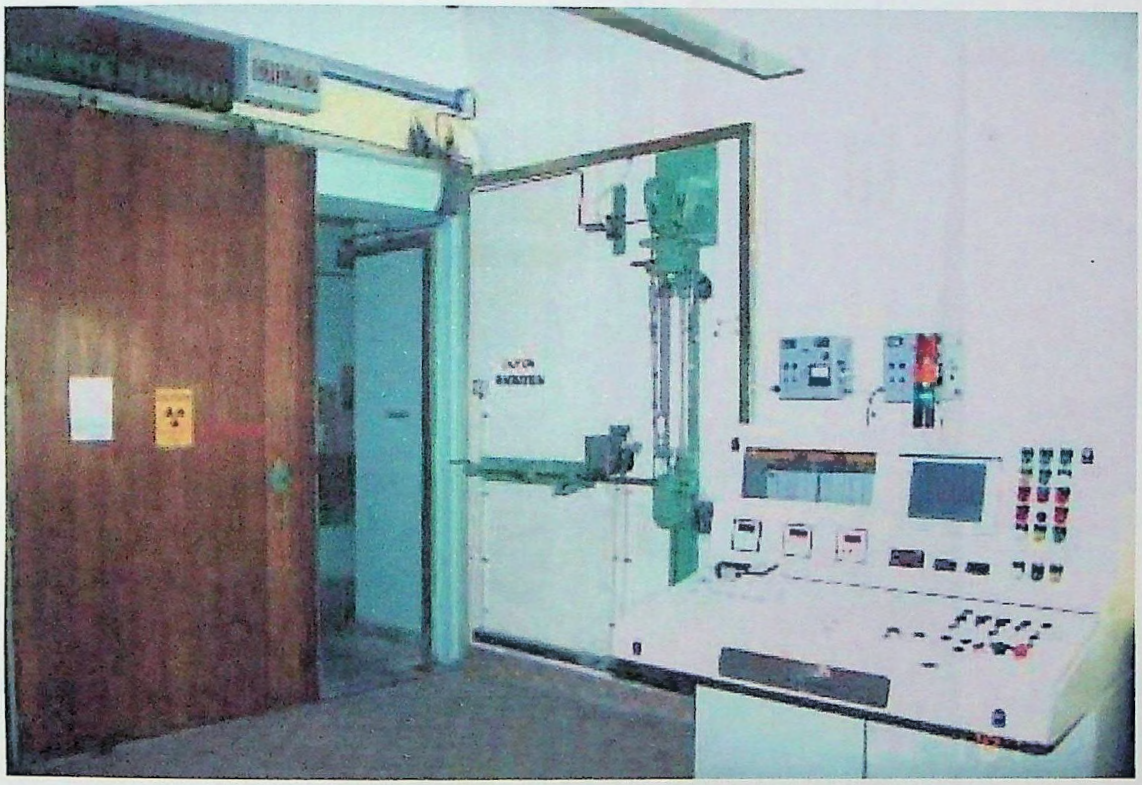


Plate 3.5. ^{60}Co gamma irradiation source plant.

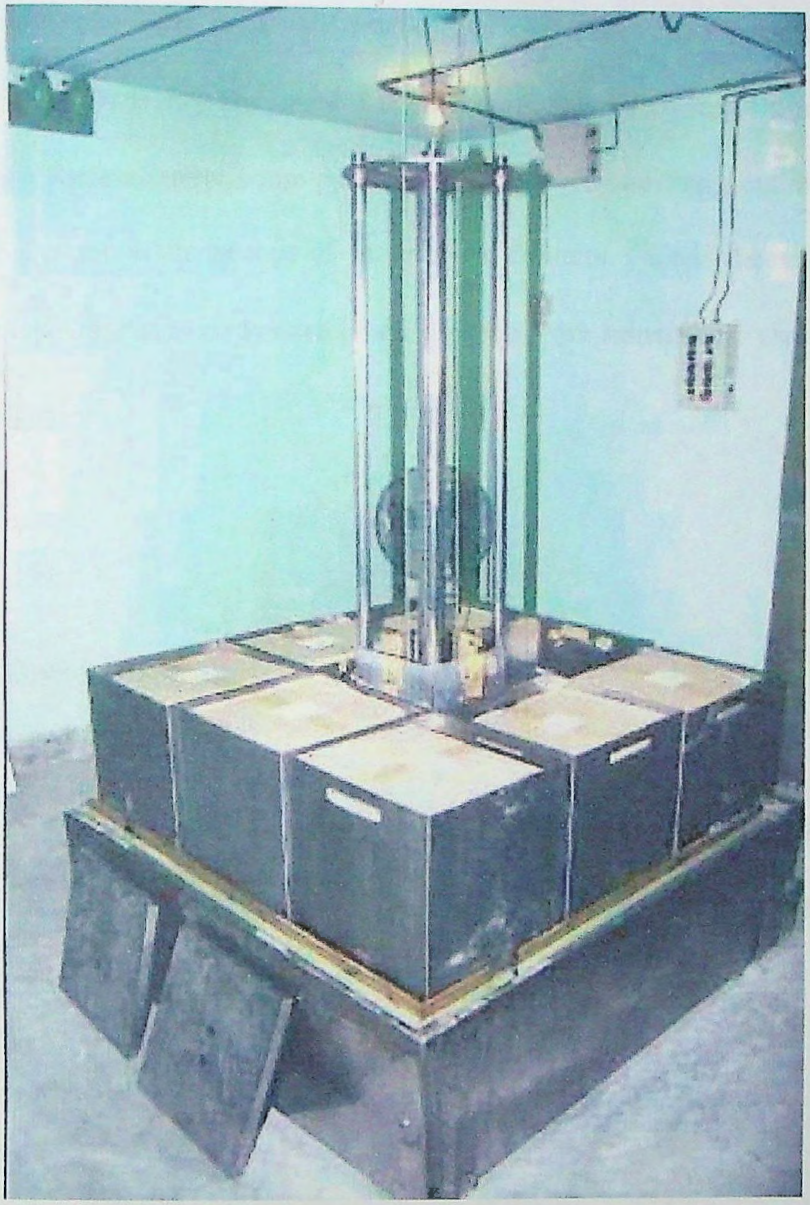


Plate 3.6. Glass vials with insects arrounding the gamma source pencils.

3.3. Definition of radiation

Radio activity is a spontaneously self disruptive emission from some unstable element. These unstable elements are called transitional elements. Their atomic weights are more than 206. In 1896, Henry-Bequerel a French scientist discovered the radioactive element Cobalt. From these element some particles and rays are emitting continuously. Most of the transitional element is the isotope of any normal element. ^{60}Co is the isotope of cobalt.

Three types of radio active rays are being emitted from transitional element; i.e. α-rays, β-rays and χ-rays.

3.4. Calculation of radiation dose

Hilchey and Cooper (1959) developed a means for calculating the dose of radiation in rads, absorbed by *T. castaneum* and *T. confusum* when irradiated with high-energy electrons.

Gamma radiation source was cobalt isotope i.e. ^{60}Co which is a deep therapy unit. Beetles were irradiated with a series of dose ranging from 1 to 5 krad, with about 1 cm layer of medium at a distance of 2 inch from the midline of the medium to the ray tube target (Plate-3.5 & 3.6). Thus, variations in subject target distance were negligible. Vials were placed in a ring on a turntable. The dose rate at 30 cm was approximately 5.955×10^4 rads/hr, as measured with a victoreen condenser meter.

3.5 Process of Radiation

There is a nucleus in every atom of an element. Each nucleus consist of newtron and proton (+ve). There are some orbits around the nucleus. Electrons are always moving with its own orbit.

3.6. Half life of ^{60}Co

After emisson of radiation from a transitional element the atoms decay overtime. The time taken for the emissions to reduce by 50% is called half-life. The cobalt (^{60}Co) has a half-life of 20 years.

3.7. Unit of radiation

There are two units of radiation (i) Curie (ii) Rutherford.

i) Curie : When 3.7×10^{10} nucleus break per second from a transitional element, so called Curie. e.g., 1 mc, 1 mc, 1 kc and 1 Mc.

ii) Rutherford : When, only 10^6 nucleus break per second from a transitional element, so called Rutherford = (1 ra)

1 krad = 1 kilo - radiation - absorption - dose = 10 Gy.

There is another unit of radiation i.e. 1 Bq. When only 1 nucleus break per second from a transitional element, so called Bacquerel.

3.8. Aim of the Work

The use of ionizing radiation for the non-chemical disinfestation of grains has been actively studied for over four decades (Tilton et al., 1966a). The early work on gamma rays led to understanding the biological effects of ionizing radiation on stored product insects (Hasan and Khan, 1998b).

The ultimate goal of the project was to determine the effects of radiation on the biology and sensitivity of two most destructive flour beetles *T. castaneum* and *T. confusum* throughout ontogeny. As a part of the radiosensitivity studies experiments were conducted to investigate the influence of age in different stages. Experiments were also designed to test mating propensity at suitable sterile doses of gamma radiation.

The research was focused on five sections; firstly, A preliminary note on the biology of *T. castaneum* and *T. confusum* (Chapter-4); Secondly, the effect of gamma radiation on growth and development (Chapter-5); thirdly, the dose mortality response to gamma radiation throughout ontogeny (Chapter-6); fourthly the time course mortality and radiosensitivity indices (Chapter-7); lastly, the mating propensity and radiation-induced sterility (Chapter-8).

Chapter: Four

STUDIES ON THE BIOLOGY OF THE FLOUR BEETLES *TRIBOLIUM CASTANEUM* AND *T. CONFUSUM*

STUDIES ON THE BIOLOGY OF THE FLOUR BEETLES *TRIBOLIUM CASTANEUM* AND *T. CONFUSUM*

4.1 INTRODUCTION

Flour beetles of the genus *Tribolium* comprise a large number of pests attacking a great variety of stored commodities (Sokoloff, 1972). They are easy to culture in large numbers and require no sophisticated equipment for maintenance. However, the biology of this genus varies greatly from one species to another. The medium is one of the main factors which regulates the rate of development of *Tribolium* throughout ontogeny. Different media have been used by the Entomologists to maintain *Tribolium* cultures, though standard media have been established for the culture of both *T. confusum* and *T. castaneum* (Park and Frank, 1948). The present study is an attempt to study the biology of *T. castaneum* and *T. confusum* in detail.

4.2. Material and Methods

T. castaneum and T. confusum beetles were cultured with standard food media which were described in the General Materials and Methods (Chapter-3) (Hasan and Selman, 1993).

Some beetles of each species were taken from the culture and kept in a petri dish for oviposition. The eggs were collected on the following day using the method of Khan and Selman (1981) and kept in petri dishes for hatching. After hatching , 100 neonate larvae of each species were collected with a fine camel hair brush and transferred to the respective food media in plastic containers (5 x 11 cm). Each set of food media consisted of three replicates. After ten days, the larvae of each species were weighed and their length and headcapsule width measured using an occulo micrometer. The mature larvae were also weighed and measured. After pupation, the larval periods were recorded. The pupae were sexed using the exogenital processes of the female (Halstead, 1963) and weighed. After eclosion, the pupal periods were recorded and the adults weighed.

After ten days, the adults of each species were paired and placed in glass vials (2.5 x 5 cm) containing the respective food medium. The eggs were sieved off at 3 day intervals for 30 days and kept in petri dishes to record the fertility rate. The length and width of the eggs were also measured. All the experiments were conducted in an incubator at 30°C and uncontrolled relative humidity.

4.3. Results and Observation

The data of the biology of *Tribolium* species cultured on standard food media are shown in Tables 4.1-4.3. The maximum 10 and 15 day old larval weight of 0.051 and 2.71 mg respectively was found in *T. confusum* followed by *T. castaneum* (Table-4.1). The larval weight for both the stages varied significantly ($P<0.001$) between the species (Appendix 8). Larval length and headcapsule width also varied significantly ($P<0.001$) both for the 10 day and mature larvae (Table 4.1)(Appendix 1).

The pupal and adult weight also varied significantly (P<0.001) between sexes and species, and these corresponded with their larval weight (Table 4.1) (Appendices 9 & 10). This table indicates that the female was heavier than the male at all stages. These results are in close conformity with the findings. Larval periods varied significantly (P<0.001) (Appendix 6) and the maximum larval period of 20.25 days was recorded in *T. confusum* followed by > *T. castaneum* (Table 4.2). Larval periods of 20.06 (30°C, 82% rh) day in *T. castaneum* using a different range of culture media. However, the pupal periods also showed a significant (P<0.001) variation between the species (Appendix 12). The maximum pupal period recorded was 8.20 days in *T. confusum* (Table 4.2).

The maximum reproductive potential of 8.30 eggs/female/day was found in *T. castaneum* compared to 5.7 egg/female/day in *T.confusum* (Table 4.3) and they varied significantly (P<0.001)(Appendix 14). The present results are more or less similar to the findings of Das (1992) who recorded 8.34 eggs laid/female/day in *T. castaneum* using the standard food medium described by (Park and Frank, 1948). The fertility rate did not vary significantly (P<0.05) (Appendix 15). The highest fertility(%) was found in *T.castaneum* and the lowest(%) in *T. confusum* (Table- 4.3).

The biological study of *Tribolium* species could provide valuable information for mass rearing and give a better understanding for the application of sterile-male release techniques.

Table 4.1. Mean length, weight and head capsule width of *T. castaneum* and *T. confusum* reared on standard food medium.

Stages	Species	Age (days)	Mean ± S.E.		
			Head capsule Length (mm)	Length (mm)	Weight (mg)
Eggs	*T. castaneum*	2	-	0.5 ± 0.001	0.0041 ±0.001
	T. confusum	2	-	0.58 ± 0.01	0.0044 ± 0.01
Larvae	*T. castaneum*	10	0.65 ± 0.07	2.14 ± 0.002	0.068 ± 0.015
		15	0.78 ± 0.01	7.45 ± 0.018	2.05 ± 0.13
	T. confusum	10	0.47 ± 0.001	2.17 ± 0.005	0.051 ±0.007
		15	0.74 ± 0.001	7.9 ± 0.012	2.17 ± 0.002
Pupae	*T. castaneum* (male)	2	1.52 ± 0.02	4.0 ± 0.008	2.15 ± 0.01
	T. castaneum (female)	2	1.5 ± 0.01	4.0 ± 0.001	2.21 ± 0.18
	T. confusum (male)	2	1.51 ± 0.01	4.1 ± 0.001	2.39 ± 0.001
	T. confusum (female)	2	1.5 ± 0.001	4.21 ± 0.002	2.41 ± 0.01
Adult	*T. castaneum* (male)	10	1.6 ± 0.001	3.27 ± 0.001	3.7 ± 0.001
	T. castaneum (female)	10	1.6 ± 0.001	3.26 ± 0.001	3.91 ± 0.012
	T. confusum (male)	10	1.6 ± 0.001	3.18 ± 0.001	3.56 ± 0.001
	T. confusum (female)	10	1.6 ± 0.001	3.19 ± 0.12	3.97 ± 0.001

Table 4.2. Developmental periods of *T. castaneum* and *T. confusum* reared on standard food medium.

Species	Developmental periods (days)		
	Larvae Mean ±SE (rang)	Pupae Mean ±SE (rang)	Incubation Mean ±SE (rang)
T. castaneum	20.00 ± 0.50 (18- 22)	7.10 ± 0.02 (6-8)	4.27 ± 0.02 (3-5)
T. confusum	20.25 ± 0.01 (18-23)	8.20 ± 0.01 (6-10)	4.86 ± 0.015 (3-7)

Table 4.3. Reproductive potential of *T. castaneum* and *T. confusum* reared on standard food medium.

Species	No. of egg/female/day Mean ± SE (rang)	% batched Mean ± SE (rang)
T. castaneum	8.3 ± 0.07 (3-11)	82.00±0.15 (75-86)
T. confusum	5.70 ± 0.082 (2-8)	78.00 ± 0.09 (73-83)

Chapter: Five

EFFECT OF GAMMA RADIATION ON THE GROWTH AND DEVELOPMENT OF *T. CASTANEUM* AND *T. CONFUSUM*

EFFECT OF GAMMA RADIATION ON THE GROWTH AND DEVELOPMENT

5.1. INTRODUCTION

Early literature reveal that many authors have already carried out work on the control of pests by gamma, X-rays and / or accelerated electron irradiation (Brower, 1978; Ducoff, 1986; Hasan and Khan, 1998). However, very little work has been done on control including the radiation effects on the growth and development of *Tribolium* species throughout ontogeny.

It has been observed that irradiation reduces the growth in *Tribolium* and this reduction is enhanced as the doses increased (Sokoloff, 1977). Rogers and Hilchey (1960) pointed out that irradiation caused a decrease in the feeding activity of *T. castaneum* larvae that may subsequently affect growth and development. It is generally agreed that the growth as well as the weight of an insect is directly correlated with their different stages. Hasan and Saleh Reza (1993) observed a significant effect of radiation on the weight of *T. anaphe* pupae and adults resulting from treating larvae of various ages. They also noted a linear relationship between the size of different treated stages and the size of the stages in the following generation. However, they did not consider the range of age-dose attributed effects.

The present investigation describes the effect of a series of gamma doses on the growth and development of *T. castaneum* and *T. confusum*.

5.2. MATERIAL AND METHODS

5.2.1. Development of mature larvae treated with gamma radiation

One hundred mature larvae (16 day old) of each species were collected from the stock cultures maintained with the specific food media. They were irradiated with the doses of 0 (control), 1-, 2-, 3-, 4- and 5-krad. The irradiated larvae were placed in plastic containers (5 x 11 cm) containing the standard food medium. There were three replicates for each set. The larval parameters like length of head capsule, length, weight were recorded just before the pupation. The larval mortality was recorded for survival percentage based on pupal formation. The data were computed for ANOVA analyses.

5.2.2. Development of pupae and adults developing from mature stage irradiated larvae.

One hundred mature larvae (16 day old) of each species were collected from the stock cultures maintained with the specific food media. They were irradiated with the doses of 0 (control), 1-, 2-, 3-, 4- and 5-krad. After treatment, they were placed in plastic containers (5 x 11 cm) containing the respective food medium. There were three replicates for each set. After pupation, the pupal parameters like length of head capsule, length, weight were recorded and kept in petri dishes for adult eclosion. After eclosion, the adult parameters were recorded as did with larvae and pupae. The data were computed for ANOVA analyses.

5.2.3. Survival percentage of pupae and adults developing from early stage irradiated larvae.

One hundred early larvae (2 day old) of each species were collected from the stock cultures maintained with the specific food media. They were irradiated with the doses of 0 (control), 1-, 2-, 3-, 4- and 5-krad. After treatment, they were placed in plastic containers (5 x 11 cm) containing the respective food media for pupation. There were three replicates for each set. After pupation, the percentage of pupal formation was recorded and kept in petri dishes for adult eclosion. After eclosion, the percentage of adult eclosion was recorded as did with pupae. The data were computed for ANOVA analyses.

5.2.4. Survival percentage of pupae and adults of developing from mature stage irradiated larvae.

One hundred mature larvae (16 day old) of each species were collected from the stock cultures maintained with the specific food media. They were irradiated with the doses of 0 (control), 1-, 2-, 3-, 4- and 5-krad. After treatment, they were placed in plastic containers (5 x 11 cm) containing the respective food media for pupation. There were three replicates for each set. After pupation, the percentage of pupal formation was recorded and kept in petri dishes for adult eclosion. After eclosion, the percentage of adult eclosion was recorded as did with early larvae. The data were computed for ANOVA analyses.

5.2.5. Survival percentage of pupae of *T. castaneum* and *T. confusum* irradiated at early and late stages.

Pupae, at 2- and 5-days old, of both sexes, were collected from the stock culture. They were irradiated in glass vials (2.4 x 5 cm) with 0 (control), 1-, 2-, 3-, 4- and 5-krad. Each test consisted of three replicates each having 60 pupae for each sex. After treatment, they were kept in separate petri dishes for eclosion. After that, the survival percentage of the pupae of each sex for all the species was recorded for each age and dose. The data were computed for ANOVA analyses.

