

Comparative effect of biopesticides against the fall armyworm *Spodoptera frugiperda* (J.E. Smith)

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Summary The current study evaluated the effect of microbial-derived insecticides (abamectin and spinosad), two microbials (*Beauveria bassiana* (Balsamo) Vuillemin, *Bacillus thuringiensis* Berliner), and three chitin synthesis inhibitors (CSIs) (chlorfluazuron, hexaflumuron and lufenuron) against *Spodoptera frugiperda* larvae. Spinosad and abamectin caused pronounced mortality against second larval instar of *S. frugiperda* using the leaf dipping method. Spinosad induced higher toxicity ($LC_{50} = 4.01$ mg/L) than abamectin ($LC_{50} = 8.33$ mg/L) one day after treatment. The treatments with *B. bassiana* and *B. thuringiensis* caused higher mortality of *S. frugiperda* larvae 7 days after treatment with LC_{50} values of 3.0×10^5 spores/ml and 8.2×10^6 cells/ml, respectively. In the case of the CSIs, hexaflumuron showed higher toxicity than chlorfluazuron and lufenuron with LC_{50} values of 0.01, 0.009 and 0.005 mg/L 3, 7 and 10 days after treatment, respectively.

Additional keywords: abamectin, *Beauveria bassiana*, *Bacillus thuringiensis*, chitin synthesis inhibitors, fall armyworm

Introduction

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) is a destructive insect species for many crops including field crops, such as maize, rice, sorghum, sugarcane, and cotton, and vegetable crops, such as tomato, potato, cucumber, and cabbage. The insect causes massive loss crop yield either in quality or quantity (Murúa *et al.*, 2006; Prasanna *et al.*, 2018). Recently, the insect has become a key pest in grain crops, especially maize, in most of the countries in America and Africa (Rwomushana *et al.*, 2018; Mendesil *et al.*, 2023). Typical insect damage symptoms include holes in plant leaves and death of a heart of young plants due to larvae feeding (Abrahams *et al.*, 2017; Capinera, 2017).

In Egypt, *S. frugiperda* was first recorded in May 2019 in a maize field in Aswan Governorate (Upper Egypt) and since then it

has been spread out throughout the country (Dahi *et al.*, 2020; Gamil, 2020; Al-Ayat *et al.*, 2022). *Spodoptera frugiperda* was first reported in West Africa in late 2016 (Goergen *et al.*, 2016), and by early 2017 the pest invaded Sub-Saharan Africa. Recent reports confirmed the occurrence of fall armyworm in 28 countries in Africa (Day *et al.*, 2017). Since the outbreak of *S. frugiperda* in Africa, the synthetic insecticides have been broadly applied for the management of this insect pest on infested crops, particularly maize (Tepa-Yotto *et al.*, 2022). As the overuse of synthetic insecticides is connected with serious problems, such as the increase of environmental pollution, adverse effects on animals and humans, and emerging resistance of insects (Yu, 1991; Prasanna *et al.*, 2018), alternative strategies, such as bioinsecticides, entomopathogenic fungi, pheromone traps, and parasitoids, have been examined and used against *S. frugiperda* (Mendez *et al.*, 2002; Gutierrez-Martinez *et al.*, 2012; Varshney *et al.*, 2021).

Many studies report the efficacy of biopesticides, such as entomopathogenic bacteria, fungi, viruses and microbial-derived insecticides (spinosad, spinetoram, and abamectin) on larvae of *S. frugiperda* (Polanc-

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zyk et al., 2000; Mendez et al., 2002; Molina-Ochoa et al., 2003; Ríos-Velasco et al., 2010; Deshmukh et al., 2020; Kulye et al., 2021; Han et al., 2023). However, no information is available on the efficacy of such products against the Egyptian strain of *S. frugiperda*. Therefore, the study focuses on examining the effect of microbial-derived insecticides (abamectin and spinosad), the microbials (*Beauveria bassiana* (Balsamo) Vuillemin) and *Bacillus thuringiensis* Berliner and three CSIs (chlorfluazuron, hexaflumuron and lufenuron) on *S. frugiperda* larvae in Egypt. Also, the latent effects of CSIs on biological aspects of this insect pest were assessed.

Materials and Methods

Source and Insect Rearing

Larvae of *S. frugiperda* were first collected from a maize field in Al-Sharqia, Egypt and reared on maize leaves until completing a life cycle (Sayed et al., 2022). Healthy male and female adults were allowed to mate and female laid eggs in plastic jars. The resulting neonate larvae were fed on fresh castor bean leaves, *Ricinus communis*, under laboratory ($28 \pm 1^\circ\text{C}$, $65 \pm 5\%$ relative humidity (RH) and 12:12 h of light and dark). The larvae were reared on castor bean leaves because the plant is available all the year and is cultivated in an area free from insecticides.

