

## Effects of Gramma Radiation on Azuki Bean Weevil, *Callosobruchus chinensis* (L.)

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### ABSTRACT

Effects of gamma radiation at various doses on eggs, larvae, pupae and adults of azuki bean weevil, *Callosobruchus chinensis* (L.) were studied. It was found that there were no significant differences between percent egg mortalities at 40 and 80 Gy and among 120, 160 and 180 Gy. As for the larvae, percent mortalities at all doses were significantly different from that of the control. Significant differences were also found among tested doses from that of the control in percent pupal mortality. After 4 and 7 days of irradiation, percent adult mortalities were found to be significantly different from those of the control at every dose. No significant differences were found among percent mortalities at all tested doses between 4 and 7 days. Percent sterilities at 100 and 120 Gy were not significantly different. When untreated male mating with treated female, there were significant differences in fecundity from the control and from among one another. In treated larvae at 100, 300 and 500 Gy, of all doses the degrees of melanization decreased with the increasing dose. The percent phenoloxidase (PO) activities of the treated larvae reduced compared with that of the control.

**Key words:** gamma radiation, azuki bean weevil, *Callosobruchus chinensis*, melanization, phenoloxidase activity

### INTRODUCTION

Irradiation of insect has received wide attention in all aspects, from the fundamentals of genetics, through the different approaches of exploratory development to technology and successful application to limited areas or to country-wide insect-pest eradication programs. Radiation studies on specific effects have been carried out on male and female germ cells in Diptera, Hymenoptera, Coleoptera and Hemiptera in order to measure variation in response and sensitivity to

radiation at different stages of oogenesis and spermatogenesis (IAEA, 1963).

Many species of stored-product pests are cosmopolitan, but other serious pests such as the khapra beetle (*Trogoderma granarium*), the larger grain borer (*Prostephanus truncatus*), and various species of legume weevil (Bruchidae) are not. Irradiation has been proposed as a possible quarantine treatment for various species of fruit fly, mango seed weevil, and codling moth but not for stored-product pests. However, irradiation has good potential for control of these pests, especially

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since the radiosensitivity of many of them is well documented (Brower and Tilton, 1985).

The genera *Bruchus* and *Callosobruchus* are particularly serious, and moderate to heavy infestations have been reported in the region, because the insects pass a major portion of their life-cycles inside the seeds (Quraishi and Metin, 1963). The most serious of these species in Asia are azuki bean weevil (*Callosobruchus chinensis*), cowpea weevil (*Callobruchus maculatus*) and graham bean weevil (*Callosobruchus analis*). The three bruchid pests have different distribution ranges. *C. chinensis* occurs in Asia, where it is a pest on azuki bean, chickpea, cowpea, mungbean, peanut, soybean and other grain legumes. The aims of the study were to investigate the effects of gamma radiation on various developmental stages and reproduction of *C. chinensis* and to determine if change in melanization of irradiated *C. chinensis* larvae could be used to indicate irradiation exposure including analysis of phenoloxidase activity.

## MATERIALS AND METHODS

### Laboratory rearing of *C. chinensis*

The culture was started with eggs of *Callosobruchus chinensis* (obtained from Stored Pest Division, DOA) on *Vigna angularis* Sayi seeds kept at room temperature of  $27 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$ , relative humidity (RH) and 10 : 14 (light : dark) photoperiod. This included the rearing of larvae and pupae as well as the adults in azuki bean seeds. All life stages were used in various experiments.

### Effects of gamma radiation on various developmental stages of *C. chinensis*

Eggs: four-day-old eggs from laboratory culture were irradiated with 0, 40, 60, 120, 160 and 180 Gy in a Cobalt 60 gamma irradiator (Gamma Cell 220) at the Office of Atoms for Peace. In each replication, 100 eggs were taken and each dose treatment was replicated three times. After

irradiation, the eggs were reared in petri dishes (1 cm tall  $\times$  9 cm diameter). The viabilities of irradiated and control egg were recorded at the end of ten days. Wherever hatching occurred, the larvae were provided with azuki bean seeds and allowed to complete development.

Larvae: Seeds of 10-day-old egg laying with larvae inside were irradiated with doses of 0, 100, 300, 500 and 800 Gy. For each dose, three replications of 100 larvae each, were used. After irradiation, the larvae were dissected from seeds, reared in petri dishes (1 cm tall  $\times$  9 cm diameter), released over azuki bean seeds and kept at  $27^\circ\text{C}$ .

