

Synergistic Effect of Gamma Radiation and Nano Silica on the Cowpea Weevil; *Callosobruchus Maculatus* Reproduction and Mortality

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Abstract: *Callosobruchus maculatus* is the most destructive stored seeds pests. The current study was planned to study the synergistic impact of inherited sterility technique and nano silica to control *C. maculatus*. The newly emerged adults were irradiated with 20Gy and different matings were made, the results revealed that when both sex was irradiated the no. of the deposited eggs was significantly declined that other matings. Different concentrations of nano silica (0.0012, 0.0025, 0.005, 0.01 and 0.015 gm/5gm cowpea) were used against normal, irradiated and F1 *C. maculatus* adults. The insecticidal studies showed that the mortality increased with nano silica concentration increase. In addition, the results exposed that the irradiation increased the susceptibility of the adults. The fecundity and the emerged generation from the infested seeds were significantly reduced in F1 and irradiated adults' experiments. Consequently, we could conclude that the combination of nano silica and gamma radiation could be considered a promising control program for *C. maculatus*.

Keywords: *Callosobruchus maculatus*, Gamma radiation, Nano silica, Mortality, Production, cowpea weevil.

1 Introduction

Cowpeas are an excellent source of vegetable protein, all over the world. The estimated global postharvest losses due to insect disruption, microbial degradation, and other factors range between 10 and 25% [1]. *Callosobruchus maculatus* is the main destructive cowpea "*Vigna unguiculata*" pests, also it can grow inside 14 leguminosae seeds [2]. The damages caused by it are regarded to the development of the stages inside the seed [3]. As stated by Singh et al. [4], in West Africa all the stored cowpea seeds become infested within 8 months. Which causes weight loss of 60% [5], and can increase to 90% [6]. In Egypt, chemical insecticides are the traditional tool that used to control *C. maculatus*, which have many problems facing them use "environmental pollution, poisonous residues on the seeds, and the developed resistance in insect" [7]. According to the investigation of Bogamuwa et al. [8] that *C. maculatus* showed resistance to numerous insecticides. Therefore, it is highly beneficial to provide alternatives to synthetic chemical insecticides.

Radiation Technology tends to be a possible alternative to pesticides for stored pest control in storages [9]. Numerous

researchers had conveyed the effect of gamma radiations on different developmental stages of *C. maculatus* [10, 11]. The using of the substerilizing doses of gamma radiation causing suppression in the F1 progeny and the produced progeny become more susceptible to surrounding circumstances and insecticides [12]. Carpenter et al. [13] announced that F1 sterility could be combined with other different control tools in laboratory and field studies.

Recently, nanotechnology proved its excellence as a new tactics for pest control. Nanoparticles represent a new generation of environmental remediation technologies that have the potential to offer cost-effective solutions to some of the most difficult environmental clean-up problems [14]. Owolade et al. [15] reported that nanoparticles exhibited a pesticidal and repellents activity against insect. Several studies proved the effectiveness of nano silica against *C. maculatus* [16], *Spodoptera littoralis* [17] and *Oryzaephilus surinamensis* [18].

Subsequently, the current investigation was designed to study the effect of gamma radiation on *C. maculatus* production and the ability of using nano silica in combination with gamma radiation to control it.

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2 Materials and Methods

2.1 *Callosobruchus Maculatus* Rearing:

The *Callosobruchus maculatus* colony were obtained from infested cowpea seeds maintained in Stored Products Department, Plant Protection Institute, Agricultural Research Center, Dokki, Giza, Egypt. The culture was reared at $28\pm 2^\circ\text{C}$ and $70\pm 2\%$ relative humidity (RH) in continuous darkness.

2.2 Nanosilica:

The nano silica obtained from Nano Gate Company, Quality Control Department, Nasr city, Cairo, Egypt, and previously characterized by Zahran and Sayed [18].

