Toxicity and Biochemical Effects of Spirotetramat and its Binary Mixtures with Nanosilica against *Aphis gossypii* Glover, *Bemisia tabaci* Gennadius and the Earthworm, *Eisenia fetida*

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ABSTRACT

Nanotechnology will make agriculture eco-friendly and profitable by reducing the concentration of insecticides used, which is appreciable from the environmental safety perspective. Toxicity of spirotetramat alone and its mixtures with nanosilica against nymphs of *Aphis gossypii* (Hemiptera: Aphididae) and adults of *Bemisia tabaci* (Hemiptera: Aleyrodidae) was evaluated through laboratory and field experiments during two years (2020 and 2021). The possible impact of treatments on *Eisenia fetida* (Oligochaeta: Lumbricidae) was also assessed. The enzymatic activity of carboxylesterase (CarE) and glutathione *S*-transferase (GST) in tested insects and earthworm was estimated. Toxicity of spirotetramat was increased when mixed with nanosilica at 250, 500, 1000 mg L^{-1}, ratios were 1.13, 1.91 & 2.59 -fold on *A. gossypii*, 1.12, 1.41 & 2.26 -fold on *B. tabaci* and 1.05, 1.17 & 1.46 -fold on *E. fetida*, respectively. Spirotetramat and nanosilica at 1000 mg L^{-1} mixture gave the highest significantly increased in CarE activity of *A. gossypii* and *B. tabaci* (11.42 and 9.33 µmol min^{-1} mg $protein^{-1}$), and decreased the GST activity (1.54 and 1.69 µmol min^{-1} mg $protein^{-1}$), respectively, relative to the control. While, the same previous mixture on *E. fetida* increased the enzyme activity of CarE and GST (13.37 and 3.36 µmol min^{-1} mg $protein^{-1}$), respectively, compared to the control. Additionally, the mixture of spirotetramat and nanosilica at 1000 mg L^{-1} recorded the highest population reduction percentages in *A. gossypii* (92.5 and 95.5 %) and *B. tabaci* (79.9 and 84.4 %) compared with spirotetramat alone (88.6 and 90.7%) for *A. gossypii*, (73.6 and 78.9%) for *B. tabaci* during 2020 and 2021 cotton seasons, respectively.

Keywords: spirotetramat; silica nanoparticles; *Aphis gossypii*; *Bemisia tabaci*; *Eisenia fetida*; insecticidal activity; biochemical effects

INTRODUCTION

The cotton aphid (*Aphis gossypii* Glover) and cotton whitefly (*Bemisia tabaci* Gennadius) are among the most damaging pests threatening cotton crop all over the world (Brown, 2010). They cause a significant yield loss through sucking phloem sap, honeydew excretion, which promote the growth of sooty mold fungus and their major role as vectors of plant viruses (Campolo *et al*., 2014 and Polston *et al*., 2014).

Soil invertebrates play a vital role in the decomposition and nutrient cycling processes that are very important for sustaining a healthy soil. Earthworms are ecosystem engineers and one of the most remarkable soil invertebrates used as bioindicators for environmental pollution. Arguably, few studies have evaluated the harmful impact of pesticide applications upon earthworm populations (Fründ *et al.,* 2011; Blouin *et al.*, 2013 and Datta *et al.*, 2016).

Chemical control plays an intrinsic role and remains the basis of integrated pest management (IPM) systems to suppress aforementioned insect pests. However, the massive use of chemical insecticides has developed high levels of resistance to many classes of insecticides (Herron and Wilson, 2011; Longhurst *et al*., 2013; Naveen *et al.,* 2017; Dângelo *et al.,* 2018 and Ma *et al*., 2019).

Spirotetramat is a phloem- and xylem-mobile insecticide derived from tetramic acid with effective control against several sucking insects including aphids, scale insects and whiteflies (Nauen *et al*., 2008). It interferes with lipid biosynthesis, resulting in inhibition of acetyl-CoA carboxylases to delay insect development (Lümmen *et al*., 2014). Compared with neonicotinoid insecticides, studies have shown that spirotetramat is less toxic to honeybees and other beneficial invertebrates, which make it a good choice for IPM programs (Brück *et al*., 2009).

The problems resulting from excessive use of traditional insecticides could be overcome with the development of new alternate pest control strategies to protect the environment from the insecticidal

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pollution, limit the resistance regenerating and increase agriculture crop productivity (Eldesouky 2019 and Tawfeek & Eldesouky, 2022). One of the most promising new approaches for pest control in recent years, the use of nanoparticles in pesticide formulations, where nanotechnology has picked up prevalence at a fast pace in various field and disciplines with special mention in environmental and agricultural systems (Khamis *et al*., 2017). Using of silica nanoparticles for pest control is emerging as a highly attractive research field toward achieving these goals (Athanassiou *et al*., 2018). Their potential action is through desiccation of insect by scratching of the insect`s cuticle or/and absorption of cuticle lipids (Benelli, 2018).

