SOME OBSERVATIONS ON THE BEHAVIOURAL PATTERN, FECUNDITY AND FERTILITY DUE TO SUB-LETHAL AND FRACTIONATED DOSES OF GAMMA-IRRADIATION ON THE RED COTTON BUG, DYSDERCUS KOENIGII F.

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G. JAYARAMAN

School of Environmental Sciences
Jawaharlal Nehru University
New Delhi-110067

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PREFACE

The research work embodied in this dissertation has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full for any other degree or diploma of any University.

G. JAYARAMAN.

ANJALI MOOKERJEE
Supervisor

B. BHATIA
Dean

School of Environmental Sciences
Jawaharlal Nehru University
New Mehrauli Road
New Delhi-110 067.

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CONTENTS

INTRODUCTION

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Insects that are harmful to animals and plants continue to pose one of the most serious problems in agriculture and human health (IAEA, 1968). Despite sophisticated equipments and continuous development of new techniques only a few species of harmful insects can be said to be controlled (Brown, 1951; Knipling, 1955; De Bach, 1964a; IAEA, 1963, 1964, 1968, 1970 & 1975). Insecticides have met with only limited success in pest eradication programmes (Muri, 1978). Resistance (Simon, 1958; Ohoshi & Simon, 1963; Brown, 1968, 1969) and deleterious environmental side-effects have limited the use of pesticides in insect control programmes (De Bach, 1951, 1960, 1964_a, 1964_c, 1974; Newson, 1967; Pimental, 1971; Moore, 1967; Huffaker, 1971a; Woods, 1974; Smith, 1978). And, thus, researchers have become increasingly aware of the potential of insect population control by alternative means (Turnipseed et al, 1975; Wilkinson et al, 1975).

Isotopes and radiation are becoming increasingly important in entomology and the growth of information on their manifold uses represent an important step towards non-chemical pest management (IAEA, 1963, 1964, 1968, 1970, 1971 and 1975). The successful eradication of the screw worm (Choclomiya hominivorax) (Baumhover et al., 1955;

Anonymous, 1973b) by mass release of radio-sterilized males has demonstrated the great potential of radiation entomology in insect pest management. Serious drawbacks, however, have often limited the successful utilization of this approach (Lachance et al.,1967). Males are usually sterilized by the induction of dominant lethal mutations in sperms by high doses of ionising radiation. In some species radiation sickness, less competitiveness, impairment of deveolpmental processes, fertility regain and somatic damage are encountered (Denell 1973).

Hence, current research is underway on several aspects of radiation entomology which are hindrance to the radio - sterile technique.

Insects provide a good system for the estimation of hereditary risks from radiation. Body malformations due to radiation (Newcombe & Mc.Gregor 1967, 1972), and genetic & non-genetic effects of ionising radiation on insects are extensively studied and these have been adequately documented by several workers (Grosch 1962, O'Brein & Wolfe 1964, and Lachance 1967). Although there is considerable information on the radiosensitivity of the various nymphal instars of the holometabolous insects, little is known of the sensitivity of the various nymphal instars of the hemimetabolous insects where the metamorphosis is gradual from instar to instar (Harwalkar & Nair, 1968).

In the present study an approach has been made with dual interest in understanding radio sensitivity of different stages together with body malformations and impairment of reproduction by radiation in understanding and elucidating different effects of radiation on the test system Dysdercus koenigii F. (Heteroptera: Pyrrhocoridae). It is commonly known as red cotton bug or cotton stainer. It has a wide distribution in India, Africa and in all other cotton growing regions in the world (Crowe, 1977). It is a severe pest on cotton in major cotton growing countires feeding on the bolls or cell-sap of cotton plants (Srivastava and Bahadur, 1958; Simon, 1965; Frohlich and Rodewald, 1969; FIU, Ministry of Agriculture, India, 1973; Rens, 1975; Atwal, 1976; Crowe, 1977).

In India they are reported attacking cotton bolls (Khan and Rao, 1960; Sohi, 1964). As early as 1926, it was noted that they spread a disease called "Stigmatomycosis" which is commonly known as "internal boll disease of cotton" (Ashby and Nowell 1926; Carter, 1962). Later studies revealed that this disease is caused by a pathogenic fungus, Nematospora gossypii which is inoculated by the infected bugs when they feed on the bolls (Clarke and Wilde, 1970a,b). This fungus stains the cotton lint.

The staining is also due to the excreta of the bugs and the body fluid of them as they get crushed in the ginning factories (Atwal, 1976). The weight, germinating capacity and oil content of the cottenseeds were reported to be reduced. Further, seed cotton with stained lint commands only half the price of the clear cotton (Crowe, 1977).

In addition to their economic impact on cotton growing industry, reports on their resistance to organic insecticides (Simon, 1958; Ohoshi and Simon, 1963) have necessiated for search of alternative non-chemical way of their control.

Information regarding varied physiological aspects, especially about neuroendocrines of Dysdercus spp. are accumulating in the recent years (Jalaja and Prabhu, 1976a, 1976b, 1977; Prabhu and John, 1975a, 1975b; Prabhu et al., 1973; Joseph and Prabhu, 1977; Geering and Coaker, 1960; Jalaja, 1974; Jalaja et al., 1973, 1976; Tiwari and Srivastava, 1975; Gopakumar et al., 1977; Brunt, 1971; Muraleedharan and Prabhu, 1978). Reduced fertility was recorded when D. koenigii nymphs were treated with compounds like cyclic AMP, prostaglandings, acetic acid and U 7118 (Datta and Banerjee, 1978). Acorus calamus oil vapours have been shown to reduce adult male fertility

(Saxena and Mathur, 1976; Koul et al., 1977). A preliminary study on the female sex pheromone which can be used for biological control of this bug has been reported (Osmani and Naidu, 1967). But no further progress has been published. Use of chemo-sterilants and juvenile hormones have been tried on various species of this insect (Saxena and Williams, 1966; Bransby and Williams, 1971, Critchley and Campion, 1971; Harwalkar and Rahalkar, 1975; Rens, 1975).

Information on the effect of radiation on D. Koenigii is scanty. Only two reports, preliminary by nature, are available in this regard. Harwalkar and Nair (1968) have studied only the effect of X-irradiation on the moulting and metamorphosis of D. Koenigii. It was shown in a Peruvian species of this bug (D. Peruvianus) that fertility is reduced by irradiation (Simon, 1968). Besides the above mentioned preliminary studies, no concrete information regarding radiation effects is available on this economically important insect.

Hence, in the present study investigations on the life cycle under the culture conditions provided during this experiment, mass rearing techniques, reproductive behaviour, and radiosensitivity of different stages of

D. Koenigii are pursued. The fecundity and fertility, mating behaviour and competitiveness of males irradiated with sub-lethal doses have also been tested.

MATERIALS AND METHODS

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I. Culture methods for mass-rearing:

(i) Diet:

(a) Germinated cotton seeds: Commercially procured cotton seeds were washed thoroughly and soaked in water for 24-36 hrs to germinate. Then these were fed to the cotton bugs (Srivastava and Bahadur, 1958; Harwalkar and Rahalkar, 1975).

(b) Soaked cotton seeds (non-germinated): Thoroughly washed cotton seeds were soaked overnight in water and fed to the bugs (Geering and Coaker, 1960: Prabhu and John, 1975a; Jalaja and Prabhu, 1976a, 1977).

(c) Minced cotton seeds (dry): Thoroughly washed and dried cotton seeds were manually minced and used as diet for rearing this red cotton bug.

(d) Minced cotton seeds + 10% Sucrose solution: Cotton seeds minced as mentioned above were mixed with 10% sucrose solution and kept in a petridish. 5ml of 10% sucrose solution was added to 10 grams of minced cotton seeds. The insects were allowed to feed on them. This diet was used throughout the experiments in later stages.

(ii) Containers:

(a) Inverted cone shaped transparent plastic containers

(Plastella No.2, 3, 4, & 5, Plastella (India), Bombay,92) were used.

Container No.5 was found to be suitable for mass stock culture. No.4 containers were used for rearing a stock of a particular age group of insects. Separate maintaenance of irradiated insects and mating behavioural studies of test insects were done with container No.3. The smallest containers (No.2) were used for incubating the eggs laid.

(b)Lining the bottom of the container: Lining the bottom of the container with suitable base material to simulate the moist soil environment is necessary for the bugs. Four lining materials were tried: 1) Whatman No.1 filter papers (Rens, 1975) 2) moist sand (Harwalkar and Rahalkar, 1975); 3) wet filter paper above the wet cotton lining of 1 cm thickness; and 4) 1-1·5 cm thick wet synthetic sponge foam. The latter two methods were developed indigenously.

The used sand, filter paper and cotton were disposed as they could not be reused. The containers and the foam lining were washed with detergent and the foam was thoroughly rinsed in running tap water for whole night. But the lining foam was reused.

The containers were covered with a piece of green net cloth.

(iii) Temperature and Relative Humidity (RH %):

The temperature of the culture cabin was maintained through out the year at 28 $\pm$ 2 ^{o}C by conditioning the temperature of the air.

The RH was maintained as follows. When the RH outside the cabin was low, the humidity was raised inside the culture cabin by keeping a trough of water with a surface area of 135 cm^2. The water evaporates according to the RH of the incoming air. Further, the RH inside the container was raised by the slow release of moisture by evaporation from the lining material. The day time RH of ambient air outside the culture cabin, culture cabin and inside culture container were recorded all through the experimental period (Table III).

(iv) Photoperiod:

The insects were reared under the illumination pattern of around 12 L and 12 D hours.

(v) Study on Fecundity, & Fertility under different diet conditions:

To ensure the bearing of different diets, recommended in literature and developed by us, it was felt worthy to record the number of eggs laid (fecundity) per female and number of eggs hatched out (fertility) from the eggs laid when they were reared under different diets. Eggs from 30 females reared under the specific diet conditions were scored and fecundity and fertility ratio was studied (Table 15).

(vi) Life cycle and Life span studies:

As a modified diet (minced cotton seeds and 10% sucrose solution) was used in the rest of the experiments and to choose a particular phase of the life cycle for irradiation it was felt necessary to study the periods of different stages and their morphological changes with which their phase in life cycle can easily be identified. One hundred eggs and fifty insects of each instar were used. The periods of different stages of the life cycle are shown in Table II.

