

Effects of ultra violet irradiation (254 nm) on egg hatching, population growth and reproductive parameters of cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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ABSTRACT: *Callosobruchus maculatus* (F.) is a serious pest of stored products. In this research, the eggs of *C. maculatus* belonging to three age groups, 1, 2 and 3 day-old were exposed to ultraviolet (UV) irradiation with 254nm wavelength (UV-C) for different durations at temperature of 25±5°C and 10:14 (L:D), without humidity control to determine irradiation effects on egg-hatching, reproduction and population growth parameters. An increase in time of exposure to UV-rays caused a gradual decrease in the percentage of hatching of eggs in all age groups of eggs, while for each dose the older eggs were more sensitive than younger ones. The percentage of hatched eggs was determined to be 95 % in control treatment, while in 1, 2 and 3-day-old eggs were 7.5, 1.67 and 0.83%, respectively. UV-irradiation in all exposure periods and group of age significantly decreased the reproduction parameters. The R_0 was 79.98±2.34 on control and the lowest of this parameter was in generation emerged from 2-day-old eggs that treated by 4 min exposure (11.07±0.42 eggs). Value of r_m , was 0.14±0.0009 on control that decreased by increasing of irradiation dose. The lowest amount of r_m was observed in 2-day-old eggs that treated by 4 min exposure (0.05±0.0012). Value of λ decreased along with increasing exposure time from 2 to 4 min. Consequently D_t and T_C increased with; increasing time of exposure from 2 to 4 min exposure time. The longest mean generation time was recorded in 3-day-old eggs which treated by 4 min exposure time (31.72±0.06 days).

Keywords: *Callosobruchus maculatus*, Egg hatching, Population growth parameters, Reproduction parameters, Ultra violet irradiation

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of several pulses including chickpea *Cicer arietinum* L. and cowpea *Vigna unguiculata* (L.) Walp. The adults lay their eggs on the seeds in the storage; larval feeding in the cotyledons causes substantial quantitative and qualitative losses (Singh, 1978; Steel, 1985; Jackai & Daoust, 1986; Ogunwolu & Odunlami, 1996; Pascual-Villalobos & Ballesta-Acosta, 2003). Over 90% of the insect damage to cowpea seeds is caused by *C. maculatus* (Caswell, 1981). Commercial quantities of cowpeas are effectively disinfested by fumigating with insecticides such as methyl bromide or phosphine gas (Mbata, 2004). However, methyl bromide causes depletion of the ozone layer has compelled the parties to the Montreal Protocol to agree to phase methyl bromide out by 2010 in developed countries, and to freeze its consumption and production, and finally to phase it out by 2015 in developing countries (Clarks, 1998). Uses of other insecticides in stored products are facing

restrictions in the world. In addition, pest populations are developing resistance to chemical insecticides (Hagstrum, 1999; Phillips, 2000). So, the use of insecticides and its concomitant impact on environment, has necessitated exploration of alternative non-toxic pest control methods. Irradiation becomes an established technique for controlling stored product insects because of residue free advantages over chemical fumigation (Tuncbilek, 1995). Pszczola (1997) demonstrated the acceptability of irradiation technology as an alternative treatment for food protection because irradiation can extend the shelf life of various fruits and vegetables, and maintain the quality of the product over a longer period of time. Hasan & Khan (1998) mentioned that irradiation does not significantly change the quality of the food material or stored seeds. In the present study, non-ionizing radiation, i.e. ultra violet radiation (UVC), was used as a possible method for controlling *C. maculatus*. This study provides new information on the effects of different doses of UVC on demography of *C. maculatus*, which has as yet not been thoroughly studied. The main objective of this research is to examine the effects of different exposure times of UVC on the egg hatching, reproduction, and population growth parameters of *C. maculatus*. A search of databases for research on UVC and *C. maculatus* did not yield any information on minimum radiation doses for suppressing infestation. Therefore, we have chosen radiation doses arbitrarily to evaluate the effect of UVC doses on the developmental stages of *C. maculatus*.

