



Susceptibility of *Callosobruchus chinensis* (Coleoptera: Bruchidae) infesting chickpea to low energy electrons

SUBADAS SINGH¹, SHASHI BHALLA², KALYANI SRINIVASAN³, TELAPROLU VENKATA PRASAD⁴,
RADHEYSHYAM PRAMOD⁵ and JISHNU DWIVEDI⁶

National Bureau of Plant Genetic Resources, New Delhi 110 012

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ABSTRACT

Low energy electrons irradiation has been reported to effectively disinfest the grains and is considered safe and environment friendly. Laboratory experiments were conducted to study the effect of low energy electrons at various doses, viz. 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy at an energy level of 500 keV on different stages of adzuki bean weevil, *Callosobruchus chinensis* (L.) infesting chickpea. Percent adult emergence from seeds infested with pupa decreased from 76.6 % in the control (unirradiated) to 6% at irradiation dose of 1.5 kGy. There was no adult emergence from the eggs oviposited by irradiated female at 0.25 kGy, whereas, untreated female produced 95.3% of adults in next generation. Egg stage was comparatively more sensitive while late larva was most tolerant stage to the treatment. The LD₉₉ values of egg, early larva, mid larva, late larva and pupa were 1.21, 1.25, 2.94, 3.64, and 1.81 kGy respectively. The estimated doses for 50 and 99% control of adults were 1.09 and 2.25 kGy respectively. Low energy electron treatment did not affect the germination of chickpea seeds.

Key words: Adult emergence, *Callosobruchus chinensis*, Chickpea, Longevity, Low-energy electrons, Mortality

The adzuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) is an important pest of grain legumes. It is widespread throughout the tropics and subtropics with a wide host range including chickpea, cowpea, faba bean, lentil, pea and soybean. Fumigation with methyl bromide (MeBr) and use of insecticides is normally practiced for the management of this pest. However, MeBr has been designated as an ozone depleting substance under the Montreal Protocol and needs to be phased out globally in the near future. In addition, the injudicious use of chemicals has led to the development of resistant strains (Badmin 1990), therefore, there is an urgent need to find an alternative strategy for controlling the stored grain insect pests. Irradiation, an approved technology by International Plant Protection Convention, seems to be a viable non-chemical, residue-free strategy. Irradiation has a potential for control of stored grain pests, as the radio sensitivity of many of them is well documented (Brower and Tilton 1985). Due to adverse effects of gamma rays on host seeds, electron beam (EB) irradiation of the material has been considered safe and more cost-effective method of disinfestation (Amjad

and Anjum 2003). The extensive studies on the effect of different doses of EB on different pests (and stages thereof) have been done by Dohino *et al.* (1997) in Japan during 1993-1998. Soft-electrons, energies lower than 300 keV with a low penetration capacity have been reported to effectively disinfest grains, pulses, spices, dehydrated vegetables, tea leaves, and some sprout seeds with considerably less quality deterioration than other disinfection techniques such as heating and irradiation with gamma-rays (Todoriki *et al.* 2002). Low energy electrons can inactivate even the advanced stage of maize weevil, *Sitophilus zeamais* Motschulsky (Imamura *et al.* 2004a), eggs, larvae, pupae and adults of *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hubner) (Imamura *et al.* 2004b) and different stages of *C. chinensis* (Reddy *et al.* 2006). Electron treatment had no inhibitory effect on the germination of soybean and adzuki bean seeds (Kikuchi *et al.* 2003, Reddy *et al.* 2006). Therefore, present study was conducted to investigate the effect of low energy electron beam irradiation on different developmental stages and reproduction of *C. chinensis*.

MATERIALS AND METHODS

C. chinensis was reared on the chickpea seeds (~12% moisture) at 27±1°C and 65±5% relative humidity at Entomology Laboratory, Plant Quarantine Division, National Bureau of Plant Genetics Resources, New Delhi. The culture has been maintained since 2001.