5.3 RESULTS AND DISCUSSION

5.3.1. Development of mature larvae of *T. castaneum* and *T. confusum* treated with gamma radiation

The mean ± SE. of head capsule, length and weight of mature larvae of *T. castaneum* and *T. confusum* are given in Table 5.1. The analyses of variance for the data for both sexes are given in Appendices 16-21. A significant ($P<0.001$) difference was found in the mature larvae for all the parameters in irradiated ones (Appendices 16-21). The mature larval weight decreased as the dose increased in both the species compared to the control (Table 5.1). The maximum reduction was observed in the larvae irradiated at the 3-krad dose level.

5.3.2. Development of pupae and adults of developing from mature stage irradiated mature larvae.

The results of parameters like head capsule, length, weight of pupae and adults for both sexes of *T. castaneum* and *T. confusum* developing from mature stage irradiated mature larvae are shown in Table 5.2. The analyses of variance for both the sexes and stages are given in Appendices 22-33. A significant ($P<0.001$) difference was found in both the sexes and stages for the parameter like head capsule, length and weight when developing from irradiated mature larvae (Appendices 22-33). The weight decreased as the dose increased in both the sexes and species compared to the control (Table 5.2). The maximum reduction was observed when treated at the 3-krad dose level (Table 5.2).

5.3.3. Survival percentage of pupae and adults developing from irradiated early larvae.

The age of larvae at the time of irradiation significantly ($P<0.001$) affected the pupal formation and adult emergence (Appendices 34-45). The data in Figures 5.1-5.2 demonstrate that the larvae of both *T. castaneum* and *T.confusum* did not complete their maturation when treated with 4- and 5-krad. As shown in Fig. 5.3-5.4, up to 10% of the pupae and adults successfully developed from the larvae irradiated with 3-krad while it was more than 35% when irradiated at 2-krad dose level. The larvae of both the species pupated at doses upto 3-krad when irradiated as early larvae. Moreover, the highest percentages of pupal and adults eclosion were observed in *T. castaneum*.

5.3.4. Survival percentage of pupae and adults developing from irradiated mature larvae.

The irradiated mature larvae significantly ($P<0.001$) affected the pupal formation and adult emergence (Appendices 46-47). As shown in Fig. 5.7-5.8, up to more than 50% of the pupae and adults successfully developed from the mature larvae irradiated with 2-krad The data in Figure 5.7-5.8 reflects that the larvae of *T. castaneum* and *T.confusum* did not complete their maturation when treated with 4- and 5-krad as did in early larvae. The larvae of both the species pupated at doses upto 3-krad when irradiated as mature larvae. Moreover, the highest percentages of pupal and adult eclosion were observed in *T. castaneum.*

5.3.5. Survival percentage of pupae irradiated at early and late stages.

None of the doses upto 3-krad prevented the pupal formation and emergence of adults from the various ages of irradiated pupae (Figs. 5.5-5.8). The results on the pupal formation and adult emergence derived from the various ages of irradiated pupae show a significant variation (Appendices 54-63). A 50% pupal formation and adult emergence was observed in both species treated either as early or late pupae at doses of up to 3-krad. The earlier ages of pupae were found to be more susceptible and this effect decreased with the increased age. These figures also show that *T. castaneum* was more radioresistant than *T. confusum* at both the ages.

Table 5.1: Effect of gamma radiation on the growth and development of mature larvae of *T. castaneum* and *T. confusum*.

Doses (krad)	*T.castaneum*			*T. confusum*		
	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)
0 (Control)	1.5±0.240	8.2±0.144	0.068±0.005	1.5±0.294	7.4±0.059	0.542±0.006
1	1.4±0.192	7.9±0.360	0.066±0.003	1.2±0.118	7.5±0.176	0.57±0.0013
2	1.2±0.240	8.1±0.240	0.065±0.002	1.2±0.353	8.0±0.294	0.41±0.053
3	1.6±0.265	8.2±0.144	0.064±0.002	1.6±0.353	7.9±0.176	0.51±0.024
4	1.5±0.240	8.2±0.144	0.063±0.001	1.2±0.412	7.8±0.118	0.47±0.041
5	1.5±0.240	8.1±0.240	0.063±0.001	1.2±0.235	7.4±0.235	0.46±0.035

Table 5.2: Effect on the growth and development of male pupae of *T. castaneum* and *T. confusum* resulting from irradiated mature larvae

Doses (krad)	*T.castaneum*			*T. confusum*		
	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)
0 (Control)	1.2±0.176	3.5±0.235	0.031±0.002	1.7±0.059	3.8±0.235	0.022±0.002
1	1.2±0.235	3.4±0.353	0.029±0.004	1.6±0.118	3.8±0.118	0.023±0.002
2	1.1±0.294	3.4±0.294	0.028±0.001	1.7±0.186	3.6±0.353	0.022±0.003
3	1.1±0.118	3.2±0.353	0.028±0.001	1.7±0.118	3.7±0.412	0.025±0.004
4	1.0±0.412	3.3±0.118	0.029±0.004	1.7±0.353	3.7±0.294	0.021±0.001
5	1.1±0.412	3.4±0.529	0.027±0.002	1.5±0.442	3.6±0.333	0.022±0.001

Table 5.3: Effect on the growth and development of female pupae of *T. castaneum* and *T. confusum* resulting from irradiated mature larvae

Doses (krad)	*T.castaneum*			*T. confusum*		
	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)
0 (Control)	1.5±0.240	3.8±0.353	0.028±0.002	1.5±0.294	4.2±0.118	0.030±0.004
1	1.4±0.192	3.6±0.118	0.026±0.001	1.5±0.274	4.1±0.353	0.028±0.002
2	1.4±0.192	3.6±0.118	0.026±0.004	1.4±0.192	4.1±0.235	0.027±0.001
3	1.3±0.240	3.6±0.118	0.027±0.001	1.3±0.176	4.3±0.294	0.027±0.001
4	1.2±0.144	3.2±0.353	0.025±0.002	1.4±0.182	4.2±0.412	0.025±0.003
5	1.2±0.144	3.3±0.235	0.025±0.002	1.3±0.176	4.2±0.412	0.026±0.001

Table 5.4: Effect on the growth and development of male adults of *T. castaneum* and *T. confusum* resulting from irradiated mature larvae

Doses (krad)	*T.castaneum*			*T. confusum*		
	Head Capsule Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)	Head Capsule Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)
0 (Control)	1.1±0.048	4.8±0.144	28±0.004	1.1±0.048	4.9±0.240	0.25±0.002
1	1.0±0.144	4.5±0.961	24±0.003	1.0±0.144	4.6±0.192	0.26±0.004
2	1.0±0.144	4.4±0.961	26±0.004	1.2±0.144	4.6±0.192	0.22±0.001
3	0.8±0.192	4.9±0.240	24±0.003	0.8±0.192	4.5±0.9641	0.21±0.001
4	0.9±0.176	4.9±0.240	25±0.001	0.9±0.176	4.5±0.961	0.21±0.002
5	0.9±0.176	4.2±0.961	26±0.001	0.9±0.176	4.7±0.144	0.23±0.004

Table 5.5: Effect on the growth and development of female adults of *T. castaneum* and *T. confusum* resulting from irradiated mature larvae

Doses (krad)	*T.castaneum*			*T. confusum*		
	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)	Head capsule length Mean ±SE (mm)	Length Mean ±SE (mm)	Weight Mean ±SE (mg)
0 (Control)	1.2±0.144	4.9±0.240	0.26±0.004	1.2±0.144	4.8±0.144	0.27±0.004
1	1.1±0.048	4.5±0.144	0.24±0.001	1.1±0.048	4.710.240	0.21±0.003
2	1.1±0.048	4.6±0.144	0.24±0.001	1.1±0.408	4.5±0.144	0.23±0.002
3	1.3±0.192	4.4±0.961	0.23±0.002	1.3±0.192	4.7±0.176	0.23±0.002
4	1.0±0.144	4.5±0.176	0.22±0.003	1.0±0.144	4.6±0.144	0.25±0.001
5	1.1±0.048	4.5±0.144	0.21±0.003	1.11±0.048	4.5±0.240	0.22±0.003

5.4.2. Growth and development irradiated as various stages.

The results were in agreement with Cork (1957), Erdman (1962) and Das (1992), in the sense that the age of larvae treated with gamma radiation had a great influence on the rate of growth and development. A reduction of mature larval weight was observed when the larvae were treated either . Yang and Ducoff (1969) reported that radiation would have less effect on the formation of pupae by 17-day old larvae, and might cause relatively less damage to the pupal tissues of the progenitors. Bergonie and Tribondeaur (1906) proposed the principle that the radiosensitivity of cells is proportional to their degree of reproductive activity and inversely proportional to the degree of differentiation.

The larvae of both the species were able to complete development at doses of 4- and 5-krad when treated as 16-day old larvae (Table 5.1). These survivors were reduced in size compared to other dose levels and the control batch. Hasan *et al.* (1989) found that the late instar larvae of *T. anaphe* were more resistant to radiation which confirms the above results.

Data on the pupal and adult growth resulting from irradiated mature larvae show a significant relationship in both the species (Appendices XXXIV-XLVII). Some factors such as the degree of differentiation of the nervous system (Webber *et al.*, 1946) and insect shape (Frings, 1952) may account for this. These results agreed with those of Das (1992) who reported that there was a reduced effect on larval growth when the larvae developed from irradiated pupae or adults compared to larvae resulting from other irradiated stages. Hasan *et al.* (1989) also found a similar result in *T. anaphe*.

The percentages of pupal formation and adult emergence developing either from irradiated larvae or pupae varied significantly (Appendices XXII-LXIII). Results also show that the percentages of pupal formation and adult emergence developing either from irradiated larvae or pupae decreased as gamma doses increased (Figs 5.1-5.8). These figures also show that *T. castaneum* was more radioresistant compared to *T. confusum* indicating the highest percentage of pupal and adult recovery. Differences in response within genera have been shown also in *Sitophilus* by several investigators, including van den Bruel and Bollaerts (1960) and Tilton *et al.* (1966a), and in *Tribolium* by Park *et al.* (1958) and Erdman (1962).

Data on the radiosensitivity of pupae indicated that the males were more resistant compared to females. This result agrees with to reports on other species (Abdu and El-Sawaf, 1974; Guerra *et al.*, 1974).

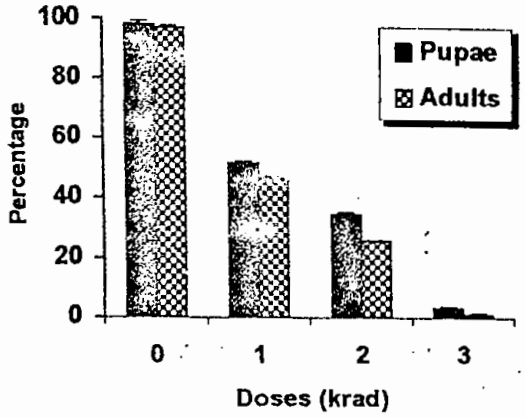


Figure 5.1: Effect of gamma radiation on the pupal formation and adult eclosion in *T. castaneum* treated as early larvae (2 day old)

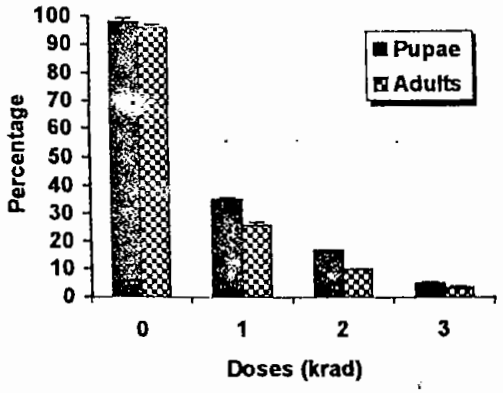


Figure 5.2: Effect of gamma radiation on the pupal formation and adult eclosion in *T. confusum* treated as early larvae (2 day old)

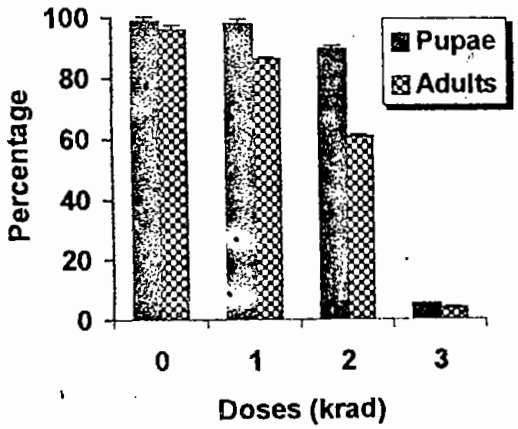


Figure 5.3: Effect of gamma radiation on the pupal formation and adult eclosion in *T. castaneum* treated as mature larvae (16 day old)

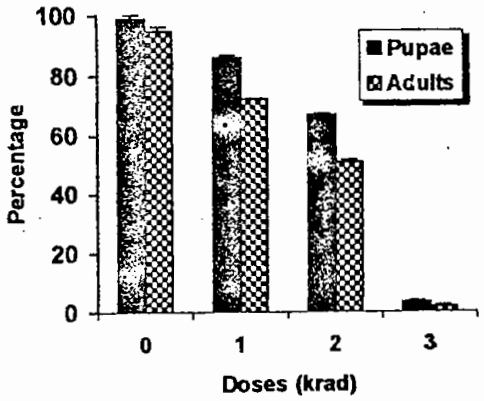


Figure 5.4: Effect of gamma radiation on the pupal formation and adult eclosion in *T. confusum* treated as mature larvae (16 day old)

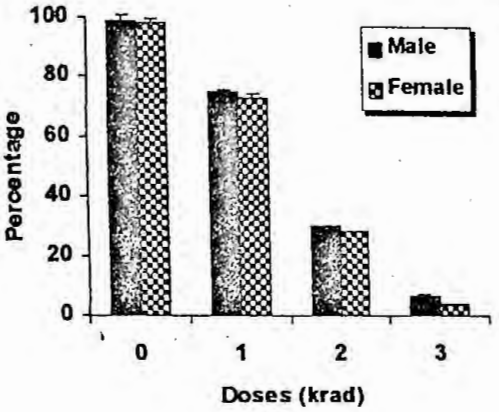


Figure 5.5: Effect of gamma radiation on the adult eclosion in *T. castaneum* treated as early pupae (2 day old)

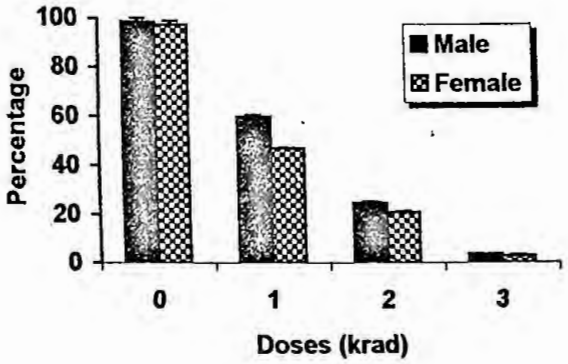


Figure 5.6: Effect of gamma radiation on the adult eclosion in *T. confusum* treated as early pupae (2 day old)

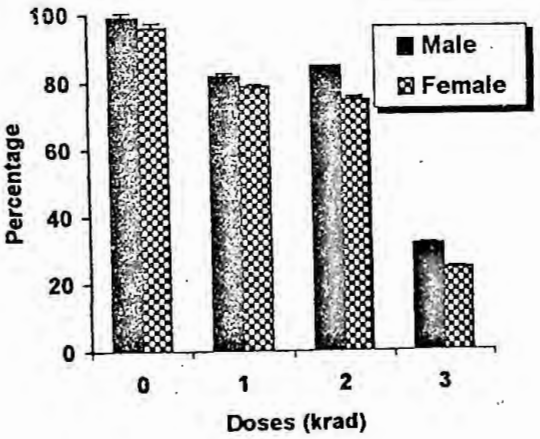


Figure 5.7: Effect of gamma radiation on the adult eclosion in *T. castaneum* treated as late pupae (5 day old)

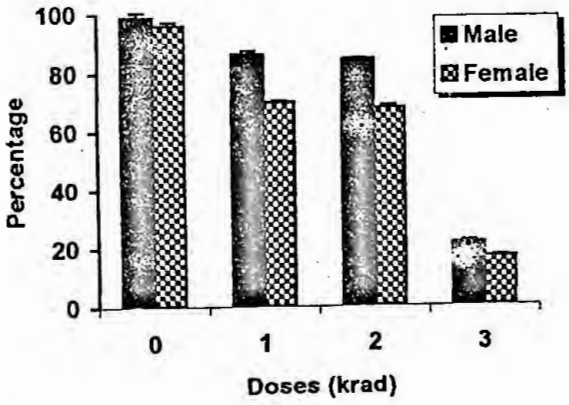


Figure 5.8: Effect of gamma radiation on the adult eclosion in *T. confusum* treated as late pupae (5 day old)

Chapter: Six

DOSE MORTALAITY RESPONSE OF *TRIBOLIUM* TO GAMMA RADIATION

DOSE MORTALITY RESPONSE OF *TRIBOLIUM* TO GAMMA RADIATION

6.1. INTRODUCTION

The species of stored product insect pests vary widely in their sensitivity to ionising irradiation (Ducoff, 1972; Hasan, 1999).The underlying causes of these differences are not known (Vardell *et al.*, 1978). No satisfactory predictor has been established for insect radiosensitivity though there have been numerous attempts (Cole *et al.*, 1959; Menhinick and Crossley, 1969; Willard and Cherry, 1975; Nakakita *et al.*, 1985; Mehta *et al.*, 1990). In general, the radiosensitivity is usually correlated with the order to which the insect belongs and also with the family of the species at least within Coleoptera (Tilton and Brower, 1973). However, it can not be predicted a priori whether an individual species will be radiosensitive or radioresistant despite suggestions to the contrary (Vardell *et al.*, 1978). For example, greater radiosensitivity seems to be related sometimes to large size (or greater body weight) (Cole *et al.*, 1959; Menhinick and Crossley, 1969; Willard and Cherry, 1975). However, body size is apparently not a consistent criterion, and, large species usually (but not always) have slower development and longer life cycles than small species and this could greatly influence their radiosensitivity (Vardell *et al.*, 1978). Tilton and Brower (1973), in a report on 27 species of grain-infesting beetles, showed that the small species of the families Curculionidae and Cucujidae were more radiosensitive than the larger species of the family Tenebrionidae. The problem is that size, development time, and many other intrinsic factors cannot be separated so that a definitive test of radio sensitivity can be conducted. Of course, large and small strains of species can be selected that have 2 or 3

fold differences in weight. However, these differences are polygenic and, in species of *Tribolium*, may be produced by as many as 280 different genes (Enfield, 1972). As a result, differences in radiosensitivity between strains may be caused by either genetic differences or size differences, and an exact determination of cause is difficult (Vardell *et al.* 1978).

To determine the dose required for commercial applications of gamma radiation, it is necessary to determine the minimum effective dose required to produce sterility in the most resistant age and metamorphic stage of those species likely to be found in the various stored products. It is also necessary to determine the minimum effective dose needed to kill those stages of insects likely to be found in packaging operations.

However, with the growing importance of *Tribolium* control and the paucity of published information on the effect of gamma radiation on the developmental stages, especially the comparison of the susceptibility of different species of *Tribolium*, the present study was designed to investigate the dose-age attribute mortality response of four stages: eggs, larvae, pupae and adults to gamma irradiation. Such an approach will be useful in fully evaluating the radiosensitivity of the different stages of *Tribolium* species and will also be useful for the selection of dose when inducing sterility.

6.2. MATERIAL AND METHODS

6.2.1. Dose-mortality response of eggs irradiated at various ages with gamma radiation.

To conduct this experiment, the eggs were collected using the method of Khan and Selman (1981). For irradiation, 60 eggs of each species at 2- and 5-days old were taken and irradiated with different doses of gamma radiation, 0 (control), 1-, 2-, 3-, 4- and 5-krad.

