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# IMPACT OF THREE APPLICATION METHODS OF SPINOSAD IN THE MANAGEMENT OF *Callosobruchus maculatus* (FABR.) (COLEOPTERA: BRUCHIDAE)

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## ABSTRACT

Cowpea beetle, *Callosobruchus maculatus* (Fabr.) is considered the most important pest of cowpea, *Vigna unguiculata* (L.) Walp., stored in tropical and sub tropical regions. Laboratory bioassays were carried out in order to evaluate the effectiveness of spinosad (Tracer 24 % SC) by using three application methods, contact, fumigant and repellent toxicity, against the cowpea beetle *C. maculates*. Regarding to the contact toxicity, when spinosad applicated by thin film residue in petri dishes(9 $cm^2$ diameter) its LC50s and LC90s recorded 122.55 and 1285.88 ppm, respectively, comperated with 645.06 and 3884.36 ppm, for grain mixing method after 24 h. The percentage of hatching eggs varied from 40.60 to 71.90 %, while the numbers of emerged adults ranged from 10 to 81 insects, comperated with 299 for control. The reduction of progeny ranged from 70.07 to 100.00 with 250 and 1500 ppm. The Main development period (period of generation) ranged from 38.00 to 60 day comperated with 24 days for control. Application of spinosad as fumigant recorded 224.599 and 1464.980 ppm (50 $cm^3$), for LC50s and LC90s , respectively. The repellent activity of spinosad by two applications methods resulted 100 % percentage repellency for adults at concentrations of 300 and 750 ppm for petri dish and jar techniques, respectively. Spinosad may be a good choice as a potent boinsecticide by thin residue film method against studied stored product insect *C. maculatus* .

**Keywords:** Cowpea beetle, spinosad, Stored grain Toxic, Repellent action

## INTRODUCTION

Spinosad is an insecticide product from Dow Agro Sciences (Indianapolis, Indiana, U.S.A.), derived via fermentation from a naturally-occurring soil actinomycete, *Saccharopolyspora spinosa* (Bacteria: Actinobacteridae). Spinosad contains two insecticidal factors, spinosyns A and D, present in an approximately 85:15% ratio in the final product (Sparks et al., 1999). Spinosad is highly active by both contact and ingestion to numerous pests in the orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and others (Bret et al., 1997). It affects nicotinic acetylcholine and gamma amino butyric acid (GABA) receptors sites of the insect nervous system, and so far has proved non-cross resistant to any other known insecticide (Salgado and Sparks,2005).In addition, spinosad exhibits low mammalian toxicity and a highly favorable environmental profile(Cleveland *et al.,*2001).Spinosad is considered a natural product and thus approved for use in organic agriculture by numerous national and international certification bodies(Cleveland,2007).Spinosad suitability as a stored grain protectant has been progressively highlighted in a series of scientific publications dating from 1999(Subramanyam *et al.,*1999, 2003;Mutambuki *et al.*,2002).Since then,spinosad has been shown to provide highly

