

# Ionizing radiation as a phytosanitary treatment against *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

Rakesh Kumar Seth<sup>1,\*</sup>, Mahtab Zarin<sup>1</sup>, Zubeda Khan<sup>1</sup>, and Ranjana Seth<sup>2</sup>

---

## Abstract

Phytosanitary irradiation (PI) has great potential to disinfest agricultural commodities of quarantine pests. Efficacy of gamma irradiation (5–500 Gy) was studied on various ontogenetic stages of the solenopsis mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), which is an economic tropical postharvest pest. As *P. solenopsis* showed a parthenogenetic mode of reproduction, studies were focused on female adults to estimate the minimum dose required for sterilization by gamma radiation. The radiosensitivity of the insect decreased as its age and developmental stage increased. The most radiotolerant stage, the 11–12 d-old gravid females required 500 Gy to completely prevent  $F_1$  egg hatch, whereas 200 Gy prevented molting of all  $F_1$  first instar nymphs to the second instar. The next lowest dose attempted was 100 Gy, whereupon 43  $F_1$  first instar nymphs—hatched from 500 eggs laid by irradiated gravid females,—molted to the  $F_1$  second instar. Therefore, a dose of ~200 Gy might suffice as a phytosanitary treatment against *P. solenopsis*. Large-scale confirmatory testing would be required to confirm a dose that could be used against this insect. Radiotolerance of potato (*Solanum tuberosum* L.; Solanales: Solanaceae)—a major host of *P. solenopsis*—was studied at 200 and 400 Gy. Color, weight, total soluble solids, titratable acidity, pH, vitamin C and  $\beta$ -carotene content were not affected compared with non-irradiated controls after 7 d storage at 5 °C.

Key Words: cotton mealybug, solenopsis mealybug, molting prevention, phytosanitary irradiation, phytosanitation, potato, nutritional quality analysis

## Resumen

La irradiación fitosanitaria (PI) tiene un gran potencial para desinfectar productos agrícolas de plagas cuarentenarias. Se estudió la eficacia de la irradiación gamma (5–500 Gy) en estadios diversos ontogenéticos de la cochinilla solenopsis, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), que es una plaga económica tropical de poscosecha. Como *P. solenopsis* mostró un modo de reproducción partenogenética, los estudios enfocaron en las hembras adultas para estimar la dosis mínima requerida para la esterilización por radiación gamma. La radiosensibilidad del insecto disminuyó a medida que su edad y estadio de desarrollo se incrementó. El estadio más radiotolerante fueron las hembras grávidas de 11–12 días de edad que requieren 500 Gy para prevenir completamente la eclosión de los huevos de la  $F_1$ , mientras que 200 Gy impidió la muda de todas las ninfas  $F_1$  del primer estadio al segundo estadio. El siguiente intento de dosis más baja fue de 100 Gy, con lo cual  $F_1$  43 ninfas de primer instar, nacido de 500 huevos puestos por las hembras grávidas irradiados, mudadas al segundo instar  $F_1$ . Por lo tanto, una dosis de ~200 Gy puede ser suficiente como tratamiento fitosanitario contra *P. solenopsis*. Pruebas a realizar que confirmen a gran escala el tratamiento serían necesarias para confirmar una dosis que podría ser utilizada contra este insecto. Se estudió la radiotolerancia - a 200 y 400 Gy de la papa (*Solanum tuberosum* L.; Solanales: Solanaceae) – un hospedero importante de *P. solenopsis*. El color, peso, sólidos solubles totales, acidez titulable, pH, contenido de vitamina C y beta-caroteno no se vieron afectados en comparación con los controles no irradiados después de un almacenamiento de 7 días a 5 °C.

Palabras Clave: algodón cochinilla, cochinilla solenopsis, prevención de la muda, irradiación fitosanitaria, fitosanidad, papa, análisis de calidad nutricional

---

Mealybugs (Hemiptera: Pseudococcidae) are important plant pests worldwide (McKenzie 1967; Williams 1985; Williams and Granara de Willink 1992; Miller et al. 2002, 2005). Mealybugs are also reported as important postharvest pests, ultimately posing quarantine barriers to agricultural trade. Hallman (2011) observed that mealybugs were the third most important quarantine pest group requiring phytosanitary treatments, after tephritid fruit flies and larvae of Lepidoptera. The minute and highly mobile crawler stage as well as the high rate of reproduction of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) makes it an especially threatening pest that they may occur on host material exported from infested areas.

The pest has been added to the European and Mediterranean Plant Protection Organization list (EPPO 2011) and the Chinese quarantine pest list (MOA 2009).

*Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), commonly known as the solenopsis or cotton mealybug, has a wide range of variation in morphological characters and biological adaptations (Hodgson et al. 2008), and is found in diverse ecological zones of many countries (Ben-Dov et al. 2009). It damages > 200 plant species from about 24 countries of tropical and subtropical regions of the world, and is a serious threat to Asian cotton fields, where three-fourths of the world's cotton is grown.

---

<sup>1</sup>Applied Entomology and Radiation Biology Laboratory, Department of Zoology, University of Delhi, Delhi- 110007, India

<sup>2</sup>Department of Zoology, Deshbandhu College, University of Delhi, Kalkaji, New Delhi-110019, India

\*Corresponding author, E-mail: rkseth57@gmail.com

Copyright © International Atomic Energy Agency 2016. Published by the Florida Entomological Society. All rights reserved.

Phytosanitary treatments are used to disinfest host commodities of insect pests before they are exported to areas free of the pests, and which are susceptible to pest invasion and establishment. Phytosanitary irradiation (PI) is a safe and effective phytosanitary treatment that is increasing in use in an increasing number of countries (Hallman et al. 2016). The measure of efficacy of PI is different from all other phytosanitary treatments in that prevention of further development or successful reproduction instead of acute mortality is the most used criterion of efficacy (FAO 2003). This study is 1 of several in this special issue of Florida Entomologist (Doan et al. 2016; Hofmeyr et al. 2016a; Kuswadi et al. 2016; Seth et al. 2016a,b; Zhan et al. 2016) that together with previous studies led to the proposal of a generic PI treatment for all mealybugs (Hofmeyr et al. 2016b).

Radiotolerance varies among arthropod taxa, and therefore, the estimated generic doses for arthropods vary from 100 Gy for Bruchinae (seed weevils) to 350 Gy for phytophagous mites (Hallman 2012). Radiotolerance may also vary among the life stages of an insect, and Hallman et al. (2010) found that it generally increases as an insect develops through successive stages of its life cycle.

The present study was carried out to assess the radiosensitivities of the various ontogenetic stages of *P. solenopsis* as measured by survival, metamorphic inhibition, and sterility, and to develop a PI treatment against the pest. Further, the influence of gamma irradiation was ascertained on the quality characteristics of potato, *Solanum tuberosum* L. (Solanales: Solanaceae), an important host on which this quarantine pest was reared.

## Materials and Methods

### MEALYBUG REARING

A culture of *P. solenopsis* was procured from the National Centre for Integrated Pest Management, PUSA Campus, New Delhi, India. Rearing conditions of  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 h L:D photoperiod were maintained in the incubators. Sprouted potatoes used for rearing the mealybugs were placed in glass jars (1 kg capacity) containing a bed of moist, sterilized soil. Adult mealybugs were then placed on the sprouted potatoes. Developmental changes in life stages (Fig. 1) were observed and recorded daily. Females started egg-laying within 11–12 d, and a total of 200–300 eggs were laid during their ovipositional period of 6–8 d. The hatching of crawlers from eggs occurred within 1–2 h after egg deposition. Female immatures had 3 instars with 7–8 d of developmental time each. Emerged crawlers ( $N_1$ ) moved toward sprouts, and when they molted to  $N_2$  (second nymphal instar) they showed a tendency to disperse. Food was replenished every wk. In case of males, the  $N_3$  (having filamentous covering around its body) lasted for 2–3 d and molted to the  $N_4$  stage enclosed within a cocoon-like covering and with a developmental period of 4–5 d. Longevity was 2–5 d for male adults and 32–37 d for female adults.

### IRRADIATION TREATMENT

Irradiation of mealybugs was conducted at the Radiobiological Unit of the Institute of Nuclear Medicine and Allied Sciences, Ministry of Defense, Delhi using a  $^{60}\text{Co}$  source (Gamma cell-5000, BRIT, BARC, Trombay, India). The dose rate was  $\sim 2$  kGy/h. Fricke dosimetry was performed and later reconfirmed by using a thermocouple chip provided through the IAEA to measure the dose values and the dose distribution.

## EXPERIMENTAL DESIGN

Various ontogenetic stages were broadly divided into 2 major groups, developing pre-imaginal stages and developed imaginal stages, to investigate the effect of irradiation on *P. solenopsis*.