¹Senior Research Fellow (mailsubadas@gmail.com),

²Principal Scientist (sbhalla@nbpgr.ernet.in), ³Principal Scientist (kalyani@nbpgr.ernet.in), ⁴Senior Scientist (tvprasad@nbpgr.ernet.in), Plant Quarantine Division, ⁵Scientific Officer (pramod@rrcat.gov.in), ⁶Engineer (jishnu@rrcat.gov.in), Raja Ramanna Centre for Advanced Technology, Indore, Madhya Pradesh 452 013

Preparation of seed samples

Seeds of chickpea cv Pusa 1103 were used in the study. Fifty pairs of freshly emerged adult weevils were released into plastic container of 15 cm in diameter containing 1 kg of seeds for oviposition and insects were removed the next day. The seeds containing single egg were separated and transferred to plastic container of 10 cm diameter and kept in incubator at $27\pm 1^\circ\text{C}$ and $65\pm 5\%$ relative humidity until further use. The stage of the insect was determined from the day of oviposition which was considered as zero days old. The different stages were ascertained as egg (0-2 days), early larvae (3-9 days), mid larvae (10-13 days), late larvae (14-18 days), pupa (19-24 days) and adult (25 days). Pre infested chickpea seeds with different days of eggs, larvae and pupa inside were used for irradiation. For each dose, three replications of 200 seeds each were used. These seeds with different stages of *C. chinensis* were taken in Petri dishes for low energy electron treatment.

Pre-infested chickpea seed samples containing different stages of *C. chinensis* were exposed to electron doses, viz. 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy at an energy of 500 keV produced by Cockroft-Walton based 20 kW DC accelerator at the Raja Ramanna Centre for Advanced Technology, Indore, India. For irradiation of different stages, samples were placed in open Petri dishes under the scanning horn of the accelerator at a distance of 15 cm in air. The samples to be irradiated were placed at one end of the conveyor belt before switching on the machine. Dose measurement was done using GAF chromic film before the irradiation. Once the desired energy and beam current established, conveyor was switched on and the sample was allowed to pass across the electron beam. The dose on the seed samples is the function of transmitted electrons from the titanium foil and speed of the conveyor belt. Based on the dosimetry experiments GAF film at 500 keV, it was measured that a beam current of 250 μA and conveyor speed of 30 m/min delivers dose of 0.25 kGy in one pass. Higher doses were given to the samples in step of 0.5 kGy by increasing the number of passes of the samples across the electron beam. The samples were exposed to the doses from 0.25 kGy to 1.5 kGy. A control in replicated trials was maintained that was not exposed to radiation. After the treatment, all the irradiated seed samples were kept in incubator at 28°C and 65 % relative humidity and number of adults emerged were counted at an interval of 24 hr and removed. The incubation was continued till no further emergence of adults was observed.

Ten pairs of freshly emerged adults were taken from culture and sexed using standard key characters. Sexed adults were kept in very fine thin polyethene bag and directly exposed to low energy electrons as before and replicated three times. The adults after exposure were transferred to the aerated plastic vials and observations were recorded at 5 hr and 2 days after treatment for mortality of adults. Observations were recorded at an interval of 24 hr for their survival and longevity. For comparing F1 progeny development of irradiated adults in second generation with

control, two pairs of surviving adults were released separately into plastic containers containing 20 fresh seeds each and observations were recorded. Control data were also recorded and all the treatments were replicated five times.

Statistical analyses were performed using Statistical Analysis Software, Version 9.2 (SAS 2009). One-way ANOVA was carried out followed by LSD using PROC GLM to find out the significant differences among the treatments at $P < 0.05$. The dose-mortality regressions and associated parameters were estimated using PROC PROBIT program. Differences among doses were considered significant if 95% confidence limits of the LD_{50} and LD_{99} values did not overlap.

Chickpea seeds cv. Pusa 1103 (300 g) were exposed to low energy electrons as described earlier, to test the effect of low energy electrons at different doses on germination, seedling vigour and vigour index. Four hundred and fifty seeds of each treatment were tested for seed germination. Twenty five irradiated seeds were arranged on the paper towel at equal distance and then another paper towel was put over the seeds. The butter paper along with paper towel with seeds was rolled up and placed in a plastic bag. Each dose along with the control (unirradiated seeds) was replicated ten times. These were kept at 26°C and 65% relative humidity in the seed germinator in an upward position for 7 days (ISTA 1996). On the 8th day, observations on germination of seeds were recorded. Seeds were considered germinated if plumules and radicals were seen emerged and calculated for per cent seed germination. For testing the seedling vigour, 100 seeds were used and same procedure as described above was adopted. On the 8th day, the shoot length (SL) and root length (RL) were measured and seedling vigour was calculated as the sum of mean of SL and RL (Perry 1977). Seedling vigour index was calculated as the product of germination percentage and seedling vigour (Abdul-Baki and Anderson 1973).