(vii) Test for monogamy and/or polygamy:

Ten virgin females and ten virgin males were used to study their mating behaviour, i.e., monogamy and/or polygamy. Each pair (1 male and 1 female) of the experimental group were introduced in a container and allowed to mate. After first mating the pairs were separated and to each male insect a virgin female was introduced again for mating. To each female which mated

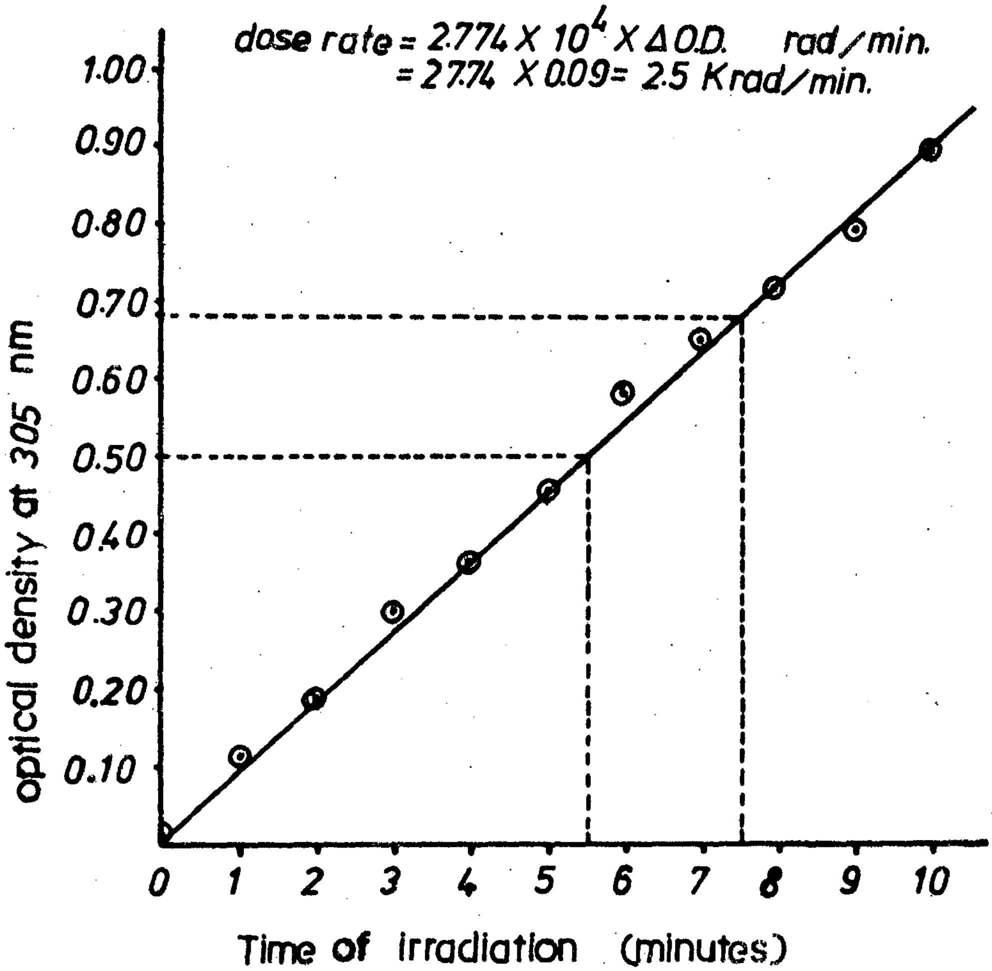


Fig. I Determination of dose-rate of the Co^{60} gamma source by actinometry (Fricke - ferrous sulphate method).

for once 2 virgin males were introduced as a means of forced mating because the females showed evasive behaviour for 2nd mating. As they were confined in a container, ultimately, they were forced to accept one of the vigorous virgin males introduced. And for third mating, males mated for 2nd time were taken and paired with third set of virgin females. Either the females did not survive for the third mating test or those which survived did not mate. The rate of fecundity and fertility were recorded at each phase of mating (Table IV).

(viii) Irradiation procedures:

In the present study irradiation was given by a Co^{60} Gamma chamber 4000 (capcity 5000 C_i) from Isotope Division, B.A.R.C., India. The dose rate was determined by actinometry (Fricke ferrous sulphate) and was found to be 2·85 - 2·53 Krad per min (Fig.1) over a period of these experiment. In different sets of experiments a dose range of 1-20 Krad was used and the exposure time varied from 21 to 420 seconds. For lower doses lead sheild was used for lowering dose rate. Irradiation to elucidate the radio-sensitivity and lethal dose-50% (LD_{50}) of fourth and fifth instar male nymphs was done as per Lee et al., (1968) and Wiend et al., (1975). The insects were collected from the stock culture whose stage of life cycle was known, and were confined in test tubes plugged with cotton and irradiated. After irradiation, the nymphs were returned back to the culture cabin and confined in separate containers. Mortality and morphological abnormalities were recorded daily until they metamorphosed into adults. Non-irradiated nymphs of the same age and number were used as controls in all experiments.

(a) Early fourth instar nymphs: These nymphs (0-1 day after moulting), each experimental lot consisting of 30 insects, were irradiated with 1, 2, 4, 6, 8, 10 and 12 Krad.

(b) Late fourth instar nymphs: These nymphs (5-6 days after moulting), each experimental lot consisting of 30 insects, were irradiated with 1, 2, 4, 6, 8, 10 and 12 Krad.

(c) Early fifth instar nymphs: These nymphs (0-1 day after moulting), each experimental lot consisting of 30 insects, were irradiated with 1, 2, 3, 4, 6, 8, 10 and 12 Krad.

(d) Late fifth instar nymphs: These nymphs (5-6 days after moulting), were irradiated with 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 Krad.

(e) Derivations of radio-sensitivity: Dose effect curves were established for fourth and fifth instars for both early and late stages of the phases with doses ranging from 1-20 Krad. The dose-effect curve, to establish the best linear regression line through a variety of test point data (in X and Y coordinates) was calculated (Texas Instruments Inc., 1977).

The following formula was used to find out the slope (m) of the best fit line:

$$m = \frac{\dfrac{\sum_{i=1}^{n} x_i y_i}{n} - \bar{X}\bar{Y}}{\sigma X^2}$$

where, m=the slope of the line
n=no. of observations
σ= Standard Deviation.

The value (b) for Y intrcept in Y coordinate was calculated by

$$b = \bar{Y} - m\bar{X}$$

b = 'Y' intersecept

LD_{50} values on the Y intercept were predicted by using the equation

$$X = \frac{Y - b}{m}$$

The accuracy of the LD_{50} value prediction was determined by finding out correlation coefficient (r). The absolute value of (r) is 1. A value close to 1 indicates a high correlation or indicates how well the data points correspond or correlate to the line drawn.

The formula for (r^2) is:

$$r^2 = \frac{m^2 \sigma_x^2}{\sigma_y^2}$$

From this (r) can be calculated.

(ix) Radiation induced sterility in males: Late fifth instar male nymphs, each experimental batch consisting of 30 insects, were irradiated with 1, 2, 3, 4, 5 and 6 Krad as these are found to be sub-lethal doses. When these nymphs metamorphosed into adults, each one of them were confined with a virgin female and allowed to pair. After mating, eggs scored per female (fecundity) and eggs hatched per group of eggs (fertility) were taken as index of radiation induced impairment on reproduction. Fertility and fecundity of untreated insects were taken as control.

(x) Mating competitiveness of irradiated male bugs: Late fifth instar male nymphs, each batch consisting of ten insects, were irradiated at 1, 2, 3, 4, 5 and 6 Krad. Control insects were not irradiated. The competitiveness of males was measured as in standard competition tests widely used for measuring vigour (sterile males: untreated males: untreated females = 1 : 1 : 1).

Eggs deposited were scored, counted and the percentage of hatching as related to the competitiveness of mating in the treated males, were recorded.

The competitiveness of the treated males was calculated in the following way (Haisch 1970).

$$e = \frac{q - f}{n(f-p)}$$

Where, e = competitiveness;

q = hatching rate (%) of eggs of control group;

p = hatching rate (%) of eggs of a group consisting of normal females and irradiated males;

f = hatching rate (%) of eggs of a group consisting of normal pairs and irradiated males; and

n = number of irradiated males/number of normal males.

(xi) Mating behaviour: In another set of experiments, the insects were irradiated as mentioned above and they were marked with white camlin water paint on the ventral side of the abdomen in between the hind legs. Each batch (ten insects per batch), of these treated and marked insects were combined with 10 untreated males and 10 untreated females. The mating behaviour of treated males compared to normal males was observed.

RESULTS

RESULTS

I. Culture methods:

Soaking cotton seeds for 36 hrs in water for germination was found necessary. Drying-off of tender hypocotyl of germinating seedlings within 12 hrs was observed. The food was not found to serve for more than a day. Problems of fungal contamination and foul smell emission were encountered while using soaked cotton seeds. Minced cotton seeds did not pose the above mentioned problems. The food served for 3 days. The diet consisting of minced cotton seeds and 10% sucrose solution stood serving for 2 days. Fungal growth in the medium became prominent during third day.

The egg yield per female and their hatchability, when the insects were reared under different diets, are shown in Table I. The fecundity was found to be significantly higher in females reared on a diet consisting of minced cotton seeds with 10% sucrose solution and it was found to be significantly low when they were fed on soaked cotton seeds (Table I). As far as fertility is concerned, excepting the diet consisting of soaked cotton seeds, other three diets did not show significant difference. The diet consisting of minced cotton seeds with 10% surose solution enhanced egg yield and thereby the number of insects in the progeny. Hence, this diet was chosen to be the best. The duration of each phase of the life cycle of this bug under this diet condition is shown in Table II. The males live longer than the females.

Normally the eggs, when laid, were pure white in colour. During the incubation period they turned to golden yellow and ultimately orange red. The first instar nymphs, immediately after hatching, were orange in colour and turned out to pure red in 18-24 hrs. The wing pads were absent until third instar and seen from fourth instar onwards. The length of the fourth instar was around 4-5 mm. The fifth instar grewn upto 8 mm. The pharate adults, both make and female, were pale in colour immediately after ecdysis. They became red in 10-18 hrs. The wings have 2 black spots. Males are 8-11 mm in length and females 10-12 mm. The sexual dimorphism can be made out right from the fourth instar onwards. The females are bigger and the end of the abdomen is round or oval while that of males are conical and elongated (PlateI).

The foam lining at the bottom of the culture container was found to retain more moisture for longer period. The RH outside the culture cabin was found to fluctuate between 21% and 77% through the experimental duration of 8 months. The variation inside the culture cabin was lesser (56-85%) as it was air conditioned. The RH was kept between 67-91% inside the culture containers (see Materials and Methods) (Table III). The required humidity is 70-90% (Sharma et al., 1975).

II. Test for Monogamy in Females:

The results of normal and/or force-mating are shown in Table IV. Females were found to be monogamous, i.e., mate only once in normal conditions. When force-mated, the fecundity was highly reduced (180 in normal mating to 36 in force mating). Males were found to be vigorous in mating though a slight decrease in their mating vigour was seen in consequent matings. Hence it is concluded that males are polygamous.

III. Radio-sensitivity:

Early 4th and 5th instar male nymphs were found to be more radio-sensitive than late fourth and fifth instar male nymphs (Tables V-VIII). The early phase of 5th instar was more or less equally radio-sensitive to the late fourth instar nymphs. Further, the results showed that radio-resistance was acquired during the course of development. Also, this is evident from the wing deformity records shown in Tables V-VIII. In the irradiated early instars, more abnormal wings were noticed when they moulted to adulthood in comparison to the later stages of the instars irradiated. Both the stages of 4th instar nymphs showed more wing abnormalities than the fifth ones when irradiated.

The results of the calculations for the slope of linear regression line (m) of Figs.II-V and correlation coefficient are given in Table IX. From this, the LD_{50} dose for early and late fourth instars were found to be 2·76 krad and 5·90 krad respectively. For early and late 5th instars the LD_{50} dose was 5·62 and 14·10 krad respectively (Table IX).

The mortality is linearly related to the dose, as the correlation coefficients of the data of Figs.II-V lie between 0·96 and 0·98.

