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Effectiveness of plant growth regulators and biocides against some stored grain insects

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Abstract

Use of synthetic insecticides poses a serious hazard to humans and wildlife because of their adverse effects on the environment. So, the present study aimed to investigate newer approaches for controlling certain economic stored product insects, that achieve the efficient insecticidal activity, meanwhile leading to no detrimental effects to humans and the environment this respect, the following approaches were investigated: Azadirachtin revealed remarkable efficient on the metamorphosis of Trogoderma *granarium* Everts (Coleoptera: Dermestidae). These effects were somewhat similar to those of the synthetic chitin synthesis inhibitor, hexaflumuron, and were dependent on concentration. Azadirachtin showed significant ovicidal action against *Callosobruchus maculatus* (Fabricius), (Coleoptera:Chrysomelidae) but the action was generally less powerful than that of the IGR, hexaflumuron. The biocide, *Bacillus thuringiensis* (B.T.) exerted a weak entomopathogenic effect against adults of *Rhyzopertha dominica* (Fabricius) (Coleoptera:Bostrichidae) at concentrations ≥ 500 mg/kg grains. The effect was also poorly exhibited against larvae of *T. granarium*. The considerable entomo-pathogenic effect of the fungal bioinsecticide, *Beauveria bassiana* against adults of *R. dominica* was obtained at the concentration, 25 ml/kg grains (750 $\times 10^6$ conidia/kg grains) at 65% relative humidity, hence 100% reduction of progeny was achieved. However, the effect was poorly exerted against larvae of *T. granerium* (at 25 ml/kg grains and 65% R.H., 18% reduction of progeny was observed). In all cases, the effect was slightly improved by raising the humidity.

Introduction

Stored grain of almost any kind is subject to attack by insects, which are highly specialized and in most cases are of small size with high reproductive potential. Therefore, they are easily concealed in grain and have been carried to all parts of the world. Under storage conditions, Insect infestation causes loss above 50 to 60% under extreme situations (Kumar *et al*., 2017; Liu *et al*., 2020 and Poonam Jasrotia *et al*., 2022). World Health Organization (WHO) and United Nation Environment Programme (UNEP) reported that pesticides are responsible for poisoning three million people and cause death for 200,000 each year

(Yadav *et al*., 2015). Once established in a commodity they are usually difficult to control (Koehler, 2003). The potential hazards for mammals from conventional insecticides, the ecological consequences, and the increase of insect resistance to these insecticides have led to a search for newer advantageous classes of insecticides. Neem insecticides are derived from the seeds of the Indian neem tree, *Azadirachta indica* (Meliaceae) (Stone, 1992 and Kwasi *et al.,* 2011). Subsequent experiments in Germany demonstrated that azadirachtin also disrupted insect moulting at very low doses and interfered with reproduction in adult insects. (Mordue and Blackwell, 1993). Neem acts in many ways as like an insect growth regulator, feeding deterrent, oviposition deterrent, sterilizer, and inhibitor of chitin synthesis (Mondal and Chakraborty, 2016).

A comparative evaluation for the efficacy of *Bacillus thuringiensis* and neem seed oil on *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) has been carried out in the field and store. These two preparations were almost equally effective on the potato tuber moth infestation. In the store, neem seed oil (500ppm) was highly protective and was as effective as sevin. A combination of both neem and B.t (Delfin) significantly protected the tubers. This suggests the possible use of either neem seed oil or B.t. in combating the insect pest in the field or during storage (Salama and Salem 2000). IGRs appear to be effective against target insects with relative safety to mammals and the environment. IGRs have been tested and evaluated against insect pests in agriculture, forestry, and public health (Mian *et al*., 1990). According to the Pesticide Manual (2000), of the British Crop Protection Council, hexaflumuron acts as a chitin synthesis inhibitor, whereas "Pyriproxyfen" is a juvenile hormone mimic.

Beauveria bassiana conidia were formulated as a dustable powder (DP), as oil suspensions (OS), and as a novel hydrogenated rapeseed oil pellet. These formulations were assessed against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) to study their potential use in the control of stored grain pests (Hidalgo *et al.*, 1998). *B. bassiana* with fipronil produced the highest mortality against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) , *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) (Wakil *et al*., 2022). The occurrence of entomopathogenic fungi isolated from stored grain insect pests included the ascomycetes *B. bassiana.* The cadavers of *T. castaneum* were significantly infected with the fungi followed by, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) , *R. dominica*, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) (Wakil *et al*., 2014). The findings revealed that *B. bassiana* and IGRs, e.g. pyriproxyfen are effective for the control of grain pests and can be used in integrated pest management (Bilal *et al.*, 2017).