MATERIALS AND METHODS

Insect cultures

Callosobruchus maculatus was reared on mung bean (*Vigna radiate* L.) seeds. The cultures were maintained at a mean room temperature of 25±5°C with a photoperiod of 10:14 (L:D) without any humidity control. The insect had been kept in our laboratory culture for over 3 years and were maintained at above condition. All experimental procedure was carried out under the same environmental conditions as the culture. The eggs of *C. maculatus* were collected by placing large numbers of beetles on the rearing medium. After 24h the adults were separated and the seeds were selected that only one egg had been laid on them. If there were more than one egg on a seed, the other eggs were omitted with needle. The seeds were kept in a beaker to obtain the eggs of 2 and 3 day- old eggs.

UV-irradiation technique of eggs

The UVC radiation source was a 15W germicidal lamp, GE15T8 measuring 40×2cm. This lamp produced UVC at a wave length of 253.7 nm. To determine the effect of UV on egg hatching, the seeds were placed on a surface 12 cm from the lamp, as the eggs were in front of the lamp. Eggs were irradiated for 2, 4, 8, 16, 24, 32 and 40 minutes. Exposure period was determined using stop watch. At the end of the exposure period the UV-lamp was turned off and the seeds were removed immediately. The experiment was conducted for one-day, two-day and three-day old eggs similarly and for each exposure period 120 eggs irradiated. After exposure, irradiated and non-irradiated control eggs of different age groups were kept separately at 25±5°C until the death of all individual members of the cohort. Two important life table parameters (survivorship and life expectancy) were calculated by the following formula (Carey, 1993; Maia, 2000):

$$l_x = \frac{N_x}{N_o} \qquad e_x = \frac{T_x}{l_x}$$

Where x is unite of age, l_x is age-specific survival rate or the fraction of individuals of the initial cohort alive at age x , N_x is number alive at age x , N_o is starting number of individuals in the cohort, e_x is life expectancy at age x , T_x is the number of time units lived by the cohort from age x until all individuals die: $T_x = \sum L_x$, where L_x is the fraction of individuals alive during the interval between x and $x+1$.

Reproduction and population growth parameters

In order to calculate demographic parameters of *C. maculatus*, a large number of 1, 2 and 3 day-old eggs of this pest were irradiated for 2 and 4 minutes. After emerging of adults, 30 newly emerged (less than 24h old) females and the same number of males were differentiated by the method of Bandara and Saxena (1995), and a pair of female and male was introduced into a petri dish. Each petri dish contained 5gr mung bean, then the petri dishes were placed in a 25±5°C temperature and photoperiod 10:14 (L:D). The number of eggs laid by each female was recorded daily until the last female died. Eggs after counting were removed. The factors that are essential for calculating of population parameters included: the age of females in days (x), the number of females alive at age x (l_x), and the mean number of eggs laid per female alive per day (mx).

Statistical analysis

Standard demographic parameters were calculated from daily records of mortality, fecundity and fertility of cohorts of *C. maculatus* females. The reproduction (gross fecundity and fertility rates, net fecundity and fertility rates, mean number of eggs and fertile eggs per female per day) and population parameters [intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T_c), finite rate of increase (λ) and doubling time (D_t)] were calculated using formulae suggested by Carey (1993). The statistical differences in R_0 , T_c , λ , D_t and r_m values were tested using jackknife procedure to estimate the variance for r_m and the other population parameters (Meyer *et al.*, 1986). This procedure is used mostly to estimate variance and bias of estimators. It is based on repeated recalculation of the required estimator, missing out each sample in turn (Maia, 2000). It is used to quantify uncertainty associated with parameter estimates, as an alternative to analytical procedures, in cases for which the last ones require very complicated mathematical derivation (Maia, 2000). Algorithms for jackknife estimation of the means and variances are described only for r_m . Similar procedures were used for the other parameters (R_0 , T_c , λ and D_t). The steps for the application of the method are the following (Maia, 2000):

(a) Estimation of r_m , R_0 , T_c , λ and D_t considering the survival and reproduction data for all the n females, referred to as true calculation. At this point, called step zero, estimates obtained are denoted as $r_m(\text{all})$, $R_0(\text{all})$, $T_c(\text{all})$, λ (all) and $D_t(\text{all})$ (Maia, 2000).

(b) Repeat the procedure described in part (a) for n times, each time excluding a different female. In so doing, in each step i , data of $n-1$ females are taken to estimate parameters for each step, now named $r_m(i)$, $R_0(i)$, $T_c(i)$, λ (i) and $D_t(i)$ (Maia, 2000).