Tested insecticide compounds

The tested insecticides were: spinosad (98%) and hexaflumuron (95%) (Dow Agro-Sciences LLC, USA); abamectin (98%) and lufenuron (94%) (Syngenta, Switzerland); chlorfluazuron (95%) (Simonis BV, Netherlands). The microbial compounds were produced as follows:

Culture of *Beauveria bassiana*

The original source of *B. bassiana* fungus was Bioinsecticides Production Unit, Agriculture Research Center, Giza, Egypt. The fungal strain was cultured and maintained following a protocol described by Mohamed et al. (2018). The fungal spores were prepared

and the concentration was calculated using a haemocytometer and adjusted to 1.0×10^4 , 1.0×10^5 , 1.0×10^6 , 1.0×10^7 , 1.0×10^8 and 1.0×10^9 spores/ml to be used in the bioassay experiments.

Bacillus thuringiensis

Bacillus thuringiensis subsp. kurstaki was used as a Protecto product (9.4% WP contain 32,000 international unit/mg) produced by Bioinsecticides Production Unit, Agriculture Research Center, Giza, Egypt. The six concentrations of *B. thuringiensis* (3.0×10^5 , 3.0×10^6 , 3.0×10^7 , 3.0×10^8 , 3.0×10^9 and 3.0×10^{10} cells/ml).

Bioassays

Bioassays were conducted by using the leaf dipping method according to Insecticide Resistance Action Committee (IRAC) method (IRAC, 2018). The concentrations of tested insecticides, spinosad, abamectin, chlorfluazuron, hexaflumuron and lufenuron were prepared in acetone and tested at 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/L. Acetone was used as solvent because the active ingredients of insecticides are not soluble in water. The concentrations of *B. bassiana* and *B. thuringiensis* were prepared in distilled water. The fungus (*B. bassiana*) was tested at 1.0×10^4 , 1.0×10^5 , 1.0×10^6 , 1.0×10^7 , 1.0×10^8 and 1.0×10^9 conidia/ml, while *B. thuringiensis* was tested at 32.0×10^4 , 32.0×10^5 , 32.0×10^6 , 32.0×10^7 , 32.0×10^8 and 32.0×10^9 cells/ml.

The castor bean leaves were cut into small pieces (4×4 cm). The pieces were immersed for five seconds in each concentration and then left to complete evaporation of solvent. Three treated pieces were transferred to each plastic cup (8 cm diameter $\times$ 5 cm high). Ten newly molted second instar larvae of *S. frugiperda* were introduced to each cup. The second larval instar was chosen to give sufficient time to complete the experiment before the larvae turned into pupae. The cups were covered with cheese cloth and kept under above mentioned insect rearing environment. Three replicates were used in each tested concentration. An additional series of castor bean leaves were

treated with pure acetone (99%) or distilled water alone and served as a control. After 24 h, the treated leaf pieces were discarded and fresh untreated leaf pieces were introduced daily for 10 days.

Mortality percentages were recorded 1, 3, 5, 7 and 10 days after treatment. Additionally, the larvae fed on the treated leaves with CSIs were examined daily until complete pupation and adult emergence and percentages of pupation, adult emergence and malformation were calculated.

Data Analysis

Abbott's formula (1925) was used for correction of mortality data. Values of LC_{50} were calculated using probit analysis (Finney, 1971). Percentages of pupation, adult emergence and malformation were analyzed with ANOVA using Tukey's HSD test at a significance level <0.05 (SPSS, Chicago, IL, USA).

Results

The mortality of larvae in control treatments, both water and acetone did not exceed 10 and 15%, respectively; the mean of these mortalities was used to correct the larvae mortalities by Abbot for the insecticide treatments, and for the estimation of corresponding LC_{50} values.

Toxicity of the two microbial-derived insecticides (abamectin and spinosad) expressed as LC_{50} values against the second larval instar of *S. frugiperda* 1 and 3 days after treatment is summarized in Table 1. Both insecticides showed pronounced toxicity in leaf dipping application; spinosad showed higher toxicity ($LC_{50} = 4.01$ mg/L) than abamectin ($LC_{50} = 8.33$ mg/L) one day after treatment. However, both compounds showed similar toxicity 3 days after treatment where LC_{50} values were 0.18 and 0.19 mg/L for spinosad and abamectin, respectively.

The LC_{50} values of the entomopathogenic fungus *B. bassiana* and the bacterium *B. thuringiensis* against *S. frugiperda* second instar larvae 3, 5 and 7 days after treatment are presented in Table 2. Both biological control agents displayed different levels of insecticidal effect which enhanced with the increase of concentration and time after treatment. The toxicity of *Beauveria bassiana* and *B. thuringiensis* was high 3 days after treatment as their LC_{50} values were 3.9×10^7 spores/ml and 2.6×10^8 cells/ml, respectively. The effect of both biological control agents increased significantly 5 and 7 days after treatment. Five days after treatment, the LC_{50} values for *B. bassiana* and *B. thuringiensis* were 1.2×10^6 spores/ml and 2.2×10^7 cells/ml, whereas the values decreased to 3.0×10^5 spores/ml and 8.2×10^6 cells/ml, after

Table 1. Comparative toxicity of microbial-derived insecticides against second instar larvae of *Spodoptera frugiperda* 1 and 3 days after treatment.