### Pre-imaginal Stages

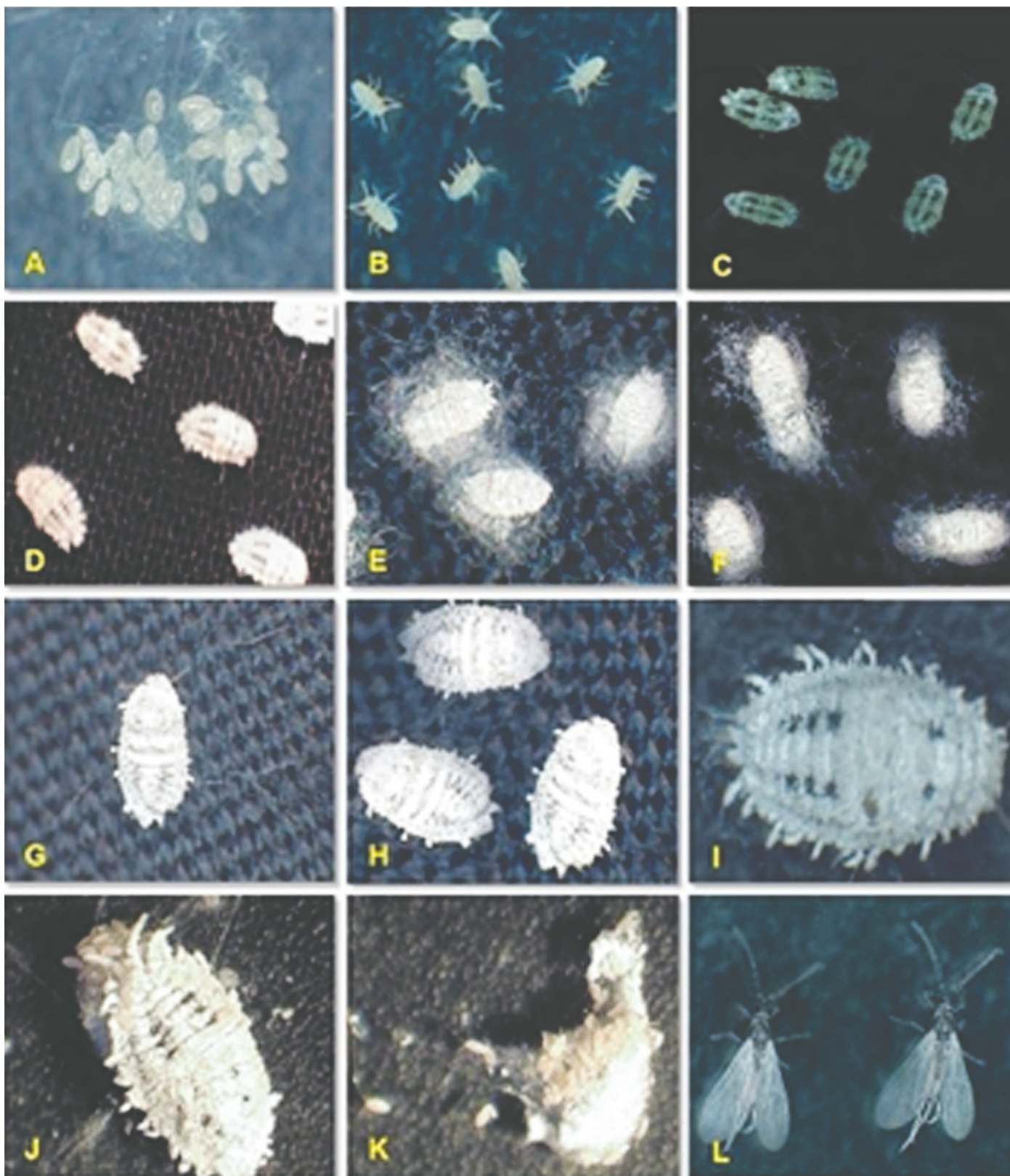
The bio-efficacy of gamma radiation was evaluated on the development and reproductive behavior of *P. solenopsis*, irradiated as crawlers ( $N_1$ ), second instars ( $N_2$ ), third instar females ( $N_3$ -♀), and third and fourth instar males ( $N_3$ -♂ and  $N_4$ -♂) (Fig. 1) with doses ranging from 5 to 500 Gy plus non-irradiated controls. A group of  $\sim 50$  individuals for  $N_1$  and a group of 15–20 individuals for subsequent ontogenetic stages per replicate were evaluated for their biological characteristics related to metamorphosis and adult formation in response to irradiation. The pre-imaginal stages ( $N_1$ -crawlers,  $N_2$ -second instar nymphs and  $N_3$ -♀-third instar female nymphs) were treated with 5–200 Gy to assess the sterilizing potential of gamma radiation. The reproductive behavior (egg laying and hatching) of the resultant adults was monitored daily; 7 individual females constituted each replicate. Effective doses for 50, 90, and 99.9% response ( $\text{ED}_{50}$ ,  $\text{ED}_{90}$ , and  $\text{ED}_{99.9}$ ) with respect to inhibition in metamorphic development and adult formation were determined via linear regression, and induced sterility in resultant adults from treated pre-imaginal stages was analyzed via probit analysis (PoloPlus, Petaluma, California).

### Imaginal Stages

To investigate the bio-efficacy of gamma radiation on reproductive behavior of imaginal stages of *P. solenopsis* (which exhibited parthenogenesis), only female mealybugs in 3 age groups—(i) freshly formed females (0–1 d-old), (ii) pre-gravid females (5–6 d-old), (iii) gravid females (11–12 d-old) (Fig 1.)—were exposed to gamma radiation (5–500 Gy), and their progeny were compared to those of non-irradiated controls. Radiation efficacy was further evaluated on the development and level of inherited sterility of  $F_1$  progeny derived from the P (parent) female imaginal stage irradiated with 5–200 Gy. Thus the effective doses ( $\text{ED}_{50}$ ,  $\text{ED}_{90}$  and  $\text{ED}_{99.9}$ ) inducing 50, 90 and 99.9% sterility in the parent generation and their carry-over impacts—metamorphic inhibition and inviability in  $F_1$  progeny due to sub-sterilized parent mealybugs—were determined.

### Physico-chemical and Nutritional Quality Analysis of Irradiated Potatoes

Several physico-chemical (weight loss, color change, total soluble solids, acidity, and pH) and nutritional (vitamin C and  $\beta$ -carotene) characteristics of potatoes irradiated with 200 and 400 Gy and stored for 7 d at  $5^\circ\text{C}$  were examined as per the following methods. The percentage weight loss of the irradiated potatoes after 1 wk of storage at  $5^\circ\text{C}$  was calculated. The color properties of the potatoes were measured by using an X-Rite CA22 spectrophotometer (X-Rite Inc., Grand Rapids, Michigan, USA). Color was recorded using the CIE-Lab, where the  $L^*$  value indicates lightness, the  $a^*$  value indicates chromacity on a green (–) to red (+) axis, and the  $b^*$  value indicates chromacity on a blue (–) to yellow (+) axis (Ranganna 1986). Total soluble solids were determined using an Abbemat refractometer (Anton Paar Instruments, Seelze-Letter, Germany) at  $20^\circ\text{C}$  and expressed as a percentage by mass of total soluble solids of the total contents (DGHS 2005). Acidity was determined by titrating samples (diluted with distilled water) with 0.1 N NaOH solution using a phenolphthalein indicator. The percentage of titratable acidity was calculated (AOAC 2012). Determination of pH of



**Fig. 1.** Life cycle of *Phenacoccus solenopsis*: A) eggs, B) first instars (crawlers;  $N_1$ ), C) second instars ( $N_2$ ), D) third instar females ( $N_3^-$ ), E) third instar males ( $N_3^+$ ), F) fourth instar males ( $N_4^+$ ), G) 0-1 d-old female adult, H) 5-6 d-old (pre-gravid) female adult, I) 11-12 d-old (gravid) female adult, J) gravid female with ovisac, K) crawlers emerging from ovisac, and L) male adults.

homogenized potato was done by a pH meter (Orion-420, ThermoScientific, Waltham, Massachusetts, USA) (DGHS 2005). Vitamin C was extracted and titration performed using 2,6-dichlorophenol indophenol

dye (AOAC 2012).  $\beta$ -Carotene was extracted and the quantification was carried out by high performance liquid chromatography (Shimadzu Scientific Instruments, Kyoto, Japan). The  $\beta$ -carotene was detected by

absorbance at 436 nm. The concentration of carotene in the samples was determined by comparing the sample area of the peak with the standard  $\beta$ -carotene (AOAC 2012).

## STATISTICAL ANALYSIS

The data obtained in the above experiments were usually replicated 10 times; any variation in replicate number is mentioned in the text. Effective doses  $ED_{50}$ ,  $ED_{90}$ , and  $ED_{99.9}$  with respect to metamorphic inhibition and adult formation were analyzed with linear regression. Prevention of reproduction was analyzed with probit analysis (Polo-Plus, Petaluma, California) of the dose-response data. The data were subjected to analysis of variance (ANOVA). A probability of  $P < 0.05$  was considered significant. Percentage data were transformed using arcsine  $\sqrt{x}$  value before ANOVA. Means were separated at the 5% significance level by least significant difference (LSD) test among the different treatments (SPSS 22.0, Armonk, New York, USA).

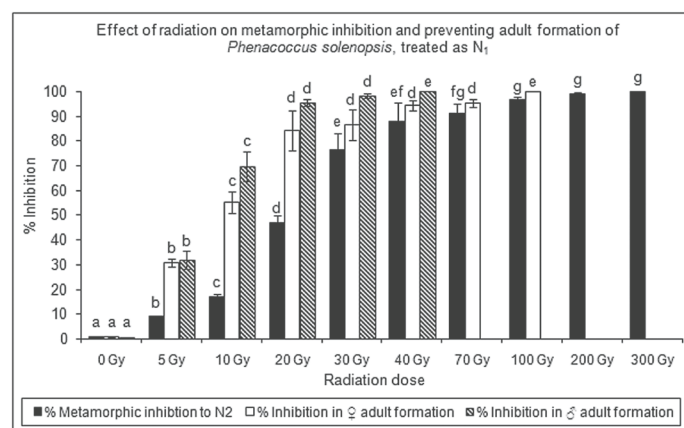
## Results

### EFFECT OF GAMMA RADIATION ON DEVELOPMENT AND SURVIVAL OF THE PRE-IMAGINAL STAGES

#### Effect of Irradiation of Different Pre-imaginal Stages on Metamorphic Inhibition

Figures 2–6 show the extent of reduction in developmental success of the different pre-imaginal stages of *P. solenopsis* as the radiation dose was increased from 5 to 200 Gy. Males were generally more radiosensitive than females for this characteristic. Radiosensitivity was inversely proportional to developmental stage.

Effective doses ( $ED_{50}$ ,  $ED_{90}$ , and  $ED_{99.9}$ ) for metamorphic inhibition of the irradiated  $N_1$  to the  $N_2$  were statistically calculated as 36 Gy, 146 Gy and 174 Gy, respectively. Among the various pre-imaginal stages, the third instar female nymph ( $N_3$ -♀) was found to be the most radio-resistant as ED values for 50, 90 and 99.9% inhibition of metamorphosis of the irradiated  $N_3$ -♀ to the female adult were determined as 140 Gy,



**Fig. 2.** Effect of various doses of gamma radiation applied to *Phenacoccus solenopsis*  $N_1$  nymphs (crawlers) on the prevention of metamorphosis into either  $N_2$  and subsequently into either adult males or adult females. Means followed by the same letter within each of the 3 molts are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in the graphs are back transformed. Number of replicates = 10. Fifty crawlers within each treatment regimen constituted 1 replicate.