(c) In each step i , pseudo-values are calculated for each parameter, subtracting the estimate in step zero from the estimate in step i . For instance, the pseudo-values of r_m , $r_m(j)$, was calculated for the n samples using the following equation (Maia, 2000):

$$r_{m(j)} = n r_{m(\text{all})} - (n-1) r_{m(i)}$$

d) After calculating all the n pseudo-values for r_m , jackknife estimate of the mean ($r_m(\text{mean})$), variance (VAR $r_m(\text{mean})$) and standard error (SEM $r_m(\text{mean})$) calculated, respectively, by the following equations (Maia *et al.*, 2000):

$$r_{m(\text{mean})} = \frac{\sum_1^n r_{m(j)}}{n}$$

$$VAR_{r_m(\text{mean})} = \frac{\sum_1^n (r_{m(j)} - r_{m(\text{all})})^2}{(n-1)}$$

$$SEM_{r_m(\text{mean})} = \sqrt{\frac{VAR_{r_m(\text{mean})}}{n}}$$

The differences in development, reproduction and population parameters were compared using one-way analysis of variance (ANOVA). If significant differences were detected, multiple comparisons were made using the Student-Newman-Keuls (SNK) at $P < 0.05$. Statistical analysis was carried out using Minitab software (MINITAB, 2000). A dendrogram of different age groups of eggs of *C. maculatus* based on reproductive and population growth parameters on different exposure time of UV-irradiation was constructed after cluster analysis by Ward's method using the statistical software SPSS 13.0 (SPSS, 2004)

RESULTS AND DISCUSSION

Results

Effect of UV-irradiation on egg hatching

Analysis of variance for ultra violet irradiation effect on egg hatching of *C. maculatus* are shown in Table 1. The results were showed that egg hatching of *C. maculatus* under different UV-irradiated time were differed significantly at 1% probability levels. It was found that UV-irradiation reduced hatching of eggs of all age groups, the effect gradually increased with increasing exposure periods (Fig1). All exposure periods of UV-irradiation reduced the hatching of eggs in comparison to control. Our results indicated that, for each exposure duration, the hatching rate was decreased as the age of irradiated eggs increased from 1 to 3 days (Fig1).

UV-irradiation had a pronounced effect on the age-specific survivorship rate (l_x) of *C. maculatus*. The survivorship (l_x) at first day of life was estimated to be 95, 67, 61, 36, 27, 22, 10 and 7% on control and 1day-old treated eggs by 2, 4, 8, 16, 24, 32 and 40 min. This parameter was 39, 13, 9, 5, 3, 1, 1 and 40, 24, 9, 10, 5, 2, 0% in 2 day and 3day-old treated eggs, respectively. The life expectancy (e_x) at the first day of life was estimated to be 48.22, 35.3, 33.45, 20.74, 17.15, 14.10, 9.48 and 8.49 days on control and 1day-old eggs that treated by mentioned doses. This parameter in 2 and 3day-old eggs calculated 24.80, 13.50, 11.23, 5.02, 8.96, 8.24, 9.04 and 25.02, 16.86, 10.10, 10.41, 8.72, 7.15, 6.83 days on the examined doses. The life expectancy values at the beginning of adult emergence were 18.97, 20.91, 19.63, 19.76, 21.20, 19.77, 21.27, 18.35 days in 1day- old eggs, 19.91, 18.29, 14.50, 9.96, 18.25, 19, 7 days in 2 day- old eggs 21.68, 17.38, 20.12, 18.93, 18.90, 16.50, 17.50 days in 3day-old eggs at 2, 4, 8, 16, 24, 32 and 40 min exposure time, respectively. Therefore, the results indicated that irradiation decreased the survivorship and the life expectancy on treated generation in comparison to control.