Insecticide	Time (days)	LC_{50}^a (mg/L) (Confidence limits)	LC_{90}^b (mg/L) (Confidence limits)	Slope ^c ± SE	(χ^2) ^d	P^e
Abamectin	1	8.33 (4.49-21.65)	684.71 (163.36-7321.31)	0.67±0.08	4.11	0.391
	3	0.19 (0.12-0.30)	4.47 (2.74-10.96)	0.94±0.07	13.9	0.031
Spinosad	1	4.01 (2.67-7.32)	151.90 (51.32-986.94)	0.81±0.11	5.40	0.144
	3	0.18 (0.13-0.23)	3.53 (2.37-5.98)	0.99±0.09	5.03	0.284

^{a,b}The concentration causing 50 and 90% mortality.

^c Slope of the concentration-mortality regression line ± standard error.

^d Chi square value.

^e Probability value.

Table 2. Comparative toxicity of *Beauveria bassiana* and *Bacillus thuringiensis* against second instar larvae of *Spodoptera frugiperda* 3, 5 and 7 days after treatment.

Insecticide	Time (days)	LC ₅₀ ^a (spores/ml) (Confidence limits)	LC ₉₀ ^b (spores/ml) (Confidence limits)	Slope ^c ± SE	(χ^2) ^d	P ^e
<i>Beauveria bassiana</i>	3	3.9x10 ⁷ (1.3x10 ⁷ - 1.5x10 ⁸)	6.5x10 ¹² (2.1x10 ¹¹ - 5.1x10 ¹⁵)	0.25±0.04	5.10	0.164
	5	1.2x10 ⁶ (4.0x10 ⁵ - 3.5x10 ⁶)	4.5x10 ¹¹ (3.1x10 ¹⁰ - 4.3x10 ¹³)	0.23±0.03	4.12	0.389
	7	3.0x10 ⁵ (1.2x10 ⁵ - 6.8x10 ⁵)	3.2x10 ⁹ (7.4x10 ⁸ - 2.5x10 ¹⁰)	0.32±0.03	1.44	0.836
<i>Bacillus thuringiensis</i>	3	2.6x10 ⁸ (8.5x10 ⁷ - 1.3x10 ⁹)	5.6x10 ¹³ (1.4x10 ¹² - 8.9x10 ¹⁶)	0.24±0.04	0.68	0.879
	5	2.2x10 ⁷ (8.9x10 ⁶ - 4.9x10 ⁷)	2.3x10 ¹¹ (3.2x10 ¹⁰ - 6.3x10 ¹²)	0.32±0.04	0.62	0.893
	7	8.2x10 ⁶ (3.2x10 ⁶ - 1.8x10 ⁷)	5.9x10 ¹⁰ (1.5x10 ¹⁰ - 4.0x10 ¹¹)	0.33±0.03	7.29	0.121

^{a,b} The concentration causing 50 and 90% mortality.

^c Slope of the concentration-mortality regression line ± standard error.

^d Chi square value.

^e Probability value.

7 days, respectively.

Toxicity of the three CSIs against second larval instar of *S. frugiperda* 3, 7 and 10 days after treatment expressed as LC₅₀ values is summarized in Table 3. CSIs had a strong toxicity against *S. frugiperda* larvae. Hexaflumuron displayed the greatest insecticidal effect with LC₅₀ values of 0.01, 0.009 and 0.005 mg/L 3, 7 and 10 days after treatment, respectively. Chlorfluazuron and lufenuron were highly effective 10 days after treatment as their LC₅₀ values were 0.09 and 0.06 mg/L, respectively, while both compounds showed moderate toxicity 3 and 7 days after treatment.

The delayed effect of CSIs on pupation, emergence and malformation of pupae and adults is presented in Table 4. Pupation and adult emergence percentages of treated larvae decreased significantly with increasing concentrations of the tested CSIs compared to untreated larvae (95.0 and 94.9 %). The treatment with chlorfluazuron at 1.0 mg/L and hexaflumuron at 0.5 mg/L caused complete inhibition of pupation. Moreover, treatments with 0.25 mg/L of chlorfluazuron and hexaflumuron, and with 2.5 mg/L of lufenuron could induce complete

suppression of adult emergence. Also, the treatment with the CSIs induced malformation of pupae and adults. Chlorfluazuron and lufenuron at 0.25 mg/L caused 50.0% malformation of pupae. Chlorfluazuron at 0.1 mg/L, hexaflumuron at 0.05 mg/L and lufenuron at 1.0 mg/L resulted in 33.3, 25.0 and 33.0% malformation of adults, respectively.

Discussion

The insecticidal effects of spinosad, abamectin, *B. bassiana*, *B. thuringiensis*, chlorfluazuron, hexaflumuron and lufenuron have been reported against *S. frugiperda* strains present in some countries around the world (Polanczyk et al., 2000; Mendez et al., 2002; Eriksson, 2019; Kulye et al., 2021). However, this is the first study on the toxicity of these compounds or products against *S. frugiperda* strain present in Egypt.

The treatment of castor bean leaves with spinosad and abamectin induced pronounced mortality of second larval instar of *S. frugiperda* 3 days after treatment with LC₅₀ values less than 0.2 mg/L. In agreement

Table 3. Comparative toxicity of three chitin synthesis inhibitors against second instar larvae of *Spodoptera frugiperda* 3, 7 and 10 days after treatment.