IV. Radiation induced sterility in males:

The results are shown in Table X. Reduction in fecundity and fertility is taken as index to radiation induced male sterility. This was found to be dose dependent and reduced fertility was achieved at higher doses. No linear relationship was found between dosage and sterility (Fig VI). Decrease in fertility was drastic due to 1 krad irradiation which gave 37% decrease in fertility. Comparatively slow decrease in fertility was seen at doses higher than 1 krad i.e., between 2-3 krad (Fig VI). Then again at 4 krad there was a steepfall and at 6 krad dosage, residual fertility of 7·5% was recorded (Table X).

V. Mating behavious and Mating competitiveness:

In control insects mating took place about 2 days after metamorphosis. Males were noticed to be vigorous enough to mate 1 day after metamorphosis. No remarkable specific courting behaviour was noticed in both control and irradiated insects. No abnormal mating behaviour, except being inactive at higher doses, was noticed among irradiated males. Insects were noted to pair continuously for 48 hrs. Females laid, 7-16 hrs after the completion of mating, around 180 eggs in a single batch or in 1-6 batches.

The mating competitiveness of irradiated males were found to be decreased due to irradiation treatment (Table XI). But the decrease was low at lower doses, i.e., from 1-4 krad and a drastic decrease was noticed thereafter (5 and 6 krad; Fig VII). No significant decrease of 'vigour' was recorded between insects irradiated at 1 krad and 4 krad. Competitiveness was found to be minimum (0·13) at 6 krad.

Table I. Fecundity and Fertility of Dysdercus Koenigii reared on different diets. (Eggs from 30 females were scored under each condition; values represent mean ± S.D.*)

Diet	Fecundity	Fertility	Fertility (%)
A	145·47 ± 11·28	130·30 ± 11·14	89·57 ± 3·87
B	125·33 ± 11·93	105·77 ± 15·76	81·53 ± 8·28
C	158·90 ± 12·68	141·93 ± 12·81	89·31 ± 3·79
D	184·77 ± 18·94	165·57 ± 20·22	89·54 ± 3·54

A=Germinated cotton seeds

B=Soaked cotton seeds (non-germinated)

C=Minced cotton seeds (dry)

D=Minced cotton seeds + 10% sucrose solution

* S.D. = Standard deviation:

Table II. Duration of each phase of the life cycle of *Dysdercus koenigii* maintained under the culture conditions adopted in this course of experiments.

No. of eggs or insects	Phase of Life Cycle	Duration in days (mean ± S.D.)
100	Eggs to I instar nymphs	6·2 ± 2·79
50	I to II instar nymphs	4·8 ± 2·56
50	II to III instar nymphs	5·3 ± 2·44
50	III to IV instar nymphs	5·6 ± 1·50
50	IV to V instar nymphs	7·2 ± 1·32
50	V instar to Adult	6·3 ± 1·51
50	Adult male life span	9·5 ± 2·68
50	Adult female life span	7·8 ± 2·03

Egg to Adult stage = 35·4 ± 2·10 days

Table III. Record of relative humidity (RH %) maintained during the course of experiments. (Values represent record of day time RH only)

Month	Relative Humidity in % (Mean ± S. D.)		
	Outside the culture cabin	Inside the culture cabin	Inside the culture container
March	34·74 ± 2·18	56·10 ± 3·04	74·00 ± 3·99
April	29·30 ± 2·48	69·63 ± 3·39	77·40 ± 3·90
May	21·80 ± 1·75	62·97 ± 2·44	71·90 ± 3·35
June	30·40 ± 2·42	57·50 ± 2·95	67·10 ± 2·51
August	71·87 ± 7·57	75·90 ± 8·62	88·74 ± 1·66
Sept.	77·27 ± 3·47	85·93 ± 2·42	90·97 ± 1·20
Oct.	73·29 ± 2·99	74·35 ± 2·92	85·23 ± 3·97
Nov.	62·67 ± 3·00	72·53 ± 3·08	80·17 ± 2.27

Table IV. Test for monogamy and/or polygamy in adult virgin females and males of Dysdercus koenigii. Fecundity and fertility were recorded for normal and/or force matings.

Sex	No. of insects	I mating A	I mating B	II mating A	II mating B	III mating A	III mating B
Females	10	180·10 ±10·88	160·80 ±12·85	35·80 ±12·38	30·70 ±12·45	-	-
Males	10	180·10 ±10·88	160·80 ±12·85	172·60 ±22·77	156·00 ±21·54	162·60 ±16·44	119·10 ±14·22

A = Fecundity; B = Fertility.

I mating = normal for both the sexes.

II mating = Females : forced mating, i.e., individual females, after oviposition due to I mating, were confined with 2 virgin males in container for mating.
Males : normal mating. Individual males, after first mating, were confined with one virgin female each in container for mating.

III mating = Females : did not survive or those which survived did not mate.
Males : normal mating. Individuals, after second mating, were confined with one virgin female each in container for mating.

Table V. Mortality of early fourth instar nymphs of <u>Dysdercus koenigii</u> at given doses of Gamma-irradiation.

Dose (Krad)	No of insects studied	Insects moulted into fifth instar NO.	%	Insects died NO.	%	Insects metamorphosed into adults NO.	%	Insects died NO.	%
0	30	29	96·67	1	3·33	27	90·00	3	10·00
1	30	27	90·00	3	10·00	20	66·67	10	33·33
2	30	24	80·00	6	20·00	14	46·67	16	53·33
4	30	18	60·00	12	40·00	11	36·67	19	63·33
6	30	13	43·33	17	56·67	4	13·33	26	86·67
8	30	8	26·67	22	73·33	0	0·00	30	100·00
10	30	4	13·33	26	86·67	0	0·00	30	100·00
12	30	5	16·67	25	83·33	0	0·00	30	100·00

Wing deformities : 1 krad : 26% adults showed abnormal wings.
2 Krad : 45% adults showed abnormal wings.
4 krad onwards nearly 80% adults showed abnormal wings.

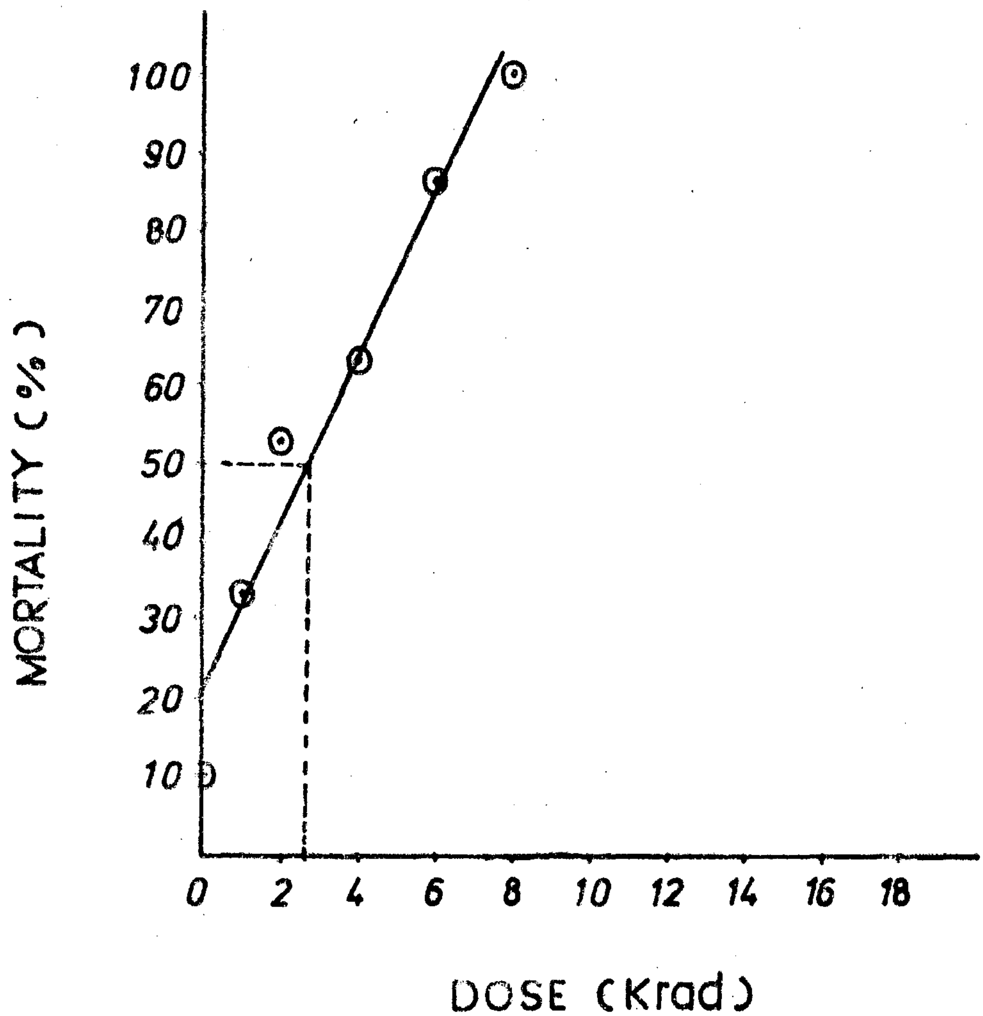


Fig. II LD_{50} gamma radiation dose for early fourth instar nymphs of D. koenigii (LD_{50} = 2.76 Krad).

Table VI. Mortality of late fourth instar nymphs of *Dysdercus koenigii* at given doses of Gamma-irradiation.

Dose (Krad)	No of insects studied	Insects moulted into fifth instar NO.	Insects moulted into fifth instar %	Insects died NO.	Insects died %	Insects metamorphosed into Adults NO.	Insects metamorphosed into Adults %	Insects died NO.	Insects died %
C	30	30	100·00	0	0·00	29	96·67	1	3·33
1	30	30	100·00	0	0·00	27	90·00	3	10·00
2	30	28	93·33	2	6·67	27	90·00	3	10·00
4	30	24	80·00	6	20·00	16	53·33	14	46·67
6	30	22	73· 00	8	26·67	12	40·00	18	60·00
8	30	18	60·00	12	40·00	13	43·33	17	56·67
10	30	13	43·33	17	56·67	6	20·00	24	80·00
12	30	5	16·67	25	83·33	0	0·00	30	100·00

Wing deformities : 2 krad : 20% adults showed wing deformities.
4 krad : 50% adults showed wing deformities.
6 krad and above : more than 50% adults showed wing deformities.