effective and long-lasting control of numerous key stored product pests on various seeds (Toews *et al.,* 2003; Chintzoglou et al., 2008). One of the main problems that occur during storage is the attack of insect pests, notably the cowpea *C. maculatus.* (Sanon *et al.*, 2002). The losses arise from larvae penetration and feeding within the seeds, which leads to weight loss, as well as lower nutritional value, germination potential and cleanliness (Barbosa et al., 2002). In addition, the mite infestation and infection by micro organisms, especially fungi, contribute to the increase of the grain mass temperature, affecting the product's quality (Sari et al., 2003). Chemical control with protect synthetic insecticides (organophosphates and pyrethroids) and fumigants (phosphine) is a common practice used to control pests of stored seeds. However, due to the accumulation of residues in seeds, the selection of resistant insect population and other side effects, alternative approaches in Integrated Pest Management (IPM) have been considered. In this context several biotic agents and constituent bioactive substances, also called bio-insecticides, have been tested and considered promising for the control of agricultural and urban pests (Arruda and Batista, 1998; Martinazzo et al., 2000; Kemabonta and Odebiyi, 2005). Control of stored product insects is best achieved through an integration of physical, chemical, and biological methods (Hagstrum et al., 1999; Phillips and Throne, 2010). However, in practice there is still a strong reliance on the use of chemicals applied to seeds at the time of storage. These chemicals are known as grain protectants and they provide protection to stored seeds for 4 to 12months of storage. To control an existing infestation, especially in grain that is not treated with a protectant, fumigants such as phosphine are used. Existing chemical control products are few, and of these many are under intense scrutiny due to concerns about human safety, insect resistance, environmental impacts, and presence of chemical residues in raw and processed foods (Daglish, 2006). Alternative chemical control options to protect grain that do not suffer from the concerns outlined above are urgently needed, and spinosad is one such product that fills this void. After global launch imminent, this paper attempt to estimate toxicity of spinosad in different three application methods, contact, fumigant and repellent effect against one from serious stored product insects, the cowpea beetle *C. maculatus*.

## MATERIALS AND METHODS

**Bioinsecticide:** Spinosad (Tracer 24 % Suspension concentrate)
**Source:** Nile valley for Agricultural Development, Giza, Egypt.
**Insects:**

The experiments were conducted at the Laboratory of Agricultural Research Station, Sakha, Department of Stored Product Insects, at 28.5 ± 1.6 $C^{o}$, 52.6 ± 7.4% relative humidity and 12 h photophase. The insects were reared for several generations in cowpea cv. and maintained for the next experiments. The seeds packed in glass containers closed with perforated plastic lids lined on the inside with a fine cloth to allow gas exchange. They were confined for three days for oviposition, before being removed. The containers were stored until the emergence of the F1 generation. Clean and dry seeds, used for experiments, were placed in plastic bags and kept in a freezer at -10 $^{o}$C for seven days, to eliminate possible insect infestation from the field. Then, the seeds were transferred to glass fasks and kept in the laboratory for 10 days in order to reach the equilibrium moisture content.

**Contact toxicity tests**

**filter paper method:**

The contact toxicity on filter papers was conducted using filter paper discs (Whatman No. 1, 9 cm diameter) (Tapondjou et al., 2005). Spinosad was tested at concentrations of 100, 200, 300, 400 and 500 ppm/$cm^2$, and 1 mL of each solution was dispensed on the surface of the paper that was then placed in glass petri dishes. After 10 min, once the solvent had been evaporated, 10 unsexed adults were deposited into each disc and stored in darkness at 26 ± 2 $^0$C and 70 – 85 % RH (Olivero-Verbel et al., 2010). Three replicates were used for each concentration, repeating each assay twice. Mortality was recorded after 24 h. Insects were considered dead when no leg or antennal movements were recorded.

**Grain mixing method:**

Preliminary tests were performed to define the concentrations of spinosad (250, 500, 750, 1000 and 1500 ppm/20 g seeds). Each treatment consisted of 20 g of cowpea cv. seeds infested with ten female of *C. maculatus* (1-3 day old) packed in 250 mL glass containers with a perforated lid, coated with thin fabric (voile) to allow gas exchange (Mutambuki, K. et al, 2002). The concentrations of spinosad were added to the seeds with an automatic pipettor, in glass containers, and subjected to manual agitation for 2 min. After 24, 48, 72 h from the experiment assembly, mortality percentages were evaluated. Eggs were counted at 12 days and the insects hatched 23 days after confinement. The lethal concentrations (LC50 and LC90) of spinosad were estimated using the Probit analysis program (Finney 1971).