Pupae: Seeds of 22-day-old egg laying with pupae inside were irradiated with doses of 0, 100, 300, 500 and 800 Gy. Each group of irradiated pupae consisted of 100 individuals dissected from seeds. Replications were conducted the same way as in the larvae.

After hatching, counts of dead and living insects were recorded every day, together with development and reproduction changes.

Adults: Approximately two-day-old adults of *C. chinensis* were irradiated at 0, 300, 600 and 800 Gy. After irradiation, they were mated into their groups. Data on insect emergence, insect deformation and longevity were recorded.

Percent mortality was corrected using Abbott's formula. Duncan's new multiple range test (DNMRT) was used in statistical analysis.

### Effects of gamma radiation on reproduction of *C. chinensis* females

Mature females were irradiated with doses of 0, 40, 60 and 80 Gy while males were irradiated with 0, 50, 100 and 120 Gy respectively. Treated and untreated adults were held in the same conditions previously described. Fecundities and percent sterilities of female were recorded from the following crosses for each radiation dose. Three replications were administered.

1. In each replication, 10 untreated males were crossed with 10 untreated females (The

control)

**UTM X UTF**

2. 10 treated males of different doses were crossed with 10 untreated females

**TM X UTF**

3. 10 treated females of different doses were crossed with 10 untreated males

**TF X UTM**

Where UT stands for untreated, T stands for treated, M stands for male and F stands for female.

The fecundity was determined by counting the eggs laid and the sterility by the percentages of unhatching egg. The numbers of adult emerged in each case were recorded as percentages of the number emerged from the controls.

**Effects of gamma radiation on melanization of 10 days old *C. chinensis***

Mungbean seeds of 10 day-old egg laying with larvae inside were irradiated with doses of 0, 100, 300 and 500 Gy. After irradiation, the larvae were reared singly in petri dishes. The larvae were cold-killed by placing in freezer (-20±1°C) for 24 hours after irradiation of 1, 2, 3 weeks. They were then removed from the freezer and kept at room temperature. Melanization was evaluated visually. The observations on melanization process were made using stereomicroscope (30X). The color of melanized body portion (black color) was shown by photo.

**Effects of gamma radiation on phenoloxidase (PO) activity in *C. chinensis***

Control and irradiated 10-day-old larvae with 300 and 500 Gy were killed by placing them in a freezer (-20±1°C) for a few days. Larvae were then removed from the freezer until used in the experiments. PO activity was to be determined by homogenizing each larval instar individually in 150 µl 0.1 M phosphate buffer solution (pH 6.5) in glass apparatus. The tissue homogenate was added with polyvinylpyrrolidone (oxidation protection)

and centrifuged at 12000 x g for 10 min. The supernatant was used as the enzyme source. Enzyme preparations were kept on ice until tested to avoid possible auto oxidation. The substrate was 3 mg/ml of L-dihydroxyphenylalanine (2-methyl dopa, Sigma) in 0.1 M phosphate buffer solution (pH 6.5). One hundred microlitre of the supernatant was added to 150 µl substrate solution and 750 µl 0.5 M phosphate buffer, mixed for few seconds and incubated at 25°C for 30 min. The intensity of the red color produced was measured by light absorption at 490 nm using a 6400 Spectrophotometer (Jenway). One unit of PO activity was defined as the amount of enzyme, at pH 6.5, producing a change of one absorbance unit a 490 nm. Specific activity was measured as units per milligram of protein (units/mg protein). Data from spectrophotometric analyses were statistically analyzed at 0.05 level of significance and means separated by the Duncan's new multiple range test (DNMRT).

**RESULTS AND DISCUSSION**

**Effects of gamma radiation on various developmental stages of *C. chinensis***

**Eggs**

Results on egg irradiation at different doses after 10 days are shown in Table 1. It was found that the mortality of 4 days old egg increased with the increasing dose. Percentages of mortality at 0, 40, 80, 120, 160 Gy were 0, 40.6, 75.2, 93.7, 98.3 respectively and no egg hatched after treated with 180 Gy. There were no significant differences between percent mortalities of egg irradiated at 40 and 80 Gy and among those at 120, 160 and 180 Gy. Percent egg mortalities at every dose were also found to be significantly different from that of the control.