Each set consisted of three replicates. After irradiation, they were kept in petri dishes for hatching. The percentage of hatching was recorded for each dose, age and species group. The data were analysed for probit mortality following the methods in Busvine (1971).

6.2.2. Dose-mortality response of larvae irradiated as larvae of various ages.

Sixty Two-day old and mature larvae (16-day old) of sixty of each species were collected from the stock cultures maintained with the specific food media. They were irradiated with the same doses as the eggs. After treatment, they were placed in plastic containers (5 x 11 cm) containing the respective food media. There were three replicates for each set. The larval mortality was recorded as survival percentage based on pupal formation. The 2-day old larvae became moribund at doses of 4- and 5-krad and died within 4-5 days. The data were analysed for probit mortality following the methods in Busvine (1971).

6.2.3. Dose-mortality response of pupae of *Tribolium* species irradiated at various ages.

Pupae, at 2- and 5-days old, of both species, were collected from the stock culture. They were irradiated in glass vials (2.4 x 5 cm) with same doses. Each test consisted of three replicates each having 60 pupae for each sex. After treatment, they were kept in separate petri dishes for eclosion. The survival percentage of the pupae of each sex for both the species was recorded for each age and dose. The data for both sexes were analysed following the methods in Busvine (1971).

6.2.4. Dose-mortality response of adults of *Tribolium* species irradiated at various ages.

In this experiment, 9- and 16-day old adults of both sexes were collected from the stock culture for irradiation, and the dose used here was based on its effectiveness against *Tribolium* adults, 0 (control), 1-, 2-, 3-, 4- and 5-krad (Cornwell, 1960; Sokoloff, 1972; Brower, 1975). There were three replicates each having 60 adults in each age and dose group for each of the species. After irradiation, they were kept in petri dishes containing the respective food media. The survivorship of the adults was recorded based on the mortality seven days after irradiation for both sexes. The data for both the sexes were analysed following the methods in Busvine (1971).

6.3. RESULTS AND DISCUSSION

6.3.1. Dose-mortality response of eggs of *Tribolium* species irradiated at various ages with gamma radiation.

The results obtained for the effect of gamma radiation on 2- and 5-day old eggs (Appendix 66-67 and Fig. 6.1) showed that the hatchability of irradiated eggs was affected by the dose levels used, and hatching was completely inhibited at a dose of 4- and 5-krad. The susceptibility of the eggs increased with age, unlike with ionising radiation, where the younger ages are most sensitive (Mehta *et al.*, 1990).

The results show that the slope of the response curve increases as the age increases in most cases (Table 6.1). This may support the ideas of others that age plays an important role in influencing susceptibility (Johnson, 1987; Hasan *et al.*, 1989; Hasan and

Saleh Reza, 1993). In considering the radio-resistance of the egg, *T. castaneum* was found to be more resistant to gamma radiation than *T. confusum.*

The results were in agreement with the findings of Das (1992) that the egg stages are more radio-susceptible than the other stages. A comparison of hatchability between different age-groups of eggs treated at the same doses showed that the age at the time of irradiation was a major influence on the mean larval hatch and that the eggs exposed to gamma radiation at an early stage of embryonic development were more susceptible to radiation injury than the older ones. Apart from age the susceptibility of eggs to gamma-radiation was found to be dose dependent: as the dose increased the hatching percentage decreased. The doses tested, failed to produce an immediate lethal effect on the embryos except for 4- and 5-krad. These results show that the susceptibility of *Tribolium* eggs to gamma radiation was correlated with the development of the eggs as well as with the dose of radiation applied. Calderon *et al.* (1985) noted that the eggs of *T. castaneum* during early embryonic development appeared to be much more radio-sensitive than the eggs preceding hatching. Hussain *et al.* (1994) also observed a deleterious effect on the embryonic development, fecundity and fertility of *Callosobruchus chinensis* L. adults developing from irradiated eggs.

There is a threshold at which maximum biological effectiveness is manifested and above this threshold the increase in the damage to the biological material will not be produced at the same rate. This hypothesis fits with the present studies when 1-3 day old eggs were irradiated. It is concluded that merely increasing the dose at the same rate will not produce biological effects at the same rate as the lower doses. It is also known that sublethal doses of irradiation can produce short or long-term changes in physiology, developmental rate,

viability, longevity, behaviour, and fertility (Tilton and Brower, 1983). Sublethal doses applied to the eggs may depress the metabolic rate and hatching is often delayed (Tipton and Amand, 1954). Repair of cellular or tissue damage may also cause a delay in hatching even when the metabolic rate is not depressed.

Eggs of insect species belonging to Diptera, Coleoptera, Hymenoptera and Orthoptera have all been subjected to radiosensitivity studies by various workers and it is generally concluded that the eggs in early embryonic development are more susceptible to radiation injury than those exposed at a later stage (Tilton *et al.*, 1966b; Brower, 1972; Bughio, 1977; Younes and Ahmed, 1977).

6.3.2. Dose-mortality response of larvae of *Tribolium* species irradiated as larvae of various ages.

The results of the dose-mortality response of larvae of *T. castaneum* and *T. confusum* species treated as early (2-day) and mature (16-day old) stages are summarised in Appendices 68-69 and Table 6.1.

As shown in Appendices 68-69, the survival percentage of larvae of *T. castaneum* and *T. confusum* was significantly affected by the different dose levels of gamma irradiation. It also shows that the early larvae were more sensitive than the mature ones (Table 6.1). On the otherhand, significant results were observed in both the age groups which clearly indicates the existence of heterogeneity (Table 6.1). In considering radio-sensitivity differences between the species, *T. castaneum* was the least sensitive than *T. confusum* at all age groups indicated the higher values of LD_{50} (Table 6.1). Fig 6.4 also shows dose-age dependent mortality in both the species

Figures 6.1-6.4 indicates that the mortality of irradiated larvae is directly proportional to the dose of radiation and that the failure to pupate is the measure of total mortality. The age of larvae at the time of irradiation severely affected pupation (Appendices 68-69). It has also been found that the dose required for 50% kill increased as the age increased (Table 6.1). These findings support the results of Hasan *et al.* (1989) who worked on *T. anaphe* with the same dose levels. Ahmed *et al.* (1971) also found that the late instar larvae of *E. cautella* were more resistant to radiation and that higher doses were required to inhibit pupation and adult emergence. In the present experiment, variation was observed in the susceptibility of the species indicating the discrimination of LD_{50} values (Table-6.1). In this respect, *T. confusum* was found to be more radio-resistant than the others (Fig 6.4). When *Trogoderma inclusum* and *T. variabilis* larvae were irradiated with 5-krad or more, the development of adults from such larvae was prevented. Results also indicate that the formation of pupae by irradiated larvae is common, especially when mature larvae or prepupae are treated. However, it is well known that irradiation damage to the cells and tissues is often severe enough to prevent the formation of the imago within the pupal cuticle, a complex process that involves considerable cell division and reorganisation.

6.3.3. Dose-mortality response of pupae of irradiated at various ages.

The pupae either male or female appeared somewhat more resistant to gamma radiation than did the larvae or eggs. Appendices 72-79 give the mortality of pupae of both sexes of various ages exposed to different levels of gamma radiation.

The Λ^2 for goodness of fit for the regression equation for both sexes revealed the existence of considerable heterogeneity in most of the species and age groups indicating the higher

level of significance except the late *T. castaneum* and early *T. confusum* female pupae (Table 6.1). Other stages of *T. castaneum* was more radio-resistant than *T. confusum*, bearing the higher and lower LD_{50} values respectively for both the sexes. It also shows that the male pupae were more resistant than the female reflecting the higher values of LD_{50} for both the species.

The time sequence mortality in pupae is very difficult to determine and the failure to emerge is usually used to assess overall pupal mortality. The present results showed that adult emergence from either male or female irradiated pupae decreased as the dose increased (Appendices 72-79). It is generally known that pupal radiosensitivity is directly correlated with age of the pupae unless diapause or resting periods occur. The late pupae of *Tribolium*, either male or female, were more radio-resistant than the early ages, indicating higher LD_{50} values. Brown *et al.* (1972) obtained similar results in their work with *Sitophilus zeamais*, while in *S. granarium*, they found that adult emergence from irradiated pupae occurred with doses as high as 20-krad. Similar results were also obtained with the alfalfa weevil, *Hypera postica* (Gyllenhal), when pupae of five different ages were exposed to gamma radiation. The youngest of the five pupal ages was the most sensitive, with a general decrease in mortality with an increase in pupal age (Burgess and Bennett, 1972). When pupae of the Indian meal moth were irradiated at seven different ages, the percentage of adult emergence was dependent on both pupal age and total dose (Brower, 1976). An identical result was obtained with the pink bollworm, *Pectinophora gossypiella* (Saunders) irradiated at four different ages (Ouye *et al.*, 1964). So, differences in age clearly affect, pupal mortality on radiosensitivity in this study. However, it has been found that the pupal stages were less radio-sensitive than the other stages like the eggs and

larvae. Similar findings were also reported by Khalequzzaman and Hasan (1989) and Cogburn *et al.* (1966) while working on *T. anaphe* and *Plodia interpunctella* (Hubner) respectively. The present results also show that in *T. castaneum* both males and females were the most resistant followed by *T. confusum* reflecting the higher values of LD_{50} (Table 6.1). In an earlier report (Brower and Tilton, 1973), it was demonstrated that the mortality rates for adults emerging from treated pupae of *T. madens* increased greatly at doses of 10-krad or more. However, no difference in adult mortality response was observed between the control and 5-krad treatment. Adults that emerged from the pupae of *T. confusum* irradiated at 10-krad to 30-krad, died within 10 days of exposure. These findings differ from the results reported in the present study where a great variation was found between the control batch and the 5-krad dose level. These differences may be due to species variation. However, there is evidence that the rate of irradiation needed to cause death in *Laemophloeus* depends on the species which vary considerably in response (Cornwell *et al.*, 1957). Differences in response within genera have also been reported in *Sitophilus* by several investigators, including van den Bruel and Bollaerts (1960) and in *Tribolium* by Park *et al.* (1958) and Erdman (1962). Figure 6.7 shows that adult emergence from irradiated pupae was greatly influenced by sex i.e., male pupae were more resistant than the females. These findings support the works of Pendlebury *et al.* (1966) while working with *E. cautella*.

6.3.4. Dose-mortality response irradiated at various ages.

The results for the dose-mortality response of adults of both sexes of *Tribolium* species treated at 9- and 16- day old are reflected in Appendices 80-87 and Figure

Appendices 80-87 show that the survival percentage of adults of both sexes was significantly affected by gamma radiation. As shown in Table 6.1, *T. castaneum* was found to be more resistant either for male or female followed by *T. confusum* (Table 6.1). The insignificant results for the Λ^2 values of both sexes in *T. confusum* revealed the existence of homogeneity in both stages except the early female (Table 6.1). As is reflected in Figure 6.4, male adults were more radioresistant than the females as indicated by the higher values of LD_{50} in both stages and species groups.

It is evident from the results that the adults are more radio-resistant than the other stages in the developmental sequence. Survival of *Tribolium* adults either male or female was significantly affected by the radiation treatments in all the species (Appendices 80-87). Similar results were reported by Mehta *et al.* (1990) while working on the susceptibility response of *T. confusum* adults to gamma radiation. Brower (1975c) also reported a significant reduction in the adult life span of *T. destructor* at doses of 10-krad or more. According to Riemann and Flint (1967), reduced longevity was one of the most commonly observed responses to somatic damage. The difference in post irradiation response between males and females is quite a common phenomena (Tilton and Brower, 1983). These results demonstrate that male adults are much more radio-resistant than the females indicating the higher values of LD_{50} in both the species and stage groups (Table 6.1). It may be speculated that reduced metabolic rate or stimulation of repair systems might have been responsible for this differentiation. The results also showed that *T. destructor* was the most radio-resistant while *T. freemani* was the least as observed and explained for the pupae. However, several studies have shown that even closely related species can differ in response (Tilton *et al.*, 1966a; Brower and Tilton, 1973; Brower, 1975). Even different

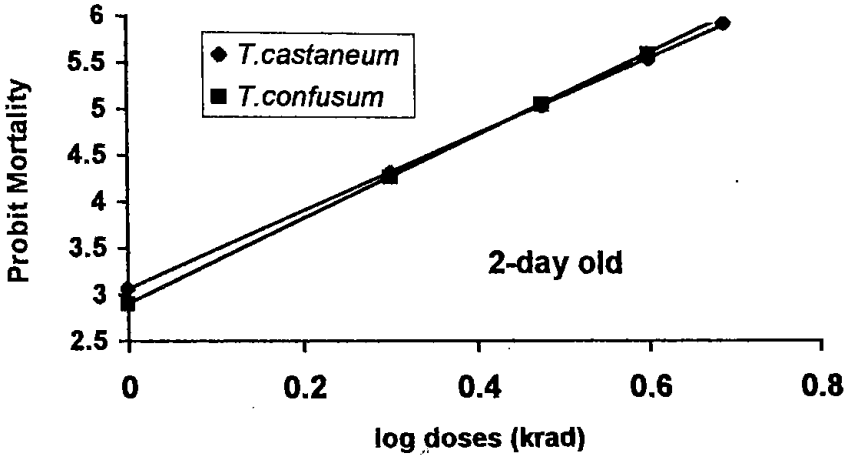
strains of the same species can have clearly detectable differences in mortality after radiation (Tilton and Brower, 1983). A study of 35 laboratory and wild strains of the granary weevil from different parts of the world showed marked differences in the mortality rate after adult irradiation (Cornwell, 1966). He reported that the most susceptible laboratory strain had an LD_{50} of 3.95 krad whereas the most resistant wild strain had an LD_{50} of 7.1 krad. Shipp (1966) also observed that six strains of the granary weevil from Australia exhibited differences in post irradiation mortality. Differences in the lethal effects of irradiation were shown for four and five strains of the *T. castaneum* and *T.confusum* respectively.

From these results, it can be concluded that the younger immature stages of both the species of *Tribolium* are more sensitive to radiation, and resistance to radiation increased with developmental stage. Doses up to 3-krad could be considered as sublethal doses for the population leading to suppression of *Tribolium* species. This also supports the idea of others (Cornwell *et al.*, 1957; Park *et al.*, 1958 and Erdman, 1962) that radio-sensitivity differs markedly within a single species and genus.

Table 6.1: Dose-mortality response of *Tribolium* spp. treated with gamma radiation throughout ontogeny.

Species	Stages	Age (days)	LD_{50} (Krad)	95% Conf. Limits Lower	Upper	Regression Equation	χ^2 Values (3 df)
T.castaneum	Eggs						
	Early	2	0.643	0.452	1.139	3.065 + 4.196 X	32.531***
	Late	5	1.158	0.757	2.913	2.065 + 5.418 X	16.989***
	Larvae						
	Early	2	1.906	1.497	2.426	3.497 + 5.361 X	21.018***
	Late	16	3.383	1.579	5.212	4.434 + 4.005 X	1.284*
	Pupae Male						
	Early	2	3.844	2.641	23.294	4.400 + 0.670 X	2.544*
	Late	5	4.236	3.599	4.2462	4.574 + 0.223 X	8.183**
	Pupae Female						
	Early	2	3.820	1.235	7.889	3.931 + 0.991 X	1.067*
	Late	5	4.595	2.817	8.096	4.093 + 0.330 X	0.296NS
	Adults Male						
	Early	9	10.628	5.070	15.021	3.544 + 1.192 X	1.692*
	Late	16	12.931	9.858	21.821	4.319 + 0.433 X	2.210*
	Adults Female						
	Early	9	8.122	5.765	14.101	3.922 + 0.913 X	2.411*
	Late	16	10.311	5.1676	28.574	4.318 + 0.187 X	1.696*
T.confusum	Eggs						
	Early	2	0.780	0.379	2.254	2.902 + 4.565 X	23.731***
	Late	5	0.901	0.591	2.730	2.791 + 4.257 X	13.193***
	Larvae						
	Early	2	0.727	0.232	1.823	1.650 + 8.697 X	61.054***
	Late	16	2.299	1.799	5.938	4.657 + 3.011 X	9.943**
	Pupae Male						
	Early	2	3.319	1.237	6.955	3.737 + 0.371 X	2.222*
	Late	5	4.319	1.237	8.955	3.737 +0.371 X	2.254*
	Pupae Female						
	Early	2	3.124	0.868	5.965	4.006 + 0.916 X	0.730NS
	Late	5	4.059	1.320	9.029	4.419 + 0.429 X	7.371**
	Adults Male						
	Early	9	8.852	3.065	12.759	4.419 + 0.508 X	0.925NS
	Late	16	10.025	5.912	17.516	4.394 + 0.383 X	0.160NS
	Adults Female						
	Early	9	7.101	5.581	14.149	4.208 + 0.423 X	2.527*
	Late	16	9.790	6.612	23.903	4.023 + 0.911 X	0.308NS

*P<0.05; **P<0.01; ***P<0.001 and NS- not significant



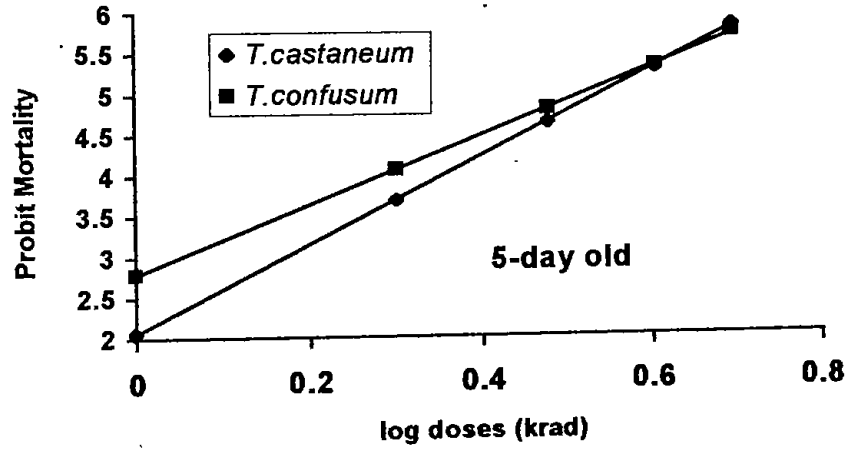


Figure 6.1: Probit regression lines for the mortality of eggs of *T. castaneum* and *T. confusum* irradiated at 2- and 5- days old.