The present study was planned to evaluate the insecticidal potency of the above-mentioned approaches (Neem insecticides, IGRs, *B. thuringiensis* and *B. bassiana*) against certain economically stored grain insects.

Materials and methods

1. Pesticides used:

1.1. Azadirachtin (Neemix 4.5% EC): Thermo Trilogy, Grace Drive, Columbia.

1.2. Hexaflumuron (Cousult 10% EC): Dow Agro Sciences.

1.3. Pyriproxyfen (Admiral 10% EC): Sumitomo chemical Company.

1.4. The bioinsecticide:

1.4.1. *Bacillus thuringiensis* **(**Xentari 13.3% W.G.)**:** Abbott Laboratories. Chemical and Agricultural Products Division, North Chicago, 1L 60064, USA, provided by Bayer Company.

1.4.2. *Beauvaria bassiana* **(**Biofly, a commercial liquid preparation containing 3×10^7 conidia/ml**):** El-Nasr Company for Fertilizers and Pesticides, Egypt.

2. Insects tested:

The original strains of all tested insects were obtained from the Department of Stored Product Pests, Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt.

2.1. Cowpea weevil *Callosobruchus maculatus*:

Insects were maintained in small glass jars containing 150-200 unsexed adults and approximately 200 gm of cowpea seeds each. Jars were covered with muslin and kept in position with rubber bands. The cowpea seeds used for insect culture and the experiments were previously sterilized by freezing at -18°C for one week to kill any prior insect infestation, then stored in sealed polyethylene bags at 5°C until required for experiments. Newly emerged adults (0-24 hrs old) were used in the experiments. To obtain the adults, the culture medium was sieved to remove the old beetles therein and the emerging insects were collected for experiments.

2.2. Khapra beetle *Trogoderma granarium*:

Females lay eggs (30-60 eggs, each) inside grains or between them. Proper conditions for growth: 35°C; 70% RH. The larval period ranges from 16-19 days, whereas the life span duration ranges from 24-27 days. Insects were maintained in the laboratory at 70 ± 5 RH., in small jars each containing 200-400 adults and 400 gm of crushed wheat grain. Jars were covered with muslin cloth and fixed with rubber bands to be tightly closed. Newly emerged adults (0-24 hrs old) were used in the experiments.

2.3. Lesser grain borer *Rhizopertha dominica*:

The female lays 300-500 eggs singly or in clusters in the loose grain. They hatch in a few days. The larvae molt 2-4 times. They may feed on the flour produced by the boring of the adults or may bore directly into kernels that have been slightly damaged. They complete their growth within the grain, transform into white pupae and the adults cut their way out. The life cycle takes only a month or two, depending on the temperature. Both adults and larvae feed within the interiors of nearly all grains including rice and the kernels are reduced to mere shells. About 300 adults (1-3 days old) were added to 150 gm wheat kernels + 10 gm wheat flour in a small jar and covered with muslin. Jars were maintained under conditions of 34 ± 3°C and 70 ± 1 RH.

3. Preparation of grains and determination of their moisture content:

Wheat grains, *Triticum aestivum* var., Sakha 61 and cowpea seeds, *Vigna unguiculate* (L.), var., Azmerly, were used as a medium, carrying the tested insects. Grains were sterilized by freezing at -18°C for 1 week to kill off any prior infestation. The moisture contents of these grains were measured by oven-drying duplicate samples each of 5 gm, at 130°C for 1 hour, then calculated from the following formula:

$$\%\ \text{Moisture content} = \frac{(\text{Initial grain wt} - \text{Final grain wt})}{\text{Initial grain wt}} \times 100$$

The grains were stored in sealed polyethylene bags in a refrigerator at 5°C as required for experiments.

4. Growth regulating effect:

The effect of azadirachtin on the metamorphosis of the khapra beetle *T. granarium*, in comparison to that of the conventional IGRs, hexaflumuron and pyriproxyfen was evaluated. Wheat grains were sterilized by heating in an oven at 60°C for 24 hrs. Sterile grains were treated with the tested toxicants as described before. 100 larvae (2nd instar) were transferred to a petri-dish (9 cm in diameter) containing 10 gm of treated grains. Another petri-dish containing 10 gm of non-treated grains carrying the same number of insects, served as control. Three replicates were made for each treatment. Dishes were kept under complete darkness and laboratory conditions for 30 days. Throughout and after this period the following parameters were recorded: % of abnormal larvae, larval period, % of pupation, % of abnormal pupae, pupal period, % of adult emergency, and % reduction of F1 progeny, calculated as follows:

$$\%\ \text{Reduction in progeny} = [(C-T)/C] \times 100$$

(El-Zun, 1998).