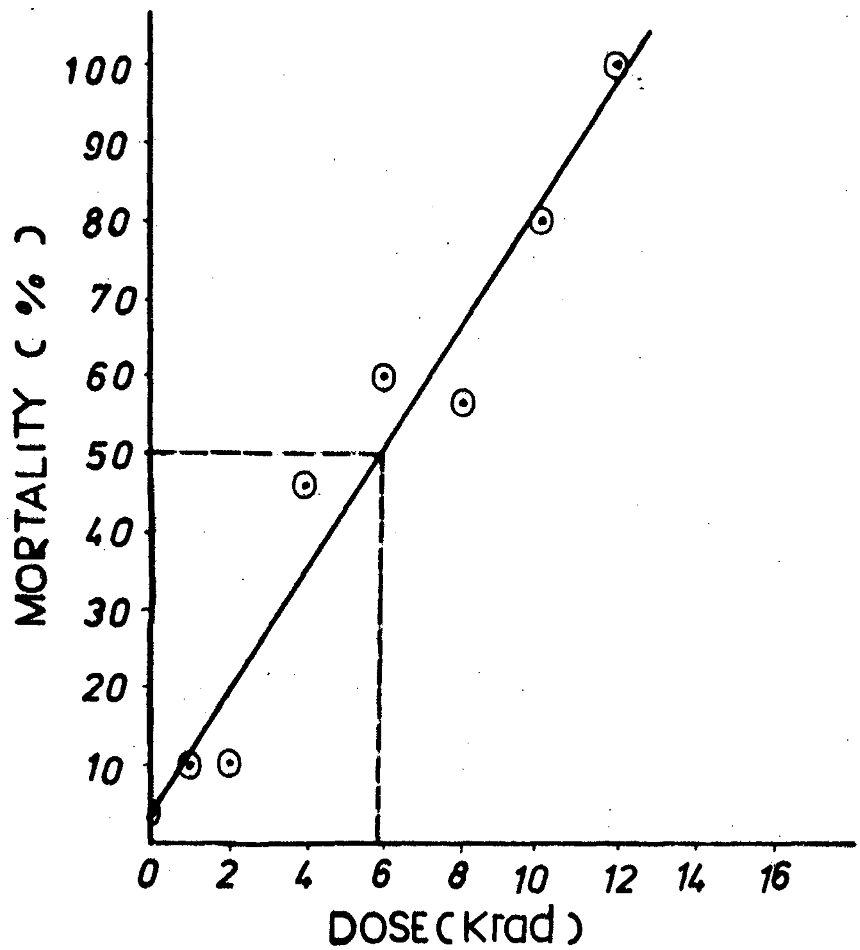


Fig. III LD_{50} gamma radiation dose for late fourth instar nymphs of D. koenigii (LD_{50} = 5.90 Krad).

Table VII. Mortality of early fifth instar nymphs of *Dysdercus koenigii* at given doses of Gamma-irradiation.

Dose (Krad)	No. of insects studied	Insects metamorphosed into adults		Insects died	
		No	%	No	%
0	30	30	100·00	0	0·00
1	30	27	90·00	3	10·00
2	25	25	83·33	5	16·67
3	30	21	70·00	9	30·00
4	30	17	56·67	13	43·33
6	30	12	40·00	18	60·00
8	30	7	23·33	23	76·67
10	30	4	13·33	26	86·67
12	30	3	10·00	27	90·00

Wing deformities:

1-3 Krad : 5% of adults.

4-6 Krad : 25-50% of adults

8 Krad : Above 50% of adults

10-12 Krad : All adults

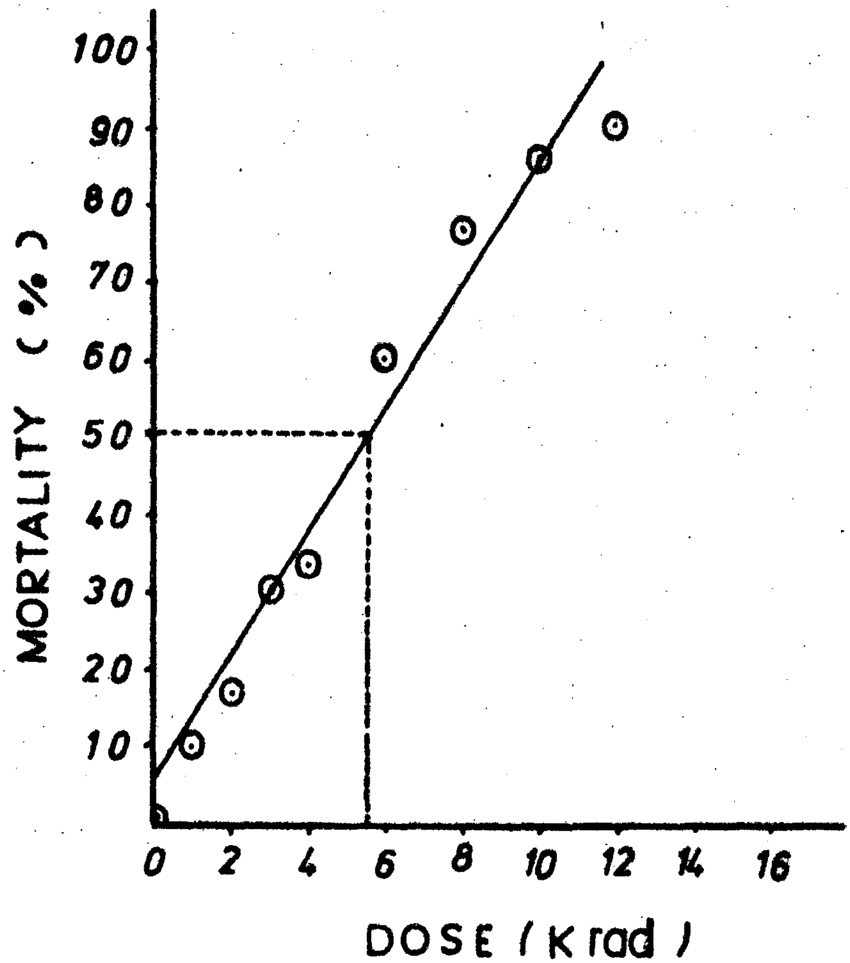


Fig. IV LD_{50} gamma radiation dose for early fifth instar nymphs of D. koenigii (LD_{50} = 5.62 Krad).

Table VIII. Mortality of late fifth instar nymphs of *Dysdercus koenigii* at given doses of Gamma-irradiation

Dose (Krad)	No.of Insects studied	Insects metamorphosed into adults NO.	%	Insects died NO.	%
C	45	44	97·78	1	2·22
1	15	14	93·33	1	6.67
2	15	14	93·33	1	6·67
4	15	12	80·00	3	20·00
6	15	13*	86·67	2	13·33
8	15	11**	73·33	4	26·67
10	15	12**	80·00	3	20·00
12	15	10**	66·67	5	33·33
14	15	6@	40.00	9	60·00
16	15	7@	46·67	8	53·33
18	15	4@	26·67	11	73·33
20	15	4@	26·67	11	73·33

* Nearly 10% adults showed wing deformities.

** Nearly 25-50% adults showed wing deformities.

@ Above 50% of adults showed wing deformities.

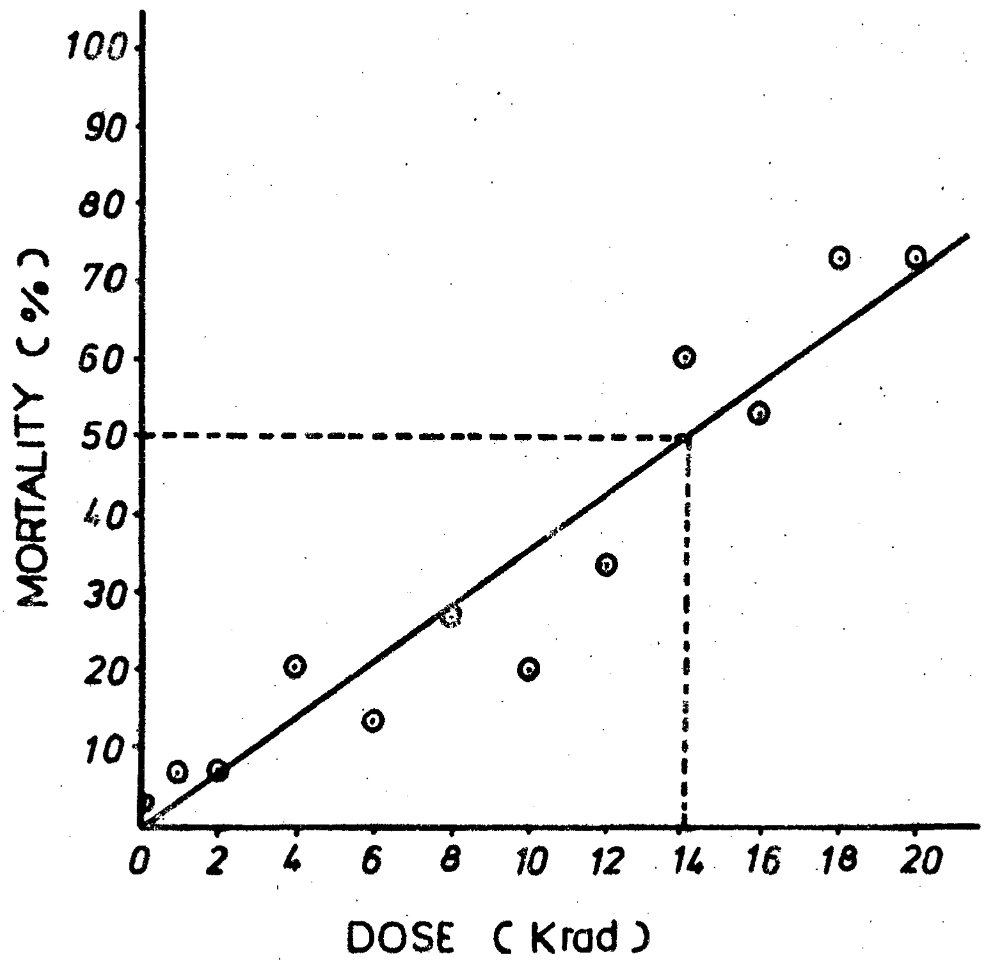


Fig. V LD_{50} gamma radiation dose for late fifth instar nymphs of D. koenigii (LD_{50} = 14.10 Krad).

Table IX. Values of the slope of linear regression line and its correlation coefficient for Figs. II-V and Tables V-VIII.

Table	Fig.	Slope in degrees (m)	Intercept (b)	Correlation coefficient (r)	LD50 (Krad)
V	II	84·6	20·93	0·974	2·76
VI	III	82·8	3·31	0·976	5·90
VII	IV	82·9	4·93	0·982	5·62
VIII	V	74·7	1·34	0·960	14·10

Table X. Radiation induced sterility in adult male *Dysdercus koenigii* (Irradiated virgin males were crossed individually with untreated virgin females. Reduction in fecundity and fertility of females were taken as index for induced male sterility).

Dose (Krad)	No.of insects	Fecundity (mean ± S.D.)	Fertility (mean ± S.D.)	Fertility %* (mean ± S.D.)
0	30	184·77±19·77	165·57±20·22	89·54±3·54
1	30	133·71±11·64	70·29± 5·92	52·62±2·27
2	30	105·37±10·19	48·83± 7·36	46·39±5·11
3	30	76·17±11·17	27·60± 5·67	36·19±4·73
4	30	52·13± 7·43	8·87± 3·23	16·77±5·10
5	30	22·27± 4·25	3·20± 1·47	14·16±5·24
6	30	17·97± 3·72	1·33± 1·14	7·48±6·40

*Male insects irradiated at late fifth instar stage.