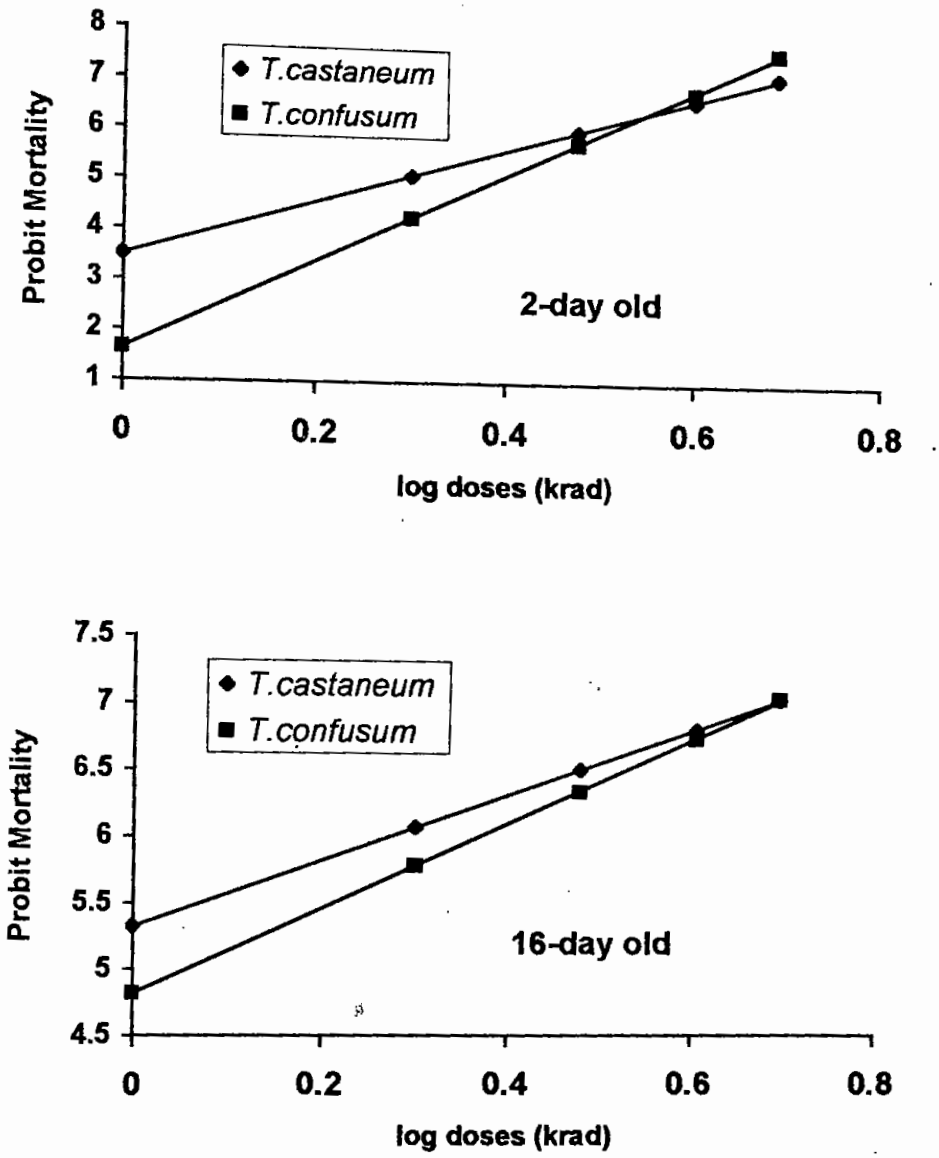


Figure 6.2: Probit regression lines for the mortality of larvae of *T. castaneum* and *T. confusum* irradiated at early (2-) and mature stages (16- day old).

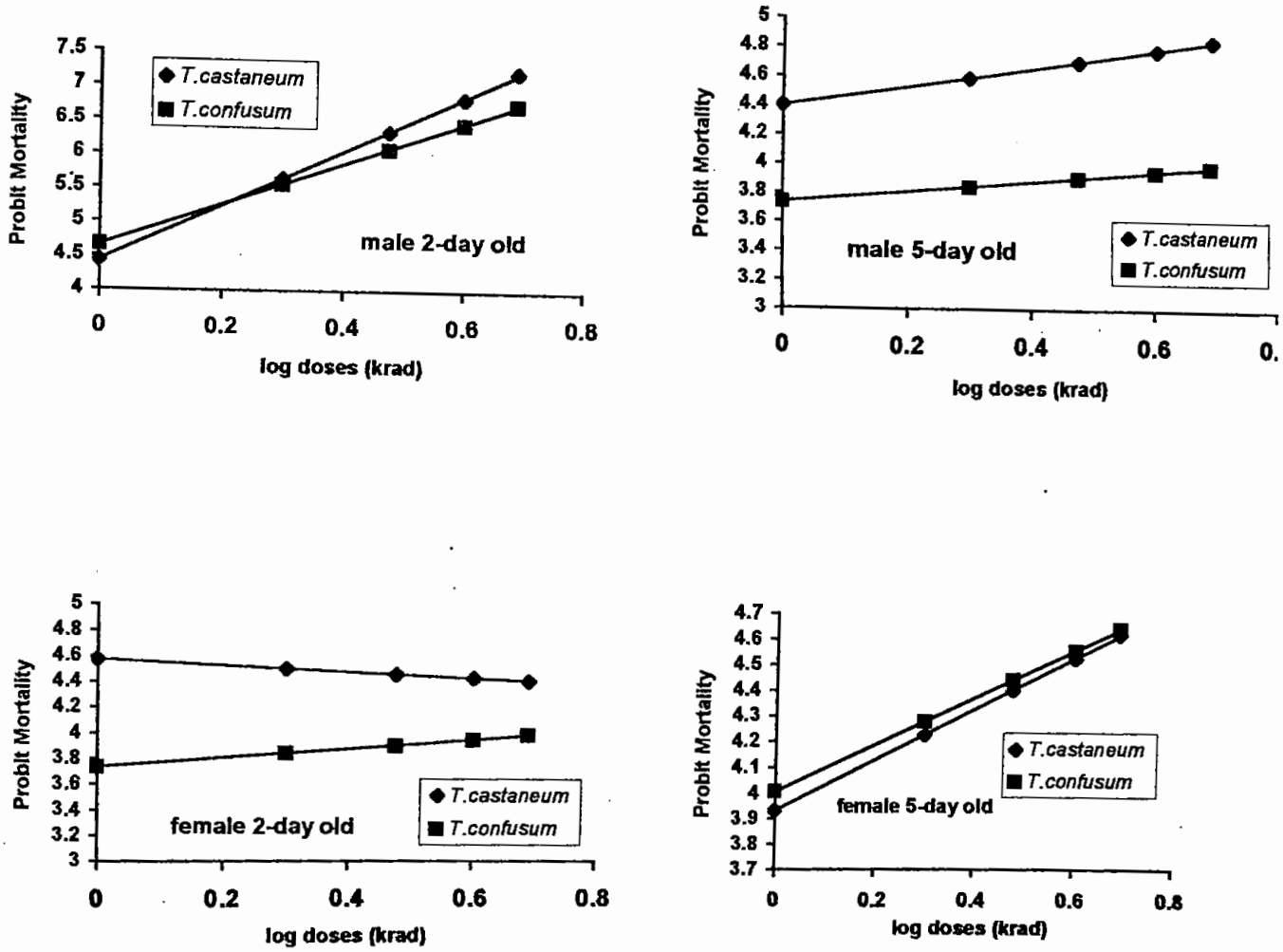


Figure 6.3: Probit regression lines for the mortality of pupae of *T. castaneum* and *T. confusum* irradiated at early (2-) and late stages (5- day old).

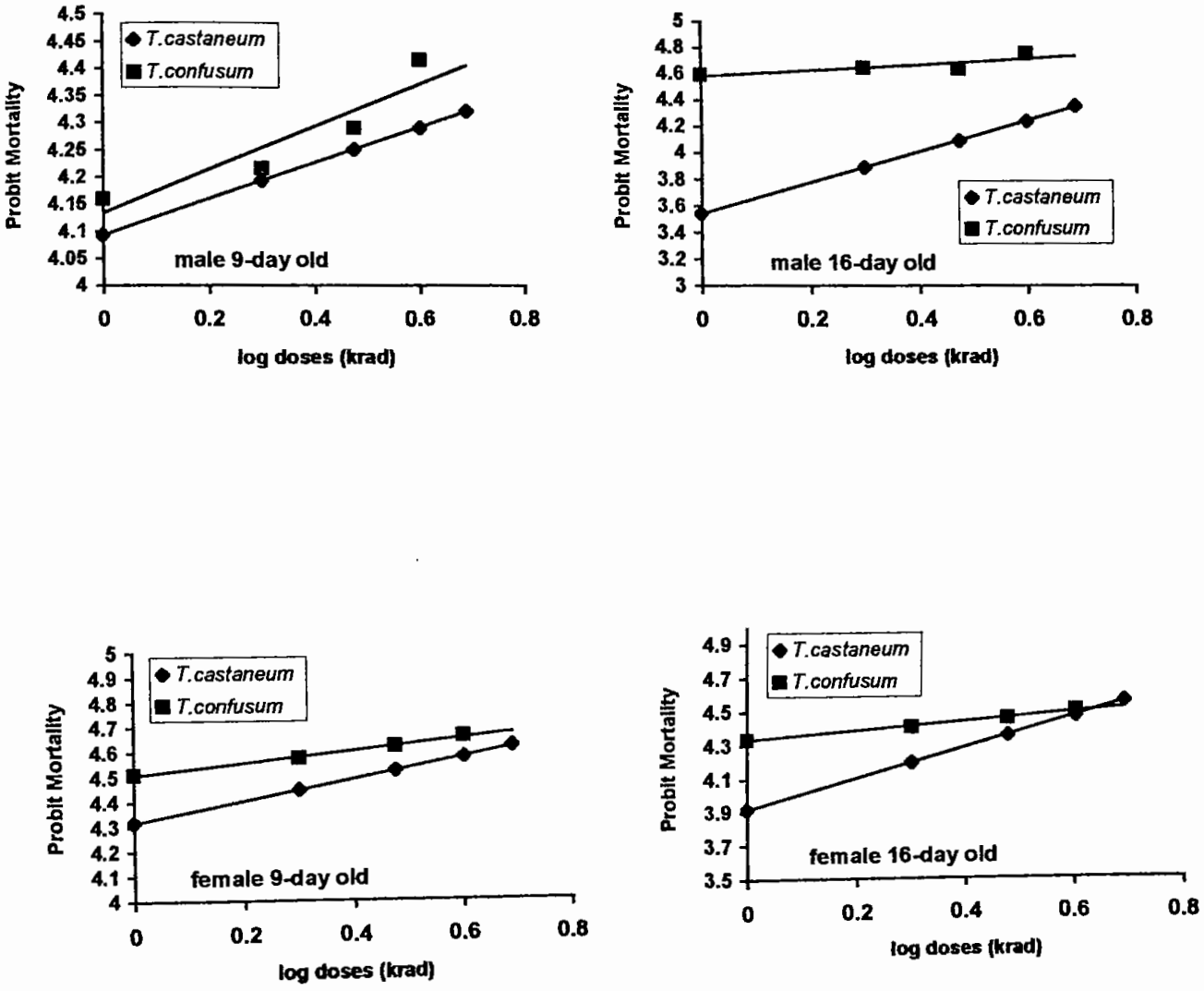


Figure 6.4: Probit regression lines for the mortality of adults of *T. castaneum* and *T. confusum* irradiated at early (9-) and late stages (16- day old).

Chapter: Seven

TIME COURSE MORTALITY AND RADIOSENSITIVITY INDICES IN ADULT OF *T. CASTANEUM* AND *T. CONFUSUM* DEVELOPING FROM IRRADIATED MATURE LARVAE

TIME-COURSE MORTALITY AND RADIOSENSITIVITY INDICES IN ADULTS OF *T. CASTANEUM AND T. CONFUSUM* DEVELOPING FROM IRRADIATED MATURE LARVAE

7.1. INTRODUCTION

It is of practical importance that insects which are sterilised and released into a wild population for control purposes should survive nearly as long (White, 1971). Longevity may affect competitiveness and indeed it has been used to measure sexual aggressiveness (Baumhover, 1965). However, so many factors affect longevity that it is questionable if it is a satisfactory measure of an insect "fitness".

The different factors that may influence the reduction in life-span after irradiation have been studied in *Tribolium* as in various other organisms (Ducoff and Bosma, 1967). The authors found that the mortality period after X-irradiation was doubled for beetles reared at 22°C compared with that for beetles kept at 30°C. The general effect of radiation, especially of sizeable doses, is to shorten the mean life span of the treated population (Tilton and Brower, 1983). The mean post irradiation longevity is usually inversely related to the rate of the radiation dose. Quite often the effects of radiation are measured by the LT_{50} or LT_{95}; that is, the time it takes 50 or 95% of the exposed population to die. Several researchers have proposed that LT_{50} or ST_{50} (survival time for 50%) be used as a sort of universal indicator of radiosensitivity for comparison of different species (Willard and Cherry, 1975).

However, there are many reports that deal with radiation effects in terms of survivorship in *Tribolium* species, and the more complete studies have been summarised by Ducoff (1972). One aspect greatly ignored is the difference in radiosensitivity between the species, which differ in their time-course of mortality.

The present study was concerned with the investigation of some parameters (longevity, age, sex) of adults *T. castaneum* and *T. confusum* developing from irradiated mature larvae and to evaluate the relevance of these parameters to the radiosensitivity index (RI).

7.2. MATERIAL AND METHODS

Approximately 1000 mature larvae of *T. castaneum* and *T. confusum* were collected from the stock culture maintained with standard respective food media (Hasan and Selman, 1993). The larvae of each species were kept in glass vials (2 x 2.5 cm) for irradiation. The larvae were irradiated with 0 (control), 1-, 2-, 3-krad dose levels from a ^{60}Co source at dose rate of approximately 5.955 krad/h at 30 cm distance from the midline of the medium to the ray tube After irradiation, they were placed in petri dishes containing food media for pupation. After pupation, the pupae were sexed (Halstead, 1963) and kept in separate petri dishes for eclosion. After eclosion, 100 adults of each species and sex were kept in petri dishes containing respective food media for observing their longevity. Each test consists of three replicates and the longevity observation was made for 24 weeks. The food media were changed after 4 weeks for all the species to avoid conditioning (Mondal, 1992). The LT_{50} of *T. castaneum* and *T. confusum* adults was computed from the data obtained on the percentage of kill at each of the dose level tested through probit analysis with 95% confidence limits (Busvine, 1971).

The experiments were conducted at 30±1°C and 70% r.h.

7.2.1. Data processing

7.2.1.1. Mean Survival Times (MST)

The mean survival time (MST) for a group of beetles exposed to a dose of radiation was calculated in the following model:

$$MST = 1/n \Sigma(t \times Y_t)$$

Where, n = number of beetles in the group, t = week, and Y_t = number of beetles that die at week t.

7.2.1.2. Radiosensitivity Indices *(RI)*

The radiosensitivity index expresses a time response to radiation exposure in terms of a species longevity which was followed (Willard, 1970):

$$RI = LT_{50} / MST$$

The LT_{50} represents the time required, following irradiation, for 50% of a population to die. This parameter(RI), like LT, has the advantage of broad applicability for comparative purposes, predictive potential and statistical value.

A curvilinear regression analysis for the radiosensitivity data were also fitted using a software package *Fig-P* which describes a prediction curve given by the following third degree polynomial equation.

$$Y(RI) = a + bx + cx^2 + dx^3$$

where, a, b, c, d are the coefficients and x is the dose level.

7.3. RESULTS AND DISCUSSION

Figure7.1 & 7.2 show the mean mortality percentage of adults of both sexes resulting from irradiated mature larvae. The mean adult longevity of both sexes decreased in all the species as the irradiation doses increased (Figures 7.1 & 7.2). though the longevity in *T.castaneum* and *T. confusum* were not significantly varied in both the sexes (Appendices 89-90). However, *T. castaneum* showed the higher longevity compared to *T. confusum* in all the dose levels and both the sexes (Figure 7.3). These figures also indicate that the rate of mortality of irradiated insects is dependent on the dose of irradiation. As shown in these figures, none of the doses from either 1- or 2-krad exhibited the 100% mortality with 24 weeks for both the species. However, there was a less remarkable difference between the species in their life-span. It was also observed that male adults were slightly longer-lived than the females.

Figure 7.4 represents the results of relationship between doses and lethal mortality for 50% in *T. castaneum* and *T. confusum* adults resulting from irradiated mature larvae. This figure clearly shows a significantly negative correlation between these parameters for both the species and sexes. These results showed the significant ($P<0.001$) differences within the sex, species, dose levels and stages though their interactions varied non significantly. *T. castaneum* was the longer-lived than *T. confusum* as found for both the sexes (Figure 7.1 & 7.2).

LT_{50} values for both the species decreased as the dose increased which confirm the results of mean survival times that *T. castaneum* is more resistant than *T. confusum*.

Highest values of LT_{50} (Apendix table 89 & Fig. 7.3). It was also observed that males are longer-lived than the females showing higher LT_{50} values (Apendix table 89 & Fig. 7.3).

The results of the experiments are presented in Figure 7.4 with radiosensitivity indices (RI) curves predicted using the model described in materials and methods (Willard, 1970). This figure shows that the RI values are not varied widely among the species and the sexes, but it increased with the increasing dose levels. It also indicates that *T. confusum* was more radio-sensitive at the higher doses than *T. castaneum* (Figure 7.4).

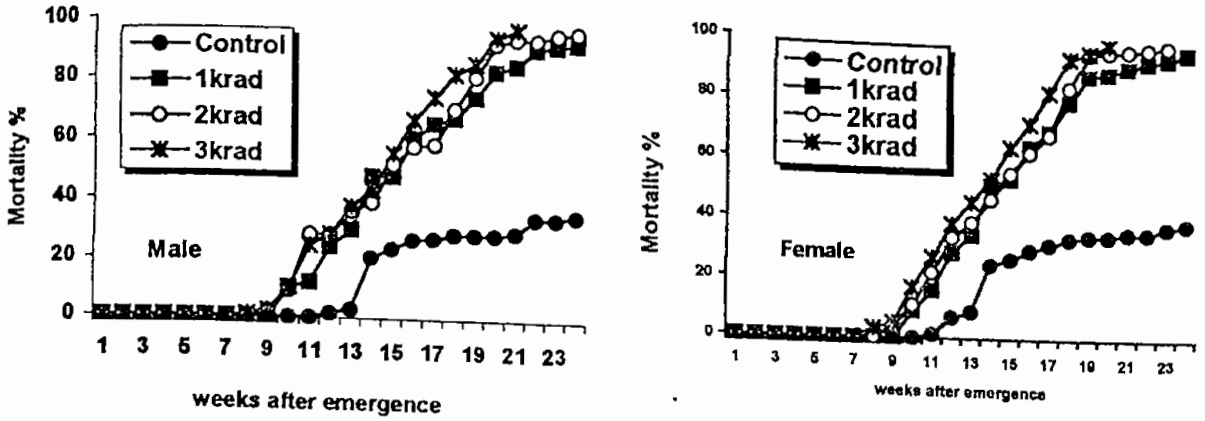


Fig. 7.1. Time-course mortality in *T. castaneum* adults developing from gamma irradiated mature larvae.

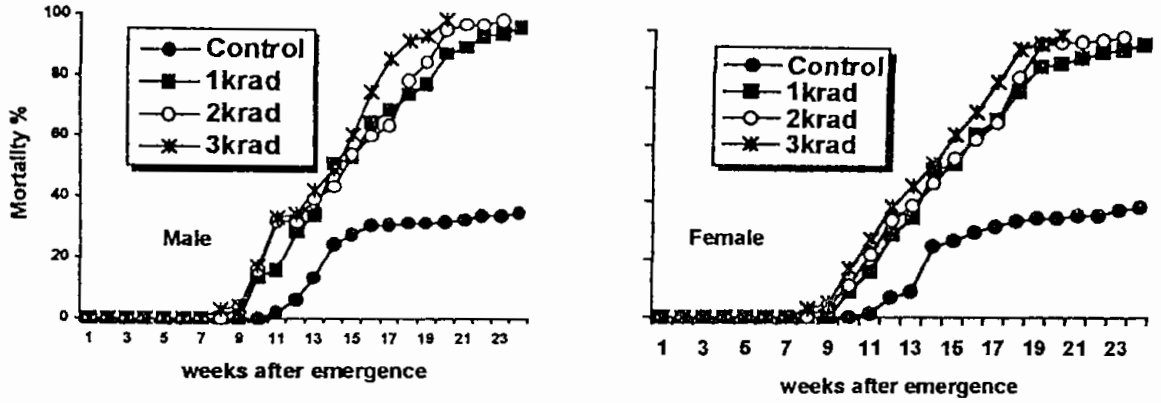


Fig. 7.2. Time-course mortality in *T. confusum* adults developing from gamma irradiated mature larvae.