RESULTS AND DISCUSSION

Adult emergence data from low energy electron treatment of different stages of *C. chinensis* are presented in Table 1. The per cent adult weevils that emerged from all the treated samples was significantly lower than those from untreated ones. The adult emergence of all the stages decreased with an increase in treatment dose. Egg stage was most susceptible to irradiation. At an irradiation dose of 0.25 kGy the per cent emergence from irradiated eggs decreased from 72.1% in the control to 8.5%. At all the doses, emergence of *C. chinensis* adults was found to gradually increase as the age of the larvae increased. Among the immature stages, late larvae was most tolerant stage to irradiation even the highest dose of 1.5 kGy did not markedly reduce adult emergence, while the per cent adult emergence from pupa decreased from 76.6 % in the control to 6% at 1.5 kGy.

Effect of low energy electron treatment at different doses at the energy level of 500 keV on adult *C. chinensis*

Table 1 Susceptibility of different stages of *C. chinensis* infesting chickpea seeds to low energy electrons

Radiation dose at 500 keV	Percent adult emergence from chickpea seeds infested with				
	Egg	Early larvae	Mid larvae	Late larvae	Pupa
0.25 kGy	8.5±1.1b	15.0±1.5b	42.1±2.2b	66.8±2.3b	19.0±1.7b
0.5 kGy	7.5±1.0b	7.0±1.0c	36.5±3.1c	61.3±3.8c	13.7±2.1c
0.75 kGy	5.7±0.4c	5.5±0.8d	33.5±3.0c	59.1±3.2c	11.4±2.3c
1.0 kGy	2.4±0.3d	4.5±0.8e	31.2±2.9cd	52.5±4.1d	10.8±1.0c
1.25 kGy	2.1±0.9d	2.7±0.7f	28.1±2.0d	48.0±2.8e	6.5±1.1d
1.5 kGy	1.9±0.9d	2.1±0.9f	21.5±1.9e	37.9±3.0f	6.0±1.1d
0.0 kGy (Control)	72.1±3.6a	78.9±4.2a	85.4±6.3a	91.6±4.9a	76.6±3.9a

Means ± SE followed by the same letter in a column are not significantly different at P<0.05 (ANOVA followed by LSD post test); About 200 seeds of chickpea infested with egg, early larvae, mid larvae, late larvae and pupal stages of *C. chinensis* were irradiated at different doses; replicated three times

depicted in terms of per cent adult mortality, longevity, fecundity and progeny development is given in Table 2. Per cent adult mortality at all doses was significantly higher than control 2 days after treatment and increased with the increase in radiation dose. Adult longevity was reduced and decreased with the increase in radiation dose. It was 9, 7, 6, 6, 4 and 3.5 days at 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy respectively as compared to 11 days in control. Similarly, the fecundity of the treated adults was also adversely affected. The mean number of eggs laid per female was maximum at 0.25 kGy and no eggs were laid by females treated at 1.5 kGy compared to 89.8% in control. There was no adult emergence from the eggs oviposited by irradiated female at 0.25 kGy, whereas, untreated female produced 95.3% of adults of next generation.

The regression estimates obtained after probit analysis are presented in Table 3. Although, all the immature stages were significantly affected by the low energy electron treatment, the egg stage (0-2 days) was the most sensitive. The LD₉₉ values of egg, early larva, mid larva, late larva and pupa were 1.21, 1.25, 2.94, 3.64, and 1.81 kGy respectively. The estimated doses for 50 and 99% control of adults were 1.09 and 2.25 kGy respectively. The results showed that 99% of *C. chinensis* adults may be controlled at 2.25 kGy. Mid larva and late larval stages inside the chickpea seeds were more tolerant (LD₉₉ of 2.94 and 3.64, respectively) to electron treatment as the electrons with low

penetration did not reach larvae inside the host commodity (Imamura *et al.* 2004a).