301 Gy and 340 Gy, respectively. These doses were much higher than the ED values observed for metamorphic inhibition in other ontogenetic stages ( $F = 65.9$  among  $ED_{50}$ ;  $F = 18.9$  among  $ED_{90}$ ;  $F = 16.4$  among  $ED_{99.9}$ ;  $df = 5, 54$ ;  $P < 0.05$ ) (Table 1).

#### Effect of Irradiation of the Different Pre-imaginal Stages on Prevention of Adult Formation

Development of irradiated crawlers to the adult stage decreased with increasing dose of radiation (Fig. 2). Doses of 40 Gy and 100 Gy completely prevented male and female adult formation, respectively, when treated as crawlers. A dose of 100 Gy applied to the  $N_2$  completely inhibited male and female adult formation (Fig. 3). Both development and survival of irradiated  $N_3$ -♀ to the adult were prevented by 300 Gy (Fig. 4). A dose of 200 Gy applied to the  $N_3$ -♂ completely inhibited male adult formation (Fig. 5), while 400 Gy inhibited  $N_4$ -♂. A dose of 300 Gy applied to the  $N_4$ -♂ completely inhibited male adult formation (Fig. 6).

Therefore, a decrease in radiation impact was evident with an increase in ontogenetic stage. Effective doses for 99.9% ( $ED_{99.9}$ ) in male adult formation from irradiated  $N_1$ ,  $N_2$ ,  $N_3$ -♂ and  $N_4$ -♂ were computed as 32 Gy, 85 Gy, 161 Gy and 270 Gy, respectively. Whereas, the effective doses for 99.9% check ( $ED_{99.9}$ ) in female adult formation from irradiated  $N_1$ ,  $N_2$ ,  $N_3$ -♀ were 74 Gy, 149 Gy and 340 Gy, respectively, they were relatively higher doses than the ED values needed to prevent male adult formation (Table 2).

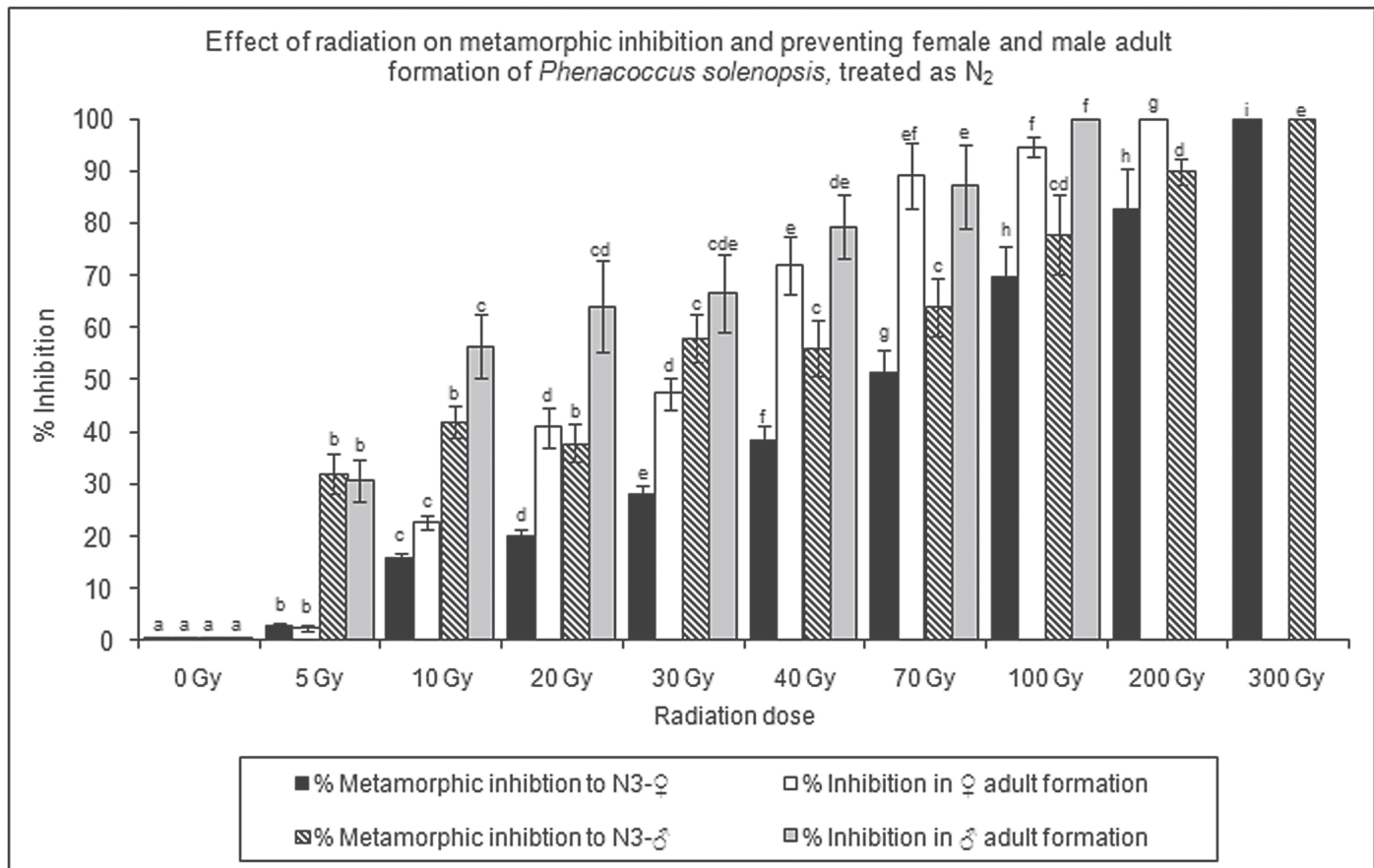
#### Effect of Irradiation in Pre-imaginal Stages on Reproductive Behavior

Oviposition rate and egg viability exhibited an inverse relationship with increasing doses and with the progression of ontogenetic development. Egg fertility in adults developed from irradiated crawlers ( $N_1$ ) was drastically affected with no egg hatch at 30 Gy. The radiosensitivity of first instar nymph ( $N_1$ ) was not markedly different from that of second instar nymph ( $N_2$ ) in terms of reproductive inhibition. For instance, a dose of 20 Gy resulted in 8.5% and 10.9% fertility in adults that developed from  $N_1$  and  $N_2$  respectively. Further, in case of third instar female nymphs ( $N_3$ -♀), the dose of irradiation was negatively correlated with ovipositional performance, and with the fertility of adults derived from  $N_3$ -♀ ( $F = 278.7$ ;  $df = 4, 45$ ;  $P < 0.05$ ). Thus, for example, the 20 Gy dose resulted in 18.3% fertility in the resultant adult, which revealed that the older developmental stages were the more radio-resistant (Table 3). A dose of 40 Gy applied to third instar female nymphs resulted in 63.3% adult formation, all of which were completely sterile. The effective doses applied to the  $N_1$ ,  $N_2$  and  $N_3$ -♀ stages for inducing almost complete sterility ( $ED_{99.9}$ ) were 25.7 Gy, 27.3 Gy and 35.5 Gy, respectively (Fig. 7).

### EFFECT OF GAMMA RADIATION ON REPRODUCTIVE BEHAVIOR OF IMAGINAL STAGES AND THEIR $F_1$ PROGENY

#### Oviposition and Fertility of Adults Irradiated in Different Age Groups

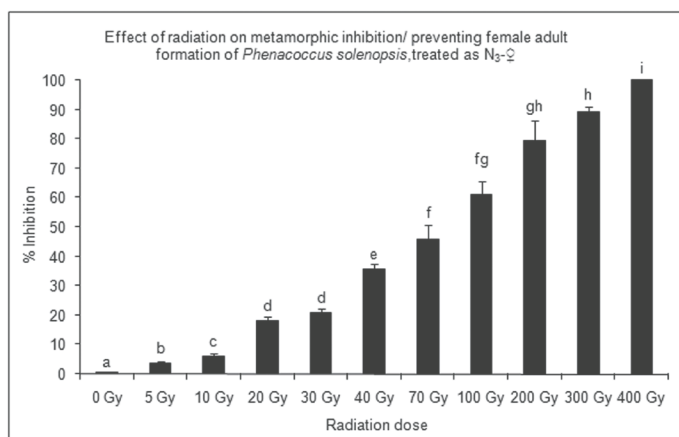
The oviposition rate of irradiated female adults in different age groups decreased with increasing dose. For example, in the case of irradiated 5–6 d female adults, the total eggs laid were observed to be 248 at 5 Gy, which was almost the same as the total eggs laid in the control (256); whereas, an 18–67% reduction in total laid eggs was observed within the dose range of 10–500 Gy when compared to the control ( $F = 734.8$ ;  $df = 11, 108$ ;  $P < 0.05$ ). Further, in case of 11–12



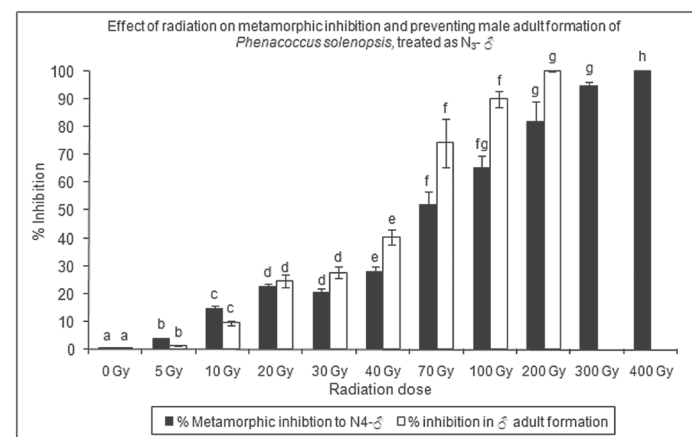
**Fig. 3.** Effect of various doses of gamma radiation applied to *Phenacoccus solenopsis* N<sub>2</sub> nymphs on the prevention of metamorphosis into either N<sub>3</sub>-♂s and N<sub>3</sub>-♀s or subsequently into adult males and females. Means followed by same letter within each of the 4 molts are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in graph are back transformed. Number of replicates = 10. Fifteen N<sub>2</sub> within each treatment regimen constituted 1 replicate.