**Fumigant toxicity tests:**

Fumigation bioassays without seeds were carried out with 10 adults exposed in 50 ml conical flasks sealed with glass adaptors fitted with injection septa. Filter papers (Whatman Number 1) were placed below the septa to capture the injected spinosad and to produce a large surface area for evaporation. (Arruda and Batista (1998). Each flask had its volume measured by the amount of water it could contain. Different volumes concentrations (V/ V) of bio- insecticide spinosad were injected through the septa into the conical flasks using a gas syringe. Flasks were held at 28.0 ±1.0 ºC & 60.0 ±5.0 % R.H in a constant temperature room during the exposure periods. At least 5 concentrations were tested from 100 to 500 ppm/$cm^2$. Three replicates were prepared for each concentration and control. Adults of *C. maculatus* (1-3 day old) were exposed to treatments for 24, 48 and 72 hours for each concentration. After each exposure period, insects were removed and put into clean vials and mortality determined immediately. Similar units, without spinosad used as control containing the same number of insect and maintained at the same conditions. Insect showing any movement were considered to be alive. Mortality counts were recorded at the same exposure periods that conducted in treatments. The percentage mortality was calculated after each exposure period for each concentration by Abbott's equation (1925). The LC 50 and LC 90 values were calculated by probit analysis (Finney 1971).

**Repellent effect tests**

**Petri dishes method:**

The repellent activity was measured using the area preference method (Olivero-Verbel *et al.,* 2010). A volume of 0.5 mL of spinosad was uniformly applied to a half-filter paper disk to obtain the desired spinosad volume per unit area of 100, 200, 300, 400 and 500 ppm/$cm^2$. The other half of the filter paper was treated with an equal volume of water as a vehicle control. Test areas consisted of 9 cm Whatman No. 1 filter paper cut in half. The treated and control half disks were air-dried for 10 min to remove the solvent, re-attached with adhesive tape, and kept in 90 mm glass Petri dishes. Ten adults of *C. maculatus* of both sexes were released at the centre of each filter paper disk. Dishes were covered and placed in darkness at 26 ± 2 $^0$C and relative humidity of 70-85%. The numbers of *C. maculatus* specimens on treated and untreated portions of the experimental paper halves were counted for each dish after 2, 8 and 12 h exposure. Percentage repellency (PR) for a given treatment time was obtained using the formula: PR = [(Nc _ Nt) **/** (Nc + Nt)] x 100, where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively. Three replicates were used for each tested concentration of spinosad, and each assay was repeated twice.

**jar method:**

Concentrations 250, 500, 750, 1000 and 1500 ppm of spinosad were tested. Bioassays were conducted in arenas made of two 120 mL plastic containers connected to a central plastic box through plastic tubes. In one of the boxes, 20 g of cowpea seeds cv. without the spinosad (control) was placed (Tapondjou, *et al.* 2005). The same amount of seeds impregnated with the respective concentrations of spinosad were placed in the other box. Ten adults of *C. maculatus* (1- 3 day old), were released in the central box. The completely randomized design was used with two treatments (concentration of spinosad and control) and 10 repetitions. After 2, 8 and 12 h, the insects attracted to each box were counted and discarded, and the seeds transferred to other plastic containers. Percentage repellency (PR) for a given treatment time was obtained using the formula: PR = [(Nc _ Nt) / (Nc + Nt)] x 100, where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively. Three replicates were used for each tested concentration of spinosad, and each assay was repeated twice.

## RESULTS AND DISCUSSION

**Contact toxicity tests**

**Petri dishes method:** According to Table 1, the LC50s LC90s of the spinosad recorded 122.55 and 1285.88 ppm /$cm^2$, respectively. Mortality rate of *C. maculatus,* adults, increased with the increase of concentrations. The results of upper confidential level (UCL) and lower confidential level (LCL) values are 24.410 - 188.408 and 622.45 – 40366.29 for LC50s and LC90s, recpectively.