The results of the present study clearly demonstrate that low energy electron treatment can be an effective strategy for the management of *C. chinensis* infesting chickpea. Egg was found to be the most susceptible stage, followed by early larva, pupa, adult, mid larva and late larval stage. As the development of the pest is inside the chickpea seed and the electron penetration depends on seed size, therefore, results are different from the report of Reddy *et al.* (2006) who found 18 day old stage (fully developed adults ready for emergence from greengram seeds which are comparatively smaller in size than chickpea seeds) was more tolerant. Our results confirmed the high radio-sensitivity of the eggs stage. Eggs, which are glued to the surface of the seeds, were directly exposed to the electron beam, unlike the other stages. Reproduction of stored product Coleopteran can be prevented with doses ranging from a low of 0.05 kGy for some species to a high of 0.4 kGy for depressed flour beetle, *Palorus subdepressus* (Woollaston) (Hallman 2012). Because the electron beam radiation energy decrease with increasing depth of the commodity (Hayashi 1998), the position of insect on or inside the seed could also affect the sensitivity. Low energy electrons at 500 keV could not completely inactivate the mid larval and late larval stages of *C. chinensis* inside the chickpea seeds because the electrons with low penetration did not reach

Table 2 Effect of low energy electrons on adult mortality, longevity and fecundity of *C. chinensis*

Radiation dose at 500keV	Adult mortality (%)		Adult longevity (days)	No. of eggs/ female	Per cent adults emerged (second generation)
	After 5 hrs	After 2 days			
0.25 kGy	0.0±0.0a	10.0±0.8b	9±0.28a	19.0±1.1b	0.0±0.0a
0.5 kGy	0.0±0.0a	16.6±0.5c	7±0.05b	14.9±1.7b	0.0±0.0a
0.75 kGy	0.0±0.0a	28.3±2.1d	6±0.35c	2.3±0.6c	0.0±0.0a
1.0 kGy	26.6±1.9b	58.3±3.6e	6±0.28c	1.1±0.05c	0.0±0.0a
1.25 kGy	38.3±1.4c	68.3±4.2f	4±0.26d	0.4±0.05c	0.0±0.0a
1.5 kGy	60.0±3.6d	80.0±4.1g	3.6±0.23e	0.0±0.0d	0.0±0.0a
0.0 kGy (Control)	0.0±0.0a	0.0±0.0a	11±0.05f	89.8±2.8a	95.3±4.6b

Newly emerged adults (2-day-old) of *C. chinensis* were irradiated in triplicate; each replicate with twenty adults; two pairs of surviving adults were released in twenty seeds in triplicate; Means ± SE followed by the same letter in a column are not significantly different at P ≤ 0.05 (ANOVA followed by LSD post test)

Table 3 Electron beam radiation dose-mortality regression estimates for *C. chinensis* life stages infesting chickpea

Life stage	Correlation coefficient (R ²)	Heterogeneity (χ^2)	Slope \pm S.E.	LD ₅₀ (kGy)	95% Fiducial limits(LFL-UFL)	LD ₉₉ (kGy)	95% Fiducial limits (LFL-UFL)
Adults	0.974	18.091	0.024 \pm 0.4	1.09	0.891-1.249	2.25	1.937-2.886
Pupa	0.954	48.677	0.20 \pm 1.1	0.29	0.281- 0.311	1.81	1.722-2.143
Late larva	0.971	24.306	0.008 \pm 0.2	1.21	1.191-1.285	3.64	2.112-3.907
Mid larva	0.964	59.599	0.10 \pm 0.1	0.66	0.541-0.834	2.94	2.704-3.145
Early larva	0.741	23.314	0.002 \pm 0.01	0.32	0.301-0.430	1.25	1.039-1.562
Egg	0.959	57.844	0.09 \pm 0.01	0.18	0.151-0.224	1.21	0.990-1.588

LFL, Lower fiducial limit; UFL, upper fiducial limit; LD₅₀, lethal dose for 50% kill; LD₉₉, lethal dose for 99% kill.

