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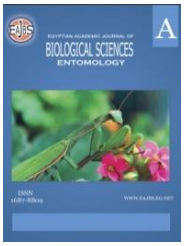

Sublethal Effects of Two Insecticides, Deltamethrin, Thiamethoxam and the Botanical Insecticide (*Foeniculum vulgare* Mill.) on *Callosobruchus maculates* (Fabr.) (Coleoptera: Bruchidae)

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ABSTRACT

The cowpea beetle *Callosobruchus maculates* (Fabr.) is a serious pest that infests pulse seeds in storage. The current study aimed to assess the sublethal effects of two insecticides deltamethrin, thiamethoxam and a botanical power of fennel (*Foeniculum vulgare* Mill) on *C. maculatus* development and its essential enzymes. Thiamethoxam, deltamethrin insecticides and fennel plant powder activities against the cowpea beetle, *C. maculatus,* were assessed throughout direct contact application. Thiamethoxam showed the LC_{50} value of 21.05 ppm followed by deltamethrin (LC_{50}= 85.1 ppm) while, the least effect was recorded with fennel seeds powder with LC_{50} value 36325.8 ppm. Concerning sublethal effects, sublethal concentrations of thiamethoxam (LC_{10}, LC_{25}, LC_{50} and LC_{75}) significantly reduced egg-laying. While LC_{75} of deltamethrin significantly decreased egg-laying related to control. In contrast, fennel seeds powder failed to turn down the number of eggs laid by *C. maculatus* females compared with the control. Complete protection to deltamethrin against *C. maculatus* adults was maintained after 24 h till 7 months and to thiamethoxam from 24 h till 4 months. Biochemical studies showed that the activity of acetylcholine esterase (AChE), α, ß esterase and glutathione-S-transferase (GST) showed a significant decrease in sublethal concentrations of thiamethoxam and deltamethrin related to the control. Regarding the effect on germination, no adverse effect was noticed due to the chemical and botanical seed protectants application.

INTRODUCTION

In Egypt, Faba bean (*Vicia faba* L.) is one of the most important crops for human consumption and represents meat and skim-milk substitutes. Due to its nutritive value, faba bean is considered superior to field peas or other legumes (Duke, 1981). It is the fourth most important pulse crop in the world. Faba beans are used as seeds and green vegetables for human and animal consumptions as an important source of dietary protein (Mahmoud, 2020). The chemical composition of faba bean is rich in protein and its air-dry seed contains

28% protein, 48% carbohydrates, 3.6% fats, 2% glucose and 3.5% mineral salts (Baginsky *et al.,* 2013). The major stored legume pests are the bruchid beetles. Beetles of the family Bruchidae, show host specificity to a particular genus of legume (Hill, 2002). These pests attack the endosperm, causing loss of weight, reduction in nutrients and seed germination. Ultimately, the specific gravity of the seed and the market value of the product will decrease (Ragaa 2003). However, the cowpea beetle, *C. maculatus* is a serious pest that infests the pulse seeds in storage. The estimated losses due to bruchids in various pulses ranged from 30 to 40 percent within six months and the post-harvest seed losses due to bruchids can reach even 100 percent during severe periods of infestation (Kaur *et al.,* 2020). One pair of this insect cause the seed's weight loss of 61.4% through four weeks, besides the infestation seeds, are not suitable for human nutrition (Boeke, 2004).

Synthetic insecticides have played a historical role in agriculture development and particularly in postharvest grain storage (Ogendo & Hagstrum 2004; Mbata & Fadamiro 2005). Organophosphorous was registered to control stored product insects included *C. maculatus* then replaced by pyrethroids, which were shown to be very efficient against arthropods, especially deltamethrin (Boyer *et al.,* 2012). Pyrethroids insecticides act on the insect nervous system, by altering the normal functioning of the para-type voltage-gated sodium channel, resulting in insect paralysis and death (Tsagkarakou *et al.,* 2009; Zongo *et al.* 2020). Extensive use of organophosphate and pyrethroids insecticides has resulted in the rapid development of insecticide resistance. Novel insecticides with different modes of action were needed to overcome organophosphate and pyrethroids developing resistant strains.