d-old gravid females—where 500 Gy caused only a 42.1% reduction in number of eggs laid compared with the control—the relative impact of irradiation was relatively less than that observed in case of irradiated younger females in which the reduction in number of eggs laid reached

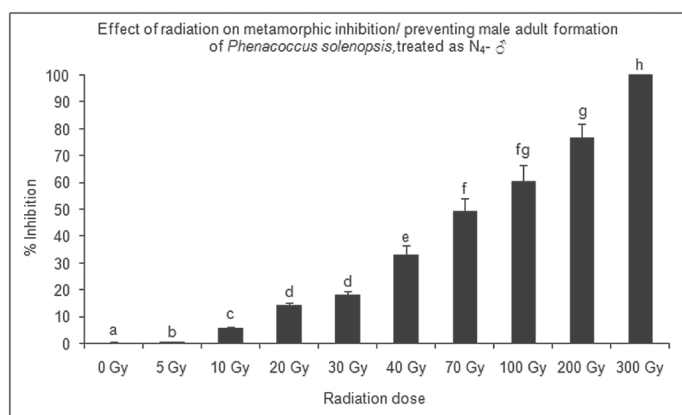
a low of 72% for 0-1 d-old females ( $F = 112.8$ ;  $df = 11, 108$ ;  $P < 0.05$ ). Irradiation drastically affected the fertility of 0-1 d-old female adults ( $F = 512.3$ ;  $df = 6, 63$ ;  $P < 0.05$ ). For instance, 70 Gy or more absolutely inhibited hatching of eggs laid by irradiated 0-1d-old females. Irradia-



**Fig. 4.** Effect of various doses of gamma radiation applied to *Phenacoccus solenopsis* N<sub>3</sub>-♀ nymphs on the prevention of metamorphosis into female adults. Means followed by same letter within the last molt are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in the graphs are back transformed. Number of replicates = 10. Fifteen N<sub>3</sub>-♀s within each treatment regimen constituted 1 replicate.



**Fig. 5.** Effect of various doses of gamma radiation applied to *Phenacoccus solenopsis* N<sub>3</sub>-♂ nymphs on the prevention of metamorphosis into either N<sub>4</sub>-♂ males or adult males. Means followed by the same letter within each of the 2 molts are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in the graphs are back transformed. Number of replicates = 10. Fifteen N<sub>3</sub>-♂s within each treatment regimen constituted 1 replicate.



**Fig. 6.** Percent inhibition of development of *Phenacoccus solenopsis* N<sub>4</sub>-♂ nymphs into male adults by various doses of gamma radiation applied to the N<sub>1</sub>-♂ nymphs. Means followed by same letter are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in the graphs are back transformed. Number of replicates = 10. Fifteen N<sub>4</sub>-♂s within each treatment regimen constituted 1 replicate.

tion of pre-gravid (5–6 d-old) females resulted in 100% egg inviability at 200 Gy or more, whereas 11–12 d-old females irradiated with around 400 Gy produced no viable eggs (complete sterility) (Table 4).

After the dose response test on fertility of females, the ED<sub>99.9</sub> doses for sterility were computed as 52, 138 and 374 Gy for females irradiated at the ages 0–1d, 5–6d (pre-gravid), and 11–12d (gravid), respectively, ( $F = 259.5$ ;  $df = 2, 27$ ;  $P < 0.05$ ) (Fig. 8).

#### Parent (P) Irradiation-Induced Effects on Metamorphosis and Development in F<sub>1</sub> Progeny

Prevention of development of the F<sub>1</sub> N<sub>2</sub> from the N<sub>1</sub> could be considered as a criterion for efficacy of phytosanitary irradiation treatment against this species, as  $> 400$  Gy would be required to prevent egg hatch when the irradiated gravid female contained eggs that were ready to hatch (Table 4). Effective doses for 50%, 90% and 99.9% metamorphic inhibition of F<sub>1</sub> generation N<sub>1</sub> nymphs to N<sub>2</sub> nymphs were computed as 75, 150, and 169 Gy, respectively, when the most radio-resistant (11–12 d) female parents were irradiated (Table 5). The percentages of

irradiated F<sub>1</sub> N<sub>1</sub> nymphs that developed to the N<sub>2</sub> stage for 0, 5, 10, 20, 30, 40, 70, 100, and 200 Gy doses applied to 11–12 d-old adult females were 98, 90, 84, 81, 71, 67, 44, 8.6, and 0%, respectively. F<sub>1</sub> adult formation in descendants from sub-sterilized 0–1d male adults also were significantly reduced with increasing doses of radiation as compared to the control ( $F = 58.5$ ;  $df = 5, 54$  for female adults; and  $F = 21.2$ ;  $df = 4, 45$  for male adults;  $P < 0.05$ ).

Differential responses of male and female development due to irradiation were observed. For example, 30 Gy resulted in 10.6% F<sub>1</sub> female adult formation, whereas it completely inhibited F<sub>1</sub> male adult formation (Table 6). F<sub>1</sub> adult formation in descendants from irradiated 5–6 d-old P females, was significantly reduced with increasing doses of radiation (5–70 Gy) as compared to the control ( $F = 70.6$ ;  $df = 6, 63$  for female adult and  $F = 25.9$ ;  $df = 5, 54$  for male adult;  $P < 0.05$ ). Furthermore, in the case of the F<sub>1</sub> progeny of 11–12 d-old female mealybugs, there was a dose-dependent decrease in adult male ( $F = 40.0$ ;  $df = 6, 63$ ;  $P < 0.05$ ) and adult female ( $F = 62.7$ ;  $df = 7, 72$ ;  $P < 0.05$ ) development, with an apparently greater radiosensitive response by the males. Thus, for example, 70 Gy resulted in 2.2% F<sub>1</sub> female adult formation, while it completely inhibited F<sub>1</sub> male adult formation.

The estimated effective radiation doses used to treat P generation females for 99.9% inhibition of survival of F<sub>1</sub> progeny up to the F<sub>1</sub> male adult were 28, 35, and 61 Gy when applied to 0–1, 5–6, and 11–12 (gravid) d-old P generation females, respectively. Likewise, the ED<sub>99.9</sub> of P generation females for 99.9% inhibition of survival of F<sub>1</sub> progeny up to F<sub>1</sub> female adult were 36, 55, and 79 Gy when treated as 0–1, 5–6, 11–12 d-old P generation females, respectively (Fig. 9). Gravid (11–12 d-old) females were found to require quite high radiation doses to inhibit 50, 90, and 99.9% of F<sub>1</sub> male and female adult formation ( $F = 46.0$  among ED<sub>50</sub>;  $F = 64.8$  among ED<sub>90</sub>;  $F = 66.2$  among ED<sub>99.9</sub>;  $df = 5, 54$ ;  $P < 0.05$ ). The radio-sensitivity of the parents of with respect to the formation of adult male F<sub>1</sub> progeny was evidently higher than their radio-sensitivity with respect to the formation of adult female progeny.

#### Impact on F<sub>1</sub> Reproduction Induced by Sub-sterilizing Irradiation of the Parent

The F<sub>1</sub> oviposition rates along with their oviposition periods were affected due to the sub-sterilization of P (parent) generation mealybugs. For instance, in the case of 11–12 d-old females, 5–70 Gy caused 22.6–93.6% reduction in total eggs laid per F<sub>1</sub> female compared to the

**Table 1.** Prevention of molting to the subsequent developmental stage in *Phenacoccus solenopsis* by gamma irradiation of the various pre-imaginal stages.

Pre-imaginal stage irradiated and the target stage to be prevented	Estimated dose* (Gy) to prevent 50, 90, and 99.9% from reaching the succeeding stage			Linear regression equation
	ED <sub>50</sub>	ED <sub>90</sub>	ED <sub>99.9</sub>	
N <sub>1</sub> to N <sub>2</sub>	36 ± 3a	146 ± 10a	174 ± 12a	y = 0.363x + 36.86 R <sup>2</sup> = 0.5371
N <sub>2</sub> to N <sub>3</sub> -♂	55 ± 4b	212 ± 18b	251 ± 21b	y = 0.254x + 36.07 R <sup>2</sup> = 0.7223
N <sub>2</sub> to N <sub>3</sub> -♀	106 ± 8c	229 ± 16c	260 ± 19b	y = 0.323x + 15.87 R <sup>2</sup> = 0.8696
N <sub>3</sub> -♂ to N <sub>4</sub> -♂	114 ± 8cd	242 ± 16cd	273 ± 18bc	y = 0.314x + 14.01 R <sup>2</sup> = 0.8771
N <sub>3</sub> -♀ to ♀ adult	140 ± 11d	301 ± 24d	340 ± 27c	y = 0.248x + 15.32 R <sup>2</sup> = 0.8702
N <sub>4</sub> -♂ to ♂ adult	120 ± 9cd	240 ± 18cd	270 ± 21b	y = 0.333x + 9.94 R <sup>2</sup> = 0.9047
F-value	F = 65.9	F = 18.9	F = 16.4	for slopes: F = 9.03

\* ED<sub>50</sub>, ED<sub>90</sub>, and ED<sub>99.9</sub> are the effective doses that prevent molting and induce either 50, 90 or 99.9% mortality, respectively. Means followed by same letter within a column are not significantly different at  $P < 0.05$  level (ANOVA,  $df = 5, 54$ ; followed by LSD post-test). Number of replicates = 10. Fifty insects for N<sub>1</sub>, and 12–15 insects each for the N<sub>2</sub>, N<sub>3</sub>♂, N<sub>3</sub>♀ and N<sub>4</sub>-♂ instars constituted 1 replicate.

**Table 2.** Irradiation doses applied to various nymphal stages of *Phenacoccus solenopsis* required to prevent either 50, 90 or 99.9% of the irradiated nymphs from ultimately developing into adults.