Table 1. Analysis of variance for ultra violet irradiation effect on egg hatching

Source of Variation	df.	Mean of Square (MS)
A factor (Ultra violet irradiation)	1	463.25**
B factor (Egg old)	2	359.77**
C factor (Irradiation time)	6	1025.14**
A x B factor	2	785.69**
A x C factor	6	1156.32**
B x C factor	12	825.83**
A x B x C factor	12	1489.66**
Replication	3	541.31 [†]
Error	123	145.23
%C.V.	18.64	

* and **: Significant at 5% and 1% probability levels, respectively

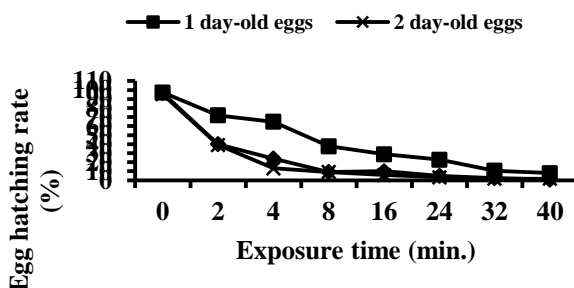


Figure1. Egg hatching rate (%) of *C. maculatus* under different UV-irradiated time

Effect of UV-irradiation on reproductive parameters

No significant difference was observed on the gross fecundity rate (i.e. the mean number of eggs per female per generation) when eggs of different ages were exposed to UV-irradiation (Table 2). Net fecundity was 79.98±2.34 eggs on control, however decreased significantly by increasing exposure time. In the other hand the lowest value of net fecundity was 52.93±5.08, 11.07±0.42 and 17.96±0.63 in day 2 day and 3 day-old eggs respectively (Table 2).

A significant reduction in gross fertility rate of *C. maculatus* was observed when eggs of different ages were exposed to UV-irradiation. The effect gradually increased with increasing exposure periods (Table 2). The gross fertility was 94.64±2.81 eggs on control, the lowest value of gross fertility rate observed on 2 day- old eggs that were irradiation by 4 min exposure irradiation (12.7±0.57). Net fertility rate reduced significantly by UV-irradiation (Table 2). For all exposure periods of UV-irradiation, significantly reduced the mean number of eggs per female per day (P<0.05). This parameter was 11.36±0.013 in control and reduced with increasing the exposure period. The lowest number of eggs laid per day was obtained on 2 day-old eggs, which were irradiated by 4 min exposure (Table 2). The number of hatched eggs laid per female per day was 10.79±0.012 on control. By 4 min exposure time of irradiation this parameter decreased to 6±0.007, 1.43±0.002 and 2.45±0.002 on 1, 2 and 3 day-

old eggs (Table 2). Our results showed at the same dose of UV-irradiation among three different ages of eggs, the 2 day-old eggs were most sensitive.

Effect of UVC radiation on the population growth parameters

Effect of different exposure time of UVC-irradiation on the population growth parameters are presented in Tables 3. At all age groups of eggs, intrinsic rate of increase (r_m), finite rate of increase (λ) and the net reproductive rate (R_0) of *C. maculatus* decreased with; increasing time of exposure from 2 to 4 min while the mean generation time (T_c) and doubling time (D_t) increased within these irradiation doses. Net reproductive rate was 38.32 ± 0.1 on control, that gradually decreased as the duration of exposure to radiation increased. The lowest value of net reproductive rate among our treatment was observed on 2 day-old eggs that treated by 4 min exposure (5.19 ± 0.21) (Table 3). The lowest amount of r_m in 1, 2 and 3-day-old eggs were 0.11 ± 0.0009 , 0.05 ± 0.0012 and 0.077 ± 0.0008 respectively by 4 min exposure time while it was 0.14 ± 0.0009 on control (Table 3). Also changes in amount of λ indicated similar effects of the irradiation (Table 3). Consequently values of T_c and D_t on untreated population were lower than those exposed to UV radiation (Table 3). Our results indicated that UVC radiation was more effective in 2 and 3 day than 1day-old eggs. However, no significant difference observed in amount of R_0 , r_m , λ in 2 and 3 day old eggs by 2 min exposure, while the value of these parameters were least significantly in 2day old eggs irradiated by 4 min exposure time. The lowest r_m , λ and R_0 and highest D_t , T_c occurred at 2day-old eggs, suggesting that this is the most sensitive age for controlling of *C. maculatus* by irradiation. Among the enclosed adults from the irradiated eggs, a proportion of the individuals were severely deformed in their wings, elytra and bodies. Deformity was observed at high doses (24, 32 and 40 min) in three age groups of eggs.