Neonicotinoids are registered to overcome organophosphate, and pyrethroids developed resistant strains. Neonicotinoids act as nicotinic acetylcholine receptors (nAChR) agonists at the postsynaptic. Neonicotinoids sensitivity and specificity (structure-activity relationships) across a broad range of insects are contributed to the high-affinity binding site. Neonicotinoids are used for seed treatment and have been proved effective against several pest categories (Yue *et al.,* 2003; Tomizawa and Casida, 2003; Tsaganou *et al.,* 2014; Yan *et al.,* 2016). Thiamethoxam is a second-generation neonicotinoid and it belongs to the thianicotinyl subclass. Worldwide, including Egypt, thiamethoxam provides excellent control of a broad range of economically important pests, such as aphids, whiteflies, thrips, rice hoppers, Colorado potato beetle, flea beetles, wireworms, leaf miners as well as some lepidopterous species (Mohamed *et al.,* 2015; Tomizawa and Casida, 2003; Zhang *et al.,* 2018; Kayis *et al.,* 2019).

Using plant material for grain preservation, particularly cowpea, is considered a promising alternative to synthetic insecticides for several decades (Boeke *et al.,* 2004). Plant-derived protectants such as seeds powders, water, or organic solvents extracts are used to control stored grain insects. Over 2400 plant species contained some active ingredients that can reduce the activities of storage pests (Ahmeed, 1995; Kayode, 2006). Fennel, *F. vulgare* Mill. (Apiaceae), is an aromatic plant indigenous of the Mediterranean region well known for its pharmacological properties as carminative, digestive, lactogogue and diuretetic (Manzoor *et al.,* 2012). Fennel essential oil has been already shown to have acaricidal effects (Lee, 2004), antifungal properties (Singh *et al.,* 2006), insecticidal and repellent effects against many insects (Bertoli *et al.,* 2012).

Successful stored pest control depends on the persistence of the efficacy of the insecticidal materials; therefore, the assessment of the sublethal effects of an insecticide is important, sublethal concentrations of insecticides affect the physiology, behaviour, development, and population growth of treated insects (Desneux *et al.,* 2007). Several studies on the sublethal effects of insecticides have been reported for several insects but rarely investigated in stored-product insects (Guedes *et al.,* 2011; Mokbel *et al.,* 2020).

So, the current work provides some information about the susceptibility of *C. maculatus* to deltamethrin, thiamethoxam insecticides and fennel (*F. vulgare*) as a botanical insecticide, assesses their sublethal effects on insect development, various reproductive activity parameters (fecundity and hatchability percentage) and critical enzyme activities such as AchE, CarE and GST. Furthermore, the present study was carried out to investigate the viability of faba bean (in terms of seed germination) post- treatment.

MATERIALS AND METHODS

Chemicals and Insecticides Tested:

Two commercial formulations of thiamethoxam (Actara 25% WG, Syngenta) and deltamethrin (2.5% WP, Kafr El-Zayat Pesticides and Chemicals Company) and fennel seeds *F. vulgare* were purchased from an herb shop, Cairo, Egypt. Seeds were ground well in an electric mill, sieved using a 300-mesh sieve, and kept in tightly clean plastic jars and kept in the freezer.

Acetylthiocholine iodide (ATChI), Triton X-100, fast blue RR salt, glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma–Aldrich. 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) was obtained from Roth, ß-naphthyl acetate and α-naphthyl acetate were obtained from Mpbio.

1. Insect Cultures:

Cultures of the cowpea beetle, *C. maculatus* (F.) were reared on faba bean (variety Giza 2) at 27 °C and 65 ± 5% relative humidity (RH) in the Stored Grains pest Research Department, Plant Protection Research Institute, Agriculture Research Center. Before each trial, faba bean was fumigated with phostoxin tablets for at least three days to exclude infestation by insects.

2. Bioassays:

To estimate the insecticidal efficiency of thiamethoxam, deltamethrin and fennel seeds powders against *C. maculatus* adults. A sample of 10 g of fresh faba bean seeds was placed in a glass tube (1x7.5 cm) and exposed to five concentrations of the tested powders and then shake manually for one minute to achieve equal distribution of the powders and uniform coating of faba bean seeds with the tested material. Twenty-five *C. maculatus* adults (1 to 2 days old) were collected randomly from the stock colony and introduced into each prepared tube containing treated and untreated grains (control). Each experiment and the control were replicated 3 times. The tubes were covered with a secured plastic cover and maintained in the incubator. Mortality was assessed after 24 h post-exposure in the treated and untreated tubes and corrected according to Abbott formula (Abbott 1925) for natural mortality in controls.

3. Effect on Some Biological Aspects of *C. maculatus*:

To determine the number of eggs laid by mated female adults of *C. maculates* exposed to each of the tested materials, glass tubes measuring 1 × 7.5 cm contained 5 gm of cleaned faba bean seeds treated with LC_{10}, LC_{25}, LC_{50} and LC_{75} (separately) of each tested material (each treatment had three replicates), two pairs of newly emerged adults were placed in each tube and covered with plastic cover and kept in the incubator at 28 ±2 °C and 75±5% RH till death. The number of eggs laid was counted and the reduction in hatchability was determined.