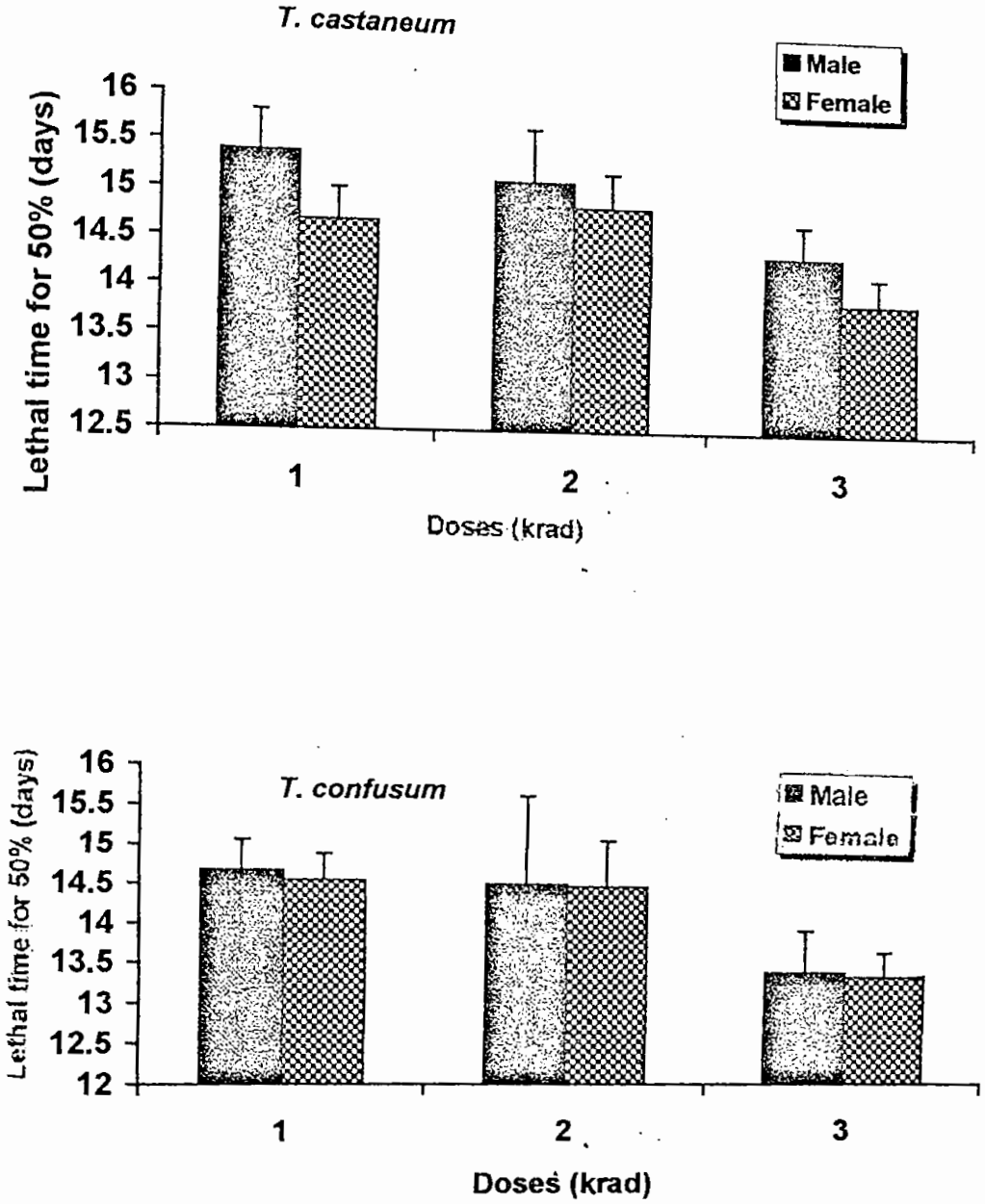


Fig. 7.3. Lethal time for 50% for both sexes of *T. castaneum* and *T. confusum* adults resulting from irradiated mature larvae. [line bar indicates 95% confidence limits]

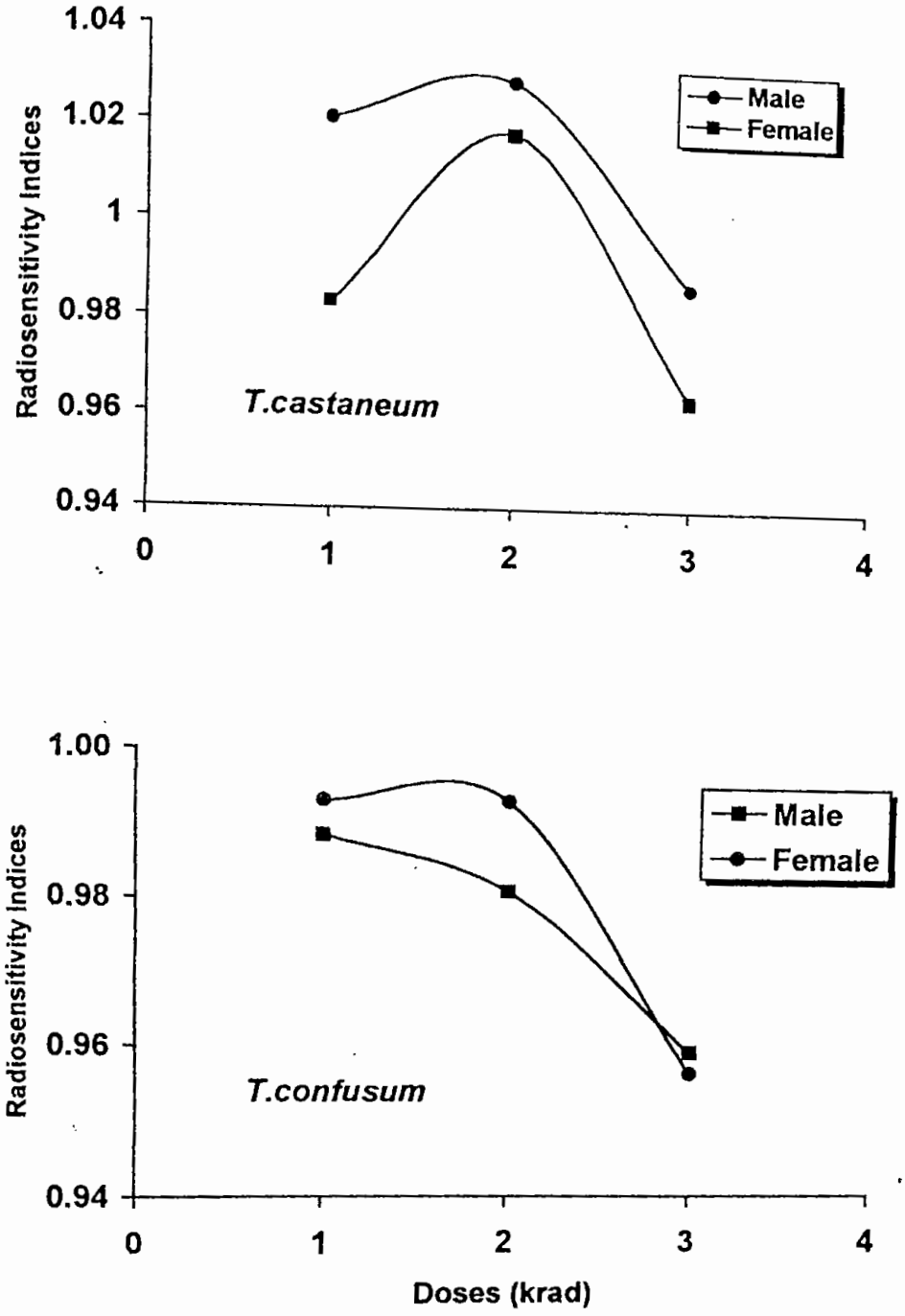


Fig. 7.4. Curvilinear regression for the radiosensitivity indices in *T. castaneum* and *T. confusum* adults developing from irradiated mature larvae.

The post irradiation longevity of adults of both sexes developing from irradiated mature larvae was less than that of control. Results also showed that irrespective of dose from 1- to 3-krad, the longevity was almost identical for both the species. Hasan (1995) reported that mature larvae were more radio sensitive than pupae and adults in terms of shortened life span of adults. These findings are in accord with Brower and Scott (1972) who worked on the spider beetle, *Gibbium psylloides*. Brower (1973) also confirmed these results while working with the depressed flour beetle, *Palorus subdepressus* (Wollaston).

Figure 7.1 shows that none of the doses 1 and 2-krad in 24 weeks time produced 100% adult mortality which emerged from irradiated mature larvae. Hasan (1995) observed that *T. castaneum* was the most radio-resistant and long-lived while *T. freemani* was the least. There was very slightly variation in their life span among all the species. These results were supported by the LT_{50} analyses where *T. castaneum* showed the highest values of LT_{50} followed by *T. confusum* (Appendix table 89 & Fig 7.3). These sorts of species variation were reported by Tilton *et al.* (1966), Brower and Tilton (1973) and Brower (1975). However, the size of the species may account for this differentiation. A rather similar effect has been suggested by Willard and Cherry (1975). It is known that the larger species develops more slowly than the smaller species (Brower, 1975). Moreover, the phylogenetic relationship appears to be more important than physical size or rate of development in determining radiosensitivity and in species *Tribolium*, may be produced by as many as 280 different genes (Enfield, 1972). But this correlation may be dependent either on the number of chromosomes possessed by the two groups as proposed by Sparrow *et al.* (1963) or on other undiscovered differences.

Results also showed that the adults either male or female resulting from irradiated mature larvae did not vary significantly in their survival rate, though the females were longer-lived than the males in both the species (Figure 7.1 & 7.2). These results support the findings of Brower (1973) who found that there was apparent difference between the sexes of *P. subdepressus* in their survival rates due to low dose of irradiation. Similar results were also observed by Jones (1967) and White and Hutt (1970) while working with *Culicoides variipennis* and *Laspeyresia pomonella* respectively.

The results of the present investigation also demonstrate that the RI values were more or less identical for both the species (Figure 7.4). This may be due to the application of sublethal doses from 1- to 3-krad. Willard (1970) indicated that the criteria reported to influence the radiosensitivity of organisms (age, weight, sex, diet environmental factors, endocrine disturbances, etc.) may act by altering the organisms' physiology, and it is the interaction of ionising radiation with the integrated physiology of an organism which results in a given effect. O'Brien and Wolfe (1963) reported that the effects of ionizing radiation on insects vary according to the dose levels, species, developmental stages and criteria employed.

The foregoing study provides useful information about the effects of gamma irradiation on the longevity of *Tribolium* species which is crucial to the application of sterile male techniques.

Chapter: Eight

EFFECT OF POTENTIAL *T. CASTANEUM* AND *T. CONFUSUM* IRRADIATED AS PUPAE AND ADULTS

REPRODUCTIVE POTENTIAL OF *T. CASTANEUM* AND *T. CONFUSUM* IRRADIATED AS PUPAE AND ADULTS

8.1 INTORODUCTION

Recently, the control of stored grain pests by using ionizing radiations has received serious attention because at appropriate doses, irradiation assures mortality to all the stages of the insects and prevents residual effects. Several insect species have been controlled successfully by using nuclear techniques which appear to be potential alternatives to chemicals for stored product pests. Recent reports suggest that irradiation is useful for inducing effective sterility in various pest insects (Hasan & Khan, 1998; Hasan, 1999; Hasan, 2000). Comparative studies of the radiation sensitivity among stored product insect pests are important for nuclear control strategy because many species may be present in infested pulses and grains and radiation dosage must be high enough to control the most resistant species present. This is crucial because sensitivity to radiation varies depending on stages and strains of the pest species (Hasan, 1995).

Sterile male release technique is one of the effective means of combating pest species where sterile but competitive males are released in the field to compete normal males, bringing about a suppression of the pest population. Such agents as radiations chemosterilants and endosymbiotic microorganisms gained much popularity in sterilization or complete breakdown in gamete formation in a number of pest species.

Unfortunately, such a promising approach like sterile male technique has not so far been applied to the flour beetles and this led to design the present investigation. The present investigation reports the effects of a range of gamma doses on the reproductive potential in both *T. castaneum* and *T. confusum*.

8.2 MATERIAL AND METHODS

8.2.1 Experimental design and mating schedules

(i) Irradiated with mature pupae

To determine the effect of radiation on the reproductive potential of *T. castaneum* and *T. confusum*, pupae were collected from the stock culture reared on standard food media. Pupae of both sexes of each *Tribolium* spp. were selected for irradiation with a substerile dose of 3-krad (Brower and Tilton, 1973; Brower, 1975). After 10 days of eclosion, the cross schedules were designed as nonirriadiated (U♂ X U♀), irradiated (T♂ X T♀) and reciprocal crosses ((T♂ X U♀ and U♂ X T♀) each having three replicates. The adults of each set were placed in small plastic containers (4 X 7 cm) containing the respective food media for oviposition. The eggs were sieved and the number were recorded for 30 days at 3-day intervals (Khan and Selman, 1981) and kept in vials for hatching. The food media were changed every three days to avoid conditioning (Mondal, 1992). The hatched larvae were counted and kept in plastic containers (5 x 11cm) containing the respective food media for the F_1 adult progenies.

(ii) Irradiated with adults (10 days old)

This experiment was conducted to determine whether irradiated both sexes of *T. castaneum* and *T. confusum* could compete sexually with normal ones when treated as 10-days old adults. Both sexes of adult beetles were irradiated with a 3-krad dose of gamma radiation. Immediately after irradiation, the irradiated (T) males and females were placed with the

unirradiated (U) pair. Three replicates were tested for each ratio. The same parameters and experimental procedures were followed for the pupae.

8.3 RESULTS AND DISCUSSION

Gamma radiation-induced changes in the reproductive potential in two species of *Tribolium* and shown in Figure 8.1-8.4. A significant reduction was observed in the reproductive abilities of *T. castaneum* and *T. confusum* when irradiated as either pupae or adults and this reduction was dependent on the irradiated/nonirradiated individuals (Appendices 92-95).

As reflected in the figures 8.1 & 8.2, the fecundity was gradually suppressed with increased gamma doses irradiated either as pupae or adults for both the *Tribolium* species. The results of the reproductive potential showed that the maximum eggs per day per female was recorded for the cross schedule U♂ X U♀ compared to crosses involving the irradiated individual for both the species and stages. As shown in Figures 8.1 & 8.2, the lowest number eggs were observed in the cross schedules T♂ X T♀ for both the species irradiated either pupae or adults.

These results clearly indicate that egg-hatchability in *Tribolium* species was adversely affected by irradiating males and females (Figures 8.3-8.4). As reflected in Figure 8.3 & 8.4, the patterns of hatchability followed the dose-dependent manner. These figures also indicate that the hatching was completely inhibited at a dose level of 3 krad. The results of the tests illustrate that the maximum 25 percentage eggs were hatched at a dose level of 1 krad for both the species and stages, while it was only 10 percent at 2 krad. As the results appear in Figures 8.3 & 8.4, the sequence of order based on the hatching percentage was cross schedule T♂ X T♀ < T♂ X U♀ < U♂ X T♀ for both the species and stages.

A significant reduction was observed in the reproductive abilities of *Tribolium* when irradiated as either pupae or adults (Appendices 96-99). In preliminary studies, found that the development of 4-day old pupae stopped when they were exposed to a radiation dose of 20 Gy (10 Gy =1-krad). Furthermore, when 8-day old pupae were exposed to a dose of 70-120 Gy, adult emergence was 20-60% lower than from untreated pupae. These results indicate that with age the insects become more tolerant to irradiation. This statement also agrees with that made by who stated that competitiveness of males is directly proportional to the age of the age of the pupae when irradiated. The present data also show that the reproductive potentialities decreased as the adding of irradiated individual in both the species and stages. Both these findings are consistent with previous results for a number of stored products pests (Abdel-Salam, 1989; Makee, 1989). **Henneberry** noticed that males from irradiated pupae elicited lower oviposition response when paired with untreated females. They also concluded that this difference probably occurred due to the lack of cupyrene sperm transferred by irradiated males during mating.

Aiming at developing a sterile male technique for flour beetles, the present experiments have shown a detailed account of gamma radiation-induced alterations in the reproductive potential. On the other hand, irradiated pupae with 3 and 3.5 Kr doses to induce 92 and 98.7% sterility respectively in *C. chinensis*, where a total suppression of the beetle population was achieved with 8:1:1 ratio of sterile males to normal males and females. Arrested development at doses of 1, 4, 10 Gy for the egg and larvae of *C. maculatus* was reported by where sterility was induced by 4 Gy but the longevity of the treated insects was not affected even by 100 Gy.

Irradiated males with 10 Kr and females with 8 Kr in *C. chinensis* and noted that the population of the insect could be suppressed significantly at ratios of 1:20 and 1:25 normal to sterile males, without affecting the mating ability of the latter. As regards of reproductive potential of the flour beetles, the present results fit with the above ones as well as those reported by Ghomomu (1989).

Previous studies have shown that populations of stored-product insects could be controlled by producing immediate mortality or by producing sterility in insects with variable doses of irradiation (Cornwell and Bull, 1960). However, this information would be helpful in determining the competitiveness values (CVs) of the treated males and females needed for a release programme designed to suppress the pest populations under laboratory as well as field conditions.

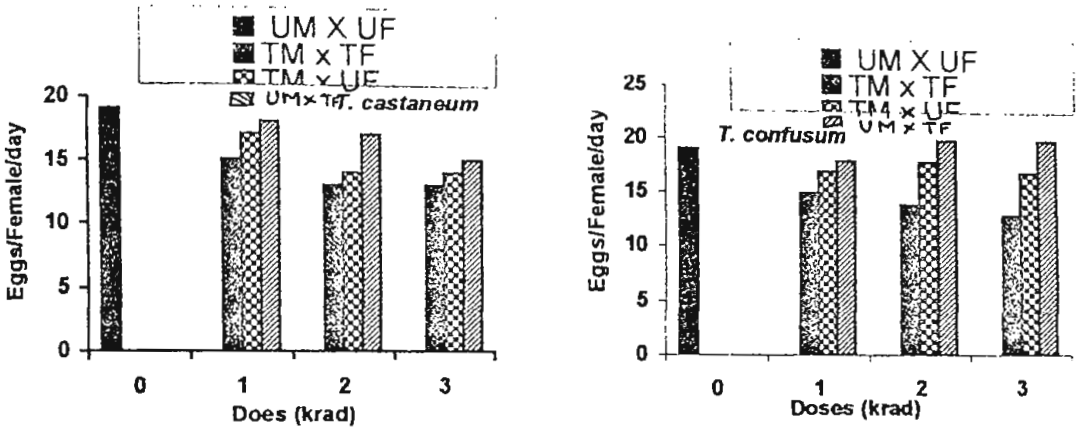


Figure 8.1: Effect of gamma radiation on the fecundity of *T. castaneum* and *T. confusum* treated as pupae.

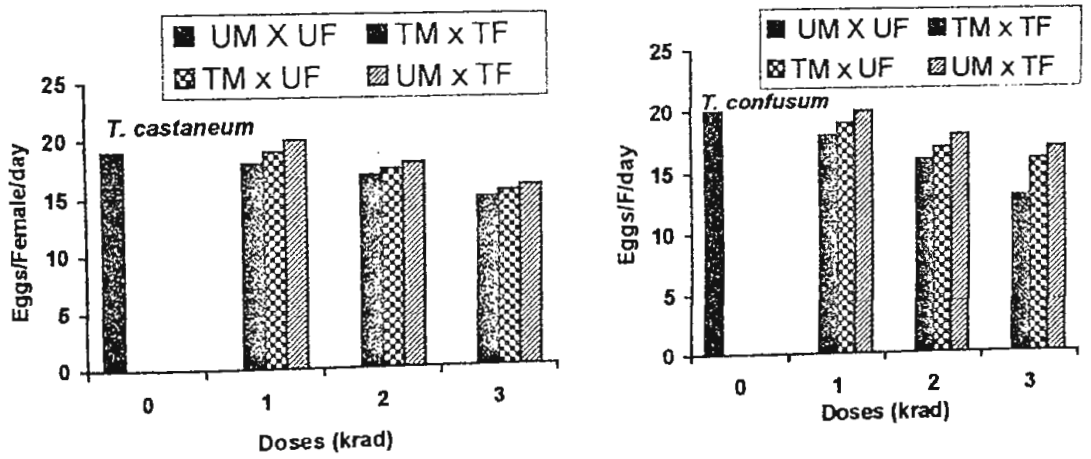


Figure 8.2: Effect of gamma radiation on the fecundity of *T. castaneum* and *T. confusum* treated as adults.