Pre-imaginal stage irradiated to prevent adult formation	Estimated dose <sup>a</sup> (Gy) for prevention of 50, 90, and 99.9% from reaching the adult stage			Regression equation
	ED <sub>50</sub>	ED <sub>90</sub>	ED <sub>99.9</sub>	
N <sub>1</sub> to ♂ adult	10.7 ± 0.78a	27.7 ± 2.0a	31.9 ± 2.3a	y = 2.3533x + 24.669 R <sup>2</sup> = 0.7653
N <sub>1</sub> to ♀ adult	11.1 ± 0.73a	62 ± 4b	74.6 ± 4.9b	y = 0.7858x + 41.246 R <sup>2</sup> = 0.5656
N <sub>2</sub> to ♂ adult	20.8 ± 1.9b	72 ± 6.7b	84.6 ± 7.9b	y = 0.7817x + 33.712 R <sup>2</sup> = 0.7148
N <sub>2</sub> to ♀ adult	48.3 ± 2.8c	129 ± 7.6c	149 ± 8.8c	y = 0.4958x + 26.023 R <sup>2</sup> = 0.6691
N <sub>3</sub> -♂ to ♂ adult	69.4 ± 4.9d	143 ± 10c	161 ± 12c	y = 0.5421x + 12.367 R <sup>2</sup> = 0.8273
N <sub>3</sub> -♀ to ♀ adult	140 ± 11e	301 ± 24d	340 ± 27e	y = 0.2484x + 15.322 R <sup>2</sup> = 0.8702
N <sub>4</sub> -♂ to ♂ adult	120 ± 9e	240 ± 18d	270 ± 21d	y = 0.3331x + 9.9394 R <sup>2</sup> = 0.9047
F-value	F = 217.5	F = 120.5	F = 115.1	for slope: F = 188.5

<sup>a</sup>ED<sub>50</sub>, ED<sub>90</sub>, and ED<sub>99.9</sub> are effective doses that prevent either 50%, 90% or 99.9% from developing into adults, respectively. Means followed by same letter within a column are not significantly different at  $P < 0.05$  level (ANOVA, df = 6, 63; followed by LSD post-test). Number of replicates = 10. Fifty insects for N<sub>1</sub>, and 12–15 insects each for the N<sub>2</sub>, N<sub>3</sub>♂, N<sub>3</sub>♀ and N<sub>4</sub>-♂ instars constituted 1 replicate.

control ( $F = 462.4$ ; df = 6, 63;  $P < 0.05$ ). Fertility of F<sub>1</sub> adults derived from P generation 0–1 d female adults was completely inhibited at 30 Gy ( $F = 200.6$ ; df = 4, 45;  $P < 0.05$ ). Furthermore, F<sub>1</sub> adults derived from irradiated P 5–6-d-old and P 11–12-d-old females were completely infertile at 40 Gy and 70 Gy, respectively (Table 7).

The doses used to irradiate 0–1, 5–6, 11–12 d-old P generation females estimated to induce 99.9% F<sub>1</sub> sterility were 26, 35, and 57 Gy, respectively ( $F = 109.1$ ; df = 2, 27;  $P < 0.05$ ) (Fig. 10).

#### PHYSICO-CHEMICAL AND NUTRITIONAL QUALITY OF POTATOES SUBJECTED TO PHYTOSANITARY IRRADIATION

No significant differences ( $P \leq 0.05$ ) were observed for weight loss, color, total soluble solids, titratable acidity, pH, vitamin C content, or  $\beta$ -carotene content between non-irradiated potatoes and those irradiated at either 200 or 400 Gy and then stored at 5 °C for 7 d (Table 8).

## Discussion

The present radiobiological investigation of *P. solenopsis* was undertaken to understand physiological or developmental responses of each of the ontogenetic stages submitted to doses of irradiation that could be used in phytosanitary irradiation treatments. This study would not only predict the efficiency of using phytosanitary irradiation, but also indicate the carry-over effects of sublethal irradiation that could be apparent due to a logistical limitation. This study might also help in steering overall phytosanitary irradiation strategy in the direction of taking advantage of the considerable sustained impacts of irradiation on pests with diffuse chromosomes, which include Hemiptera, Lepidoptera and Arachnida.

#### BIO-EFFICACY OF RADIATION ON PRE-IMAGINAL STAGES, *PHE-NACOCOCCUS SOLENOPSIS*

Metamorphosis is a physiological process implying active cell division. It is, therefore, expected to be highly susceptible to irradiation. In the present study, the mortality of the pre-imaginal stages was assessed

in terms of metamorphic inhibition due to irradiation at the different ontogenetic stages. Survival of growing nymphs to the adult stage was observed to decrease with increasing radiation doses. It showed that some irradiated crawlers developed to the adult stage at doses up to 70 Gy range but these adults did not reproduce. Similarly, Ravuiwasa et al. (2009) reported that *Planococcus minor* Maskell (Hemiptera: Pseudococcidae) treated as crawlers with 150–250 Gy survived to adult stage but were unable to reproduce. Another study conducted on *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae) demonstrated that 1.3–11.3% of adults developed from treated crawlers at 100–200 Gy, but reproduction was inhibited at 200 Gy (Doan et al. 2012).

The present study demonstrated that adult development from irradiated N<sub>2</sub> stage *P. solenopsis* ranged from 61% at 5 Gy to 3.3% at 100 Gy, whereas, oviposition was observed only at doses up to 30 Gy. Furthermore, irradiation with 30 Gy inhibited the hatching of the eggs laid by adults that had developed from treated N<sub>1</sub> and N<sub>2</sub> stages. Doan et al. (2012) reported that 8–27% of *D. neobrevipes* N<sub>3</sub> nymphs survived to the adult stage at all tested irradiation doses (100–250 Gy), and that the adults that developed from N<sub>3</sub> females irradiated with 200–250 Gy were completely sterile. In the present study some adults developed from female N<sub>3</sub> nymphs irradiated at 70–300 Gy, but none laid viable eggs.

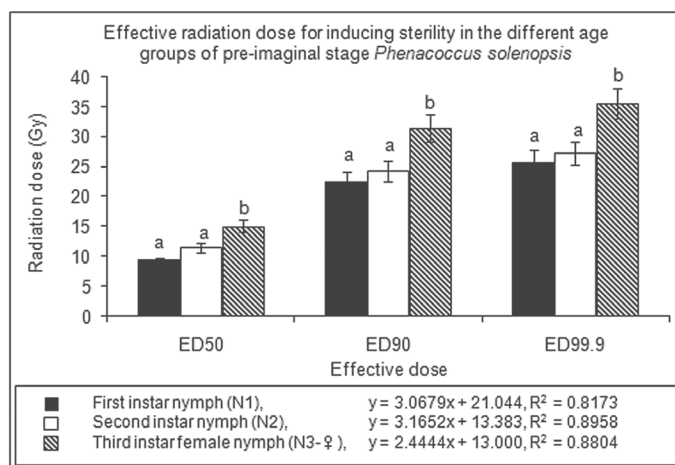
*Phenacoccus solenopsis* male nymphs were found to be more radiosensitive than female nymphs in terms of inhibition of development, which was consistent with the findings of Jacobsen & Hara (2003) and Seth et al. (2009). For example, male adult formation of irradiated crawlers of *P. solenopsis* was completely inhibited at 40 Gy, whereas the corresponding complete inhibition of female adult formation required 100 Gy.

Effective doses for 99.9% prevention of metamorphosis to the succeeding stage were 174 Gy applied to the N<sub>1</sub> stage and 340 Gy applied to the N<sub>3</sub>-♀ stage. Radiosensitivity of irradiated pre-imaginal stages of *P. solenopsis* decreased as nymphs grew and developed. The increased radiosensitivity in developing pre-imaginal stages might be attributed to the fact that these ontogenetic stages require radical cell division and differentiation as suggested in Tilton & Brower (1983) and Jacobsen & Hara (2003). Therefore, the third instar female nymph being the penultimate stage to adult development was the most radio-tolerant among various irradiated pre-imaginal stages.

**Table 3.** Reproductive performance—number of eggs laid and percent that hatched—of *Phenacoccus solenopsis* females that were gamma-irradiated while they were still in various pre-imaginal stages.

Pre-imaginal stage irradiated	Reproductive parameters	Radiation doses applied to parent generation							F-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	
First instar	Eggs laid per female	269.6 ± 19.8a	215.7 ± 16.3a	124.8 ± 7.0b	35.6 ± 2.7c	9.9 ± 1.0d	0e	—	F = 782, df = 5,54
	Egg hatch (%)	98.6 ± 0.76a	58.9 ± 2.4b	24.6 ± 2.9c	8.5 ± 1.0d	0e	—	—	F = 475, df = 4,45
Second instar	Eggs laid per female	260.2 ± 12.7a	224.4 ± 11.9b	154.3 ± 7.3c	49.5 ± 2.6d	21.4 ± 1.1e	0f	—	F = 842, df = 5,54
	Egg hatch (%)	96.9 ± 1.2a	69.9 ± 2.5b	28.7 ± 1.4c	10.9 ± 0.85d	0e	—	—	F = 668, df = 4,45
Third instar female	Eggs laid per female	242.5 ± 14.3a	215.2 ± 16.2a	157.3 ± 8.0b	119.5 ± 4.4c	80.3d ± 7.3d	60.3 ± 4.3e	0f	F = 672, df = 6,63
	Egg hatch (%)	97.5 ± 0.31a	82.3 ± 5.2b	38.7 ± 1.0c	18.3 ± 1.2d	6.9 ± 0.44e	0f	—	F = 279, df = 5,54

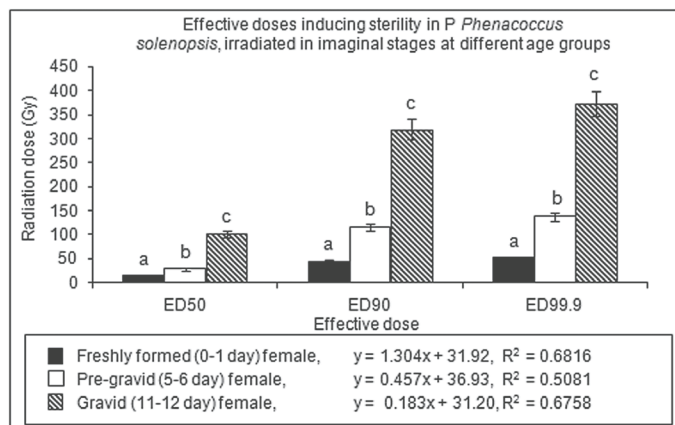
Means ± SE followed by same letter within a row are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Percentage data were arcsine transformed before ANOVA, but data in the table are back transformations. Number of replicates = 10. Average data of 7 parthenogenetic females within each treatment regimen constituted 1 replicate.