4. Percentage of Adult Emergence:

The infested treated seeds with eggs were incubated and observed regularly until adult emergence. Emerging adults were counted daily for 10 days. The reduction percentage of emerging adults also was recorded.

Residue Determination:

Tubes measuring 1x7.5 cm each containing 10 gm of faba bean seeds treated with LC_{95} of each treatment and were divided into several groups. Each group consisted of three replicates. The tubes were kept under laboratory conditions just after mixing the faba bean seeds with tested powders. Treated seeds were stored for 14 months. Every month twenty-five adults of *C. maculatus* were placed on each of the three replicates, to show the residual effect of the treatments on the treated faba bean seeds. The tubes were covered with a plastic cover fixed with a rubber band, and the tubes were kept in laboratory conditions. Mortality counts were carried out after 24 h of introducing insects. Similar three replicates of untreated faba bean seeds were used as control.

Activity of Enzymes:

1. Sample's Preparations:

The untreated and treated adults (LC_{10}, LC_{25}, LC_{50} and LC_{75} values of actara, deltamethrin as ppm and fennel seeds) were transferred to a clean jar and kept at -40 °C, fifteen insects were weighed and homogenized in ice-cold of 0.1 M phosphate buffer with different pH values according to the enzyme in ratio 1:10 (W: V), five replicates were used to record the mean enzyme activities after 24 h from exposure.

2. Acetylcholine Esterase (AChE) Activity:

Acetylcholine esterase (AChE) activity was determined as indicated by Ellman *et al.* (1961) with modifications. Treated and untreated insects were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.1% (V/V) Triton X-100 then centrifuged at 13,000g at 4 ºC for 15 min. The supernatants were transferred to new tubes. 800 µl potassium phosphate buffer (0.1 M, pH 7.5), 100µl enzyme solution was placed in a new tube, 50 µl of 0.075 M ATChI and 50 µl of 0.01 M DTNB. The reaction began with the addition of the substrate (ATChI) and the reagent (DTNB). The change in absorbance at 412 nm was recorded for 5 min at a UV/Vis spectrophotometer (V-530).

3. α- and β- Esterase Assay:

Esterase activity was determined according to Van Asperen (1962) with some modification (Cao *et al*., 2008). Insects from each treatment were homogenized in phosphate buffer (0.1 M, pH 7.0). The homogenates were centrifuged at 12000 g for 15 min at 4 °C. The supernatants were transferred to new Eppendorf tubes for the assay. A 50 µl enzyme solution was incubated with 50 µl of α or ß- NA (30 mM) for 15 min at 30 °C. The reaction was stopped by adding 50 µl of stop solution (two parts of 1% Fast Blue RR and five parts of 5% sodium dodecyl sulfate). The absorbance was measured at 600 nm for the hydrolysis of α-NA and 550 nm for ß- NA at UV/Vis Spectrophotometer (V-530). Mean levels of total esterase activity cited were based on α and ß- naphthol standard curves at a UV/Vis spectrophotometer (V-530).

4. Glutathione S- Transferase:

The glutathione-S-transferase activity was measured as indicated by Habing *et al.* (1974). The larvae were homogenized in ice-cold phosphate buffer (0.1 M, pH 6.5).
The homogenates were centrifuged at 12000 g for 15 min at 4 °C and the supernatants were transferred and kept at -40 °C for the assay. The reaction solution contained 100 µl supernatant, 10 µl CDNB (30 mM), and 10 µl GSH (50 mM). Enzyme activity was determined by continuous monitoring of the change in absorbance at 340nm for 3 min at 25 °C at a UV/Vis spectrophotometer (V-530).

Seeds Germination:

Germination tests were carried out according to International Standard Methods (ISM) (Anonymus, 1966) to find out the effect of the chemical and botanical seed protectants application on the germination percentage of treated faba bean.

Statistical Analysis:

Percentage mortality was corrected by Abbott formula (Abbott, 1925). Toxicity values (LC_{10}, LC_{25}, LC_{50}, LC_{75} and LC_{95}) were calculated by probit analysis **(**Finney 1971) using Ldp-line software.