**Table (1):Toxicity ratios and lethal concentration of spinosad applied by contact methods (thin film) on adults of C. *maculatus*. after 24 h.**

| Conc (ppm) | Mortality % | Slope | LC 50 (ppm/cm$^2$) | CL 95% | LC 90 (ppm/cm$^2$) | CL 95% | $X^2$ |
|---|---|---|---|---|---|---|---|
| 100 | 46.60 | 1.255 | 122.55 | 24.410 - 188.408 | 1285.88 | 622.45 – 40366.29 | 1.623 |
| 200 | 60.00 | | | | | | |
| 300 | 66.67 | | | | | | |
| 400 | 73.73 | | | | | | |
| 500 | 80.00 | | | | | | |

**CL: Confidence limits at 95 %.** **$X^2$: Sum squares**

**Grain mixing method:**

According to Table 2, the LC50s LC90s of the toxicity recorded 645.06 and 3884.36 ppm, respectively. Mortality rate of *C. maculatus.* Adults, increased with the increase of concentrations tested. The results of upper confidential level (UCL) and lower confidential level (LCL) values are 453.15 – 886.532 and 2087.42 – 21766.45 for LC50s and LC90s, recpectively.

**Table (2): Toxicity ratios and lethal concentration of spinosad applied by contact methods (mixing with feeding medium) on adults of *C. maculatus*. after 24 h.**

| Conc (ppm) | Mortality % | Slope | LC 50 (ppm/20 g seeds) | CL 95% | LC 90 (ppm/20 g seeds) | CL 95% | $X^2$ |
|---|---|---|---|---|---|---|---|
| 250 | 26.67 | 1.64 | 645.06 | 453.15 – 886.532 | 3884.36 | 2087.42 – 21766.45 | 0.583 |
| 500 | 43.33 | | | | | | |
| 750 | 50.00 | | | | | | |
| 1000 | 60.00 | | | | | | |
| 1500 | 76.67 | | | | | | |

**CL: Confidence limits at 95 %.** **$X^2$: Sum squares**

The results for the number of eggs and emerged insects indicated that, in general, the higher concentration caused the higher mortality of spinosad, the lower number of eggs and emerged insects in Table 3. Studies have shown that bio-insecticide tested can effectively control eggs, larvae, pupae and adults of *C. maculatus.* Spinosad at concentrations of 100, 200, 300, 400 and 600 ppm resulted in 35.63, 52.03, 61.64, 68.08 and 72.74 % mortality of adults, respectively, the corresponding viable eggs values were, 131, 94, 61, 30 and 19 eggs (344 for control), respectively. The percentages hatched eggs listed in table 3 were 71.9, 63.5, 57.23, 43.27 and 40.6 % hatched. (85.1 % for control). The number of emerged adults were, 81, 57, 35, 10 and 00.00 ( 299 for control). The percentages of emerged adults were, 61.8, 61.4, 57.17, 37.17 and 00.00 % emerged adults. (86.93 % for control). The percentages reduction of progeny were, 70.07, 81.17, 88.43, 96.37 and 100.00 % reduction. Spinosad at 600 ppm resulted in 100 % mortality of adults besides reducing viable eggs and emerged insects by 100 %.

**Table (3):Offespring reduction (%) of *C. maculatus* in cowpea seeds treated with spinosad applied by grain mixing method.**

| Conc | T.N of hatched eggs | % hatchability | T.N of adult emergance | % of adult emergance | % adult reduction | Productively index | Main development period |
|---|---|---|---|---|---|---|---|
| 250 | 131.00 | 71.90 | 81.00 | 61.80 | 70.07 | 84.77 | 38.00 |
| 500 | 94.00 | 63.5 | 57.00 | 61.40 | 81.17 | 74.87 | 40.00 |
| 750 | 61.00 | 57.23 | 35.00 | 57.17 | 88.43 | 67.37 | 40.00 |
| 1000 | 30.00 | 43.27 | 10 | 37.77 | 96.37 | .50.80 | 51.00 |
| 1500 | 19.00 | 40.60 | 0.00 | 100.00 | 100.00 | 100.00 | 60.00 |
| Control | 344.00 | 85.10 | 299.00 | 86.93 | -- | -- | 24.00 |