2.3 Irradiation Technique:

Newly emerged *Callosobruchus maculatus* adults were irradiated with 20Gy [10] using Gamma cell-40 (Co^{60} irradiation unit) with a dose rate of 1.107kGy/h placed at National Center for Radiation Research and Technology (NCRRT),

2.4 Effect of gamma radiation on the fecundity of *Callosobruchus maculatus* (No. of eggs laid/ ♀):

1 pair was placed in jar contain 5gm cowpea and kept at $28\pm 2^\circ\text{C}$, $70\pm 2\%$ RH. for mating. The no. of eggs was counted after 1 week. 3 replicates were performed for each crossing combination as follows

irradiated ♂ X unirradiated ♀

unirradiated ♂ X irradiated ♀

irradiated ♂ X irradiated ♀

unirradiated ♂ X unirradiated ♀ (Control).

2.5 Bioassay of nanosilica (SNPs) on *Callosobruchus maculatus*:

Susceptibility of *C. maculatus* adults (unirradiated adults, irradiated adults and F1 of the irradiated adults) was conducted by mixing 5 different weights of nanosilica (0.0012, 0.0025, 0.005, 0.01 and 0.015 gm) with 5gm cowpea in small jars. 10 newly emerged adults were added in each jar. The jars were covered with muslin cloth for sufficient ventilation, 3 replicates were done for each conc. And incubated at $28\pm 2^\circ\text{C}$, $70\pm 2\%$ RH. The mortality was counted till get 100 mortality in each group. LC_{50} and LC_{90} values were calculated depend on the accumulative mortality after 2days. using a software package Ldp-line a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt.

2.6 Effect of nanosilica and gamma radiation on *Callosobruchus maculatus* progeny:

The no. of eggs laid and the no. of the produced adults from the different treatments of the bioassay experiment were counted for each conc. and replicate.

2.7 Statistical Analysis:

The data were statistically evaluated by analysis of variance (F) followed by Tukey Pairwise Comparisons test to examine the significant differences between the treatments. The statistical Minitab program was used for all analyses.

3 Results

Data arranged in Table (1) exposed the effect of *C. maculatus* adults' irradiation with 20Gy on the fecundity of different mating. As shown from the data the no. of produced egg after 1week was significantly decreased ($p<0.05$) when irradiated females crossed with normal or irradiated males. While the no. of produced egg when irradiated male mate with unirradiated females was non-significantly decreased when compared with the control.

Table (1): Effect of gamma radiation on *Callosobruchus maculatus* fecundity:

| Groups | No. of eggs after 1 week |
|-------------------------------------------------------------|--------------------------|
| unirradiated ♂ X unirradiated ♀ (Control) | 52 ± 1.5^A |
| irradiated ♂ X unirradiated ♀ | 45.33 ± 2.3^A |
| unirradiated ♂ X irradiated ♀ | 35.67 ± 1.67^B |
| irradiated ♂ X irradiated ♀ | 18 ± 2.3^C |

- Values represent the mean \pm S.E of 3 replicates.
- Means that do not share a letter are significantly different ($p<0.05$) (Tukey Pairwise Comparisons).

The percentage mortalities of unirradiated *C. maculatus* adults treated with different concentration of nano silica were displayed in Table (2). The results revealed that there was not mortality in the first day in all used concentration except 0.015gm of nano silica. Moreover, the percent mortality was increased with increasing the time of the experiment. In addition, a significant increase ($p<0.05$) in the adults' mortality with increasing the nano silica concentration. The maximum mortality was obtained after 4 days with 0.015 gm nano silica.

Table 2: Effect of nano silica on the accumulative percentage mortalities of unirradiated adults of *Callosobruchus maculatus*.

| Concentrations (gm nano silica/5gm cowpea) | Percent Mortality | | | |
|--------------------------------------------|-------------------|----------------------|--------------------|----------------------|
| | 1 Day | 2 Days | 3 Days | 4 Days |
| 0 | 0 ± 0^B | 0 ± 0^C | 0 ± 0^D | 0 ± 0^E |
| 0.0012 | 0 ± 0^B | 6.667 ± 3.33^{BC} | 10 ± 0^{CD} | 33.33 ± 8.81^D |
| 0.0025 | 0 ± 0^B | 6.667 ± 3.33^{BC} | 13 ± 3.33^C | 60 ± 5.77^C |
| 0.005 | 0 ± 0^B | 20 ± 5.77^{AB} | 56.667 ± 3.33^B | 73.33 ± 3.33^{BC} |
| 0.01 | 0 ± 0^B | 20 ± 0^{AB} | 70 ± 0^A | 83.33 ± 3.33^{AB} |
| 0.015 | 6.66 ± 3.33^A | 26.667 ± 3.33^A | 76.667 ± 3.33^A | 96.667 ± 3.33^A |

- Values represent the mean \pm S.E of 3 replicates.
- Means that do not share a letter (in the same column) are significantly different ($p<0.05$) (Tukey Pairwise Comparisons).