Where:

C = No. of adults emerged in control.

T = No. of adults emerged in treatment.

5. Ovicidal action:

The ovicidal action of azadirachtin, hexaflumuron, and pyriproxyfen was evaluated against cowpea weevil, *C. maculatus.* The effect on metamorphosis when exposing eggs to these toxicants was studied as well. Serial concentrations of the pesticides were prepared in water. 20 gm of cowpea seeds carrying 230-250 eggs were immersed in the dilution of each concentration for one minute. Seeds carrying the eggs were left to dry, transferred to a petri-dish, and stored under complete darkness and laboratory conditions for 4-5 days. Three replicates were made for each concentration. After this period, egg-hatching was examined and % of hatchability was calculated. Survival of emerged larvae were allowed to complete their life cycle. Hence, the following parameters were determined: Larval/pupal period, adult emergency (%), and reduction of progeny (%). It is known that larvae and pupae of cowpea weevil infest seeds internally, therefore, the total period of larval and pupal stages could be examined.

6. The entomopathogenic effect of the tested microbials:

The entomopathogenic of B.T. and the fungus, *B. bassiana* against adults of the lesser grain borer, *R. dominica*, and larvae of *T. granarium* was evaluated. The biocides were diluted in water and mixed thoroughly with sterile wheat grains (8.5% moisture content) to form serial concentrations as described before. 50 adults of *R. dominica* or 100 larvae (2nd instar) of *T. granarium* were confined to 10 gm of treated grains (For each concentration) in a 9 cm-petri dish and this was replicated four times. In control trials, grains were mixed only with water. Dishes were kept under complete darkness at 30° ± 2°C and 70 ± 5 RH. for 21 days (BT. treatments) or 11 days (Fungal treatments). During these durations, mortality counts were periodically recorded. At the end of each period, survival and dead parent adults were removed and offspring were allowed to complete their life cycle. Ultimately, No. of emerged adults was recorded and the percent, of reduction in F_1 adult progeny, was calculated as described before. The entomopathogenic effect of the fungal preparation, *B. bassiana* was carried out at three levels of ambient relative humidity (RH.) i.e., 65, 85, and 100%. Dishes containing insects confined to treated grains were kept for 11 days in tightly-closed glass jars (50 × 30 × 25 cm). Relative humidity was adjusted inside the jars by the method of Solomon (1951) with some modifications. Concentrations of pure KOH were prepared in distilled water, i.e., 27.3, 15.8, and 0.0%, to attain 65, 85 and 100 % RH., respectively. 100 ml of each concentration was transferred to a 100 ml glass beaker. One beaker was placed inside each jar and this represented one replicate of biocidal treatments. The attained R.H was checked by a hygrometer placed inside the closed jar. Throughout the test period, the temperature ranged from 26-30°C.

7. Statistical analysis:

The results were analyzed by one-way analysis of variance ANOVA followed by the Least Significant Difference (LSD test). P values of ≤ 0.05 were considered significant. The lethal concentration for 50% mortality LC_{50} was determined by log probit analysis. Data were analyzed by chi-square values. The analysis of data was calculated with SPSS program version 24.0 windows (SPSS Inc., IBM Corp.).

Results and discussion

1. Growth regulating effect of Azadirachtin on *T. granarium*:

The effect of azadirachtin on the development and metamorphosis of khapra beetle, *T. grannasium* was investigated. Larvae (2nd instar) were confined to treated grain for 30 days. Hexaflumuron (A chitin synthesis inhibitor) and pyriproxyfen (A juvenile hormone mimic) were evaluated for comparison as standard insect growth regulators (IGR). LC_{50} of azadirachtin against adults of *C. maculatus*, and *T. granarium* after 24 hrs. exposure was found to be > 1500 mg a.i/kg grains (Table 1).

Table (1): Toxicity of Azadirachtin against adults of cowpea weevil *Callosobruchus maculatus* and khapra beetle *Trogoderma granarium* after 24 hrs. exposure to treated grains.

Toxicant	LC_{50} (mg/kg grains)	
	Callosobruchus maculatus	***Trogoderma granarium***
Azadirachtin	> 1500	> 1500

Results are recorded in Tables (2-4). Data presented in Table (2) show that azadirachtin at concentrations 0.25 mg/kg grain caused significant disruption in normal life processes of *T. granarium* and appeared as lethal morphogenetic effects, abnormalities and arrested development, ultimately resulting in suppression of F1 progeny. The morphogenetic activities of azadirachtin were less powerful than those of the standard IGRs particularly pyriproxyfen whose activity was remarkably observed. In all cases, the effects were dose dependent. At 0.5 mg/kg grain, reductions of progeny were 46, 43 and 100% for azadirachtin, Hexaflumuren and pyriproxyfen, respectively. At 4 mg/kg grain (i.e the highest tested concentration), these percentages were 75, 82 and 100% for Azadirachtin, Hexaflumuren and pyriproxyfen, respectively.