The experiment showed the eggs of *C. chinensis* with 100 percent mortality at 180 Gy which was in agreement with Sutantawong (1991) who found that a dose of 180 Gy caused 100%

mortality in *C. maculatus* eggs.

### Larvae

Results on larval irradiation at different doses are shown in Table 2. The mortalities of 10 days old *C. chinensis* larvae were also found to vary directly with the radiation doses. Larval mortalities of full-grown larvae following irradiation at 0, 100, 300, 500 and 800 Gy were 0, 27.37, 83.86, 96.14 and 100 % respectively. At every dose, percent larval mortalities were noticed to be significantly different from that of the control. While there was no significant difference between

**Table 1** Effects of gamma irradiation on percent mortalities of 4-day-old egg of *C. chinensis* 10 days after irradiation.

Dose (Gy)	% mortality <sup>1/</sup>
0	0 a
40	40.6 b
80	75.2 b
120	93.7 c
160	98.3 c
180	100 c

<sup>1/</sup> Means in column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

**Table 2** Effects of gamma irradiation on the mortalities of 10-day-old *C. chinensis* larvae 10 days after irradiation.

Dose (Gy)	% larval mortality <sup>1/</sup>
0	0 a
100	27.37 b
300	83.86 c
500	96.14 d
800	100 d

<sup>1/</sup> Means in column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

percent larval mortalities at 500 and 800 Gy, both were significantly different from those at 100 and 300 Gy.

In the experiment, a dose of 800 Gy caused 100% larval mortality while OAEP (1968) and Quraishi and Metin (1963) reported 16000 rad (160 Gy) and 200 Gy respectively to cause 100% mortality of 16 days old *C. chinensis* larvae after hatching from eggs. Yet, the result was similar to that reported by Sutantawong (1991) which found 500 Gy to cause 100% mortality of 7-10 days old *C. maculatus*. It was possible that the different result from that of Quraishi and Metin (1963) was in the different ages of larvae used. The 8-day-old larvae in their experiment, compared to the 10-day old as in this study, were more sensitive to radiation as suggested by Molin (2001). Hence, lower dose (200 Gy) than the dose in the experiment was required to cause 100% mortality.

### Pupae

The results on the pupal irradiation are shown in Table 3. The mortality of pupal irradiation also increased with increasing dose. Percentages of pupal mortality at 0, 100, 300, 500 and 800 Gy were 0, 44.33, 69.41, 92.09 and 100 % respectively. Some pupae were found dead inside the seeds, hence they were not used in the treatments.

**Table 3** Effects of gamma irradiation on the mortalities of 4-day-old *C. chinensis* pupae 10 days after irradiation.

Dose (Gy)	% pupal mortality <sup>1/</sup>
0	0 a
100	44.33 b
300	69.41 b
500	92.09 c
800	100 c

<sup>1/</sup> Means in column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

Significant differences were found among all doses from that of the control in percent pupal mortality. It was also found that between 500 and 800 Gy, the percent pupal mortality did not significantly different from each other. There was no significant difference of percent pupal mortality at 100 and 300 Gy as well.

The result was similar to those of Sutantawong (1991) and Quraishi and Metin (1963) which reported 100 percent pupal mortality of *C. maculatus* and *C. chinensis* found at 500 and 470 Gy respectively. This study also agreed with Bhuiya *et al.* (1985) who found 100 percent pupal mortality of *C. chinensis* at 800 Gy.

### Adults

Table 4 expresses the adult mortality to increase when irradiated at higher doses. Four days after irradiation, the 2-day old adults irradiated at 0, 300, 600 and 800 Gy were noticed to have percentages of mortality of 0, 16, 86.5 and 97 respectively, while after 7 days after irradiation percent mortalities were 0, 81, 100 and 100 respectively. At every dose after 4 and 7 days of irradiation, percent adult mortalities were found to be significantly different from those of the control. While percent mortalities at 600 and 800 Gy were not significantly different from each other, they

**Table 4** Effects of gamma irradiation on the mortalities of 2-day-old *C. chinensis* adults 4 and 7 days after irradiation.

Dose (Gy)	% mortality after irradiation <sup>1/</sup>	
	4 days	7 days
0	0 a	0 a
300	16 b	81 b
600	86 c	100 b
800	97 c	100 b

<sup>1/</sup> Means in each column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

were from that at 300 Gy 4 days after irradiation. No significant differences among tested doses after 7 days of irradiation were noticed.