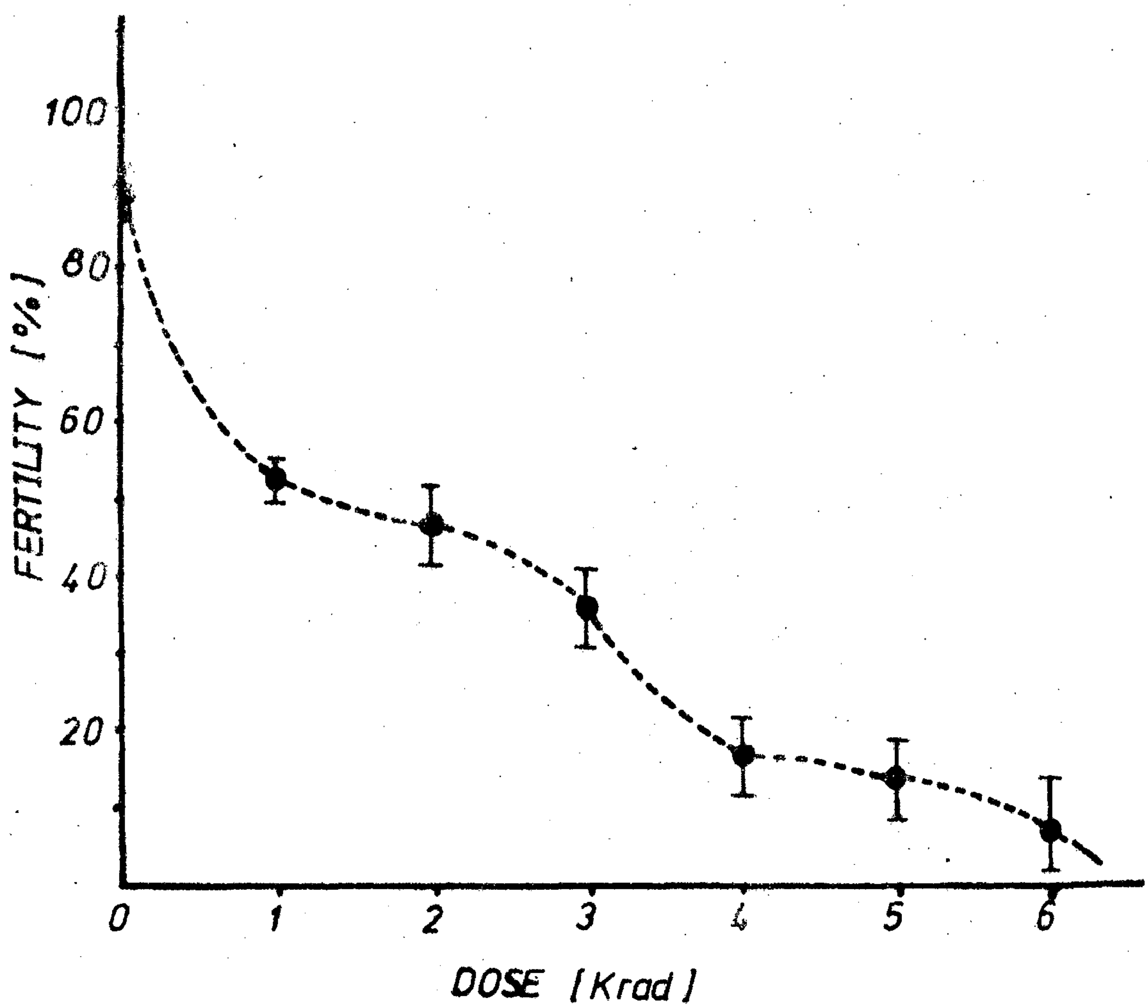


Fig. VI Influence of increasing gamma dose given to late fifth instar male nymphs of D. koenigii on egg hatch. The radiated nymphs were paired with normal females at 1:1 ratio when they attained adulthood. The hatchability of eggs laid by the females was taken as an index of radiation induced sterility in male bugs.

Table XI. Mating competitiveness of radiosterilized male *Dysdercus koenigii* (10 treated males : 10 untreated males : 10 untreated females; all virgins; males irradiated at late fifth instar nymphal stage).

Dose (Krad)	0	1	2	3	4	5	6
Fecundity	1647	1246	1113	1017	917	1192	1206
Fertility	1497	896	781	669	522	812	982
Recorded hatch-rate(%)	90·89	72·91	70·17	65·78	56·90	68·12	81·22
Expected hatch-rate(%)	100	50	50	50	50	50	50
Competitiveness	1·00	0·89	0·87	0·85	0·85	0·42	0·13

*Competitiveness is calculated by formula af Haisch (1970,1971).

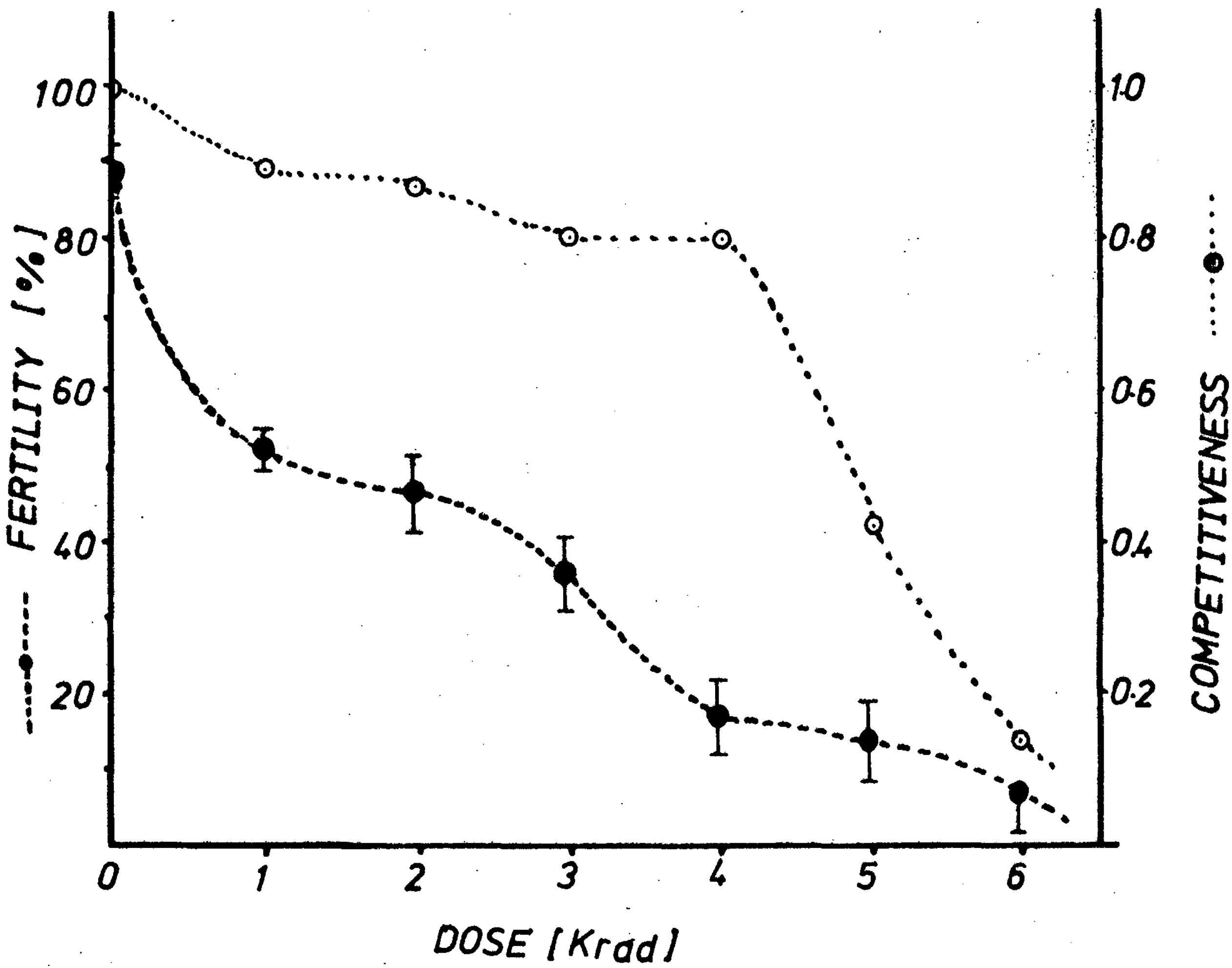


Fig. VII Influence of radiation induced sterility on the mating competitiveness of male D. koenigii. Competitiveness was calculated according to Haisch (1970).

PLATE - I

(a) A normal female adult Dysdercus koenigii.

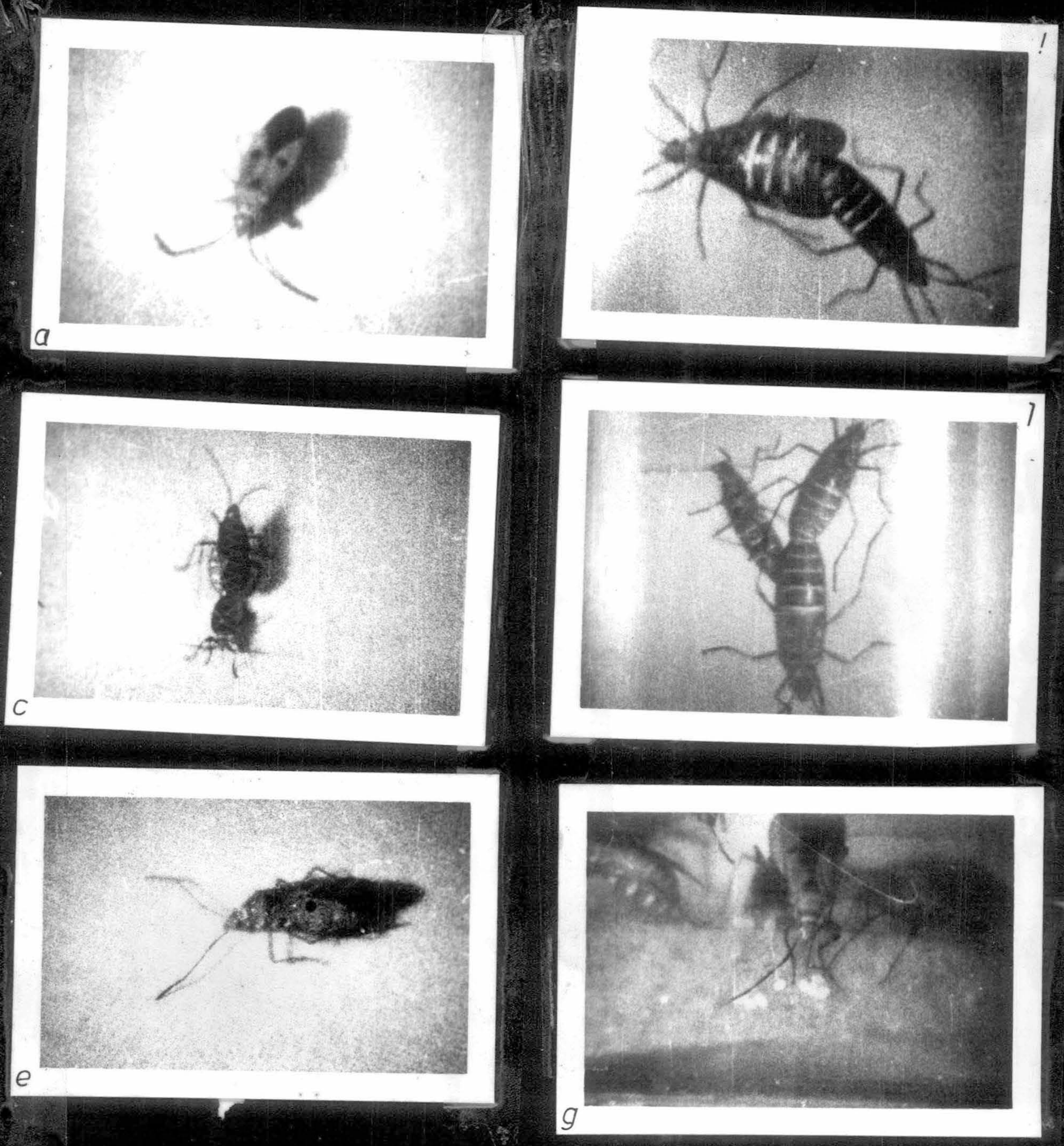
(b) A normal male (adult) Dysdercus koenigii.