[a] **PR = [(NC _ NT)/(NC) x 100] as PR = percentage of oviposition reduction; NC = number of eggs in the control and NT = number of eggs in the treatment.**

**Fumigant toxicity tests:**

According to Table 4, the LC50s LC90s of the spinosad recorded 224.599 and 1464.980 ppm, respectively. Mortality rate of *C. maculatus* adults increased with the increase of concentrations tested. The results of upper confidential level (UCL) and lower confidential level (LCL) values are 142.366 – 306.714 and 865.47 – 4783.849 for LC50s and LC90s, recpectively.

**Table (4): Toxicity ratios and lethal concentration of spinosad applied by fumigant methods.on adult *C. maculatus.* after 24 h.**

| Conc (ppm) | Mortality % | Slope | LC 50 (ppm) | CL 95% | LC 90 (ppm) | CL 95% | $X^2$ |
|---|---|---|---|---|---|---|---|
| 100 | 30.00 | 1.57 | 224.599 | 142.366 – 306.714 | 1464.980 | 865.47 – 4783.849 | 0.237 |
| 200 | 46.67 | | | | | | |
| 400 | 63.33 | | | | | | |
| 600 | 73.33 | | | | | | |
| 800 | 83.33 | | | | | | |

**CL: Confidence limits at 95 %.** **$X^2$: Sum squares**

**Repellent activity of spinosad**
**Petri dishes.**

The repellent activity of spinosad was increased when insects were exposed for a longer time. Spinosad showed repellent activity to *C.maculatus* adults when applied by petri dish method at concentrations ranging from 100 to 500 ppm/$cm^2$ during 2, 8, 12 h of exposure. After 2 h, the PR values for these five concentrations ranged from 20 to 100 % . The previous values were recorded 60 to 100 % at 8 h of exposure. After 12 h of exposure the PR values resulted 93.33 to 100 %.(Table 5).

**Table (5): Repellent effect of spinosad on adults of *C. maculatus* in Petri dishes. without feeding medium.**

| Conc | Adult individual attracted | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | After 2 h | | | After 8 h | | | After 12 h | | |
| | Control | spinosad | PR | Control | Spinosad | PR | Control | spinosad | PR % |
| 100 | 18 | 12 | 20 | 24 | 6 | 60 | 29 | 1 | 93.33 |
| 200 | 24 | 6 | 60 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |
| 300 | 27 | 3 | 80 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |
| 400 | 30 | 0.00 | 100 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |
| 500 | 30 | 0.00 | 100 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |

**PR (percentage repellency) = NC-NT/ (NC+NT) X 100, = % , where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively.**

**Jar methods**

The repellent activity of spinosad was increased when insects were exposed for a longer time. Spinosad showed repellent activity to *C.maculatus* adults when applied by jar method at concentrations ranging from 250 to 1500 ppm/$cm^2$ during 2, 8, 12 h of exposure. After 2 h, the PR values for these five concentrations ranged from 6.67 to 100 %. The previous values were recorded 33.33 to 100 % at 8 h of exposure. After 12 h of exposure the PR values resulted 66.67 to 100 %.(Table.6).

**Table (6): Repellent effect of spinosad on adult *C. maculatus* in cowpea seeds supplied with feeding medium.**

| Conc ppm | Adult individual attracted | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | After 2 h | | | After 8 h | | | After 12 h | | |
| | Control | spinosad | PR % | control | spinosad | PR % | Control | spinosad | PR % |
| 250 | 16 | 14 | 6.67 | 20 | 10 | 33.33 | 25 | 5 | 66.67 |
| 500 | 20 | 10 | 33.33 | 25 | 5 | 66.67 | 29 | 1 | 93.33 |
| 750 | 22 | 8 | 46.67 | 28 | 2 | 86.67 | 30 | 0.00 | 100 |
| 1000 | 30 | 0.00 | 100 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |
| 1500 | 30 | 0.00 | 100 | 30 | 0.00 | 100 | 30 | 0.00 | 100 |