Table 2. Effect of UV-irradiation on reproductive parameters of *C. maculatus*

Reproductive parameters	Exposure Time (min)	Age of eggs (days)		
		1	2	3
GFcR Gross fecundity rate	0 Control	99.68±4.06 a	99.68±4.06 a	99.68±4.06 a
	2	101.63±2.00 Aa	89.82±4.28 Ba	105.97±6.94 Aa
	4	100.27±4.54 Aa	95.27±4.47 Aa	102.22±3.72 Aa
NFcR Net fecundity rate	0 Control	79.98±2.34 a	79.98±2.34 a	79.98±2.34 a
	2	60.08±1.31Ab	32.19±1.47Bb	57.76±2.03Ab
	4	52.93±5.08 Ab	11.07±0.42 Bc	17.96±0.63 Bc
GFfR Gross fertility rate	0 Control	94.64±2.81 a	94.64±2.81 a	94.64±2.81 a
	2	68.49±1.35 Ab	35.12±1.75 Cb	43.46±1.53 Bb
	4	60.24±2.53Ab	12.70±0.57Cc	24.47±0.95Bc
NFfR Net fertility rate	0 Control	76.02±2.20 a	76.02±2.20 a	76.02±2.20 a
	2	40.49±0.88Ab	12.59±0.57Cb	23.50±0.83Bb
	4	34.64±2.72 Ab	1.57±0.11 Bc	4.30±1.16 Bc
Mean eggs per female per day	0 Control	11.36±0.013 a	11.36±0.013 a	11.36±0.013 a
	2	11.20±0.008Ab	10.75±0.02Bb	10.74±0.01Bb
	4	10.01±0.01 Ac	8.76±0.01 Cc	10.21±0.008 Bc
Mean fertile eggs per female per day	0 Control	10.79±0.012 a	10.79±0.012 a	10.79±0.012 a
	2	7.56±0.005Ab	3.42±0.006Cb	4.40±0.004Bb
	4	6.00±0.007 Ac	1.43±0.002 Cc	2.45±0.002 Bc

Means followed by the different letters in the columns are significantly different (P<0.05). Means row followed by the different upper case letters in the rows are significantly different (P<0.05) (SNK)

Discussion

Understanding the demographic parameters of a pest is essential to develop an integrated pest management strategy. These parameters provide population growth rate of an insect pest in the current and next generations (Frel, 2003). As a population endpoints, r_m has been successfully utilized to determine the effect of several different xenobiotic compounds, such as pesticide, metal and radiation on aquatic invertebrates (Mbata, 2004). Our results showed that it is possible for ultra violet radiation to reduce the egg hatching, reproductive and population growth parameters of *C. maculatus*. The results also showed that the younger eggs (1day-old) of *C. maculatus* were more resistance than the older ones, which corroborate with the findings of Faruki, (2007) who reported that older eggs of *Tribolium castaneum* and *T. confusum* were more sensitive to UV-rays than younger eggs. Also, the present findings corroborate with the findings of Ayvaz, (2007) who observed that older eggs of *Ephestia (Cadra) cautella* were highly sensitive to UV-rays than younger eggs. Calderon, (1985) exposed the 1 to 4 day-old eggs *T. castaneum* to UV-irradiation. They reported that sensitivity to irradiation was augmented as the age of irradiated eggs increased from 1 to 4 days. Seidel, (1940) found that during early embryonic organization injury to the

peripheral parts of the eggs by UV- exposure did not impede the viability of the embryonic regions became more specialized, and different organ fields can no longer replace each other. Thus, damaging of the surface tissue of the eggs can be fatal at the advanced stages of development by non-penetrating radiations like UV-rays. However, our results contrast with the findings of Faruki, (2005) who observed that the younger eggs of *Cadra cautella* were more sensitive than older ones.