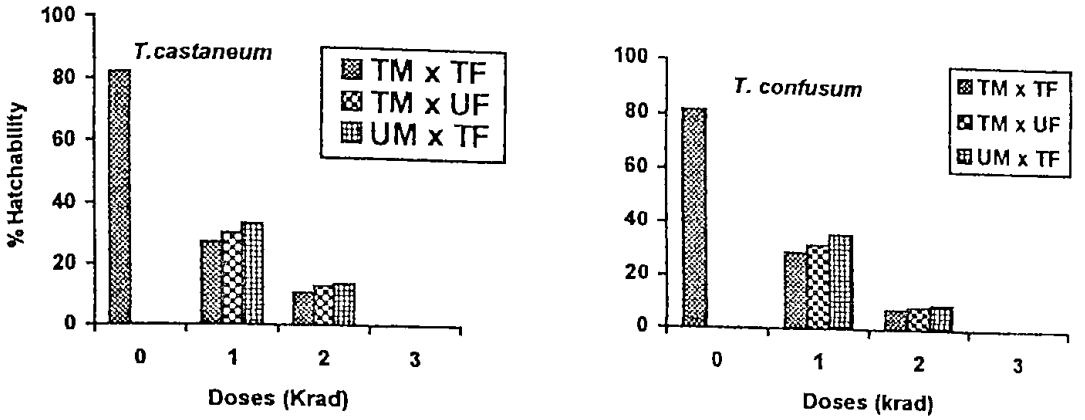


Figure 8.3: Effect of gamma radiation on the hatchability of *T. castaneum* and *T. confusum* treated as pupae.

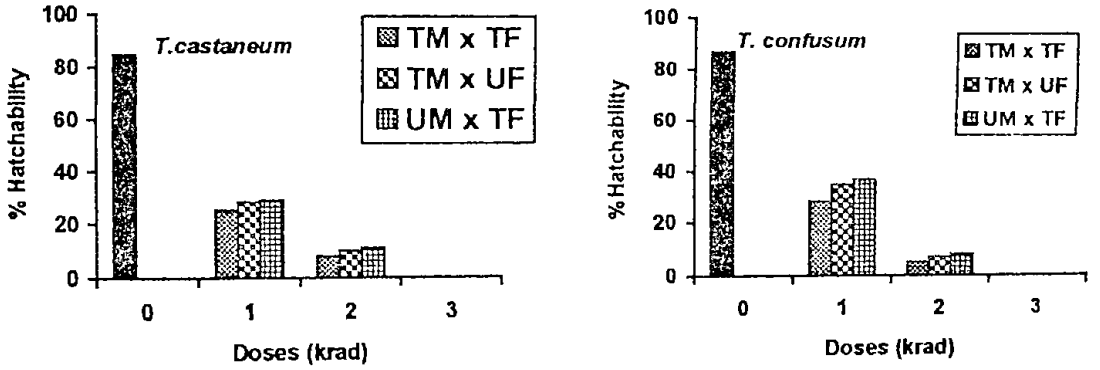


Figure 8.4: Effect of gamma radiation on the hatchability of *T. castaneum* and *T. confusum* treated as adults.

Chapter: Nine

GENERAL DISCUSSION

GENERAL DISCUSSION

It is necessary to investigate the radiation effects on parameters like growth, stage, longevity of the insect which are the prime factors for the successful implementation of sterilisation techniques (Baumhover, 1965; White, 1971; Ducoff, 1972; Murad and Ahmed, 1977; Tilton and Brower, 1983). The dose rate can be important in modifying the effects, especially when low or high rates are used. Tilton and Brower (1983) reported that sublethal doses of irradiation can produce short- or long-term changes in physiology, developmental rate, viability, longevity, behaviour and fertility. So, sublethal and lethal doses ranging from 1 to 20 krad were used in this study to determine the optimal level.

Biological studies of insects play an important role prior to conducting laboratory or field experiments. This is important especially when sterile male release techniques require experiments which involve mass rearing. The present biological studies on *T. castaneum* and *T. confusum* provide useful information which may be used in conducting experiments on a wide scale in the laboratory.

Ionising radiation reduces the rate of development and may completely or partially inhibit metamorphosis. It has been shown with *Ephestia* that inhibition of pupation in irradiated larvae results not from a disturbance of the DNA of the hypodermis as was formerly believed (Kuzin *et al.*, 1965), but from the absence of the pupation hormone, ecdysone. Its absence is believed to be the result of irradiation damage to neurosecretory cells.

The results of the investigation indicate that the gamma radiation significantly affected growth and development in both *T. castaneum* and *T. confusum* (Appendices 16-63). This

growth rate of *T. castaneum* and *T. confusum* varied when developing from different irradiated stages i.e., eggs, pupae and adults. The results also show that growth rate decreased as the dose increased. The nutritional state of larvae resulting from different irradiated stages may account for these differences. It is well known that insects which are exposed to ionising radiation frequently become temporarily lethargic (Proverb and Newton, 1962). and many authors have reported that irradiation of larvae may cause a permanent cessation of development (Whiting, 1950; Bletchly and Fisher, 1957; Cornwell *et al.*, 1957; Hasan *et al.*, 1989; Das, 1992). However, there is a lack of comparable published data, due to which, it was not possible to compare extensively the present findings with others.

The present findings show that the egg stages were very sensitive to gamma radiation as compared to the other stages indicating the lowest values of LD_{50} (Table 6.1 and Fig. 6.1) and this sensitivity reduced as development proceeded in both the species above table also shows that the earlier aged eggs were more susceptible than the late aged. It shows that the eggs of *T. castaneum* were more radio-resistant than *T. confusum*, having the highest values of LD_{50}. The early works with stored-product pests clearly established the magnitude of the changes in radiosensitivity that take place during embryonic development, changes that have been correlated with embryogenesis for several species of insects (Calderon, *et al.*, 1985; Hussain *et al.*, 1994). Eggs of the yellow mealworm beetle, *T. molitor* L., increased in resistance during the first day of development, then had a plateau in sensitivity for 2 days before beginning the sudden and profound increase in resistance that marked growth of the fully formed embryo (Brower, 1972). There was a 250-fold increase

in resistance as eggs matured from 0.5 to 7.5 days as measured by the dose required to produce an LD50. Dose rate also can produce a measurable effect when a sensitive criterion such as percentage egg hatch is used. In the present study, a dose of 4 and 5-krad completely prevented egg hatching in both the species and age groups (Appendices 64-67). In addition, the hatching percentage increased as the dose levels decreased from 3 to 1 krad. These results are in agreement with the findings of Brown and Davis (1973) who noted that the eggs of T. castaneum irradiated at doses of 0.36-, 1.1-, and 2.6-krad showed the greatest decrease in hatching at 2.6-krad though the differences were small. The present findings also show that the young larvae were more sensitive to radiation than older larvae as measured by the LD50 values (Table 6.1 and Appendices 68-69). A study of stage sensitivity with all four larval instars of the granary weevil showed that the increase in radioresistance was minimal during the larval period (Cornwell, 1966). If larval mortality or failure to pupate had been measured instead of failure of adult emergence, radioresistance would probably have increased with each instar. this illustrates the importance of selecting the right criterion to measure the effect under study. However, a series of other studies by indicated that interruption of the mitotic processes may be the most important effect of larval irradiation. The results also show that T. castaneum larvae were more resistant than T. confusum, indicating the higher LD50 values (Table 6.1 and Appendices 68-69). These species variations in terms of radio-sensitivity have been reported by several workers (Park et al., 1958; Erdman, 1962; Brower, 1975). Irradiation of insect larvae may lead to one or more of a number of responses in addition to simple lethality. These include delay in pupation (Bourgin et al.,

1956), developmental abnormalities in the adult (Ducoff and Bosma, 1966), death during the pupal stage or failure to emerge (Vinson *et al.*, 1969), and imaginal death soon after eclosion (Yang and Ducoff, 1969). The relationships between these responses and larval death are not clear, and differ among the various orders. Furthermore, few investigations have furnished data on all of these responses.

The mortality patterns in pupae of *Tribolium* as well as other pest insects is very difficult to be determined and the failure to emerge is usually used to assess overall pupal mortality. Because the pupal stage is such an important stage physiologically and morphometrically, it is not surprising that radiation often disrupts these processes to the extent that gross physical malformations occur (Tilton and Brower, 1983). The present findings show that the earlier pupae, either male or female, were more sensitive, with a general decrease in mortality and an increase in pupal age (Table 6.1). Burgess and Bennett (1972) obtained a similar result while working with the alfalfa weevil, *Hypera postica* (Gyllenhal), when pupae of five different ages were irradiated. They also found that the youngest of the five pupal ages was the most sensitive, with a general decrease in mortality with increase in pupal age. In this work, the most prevalent syndrome of pupae either male or female which failed to eclose was an incomplete casting of the pupal skin and they usually remained soft until death. Usually the skin was split and clung to all parts of the body rather than any one body region. At the highest doses like 5-krad, most of the pupae of both sexes failed entirely to become adultoid and few of them were able to survive (Appendices 72-79). The production of abnormalities was also dose dependent both with eggs and larvae. Similar age and dose dependent mortality patterns have been observed in several insect species (Ouye *et al.*, 1964; Burgess and Bennett, 1972; Khalequzzaman and Hasan, 1989).

The present results show that the male pupae are more radioresistant than the female pupae indicating the higher values of LD_{50} (Table 6.1). These results are in agreement with the findings of Brower (1976) who found that female pupae of Indian meal moths, *Plodia interpunctella* were more radiosensitive than the male. The data of the present results also show that there was often a substantial delay in adult emergence when pupae were irradiated with sublethal doses and this delay was related to the dose and age of the pupae at the time of treatment. The variation of metabolic rate may be a considerable factor in this difference. In many ways the pupal stage is analogous to an embryonic stage in that periods of intense cell division and differentiation occur (Hasan and Khan, 1998). Therefore, it is not surprising that the overall pattern of radiosensitivity is similar to the pattern exhibited by developing eggs or larvae. Most studies of pupal radiosensitivity have not been precise enough or at least are not correlated enough with the morphogenetic changes occurring, to reveal the tiny details in the pattern of radiosensitivity. However, in general, pupal radiosensitivity either in males or females is directly correlated with the age of the pupae unless diapause or resting periods occur. Tilton and Brower (1983) suggested that pupae can be segregated by time elapsed after pupation or by visual clues that are indicative of internal development.

The present investigation shows that adult *Tribolium*, either male or female, are more radioresistant than the other stages as indicated by the higher values of LD_{50} (Table 6.1; Fig 6.4 & Appendices 80-87). *T. castaneum* adults show more radioresistance than *T. confusum* as was found in other stages (Table 6.1). Several studies indicated that even closely related species can differ in response (Tilton and Brower, 1983). Differences in the

lethal effect of irradiation were shown for four and five strains of *T. castaneum* and *T. confusum* respectively (Shipp, 1966). Also observed that the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) was more radiosensitive than the very similar merchant grain beetle, *O. mercator* (Fauvel). However, the effects of acute doses on adults may be summarised as follows: (i) very high doses are required for immediate death (ii) the adult becomes resistant with advancing age. Changes in the susceptibility of adults have been reported in a number of species; the differences are small and difficult to detect without critical study using doses which span the LD_{50}.

It is well known that the survival times of irradiated males are very important requirements for an effective sterile release programme. Moreover, the irradiated insects must be able to survive the irradiation dose which causes the required sterility. The somatic cells in young metamorphic stages, as has been conclusively shown in the literature, are very radiosensitive. Thus, when young metamorphic stages are exposed to the level of irradiation which causes the required level of sterility the somatic cells will be severely damaged, and survival will be very low (Makee, 1989). This damage fully depends on the dose. Therefore, only the effects of low and moderate doses of irradiation on the survival of adults *T. castaneum* and *T. confusum* of both sexes resulting from treated mature larvae were considered (Chpater-7).

In the present experiments adult longevity was adversely affected by the irradiation of mature larvae (Figs. 7.1-7.2). In all cases irradiation exposure of mature larvae significantly decreased the length of life of the resulting adults in both the species (Figs. 7.1-7.2). Probit analyses indicate a significant regression between dose delivered to different stages and

adult longevity. A study by Bergonie and Tribondeau (1906) concluded that radiosensitivity of cells was related directly to their proliferative activity and inversely to their degree of differentiation. Thus the fully differentiated, non-proliferating cells of the adult insect are, or appear to be, very radioresistant, even though they may suffer latent damage in dividing cells.

The present findings also indicate that *T. castaneum* shows a higher rate of mean survival time than that of *T. confusum* (Fig. 7.3). These species variations in terms of their longevity were noted by several workers (Ducoff *et al.*, 1971; Ducoff, 1972; Soliman, 1972; Brower, 1975; Mehta *et al.*, 1990). Titon and Brower (1983) reported that even different strains of the same species can have clearly detectable differences in their longevity after radiation. A study of 35 laboratory and wild strains of the granary weevil from different parts of the world showed marked differences in their survival rate after adult irradiation (Corwell, 1966). Reduced longevity is one of the most commonly observed responses caused by somatic damage (Proverbs and Newton, 1962). In certain adult insects, the midgut epithelium is renewed periodically, but since radiation and some chemosterilants inhibit mitosis the degenerated cells cannot be replaced. This leads to early death in the adult (Riemann and Flint, 1967). Irradiation may shorten the life span of an insect by increasing its susceptibility to attack by microorganisms (Jafri, 1965).

As is known, determination of the comparative radiosensitivity of the different biological strains of laboratory insects should be a prerequisite in sterile male release techniques (SMRT). Figure 7.4 reflect the radiosensitivity indices (RI) for *T. castaneum* and *T. confusum* treated at mature larvae. These values ranged widely among the species. Though, the index values for both sexes of both the species were in close agreement even though the

mean longevity varied widely in many cases. However, these index values were dose dependent i.e., decreased as the dose increased.

Perhaps the species level of taxonomic organisation may be the pivotal point where the generalisation relating radiosensitivity to phylogeny breaks down and other criteria become more important as indicators of radiosensitivity. Willard (1970) pointed out that the criteria reported to influence the radiosensitivity of organisms (age, weight, sex, diet, environmental factors, endocrine disturbances, etc.) may act by altering the organisms physiology and it is the interaction of ionising radiation with the intergrated physiology of an organism which results in a given effec. O'Brien and Wolfe (1963) reported that the effects of ionising radiation on insects vary according to the order, genus, species, developmental stages and criteria employed. Weight is the foremost of the parameters generally believed to have the greatest influence on radiosensitivity (Baxter and Tuttle, 1957; Odum, 1959; Wharton and Wharton, 1959; Menhinick and Crossley, 1969). Although not conclusive, an earlier study (Willard, 1970) suggested a curvilinear relationship between the RI and both age and stage.

From the foregoing discussion, it should be noted that radiation sterilisation of *Tribolium* with a dose up to 2-krad given to mature larvae, caused no appreciable shortening of life span compared with dose of 3-krad.

The most often-studied effects of adult irradiation are those pertaining to reproductive potential. The existence of broods of lesser viability or fecundity from irradiated males may be explained on a basis of differential germ cell sensitivity (Clark, 1960). Gonen (1975) observed no differences between fertility in males and females treated at similar doses.

Generally, the studies of male fertility have been concerned with measurements of the ability of the irradiated males to compete with various ratios of normal males (Bushland and Hopkins, 1953; Davis *et al.*, 1959; Proverbs and Newton, 1962 and others).

In SMRT, the adverse effects of high doses of radiation on the mating competitiveness and behaviour of insects have stimulated us to concentrate greater efforts in recent years on the use of minimum doses that produce a high degree but not necessarily complete sterility (Knipling, 1970). This is because high doses of radiation to induce complete sterility sometimes cause severe physiological and somatic damages (North and Holt, 1968) and are also more expensive. The role that the release of partially sterile insects capable of transmitting sterility effects to the F_1 generation can play in the suppression of certain pests has been appraised.

The reproduction potential of unirradiated females was significantly affected by mating irradiated males treated either as pupae or adults (Appendices 92-99).

Gamma radiation-induced changes in the reproductive potential in two species of *Tribolium* are shown in Figure 8.1-8.4. A significant reduction was observed in the reproductive abilities of *T. castaneum* and *T. confusum* when irradiated as either pupae or adults and this reduction was dependent on the irradiated/nonirradiated individuals.

As reflected in the figures 8.1 & 8.2, the fecundity was gradually suppressed with increased gamma doses irradiated either as pupae or adults for both the *Tribolium* species. The results of the reproductive potential showed that the maximum eggs per day per female was recorded for the cross schedule U♂ X U♀ compared to crosses involving in irradiated individual for both the species and stages. The lowest number eggs were observed in the cross schedules T♂ X T♀ for both the species irradiated either pupae or adults.

These results clearly indicate that egg-hatchability in *Tribolium* species was adversely affected by irradiating males and females (Figures 8.3-8.4). As reflected in Figure 8.3 & 8.4, the patterns of hatchability followed the dose-dependent manner. These figures also indicate that the hatching was completely inhibited at a dose level of 3 krad. The results of the tests illustrate that the maximum 25 percentage eggs were hatched at a dose level of 1 krad for both the species and stages, while it was only 10 percent at 2 krad. As the results appear in Figures 8.3 & 8.4, the sequence of order based on the hatching percentage was cross schedule T♂X T♀ < T♂X U♀ < U♂X T♀ for both the species and stages.

The application of 3-krad to adults resulted in F_1 progeny which had a high level of sterility in both the species (Figs 8.1-8.4). Thus there are several advantages in using partially sterile males. Firstly, the competitiveness of semi-sterile male parents is higher. Secondly, the cost will be lower as a result of treating at least two generations and also being able to apply lower doses.

It is well known that high mating ability and frequency of mating are very important requirements for effective sterile male control. Moreover, adults are sexually more vigorous at earlier ages compared to late ages (Makee, 1989). Tilton and Brower (1987) reported that sterile males of *C. maculatus* irradiated at 3~5 days and as newly emerged adults were adequate in their sexual competitiveness with normal males. However, larger scale experiments should be carried out in small warehouses to evaluate the practical use of the sterile-male technique for control of these pest. The eradication of *Tribolium* spp. as well as others by this technique must be more feasible in areas such as Bangladesh, where the pests is restricted to warehouses.

From the foregoing discussion, it could be concluded that the sterile-male technique is a most promising method for the control of Tribolium as well as other stored product insect pests. Immediate 9direct killing) and long-term benefits (reduction in the F1 progeny) can be achieved. This technique can solve several problems which may occur as a results of applying other techniques. However, it should not be looked at a short-term method which may give an immediate satisfactory result but which has long-term side effects. Concerning long-term benefits, low-doses could be effective in suppressing the F1 progeny as found in these experiments (Chapter 8). For commercial adaptation, research into the economic feasibility is needed. Experience argues that the major problem encountered in using this technique is the cost benefit ratio as compared to the alternatives, such as pesticides i.e., it is an economic problem rather than an ecological one. As was described earlier, low doses i.e., sub-sterilising doses could be effective and economically feasible, if these techniques are considered for a long term investment. However, commercialisation of radiation depends on the economics of the process. Cost is usually cited as a major factor in the general reluctance to accept the processes (Watters, 1985).

The cost of this technique could be reduced if an irradiation device is installed on mobile trucks or rail cars. By moving such facilities to locations where they are needed, maximum use can be made of the irradiator, which reduces the use cost proportionately. Indeed, it would be applicable in tropical as well as developing countries where most of the warehouses are located near railway stations, so that, grains can easily be handled. Therefore, it would be useful to examine the practical possibility in future investigations.

In the light of these findings, in order to enhance the efficacy of the sterile-male technique the following criteria should be considered:

- Sub-sterile dose of gamma irradiation (3 krad) should be exposed.
- The ratio of semi-sterile males to unirradiated males should be high.
- Irradiated males should be released promptly after the treatment so they can mate with several unirradiated females during their life span.