**PR (percentage repellency) = NC-NT/ (NC+NT) X 100, = % , where Nc and Nt were the number of insects on the untreated (control) and treated areas, respectively.**

Beeman and Speirs (1986) found that avermectin $B_1$ (Abamectin) was extremely effective against 6 beetles and 3 moth pests of stored products. At dose 320 ppb in wheat, all adults of 3 species of Coleoptera were killed in 3 weeks. For most of the Coleoptera and Lepidoptera, 96-100% Suppression of progeny was achieved at doses of 10-160ppb. Abo Arab and El-Hamady (1998) carried out studies to evaluate the efficiency of avermectin as a protectant against three important stored grain insects, namely, the rust red flour beetle, *Tribolium castaneum* (Herbest); the rice weevil, *Sitophilus oryzae* L. and the cowpea weevil, *Callosobruchus maculatus* F. using the technique of exposure to feeding medium. Avermectin exhibited considerable toxicity nearly equal to that of malathion. *C. maculatus* showed the highest susceptibility to avermectin followed by *S. oryzae* and *T. castaneum* (LC50's

0.094, 1.18 and 1.75 mg a.i./100 gm of grain, respectively). The compound also showed potential toxicity to the immature stages inducing reduction in the progeny. Thus, number of offspring and number of eggs (laid by *C. maculatus*) or their hatchability were greatly reduced. Subramanyam *et al.* (2003) carried out laboratory and field tests on wheat and maize have shown that spinosad is effective against the lesser grain borer (*Rhizopertha dominica*), rice weevil (*S. oryzae*), flat grain beetle (*Cryptolestes pusillus*), rusty grain beetle (*Cryptolestes ferrugineus*), confused flour beetle (*Tribolium confusum*) and larvae of the Indian mill moth (*Plodia interpunctella*) at 1 mg/kg grain. Flinn *et al.* (2004) evaluated the effects of controlled aeration and a commercial biological insecticide, spinosad in suppressing insect populations in stored wheat. They stated that is the first report comparing the field efficacy of spinosad and aeration in managing insects in farm bins, they suggest that spinosad is very effective in suppressing *R. dominica* and *T. castaneum* populations in stored wheat. Many research workers determined the efficacy of spinosad (a biopesticide) in laboratory bioassays against a range of stored product insect species (Daglish and Nayak, 2005; Kljajic and Peric, 2007; Daglish (2008), Athanassion *et al.* (2009). El-Madawy (2013) evaluated spinosad as contact, repellent and fumigant agent against *T. castaneum* and *R. dominica* in laboratory. She reported that spinosad was effective against the two tested insect species at the all rates of concentration and exposure periods. Mortaliy increased with the increasing of concentration and exposure periods with the tested insects where 12.5 ppm of spinosad acheved 9 and 23% mortality after 24 and 72 h of treatment, while % mortality reached 37 and 87% at 24 and 72 h by 100 ppm with *T. castaneum* post-treatment. Also, the percentage repellency ranged from 74 to 34% and 94 to 100% against *T. castaneum* and *R. dominica* through the time of exposure (24 h), respectively. For spinosad as fumigant agent, El-Madawy (2013) found that spinosad had an insecticidal effect on *R. dominica* increased with the increasing of concentrations and exposure periods while the same insecticide does not have any effect on *T. castaneum* adults at the tested concentration. Also, spinosad completely prevented laying eggs till four weeks after treatment for *T. castaneum*. We reviewed the previous available references which have not included the fumigation and repellent methods according to our information, but we conducted those methods (repellent and fumigation) to evaluate their action as two common bioassay techniques where the current laboratory experiments were conducted to select the suitable method for controlling cowpea beetle, *C. maculatus* which attacks some of the important legume seeds in field and through storage. Three bioassay investigations were used for this purpose, contact (thin film residue and feeding medium mixing), repellent and fumigation techniques. Our findings obtained clearly showed that the bioinsecticide spinosad achieved a good potency against *C. maculatus* with the all tested methods and levels of concentrations. A different action was found between the three techniques due to the type of application where contact with spinosad deposit varied from one method to another. Through exposure of an insect to the spinosad residue, it picks different amounts of insecticide (more or little) according to the method used. In this study, the contact method (thin film)