Table 5 shows the percent mortalities of *C. chinensis* adult between 4 and 7 days after irradiation at various doses. It was revealed that there were significant differences in percent mortality between 4 and 7 days at 300 and 600 but not at 800 Gy according to t-test analysis.

According to the results of Table 4 & 5, 800 Gy at 4 and 7 days or 600 Gy at 7 days after irradiation should be recommended as the killing doses and times for complete control of this insect. However, from an economical point of view, 600 Gy at 7 days of irradiation is likely to cause less expense since higher dose means greater cost while longer checking days does not increase as much expense.

Similar results were reported by Sutantawong (1991) who revealed a dose of 1000 Gy 7 days after irradiated to give *C. maculatus* 100% mortality. The study also agreed with Kovacs and Kiss (1985) who reported that with a treatment of 0.8 kGy all imagoes of *T. confusum* were dead 7 days after irradiation.

As stated by Molin (2001), the effect of radiation on insects are many and varied depending primarily on the species, stage, age and physical factor and the younger metamorphic stages of insect are most radiosensitive than the older stages.

**Table 5** Effects of gamma irradiation on the mortalities of 2-day-old *C. chinensis* adult between 4 and 7 days after irradiation.

Dose (Gy)	% mortality after irradiation		
	4 days	7 days	t value
300	16	81	-6.40*
600	86	100	-2.96*
800	97	100	-1.73

\* Significant at .05 level.

Thus, eggs, larvae and young pupae are quite easily killed within a reasonable period of time after treatment, the old pupae and young adults are relatively resistant to the lethal effects of irradiation. Eggs are most sensitive to radiation. This was to explain egg mortality occurring at lower doses as in this study compared to the mortalities of other stages at higher doses. Physical factors also have an influence on the effects of radiation on insects. The differences in temperature and humidity, hence, caused different results to even 2-day old difference in age as between larval mortality in the study of Quraishi and Metin (1963) and this experiment.

#### Effects of gamma radiation on fecundity and sterility of *C. chinensis* females

The effects of gamma irradiation with 2-day-old *C. chinensis* males (TM) on the fecundities and sterilities of untreated female (UTF) compared with the control (UTF X UTM) are shown in Table 6. It was found that the numbers of egg/female at each dose of untreated females were not significantly different from the control or from one another. While the percent sterilities at 100 and 120 Gy were not significantly different they differed from that at 50 Gy.

**Table 6** Effects of gamma irradiated 2-day-old *C. chinensis* males mated with untreated females (TM  $\times$  UTF) on the fecundities and sterilities of untreated female.

Dose (Gy)	Fecundity <sup>1/</sup> (Eggs / female)	% Sterility <sup>2/</sup>
0	32.93 a	76.15 a
50	33.23 a	79.67 b
100	28.63 a	90.40 c
120	28.53 a	91.47 c

<sup>1/2/</sup> Means in each column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

When untreated male (UTM) mating with treated female (TF) (Table 7), it was revealed that at various doses of radiation the fecundity of treated female decreased resulting in increased percent sterilities from 0 to 80 Gy with dose of 80 Gy gave 100% sterility in female. When female exposed to this dose was paired with a normal adult of the opposite sex, eggs were laid on the seeds but the eggs failed to hatch. It was also found that there were significant differences in fecundity from the control and among one another at all doses. Percent sterilities at every tested dose were significantly different from that of the control (0 Gy) but not among one another.

In sterility of female, the results agreed with that of Brower and Tilton (1985) who showed the doses required to sterilize stored-product insects to vary widely from 70 Gy for the cowpea weevil, *C. chinensis* and the female appeared more susceptible than the male in all cases in the same insects (OAEP, 1968). The result also agreed with Sutantawong (1991) who suggested that female of *C. maculatus* was susceptible than male at 40, 60 and 80 Gy.

According to the results in Table 6 & 7, the lower dose in obtaining complete mortality in females than males was required, hence, irradiated

**Table 7** Effects of gamma irradiated 2-day-old *C. chinensis* females mated with untreated males (TF  $\times$  UTM) on their fecundities and sterilities.

Dose (Gy)	Fecundity <sup>1/</sup> (Eggs / female)	% Sterility <sup>2/</sup>
0	32.93 a	76.15 a
40	24.93 b	98.34 b
60	21.22 c	99.67 b
80	8.66 d	100 b

<sup>1/2/</sup> Means in each column followed by the same letters are not significantly different at 5% level as determined by DNMRT.