**Fig. 7.** Estimated doses to induce 50, 90, and 99.9% sterility in *Phenacoccus solenopsis* irradiated in different pre-imaginal stages. Means followed by same letter within a level of control are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Number of replicates = 10. Average of the data of 7 females within each treatment regimen constituted 1 replicate.

Phytosanitary irradiation differs from all other commercial treatments in 1 important technical consideration that the end point of the treatment may be prevention of further biological development and/or reproduction, rather than acute mortality (Hallman 2011). Irradiation protracted the developmental period of male and female mealybugs proportionately to the doses applied. However, radiation-mediated delay in development of male mealybugs was greater than in female mealybugs. This protraction of the developmental period due to irradiation could have a physiological effect on its later development, possibly resulting in sterility of subsequent adults (Ravuiwasa et al. 2009). In agreement with this suggestion, the present study concluded that reproductive sterilization was induced in adults that developed from irradiated pre-imaginal stages at estimated doses of 25 to 36 Gy.

#### BIO-EFFICACY OF RADIATION ON IMAGINAL STAGES, *PHENACOCUS SOLENOPSIS*

The target of a phytosanitary treatment must be the most tolerant life stage of quarantine pests that can infest the shipped commodity,

**Fig. 8.** Estimated doses to induce 50, 90, and 99.9% sterility in *Phenacoccus solenopsis*, irradiated as adults in 3 age groups. Means followed by same letter within a level of control are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Number of replicates = 10. Average of the data of 7 females within each treatment regimen constituted 1 replicate.

**Table 4.** Reproductive performance—number of eggs laid and percent that hatched—of *Phenacoccus solenopsis* females gamma-irradiated as adults in different age groups.

Age group of adult females when irradiated	Reproductive parameters	Radiation doses												F-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy	
Freshly formed female (0–1 d-old)	Eggs laid per female	224.8 ± 17.1a	220.7 ± 8.8a	187.7 ± 14.6ab	185.4 ± 17.1ab	190.1 ± 9.8ab	166.7 ± 14.6bc	132.4 ± 14.2c	141.3 ± 7.3c	121.5 ± 13.8c	96.6 ± 7.8cd	62.3 ± 12.1d	60.6 ± 6.6d	F=478.3 df=11,108
	Egg hatch (%)	97.5 ± 0.3a	60.2 ± 3.8b	20.8 ± 2.0c	13.6 ± 1.1d	8.2 ± 0.9e	1.4 ± 0.1f	0g	—	—	—	—	—	F = 512.3 df = 6,63
Pre-gravid female (5–6 d-old)	Eggs laid per female	256.9 ± 24.7a	248.3 ± 17.1a	199.9 ± 15.6ab	202.2 ± 14.7ab	209.3 ± 18.8ab	173.0 ± 15.3b	159.6 ± 9.1bc	139.6 ± 11.5c	108.5 ± 10.6d	91.2 ± 5.9d	95.6 ± 7.0d	85.0 ± 5.1d	F = 734.8 df=11,108
	Egg hatch (%)	97.8 ± 0.4a	87.4 ± 5.7ab	75.0 ± 5.1b	51.5 ± 2.8c	12.3 ± 0.8d	6.4 ± 1.4e	2.7 ± 0.6f	1.2 ± 0.3g	0h	—	—	—	F = 238.3 df = 8,81
Gravid female (11–12 d-old)	Eggs laid per female	268.6 ± 28.4a	267.5 ± 10.5a	244.1 ± 12.3b	225.7 ± 28.5bc	247.1 ± 9.6bc	240.8 ± 18.6bc	239.1 ± 24.3bc	202.1 ± 13.9bc	209.6 ± 16.3bc	181.3 ± 15.7bc	180.2 ± 15.1bc	155.2 ± 12.5c	F = 112.8 df=11,108
	Egg hatch (%)	97.6 ± 0.5a	92.4 ± 1.9ab	84.6 ± 5.9b	69.4 ± 2.7c	60.9 ± 2.8d	43.7 ± 3.7e	28.1 ± 2.6f	15.9 ± 1.9g	4.9 ± 1.2h	2.4 ± 0.9i	1.1 ± 0.6i	0j	F = 191.0 df=11,108

Means followed by the same letter within a row are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test); percentage data were arcsine transformed before ANOVA, but data in table are back transformed. Number of replicates = 10. Average data of 7 parthenogenetic females within each treatment regimen constituted a replicate.

regardless of whether that stage is less commonly found than others. The radiotolerance of an arthropod species increases progressively as the successive immature stages develop (Hallman et al. 2010). When the adult stage can occur in the commodity, then the measure of treatment efficacy should be the prevention of reproduction of adult. The adult female mealybug is a serious surface quarantine pest. As thelytokus parthenogenesis was observed to be the main reproductive mode in some mealybug species including *P. solenopsis*, the female adults necessarily were the primary focus of the present study. Furthermore, our elucidation of development and reproduction of the  $F_1$  progeny due to sub-sterilizing doses applied to P generation adults—together with our findings on radiosensitivity—are useful for developing PI treatments that would be efficacious for large consignments of various host commodities.

In similar investigations involving parthenogenetic female adults, *D. neobrevipes* (Doan et al. 2012) and *P. minor* (Ravuiwasa et al. 2009) were studied to determine the most tolerant stage and the minimum dose to control these mealybug species. The measure of efficacy for a PI treatment may be prevention of adult emergence, induction of adult sterility, prevention of further  $F_1$  development, or  $F_1$  sterility. The present study quantified the degree of sterility induced in female mealybugs by irradiation of females of different age groups. As the females aged, their radio-tolerance increased. Hence, the  $ED_{99.9}$  doses were 52, 138, and 374 Gy for 0–1, 5–6, and 11–12 d-old females, respectively.

The present study demonstrated that 4.8% crawlers emerged from eggs laid by gravid female of *P. solenopsis* irradiated with 200 Gy, but none of them developed into second instar nymph ( $N_2$ ). Hence, 200 Gy might serve as a phytosanitary treatment against this mealybug. Large-scale confirmatory testing in which many thousands of gravid adult females are irradiated at a single dose that allows no  $F_1$   $N_2$  nymphs to be produced would normally be required to substantiate a confirmatory test for commercial approval and use (Schortemeyer et al. 2011).

The studies conducted on another parthenogenetic species, *P. minor*, found that a dose of 100 Gy caused complete sterility in the parent female mealybug (Ravuiwasa et al. 2009). The higher radioresistance of *P. solenopsis* might be attributed to its ovoviviparous nature, exhibiting embryonic development within the female body; therefore, egg hatch could be observed within 1–2 h of egg laying.

In a similar finding, 200–250 Gy for inducing adult sterility was reported in asexually reproducing and ovoviviparous mealybug species, *D. neobrevipes* (Doan et al. 2012). Irradiation affected the physiology of insects as was reflected in their impaired reproductive behavior—viz., prolonged pre-oviposition period, shortened oviposition period and enhanced degree of sterility—and reduced survival, which would result in successful phytosanitary radiation treatments. Furthermore, irradiation was observed to delay the onset of oviposition in adults developed from pre-imaginal and imaginal stages of *P. solenopsis*. Similarly, Doan et al. (2012) indicated that female survivors that developed from irradiated  $N_1$ ,  $N_2$ ,  $N_3$ -♀ nymphs, and irradiated adult females of *D. neobrevipes* showed delayed reproduction.

The order of radio-tolerance among different life stages in terms of induced sterility in the adult stage of *P. solenopsis* was  $N_1 < N_2 < N_3$  ♀ < adult. This pattern of radioresistance was consistent with the other parthenogenetic mealybugs (Ravuiwasa et al. 2009; Doan et al. 2012).

Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security; therefore, live but inviable insects may occur with the exported commodity, eliminating the value of inspection for live target pests and placing reliance on verification of treatment application and efficacy.

Studies on mechanisms behind tolerance to ionizing radiation in mealybugs were reported by Chandra (1963a, b). Cytological examina-

**Table 5.** Estimated gamma radiation doses (Gy) applied to P generation *Phenacoccus solenopsis* adult females of different age groups to prevent the metamorphosis of  $F_1$  first instars ( $N_1$ ) into second instars ( $N_2$ ).

Age group of P generation adult females when irradiated	Estimated dose (Gy) to prevent the molt of $F_1$ $N_1$ to $N_2$			Regression equation
	ED <sub>50</sub>	ED <sub>90</sub>	ED <sub>99.9</sub>	
Freshly formed female (0–1 d-old)	21.1 ± 1.2a	37.5 ± 2.1a	41.6 ± 2.3a	y = 2.436x – 1.47 R <sup>2</sup> = 0.96
Pre-gravid female (5–6 d-old)	34.2 ± 1.9b	61.1 ± 3.4b	67.7 ± 3.8b	y = 1.490x – 1.05 R <sup>2</sup> = 0.99
Gravid female (11–12 d-old)	74.7 ± 4.9c	150 ± 10.1c	169 ± 11.6c	y = 0.528x + 10.57 R <sup>2</sup> = 0.88
F-value	F = 218.1	F = 247.3	F = 270.6	for slope: F = 279.4

ED<sub>50</sub>, ED<sub>90</sub>, and ED<sub>99.9</sub> are effective doses that prevent 50%, 90%, 99.9%, of  $F_1$   $N_1$  to molt to  $N_2$ , respectively. Means followed by same letter within a column are not significantly different at  $P < 0.05$  level (ANOVA, df = 2, 27; followed by LSD post-test).

Number of replicates = 10. Fifty  $F_1$   $N_1$  nymphs from each treatment regimen constituted 1 replicate.

**Table 6.** Survival to the adult stage of the  $F_1$  progeny of *Phenacoccus solenopsis* P generation adult females that were gamma-irradiated in different age groups.