Table (3) disclosed that only 0.01 and 0.015gm nano silica

caused *C. maculatus* mortality after 1day of the treatment. The illustrated percentage mortalities of the irradiated *C. maculatus* adults showed a significant parallel correlation with increasing the nano silica concentration. The highest mortality was 96.667, which was after 3 days of using 0.015 gm nano silica.

Table 3: Effect of nano silica on the accumulative percentage mortalities of irradiated adults of *Callosobruchus maculatus*.

| Concentrations (gm nano silica/5gm cowpea) | Percent Mortality | | |
|--------------------------------------------------|-------------------------|--------------------------|---------------------------|
| | 1 Day | 2 Days | 3 Days |
| 0 | 0±0 ^C | 0±0 ^D | 0±0 ^D |
| 0.0012 | 0±0 ^C | 6.667±6.6 ^{CD} | 33.33±8.8 ^C |
| 0.0025 | 0±0 ^C | 33.33±8.8 ^{BC} | 60±10 ^{BC} |
| 0.005 | 0±0 ^C | 53.33±3.33 ^{AB} | 73.33±6.667 ^{AB} |
| 0.01 | 20±5.77 ^B | 66.67±6.6 ^A | 90±5.77 ^{AB} |
| 0.015 | 43.33±6.67 ^A | 70±5.77 ^A | 96.667±3.33 ^A |

- Values represent the mean ± S.E of 3 replicates.
- Means that do not share a letter (in the same column) are significantly different (p<0.05) (Tukey Pairwise Comparisons).

Table (4) displayed that the percent mortality of F1 *C. maculatus* adults was time and concentration dependent. The data revealed that there was a significant increase in the mortality percent with increasing nano silica concentration. The mortality reached 100% after 2 days of using 0.015gm nanosilica.

Table 4: Effect of nano silica on the accumulative percentage mortalities of F1 of irradiated *Callosobruchus maculatus* adults.

| Concentration s (gm nano silica/5gm cowpea) | Percent Mortality | | |
|---------------------------------------------------------|------------------------|-----------------------------|-------------------------|
| | 1 Day | 2 Days | 3 Days |
| 0 | 0±0 ^C | 0±0 ^D | 0±0 ^D |
| 0.0012 | 6.67±3.3 ^C | 13.33±3.3 ^C D | 36.67±6.67 ^B |
| 0.0025 | 10±5.7 ^C | 33.33±6.7 ^C | 53.33±8.8 ^B |
| 0.005 | 13.33±3.3 ^C | 56.67±6.6 ^B | 80±5.7 ^A |
| 0.01 | 40±5.7 ^B | 76.67±3.3 ^B | 100±0 ^A |
| 0.015 | 63.3±6.7 ^A | 100±3.3 ^A | 100±0 ^A |

- Values represent the mean ± S.E of 3 replicates.
- Means that do not share a letter (in the same column) are significantly different (p<0.05) (Tukey Pairwise Comparisons).

Table (5) and Fig. (1) represented the LC₅₀ and LC₉₀ of nanosilica of unirradiated, irradiated and F1 *C. maculatus*. The results showed that F1 *C. maculatus* had the lower LC₅₀ and LC₉₀ followed by the irradiated adults then the unirradiated *C. maculatus*.