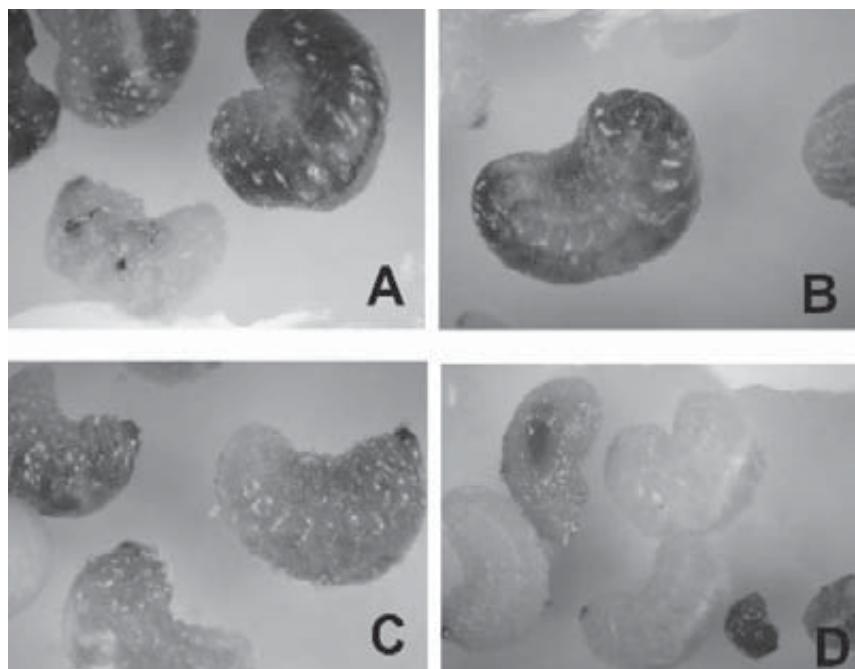
females should be employed in SIT (Sterile Insect Technique) program. However, since the females in the nature are needed to reduce their fecundities, it is more practical to release the irradiated males in order to mate with these wild females even though, in economical point of view, the expense will be higher.

In most SIT program, however, both sexes are released and the response of both males and females to the sterilizing doses has to be assessed. In some species the males are the more sensitive sex, e.g., the screw-worm, *Cochliomyia hominivorax*, in other species the females are, e.g., the medfly, *Ceratitis capitata*. In case where the male is the more sensitive sex it would be very advantage to have a system for the removal of females so that a lower radiation dose could be given to the male (Rechcigl and Rechcigl, 1998).

As suggested by Molin (2001), low radiation doses cause insect sterilization or genetically deformed gametes, which higher doses required to induce insect death. In these experiments, 600-800 Gy caused complete mortality while only 100-120 Gy induced complete sterility in *C. chinensis* adults. Higher radiation doses cause multiple breaks in the chromosomes, thus causing death or sterility.

#### Effects of gamma radiation on melanization process of 10 days old *C. chinensis* larvae

Figure 1 presents the color of *C. chinensis* at different doses. When larvae were treated with various doses (0-500 Gy), the color variation of the larvae ranged from black to creamy white. The degree of melanization in treated larvae decreased with the increasing doses.



**Figure 1** Degrees of darkening in the larvae of azuki bean weevil, *C. chinensis* 3 days after treatment.

- A. Control (30X)
- B. Treated with 100 Gy (30X)
- C. Treated with 300 Gy (30X)
- D. Treated with 500 Gy (30X)

The results similarly agreed with Kongrat-aron (2002) who reported melanization occurred in untreated young larvae of cigarette beetle larvae after killing by freezing. Some parts of larvae body became dark brown to black while the rest of the body was yellow-white or grayed-yellow. In the treated young larvae, non-melanization to slight melanization occurred at 100, 300 and 500 Gy. The degree of melanization in treated young larvae decreased with the increasing dose. Ignatiowicz and Banasik-Solgala (1997) reported that after the irradiation treatment with doses ranging from 0.1 to 0.5 Gy, the melanization process was significantly inhibited in young larvae cold-killed after irradiation and old larvae of khapra beetle melanized slowly after their death, so that some visible in the body color were noted as late as after 24 hours. With similarity of this study to such report, the change in melanization of the azuki bean weevil, *C. chinensis* larvae could not be used for indicating previous exposure of these insects to irradiation, because of the great variability in response of the melanization process to the irradiation treatment.