Table 4 Effect of electron beam irradiation on the physiological parameters of chickpea seeds

Radiation dose at 500keV	Per cent germination	Mean shoot length (cm)	Mean root length (cm)	Seedling vigour	Vigour Index
0.25 kGy	93.0 (75.2)a	18.0 \pm 2.0b	14.8 \pm 2.0a	32.8 \pm 1.8b	3132.7 \pm 16.7c
0.5 kGy	95.3 (78.0)a	21.1 \pm 1.9b	14.6 \pm 1.1a	35.7 \pm 1.1b	3327.6 \pm 57.7d
0.75 kGy	90.0 (71.9)a	17.4 \pm 1.0b	14.2 \pm 2.1a	31.6 \pm 1.9b	2845.3 \pm 25.9c
1.0 kGy	89.3 (71.0)a	10.2 \pm 2.2a	13.7 \pm 2.3a	23.9 \pm 1.6c	2138.7 \pm 57.7b
1.25 kGy	88.6 (70.9)a	9.5 \pm 1.7a	11.3 \pm 1.0b	20.8 \pm 1.0c	1849.0 \pm 27.1a
1.5 kGy	88.0 (69.9)a	11.2 \pm 1.1a	9.0 \pm 1.0b	20.2 \pm 1.3c	1782.8 \pm 46.1a
0.0 kGy (Control)	93.3 (75.9)a	13.4 \pm 1.5a	16.5 \pm 1.1a	29.9 \pm 2.5a	2798.1 \pm 50.0c

Data in the parenthesis are square root transformed (SQRT) values; Means \pm SE followed by the same letter in a column are not significantly different at P<0.05 (ANOVA followed by LSD post test)

larvae inside the host commodity. In organisms, radiation most easily affects sites of ongoing cell division, which in the adult insect include the gonads and midgut (Hallman 2013). Besides mortality, other effects of radiation on insect include reduced longevity, fecundity, reduction of egg hatch, delay in development and reduced feeding (Ahmed *et al.* 1981). In our study, adult mortality, longevity, fecundity and development of second generation weevils were found to be significantly affected following electron treatment. The studies are in conformity with the earlier reports of Hayashi *et al.* (2004). The higher doses/ energy levels are required to kill the adults while the sterility is caused even at lower doses. The sterility effect caused in the insects reduces the fertility and thus affecting the population build up (Vreysen *et al.* 2010). The sterility caused by lower doses of irradiation is still equally beneficial rather than mortality. Thus, the treatment could bring about substantial control of adzuki bean weevil infesting chickpea.

The results in the Table 4 indicate the safety of low energy electron treatment to chickpea seeds. Electron treatment had no adverse effect on seed germination. The percentage germination ranged from 88 to 95.3% at different doses which were at par with that of untreated control (93.3%). It is expected that soft-electrons could disinfest grains and pulses with minimal quality deterioration (Hayashi *et al.* 2002). Even the highest tested dose (1.5 kGy at 500 keV) of the low energy electron treatment had no adverse effect on seed germination and hence can be used for treating the seed material and that meant to be used for sprouts as also reported for adzuki bean seeds (Reddy *et al.* 2006). The shoot length was significantly better than

control at doses <1kGy while root length was at par with control at doses \leq 1kGy. The seedling vigour was also significantly better than control at doses <1kGy while vigour index was at par/ significantly better than control at doses <1kGy. This could be attributed to the stimulatory effect of irradiation on some metabolic processes and enzyme activity. Relatively low doses usually alter growth characteristics whereas, very low doses have been shown to stimulate plant growth (Munjeeb and Greij 1976). Bhat and Sridhar (2008) investigated the effect of electron beam irradiation on the quality characteristics of a legume, *Mucuna pruriens* L. and commented that seeds loose germination capacity at about 2.5 kGy of electron beam irradiation. However, radio sensitivity varies from species to species and even among genotypes of the same species (Ahmad and Qureshi 1992). The morphological, structural and functional changes depend on the strength and duration of radiation stress. In the present study, low energy electron treatment did not affect germination and this may be attributed to the low penetrating power of electrons that may not reach the seed embryo, thus leaving it unaffected (Reddy *et al.* 2006). Thus electron beam irradiation has potential to be used as a mitigation measure against *C. chinensis* in chickpea.

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