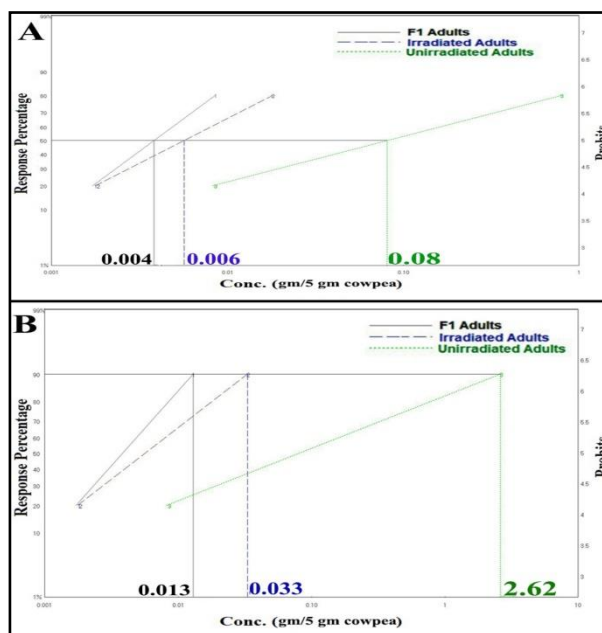


Fig. 1: Lethal concentration line of Silica after 2 days. A: LC₅₀ B: LC₉₀

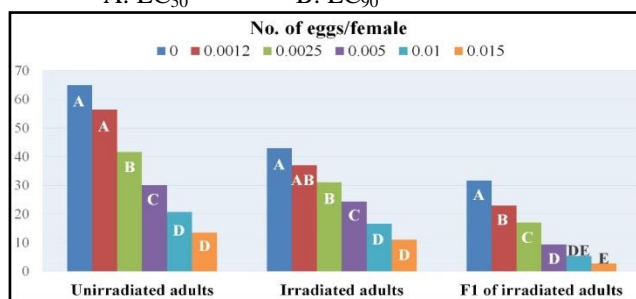


Fig.2: Effect of nanosilica and gamma radiation on no. of eggs/female.

- Values represent the mean ± S.E of 3 replicates.
- Means that do not share a letter (in the same group) are significantly different (p<0.05) (Tukey Pairwise Comparisons).

Data in Table (6) displayed the no. of the adults produced from the treated seeds for the studied groups (unirradiated, irradiated and F1). The results indicated that there was a significant decline in the new progeny resulted with increasing the nano silica concentration. Moreover, when using 0.015gm nano silica for controlling F1 *C. maculatus* adults, the no. of the generation was reduced 98.97%.

4 Discussions

Callosobruchus maculatus is the main cowpea seeds pest over the entire world, causing export deficits and significant losses, as well as making the local cowpea market unappealing and worthless. Radiation offers a solution for this issue. Gamma radiation could be used in pest control to produce sterile males (sterile insect technique, SIT), or by using lower doses to cause partialmale sterility (inherited sterility, IS or F1), which is more favor to be used than complete sterility [12].

Table 5: Comparing LC₅₀ and LC₉₀ of Silica after 2 days.

| Treatment | LC ₅₀ Upper and lower limit | LC ₉₀ Upper and lower limit | Line Slope | Resistance Ratio (RR) | | Index | |
|---------------------|----------------------------------------|----------------------------------------|---------------|-----------------------|------------------|------------------|------------------|
| | | | | Lc ₅₀ | Lc ₉₀ | Lc ₅₀ | Lc ₉₀ |
| Unirradiated adults | (0.0348 – 0.6156) | (0.409 – 266.79) | 0.8484±0.1862 | 21.316 | 201.61 | 4.691 | 0.496 |
| Irradiated adults | (0.0034 – 0.0103) | (0.0268 – 0.1948) | 1.6682±0.1627 | 1.5 | 2.54 | 66.67 | 39.39 |
| F1 adults | (0.0022 – 0.006) | (0.0105 – 0.0362) | 2.4221±0.1854 | 1 | 1 | 100 | 100 |

Table 6: Effect of gamma radiation and nanosilica on the no. of the produced adults:

| Concentrations (gm nano silica/5gm cowpea) | Unirradiated adults | | Irradiated adults | | F1 of irradiated adults | |
|-----------------------------------------------|------------------------|-------------|-------------------------|-------------|-------------------------|-------------|
| | No. of produced adults | % reduction | No. of produced adults | % reduction | No. of produced adults | % reduction |
| 0 | 65±1.15 ^A | 0 | 38.67±0.88 ^A | 40.51 | 27±1 ^A | 58.46 |
| 0.0012 | 52.67±1.2 ^B | 18.97 | 26.33±0.67 ^B | 59.48 | 17.67±0.88 ^B | 72.82 |
| 0.0025 | 38±1 ^C | 41.53 | 27.33±1.2 ^B | 57.94 | 11.33±0.88 ^C | 82.56 |
| 0.005 | 26±1.15 ^D | 60 | 20±1 ^C | 69.23 | 6.67±0.67 ^D | 89.74 |
| 0.01 | 16±1 ^E | 75.38 | 12±0.57 ^D | 81.53 | 3±0 ^E | 95.38 |
| 0.015 | 10±0.57 ^F | 84.61 | 7.67±0.88 ^E | 88.2 | 0.67±0.67 ^E | 98.97 |