Ignatiowicz and Ibrahim (1996) found that the melanization in irradiated young larvae of the confused flour beetle, *Tribolium confusum* DUAVL., was reduced in the first week after treatment and completely inhibited in the second week. Great variation of melanization in the untreated old larvae partially obscured the effects of gamma radiation on this process. However, the melanization was considerably reduced in all experiments involving old larvae. Banasik-solgala and Stanislaw (1997) found that the degree of melanization differed significantly between treated and untreated the Indian meal moth (*Plodia interpunctella* HBN), the Mediterranean flour moth (*Ephestia (Anagasta) kuehniella* ZELL) and the almond moth (*Cadra cautella* WLK).

### Effects of gamma radiation on phenoloxidase (PO) activity in *C. chinensis*

Results of the experiment on the effects of radiation on PO in larvae of azuki bean weevil, *C. chinensis*, indicated variability of the response of enzyme activity to gamma radiation. Three sets of experiment were conducted to determine PO activity spectrophotometrically. In this assay, the frozen larvae were used to increase the activity of the PO system (Mansour and Franz, 1996). The results of PO activity measurement are shown in Table 8.

The preliminary analysis of PO in the irradiated larvae at 0, 100, 300 and 500 Gy showed low activities of PO which was difficult to detect because the larvae had small sizes that were not easy in homogenizing. At 0, 100, 300 and 500 Gy, PO activities of larva were found to be 2.500, 1.867, 0.851 and 0.409 units/mg protein respectively.

The values obtained for PO activity showed that with the increasing dose from 0 to 100, 300 and 500 Gy, PO activity decreased. The PO activity was found to be significantly different between 0 and 500 Gy, while not differed from those at 100 and 300 Gy. No significant differences were found among PO activities at 100, 300 and 500 Gy.

Percent reduction of PO activity also decreased with the increasing dose where the highest (83.67%) was at 500 Gy and lowest (25.33%) at 100 Gy.

Irradiation was found to reduce or eliminate melanization after death and reduced phenoloxidase activity in the fourth instar of *C. chinensis* larvae. The irradiation dose at which melanization was essentially prevented was from 100 Gy up.

The result agreed with Lupa and Ignatiowicz (1999) who showed the highest doses to inhibit enzyme activities in larvae of Mediterranean flour moth and the confused flour beetle after irradiated. There was a decreasing of PO activity and significant difference when larvae were treated

**Table 8** Phenoloxidase (PO) activity and percent reduction of irradiated 10-day-old larvae of *C. chinensis* at different radiation doses.

Dose (Gy)	Mean of PO activity (units/mg protein) <sup>1/2</sup> (% Reduction)
0 (control)	2.500 a
100	1.867 ab (25.32)
300	0.851 ab (65.96)
500	0.409 b (83.67)

<sup>1/</sup> Means followed by the same types of letter in the same columns are not significantly different from one another as determined by DNMRT at 0.05 level.

<sup>2/</sup> Units of phenoloxidase at 490 nm.

with 0.3 (kGy) or higher dose of gamma radiation.

Lupa (2000), also reported that the phenoloxidase activity in fourth instar of kaphra beetle (*Trogoderma granarium*) larvae was inhibited by 50 and 150 Gy after 1 week and larvae irradiated with 100 and 300 Gy exhibited higher enzyme activity compared to the control. Surisan (2004) reported the changes in the effect of radiation on PO activity on each instar of cotton bollworm. At 75 and 150 Gy the irradiated insects were found to have PO activities significantly less than that of the control and percent reduction of PO activity also increased from 4<sup>th</sup> to 1<sup>st</sup> instar. According to Hara *et al.* (1991) the active units of cuticle-bound phenoloxidase should be measured during metamorphosis along with hemolymphatic phenoloxidase activity in order to better understand the physiological role of phenoloxidase in cuticle tanning and sclerotization.

## CONCLUSION

The egg, larval, pupal and adult mortalities increased with the increasing dose with the egg being the most sensitive. Sterilities of irradiated male mating with untreated female were

significantly different at all doses while those of irradiated female mating with untreated male significantly different between 0 and other doses. Lower dose was used for female than for male in obtaining complete mortality. Melanization in irradiated larvae decreased with increasing dose and could not be used in detecting radiation exposure due to great variability in response of the process to irradiation. Percent PO activity decreased with the increasing dose.

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