(c) Early phase of mating.

(d) Late phase of mating.

(e) Gravid female after mating.

(f) A batch of Dysdercus koenigii eggs.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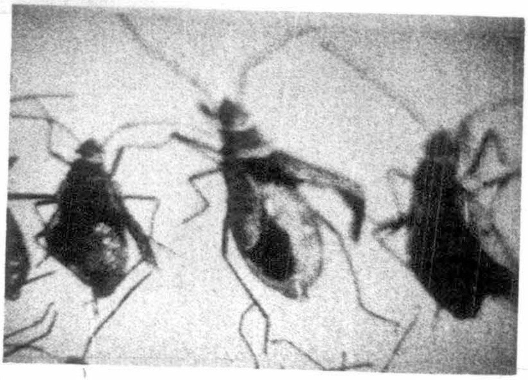
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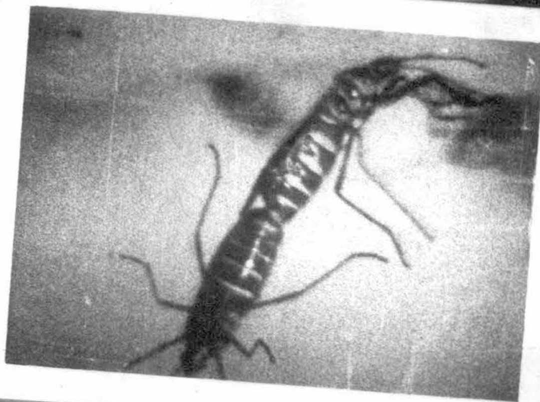
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PLATE -I (contd.)

(g) Parental care of eggs by a female bug after laying eggs.

(h) Effect of radiation on early fifth instar nymphs (note the wing deformities).

(i) Normal mating of control insects (ventral view).

(j) Mating between irradiated male and normal female.

(k) Irradiated male marked with white paint on the ventral side of the abdomen.

(l) Test for mating competitiveness. The photograph shows the ventral view. Normal female (marked with white paint on the ventral side of the abdomen) is deen mating with irradiated male (marked with white paint). A normal male is seen competing for mating.

DISCUSSION

Since the present type of experiments require a large number of insects for test, mass rearing of the insect becomes important (Knipling, 1964, 1967). The mass breeding must be economical, non-time consuming, and simpler (Smith, 1966; Briton et al., 1969; Howell, 1970, 1972).

Among the different diets tried in the present study cotton seeds constituted the major-and in three cases-the only ingredient. Geering and Coker (1960) have suggested that cotton seeds are the best diet for higher fecundity in Dysdercus species. Among the four diets tried, the one developed by us (minced cotton seeds 10% sucrose solution) was found to serve the best in terms of 'serving capacity' and egg yield. Using germinated cotton seeds (Srivastava and Bahadur, 1958; Harwalker and Rahalkar, 1975) was found to be unsuitable as the hypocotyl of the seedlings dries off unless a wet sand base is given. If sand base is given, separation of eggs from sand grains becomes difficult. Adding to that, foul smell and fungal contamination which were encountered in this method are highly undesirable. Soaked cotton seeds (Geering and Coker, 1960; Prabhu and John, 1975; Jalaja and Prabhu, 1976a, 1977) also posed the problem of foul smell and fungal contamination. The egg yield was comparatively low (minimum) when this diet was used. (It must be noted that these diets were used when small colonies for physiological studies were maintained). These problems were overcome when dry minced cotton seeds were used. Further a modification of this diet (addition of 10% sucrose solution) yielded the maximum number of eggs. The fungal growth was not a problem as the contamination was noted at the expiry of the diet, i. e., by the third day. Hence this diet was preferred for mass rearing.

Lining the bottom of the container with Whatman no.1 filter paper (Rena, 1975) and wet cotton was found to be unsuitable as the water retaining capacity was very low. Using wet sand is recommended by Harwalkar and Rahalkar (1975). All these lining materials form a base for fungal growth. Synthetic sponge foam was found to be the best lining device as it retained water for longer period and did not form a base for fungal growth. The necessity to dispose the used filter paper and cotton every two days makes this un-economical. The foam lining is reusable after thorough washing and is suitable for use in the present kind of studies. The temperature and RH maintained in the present work is comparable to the recommendations of Sharma et al, (1975). The life cycle studies, when a different diet is used, is necessary for irradiation studies

(Hatmosoewarno, 1971; Katiyar & Ferrer 1968). The present life cycle studies aided in choosing a particular instar of known age.

Monogamy in females is a conducive condition for sterile-male technique (Knipling, 1955, 1964). In the present study females were found to be monogamous and males polygamous. Hence this species is highly desirable for control by sterile-male technique. For radiation studies only males were chosen for their polygamous nature.

It is evident from the present studies that gamma-irradiation can affect the pre-imaginal (pre-adult) development of *D. koenigii* by inhibiting moulting, preventing adult emergence, inducing morphological abnormalities like wing deformation and adversely affecting reproduction. It is observed that the early stages of fouth and fifth instar nymphs of *D. koenigii* are highly radio-sensitive. In developmental stages, radiation affects the differentiation processes of wings, epidermal cells and gonadal cells.

Increase in age results in increased differentiation. Nothel (1968) has shown in *Drosophila* that increasing age with increase in differentiated cell leads to decreased radio sensitivity. The present work shows that early stages, i.e., less differentiated stages, are more prone to radiation damage. These observations closely resemble the reports of Karwalkar and Nair (1968) in X-irradiated D. koenigii, Karwalkar and Rahalkar (1975) in Metepa treated D. koenigii nymphs and of Economopoulous (1971) in tretamine-treated Oncopeltus fasciatus, a closely related hemipteran. This aid us to conclude that less differentiated cells or cells at the process of differentiation are much prone to radiation than fully differentiated ones.

In hemipteran insects, nymphal epidermal cells exhibit intense mitotic activity at a particular period of previous moult (Wigglesworth, 1954). The report on the inhibition of pupation of Ephestia Kuhniella confirms this phenomenon (Kusin et al, 1968).

The fact that many nymphs treated at a particular dose of radiation during the terminal (late) period of an instar successfully moulted into the next stage, where as those treated during the early period failed to do so and died, suggests that radiation, in some way, interferes with the production of moulting hormone or some step in the process of moulting or metamorphoses. This view is strengthened by the observations of Karwalkar and Nair (1968), Karwalkar and Rahalkar (1975), Economopoulous (1971) and Walker (1971).

The sensitivity of the various developmental stages of the insect to ionising radiation is rather simply measured by survival to adulthood (Nothel, 1963). At higher doses of radiation, progressively lesser number of nymphs metamorphosed into adults in the present work. This effect is comparable with the effect of juvenile hormone analog reported by Saxena and Williams (1966). This clearly shows that radiation affects or interferes with hormonal activity of the early stages of the insect.

In the present study, higher percentage of wing deformities at early stages show that they are more prone to radiation. The developmental changes due to radiation reflects the preadult differentiation of the tissues and their radiosusceptibility. In Dolichodorus species similar effect has been reported (Yeressian and Cause, 1963). A steady increase in radio-resistance with age was noticed in Diatraea saccharalis (Walker 1971). In the present investigation, both the stages of fourth instar nymphs showed more wing abnormalities than the fifth ones and as the early 5th instar, too, was more radio sensitive, it can be hypothesised that the cells of early stages, as they differentiate due to hormonal activity, are more susceptible to radiation.

In all stages the radiation effect seems to be linearly related to the dosage. This is in agreement with the findings on other insects (Lochereau et al, 1975; Suentord, 1975;

Rahalkar et al, 1975; Anwar, 1968; Maksimovic, 1971; Hooper, 1971).

Present study shows that the effects of ionising radiation is characterised by inhibitions of fertility in direct proportion to exposure dosage.

But a drastic decrease due to one krad irradiation and a steady decrease beyond that till 4 krad, where, again a steep fall was noticed, suggest that impairment of reproduction is not linearly related to dose. Studies on other insects, too show a similar trend (Huignard, 1971; Katiyar and Ferrer, 1968; Boller et al, 1975; Rahalkar et al, 1975; Lecherenu et al, 1975).

In D. koenigii, the fertility and fecundity are reduced at 4 krad onwards when compared to 9 krad for mediterranean fruitfly (Hooper, 1971), 15 krad for Trogoderma granarium (Kansu, 1962) and Lasioderma serricorne (Harvey, 1963), 30 krad for Pectinophora gossypiella (Ouye et al, 1964), 40 krad for the codling moth (Proverbs, 1964) and 45 krad for the mediterranean flour moth (Hull, 1963). In Rhodnius species, a hemipteran related to D. koenigii, Baldwin and Shaver (1963) have reported that 20 krad can bring down the fertility and fecundity of this insect drastically.

The data obtained on the relationship between gamma-irradiation dose and the reduction in fertility in male D. koenigii in the present study are different from those obtained by Simon (1968) who reported that a dose rate of 5 and 10 krad was necessary to obtain the same results in D. Peruvianus.

It is a well established fact that radiation-produced dominant lethal mutations in the insect sperm renders the reduction in fertility (Riemann and Thorson, 1969; Denell, 1973). The less dosage needed for D. koenigii when compared to other insects and also when compared to a Peruvian species of the same genus , shows that the chromosomes of these insects are more susceptible for the induction of dominant lethal mutations by radiation than others.

The males exposed to 4 krad reduced the percentage of egg hatching, more than those subjected to lesser exposures used in this study. However, the competitiveness was drastically reduced at 5 & 6 krad, as the insects exposed to this dosage of radiation were not able to compete more efficiently with normal males. The data of Simon (1968) shows that at 5 and 10 krad the competitiveness of D. Peruvianus is reduced to 0.31 and 0.008 respectively. But, the present study on D. koenigii shows that at the dose of 4 krad the competitiveness is maintained at 0·85 and the fertility too is low. In Rhodnius prolixus the optimum dose for reduced fertility without any impairment of competitiveness was reported to be 10 krad (Gomes Nonus, 1971). Boller et al, (1975) have shown that a 10 krad exposure has not significantly reduced the competitiviness of Rhagoletis cerasi whereas the fertility was reduced, and at higher doses the vigour was reduced. Similar trend is noticed in the present work, too.

Reduction of competitiveness at higher doses has been attributed to the somatic damage by irradiation (Grosch, 1975). In the present work also more somatic abnormalities like wing deformities were noted at doses above 4 krad. The less competitiveness is due to the above mentioned factor.

CONCLUSIONS:

The overall radiation response of the living system can be measured by fitness components (Nothel, 1968). In insects, these are mainly developmental phenomenon, reproductive capacity and fitness for competitive mating.