Chapter: Ten

LITERATURE CITED

10.1. LITERATURE CITED

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Chapter: Ten

APPENDICES

10.2. APPENDICES

Appendix Table 1: Analysis of variance for the head capsule length of 10 days old and mature larvae of *T. castaneum* and *T. confusum*.

Source	DF	SS	MS	F values
Species	1	0.042	0.042	66.363***
Age	1	0.118	0.118	186.426***
Species*Age	1	0.015	0.015	24.341***
Error	8	0.005	0.00006	
Total	11	0.180		

Appendix Table 2: Analysis of variance for the head capsule length of pupae of *T. castaneum* and *T. confusum* both sexes.

Source	DF	SS	MS	F values
Species	1	0.0192	0.019	0.824 NS
Sex	1	0.011	0.011	0.463 NS
Species*Sex	1	0.015	0.0147	0.631 NS
Error	8	0.187	0.023	
Total	11	0.232		

Appendix Table 3: Analysis of variance for the head capsule length of both sexes of *T. castaneum* and *T. confusum* adults.

Source	DF	SS	MS	F values
Species	1	0.017	0.017	0.782 NS
Sex	1	0.041	0.041	1.854 NS
Species*Sex	1	0.017	0.016	0.727 NS
Error	8	0.176	0.022	
Total	11	0.272		

Appendix Table 4: Analysis of variance of eggs of *T. castaneum* and *T. confusum*.

Source	DF	SS	MS	F values
Species	1	0.009	0.009	1.231 ^{NS}
Replication	2	0.007	0.004	0.487 ^{NS}
Error	2	0.0156	0.008	
Total	5	0.033		

Appendix Table 5: Analysis of variance for the length of 10 days old and mature larvae of *T. castaneum* and *T. confusum*.

Source	DF	SS	MS	F values
Species	1	0.173	0.173	7.612 ***
Age	1	91.411	91.411	4026.93 ***
Species*Age	1	0.132	0.323	5.828 *
Error	8	0.182	0.023	
Total	11	91.898		

Appendix Table 6: Analysis of variance for the length of both sexes pupae of *T. castaneum* and *T. confusum*.

Source	DF	SS	MS	F values
Species	1	0.072	0.072	3.176 ^{NS}
Sex	1	0.009	0.009	0.401 ^{NS}
Species*Sex	1	0.009	0.0091	0.401 ^{NS}
Error	8	0.182	0.0227	
Total	11			

Appendix Table 7: Analysis of variance for the length of both sexes adults of *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.024	0.024	108.00***
Sex	1	0.003	0.0003	1.333 NS
Species*Sex	1	0	0	0 NS
Error	8	0.002	0.0002	
Total	11	0.026		

Appendix Table 8: Analysis of variance for the weight of 10 days old and mature larvae of *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.008	0.008	42.15***
Age	1	12.619	12.619	63099.00***
Species*Age	1	0.013	0.013	67.35***
Error	8	0.002	0.0002	
Total	11			

Appendix Table 9: Analysis of variance for the weight of both sexes pupae of *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.145	0.1452	446.769***
Sex	1	0.004	0.005	14.769***
Species*Sex	1	0.001	0.0012	3.692 NS
Error	8	0.003	0.0003	
Total	11			

Appendix Table10: Analysis of variance for the weight of both sexes adults of *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.0243	0.0243	0.9241 ^{NS}
Sex	1	0.1541	0.1541	5.859 *
Species*Sex	1	0.0075	0.0075	0.285 ^{NS}
Error	8	0.2101	0.0263	
Total	11	0.3961		

Appendix Table 11: Analysis of variance for the larval periods in *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.09	0.09	0.020 ^{NS}
Replication	2	9.12	4.56	1.027 ^{NS}
Error	2	8.88	4.44	
Total	5	18.09		

Appendix Table 12: Analysis of variance for the pupal periods in *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	1.109	1.109	13.764 ***
Replication	2	0.159	0.079	0.990 ^{NS}
Error	2	0.161	0.081	
Total	5	1.430		

Appendix Table 13: Analysis of variance for the incubation periods in *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	0.522	0.522	549.63***
Replication	2	0.0007	0.0004	0.368 NS
Error	2	0.002	0.001	
Total	5	0.524		

Appendix Table 14: Analysis of variance for the fecundity in *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	10.140	10.140	289.714***
Replication	2	0.030	0.015	0.428 NS
Error	2	0.070	0.035	
Total	5	10.240		

Appendix Table 15: Analysis of variance for the hatching percentage in *T. castaneum* and *T. confusum.*

Source	DF	SS	MS	F values
Species	1	24.00	24.00	3.692 NS
Replication	2	21.00	10.50	1.615 NS
Error	2	13.00	6.50	
Total	5	58.00		

Appendix Table 16: Analysis of variance for the effect of gamma radiation on the head capsule of mature larvae of *T. castaneum*.

Source	DF	SS	MS	F values
Replication	2	0.130	0.065	3.823*
Doses	5	0.285	0.057	3.352*
Error	10	0.170	0.017	
Total	17	0.585		

Appendix Table 17: Analysis of variance for the effect of gamma radiation on the length of mature larvae of *T. castaneum*.

Source	DF	SS	MS	F values
Replication	2	0.043	0.022	0.523^{NS}
Doses	5	0.205	0.041	0.976^{NS}
Error	10	0.417	0.042	
Total	17	0.665		

Appendix Table 18: Analysis of variance for the effect of gamma radiation on the weight of mature larvae of *T. castaneum*.

Source	DF	SS	MS	F values
Replication	2	0.0000123	0.0000062	10.000***
Doses	5	0.000057	0.000011	17.741***
Error	10	0.000062	0.00000062	
Total	17	0.000131		

Appendix Table 19: Analysis of variance for the effect of gamma radiation on the head capsule of mature larvae of *T. confusum*.

Source	DF	SS	MS	F values
Replication	2	0.1411	0.071	1.651 ^{NS}
Doses	5	0.589	0.118	2.744 ^{NS}
Error	10	0.426	0.043	
Total	17	1.1561		

Appendix Table 20: Analysis of variance for the effect of gamma radiation on the length of mature larvae of *T. confusum*

Source	DF	SS	MS	F values
Replication	2	0.063	0.032	0.643 ^{NS}
Doses	5	1.060	0.212	4.265***
Error	10	0.497	0.0497	
Total	17	1.620		

Appendix Table 21: Analysis of variance for the effect of gamma radiation on the weight of mature larvae of *T. confusum*.

Source	DF	SS	MS	F values
Replication	2	0.063	0.032	0.640 ^{NS}
Doses	5	1.060	0.212	4.240***
Error	10	0.497	0.050	
Total	17	1.620		

Appendix Table 22: Analysis of variance for the head capsule of male pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.00059	0.000295	0.585 ^{NS}
Doses	5	0.05137	0.010274	20.384***
Error	10	0.00504	0.000504	
Total	17	0.056996		

Appendix Table 23: Analysis of variance for the length of male pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.063	0.032	0.484 ^{NS}
Doses	5	0.085	0.017	0.257 ^{NS}
Error	10	0.657	0.066	
Total	17	0.805		

Appendix Table 24: Analysis of variance for the weight of male pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.308	0.154	2.610 ^{NS}
Doses	5	0.198	0.041	0.694 ^{NS}
Error	10	0.586	0.059	
Total	17	1.091		

Appendix Table 25: Analysis of variance for the head capsule of male pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.000012	0.00006	7.500***
Doses	5	0.000028	0.0000056	0.700 NS
Error	10	0.000080	0.0000080	
Total	17	0.00012		

Appendix Table 26: Analysis of variance for the length of male pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.070	0.035	0.339 NS
Doses	5	0.105	0.021	0.203 NS
Error	10	1.030	0.103	
Total	17	1.205		

Appendix Table 27: Analysis of variance for the weight of male pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.203	0.102	1.062 NS
Doses	5	0.120	0.024	0.250 NS
Error	10	0.957	0.096	
Total	17	1.280		

Appendix Table 28: Analysis of variance for the head capsule of female pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.000022	0.000011	2.244 ^{NS}
Doses	5	0.0000338	0.0000068	1.387 ^{NS}
Error	10	0.000049	0.0000049	
Total	17	0.000104		

Appendix Table 29: Analysis of variance for the length of female pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.2100	0.105	1.842 ^{NS}
Doses	5	0.22000	0.044	0.771 ^{NS}
Error	10	0.5700	0.057	
Total	17	1.0000		

Appendix Table 30. Analysis of variance for the weight of female pupae of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0400	0.0200	0.041 ^{NS}
Doses	5	0.7450	0.1490	0.310 ^{NS}
Error	10	0.4800	0.480	
Total	17	1.2650		

Appendix Table 31: Analysis of variance for the head capsule of female pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0000144	0.0000072	0.163 ^{NS}
Doses	5	0.0001804	0.0000361	0.820 ^{NS}
Error	10	0.000437	0.000044	
Total	17	0.0006314		

Appendix Table 32: Analysis of variance for the length of female pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0633	0.0317	0.530 ^{NS}
Doses	5	0.1200	0.0240	0.402 ^{NS}
Error	10	0.5967	0.0597	
Total	17	0.7800		

Appendix Table 33: Analysis of variance for the weight of female pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.1033	0.0517	0.763 ^{NS}
Doses	5	0.0850	0.0170	0.251 ^{NS}
Error	10	0.6767	0.0677	
Total	17	0.8650		

Appendix Table 34: Analysis of variance for the head capsule of male adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0000303	0.0000152	1.583 ^{NS}
Doses	5	0.000118	0.0000236	2.458 ^{NS}
Error	10	0.0000957	0.0000096	
Total	17	0.0002440		

Appendix Table 35: Analysis of variance for the length of male adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0078	0.0039	0.122 ^{NS}
Doses	5	0.1778	0.0356	1.115 ^{NS}
Error	10	0.3189	0.0319	
Total	17	0.5044		

Appendix Table 36: Analysis of variance for the weight of male adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.7811	0.3906	7.153 ***
Doses	5	2.1294	0.4259	7.800 ***
Error	10	0.5456	0.0546	
Total	17	3.4561		

Appendix Table 37: Analysis of variance for the head capsule of adults pupae of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.000278	0.000139	0.278 ^{NS}
Doses	5	0.005511	0.001102	2.208 ^{NS}
Error	10	0.004989	0.000499	
Total	17	0.010778		

Appendix Table 38: Analysis of variance for the length of male adults of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.1733	0.0867	1.939 ^{NS}
Doses	5	0.3250	0.0650	1.454 ^{NS}
Error	10	0.4467	0.0447	
Total	17	0.9450		

Appendix Table 39: Analysis of variance for the weight of male adults of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0100	0.0050	0.081 ^{NS}
Doses	5	0.3400	0.0680	1.114 ^{NS}
Error	10	0.6100	0.0610	
Total	17	0.9600		

Appendix Table 40: Analysis of variance for the head capsule of female adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.000233	0.000117	0.023 ^{NS}
Doses	5	0.006600	0.001320	0.265 ^{NS}
Error	10	0.004967	0.00497	
Total	17	0.011800		

Appendix Table 41: Analysis of variance for the length of female adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.0233	0.117	2.677 ^{NS}
Doses	5	0.1600	0.0320	0.732 ^{NS}
Error	10	0.4367	0.0437	
Total	17	0.6200		

Appendix Table 42: Analysis of variance for the weight of female adults of *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.120	0.060	0.454 ^{NS}
Doses	5	0.460	0.092	0.696 ^{NS}
Error	10	1.320	0.132	
Total	17	1.900		

Appendix Table 43: Analysis of variance for the head capsule of female adults of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.001900	0.000950	1.862 ^{NS}
Doses	5	0.004600	0.000920	1.803 ^{NS}
Error	10	0.005100	0.000510	
Total	17	0.011600		

Appendix Table 44: Analysis of variance for the length of female adults of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	0.1401	0.0701	0.946 ^{NS}
Doses	5	0.1583	0.0316	0.426 ^{NS}
Error	10	0.7407	0.0741	
Total	17	1.0390		

Appendix Table 45: Analysis of variance for the weight of female adults of *T. confusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	1.281	0.641	2.233 ^{NS}
Doses	5	1.064	0.213	0.742 ^{NS}
Error	10	2.866	0.287	
Total	17	5.211		

Appendix Table 46: Analysis of variance for the pupal formation in *T. castaneum* developing from irradiated early larvae.

Source	DF	SS	MS	F values
Replication	2	0.000033	0.000017	0.037 ^{NS}
Doses	5	0.007050	0.001410	3.085 *
Error	10	0.004567	0.000457	
Total	17	0.011650		

Appendix Table 47: Analysis of variance for the adults emergence in *T. castaneum* developing from irradiated early larvae.

Source	DF	SS	MS	F values
Replication	2	4.00	2.00	1.250 ^{NS}
Doses	5	21870.09	4374.02	2733.76 ***
Error	10	16.00	1.60	
Total	17	21890.09		

Appendix Table 48: Analysis of variance for the pupal formation in *T. cconfusum* developing from irradiated early larvae.

Source	DF	SS	MS	F values
Replication	2	8.33	4.17	2.355 ^{NS}
Doses	5	22191.76	4438.35	2507.54 ***
Error	10	17.67	1.77	
Total	17	22217.76		

Appendix Table 49: Analysis of variance for the adults emergence in *T. cconfusum* developing from irradiated early larvae.

Source	DF	SS	MS	F values
Replication	2	4.33	2.17	2.237 ^{NS}
Doses	5	23260.13	4652.03	4795.90 ***
Error	10	9.67	0.97	
Total	17	23274.13		

Appendix Table 50 : Analysis of variance for the pupal formation in *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	239.3	119.7	1.395 ^{NS}
Doses	5	24089.3	4817.9	56.192 ***
Error	10	858.2	85.8	
Total	17	25186.8		

Appendix Table 51: Analysis of variance for the adults emergence in *T. castaneum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	7.00	3.50	0.813 ^{NS}
Doses	5	32622.43	6524.49	1517.32 ***
Error	10	43.00	4.30	
Total	17	32672.43		

Appendix Table 52: Analysis of variance for the pupal formation in *T. cconfusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	3.57	1.78	1.022 ^{NS}
Doses	5	27611.17	5522.23	3173.69 ***
Error	10	17.44	1.74	
Total	17	27632.18		

Appendix Table 53: Analysis of variance for the adults emergence in *T. cconfusum* developing from irradiated mature larvae.

Source	DF	SS	MS	F values
Replication	2	4.33	2.17	2.237 ^{NS}
Doses	5	23260.13	4652.03	4795.90 ***
Error	10	9.67	0.97	
Total	17	23274.13		

Appendix Table 54: Analysis of variance for the male adult emergence in *T. castaneum* resulting from irradiated early pupae.

Source	DF	SS	MS	F values
Replication	2	1.33	0.67	0.193 ^{NS}
Doses	5	31757.30	6351.46	1830.39 ***
Error	10	34.67	3.47	
Total	17	31793.30		

Appendix Table 55: Analysis of variance for the female adult emergence in *T. castaneum* resulting from irradiated early pupae.

Source	DF	SS	MS	F values
Replication	2	12.33	6.17	2.603 ^{NS}
Doses	5	32290.50	6458.10	2724.93 ***
Error	10	23.67	2.37	
Total	17	32326.50		

Appendix Table 56: Analysis of variance for the male adult emergence in *T. confusum* resulting from irradiated early pupae.

Source	DF	SS	MS	F values
Replication	2	29.4	14.7	0.595 ^{NS}
Doses	5	26792.4	5358.5	216.94 ***
Error	10	246.8	24.70	
Total	17	27068.6		

Appendix Table 57: Analysis of variance for the female adult emergence in *T. confusum* resulting from irradiated early pupae.

Source	DF	SS	MS	F values
Replication	2	22.33	11.17	2.160 ^{NS}
Doses	5	29891.39	5978.28	1156.34 ***
Error	10	51.67	5.17	
Total	17	29965.39		

Appendix Table 58: Analysis of variance for the male adult emergence in *T. castaneum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	9.00	4.50	2.142 ^{NS}
Doses	5	28164.16	5632.83	2682.30 ***
Error	10	21.00	2.10	
Total	17	28194.16		

Appendix Table 59: Analysis of variance for the female adult emergence in *T. castaneum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	13.00	6.50	1.756 ^{NS}
Doses	5	24565.00	4913.00	1327.83 ***
Error	10	37.00	3.70	
Total	17	24615.00		

Appendix Table 60: Analysis of variance for the male adult emergence in *T. confusum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	7.00	3.50	1.129 ^{NS}
Doses	5	22572.96	4514.59	1456.31 ***
Error	10	31.00	3.10	
Total	17	22610.96		

Appendix Table 60: Analysis of variance for the female adult emergence in *T. confusum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	7.00	3.50	1.060 ^{NS}
Doses	5	26920.54	5384.11	1631.54 ***
Error	10	30.00	3.30	
Total	17	26960.54		

Appendix Table 61: Analysis of variance for the female adult emergence in *T. confusum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	7.00	3.50	1.060 ^{NS}
Doses	5	26920.54	5384.11	1631.54 ***
Error	10	30.00	3.30	
Total	17	26960.54		

Appendix Table 62: Analysis of variance for the female adult emergence in *T. confusum* resulting from irradiated late pupae.