Insecticide	Time (days)	LC ₅₀ ^a (mg/L) (Confidence limits)	LC ₉₀ ^b (mg/L) (Confidence limits)	Slope ^c ± SE	(χ ²) ^d	P ^e
Chlorfluazuron	3	0.34 (0.25-0.55)	7.81 (3.31-31.27)	0.95±0.11	3.99	0.263
	7	0.34 (0.24-0.45)	13.37 (6.36-39.0)	0.80±0.08	7.96	0.093
	10	0.09 (0.06-0.12)	7.35 (3.34-24.32)	0.66±0.07	7.44	0.114
Hexaflumuron	3	0.01 (-)	1.26 (-)	0.63±0.13	6.65	0.036
	7	0.009 (0.003-0.018)	1.09 (0.41-9.69)	0.62±0.13	4.92	0.085
	10	0.005 (-)	0.43 (-)	0.67±0.14	6.50	0.039
Lufenuron	3	0.43 (0.28-0.76)	101.18 (28.26-777.28)	0.54±0.07	2.09	0.719
	7	0.29 (0.20-0.42)	32.84 (13.51-120.87)	0.62±0.06	3.02	0.697
	10	0.06 (0.03-0.12)	85.03 (17.76-1528.62)	0.41±0.06	2.51	0.642

^{a,b}The concentration causing 50 and 90% mortality.^c Slope of the concentration-mortality regression line ± standard error.^d Chi square value.^e Probability value.

with the present results, spinosad has been reported to induce high toxicity against larvae of *S. frugiperda* with LC₅₀ of 0.557 mg/L 4 days after treatment (Hardke *et al.*, 2011). Furthermore, Adamczyk *et al.* (1999) evaluated the toxicity of spinosad against third instar larvae *S. frugiperda* and found the LC₅₀ value of this compound to be 4.4 mg/L, which is similar to the obtained value (LC₅₀ = 4.01 mg/L) in our study. The results may support the potential use of spinosad for the management of the fall army worm as it interferes with nicotinic acetylcholine and γ-aminobutyric acid (GABA) receptors through pathways dissimilar from those of other insecticides, i.e., it stimulates the nervous system of insects, causing uncontrolled movement, paralysis, and death (Salgado, 1998; De Deken *et al.*, 2004). On the other hand, abamectin showed higher toxicity against the second larval instar of *S. frugiperda* in this study than that demonstrat-

ed by Ahissou *et al.* (2021) who reported an LC₅₀ value of 58.5-429.9 mg/L against third instar larvae two days after treatment using the IRAC leaf bioassay protocol. These discrepancies in toxicity could be attributed to differences in insect strain, larval stage, time after treatment and assay method. Abamectin binds with γ-aminobutyric acid (GABA) receptors, leading to open chloride channel and thus allowing more chloride ions to enter the nerve cell and disturb the transportation of nerve pulses, consequently, resulting in insect paralysis and stop of feeding (Rohrer and Arena, 1995). Gutierrez-Moreno (2017) and Sisay *et al.* (2019) stated that spinosad and abamectin had the potential for the control of *S. frugiperda* in crop fields.

The *B. bassiana* strain caused high larval mortality of *S. frugiperda* 7 days after treatment with LC₅₀ 3.0×10⁵ spores/ml which was greater than that reported by Ramanujam *et al.* (2020) against the second larval stage

Table 4. Effect of chitin synthesis inhibitors on pupation and adult emergence of *Spodoptera frugiperda*.

Conc. (mg/L)	Pupation %	Pupal deformation %	Adult emergence %	Adult deformation %	Survival %
Chlorfluazuron					
0.0	95.0±2.1a	0.0±0.0c	94.9±2.0a	0.0±0.0b	90.0±1.0a
0.01	60.0±4.1b	8.3±0.2bc	66.6±6.7a	8.0±2.9ab	46.7±2.8b
0.05	40.0±2.0bc	12.5±1.0abc	50.0±2.1ab	25.0±1.0ab	20.0±2.5bc
0.1	26.7±5.0bc	25.0±2.0ab	50.0±7.0ab	33.3±5.8a	13.3±2.4c
0.25	20.0±2.1cd	50.0±2.1a	0.0±0.0b	0.0±0.0b	0.0±0.0d
0.50	6.7±2.4de	16.7±2.4abc	0.0±0.0b	0.0±0.0b	0.0±0.0d
1.0	0.0±0.0e	-	-	-	-
Hexaflumuron					
0.0	95.0±2.1a	0.0±0.0a	94.9±2.0a	0.0±0.0a	90.0±1.0a
0.01	40.0±1.2b	0.0±0.0a	83.3±6.0ab	16.0±3.0a	33.3±3.1b
0.05	33.3±2.3bc	25.0±2.0a	66.7±5.8ab	25.0±2.0a	20.0±1.6bc
0.1	13.3±3.1bcd	33.0±6.0a	33.0±1.2ab	16.6±3.1a	6.7±2.4cd
0.25	7.0±1.2cd	17.0±2.4a	0.0±0.0b	0.0±0.0a	0.0±0.0d
0.50	0.0±0.0d	-	-	-	-
Lufenuron					
0.0	95.0±2.1a	0.0±0.0d	94.9±2.0a	0.0±0.0a	90.0±1.0a
0.01	60.0±2.5b	8.0±3.4c	50.0±1.2b	0.0±0.0a	30.0±1.6b
0.05	53.3±4.7bc	9.7±0.9bc	44.4±3.9bc	0.0±0.0a	23.3±0.6bc
0.1	46.7±6.2bc	25.0±0.8b	38.9±2.2bc	17.0±3.1a	16.7±2.3bc
0.25	46.6±5.0bc	50.0±2.0a	16.6±1.0bcd	0.0±0.0a	5.0±0.4cd
0.50	20.0±4.1cd	0.0±0.0d	11.1±2.3bcd	0.0±0.0a	3.3±1.1d
1.0	20.0±2.0cd	0.0±0.0d	8.3±1.2cd	33.0±1.1a	1.7±0.6d
2.5	13.3±2.4d	0.0±0.0d	0.0±0.0d	0.0±0.0a	0.0±0.0d