(a) From the present study, it can be derived that the early, less differentiated stages are more prone to radiation and this can be either by direct action of radiation on the target cells or the impairment of hormonal functions.

(b) The reproductive capacity is affected mainly by dominant lethal mutations induced in the sperm cells.

And (c) the third radiation effect is restricted to the particular component, i.e, mating competitiveness. The lesser the dosage, the lesser the somatic damage, and hence the more they are competitive.

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SUMMARY

SUMMARY

i) Experiments were carried out to choose a suitable diet and to improve culture techniques in connection with the radiology studies of *D. koenigii.* Several forms of diet were tried and minced cotton seeds with 10% sucrose solution was found to be apt. Improved culture maintenance methods were reported.

ii) Females were found to be monogamous and males polygamous.

iii) The radio-sensitivity of *D. koenigii* was dose dependent and radio-resistance increased with age. The early stages of 4th and 5th instar nymphs were more radio-sensitive than the respective later stages. The LD_{50} dose was found to be 2·76, 5·90, 5·62 and 14·10 krad for early 4th, late 4th, early 5th and late 5th instars, respectively.

iv) Fecundity, fertility and sexual competitiveness of the irradiated males were determined. Late 5th instar male nymphs irradiated with 4 krad showed highly reduced fertility with full competitiveness . 7 krad dose and 4 krad drastically reduced the fertility.

REFERENCES

REFERENCES

Anonymous, (1973) The screw worm strikes back. Nature, 242, 493 - 494.

Anwar, M. (1968) Some effects of gamma irradiation on Spodoptera exigua. In "Isotopes and radiation in entomology," (Proc. Sym. Vienna, 1967). IAEA, Vienna, pp - 109-121.

*Ashby, S.F., Nowell, W. (1926) 'The fungi of stigmatomycosis". Ann. Botany (London), 40, 69-83.

Atwal, A.S. (1976) "Agricultural pests of India and South-East Asia" Kalyani Pub., New Delhi, pp.294.

BALDWIN + SHAVER (1963) Cited in Kilgore and Doutt (1967) "Pest control: Biological, physical + selected chemical methods" Academic press, New York.

Baumhover, A.H., Graham, A.J., Bitter, B.A., Hopkins, D.E., New, W.D., Dudley, F.H., Bushland, R.C., (1955) Screw worm control through release of sterilised flies. J. Econ. Entomol., 48, 462-466.

Boller, E.F., Remund, U., Zehnder, J.(1975) Sterilization and its influence on the quality of the European cherry fruitfly, Rhagoletis cerasi. In "Sterility principle for insect control," (Proc. Sym. Innsburck, 1974). IAEA, Vienna, pp. 179-189.

Bransby-Williams, W.R. (1971) Juvenile hormone activity of ethyl farnesoate dihydrochloride with the cotton stainer Dysdercus cardinalis. Bull. Ent. Res., 61, 41-48.

Briton, F.E., Proverbs, M.D., Carty, B.E. (1969) Artificial diet for mass production of the codlingmoth Carpocapsa pomonella, Can. Entomol., 101, 577.

Brown. A.W.A. (1951). "Insect control by chemicals," 817 pp. Wiley, New York.

Brown. A.W.A., (1963) Insecticide resistance comes of age. *Bull. Ent. Soc. Amer.*, *14*, 3-9.

Brown. A.W.A. (1969) Insecticide resistance and future control of insects. *Canad. Med. Ass. J.*, *100*, 216-220.

Brunt, A.M. (1971) The histology of the first batch of eggs and their associated tissues in the ovariole of *Dysdercus fasciatus* as seen in the light microscope. *J.Morph.*, *134*, 105-130.

*Bull, J.O. (1963) IAEA Technical Report, Series *21*, 47. Cited in "Pest control: Biological, physical and selected chemical methods," (Kilgore, W.W., Doutt R.L., Eds.), Academic Press, New York, pp. 193.

Carter, W. (1962) "Insects in relation to plant diseases" Interscience (John Wiley), New York; pp.73.

Clarke, R.G., Wilde, G.E. (1970a) Association of the green stink bug and the yeast-spot disease organism of Soybeans-I. *J. Econ. Entomol.*, *63*, 200-204.

Clarke, R.G., Wilde, G.E., (1970b) Association of the green stink bug and the yeast - spot disease organism of Soybeans-II. *J.Econ. Entomol.* , *63*, 355-357.

Critchley, B.R., Campion, D.G. (1971) Effects of a juvenile hormone analogue on growth and reproduction in the cotton stainer *Dysdercus fasciatus*, Bull. Ent. Res., *61*, 49-51.

Crowe, T.J. (1977) Pyrrhocoridae : Dysdercus Spp.; In "Diseases, Pests and Weeds in Tropical Crops" (Kranz, J., Schmutterer, H., Koch, W., Eds.,) Verlag Paul Parey, Berlin. pp.298-300.

Datta, S., Banerjee, P. (1978) Prostaglandins, C-AMP, U7118 and Acetic Acid as Insect Growth Regulators and Sterilants. Indian J.Exp. Biol., 16 (8), 880-883.

De Bach,p.(1951) The necessity for an ecological approach to pest control on citrus in California, J.Econ.Entomol., 44, 443-447.

De Basch,p. (1960) Biological control of scales and mealybugs (Coccidae). In "Handbook on Biological control of plant Pests." (Westcott,C., ed.) Brooklyn BotanicalGarden, pp. 19-27.

DeBach,P. (1964a). In "Biological control of Insect Pests and Weeds" (P.De Bach,p. ed.). Reinhold, New York and chapman and Hall, London.

De Bach, p. (1964c) Some ecological aspects of insect eradication. Bull. Entomol. Soc. Am., 10, 221-224.

De Bach, P.(1974) "Biological Control by Natural Enemies." London-New York: Cambridge Univ.

Denell, R.E.,(1973) Use of male sterilization mutations for insect control programmes. Nature, 242, 274-275.

Economopoulous, A.P.(1971) Effects of tretamine on fourth and fifth instar Oncopeltus fasciatus nymphs: development of eggs from tretamine treated females. In "Sterility principle for insect control or eradication," (Proc. Sym. Athens, 1970). IAEA, Vienna, pp.259-270.

F.I.U., (1973) "Pest Management and Plant Protection Training." Farm Information Unit., Ministry of Agric., Govt. India; pp.159.

Frohlich, G., Rodewald, W. (1969) "Pests and Diseases of Tropical Crops and their Control" Pergamon Press, Oxford. pp. 251-252.

Geering, Q.A., Coaker, T.H. (1960) The effects of different plant foods on the fecundity, fertility and development of a cotton stainer, *Dysdercus superstitiosus*. *Bull. Entomol. Res.*, *51*, 61-76.

Gomez-Nunez, J.C. (1971) Sterility and chaga's disease vector control. In "Sterility principle for insect control or eradication," (Proc. Sym. Athens, 1970). IAEA, Vienna, pp. 157-165.

Gopakumar, B., Ambika, B., Prabhu, V.K.K (1977) Juvenomimetic activity in some south Indian plants and the probable cause of this activity in *Morus alba*. *Entomon*, *2*, 259-261.

Grosch, D.S. (1962) Entomological aspects of radiation as related to genetics and morphology. *Ann. Rev. Entomol.*, *7*, 81-106.

Grosch, D.S. (1975) Combined effects of radiation and chemical agents in altering the fecundity and fertility of a braconid wasp. In "Sterility principle for insect control", (Proc. Sym. Innsbruck, 1974). IAEA, Vienna, pp.243-259.

Haisch, A.(1970) Some observations on the decreased vitality of irradiated Mediterranean fruitfly. In "Sterile Male Technique for control of Fruit Flies." (Proc. Panel, Vienna, 1969). IAEA, Vienna; pp.71-77.

Haisch, A., Boller, E.F. (1971) Genetic control of the European fruit fly, Rhagoletis cerasi. In "Sterility principle for insect control or eradication." (Proc. Sym. Athens, 1970). IAEA, Vienna; pp.67-75.

Harvey. J.M.(1963) U.S. At. Energy Comm., Cited in Kilgore and Doutt (1967) "Pest control: Biological, physical and selected chemical methods." Academic Press, New York. pp-186.

Harwalkar, M.R., Nair, K.K. (1968) Effect of X-irradiation on postembryonic development of the red cotton bug, Dysdercus Koenigii. Ann. Ent. Soc. Amer., 61, 1107.

Harwalkar, M.R., Rahalkar, G.W.(1975) Effects of insect Chemosterilants on the development of the red cotton bug Dysdercus Koenigii. In "Sterility principle for insect control" (Proc. Symp. Innsbruck, 1974), IAEA, Vienna. pp.329-336.

Hartosoewarno, S. (1971) Biology of Scirpophaga nivella and a simple method of rearing. In "Sterility principle for insect control or eradication,"(Proc. Sym. Athens, 1970). IAEA, Vienna, pp. 303-311.

Hooper, G.H.S (1971) Gamma sterilization of the mediterranean fruitfly. In "Sterility principle for insect control or eradication," (Proc. Sym. Athens, 1970). IAEA, Vienna, pp. 87-95.

Howell, J.F. (1970) Rearing the codling moth on an artificial diet. J. Econ. Entomol., 63, 148.

Howell, J.F. (1972) Rearing of codling moth on a soya, wheat germ, starch medium. J. Econ. Entomol., 65, 636.

Huffaker, C.B. (1971a) The ecology of pesticide interference with insect populations. In "Agricultural Chemicals--Harmony or Discord for food, people and the Environment." (Swift, J.E. ed;). Berkeley: Univ. Calif., pp. 92-104.

Huignard, J. (1971) Study, using the gamma irradiation technique, of the migration and utilization of spermatozoa in the bean weevil, Acanthoscelides obtectus. In "Sterility principle for insect control or eradication," (Proc. Sym. Athens, 1970). IAEA, Vienna, pp.203-216.

IAEA, (1963). "Radioisotopes and Radiation in Entomology" (Proc.Sym. Bombay, 1962). International Atomic Energy Agency, Vienna.

IAEA, (1964) "Radiation and Radioisotopes Applied to Insects of Agricultural Importance" (Proc. Sym. Athens, 1963). International Atomic Energy Agency, Vienna.

IAEA, (1968) "Isotopes and Radiation in Entomology" (Proc. Sym. Vienna, 1967). International Atomic Energy Agency, Vienna.

IAEA, (1970) "Sterile - male technique for control of fruitflies." (Proc. Panel, Vienna, 1970) 175 pp. International Atomic Energy Agency, Vienna.

IAEA, (197?) "Sterility Principle for Insect Control or Eradication" (Proc. Sym. Athens, 1970). International Atomic Energy Agency, Vienna.

IAEA, (1975) "Sterility Principle for Insect Control" (Proc. Sym. Innsbruck, 1974). International Atomic Energy Agency, Vienna.

Jalaja, M., Muraleedharan, D., Prabhu, V.K.K. (1973) Effect of extirpation of median neurosecretory cells in the female red cotton bug, *Dysdercus cingulatus*. *J. Insect physiol.* , *19*, 29-36.

Jalaja, M. (1974) Complete inhibition of vitellogenesis after extirpation of median neurosecretory cells in *Dysdercus cingulatus*, *Curr. Sci.* , *43*, 206-207.