In the present test, the mortality of the irradiated eggs was directly proportional to the dose of radiation i.e. mortality gradually increased with the increasing of doses. This findings agreed with the results of Faruki & Khan (1993), working with *C. cautella* using UV- rays mentioned that larval mortality was positively correlated with radiation doses. Guerra, (1968) reported that when eggs of *Heliothis virescens* and *H. zea* were exposed to UV-rays the percentage of egg hatching was gradually decreased with increasing time of exposure of 20 minutes. Ghanem & Shamma (2007) were observed no eggs hatched when eggs of different ages (0, 24 and 48h) used were exposed to 3, 8 and 12 minutes UV-irradiation. They reported that microscopic examination showed the chorions of the eggs were damaged and the inner contents had leaked out. This effect was brought about by the thinness of the chorion and delicacy of the eggs in general. According the results, highest mortality was recorded in embryonic stage. Typically, the embryonic stage of an animal is a period of higher radio sensitivity and insects are no exception (Tilton & Brower, 1983).

Our results clearly showed that UV-irradiation reduced the gross fertility rate, net fecundity rate, net fertility rate and the mean number of fertile eggs per female per day. Faruki, (2005) recorded that the fecundity and fertility of *Alphitobius diaperinus* resulting from UV- irradiated of 2nd and 3rd instar larvae were reduced significantly. Hassan, (1998), also observed reduced fertility in eggs of *Exorista sorbillans* developing from UV-irradiated pupae. Some researchers reported, reduction in fecundity and fertility by gamma irradiation (Aye, 2008; Ayvaz, 2007; Ayvaz & Tuncbilek, 2006). The reduced fecundity and fertility of adults indicates the disturbances in oogenesis and spermatogenesis. To discuss the reduced fecundity in *E. sorbillans*, Hassan, (1998) stated that reduction may probably occurs due to both reduced transfer of active sperms by irradiated males to females and limited production of oocytes in the irradiated females. Insect fertility requires the presence of sufficient quantities of both eupyrene sperm (active sperm) and accessory gland secretions. Reduction in either of these two components will cause low fertility in insects (Saour & Makee, 1997). Snow, (1972) reported that sterilization significantly affects the type and quality of the sperm transferred by treated males. Knipling (1970) suggested that reduced fertility resulting from chromosomal translocations. Radiation did not affect on gross fecundity rate, even in 1 and 3 day old eggs, adults from irradiated eggs laid more eggs than control, but most of those eggs were unable to hatch. Therefore, the emergence of a new generation of *C. maculatus* was strongly inhibited by irradiation. Ayvaz, (2007) reported that gamma irradiation did not affect on the fecundity of either irradiated or un irradiated females mated to irradiated males in P, F₁ and F₂ generations of *Ephestia kuehniella* Zeller. They explained the additional number of eggs laid by irradiated and released *E. kuehniella* may provide additional host material for the egg parasitoids, *Trichogramma* spp., that could increase in number and be available for controlling subsequent generations. Therefore, perhaps additional number of eggs laid by irradiated parents was effective for releasing of parasitoids. However, much more research is needed. UV-irradiation had a significant influence on the reproductive parameters of *C. maculatus*. No published data are available concerning the effect of UV-irradiation on demographic parameters of *C. maculatus*. However effects of UV-irradiation on intrinsic rate of increase and population growth parameters of *C. maculatus* emphasize its suitability as pest control method. UV-rays produced adult deformities at long exposure periods (24, 32 and 40 min). Some of the adults that emerged from treated eggs had incomplete elytra, widely spreaded and crumpled wings, small winged. Faruki, (2005) observed when second and 3rd instar larvae irradiated by UV-rays, some of the larvae failed to shed their exuviae during molting. Larval-pupal and pupal-adult intermediate forms were common at each exposure period showing various morphological deformed characters. Some of the adults that emerged from treated larvae had incomplete elytra and crumpled wings, and short abdomen. Aye, (2008) reported that among the enclosed adults from the irradiated pupae (by gamma) of *Plodia interpunctella* (Hübner), a high proportion of individuals was severely deformed in their wing, legs and bodies. Ayvaz & Tuncbilek (2006) irradiated last instar larvae with 200 Gy doses of gamma radiation, none of the adults were normal in appearance.

The present tests showed that UV-irradiation caused a significant reduction in egg hatching and reproductive parameters. The significantly reduced hatching, fecundity, fertility and intrinsic rate of increase (r_m) of eggs resulting from UV-irradiation eggs revealed that UV-irradiation is promising, safe and effective for the control of storage pests. Thus, UV-irradiation can be used with other control methods such as insecticides and biological control in integrated pest management (IPM). However, much more comprehensive research is need

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