Age group of P generation adult females when irradiated	Sex of $F_1$ adults formed	Percent survival to adulthood <sup>a</sup> of the $F_1$ progeny of a parent irradiated as an adult								F-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	100 Gy	
Freshly formed female (0–1 d-old)	Male	21.9 ± 2.0a	18.0 ± 2.6a	8.6 ± 0.49b	5.2 ± 0.36c	0d	—	—	—	F = 21.2 df = 4,45
	Female	62.8 ± 2.9a	50.0 ± 2.2b	32.6 ± 1.8c	17.6 ± 1.3d	10.6 ± 0.83e	0f	—	—	F = 58.5 df = 5,54
Pre-gravid female (5–6 d-old)	Male	17.1 ± 1.5a	16.1 ± 1.2ab	14.2 ± 1.4ab	12.6 ± 0.99b	3.3 ± 0.24c	0d	—	—	F = 25.9 df = 5,54
	Female	72.1 ± 3.4a	56.6 ± 3.13b	39.9 ± 2.1c	28.0 ± 1.4d	14.5 ± 0.81e	3.2 ± 0.21e	0f	—	F = 70.6 df = 6,63
Gravid female (11–12 d-old)	Male	19.4 ± 1.4a	18.7 ± 1.4a	15.7 ± 1.2ab	11.3 ± 1.2b	8.0 ± 0.45c	3.3 ± 0.24d	0e	—	F = 40.0 df = 6,63
	Female	74.5 ± 5.8a	58.6 ± 2.5b	46.6 ± 2.5c	35.3 ± 2.6d	22.0 ± 1.72e	9.6 ± 0.82f	2.2 ± 0.17g	0h	F = 62.7 df = 7,72

<sup>a</sup>Means followed by the same letter within a row are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Data were arcsine transformed before ANOVA, but data in table are back transformed. Number of replicates = 10. Fifty  $F_1$  eggs from each treatment regimen constitute 1 replicate.

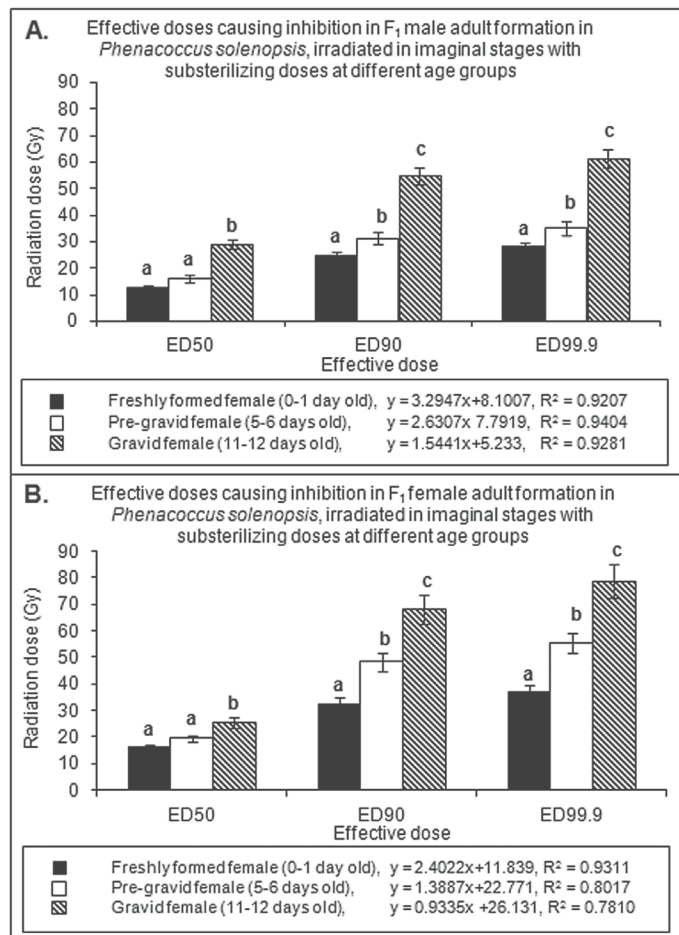
**Table 7.** Reproductive performance—number of eggs laid and percent that hatched—of the  $F_1$  female progeny of *Phenacoccus solenopsis* P generation adult females that were gamma-irradiated in different age groups.

Age group of P generation adult females when irradiated	Reproductive parameters	Radiation doses							F-value
		0 Gy	5 Gy	10 Gy	20 Gy	30 Gy	40 Gy	70 Gy	
Freshly formed female (0–1 d-old)	Eggs laid per female	264 ± 18.5a	188 ± 14.7b	120 ± 8.9c	87 ± 6.8d	24 ± 1.0e	—	—	F = 562.3, df = 4, 45
	Egg hatch (%)	98.3 ± 0.29a	67.4 ± 3.1b	26.5 ± 2.5c	13.3 ± 2.4d	0e	—	—	F = 200.6, df = 4, 45
Pre-gravid female (5–6 d-old)	Eggs laid per female	291.0 ± 17.9a	253.2 ± 21.9a	143 ± 8.4b	110 ± 7.5c	88 ± 5.8d	49 ± 2.3e	0f	F = 365.7, df = 5, 54
	Egg hatch (%)	98.4 ± 0.40a	81.7 ± 3.2b	34.2 ± 2.1c	28.1 ± 1.1d	11.0 ± 0.98e	0f	—	F = 175.5, df = 5, 54
Gravid female (11–12 d-old)	Eggs laid per female	266 ± 23.2a	206 ± 25.5ab	202 ± 20.8ab	164 ± 19.9bc	125 ± 8.5c	89 ± 4.2d	18 ± 2.1e	F = 462.4, df = 6, 63
	Egg hatch (%)	96.2 ± 0.54a	81.9 ± 1.7b	73.4 ± 2.5c	56.3 ± 3.2d	19.3 ± 1.4e	8.4 ± 1.0f	0g	F = 287.7, df = 6, 63

Means followed by same letter within a row are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test); percentage data were arcsine transformed before ANOVA, but data in table are back transformed. Number of replicates = 10, and the average data of 7 parthenogenetic females within each treatment regimen comprised 1 replicate.

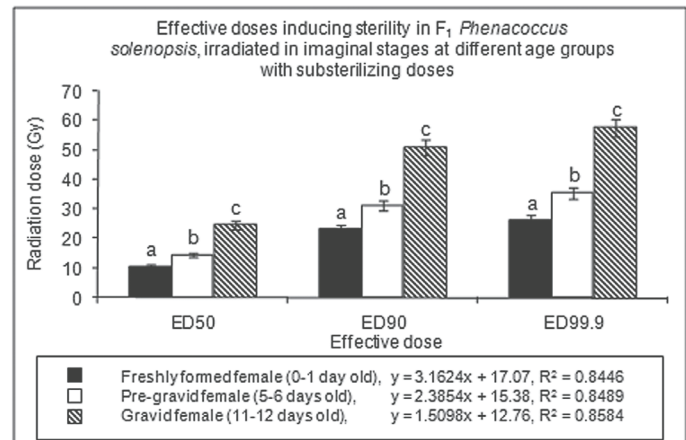
tion of the progeny obtained from paternal irradiation with 1.2 kGy revealed very small chromosome fragments that appeared to retain their individual chromosome identity such as replication, metaphase alignment, and anaphase segregation. Mohan et al. (2012) suggested that there was highly efficient protection of radiation-induced chro-

mosome ends (telomeric repeats) that would prevent the onset of fusion-bridge-breakage-fusion cycles, and that this machinery was complemented by the diffuse centromere property in mealybug chromosomes. This property of the mealybug centromere is distributed throughout the chromosomes.



**Fig. 9.** Estimated doses of gamma radiation applied to 3 different age groups of *Phenacoccus solenopsis* females for preventing their offspring from developing to the adult stage, i.e., prevention of *F*<sub>1</sub> adult formation: A. Doses for preventing *F*<sub>1</sub> male adults, and B. Doses for preventing *F*<sub>1</sub> female adults. Means followed by same letter within a level of control are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Number of replicates = 10. Average of the data of 7 females within each treatment regimen constituted 1 replicate.

A dose range of 250 Gy has been suggested for phytosanitary irradiation of mealybug species (Hofmeyr et al. 2016). A generic treatment comprising at least these 4 families (Aleyrodidae, Coccidae, Diaspididae, and Pseudococcidae) of Hemiptera might be ~250 Gy (Hallman et al. 2010). Many aleyrodids, coccids, diaspidids, and pseudococcids are quarantine pests of commodities currently treated by the APHIS (2016) generic dose of 400 Gy and would be prime candidates for research aimed at lowering the doses applied to host commodities.



**Fig. 10.** Estimated doses of gamma radiation applied to 3 different age groups of *P. Phenacoccus solenopsis* females to induce 50, 90, and 99.9% *F*<sub>1</sub> sterility, i.e., prevent 50, 90, and 99.9% of *F*<sub>2</sub> egg hatch. Means followed by same letter within a level of control are not significantly different at  $P < 0.05$  level (ANOVA followed by LSD post-test). Number of replicates = 10. Average of the data of 7 females within each treatment regimen constituted 1 replicate.

## SENSORY AND NUTRITIONAL QUALITY OF IRRADIATED POTATOES

Present study showed that irradiation at 200 and 400 Gy did not affect the quality of treated potato. Janave & Thomas (1979) reported a slight decrease in  $\beta$ -carotene content in irradiated potatoes. Blessington et al. (2007) noted that storage itself had a much greater detrimental effect on nutritive value of potato than did irradiation at 200 Gy.

As per earlier reports on phytosanitary irradiation on various mealybug species, > 200 Gy has been suggested as an effective quarantine dose for *Pseudococcus comstocki* Kuwana (Hemiptera: Pseudococcidae) (Dohino & Masaki 1995) *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) (Jacobson & Hara 2003), *P. minor* (Ravuiwasa et al. 2009) and *D. neobrevipes* (Doan et al. 2012), whereas  $\leq 200$  Gy was reported as an effective phytosanitary treatment dose for the solenopsis mealybug (Huang et al. 2014). Our data to some extent coincide with those of Huang et al. (2014), with respect to mortality, adult formation, and adult reproductive performance. The results indicated that at least 374 Gy would be required to completely sterilize (prevent *F*<sub>1</sub> egg hatch) when applied to the gravid female of *P. solenopsis* (the most tolerant among all stages). However, the phytosanitary dose could be reduced to around 170 Gy if the measure of efficacy was the prevention of development of *F*<sub>1</sub> *N*<sub>1</sub> into *F*<sub>1</sub> *N*<sub>2</sub> nymphs. Large scale testing is necessary to confirm the final PI dose.

## Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine

**Table 8.** Physiochemical and nutritional parameters of irradiated and non-irradiated potatoes after 7 d of storage at 5 °C. Values within each column were not significant at  $P < 0.05$  (ANOVA,  $n = 5$ ;  $df = 2,12$ ).