Values in columns within each compound followed by the different letters are significantly different ($P < 0.05$).

of *S. frugiperda* ($LC_{50} = 1.9 \times 10^7$ spores/ml). The current results are in agreement with those by Garcia *et al.* (2011) reporting that 1×10^9 conidia/ml of *B. bassiana* induced 96.6% mortality on second instar larvae of *S. frugiperda*. although they did not determine LC_{50} values. Morales-Reyes *et al.* (2013) and Ramanujam *et al.* (2020) reported that mortality ranged between 10 and 65% at two concentrations of *B. bassiana* (1×10^6 and 1×10^7 conidia/ml). The *B. bassiana* conidia start germination when they contact insect body and germinated spores penetrate into insect body via cuticle joints. After penetration inside the insect's body, the fungus starts to invade other insect tissues and continues a vegetative growth producing toxic

compounds, eventually leading to insect's death (Logrieco *et al.*, 2002).

Bacillus thuringiensis is among the most used microbial biopesticides for Lepidoptera pest control due to its high efficacy and less adverse effects on mammals and non-target organisms. In the current study, *B. thuringiensis* showed a pronounced toxicity against *S. frugiperda* larvae with an LC_{50} value of 8.2×10^6 cells/ml after 7 days. Similar results against the second larval stage of *S. frugiperda* were found by Polanczyk *et al.* (2000) for *B. thuringiensis* at LC_{50} value as 8.6×10^6 cells/ml. Hernandez (1988) stated that *B. thuringiensis kurstaki* caused 70% mortality of the second larvae stage of *S. frugiperda* at a concentration of 3×10^7 cells/ml. Capal-

bo *et al.* (2001) found that the application of *B. thuringiensis* against *S. frugiperda* achieved complete mortality of neonate larvae in field trials. Furthermore, other strains of *B. thuringiensis* have been shown to possess toxicity against *S. frugiperda* larvae (Dos Santos *et al.*, 2009; Loto *et al.*, 2019; Varshney *et al.*, 2021). A protoxin large protein (about 130-140 kDa) of *Bt* solubilises in the insect gut and cleaves by a gut protease to yield a delta-endotoxin (about 60kD) which binds to the midgut epithelial cells, making openings in the membranes and resulting in an equilibration of ions. Consequently, the gut is quickly immobilised, the epithelial cells lyse, the larva stops feeding, and the gut pH is dropped by equilibration with the blood pH. This lower pH allows the bacterial spores to sprout, and the bacterium can then invade the host, inducing a lethal septicaemia (Sanchis and Bourguet, 2008; Schünemann *et al.*, 2014).

The CSIs induced strong toxicity against *S. frugiperda* second instar larvae with hexaflumuron being more effective than chlorfluazuron and lufenuron. The LC_{50} value of lufenuron was 0.29 mg/L 7 days after treatment, which was similar to that (LC_{50} = 0.23 mg/L) obtained by Nascimento *et al.* (2016). But lower than that reported by Eriksson (2019) (LC_{50} = 0.12 mg/L) on the third larval stage 4 days after treatment. Moreover, novaluron has shown high toxicity against *S. frugiperda* larvae with LC_{50} value (0.166 mg/L) (Hardke *et al.*, 2011). Beside their effect on larval mortality, the tested CSIs (chlorfluazuron, hexaflumuron and lufenuron) induced significant malformation in pupae and adults, and reduced adult emergence. These results coincide with the results of earlier studies on the activity of CSIs against lepidopteran insects (Whiting *et al.*, 2000; Butter *et al.*, 2003; Biddinger *et al.*, 2006). The recorded malformation effect and inhibition of adult emergence of CSIs are probably due to their inhibitory effects on the chitin synthesis, which adversely affect insect metamorphosis (Khajepour *et al.*, 2012; Hamadah *et al.*, 2015). CSIs inhibit chitin formation in the procuticle and the deposition of epicuticle, causing and unsuccessful molt

and decess. Likewise, CSIs have been confirmed to decrease egg fertility and hatching (Haroardottir *et al.*, 2019).