exhibited the highest action against *C. maculatus* followed by feeding medium mixing and fumigation. Although fumigation method had the lower effect (based on LC50) we lean to applicate this method since it minimizes direct grain pollution and kills the immature stages either inside grain or outside. Finally, consequently, the current study suggested that spinosad may comply well with the criteria of the proper protectant against stored grain insects.

## CONCLUSION

The present results confirmed the importance of using spinosad as a promising alternative for the management of *C.maculatus* in stored cowpea seeds, since the release of use for this compound is more easily obtained. The contact, fumigation and repellency effects, combined with low mammalian toxicity, rapid degradation in the environment, efficiency in pest control and safety for applicators and consumers, reopen the need for continued research on spinosad.

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## تاثير ثلاثة انواع من طرق التطبيق للمبيد الحيوى سبينوساد فى مكافحة خنفساء اللوبيا *Callosobruchus maculatus* (FABR.), (Coleoptera: Bruchidae)

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تختلف فاعلية اى مبيد طبقا لطريقة التطبيق, لذا درست ثلاثة طرق تطبيق للمبيد الحيوى سبينوساد (تراسر اس سى 24 %). الطريقة الاولى , التطبيق المباشر ( فى اطباق بترى او خلطا مع البذور) , الطريقة الثانية التبخير ثم التاثير الطارد (طريقة اطباق بترى او البرطمانات), ضد خنفساء اللوبيا *Callosobruchus maculatus* (FABR.).

عند تطبيق سبينوساد تطبيقا مباشر فى اطباق بترى سجلت قيم ال LC90 s 122,55 LC50 s و 1285,88 جزء فى المليون, على الترتيب, مقارنة ب 645,06 و 3884,36 جزء فى المليون, عند الخلط المباشر مع بذور اللوبيا. سجلت النسب المئوية لفقس البيض عند تركيزى 250 و 1500 جزء فى المليون 40,60 و 71,90 % , مقارنة ب 85,10 % للمقارنة. تراوح متوسط خروج الحشرات من 81 الى صفر (تعداد الحشرات المنبثقة) عند نفس التركيزين., مقارنة بمتوسط 299 حشرة فى المقارنة. سجلت النسب المئوية للانخفاض فى خروج الحشرات نسبا تراوحت من 70,07 الى 100,00 % عند التركيزين 250 و 1500 جزء فى المليون . لوحظ تاثير هام للمبيد الحيوى سبينوساد تمثل فى اطالة فترة الجيل للحشرة, حيث تراوحت فترة الجيل من 38 يوم الى 60 يوم مقارنة ب 24 يوم للمقارنة. عند تطبيق سبينوساد كمادة تبخير سجلت قيم ال LC90 s 224,59 LC50 s و 1464,98 جزء فى المليون . وفيما يتعلق بالتاثير الطارد سجلت طريقتى المعملة, اطباق بترى والبرطمانات طرد كامل للحشرات (100 %) عند تركيزى 300 و 750 جزء فى المليون, لكلا الطريقتين على الترتيب.

من نتائج الدراسة يمكن ان يمثل المبيد الحيوى سبينوساد اختيارا جيدا بطريقة التطبيق المباشر للاسطح, حيث تميز بالفاعلية مع وجود صفة هامة كونة مبيد حيوى.

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