Table (2): Effect of Azadirachtin mixed with wheat grains at various concentrations on the metamorphosis of khapra beetle* *Trogoderma granarium*.

Concentration (Mg/kg grains)	3rd instar larvae*		Pupal stage			Progeny	
	Abnormal larvae (%)	Larval period (Days) Mean±SD	Pupation (%)	Abnormal pupae (%)	Pupal period (Days) Mean±SD	Adult emergency (%)	Reduction (%)
0.25	0.0 d	19 ±2b	90 a	17 c	3.5 ± 0.58 b	66 b	23.0 e
0.50	0.0 d	19 ±1b	90 a	27 b	3.5 ± 0.58 b	46 c	46.0 d
1.00	3.0 c	19 ±1b	89 a	30 b	5.5 ± 0.58 a	42 d	52.0 c
2.00	7.0 b	22 ±1a	87 b	53 a	5.0 ± 0.58 a	31 e	65.0 b
4.00	11.0 a	22 ±1a	73 c	55 a	5.5 ± 0.58 a	26 f	75.0 a
Control	0.0 d	19 ±2b	90 a	0.0 d	3.0 ± 1.00 b	86 a	0.0 f

In the same column, values followed by a common letter are not significantly different.

*** A number of 400 larvae (2nd instar) were initially exposed to treated grains, the exposure on the same treated grain continued up to adult emergence.**

Table (3): Effect of hexaflumuron mixed with wheat grains at various concentrations on the metamorphosis of khapra beetle* *Trogoderma granarium*.

Concentration (Mg/kg grains)	3rd instar larvae*		Pupal stage			Progeny	
	Abnormal larvae (%)	Larval period (Days) Mean ± SD	Pupation (%)	Abnormal pupae (%)	Pupal period (Days) Mean ± SD	Adult emergency (%)	Reduction (%)
0.25	0.0 d	20.0 ± 2a	89 a	21 e	4 ± 1 bc	74 b	14 e
0.50	2.0 d	20.0 ± 2a	85 b	32 d	4 ± 1 bc	52 c	43 d
1.00	10.0 c	20.5±0.58a	80 c	40 c	5 ± 1 ab	43 d	55 c
2.00	13.0 b	21.0 ± 1a	77 d	55 b	6.5 ± 0.58 a	29 e	71 b
4.00	20.0 a	22.0 ± 2a	56 e	63 a	6.5 ± 0.58 a	25 f	82 a
Control	0.0 d	19.5±0.58a	90 a	0.0 f	3.0 ± 1.0 c	86 a	0.0 f

In the same column, values followed by a common letter are not significantly different.
*** A number of 400 larvae (2nd instar) were initially exposed to treated grains, the exposure on the same treated grain continued up to adult emergence.**

Table (4): Effect of Pyriproxyfen mixed with wheat grains at various concentrations on the metamorphosis of khapra beetle* *Trogoderma granarium*.

Concentration (Mg/kg grains)	3rd instar larvae*		Pupal stage			Progeny	
	Abnormal larvae (%)	Larval period (Days) Mean ± SD	Pupation (%)	Abnormal pupae (%)	Pupal period (Days) Mean ± SD	Adult emergency (%)	Reduction (%)
0.25	0 a	24.5 ±2.5d	90 a	52b	9.5 ± 0.58a	24 a	71 b
0.50	0 a	30.0 ±2.5c	88 a	100a	-	0 c	100a
1.00	0 a	32.5 ±2b	79 b	100a	-	0 c	100a
2.00	0 a	32.5 ±2b	51 c	100a	-	0 c	100a
4.00	0 a	40.0 ±2a	5 d	100a	-	0 c	100a
Control	0 a	19.5±0.58e	90 a	0.0c	3 ±1b	86 b	0 c

In the same column, values followed by a common letter are not significantly different.
***A number of 400 larvae (2nd instar) were initially exposed to treated grains, the exposure on the same treated grain continued up to adult emergence**

Figure (1): Abnormalities of 3 rd instar larvae of *Trogoderma granarium* due to exposure to the toxicant at concentration, 4 mg/kg grain.
(A): Normal; (B): Azadirachtin; (C): Pyriproxyfen and (D): Hexaflumuron.