Conclusion

Based on the outcome of the present study, the non-conventional insecticidal substances, spinosad, abamectin, *B. bassiana*, *B. thuringiensis*, chlorfluazuron, hexaflumuron and lufenuron, revealed a promising toxicity against *S. frugiperda* larvae in Egypt with hexaflumuron being the most effective one in terms of LC_{50} . Therefore, these products may be useful for the IPM management of this invasive insect. The use of such products with diverse mechanisms of action is highly important to delay the development of insect resistance. Also, the use of naturally based products is expected to minimize the impact on non-target organisms, mammals and the environment.

Conflict of Interest

The authors declare that they have no conflict of interest.

Literature Cited

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18:265–267.
- Abrahams, P., Bateman, M., Beale, T., Clotey, V., Cock, M., Colmenare, Y, Corniani, N., Day, R., Early, R., Godwin, J., *et al.* 2017. Fall armyworm: impacts and implications for Africa. CABI, UK, 2017.
- Adamczyk, J.r., Leonard, B.R. and Graves, J.B. 1999. Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassay studies. *Florida Entomologist*, 82: 230-236.
- Ahissou, B.R., Sawadogo, W.M., Bokonon-Ganta, A.H., Somda, I., Kestemont, M.P. and Verheggen, F.J. 2021. Baseline toxicity data of different insecticides against the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and control failure likelihood estimation in Burkina Faso. *African Entomology*, 29: 435-444.
- Al-Ayat, A.A., Atta, A.A. and Gad, H.A. 2022. Biology and nutritional indices of the fall armyworm *Spodoptera frugiperda* fed on five Egyptian host

- plants as a new invasive insect pest in Egypt. *Journal of Crop Protection*, 11: 499-506.
- Biddinger, D., Hull, L., Huang, H., Mcpherson, B. and Loyer, M. 2006. Sublethal effects of chronic exposure to Tebufenozide on the development, survival and reproduction of the tufted apple bud moth. *Journal of Economic Entomology*, 99: 834-842.
- Butter, N.S., Gurmeet, S. and Dhawanm, A.K. 2003. Laboratory evaluation of the insect growth regulator lufenuron against *Helicoverpa armigera* on Cotton. *Phytoparasitica*, 31: 56-60.
- Capalbo, D.M.F., Valicenti, F.H., Moraes, I.O., De Oliveira Iracema and Pelizer, L.H. 2001. Solid-state fermentation of *Bacillus thuringiensis tolworthi* to control fall armyworm in maize. *Electronic Journal of Biotechnology*, 4: 9-10.
- Capinera, J. 2017. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Insecta: Lepidoptera: Noctuidae). <http://edis.ifas.ufl.edu/in255>.
- Dahi, H.F., Salem, S.A., Gamil, W.E. and Mohamed, H.O. 2020. Heat requirements for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. *Egyptian Academic Journal of Biological Sciences*, 13: 73-85.
- Day, R., Abrahams, P., Bateman, M., Beale, T., Clotthey, V., Cock, M. and Witt, A. 2017. Fall armyworm: Impacts and implications for Africa. *Outlooks on Pest Management*, 28: 196-201.
- De Deken, R., Speybroeck, N., Gillain, G., Sigue, H., Batawi, K. and Van Den Bossche, P. 2004. The macrocyclic lactone "spinosad," a promising insecticide for Tsetse fly control. *Journal of Medical Entomology*, 41: 814-818.
- Deshmukh, S., Pavithra, H.B., Kalleshwaraswamy, C.M., Shivanna, B.K., Maruthi, M.S. and Mota-Sanchez, D. 2020. Field efficacy of insecticides for management of invasive fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on maize in India. *Florida Entomologist*, 103: 221-227.
- Dos Santos, K.B., Neves, P., Meneguim, A.M., dos Santos, R.B., et al. 2009. Selection and characterization of the *Bacillus thuringiensis* strains toxic to *Spodoptera eridania* (Cramer), *Spodoptera cosmioides* (Walker) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Biological Control*, 50: 157-163.
- Eriksson, J. 2019. Toxicity bioassays with insecticide formulations used for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). PhD Thesis, North-West Univ South Africa.
- Finney, D.J. 1971. Probit analysis, 3rd Edition. Cambridge University Press, Cambridge.
- Gamil, W.E. 2020. Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) biological aspects as a new alien invasive pest in Egypt. *Egyptian Academic Journal of Biological Sciences*, 13: 189-196.
- García, C., González, M.B. and Bautista, N. 2011. Pathogenicity of isolates of entomopathogenic fungi against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Epilachna varivestis* (Coleoptera: Coccinellidae). *Revista Colombiana de Entomología*, 37: 217-222.
- Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A. and Tamo, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. *PLoS ONE*, 11(10): e0165632.
- Gutierrez-Martinez, A., Tolon-Becerra, A. and Lastra-Bravo, X.B. 2012. Biological control of *spodoptera frugiperda* eggs using *Telenomus remus* Nixon in maize-bean-squash polyculture. *American Journal of Agricultural and Biological Sciences*, 7: 285-292.
- Gutierrez-Moreno, R.A. 2017. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* (Bt) proteins and synthetic insecticides from different corn production systems in Mexico and Puerto Rico. PhD Thesis, Michigan State University.
- Hamadah, K., Tanani, M., Ghoneim, K., Basiouny, A. and Waheeb, H. 2015. Effectiveness of Novaluron, chitin synthesis inhibitor, on the adult performance of Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *International Journal of Research Studies in Zoology*, 1: 45-55.
- Han, H., Chen, B., Xu, H., Qin, Y., Wang, G., Lv, Z., Wang, X. and Zhao, F. 2023. Control of *Spodoptera frugiperda* on Fresh Corn via Pesticide Application before Transplanting. *Agriculture*, 13: 342.
- Hardke, J.T., Temple, J.H., Leonard, B.R. and Jackson, R.E. 2011. Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomologist*, 94: 272-278.
- Haroardottir, H.M., Male, R., Nilsen, F. and Dalvin, S. 2019. Effects of chitin synthesis inhibitor treatment on *Lepeophtheirus salmonis* (Copepoda, Caligidae) larvae. *PLoS ONE*, 14: e0222520.
- Hernandez, J. 1988. Évaluation de la toxicité de *Bacillus thuringiensis* sur *Spodoptera frugiperda*. *Entomophaga*, 32: 163-171.
- IRAC, (Insecticide Resistance Action Committee). 2018. Resistance Definition, Background, Development. (Web page: <http://www.irac-online.org/about/resistance>).
- Khajepour, S., Izadi, H., Asari, M.J. and Liu, N. 2012. Evaluation of two formulated chitin synthesis inhibitors, hexaflumuron and lufenuron against the raisin moth, *Ephestia figulilella*. *Journal of Insect Science*, 12(1): 102.
- Kulye, M., Mehlhorn, S., Boaventura, D., Godley, N.,