Data analyses were expressed as mean ± standard error by using one-way ANOVA, through the SPSS computer program, statistically significant at $p < 0.05$ with least significant difference (LSD)

RESULTS

Based on the LC_{50}, the susceptibility of *C. maculatus* adults varied among tested compounds (Table 1). Thiamethoxam showed the highest toxicity followed by deltamethrin which has moderate toxicity while the least effect was recorded with fennel seeds powder with LC_{50} value of 21.05, 85.1 and 36325.8 ppm, respectively.

Table 1: Toxicological evaluation of deltamethrin, thiamethoxam and fennel seeds after 24 hr of exposure against *C. maculatus* adults

Treatments	LC_{10} ppm	LC_{25} ppm	LC_{50} ppm	LC_{75} ppm	LC_{95} ppm	Slope
Deltamethrin	2.2 (0.5-5.2)	12.5 (5.5-20.02)	85.1 (61.7-127.6)	579.4 (310.98-1821.2)	9155.6 (2607.55-101917.5)	0.81 ± 0.13
Foeniculum vulgare	11630.25 (9162.3-13859.3)	19947.8 (17274.6-22299.9)	36325.8 (33471.47-39491.67)	66150.6 (58798.06-77141.54)	156694.5 (125009.4-213839.6)	2.6± 0.2
Thiamethoxam	12.8 (10.5-14.3)	16.2 (14.5-17.4)	21.05 (19.85-22.55)	27.4 (25.1-31.8)	40.04 (33.9-53.8)	5.9 ± 0.9

The numbers of eggs per female *C. maculatus* on faba bean seeds treated with LC_{10}, LC_{25}, LC_{50} and LC_{75} of deltamethrin, thiamethoxam and fennel seeds powder are given in table (2). The application of thiamethoxam significantly affected eggs laying at all concentrations used 1.43, 0.7, 0.4, and 0.3 for LC_{10}, LC_{25}, LC_{50} and LC_{75}, respectively. While deltamethrin reduced the number of eggs laid counts in comparison with the control, but its effect was significant only for the high concentration tested LC_{50} (6.6 ppm) with complete banning of eggs laying at LC_{75}. In contrast, fennel seeds powder failed to turn down the number of eggs laid by *C. maculatus* females compared with the control. The impact of tested powders on the fecundity was clear and there was a decrease in the number of eggs laid by females as powders concentration increased compared with the control.

The suppression of progeny production is as important a parameter as adult mortality to minimize grain damage by insect pests. During current studies, the insecticide application significantly affected progeny production. Adult emergence was significantly lower at all concentrations used of deltamethrin, thiamethoxam, and fennel seeds powders than the control and decreased as powders concentration amount increased. Moreover, progeny production was significantly suppressed at the rate of LC_{75} of both deltamethrin and thiamethoxam powders.

The residual activities of the contact treatment of faba bean seeds with LC_{95} of the deltamethrin, thiamethoxam and fennel seeds powders on *C. maculatus* adult's mortality (Table 3 and Fig. 1). Different mortality rates were noted among treatments at each exposure interval from the initial (24 h) to 14 months for both deltamethrin and thiamethoxam and 3 months in the case of fennel seeds. The percentage mortality of *C. maculates* adults after exposure for different periods on faba bean seeds treated with LC_{95} of deltamethrin and thiamethoxam provided the most efficient way in preserving seeds especially in the long run, complete protection to treated faba bean seeds with maintained after 24 h till 7 and 4 months for deltamethrin and thiamethoxam, respectively. A Gradual decrease in their toxicity occurred to reach 62.7 and 22.2% after 14 months post-treatment,

for deltamethrin and thiamethoxam, respectively. In contrast, fennel seeds powder achieved high mortality of 95.3% only after 24 h, and then the effectiveness sharply declined after that period and caused the least mortality of 12% at 3 months and thereafter failed to produce any mortality against this insect species.

Table 2: Number of eggs laid and adult survivorship (Mean ±SE) of *C. maculatus* adults post exposure to faba bean seeds treated with deltamethrin, thiamethoxam and fennel seeds powders at different dosage levels.

Tested powders	Conc. (ppm)	No. / female			
		Number of eggs laid (Mean ±SE)	Reduction % of hatchability	Number of emerging adults (Mean ±SE)	Reduction % of emerging adults
Control	-	28.4±3.3	-	17.3±2.6	-
Deltamethrin	2.2	25.6 ±2.7	64.2	1.90±0.06*	88.99
	12.5	24.8±1.4	67.3	1.43±0.20*	91.7
	85.1	6.6±5.1*	94.88	0.50±0.30*	97.1
	579.4	00.0±0.00*	100	0.00±0.00*	100
Thiamethoxam	12.8	1.43±0.4*	96.6	0.40±0.30*	99.6
	16.2	0.7±0.4*	98.98	0.07±0.10*	100
	21.05	0.4±0.23*	99.7	0.03±0.03*	100
	27.4	0.3±0.13*	99.89	0.00±0.00*	100
Foeniculum vulgare	11630.25	33.3±6.6	48.8	7.10±0.98*	59.1
	19947.8	29.3±11.3	60.8	4.80±1.20*	72.2
	36325.8	28.4±1.3	73.03	3.30± 0.30*	80.7
	66150.6	26.3±5.1	76.1	2.60±0.60*	84.9

* The mean difference is significant related to control followed by LSD ($P<0.05$).