Jalaja, M., Unnithan, G.C., Prabhu, V.K.K. (1976) Ecdysterone induced ovarian inhibition in the red cotton bug, *Dysdercus cingulatus*, *Curr. Sci.*, *45*, 621-622.

Jalaja, M., Prabhu, V.K.K. (1976a) Effect of the chemosterilants apholate and metepa on the ovaries of the red cotton bug, *Dysdercus cingulatus*. *Entomon*, *1*, 43-53.

Jalaja, M., Prabhu, V.K.K., (1976b) Inhibition of vitellogenesis by allectectomy in the red cotton bug, Dysdercus cingulatus, Entomon, 1, 193-194.

Jalaja, M., Prabhu, V.K.K.,(1977) Endocrine control of the vitellogenesis in the red cotton bug, Dysdercus cingulatus. Entomon, 2, 17-29.

Joseph, A., Prabhu, V.K.K., (1977) Changes in the prothoracic glands of Dysdercus cingulatus during adult life. Entomon, 2 (2), 263-267.

°Kansu, I.A.(1962) Z.Angew. Entomol., 49, 224. Cited in "Pest control: Biological, physical and selected chemical methods," (Kilgore, W.W., Doutt, R.L., Eds.). Academic Press, New York, pp.194.

Katiyar, K.P., Ferrer, F. (1968) Rearing technique, biology and sterilization of the coffee leaf miner, Leucoptera coffeella. In "Isotopes and radiation in entomology." (Proc. Sym. Vienna, 1967). IAEA, Vienna, pp.165-173.

Khan, Q., Rao, V.P.(1960) Insect and mite pests. In "Cotton in India." Indian Central cotton committee, Bombay; 2, 217-301.

Knipling, E.F.(1955). Possibilities of insect control or eradication through the use of sexually sterile males. J.Econ. Entomol. 48, 459-462.

Knipling, E.F. (1964) The potential role of the sterility method for insect population control with special reference to combining this method with conventional methods. U.S.D.A., Agr. Res. Serv. (Tech.Bull.),33,98.

Knipling, E.F. (1967) Sterile technique-Principles involved, current application, limitations, and future applications. In "Genetics of Insect Vectors of Diseases." (Wright, J.W., Pal, R., Eds.,). Elsevier, Amsterdam, pp.587-616.

Koul, O., Tikku, K., Saxena, B.P. (1977) Mode of action of Acorus calamus oil vapours on adult male sterility in red cotton bug. Experientia, 33 (1), 29-31.

KUZIN. F.H., REEDS, A.D., WILKINS, S.H. (1968) In "Isotopes and radiation in entomology", IAEA, VIENNA.

La Chance, L.E., (1967) In "Genetics of Insect Vectors of Disease," (Wright, J., & Pal.R., Eds.) Elsevier, Amsterdam; chap. 21.

La Chance, L.E., Schmidt, C.H., and Bushland, R.C., (1967) Radiation- Induced Sterilization. In "Pest control: Biological, physical and selected Chemical methods" Kilgore and Doutt, Eds., Academic press, New York; pp. 148-193.

Lee, H.B., Hwang, Y.K., Lee, K.S. (1968) Exploratory studies on the eradication of the Korean pine caterpillar by means of radiation. In "Isotopes and radiation in entomology." (Proc. Sym. Vienna, 1967). IAEA, Vienna, pp. 273-285.

Locharenu, S., Chiravatanapong, S., Sutantawong, M., Kaochnong, P. (1975) Mating competitiveness of the radiosterilized male army worm Spodoptera exigua and the male mosquito, Aedes aegypti in field cages. In "Sterility principle for insect control," (Proc. Sym. Innsbruck, 1974). IAEA, Vienna, pp.317-328.

Maksimovic, M. (1971) Effect of Co-60 irradiation of male pupae of the gypsy moth, Lymantria dispar on biological functions of male moth. In "Sterility principle for insect control or eradication," (Proc. Sym. Athens, 1970). IAEA, Vienna, pp. 15-22.

McGregor, J.F. and Newcombe, H.B. (1972) "Dose-response relationships for yields of major eye malformations following low doses of radiation to trout sperm." Radiat. Res. 49, 155-169.

Moore, N.W. (1967) A synopsis of pesticide problem. Adv. Ecology Res., 4, 75-130.

Muir, J.R. (1978), Pest control- A perspective, in "Pest control Strategies" (Smith, E.H & Pimentel. D., eds.) (Proc. Sym. Cornell, June 1977). Academic Press, New York.

Muraleedharan, D., Prabhu, V.K.K. (1978) Food intake and midgut protease activity in the red cotton bug, Dysdercus cingulatus. Entomon, 3 (1), 11-18.

Newcombe, H.B. and McGregor, J.F. (1967) "Major congenital malformations from irradiations of sperm and eggs." Mutat. Res., 4, 663-673.

Newsom, L.D. (1967) Consequences of insecticide use on target organisms. Ann. Rev. Entomol., 12, 257-286.

Nothel, H. (1968) Correlations, interactions and differences between radiation effects on longevity and natural aging. In "Isotopes and radiation in entomology" (Proc. Sym. Vienna, 1967). IAEA, Vienna, pp. 87-103.

O'Brein, R.D. and Wolfe, L.S., (1964) "Radiation, Radio-activity and Insects." Academic Press, New York.

*Ochochi, J. and Simon, J.E. (1963) Determinacion de las dosis letales del Sevin para Dysdercus peruvianus en cepas resistentes Y susceptibles al DDT, ... La Molina. (Spanish).

Osmani, Z., Naidu, M.B. (1967) Evidence of sex attractant in female Dysdercus cingulatus. Indian J. Exp. Biol. 5, 51.

Ouye, M.T., Garcia, R.S. and Martin, D.F. (1964) "Determination of the optimum sterilising dosage for pink bollworm treated as pupae with gamma radiation. J. Econ. Entomol. 57 (3); 387-390.

*Pimentel, D. (1971) "Ecological effects of pesticides on non-target species." Washington, D.C., U.S. Off. Sci. Tech. Report.

Prabhu, V.K.K., John, M., Ambika, B. (1973) Juvenile hormone activity in some South Indian plants. Curr. Sci., 42, 725-726.

Prabhu, V.K.K., John, M., (1975a) Juvenomimetic activity in some plants. Experientia 31, 913-914.

Prabhu, V.K.K., John, M. (1975b) Ovarian development in juveniles & adult Dysdercus cingulatus affected by some plant extracts. Entomologia Exp. Appl., 18, 87-95.

°Proverbs, M.D. (1964) *Can. Entomologist*, *96*, 143. Cited in "Pest control: Biological, Physical and selected chemical methods," (Kilgore, W.W., Doutt, R.L., Eds.), Academic Press, New York, pp.196.

Rahalkar, G.W., Harwalkar, M.R., Rananavare, H.D. (1975) Laboratory studies on sterilisation of the male red palm weevil, *Rhynchophorous ferrugineus*. In "Sterility Principle for insect control," (Proc. Sym. Innsburck, 1974). IAEA, Vienna, pp 261-267.

Rana, G.R. (1975) Application of Juvenile Hormone analogue as a sterilant on four *Dysdercus* species. In "Sterility Principles for Insect control" (Proc. Sym. Innsbruck, 1974). IAEA, Vienna, pp.337-346.

°Riemann, J.G., Moen, D.J., Thorson, B.J. (1967) *J. Insect Physiol.*, 13, 407-418. Cited in "Pest control: Biological, Physical and selected Chemical methods,". (Kilgore, W.W., Doutt, R.L., Eds.). Academic Press, New York, pp.195.

Saxena, K.N., Williams, C.M. (1966) "Paper factor" as an inhibitor of the metamorphosis of the red cotton bug *Dysdercus koenigii*. *Nature*, *210*, 441-442.

Saxena, B.P. and Mathur, A.C. 1976 "Loss of fecundity in *Dysdercus koenigii* *F.* due to vapours of *Acorus calamus* L. oil." *Experientia*, *32*, No.3, 315-316.

Sharma, U., Sohni, S.L. and Sinha, D.P. (1975) "Studies on Dysdercus koenigii: Effect of temperature and Humidity on the Cerebral and Extracerebral Neurosecretory Cells during Postembryonic Development." Indian J. Exp. Biol., 13, 315-317.

*Simon, J.E., (1958) Resistencia del arrebiatado de Dysdercus peruvianus a los insecticidas organicos, Inf. Men., No. 368, E.E.A, La Molina. (Spanish).

Simon, J.E. (1968) Cria masal de Dysdercus peruvianus Y su esterilisacion mediante rayos gamma. In "Isotopes and Radiation in Entomology" (Proc. Sym. Vienna, 1967) IAEA, Vienna, pp. 287-299. (Spanish).

Smith, C.N. (Ed.) (1966) Insect colonisation and mass production. Academic Press, New York.

Smith, E.H. (1978) Integrated pest management needs - teaching, research and extension. In "Pest control Strategies" (Proc. Sym. Cornell, June, 1977). Smith. E.H. & Pimentel, D. Eds., Academic Press, New York, pp. 309-329.

Sohi, G.S. (1964). Pests of cotton; In "Entomology in India", Entomol. Soc. India, New Delhi. pp. 111-148.

Srivastava, U.S., Bahadur, J. (1958) Observations on the life history of the red cotton stainer, D. koenigii. Indian J. Entomol., 20, 228-233.

Saontoel, A.(1975) Effect of substerile radiation doses
on the progeny of treated bean weevil adults. In "Sterility principle for insect control," (Proc. Sym. Innsburck, 1974). IAEA, Vienna, pp.269-278.

Texas Instruments Incorporated, (1977) "Making Trecks into Programming." - Manual. T.I. Inc., Lubbock, Texas, U.S.A. Chapter 9.

Tiwari, R.K., Srivastava, K.P. (1975) Studies on the neurosecretory system and retrocerebral endocrine organs in the red cotton bug, *Dysdercus koenigii*. *Z. Morph. Tiere*, *81*, 355-364.

Torossian, C., Causo, R.(1968) The effect of gamma radiation on the fertility and longevity of *Dolichoderus quadripunctatus*. In "Isotopes and radiation in entomology",(Proc. Sym.Vienna, 1967). IAEA,Vienna, pp. 155-166.

Turnipseed, S.G., Todd, J.W., Campbell, W.V., (1975) Field activity of selected foliar insecticides against geocorids, nabids, and speiders on soybeans. *J.Georgia Entomol. Soc.*, *10*, 272-277.

Walker, D.W.(1971) Effect of gamma irradiation on immature sugar cane borers. In "Sterility principle for insect control or eradication." (Proc. Sym. Athens, 1970). IAEA, Vienna, pp.513-524.

Wiendl, F.M., Pacheco, J.M., Walder, J.M.M., Sgrillo, R.B., Domarco, R.(1975) A method of determining the gamma - radiation doses for the sterilization of stored product insects. In "Sterility principle for insect control," (Proc.Sym.Innsburk, 1974). IAEA, Vienna, pp.289-315.

Wigglesworth, V.B.(1954) "The physiology of insect metamorphosis." Cambridge Univ.Press, London.

Wilkinson, J.D., Biever, K.D., Ignoffo, C.M., (1975) Contact toxicity of some chemical and biological pesticides to several insect parasitoids and predators. Entomophaga, 20, 113-120.

Woods, A. (1974) "Pest Control: A Survey." New York: Halsted.

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*Original not seen.