Carboxylesterase (CarE) and glutathione *S*-transferase (GST) are substantial detoxification enzymes involved in the metabolism of xenobiotics in living organisms. Their activities have been thought-out as biomarkers of environmental contamination and chemical stress (Rodríguez-Castellano and Sanchez-Hernández, 2007).

The present study aimed to evaluate the toxicity of spirotetramat and its binary mixtures with nanosilica against *A. gossypii* and *B. tabaci* through laboratory and field experiments. Their environmental impact on *E. fetida* as a bioindicator was also assessed. Additionally, this study was estimated the effects of these mixtures on some detoxification enzymes in *A. gossypii*, *B. tabaci* and *E. fetida*.

MATERIAL AND METHODS

Insects rearing

A laboratory strain of *A. gossypii* used in this experiment was originally collected from cotton fields in Abees, Alexandria, Egypt, and reared for many generations on cotton seedlings, *Gossypium hirsutum* (L.), under laboratory conditions of 22 ± 2 ˚C, 65 ± 5 % relative humidity and 16: 8 h light: dark photoperiod.

A susceptible strain of *B. tabaci* was reared since 2000 on tomato plants, *Solanum lycopersicum* L. (Solanaceae) in greenhouse at 25 ± 7 ˚C, 65 ± 5 % relative humidity and under natural light conditions.

Earthworm maintenance

The red wiggler worms, *Eisenia fetida* (Savigny, 1826) (Oligochaeta: Lumbricidae) were collected from citrus farms at Alexandria Governorate, Egypt, with complete absence of insecticides exposure. Selected mature earthworms (with clitellum), weighing 300-600 mg were maintained in artificial soil under the conditions of 20 ± 2 °C and 35 % moisture content. According to the Organization for Economic Co-operation and Development guidelines 207, the artificial soil used throughout this study was prepared by mixing 10% sphagnum peat, 20% kaolinite clay, 68% quartz sand and 2% calcium carbonate to adjust the pH value (OECD, 1984).

Chemicals

Spirotetramat (Movento® 10 % SC), field rate = 75 ml/ 100 L water, was procured from Bayer Crop Science, Germany. Hydrophilic silica nanoparticles (SiO_2 NP) (15 nm diameter), 99 % purity was purchased from Nanotech Company, Giza, Egypt. All other chemicals used for enzymes assays were analytical grade.

Toxicity bioassays

Toxicity of spirotetramat and its mixtures with nanosilica against *A. gossypii* nymphs

A leaf-dip bioassay method described by Moores *et al.* (1996) was used to evaluate the toxicity of spirotetramat alone and its binary mixtures with silica nanoparticles against third nymphal instar of *A. gossypii*. Five serial concentrations of spirotetramat (1, 2, 4, 8 and 16 mg L^{-1}) were prepared in distilled water. Binary mixtures of spirotetramat with silica nanoparticles (250, 500 and 1000 mg L^{-1}) were also prepared in distilled water. Cotton leaf discs (5 cm diameter) were dipped in each concentration for 20 sec. Leaf discs dipped in water served as control. Treated and control leaf discs were allowed to dry for a one hour and then placed two discs in each Petri-dish (9 cm in diameter) containing filter paper. Each treatment was replicated three times. Twenty aphids' nymphs were placed per each replicate. After 72 h of treatment, the alive and dead insects were counted and the mortality percent was calculated.

Toxicity of spirotetramat and its mixtures with nanosilica against *B. tabaci* adults

The toxicity of above-mentioned treatments was also tested against adults of *B. tabaci*. The uninfected tomato seedlings were sprayed, using a hand-held sprayer 1 liter capacity, with concentrations of spirotetramat alone or its mixtures with silica nanoparticles until runoff. The treated tomato seedlings left to dry for two hours. Control plants were sprayed with distilled water alone. Each treatment was replicated three times. Twenty whitefly adults per replicate were released into the treated and control seedlings covered with glass cages with muslin in the upper. After 72 h of treatment, the alive and dead insects were counted and the mortality percent was calculated.

Toxicity of spirotetramat and its mixtures with nanosilica against *E. fetida*

The toxicity of spirotetramat alone and its binary mixtures with silica nanoparticles against the earthworm, *E. fetida* was evaluated according to the OECD guideline 207 (OECD, 1984) by the artificial soil test. Five serial concentrations of spirotetramat alone (25, 50, 100, 200 and 400 mg kg^{-1} dry soil) and its binary mixtures with each silica nanoparticles concentration (250, 500 and 1000 mg kg^{-1} dry soil) were prepared in distilled water. Each concentration (100 ml total volume) was blended with one kilogram of dry artificial soil and divided into three quantities (3 replicates/ concentration) in ventilated plastic containers. The control treatment was blended with distilled water alone. Ten mature worms were placed in each container. The mortality percent was measured after 7 days of treatment.