Table 3: Percentage mortality of *C. maculatus* adults exposed to faba bean seeds treated with LC_{95} of deltamethrin, thiamethoxam and fennel seeds powders after different periods (moths)

Time (month)	% adults' mortality (mean±SE) of exposed insects after indicated period			Control
	Deltamethrin	Thiamethoxam	*Foeniculum vulgare*	
0 (24h)	100±0.00	100±0.00	96.0± 0.0	00.0
1	100±0.00	100±0.00	68.0±0.58	00.0
2	100±0.00	100±0.00	62.7±0.89	00.0
3	100±0.00	100±0.00	12.0±0.58	00.0
4	100±0.00	100±0.00	00.0±0.00	00.0
5	100±0.00	88.0±0.58	00.0±0.00	00.0
6	100±0.00	70.7±0.67	00.0±0.00	00.0
7	100±0.00	68.0±1.00	00.0±0.00	00.0
8	97.3±0.33	54.7±0.33	00.0±0.00	00.0
9	96.0±0.00	49.3±0.89	00.0±0.00	00.0
10	94.7±0.33	46.7±0.33	00.0±0.00	00.0
11	84.0±0.00	32.0±0.00	00.0±0.00	00.0
12	82.7±0.33	30.7±0.33	00.0±0.00	00.0
13	68.0±0.58	25.3±0.33	00.0±0.00	00.0
14	62.7±0.89	22.7±0.33	00.0±0.00	00.0

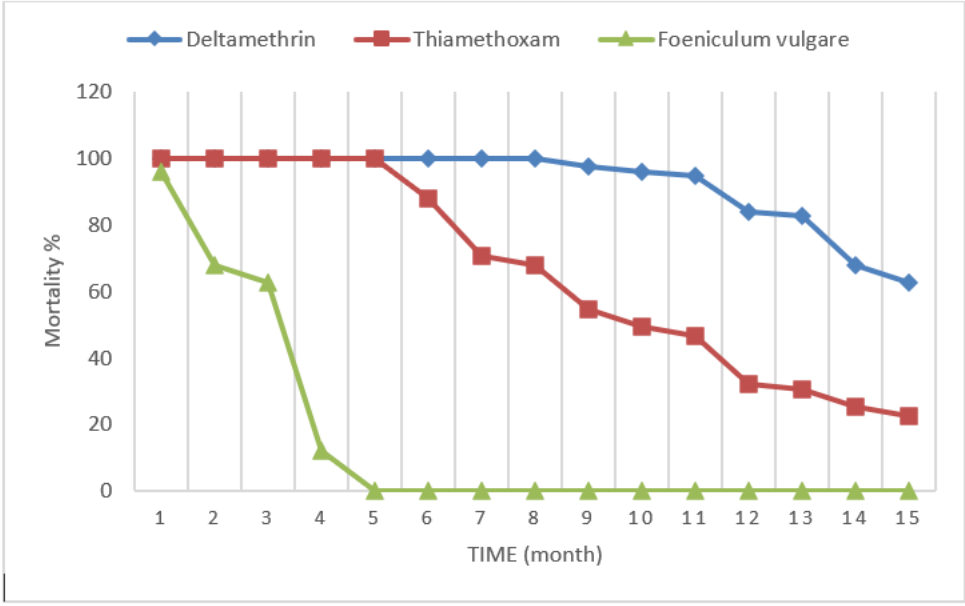

Fig.1: Residual toxicity to *Callosobruchus maculatus* adults exposed to faba bean seeds treated with LC_{95} of deltamethrin, thiamethoxam and fennel seeds powders after different periods (moths)

Data in table (4) indicated that the activity of AChE was significantly lower in all treatments related to the control associated with a much lower sublethal concentration of thiamethoxam (LC_{10}). On the other hand, the activity of α esterase observed a significant decrease in all sublethal concentrations of thiamethoxam and deltamethrin. While the activity of ß esterase showed a significant decrease at LC_{10} of thiamethoxam only and all sublethal concentrations of deltamethrin compared with control. The GST activity exhibited a much lower value at LC_{10} sublethal concentration of thiamethoxam and a significant decrease at the other treatments.