- Venkatesh, S.K., Rudrappa, T., Charan, T., Rath, D. and Nauen, R. 2021. Baseline susceptibility of *Spodoptera frugiperda* populations collected in India towards different chemical classes of insecticides. *Insects*, 12: 758.
- Logrieco, A., Moretti, A., Ritieni, A., Caiaffa, M.F. and Macchia L. 2002. Beauvericin: chemistry, biology and significance. In *Advances in Microbial Toxin Research and Its Biotechnological Exploitation*, Upadhyay, R., Ed.; Kluwer Academic: New York, NY, USA, 23–30.
- Loto, F.D.V., Carrizo, A.E., Romero, C.M., Baigori, M.D. and Pera, L.M. 2019. *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from northern Argentina: esterases, profiles, and susceptibility to *Bacillus thuringiensis* (Bacillales: Bacillaceae). *Florida Entomologist*, 102: 347–352.
- Mendesil, E., Tefera, T., Blanco, C.A., Paula-Moraes, S.V., Huang, F., Viteri, D.M. and Hutchison, W.D. 2023. The invasive fall armyworm, *Spodoptera frugiperda*, in Africa and Asia: responding to the food security challenge, with priorities for integrated pest management research. *Journal of Plant Diseases and Protection*, <https://doi.org/10.1007/s41348-023-00777-x>.
- Méndez, W.A., Valle, J., Ibarra, J.E., Cisneros, J., Penagos, D.I. and Williams, T. 2002. Spinosad and nucleopolyhedrovirus mixtures for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *Biological Control*, 25: 195–206.
- Mohamed, H.F., Sileem, T.M., El-Naggar, S.E.M., Sweilem, M., Ibrahim, A., El-sayed, O. and El-khawaga, A. 2018. Effect of gamma irradiation and/or certain entomopathogenic fungi on the larval Mortality of *Galleria mellonella* L. *Egyptian Journal of Biological Pest Control*, 28: 1–8.
- Molina-Ochoa, J., Lezama-Gutierrez, R., Gonzalez Ramirez, M., Lopez-Edwards, M., Rodriguez-Vega, M.A. and Arceo-Palacios, F. 2003. Pathogens and parasitic nematodes associated with populations of fall armyworm (Lepidoptera: Noctuidae) larvae in Mexico. *Florida Entomologist*, 86: 244–253.
- Morales-Reyes, C., Rodriguez-Contreras, J., Sanchez-Pedraza, F.E., Rosales-Escobar, O., Hernandez-Juarez, A., Felipe-Victoriano, M. and Sanchez-Peña, R.S. 2013. Activity of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda*: comparison of conidia produced on artificial media and insect hosts. Paper presented at Conference: Entomological Society of America Annual Meeting, Austin, 10–13 November, 2013.
- Murúa, G., Molina-Ochoa, J. and Coviella, C. 2006. Population dynamics of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its parasitoids in northwestern Argentina. *Florida Entomologist*, 89: 175–182.
- Nascimento, A., Farias, J.R., Bernardi, D., Horikoshi, R.J. and Omoto, C. 2016. Genetic basis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to the chitin synthesis inhibitor lufenuron. *Pest Management Science*, 72: 810–815.
- Polanczyk, R.A., Silva, R. and Fiuza, L.M. 2000. Effectiveness of *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Brazilian Journal of Microbiology*, 31: 164–166.
- Prasanna, B.M., Huesing, J.E., Eddy, R. and Peschke, V.M. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management. 1st (ed.), CIMMYT: Edo Mex, Mexico, 2018.
- Ramanujam, B., Poornesha, B. and Shylesha, A.N. 2020. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in maize. *Egyptian Journal of Biological Pest Control*, 30: 1–5.
- Ríos-Velasco, C., Cerna-Chávez, E., Sánchez-Peña, S. and Gallegos-Morales, G. 2010. Natural epizootic of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson infecting *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila México. *Journal of Research on the Lepidoptera*, 43: 7–8.
- Rohrer, S.P. and Arena, J.P. 1995. Ivermectin interactions with invertebrate ion channels. In: Clark, J.M., (ed.), *Molecular action of insecticides on ion channels*. Am. Chem. Soc. Symp. Ser., no 591. American Chemical Society, Washington, DC, 1995, pp. 264–283.
- Rwomushana, I., Bateman, M., Beale, T., Beseh, P., Cameron, K., Chiluba, M., Clottey, V., Davis, T., Day, R., Early, R., et al. 2018. Fall armyworm: Impacts and Implications for Africa; Evidence Note Update; CABI: Oxfordshire, UK.
- Salgado, V.L. 1998. Studies on the mode of action of spinosad: Insect symptoms and physiological correlates. *Pesticide Biochemistry and Physiology*, 60: 91–102.
- Sanchis, V. and Bourguet, D. 2008. *Bacillus thuringiensis*: applications in agriculture and insect resistance management. A review. *Agronomy for Sustainable Development*, 28: 11–20.
- Sayed, R.M., Ibrahim, S.S. and El-Gepaly, H.M.KH. 2022. Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), larvae to un-irradiated and gamma-irradiated entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control*, 32: 119.
- Schünemann, R., Knaak, N. and Fiuza, L.M. 2014. Mode of action and specificity of *Bacillus thuringiensis* toxins in the control of caterpillars and stink bugs in soybean culture. *ISRN Microbiology*, <https://doi.org/10.1155/2014/135675>.
- Sisay, B., Tefera, T., Wakgari, M., Ayalew, G. and Mendesil, E. 2019. The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda*, in maize. *Insects*, 10: 45.
- Tepa-Yotto, G.T., Chinwada, P., Rwomushana, I., Go-