- Values represent the mean ± S.E of 3 replicates.
- Means that do not share a letter (in the same column) are significantly different (p<0.05) (Tukey Pairwise Comparisons).
- % reduction in the produced adults was calculated with reference to the unirradiated untreated group (65 adult).

The above results indicate that the fecundity was significantly declined when females irradiated and mate with irradiated or unirradiated males. This finding was in accordance with that of Zaghoul [19] on *C. maculatus* and Supawan et al. [20] & Chiluwal et al. [21] on *C. chinensis*, That reduction in the egg laying from irradiated females might regarded to the interruption in vitellogenesis processes, hormonal, and biochemical alterations [22]. Moreover Hasan [23] stated that irradiation causes distortions follicles shape and the nuclei of the nurse cell which led to delaying in the ovary growth of *Tigriopus brevicornis*.

The results of toxicological experiments of nano silica discovered that the mortality percentages of *C. maculatus* adults “unirradiated, irradiated, F1” were in a parallel significant correlation with the concentration increase. This agreed with other studies on the insecticidal effect of nano silica, Debnath et al. [24] on *Sitophilus oryzae*, Rouhani et al. [16] on *Coleomegilla maculatus* adults, Arumugam et al. [25] on *C. maculatus*, Ali et al. [26] on *C. chinensis*, and Zahran and Sayed [18] on *Oryzaephilus surinamensis*.

The increase in the toxicity was explained by Ali et al. [26], who reported that this mortality could be due to digestive tract deficiency or surface enlargement of the integument because of dehydration or blockage of spiracles and tracheas. And the increase of the concentration significantly increased the exposed surfaces, which can interfere with the insect cuticle. Both sorption and abrasion cause damage to the insects' protective wax layer on the cuticle. He discovered that after the silica pick-up pattern in the photo was cleared, the nano particles were more dispersed throughout the insect body, affecting more surface area and raising the effects induced by the drying action of the insect treated with silica. The effect on the wax layer increased, resulting in increased water loss and, as a result, insect dehydration, which eventually led to death.

The experiment of insecticidal activity of the nano silica showed that the F1 mortality was faster that irradiated or unirradiated adults that reached 100% after 2 days. The obtained data from the toxicity lines arranged the susceptibility of the studied groups as F1 adults > irradiated adults > unirradiated adults, this mean that gamma radiation increase the adult susceptibility to insecticides. This was in parallel to the finding of Ahmadi et al. [27] who stated that combination of gamma radiation with oil is a successful tool to control *Tribolium castaneum*. In addition, Salim et al. [28] conveyed that using gamma radiation in combination with *Carum copticum* oil improved *T. confusum* adult mortality and could serve as a management strategy. The raise in F1 mortality might be owed to the inherited impact of gamma radiation to F1 progeny that makes them more sensitive to insecticide treatment [12].

The egg laying and the adult emergence were declined with increasing the nano silica concentration and the reduction was more pronounced in F1 adults.

This regarded to the combined effect of gamma radiation and the nano silica. Since gamma radiation directly affects DNA [29] or indirect effect that causes generation of free radicals which could be inherited and affect the physiological processes, fecundity, and fertility [30]. Furthermore, the nano silica toxicity could be due to their penetration through the adult or egg cuticle to the circular cavity and binding with sulfur molecules in proteins or to phosphorus molecules in DNA which cause fast denaturation of enzymes and the decline the permeability of membranes that finally lead to death [31].

The results indicated the ability of control *C. maculatus* in stories, by irradiation of the infested seeds with 20Gy and mixing them with nano silica.

5 Conclusions

From the abovementioned results, it could be concluded

that nano silica had an insecticidal activity against *C. maculatus*, which is improved by using IS technique. So, their combination could serve as an integrated control program alternative to chemicals for *C. maculatus* management.

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