Table 4: Enzymes activities at adults of *Callosobruchus maculatus* exposed to sublethal concentrations of deltamethrin, thiamethoxam and fennel.

Treatments		AChE (mmole /min/ mg protein)	Carboxylesterase (mole/min/ mg protein)		GST (mmol/min/mg protien)
			A	β	
Control		0.0409±0.0004	0.0105±0.00028	0.5933±0.0669	5.362±0.2102
Deltamethrin	**LC_{10}**	0.02440±0.0012*	0.0094±0.0017*	0. 5004±0.050*	4. 6468±0.1525*
	LC_{25}	0.01810±0.0011*	0.010±0.0024*	0.4722±0.0212*	4. 3022±0.2584*
	LC_{50}	0.0267±.0020*	0.0082±0.0004*	0.4918±0.0219*	4.4876±0.1770*
	LC_{75}	0.0166±0.0008*	0.0036±0.0002**	0.1991±0.0066*	3.2407±0.2917*
Thiamethoxam	**LC_{10}**	0.0147±0.0007*	0.0042±0.0008*	0.4920±0.0166*	2.7442±0.3473*
	LC_{25}	0.0221±0.0004*	0.0076±0.0003*	0. 6755±0.0241	4.0523±0.1640*
	LC_{50}	0.0234±0.0022*	0.0074±0.0004**	0. 5376±0.016	5.1455±0.0996
	LC_{75}	0.0208±0.0021*	0.0062±0.001**	0. 5216±0.0193	4. 0880±0.2446*
Fennel *Foenicultim vulgare*	**LC_{10}**	0.0277±0.0019*	0.0085±0.0002	0.5213±0.0203	3.8777±0.1204*
	LC_{25}	0.0237±0.0015*	0.0093±0.0001	0.5170±0.018	4.4171±0.067*
	LC_{50}	0.0243±0.0012*	0.008±0.0004	0.599±0.0415	4. 6895±0.1597*
	LC_{75}	0.0248±0.0010*	0.0091±0.0003	0.4159±0.0156*	3. 5429±0.1873*

Enzyme activity is showed as mean ± SE followed by significant different by LSD ($P<0.05$).

The germination percentages of treated faba bean seeds with tested powders concerning storage period were presented (Table 5 and Fig. 2). At the initial application, germination percentages of faba bean seeds treated with deltamethrin, thiamethoxam, and fennel seeds powders at LC_{95} values and untreated seeds (control) were observed. The

germination percentages within the seed protectants at the initial time were 96, 95 and 94% for deltamethrin, thiamethoxam and fennel seeds powders, respectively compared with 98% for control. While after 14 months of storage, the germination percentages of faba bean seeds treated with deltamethrin, thiamethoxam and fennel seeds powders were 84, 85 and 85%, respectively compared with 86% for control.

Table 5: Germination percentages (Mean± SE) of faba bean seeds after treatment with LC_{95} of deltamethrin, thiamethoxam and fennel seeds powders at initial time and after storage period.

Tested powders	Germination %	
	Mean± SE	
	Initial	After storage
Control	98±0.29	86±0.29
Deltamethrin	96±0.00	84±0.00
Thiamethoxam	95±0.25 *	85±0.25
Foeniculum vulgare	94±0.29*	85±0.25

* The mean difference is significant at the 0.05 level compare with control.