Figure (2): Abnormalities of pupae of *Trogoderma granarium* due to exposure to the toxicant at concentration, 1 mg/kg grain.
(A): Normal; (B): Azadirachtin; (C): Pyriproxyfen and (D): Hexaflumuron.

Malformations were observed especially for the pupal stage [% of deformed larvae and pupae of *T. granerium* were (11, 55); (20, 63); (0, %100) for azadirachtin, hexaflumuron and pyriproxyfen, respectively at the concentration 4 mg/kg grain]. The lower percentages of deformed larvae compared with those of pupae was certainly due to the short exposure period to the toxicants. Larvae of 2nd instar were exposed to treated grains and examination of deformed larvae was conducted on the 3rd instar larvae. Malformed larvae of azadirachtin or hexaflumuron treatments were smaller in size, conspicuously darker and setae became more brittle.

Larvae of pyriproxyfen appeared to be somewhat normal (Figure 1) and failed to pupate. Pupae of azadiracthtin and other IGRs treatments were smaller in size, damaged and occasionally appeared as adultoids completely enclosed in intact pupal exuviae (Figure 2). Pupa-adult intermediates seemed to suffer a complete inhibition of ecdysis and severe deficiencies in the process of a new cuticle deposition.

In azadirachtin or hexaflumuron treatments, most larvae succeeded to reach the pupal stage depending on the concentration tested (% pupation was 73 and 56 for azadirachtin and hexaflumuron, respectively, at the concentration, 4 mg/kg grain). Data showed also that periods of larval stages were slightly affected due to azadirachtin or hexaflumureon treatments. In contrast, these periods were remarkably prolonged for pyriproxyfen treatment and the effect was dose-dependent (At 4 mg/kg grain, larval period., for pyriproxyfen was 40 days, versus 19.5 days for control larvae).

2. Ovicidal action and growth regulation effect against *Callosobruchus maculatus*:

The ovicidal action and the effect on metamorphosis of cowpea weevil, *C. maculatus* when exposing the eggs to aqueous concentrations of azadirachtin, hexaflumuron and pyriproxyfen were evaluated. Cowpea seeds carrying eggs were immersed in the dilutions of toxicants for one minute. After 4-5 days egg-hatching was examined. survivals of hatched (Confined to treated seeds were allowed to complete their life cycl). Observations were made on larval + pupal periods, adult emergency and reduction of F_1 progeny. Results are shown in Table (5). It was clear that azadirachtin and the two synthetic IGRs exerted significant ovicidal action. The effect was dose-dependent.

Table (5): Ovicidal action and the effect on the metamorphosis of cowpea weevil *Callosobruchus maculatus when* exposing eggs* to aqueous concentrations of the tested pesticides.

Pesticides	Concentrations (ppm)											
	1250				2500				5000			
	Hatchability (%)	Larval + pupal period (days)	Adult emergency (%)	Reduction of progeny (%)	Hatchability (%)	Larval + pupal period (days)	Adult emergency (%)	Reduction of progeny (%)	Hatchability (%)	Larval + pupal period (days)	Adult emergency (%)	Reduction of progeny (%)
Azadirachtin	69 b	22 ± 2a	47 b	37 b	64 b	22 ± 2a	34 c	59 c	26 b	22 ± 2.5a	29 c	86 b
Hexaflumuron	55 c	22 ± 4a	73 a	27 c	34 d	22 ± 4a	51 d	68 b	9.0 c	22 ± 3.5a	40 b	94 a
Pyriproxyfen	60 c	24 ± 2a	18 c	80 a	44 c	24 ± 2a	13 d	89 a	26 b	25 ± 1.5a	13 d	94 a
Control	75 a	22 ± 3a	72 a	0.0 d	75 a	22 ± 3a	72 a	0.0 d	75 a	22 ± 2.5a	72 a	0.0 c

In the same column, values followed by a common letter are not significantly different.
*** A number of 700 -750 eggs were initially treated for each concentration.**
% Adult emergency = (No. of emerged adults/No. of hatched eggs) × 100

The highest effect was observed for hexaflumuron (At 5000 ppm, hatchability was 26, 9 and 26% for azadirachtin, hexaflumuron and pyriproxyfen, respectively). Durations of (larval + pupal) stages were not affected. As a general trend, the insecticidal activity of the tested compounds was not effective because both the larval and pupae phases were inside the cowpea seeds when roughly compared with those tested against *T. granarium*.