- ergen, G. and Subramanian, S. 2022. Integrated management of *Spodoptera frugiperda* 6 years post detection in Africa: a review. *Current Opinion in Insect Science*, 52: 100928.
- Varshney, R., Poornesha, B., Raghavendra, A., Lalitha, Y., Apoorva, V., Ramanujam, B., Rangeshwaran, R., Subaharan, K., Shylesha, A.N., Bakthavatsalam, N. and Chaudhary, M. 2021. Biocontrol-based management of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on Indian Maize. *Journal of Plant Diseases and Protection*, 128: 87-95.
- Whiting, D.C., Jamieson, L.E. and Connolly, P.G. 2000. Pre-and postharvest effect of lufenuron on *Epiphyas postvittana* (Lepid.: Tortricidae), *Journal of Economic Entomology*, 93: 673-679.
- Yu, S.J. 1991. Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pesticide Biochemistry and Physiology*, 39: 84-91.

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Συγκριτική δράση βιοεντομοκτόνων κατά του εντόμου *Spodoptera frugiperda* (J.E. Smith)

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Περίληψη Η παρούσα εργασία εξέτασε τη δράση εντομοκτόνων μικροβιακής προέλευσης (abamectin, spinosad), δύο μικροοργανισμών (*Beauveria bassiana* (Balsamo) Vuillemin, *Bacillus thuringiensis* Berliner) και τριών αναστολέων σύνθεσης χιτίνης (CSIs) (chlorfluazuron, hexaptenauruguronper, spofendoflumuron) σε προνύμφες του εντόμου *Spodoptera frugiperda*. Το spinosad και η abamectin προκάλεσαν μεγάλη θνησιμότητα έναντι του δεύτερου προνυμφικού σταδίου του *S. frugiperda* μετά από έκθεση με τη μέθοδο της εμβάπτισης φύλλων. Το spinosad προκάλεσε υψηλότερη τοξικότητα ($LC_{50} = 4,01 \text{ mg/L}$) από την abamectin ($LC_{50} = 8,33 \text{ mg/L}$), μία ημέρα μετά την εφαρμογή. Οι επεμβάσεις με *B. bassiana* και *B. thuringiensis* προκάλεσαν υψηλότερη θνησιμότητα των προνυμφών του *S. frugiperda*, επτά ημέρες μετά την εφαρμογή, με τιμές LC_{50} $3,0 \times 10^5$ σπόρια/ml και $8,2 \times 10^6$ σπόρια/ml, αντίστοιχα. Στην περίπτωση των CSI, το hexaflumuron έδειξε υψηλότερη τοξικότητα από το chlorfluazuron και το lufenuron με τιμές LC_{50} 0,01, 0,009 και 0,005 mg/L, 3, 7 και 10 ημέρες μετά την εφαρμογή, αντίστοιχα.

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