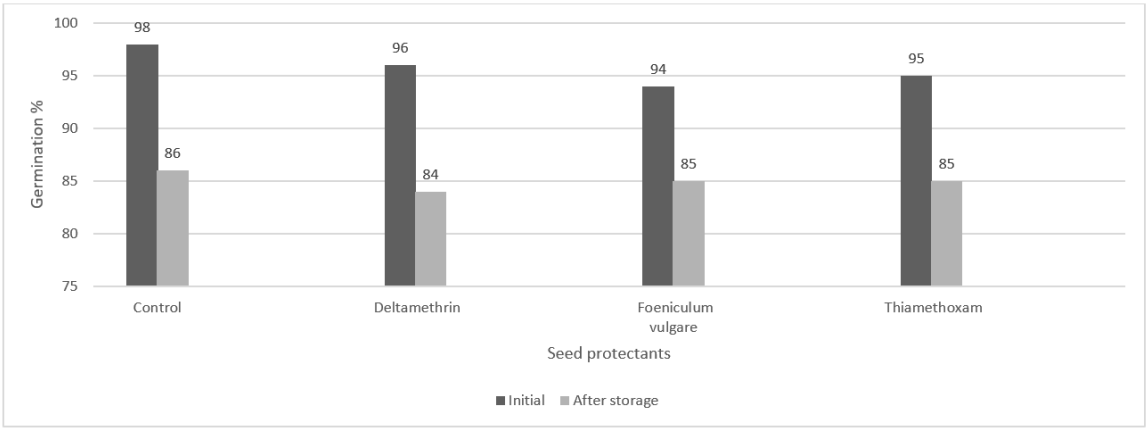

Fig.2: Germination percentages of faba bean seeds after treatment with LC_{95} of deltamethrin, thiamethoxam and fennel seeds powders at initial time and after storage period

DISCUSSION

Based on the LC_{50}, thiamethoxam proved the highest activity followed by deltamethrin, then finally *F. vulgar*e seed powder (Table, 1). Previous findings confirmed thiamethoxam superiority against several species of stored product pests. For instance, Al-Attar (2016) reported that thiamethoxam was more toxic than three pyrethroid insecticides against five coleopterans stored products pest's species. In addition, *Plodia interpunctella* larvae were susceptible when exposed to maize treated with 50 ppm of thiamethoxam (Yue et al., 2003). Similarly, *Rhyzopertha dominica* and *Sitophilus oryzae* exposed to 1–4 ppm record 90–100% control (Fang *et al*. 2002). Also, thiamethoxam was the most effective against *Tribolium confusum* young larvae (Saglam *et al*., 2013). As thiamethoxam could be used with success against a wider range of species as a grain protectant (Rumbos *et al*., 2018).

The effect of tested powders on fertility was clear; all tested powders have a concentration-dependent effect on both egg-laying and adult emergence. Egg-laying status

was a combination of some factors throughout the larval period treatments, but not because of adult treatments, only when adult females killed because of insecticide treatment, the number of laid eggs will be reduced. Similar findings with different grain protectants confirmed this notice. Regarding thiamethoxam, it provided high levels of parental mortality and progeny production suppression against examined stored-product beetle species (Rumbos *et al*., 2018). Similarly, thiamethoxam application at a low-level suppressed progeny production of *Oryzaephilus surinamensis, Rhyzopertha dominica, Tribolium castaneum* and *Sitophilus zeamais* on wheat and maize (Arthur *et al*., 2004). With deltamethrin, Stathers (2002) reported that deltamethrin prevented progeny emergence during the 8-week period. Our results confirmed several reports from multi-country which showed that deltamethrin was very effective against cowpea beetle (ZeroFly, 2015). Progeny production is a major function of any control measure, as it is crucial for long-term stored-products protection (Wakil *et a*l., 2013). In our assay, C. maculatus was found to be more susceptible to thiamethoxam and had the highest value for F1 progeny reduction and did not respond to fennel seeds powders as compared to other insecticides had the lowest value for percentage F1 progeny reduction. Ruman *et al*. (2013) stated that F. vulgare plant powder was the lowest effective powder in the average of eggs/female, emergency of adult insect numbers with averages reached 90.60 eggs/female, 66.60 insect/female compared to *Nigella sativa* and *Cyperus rotundus* powders.

The residual effectiveness is a very important consideration when insect management tactics are to be designed for stored products. Our results indicate that deltamethrin, thiamethoxam and fennel seeds powders can be applied successfully against *C. maculatus* adults to protect seeds from infestation during storage. Regarding stability, fennel seeds powder is less stable than deltamethrin and thiamethoxam powders. Loss of biological activity of fennel seeds powder was probably due to the quick degradation of its active components (volatile oil). Our findings in line with Heydarzade and Moravvej (2012) who reported that the biological activity of *F. vulgare* oil was lost in a relatively short time and did not exceed 30 h. Ngamo *et al*. (2007) showed that the persistence of the biological activity of essential oils extracted from *Hyptis spicigera* and *Lippia rugosa* L. towards four stored-product pests, including *C. maculatus*, did not exceed 24 h. The decline in the efficacy of deltamethrin and thiamethoxam insecticides occurs slowly compared with fennel seeds powder. These results support the findings of Rai *et al*. (1987) that jute bags and jute cloth made cover impregnated with deltamethrin revealed good protection values 5 months after treatment against *R. dominica* and *T. castaneum*. Mishra and Pandey (2014) also found deltamethrin 2.5 WP at 40 mg/Kg wheat most effective of 8 treatments against *S. oryzae* after 3 months of storage. Singh *et al*. (1998), Pathak *et al.* (2002) found deltamethrin effective when wheat was dusted at 3 ppm. A similar result was reported by Pathak and Jha (2001) that deltamethrin was the most effective of all treatments (chlorpyrifos methyl, etrimfos, and malathion) after 180 days of storage on treated wheat.