Dose (Gy)	Weight loss (%)	Color characteristics			Total soluble solids (%)	Titratable acidity (%)	pH	Vitamin C (mg/100g)	$\beta$ -carotene (mg/100g)
		'L' value	'a' value	'b' value					
0	5.2 $\pm$ 0.67	51.6 $\pm$ 0.15	5.60 $\pm$ 0.26	17.6 $\pm$ 0.32	6.87 $\pm$ 0.33	29 $\pm$ 0.50	6.2 $\pm$ 0.03	3.0 $\pm$ 0.12	0.091 $\pm$ 0.008
200	5.3 $\pm$ 0.83	51.9 $\pm$ 2.03	5.50 $\pm$ 0.32	18.3 $\pm$ 1.13	6.53 $\pm$ 0.17	27 $\pm$ 1.2	6.3 $\pm$ 0.08	3.1 $\pm$ 0.13	0.077 $\pm$ 0.009
400	5.9 $\pm$ 0.76	51.3 $\pm$ 1.69	5.73 $\pm$ 0.57	19.0 $\pm$ 0.53	6.83 $\pm$ 0.09	28 $\pm$ 0.9	6.3 $\pm$ 0.03	3.1 $\pm$ 0.21	0.079 $\pm$ 0.007
F-value	0.26	0.99	0.27	3.0	2.37	2.42	1.18	0.34	0.98

tine Treatments. Financial support from International Atomic Energy Agency, Vienna (vide IAEA funded Research Project, RC-15852/RB) is gratefully acknowledged. Sincere thanks are due to Guy Hallman, FAO/IAEA, Seibersdorf, Austria for his critical observations and meaningful remarks.

## References Cited

- APHIS [Animal and Plant Health Inspection Service]. 2016. Treatment Manual. [https://www.aphis.usda.gov/import\\_export/plants/manuals/ports/downloads/treatment.pdf](https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf)
- AOAC [Association of Official Analytical Chemists]. 2012. Official methods of Analysis, Washington, DC 19th Edition.
- Ben-Dov Y, Miller DR, Gibson, GAP. 2009. Scale Net. A searchable information system on scale insects. <http://www.sel.barc.usda.gov/scalenet/scalenet.htm>. Accessed on 8-VIII-2009.
- Blessington T, Miller Jr JC, Nzaramba MN, Hale AL, Redivari L, Scheuring DC, Hallman GJ. 2007. The effects of low-dose gamma irradiation and storage time on carotenoids, antioxidant activity, and phenolics in the potato cultivar Atlantic. *American Journal of Potato Research* 84: 125-131.
- Chandra HS. 1963a. Cytogenetic analysis following high dosage paternal irradiation in the mealybug *Planococcus citri*. I. Cytology of X1 embryos. *Chromosoma* 14: 310-329.
- Chandra HS. 1963b. Cytogenetic studies following high dosage paternal irradiation in the mealybug *Planococcus citri*. II. Cytology of X1 females and the problem of lecanoid sex determination. *Chromosoma* 14: 330-346.
- DGHS [Directorate General of Health Services]. 2005. Manual of methods of analysis of foods: Fruits and vegetable products. Ministry of Health and Family Welfare, Government of India, New Delhi.
- Doan TT, Nguyen TK, Vo TKL, Cao VC, Tran TTA, Nguyen HHT. 2012. Effects of gamma irradiation on different stages of mealybug *Dysmicoccus neobrevipes* (Hemiptera: Pseudococcidae). *Radiation Physics and Chemistry* 81: 97-100.
- Doan TT, Nguyen TK, Vo TKL, Nguyen TL, Cao VC, Tran TTA, Nguyen HHT. 2016. Irradiation phytosanitary treatment against the mealybugs *Dysmicoccus neobrevipes*, *Planococcus lilacinus*, and *Planococcus minor* (Hemiptera: Pseudococcidae) infesting dragon fruit in Vietnam. *Florida Entomologist* 99(special issue #2): 159-165.
- Dohino T, Masaki S. 1995. Effects of electron beam irradiation on Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Homoptera: Pseudococcidae). *Research Bulletin Plant Protection Japan* 31: 31-36.
- EPPO [European and Mediterranean Plant Protection Organization]. 2011. New Pest Records in EPPO Member Countries. EPPO Plant Quarantine Data Retrieval System—Version 5.0. Global Database, Paris, No. 2011/082.
- FAO [Food and Agricultural Organization]. 2003. Guidelines for the use of irradiation as a phytosanitary measure. ISPM #18. Food and Agricultural Organization, Rome, Italy.
- Hallman GJ. 2011. Phytosanitary applications of irradiation. *Comprehensive Reviews in Food Science and Food Safety* 10: 143-151.
- Hallman GJ. 2012. Generic phytosanitary irradiation treatments. *Radiation Physics and Chemistry* 81: 861-866.
- Hallman GJ, Henon YM, Parker AG, Blackburn CM. 2016. Phytosanitary irradiation. *Florida Entomologist* 99(special issue #2): 1-13.
- Hallman GJ, Levang-Brilz NM, Zettler JL, Winborne IC. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. *Journal of Economic Entomology* 103: 1950-1963.
- Hodgson CJ, Abbas G, Arif MJ, Saeed S, Karar H. 2008. *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootaxa* 1913: 1-35.
- Hofmeyr JH, Doan TT, Indarwatmi M, Seth R, Zhan GP. 2016a. Development of a generic radiation dose for the postharvest phytosanitary treatment of mealybug species (Hemiptera: Pseudococcidae). *Florida Entomologist* 99(special issue #2): 191-196.
- Hofmeyr JH, Hofmeyr M, Slabbert K. 2016b. Postharvest phytosanitary irradiation disinfestation of *Planococcus citri* and *P. ficus* (Hemiptera: Pseudococcidae). *Florida Entomologist* 99(special issue #2): 166-170.
- Huang F, Li X, Bei Y, Lin W, Lu Y, Wang B. 2014. Irradiation as a quarantine treatment for the solenopsis mealybug, *Phenacoccus solenopsis*. *Radiation Physics and Chemistry*, 96:101-106.
- Jacobsen CM, Hara AH. 2003. Irradiation of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) for phytosanitation of agricultural commodities. *Journal of Economic Entomology* 96(4): 1334-1339.
- Janave MT, Thomas P. 1979. Influence of postharvest storage temperature and gamma irradiation on potato carotenoids. *Potato Research* 22: 365-369.
- Kuswadi AN, Indarwatmi M, Nasution IA, Sasmita HI. 2016. Minimum gamma irradiation dose for phytosanitary treatment of *Exallomachus hispidus* (Hemiptera: Pseudococcidae). *Florida Entomologist* 99(special issue #2): 69-75.
- McKenzie HL. 1967. Mealybugs of California with Taxonomy, Biology, and Control of North American Species (Homoptera: Coccoidea: Pseudococcidae). University of California Press, Berkeley. 526 pp.
- Miller DR, Miller GL, Hodges GS, Davidson JA. 2005. Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. *Proceedings of the Entomological Society of Washington* 107: 123-158.
- Miller DR, Miller GL, Watson GW. 2002. Invasive species of mealybugs (Hemiptera: Pseudococcidae) and their threat to U.S. agriculture. *Proceedings of the Entomological Society of Washington* 104: 825-836.
- MOA [Ministry of Agriculture of the People's Republic of China]. 2009. The Government Announcement for Adding Solenopsis Mealybug, *Phenacoccus solenopsis*, in the Chinese Quarantine Pest List. No. 1147, General Administration of Quality Supervision, Inspection and Quarantine of People's Republic of China, Beijing. Available online (<http://tinyurl.com/le4yxl>). Accessed 24-II-2016.
- Mohan KN, Ge J, Kadandale JS. 2012. Mealybug as a model for studying responses to high doses of ionizing radiation, pp. 101-116 *In* Nenoi M [ed.], *Current Topics in Ionizing Radiation Research*, InTech, Rijeka, Croatia.
- Ranganna S [ed.]. 1986. Handbook of analysis and quality control for fruit and vegetable products, 2nd edition TATA McGraw-Hill Publishing Company Ltd., New Delhi, India.
- Ravuwisa KT, Lu KH, Shen TC, Hwang SY. 2009. Effects of irradiation on *Planococcus minor* (Hemiptera: Pseudococcidae). *Journal of Economic Entomology* 102: 1774-1780.
- Seth R, Zarin M, Khan Z, Seth RK. 2016a. Towards phytosanitary irradiation of *Paracoccus marginatus* (Hemiptera: Pseudococcidae): Ascertain the radiosensitivities of all life stages. *Florida Entomologist*. 99(special issue #2): 88-101.
- Seth R, Zarin M, Khan Z, Seth RK. 2016b. Phytosanitary irradiation treatment against *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). *Florida Entomologist* 99(special issue #2): 102-113.
- Seth RK, Zubeda, Zarin M, Tanwar RK, Jeyakumar P, Bambawale OM. 2009. Gamma-irradiation of *Phenacoccus solenopsis* (Homoptera: Pseudococcidae) for Phytosanitary Treatment of Agricultural Commodities. *NCIPM Newsletter* 15(1): 9.
- Schortemeyer M, Thomas K, Haack RA, Adnan Uzunovic A, Hoover K, Simpson JA, Grgurinovic CA. 2011. Appropriateness of probit-9 in the development of quarantine treatments for timber and timber commodities. *Journal of Economic Entomology* 104(3):717-731.
- Tilton EW, Brower JH. 1983. Radiation effects on arthropods, pp. 269-316 *In* Josephson ES, Peterson MS [eds.], *Preservation of food by ionizing radiation*, volume II. Chemical Rubber Company (CRC), Boca Raton, Florida.
- Williams DJ, Granara de Willink MC. 1992. Mealybugs of Central and South America. CAB International, London, England. 635 pp.
- Williams DJ. 1985. Australian Mealybugs. British Museum (Natural History), London. 431 pp.
- Zhan GP, Shao Y, Yu Q, Xu L, Liu B, Wang YJ, Wang QL. 2016. Phytosanitary irradiation of Jack Beardsley mealybug (Hemiptera: Pseudococcidae) females on rambutan (Sapindales: Sapindaceae) fruits. *Florida Entomologist* 99(special issue #2): 114-120.