At a concentration of 5000 ppm, the larvae and pupae failed to reach the full adult stage, where the percentage of offspring exit was 29, 40 and 13% for azadirachtin, hexaflumuron and pyriproxyfen, respectively. Dead or deformed larvae or pupae could not be observed because they internally infested the seeds. Anyway, the most powerful compound in this respect was the JHA, pyriproxyfen followed by azadirachtin and hexaflumuron (At 5000 ppm, adult emergencies were 13, 29 and 40% corresponding to 94, 86 and 94% reduction of F_1 progeny, for pyriproxyfen, azadirachtin and hexaflumuron, respectively).

3. The entomopathogenic effects of the tested bioinsecticides:

The acute toxicity of the tested biocides was evaluated against adults of *C. maculatus* and *T. gramarium*. LC_{50} (24 hrs.) values and their fiducial limits were calculated. Results recorded in Table (6) show that B.T. and the fungal biocide are of very low acute toxic action (LC_{50} of B.t.: >2000 mg/kg grain and for the fungus: 15.3 and 41 ml for *C. maculatus* and *T. graneraium*, respectively). Therefore, the insecticidal properties of these agents could be achieved only through their pathogenic action that requires incubation periods for the spores to manifest their pathogenicity.

Table (6): Acute toxicity of the tested bioinsecticides against adults of *Callosobruchus maculatus* and *Trogoderma granarium* after 24 hrs. exposure to treated grains.

Insect	*B.t.* LC_{50} (mg/kg grain)	*Beauveria bassiana*	
		LC_{50} (ml */kg grain)	Confidence limits
Callosobruchus maculatus	> 2000	15.3	12.7 – 18.2
Trogoderma granarium	> 2000	41	39.0 – 42.3

***ml of the commercial liquid preparation, biofly**

The entomopathogenic effect of B.t. and the fungus, *B. bassiana* against adults of the lesser grain borer, *R. dominica* and larvae of *T. granarium* was evaluated. Insects were confined to the treated grains for 21 days (B.t treatments) or 11 days (Fungal treatments) then cumulative mortalities were calculated. Reduction of F_1 adult progeny was examined as well. Result, for B.t. are recorded in Table (7). It was clear that B.t. preparation exerted weak insecticidal activity especially against larvae of *T. granarium*.

Table (7): Entomopathogenic effects of the biocide, *B.t.* against adults of the lesser grain borer *Rhyzopertha dominica* and 2nd instar larvae of *Trogoderma granarium*.

Concentration of *B.t.* (mg/kg grains)	*Rhyzopertha dominica* (Adults)		*Trogoderma granarium* (Larvae)	
	% Mortality (1)	% Reduction (2)	% Mortality (1)	% Reduction (2)
15	4.0 g	27 f	3 e	1 ef
30	16.0 f	41 e	4 e	3 ef
60	26.0 e	48 d	7 d	4 e
125	38.0 d	53 c	8 cd	13 d
250	58.0 c	71 b	10 c	22 c
500	78.0 b	75 b	12 bc	26 b
1000	96.0 a	82 a	24 a	45 a
Control	0.0 g	0.0 g	0.0 f	0.0 f

In the same column, values followed by a common letter are not significantly different.
(1) % of cumulative mortality following 21 days exposure to treated grains.
(2) % reduction of progeny.

The considerable insecticidal activity against adults of *R. dominica* was achieved only at the highest tested concentration i.e., 1000 mg/kg as cumulative mortality was 96% and reduction of progeny was 82%. The activity was poorly exhibited against the larvae of *T. granarium* even at the highest concentration (Mortality and reduction of progeny were 24 and 45%, respectively). The entomo-pathogenic effect of the fungal bioinsecticide *B. bassiana* was poorly observed and was dose-dependent, especially against larvae of *T. granarium*. The considerable effect against adults of *R. dominica* was obtained at the concentration 25 ml/kg grain (750 × 10^6 conidia/kg grain) at 65% RH., hence 100% cumulative mortality and % reduction of progeny was achieved against adults, of *R. dominica* (Table 8).

Table (8): Entomopathogenic effect of the fungal bioinsecticide, *Beauveria bassiana* against adults of the lesser grain borer, *Rhyzopertha dominica* at various levels of humidity.