Acetylcholinesterase plays a major role in the insect nervous system, which terminates nerve impulses by catalyzing the hydrolysis of the acetylcholine neurotransmitter. Several studies have established that pyrethroids can produce secondary effects on acetylcholinesterase (AChE) activity (Hossain *et al*., 2004) and has been reported that neonicotinoids reduce the AChE activity (Azevedo-Pereira *et al*., 2011; Badawy *et al*., 2015). The AChE inhibition one of the essential oils and monoterpene fumigation modes of action against stored grain insects (Kiran and Prakash, 2015). In the current paper, AChE exhibited a significant decrease at *C. maculatus* which treated with thiamethoxam, deltamethrin and fennel seed powders that agreed with Abdelsalam *et al*. (2020) who found that imidacloprid reduced acetylcholinesterase activity of the red palm weevil *Rhynchophorus ferrugineus* related to the dose (10, 15, 20 and 30 ppm). The significant

inhibition of AChE was estimated in *C. maculates* treated with cassia oil and exhibited strong inhibition when treated with *Guiera senegalensis* galls methanol extract (El-Sayed *et al*., 2015; Sombié *et al*., 2018).

The disturbance could reflect protective physiological responses such as the increment of carboxylesterases (CarE), and glutathione S-transferases (GST) that play important roles in insecticide metabolism (Yu, 2004). Esterase and GST are a group of enzymes made up of protein (85%) and they play a critical role in the detoxification of toxic compounds that enter and exit from the insect body (Tarigan, and Harahap, 2016). Esterases main roles are degradation and hydrolysis ester bond found on pyrethroids and other pesticides, esterases act as regulatory enzymes and have been reported in all organisms for degradation functions (Bhatt *et al*., 2020). GST activity has been detected in all insects and in all larval and pupal stages (Hemmingway, 2000). Insect GSTs play an important role in protecting tissues from oxidative stress (Le *et al*., 2002). GST activity is used as an indicator of insecticide stress and examined in many species (Hou et al., 2008) and can be induced or inhibited as a response to toxicants exposure (Kolawole *et a*l., 2011).

In this study, all sublethal treatments exhibited significantly decreased esterases and GST. The results were similar to the study conducted by Tarigan and Harahap (2016) who found that cardamom, cinnamon and nutmeg essential oils inhibit GST activity and reduced esterase activity on *C. maculatus* (concentration-dependent).

The results showed no adverse effect on the germination of faba bean seeds, due to the chemical and botanical seed protectants application. In all the treatments, seeds germination declined progressively with an increase in the storage period. The seeds treated with tested powders-maintained germination percent above the Minimum Seed Certification Standards (>75 %) up to the 12 weeks of storage period. The reduction in seed viability might be due to, temperature changes followed by changes in the moisture content of stored seeds during the storage periods (14 months). The present results were similar to those previously reported by El-Emam *et al*. (2010) who stated that prolonging the storage period over 12 months affected adversely seed germination. These results were also supported by Castellanos *et al*. (2017), who stated that a greater germination percentage of seeds treated with thiamethoxam doses between 200 and 300 mL 100 kg^{-1} of seeds, while doses below or above this range led to germination percentages similar to the control. This result was also supported by Almeida *et al*. (2011); Clavijo (2008); Almeida *et al*. (2009); Lauxen *et al*. (2010) who observed a similar response with the use of thiamethoxam in different seeds. In consistent with these results, Yadav *et al.* (2018) found that germination percent was found to be 84.33 and 83.00% when the seed was treated with emamectin benzoate 5SG at 40.0 g kg^{-1} seed and deltamethrin at 0.04 ml kg^{-1} seed respectively up to 9 months of storage.

Conclusions

The current study exhibited that sublethal concentrations of thiamethoxam, deltamethrin had potentially adverse effects and reduced the population growth on *C. maculatus*. Thiamethoxam may a promising insecticide and more effective to control *C. maculatus* and didn't affect seeds germination.

Ethics approval and consent to participate: The manuscript does not contain any studies involving human participants, human data, or human tissue.

Availability of data and materials: All data generated during this study are included in this published article.

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the experiment and estimated the enzyme activity and analyzing the obtained data. The two authors cooperated in write and read the final manuscript and approved it.

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