Source	DF	SS	MS	F values
Replication	2	2.33	1.17	0.493 ^{NS}
Doses	5	26731.77	5346.35	2255.84 ***
Error	10	23.67	2.37	
Total	17	26757.77		

Appendix Table 63: Probit analyses for the mortality of early eggs (2day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	6	10	10	3.72	3.077	4.43	7.86	3.065
2	0.3010	60	9	15	15	3.96	4.323	4.01	31.92	4.328
3	0.4771	60	22	36.67	37	4.67	5.052	4.68	38.22	5.067
4	0.6021	60	43	71.67	72	5.58	5.570	5.56	34.86	5.591
5	0.6910	60	59	98.33	98	7.05	5.970	6.55	28.26	5.998

$Y = 3.065407 + 4.196366\ X$
$LD_{50} = 0.643$
$\chi^2 = 32.53174$ (3 df)

Fiducial Limits
Upper =1.139
Lower =0.452

Appendix Table 65: Probit analyses for the mortality of early eggs (2 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	4	6.67	7	3.52	2.89	4.38	5.52	2.902
2	0.3010	60	10	16.67	17	4.05	4.27	4.05	30.18	4.277
3	0.4771	60	23	38.33	38	4.69	5.08	4.70	38.22	5.081
4	0.6021	60	46	76.67	77	5.74	5.65	5.73	33.48	5.651
5	0.6910	60	58	96.67	97	6.88	6.11	6.51	26.34	6.094

$Y = 2.902191 + 4.565864\ X$
$LD_{50} = 0.780$
$\chi^2 = 23.73116$ (3 df)

Fiducial Limits
Upper =2.254
Lower =0.379

Appendix Table 66: Probit analyses for the mortality of late eggs (5day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	1	1.66	2	2.95	2.11	5.15	1.14	2.065
2	0.3010	60	6	10	10	3.72	3.72	3.72	20.16	3.696
3	0.4771	60	15	25	25	4.33	4.67	4.34	36.06	4.650
4	0.6021	60	37	61.67	62	5.31	5.34	5.29	36.96	5.327
5	0.6910	60	54	90	90	6.28	5.86	6.14	30.18	5.852

$Y = 2.065 + 5.418\ X$
$LD_{50} = 1.158$
$\chi^2 = 16.989$ (3 df)

Fiducial Limits
Upper = 2.913
Lower = 0.757

Appendix Table 67: Probit analyses for the mortality of late eggs (5day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	3	5	5	3.36	2.79	4.09	4.56	2.791
2	0.3010	60	8	13..33	13	3.87	4.07	3.87	26.34	4.073
3	0.4771	60	19	31.67	32	4.53	4.82	4.55	37.62	4.823
4	0.6021	60	40	66.67	67	5.44	5.36	5.42	36.96	5.355
5	0.6910	60	51	85	85	6.04	5.77	5.99	31.92	5.768

$Y = 2.791 + 4.257\ X$
$LD_{50} = 0.901$
$\chi^2 = 13.193$ (3 df)

Fiducial Limits
Upper = 4.257
Lower = 2.791

Appendix Table 68: Probit analyses for the mortality of early larvae (2 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	8	13.33	13	3.87	3.51	3.98	16.14	3.498
2	0.3010	60	20	33.33	33	4.56	5.13	4.57	38.04	5.112
3	0.4771	60	58	96.67	97	6.88	6.08	6.51	26.34	6.057
4	0.6021	60	59	98.33	98	7.05	6.75	6.93	12.48	6.726
5	0.6910	60	59	98.33	98	7.05	7.27	7.04	5.52	7.245

$Y = 3.497 + 5.361\ X$
$LD_{50} = 1.906$
$\chi^2 = 21.01888$ (3 df)

Fiducial Limits
Upper =2.426
Lower =1.497

Appendix Table 69: Probit analyses for the mortality of early larvae (2 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	1	1.67	2	2.95	1.72	13.03	.36	1.650
2	0.3010	60	6	10	10	3.72	4.28	3.81	30.18	4.268
3	0.4771	60	54	90	90	6.28	5.78	6.15	31.92	5.811
4	0.6021	60	59	98.33	98	7.05	6.85	7.04	10.80	6.886
5	0.6910	60	59	98.33	98	7.05	7.67	6.45	2.40	7.729

$Y = 1.650 + 8.697\ X$
$LD_{50} = 0.727$
$\chi^2 = 61.054$ (3 df)

Fiducial Limits
Upper = 1.823
Lower = 0.232

Appendix Table 70: Probit analyses for the mortality of mature larvae (16 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	39	65	65	5.39	5.35	5.37	36.06	5.327
2	0.3010	60	50	83.33	83	5.95	6.11	5.92	26.34	6.072
3	0.4771	60	57	95	95	6.64	6.54	6.61	16.14	6.51
4	0.6021	60	58	96.67	97	6.88	6.85	6.91	10.80	6.817
5	0.6910	60	59	98.33	98	7.05	7.09	7.03	7.86	7.05

$Y = 4.434 + 4.005\ X$
$LD_{50} = 3.383$
$\chi^2 = 1.284$ (3 df)

Fiducial Limits
Upper = 5.212
Lower = 1.579

Appendix Table 71: Probit analyses for the mortality of mature larvae (16 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	29	48.33	48	4.95	4.81	4.96	37.62	4.819
2	0.3010	60	39	65	65	5.39	5.79	5.33	31.92	5.784
3	0.4771	60	58	96.66	97	6.88	6.36	6.66	20.16	6.348
4	0.6021	60	59	98.33	98	7.05	6.77	6.93	12.48	6.748
5	0.6910	60	59	98.33	98	7.05	7.08	7.03	7.86	7.058

$Y = 4.657 + 3.011\ X$
$LD_{50} = 2.299$
$\chi^2 = 9.943$ (3 df)

Fiducial Limits
Upper = 5.938
Lower = 1.799

Appendix Table 72: Probit analyses for the mortality of early male pupae (2 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	16	26.67	27	4.39	4.41	4.39	33.48	4.401
2	0.3010	60	23	38.33	38	4.69	4.61	4.69	36.06	4.602
3	0.4771	60	23	38.33	38	4.69	4.72	4.69	36.96	4.721
4	0.6021	60	21	35	35	4.61	4.81	4.61	36.96	4.804
5	0.6910	60	30	50	50	5	4.86	5.02	37.62	4.869

$Y = 4.400 + 0.67022\ X$
$LD_{50} = 3.844$
$\chi^2 = 2.544$ (3 df)

Fiducial Limits
Upper = 23.294
Lower = 2.641

Appendix Table 73: Probit analyses for the mortality of early male pupae (2 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	7	11.67	12	3.82	3.74	3.84	20.16	3.737
2	0.3010	60	7	11.68	12	3.82	3.84	3.82	22.2	3.849
3	0.4771	60	6	10	10	3.72	3.898	3.72	22.2	3.914
4	0.6021	60	8	13.33	13	3.87	3.94		24.3	3.961
5	0.6910	60	12	20	20	4.16	3.97	4.27	24.3	3.997

$Y = 3.737 + 0.371\ X$
$LD_{50} = 3.319$
$\chi^2 = 2.222$ (3 df)

Fiducial Limits
Upper = 6.955
Lower = 1.237

Appendix Table 74: Probit analyses for the mortality of late male pupae (5 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	16	26.67	27	4.39	4.59	4.38	34.86	4.574
2	0.3010	60	23	38.33	38	4.69	4.51	4.72	33.48	4.507
3	0.4771	60	23	38.33	38	4.69	4.45	4.72	33.48	4.468
4	0.6021	60	18	30	30	4.48	4.41	4.48	33.48	4.440
5	0.6910	60	11	18.33	18	4.08	4.39	4.11	31.92	4.419

$Y = 4.574 + -0.223\ X$
$LD_{50} = 4.236$
$\chi^2 = 8.183$ (3 df)

Fiducial Limits
Upper = 4.246
Lower = 3.599

Appendix Table 75: Probit analyses for the mortality of late male pupae (5 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	7	11.67	12	3.82	3.74	3.84	20.16	3.737
2	0.3010	60	7	11.67	12	3.82	3.84	3.82	22.2	3.849
3	0.4771	60	6	10	10	3.72	3.91	3.72	22.2	3.914
4	0.6021	60	8	13.33	13	3.87	3.94		24.3	3.961
5	0.6910	60	12	20	20	4.16	3.97	4.22	24.3	3.997

$Y = 3.737 + 0.371\ X$
$LD_{50} = 4.319$
$\chi^2 = 2.254$(3 df)

Fiducial Limits
Upper = 8.955
Lower = 1.237

Appendix Table 76: Probit analyses for the mortality of early female pupae (2 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	9	15	15	3.96	3.93	3.97	24.3	3.931
2	0.3010	60	13	21.66	22	4.23	4.24	4.22	30.18	4.229
3	0.4771	60	16	26.67	27	4.39	4.41	4.39	33.48	4.404
4	0.6021	60	17	28.33	28	4.42	4.54	4.41	34.86	4.528
5	0.6910	60	24	40	40	4.75	4.63	4.74	36.06	4.624

$Y = 3.931 + 0.991 X$
$LD_{50} = 3.820$
$\chi^2 = 1.067$ (3 df)

Fiducial Limits
Upper = 7.889
Lower = 1.235

Appendix Table 77: Probit analyses for the mortality of early female pupae (2 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	11	18.33	18	4.08	4.02	4.08	26.34	4.006
2	0.3010	60	13	21.67	22	4.23	4.29	4.22	30.18	4.282
3	0.4771	60	16	26.67	27	4.39	4.45	4.39	33.48	4.444
4	0.6021	60	19	31.67	32	4.53	4.56	4.52	34.86	4.559
5	0.6910	60	24	40	40	4.75	4.65	4.74	36.06	4.647

$Y = 4.006 + 0.916 X$
$LD_{50} = 3.124$
$\chi^2 = 0.730$ (3 df)

Fiducial Limits
Upper = 5.965
Lower = 0.868

Appendix Table 78: Probit analyses for the mortality of late female pupae (5 day old) of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	11	18.33	18	4.08	4.09	4.07	26.34	4.093
2	0.3010	60	12	20	20	4.16	4.19	4.19	28.26	4.193
3	0.4771	60	15	25	25	4.33	4.25	4.33	30.18	4.251
4	0.6021	60	15	25	25	4.33	4.29	4.33	30.18	4.292
5	0.6910	60	14	23.33	23	4.26	4.33	4.27	31.92	4.324

$Y = 4.093 + 0.330 X$
$LD_{50} = 4.696$
$\chi^2 = 0.296$ (3 df)

Fiducial Limits
Upper = 8.096
Lower = 2.817

Appendix Table 79: Probit analyses for the mortality of late female pupae (5 day old) of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	13	21.67	22	4.23	4.43	4.24	33.48	4.419
2	0.3010	60	18	30	30	4.48	4.28	4.49	30.18	4.291
3	0.4771	60	18	30	30	4.48	4.19	4.55	28.26	4.215
4	0.6021	60	10	16.67	17	4.05	4.13	4.06	28.26	4.16
5	0.6910	60	8	13.33	13	3.87	4.08	3.87	26.34	4.121

$Y = 4.419 + 0.429\ X$
$LD_{50} = 4.059$
$\chi^2 = 7.371$ (3 df)

Fiducial Limits
Upper = 9.029
Lower = 1.320

Appendix Table 80: Probit analyses for the mortality of 9 day old male adults of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	3	5	5	3.36	3.47	3.36	14.28	3.544
2	0.3010	60	11	18.33	18	4.08	3.87	4.13	22.2	3.903
3	0.4771	60	11	18.33	18	4.08	4.10	4.09	28.26	4.113
4	0.6021	60	14	23.33	23	4.26	4.27	4.25	30.18	4.262
5	0.6910	60	15	25	25	4.33	4.41	4.33	31.92	4.378

$Y = 3.544 + 1.192\ X$
$LD_{50} = 10.628$
$\chi^2 = 1.692$ (3 df)

Fiducial Limits
Upper = 15.021
Lower = 5.070

Appendix Table 81: Probit analyses for the mortality of 9 day old male adults of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	16	26.67	27	4.39	4.42	4.39	33.48	4.429
2	0.3010	60	23	38.33	38	4.69	4.58	4.69	34.86	4.592
3	0.4771	60	20	33.33	33	4.56	4.67	4.67	36.06	4.66
4	0.6021	60	24	40	40	4.75	4.74	4.74	36.96	4.66
5	0.6910	60	25	41.67	42	4.80	4.79	4.79	36.96	4.78

$Y = 4.419 + 0.508\ X$
$LD_{50} = 8.852$
$\chi^2 = 0.925$ (3 df)

Fiducial Limits
Upper = 112.759
Lower = 3.065

Appendix Table 82: Probit analyses for the mortality of 16 day old male adults of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	16	26.67	27	4.39	4.32	4.39	31.92	4.319
2	0.3010	60	17	28.33	28	4.42	4.45		33.48	4.45
3	0.4771	60	15	25	25	4.33	4.53	4.33	34.86	4.527
4	0.6021	60	23	38.33	38	4.69	4.59	4.68	34.86	4.581
5	0.6910	60	23	38.33	38	4.69	4.63	4.69	36.06	4.623

$Y = 4.319 + 0.433 X$
$LD_{50} = 12.931$
$\chi^2 = 2.21014$ (3 df)

Fiducial Limits
Upper = 21.821
Lower = 9.858

Appendix Table 83: Probit analyses for the mortality of 16 day old male adults of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	17	28.33	28	4.42	4.40	4.42	33.48	4.395
2	0.3010	60	18	30	30	4.48	4.51	4.42	34.86	4.511
3	0.4771	60	21	35	35	4.61	4.59	4.61	34.86	4.578
4	0.6021	60	21	35	35	4.61	4.63	4.63	36.06	4.625
5	0.6910	60	23	38.33	38	4.69	4.67	4.69	36.06	4.662

$Y = 4.394 + 0.383 X$
$LD_{50} = 10.025$
$\chi^2 = 0.160$ (3 df)

Fiducial Limits
Upper = 17.516
Lower = 5.912

Appendix Table 84: Probit analyses for the mortality of 9 day old female adults of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	9	15	15	3.96	3.92	3.97	24..3	3.92
2	0.3010	60	13	21.67	22	4.23	4.20	4.25	28.26	4.197
3	0.4771	60	13	21.67	22	4.23	4.36	4.23	31.92	4.359
4	0.6021	60	15	25	25	4.33	4.47	4.33	33.48	4.473
5	0.6910	60	24	40	40	4.75	4.56	4.74	34.86	4.561

$Y = 3.922 + 0.913 X$
$LD_{50} = 8.122$
$\chi^2 = 2.411$ (3 df)

Fiducial Limits
Upper = 14.101
Lower = 5.765

Appendix Table 85: Probit analyses for the mortality of 9 day old female adults of *T. confusum* treated with gamma radiation.

Dose Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	13	21.67	22	4.23	4.21	4.25	28.26	4.209
2	0.3010	60	16	26.67	27	4.39	4.33	4.39	31.92	4.336
3	0.4771	60	14	23.33	23	4.26	4.41	4.27	33.48	4.411
4	0.6021	60	15	25	25	4.33	4.46	4.33	33.48	4.46
5	0.6910	60	23	38.33	38	4.69	4.51	4.68	34.86	4.504

Y = 4.208 + 0.423 X
LD_{50} = 7.101
χ^2 = 2.527 (3 df)

Fiducial Limits
Upper = 14.149
Lower = 5.581

Appendix Table 86: Probit analyses for the mortality of 16 day old female adults of *T. castaneum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	17	28..33	28	4.42	4.32	4.43	31.92	4.318
2	0.3010	60	13	21.67	22	4.23	4.37	4.23	31.92	4.375
3	0.4771	60	15	25	25	4.33	4.40	4.33	33.48	4.408
4	0.6021	60	17	28.33	28	4.42	4.43	4.42	33.48	4.431
5	0.6910	60	20	33.33	33	4.56	4.44	4.57	33.48	4.449

Y = 4.318 + 0.187 X
LD_{50} = 10.311
χ^2 = 1.696 (3 df)

Fiducial Limits
Upper = 28.574
Lower = 5.167

Appendix Table 87: Probit analyses for the mortality of 16 day old female adults of *T. confusum* treated with gamma radiation.

Dose (Krad)	Log Dose	Number	Kill	% Kill	Corrected % Kill	Emperical Probit	Expected Probit	Working Probit	Weight	Final Probit
0	-	60	-	-	-	-	-	-	-	-
1	0	60	11	18.33	18	4.08	4.03	4.08	26.34	4.023
2	0.3010	60	13	21.67	22	4.23	4.30	4.23	31.92	4.298
3	0.4771	60	17	28.33	28	4.42	4.46	4.42	33.48	4.458
4	0.6021	60	21	35	35	4.61	4.57	4.61	34.86	4.458
5	0.6910	60	23	38.33	38	4.69	4.66	4.87	36.06	4.661

Y = 4.023 + 0.911 X
LD_{50} = 9.790
χ^2 = 0.308 (3 df)

Fiducial Limits
Upper = 23.903
Lower = 6.612

Appendix Table 88: LT_{50} values, 95% confidence limits, regression and χ^2 values for *Tribolium* adults resulting from irradiated mature larvae

Species	Dose (krad)	Sex	LT_{50} (weeks)	95% conf. limits lower	upper	Regression Equation(Y)	χ^2 values
T. castaneum	1	Male	15.40	14.99	15.82	-4.49 + 7.99x	2.76
		Female	14.69	14.37	15.03	-6.21 + 9.60x	8.41
	2	Male	15.13	14.59	15.68	14.59 15.68	16.15
		Female	14.86	14.50	15.23	-6.09 + 9.46x	11.66
	3	Male	14.38	14.06	14.72	-5.56 + 9.12x	8.91
		Female	13.90	13.62	14.18	-6.74 + 10.27x	11.23
T. confusm	1	Male	14.68	14.30	15.07	-4.73 + 8.34x	5.57
		Female	14.55	14.21	14.89	-5.39 + 8.93x	5.19
	2	Male	14.51	13.39	15.63	-6.29 + 9.59x	17.41*
		Female	14.48	13.91	15.08	-4.74 + 8.39x	18.74*
	3	Male	13.40	12.89	13.93	-5.52 + 9.33x	20.27**
		Female	13.35	13.06	13.65	-5.68 + 9.49	10.61

*P < 0.05, **P < 0.01, ***P < 0.001

Appendix table 89: Analysis variance for the time course mortality of adults of *T.castaneum* developing from mature larvae.

Source	DF	SS	MS	F values
Sex	1	79	79	0.064^{NS}
Doses	3	23809	7936	6.395^{*}
Sex* Doses	3	11	3.90	0.003^{NS}
Error	192	238339	1241	
Total	199	262238		

Appendix table 90: Analysis variance for the time course mortality of adults of *T.confusum* developing from mature larvae.

Source	DF	SS	MS	F values
Sex	1	31	31	0.023 ^{NS}
Doses	3	30893	10298	7.600 ***
Sex* Doses	3	1	0.333	0.00 ^{NS}
Error	192	260147	1355	
Total	199	291072		

Appendix table 91: Analysis variance for the time course mortality of adults of *T.castaneum* and *T. confusum* developing from mature larvae.

Source	DF	SS	MS	F values	Probability
Species	1	2941	2941	2.28	0.132
Sex	1	105	105	0.08	0.776
Doses	3	54472	18157	14.10	0.000
Species*Sex	1	5	5	0.00	0.948
Species*Doses	3	231	77	0.06	0.981
Sex*Doses	3	3	13	0.00	1.00
Error	387	498495	1288		
Total	399	556252			

Appendix Table 92: Analysis variance for the fecundity in *T. castaneum* resulting from irradiated pupae

Source	DF	SS	MS	F values
Doses	3	120.00	40.00	7.619 ***
Crosses	2	30.50	15.25	2.905 ^{NS}
Doses* Crosses	6	15.50	2.58	0.491 ^{NS}
Error	24	126.00	5.25	
Total	35	292.00		

Appendix Table 93: Analysis variance for the fecundity in *T. confusum* resulting from irradiated pupae

Source	DF	SS	MS	F values
Doses	3	31.64	10.55	2.482 ^{NS}
Crosses	2	98.00	49.00	11.529 ***
Doses* Crosses	6	75.11	12.52	2.946 ^{NS}
Error	24	102.00	4.25	
Total	35	306.75		

Appendix Table 94: Analysis variance for the fecundity in *T. castaneum* irradiated as adults

Source	DF	SS	MS	F values
Doses	3	51.42	17.14	3.718 *
Crosses	2	6.22	3.11	0.675 ^{NS}
Doses* Crosses	6	3.33	0.56	0.121 ^{NS}
Error	24	110.67	4.61	
Total	35	171.64		

Appendix Table 95: Analysis variance for the fecundity in *T. confusum* irradiated as adults

Source	DF	SS	MS	F values
Doses	3	89.00	29.67	4.031 *
Crosses	2	24.06	12.03	1.635 ^{NS}
Doses* Crosses	6	14.83	2.47	0.336 ^{NS}
Error	24	176.67	7.36	
Total	35	304.56		

Appendix Table 96: Analysis variance for the hatchability in *T. castaneum* resulting from irradiated pupae

Source	DF	SS	MS	F values
Doses	3	35091.00	11697.00	3119.20 ***
Crosses	2	30.50	15.25	4.067 *
Doses* Crosses	6	37.50	6.25	1.667 ^{NS}
Error	24	90.00	3.75	
Total	35	35249.00		

Appendix Table 97: Analysis variance for the hatchability in *T. confusum* resulting from irradiated pupae

Source	DF	SS	MS	F values
Doses	3	36429.00	12143.00	3830.59***
Crosses	2	30.50	15.25	4.811***
Doses* Crosses	6	49.50	8.25	2.603*
Error	24	76.00	3.17	
Total	35	36585.00		

Appendix Table 98: Analysis variance for the hatchability in *T. castaneum* irradiated as adults

Source	DF	SS	MS	F values
Doses	3	39101.00	13033.67	2298.70***
Crosses	2	19.50	9.75	1.720NS
Doses* Crosses	6	20.50	3.42	0.603NS
Error	24	136.00	5.67	
Total	35	39277.00		

Appendix Table 99: Analysis variance for the hatchability in *T. confusum* irradiated as adults

Source	DF	SS	MS	F values
Doses	3	39930.70	13310.20	182.83***
Crosses	2	247.40	123.70	1.699NS
Doses* Crosses	6	489.50	81.60	1.121NS
Error	24	1746.70	72.80	
Total	35	42414.30		