Concentration		Relative humidity					
		65%		85%		100%	
ml/kg Grains	Conidia/kg grains	% Mortality(1)	% Reduction (2)	% Mortality (1)	% Reduction(2)	% Mortality (1)	% Reduction (2)
0.8	**23.4 × 10^6**	3.0 d	31 d	12 f	29 e	12 f	51 f
1.6	**46.9 × 10^6**	3.5 d	34 d	18 e	37 d	18 e	63 e
3	**93.8 × 10^6**	18 c	40 c	22 d	38 d	30 d	72 d
6	**187.5 × 10^6**	20 c	43 c	26 c	44 c	34 c	77 c
12.5	**375 × 10^6**	48 b	61 b	68 b	67 b	72 b	88 b
25	**750 × 10^6**	100 a	100 a	100 a	100 a	100 a	100 a
Control	**-**	2.0 d	0.0 e	2 g	0.0 f	4.0 g	0.0 g

In the same column, values followed by a common letter are not significantly different.
(1) % of cumulative mortality following 11 days exposure to treated grains.
(2) % reduction of adult progeny.

This concentration is thought to be high. If it is desired to treat a ton (1000 kg) of grain, thus twenty five litres of the commercial formulation will be required. Against *T. granarium* larvae the effect was much lower. (At 25 ml/kg and 65% R.H., 11% mortality and 18% reductions of progeny were observed (Table 9). In all cases, the effect was slightly improved by raising the humidity. At 12.5 ml/kg grain reductions of progeny were 61 and 88% against *R. dominica* at relative humidities 65 and 100%, respectively. Against larvae of *T. granaium*, reduction of progeny increased from 13% to 33% when the humidity was raised from 65% to 100% at the same concentration.

Table (9): Entomopathogenic effect of the fungal bioinsecticide, *Beauveria bassiana* against 2nd instar larvae of *Trogoderma granarium* at various levels of humidity

Concentration		Relative humidity					
		65%		85%		100%	
ml/kg grains	**Conidia/kg grains**	**% Mortality(1)**	**% Reduction(2)**	**% Mortality (1)**	**% Reduction(2)**	**% Mortality (1)**	**% Reduction(2)**
0.8	**23.4×10^6**	0.0 e	2.0 e	0.0 e	5 e	0.0 e	3.0 g
1.6	**46.9×10^6**	0.0 e	2.0 e	0.0 e	7 e	0.0 e	8.0 f
3	**93.8×10^6**	0.0 e	3.0 d	0.0 e	8 d	0.0 e	13 e
6	**187.5×10^6**	2.0 d	5.0 d	4.0 d	14 c	7.0 d	23 d
12.5	**375×10^6**	5.0 c	13 c	11 c	16 c	19 c	33 c
25	**750×10^6**	11 b	18 b	29 b	23 b	42 b	52 b
50	**1500×10^6**	79 a	100 a	91 a	100 a	96 a	100 a
Control	**-**	0.0 e	0.0 f	0.0 e	0.0 f	0.0 e	0.0 h

In the same column, values followed by a common letter are not significantly different.
(1) % of cumulative mortality following 11 days exposure to treated grains.
(2) % reduction of adult progeny.

Use of synthetic insecticides poses a serious hazard to human and wildlife because of their adverse effects on the environment. So, the present study aimed to investigate newer approaches for controlling certain economic stored product insects, that achieve the efficient insecticidal activity, meanwhile leading to no detrimental effects to humans and the environment this respect Our study included the effect of azadirachtin on the development and metamorphosis of khapra beetle, T. granarium was investigated. Larvae (2nd instar) were confined to treat grain for 30 days. Hexaflumuron (A chitin synthesis inhibitor) and pyriproxyfen (A juvenile hormone mimic) were evaluated for comparison as standard insect growth regulators (IGR). Azadirachtin showed considerable feeding deterrent effects against larvae of khapra beetle *T. granarium* at concentrations ≥ 125 mg/kg grains. The effect was concentration-dependent.

Azadirachtin revealed remarkable effects on the metamorphosis of *T. granarium* at concentrations ≥ 4 mg/kg grains. These effects were somewhat similar to those of the synthetic chitin synthesis inhibitor, hexaflumuron, and were dependent on concentration. The results are consistent with other findings on freshly treated grain, the application rate of 5 mg/kg of azadirachtin was effective in inhibiting F1 progeny production against *R. dominica* (Rahim, 1998). Salima *et al*. (2021) found that the toxicity of azadirachtin was increased by increasing concentration and exposure time against *S. granarius*. Our results revealed that pyriproxyfen treatment At 4 mg/kg grain, larval period., was 40 days, versus 19.5 days for control larvae.

Pyriproxyfen is an insect growth regulator with juvenile hormone (JH) mimetic activity. Such JH analogues work by disrupting the development (Metamorphosis) of Juvenile insects. Late stage larval insects are most susceptible to the effects of JH analogues (Alejandro *et al.,* 2020). Browers (1971) reported that “the action of JHA sometimes prolongs larval development by delaying or preventing pupation. Lewis and Forschler (2017) found that chitin-synthesis inhibitors such as benzoylphenyl ureas were effective at killing all immature stages of a wide range of insect species including the internal grain feeders.

Our results revealed that azadirachtin at 1250-5000 ppm showed significant ovicidal action against *C. maculatus*, but the action was generally less powerful than that of the IGR, hexaflumuron (% Hatchability of azadirachtin at 5000 ppm was 26% versus 9 and 75% for hexaflumuron and control, respectively). Insect juvenile hormones and their analogues have been found to block embryonic development of treated eggs (Retnakaran, 1970) and thus, reduce oviposition. It was found that neem acted not only as an oviposition suppressant but also as an ovicide.

In laboratory experiments, Makanjuola (1989) found that all extracts of neem leaf and seed significantly reduced oviposition, percent hatched eggs and percent adult emergence of *C. maculatus* on treated cowpeas. The ovicidal actions of neem oil were also observed by Ba-Angood and Al-Sunaidy (2003). Fenoxycarb and pyriproxyfen applied topically to Indian meal moth eggs for several seconds prevented completion of embryonic development, egg hatch or early larval development depending on the time and concentration of the application (Silhacek *et al*., 1994).

The susceptibility of freshly laid eggs to direct treatment with IGRs or to transovarial activity could lead to a treatment protocal that takes advantage of effects on embryogenesis (Oberlander *et al*. 1997). Also, our stduy revealed that the biocide, B.T. exerted weak entomopathogenic effects against adults of *R. dominica* at concentrations ≥ 500 mg/kg grains. The effect was also poorly exhibited against larvae of *T. granarium* (Mortality of *R. dominica* adults following 21 days of exposure to a concentration of 500 mg/kg grains was 78% versus 12% for larvae of *T. granarium*).

The considerable entomo-pathogenic effect of the fungal bioinsecticide, *B. bassiana* against adults of *R. dominica* was obtained at the concentration, 25 ml/kg grains (750 $\times 10^6$ conidia/kg grains) at 65% relative humidity, hence 100% reduction of progeny was achieved. However, the effect was poorly exerted against larvae of *T. granerium* (At 25 ml/kg grains and 65% RH, 18% reduction of progeny was observed). In all cases, the effect was slightly improved by raising the humidity. Arthur and Brown (1994) evaluated B.t. formulations for insect control in stored peanuts after 8 months of treatment, *T. castaneum* populations in treated peanuts were not significantly different from the untreated controls. Beegle (1996) and Mikel *et al*. (2020) tested the efficacy of 36 isolates, representative of all available subspecies of B.t. against larvae and adults of *R. dominica*. They found that spore-crystal complexes of B.t. subsp. darmstadiensis isolates from Germany were effective against larvae. Isolates of the same subspecies from the USA and Japan were not effective. The degree of control achieved by even the best isolate was not economically satisfactory.

Kaelin *et al.* (1999) tested three B.t. strains against larvae of the cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). The responses to spore/crystal suspensions from all strains were in the range of 60-80% mortality after 7 days. The purified parasporal crystals showed a slightly lower insecticidal activity. Larvae surviving after exposure to sublethal levels of B.t. crystal proteins for 3-10 days could complete their life cycle on a normal diet without retardation. The researchers suggested that some adverse biological effects induced in *L. serricorne* by sublethal amounts of B.t. crystal proteins may be reversible and that larvae could recover from initial exposure.

In conclusion, Kaelin *et al.* (1999) concluded that neither B.t. strains isolated from stored tobacco more two B.t. commercial products reached satisfactory levels of mortality and potency at the required times and doses. However, Mummigatti *et al.* (1994) and Sedlacek (1996) have reported on the susceptibility of stored grain coleopteran pests to B.t. subsp. tenebrions and other strains. B.t. is a grams-positive sporulating bacterium that has been used as a biocontrol agent for more than 30 years. More than 30 different B.t. subspecies and insecticidal crystal proteins have been described which differ in insect host range, with over 90 genes cloned and sequenced (Cannon, 1996). B.t. preparations were exploited as biological control agents against susceptible species of insects of the orders, Lepidoptera, Diptera, Homoptera and very recently Coleoptera (El-Hamady, 1997).

The findings revealed that *Beauveria bassiana* and IGRs, e.g., pyriproxyfen are effective in the control of grain pests and can be used in integrated pest management (Bilal *et al.*, 2017). Wakil *et al.* (2014) reported that the cadavers of *T. castaneum* were significantly infected by *B. bassiana*. Searle and Doberski (1984) found that relative humidity and temperature played a major role in the infection of adult *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) by *B. bassiana*. At 100 % R.H., they recorded 100% mortality at day 20 after inoculation.

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