Biochemical assays

Homogenate preparation

A. gossypii, *B. tabaci* and *E. fetida* were collected after 72h of treatment by LC_{50} values of spirotetramat alone and its mixtures with silica nanoparticles. Samples were homogenized with 10 volumes (w/v) of ice cold 0.1 M phosphate buffer (pH 8.0) using a Polytron homogenizer (Tekmar tissumizer) for 60 sec. The homogenate was centrifuged at 5000 rpm for 30 min at 4 °C using Janetzki K23 cooling centrifuge. The obtained supernatants were used for measuring protein content and the activities of CarE and GST enzymes.

Protein content

The protein content was determined according to Bradford (1976) by using Bovine Serum Albumin.

Enzymes activity measurements

Carboxylesterase activity (CarE) was determined according to Van Asperen (1962) method using α-naphthyl acetate (α-NA) as a substrate. The activity of CarE was expressed as μmol naphthol min^{-1} mg $protein^{1}$. Treatments were replicated five times.

Glutathione-s-transferase (GST) was determined according to Vessey and Boyer (1984) method using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The activity of GST was expressed as μmol min^{-1} mg $protein^{-1}$. Treatments were replicated five times.

Field experiment

The efficacy of spirotetramat and silica nanoparticles against *A. gossypii* and *B. tabaci* was evaluated in cotton fields during the two seasons of 2020 and 2021. Cotton variety Giza 86 was planted at Abou Hommos, Beheira Governorate, Egypt, adopting normal agronomic practices. Spirotetramat was used alone at field rate and it used at half field rate mixed with nanosilica at concentrations (250, 500 and 1000 mg L^{-1}), while the control plots were sprayed with water alone. The treatments were designed in a randomized complete block design (RCBD) with four replicates (1/24 feddan, 175 m^2 for each). Treatments were sprayed by Knapsack sprayer equipment (CP_3) at the rate of 200 liter/feddan on 9th June and 2nd June at the cotton seasons of 2020 and 2021, respectively. The mean numbers of *A. gossypii* and *B. tabaci* adults were randomly counted on ten labeled plants per replicate during the early cotton growth period. Counts were recorded just prior to application and after 1, 3, 7 and 10 days from application and the reduction percentages were measured according to the Henderson and Tilton (1955) equation as below:

$$\text{ction percentages} = 100\left[1-\frac{Ta \times Cb}{Tb \times Ca}\right]$$

Where: Ta = insect counts after treatment, Tb = insect counts before treatment,

Cb = insect count for control before treatment, Ca = insect count for control after treatment.

Statistical analysis

LC_{50}, LC_{90} and slope of the concentration-mortality regression line values for insecticide and insecticide/nanosilica combinations were calculated by probit analysis (Finney, 1971). Mean values of treatments in the enzymes assay and field experiment were compared for significance using analysis of variance (ANOVA) test with $LSD_{0.05}$ (CoStat Statistical Software, 2005).

RESULTS AND DISCUSSION

RESULTS

Toxicity of spirotetramat and its mixtures with nanosilica on *A. gossypii* and *B. tabaci*

LC_{50} values presented in Table 1 reveals that the nanosilica alone didn't record any toxicity on *A. gossypii* and *B. tabaci*, whereas it increased the toxicity of spirotetramat after 72h of treatment. Where, LC_{50} value for spirotetramat alone on *A. gossypii* was 8.17 mg L^{-1}. When spirotetramat was mixed with nanosilica at 250, 500 and 1000 mg L^{-1}, LC_{50} values were found to be 7.26, 4.28 and 3.15 mg L^{-1}, respectively. However, LC_{50} value for spirotetramat alone on *B. tabaci* was 10.30 mg L^{-1}. While LC_{50} values for the binary mixtures of spirotetramat with nanosilica concentrations became 9.18, 7.32 and 4.56 mg L^{-1}, respectively.

Toxicity of spirotetramat and its mixtures with nanosilica on *E. fetida*

Table 2 shows that the LC_{50} value for spirotetramat alone on *E. fetida* was 217.11 mg L^{-1}. When spirotetramat was mixed with nanosilica at 250, 500 and 1000 mg L^{-1}, LC_{50} values became 206.70, 185.52 and 148.36 mg L^{-1}, respectively. Nanosilica alone didn't record any toxicity on *E. fetida*. Based on LC_{50} values, spirotetramat and nanosilica mixtures were less toxic on

Table 1. Toxicity of spirotetramat alone and its mixtures with nanosilica against *Aphis gossypii* nymphs and *Bemisia tabaci* adults after 72 h of treatment

Treatments	*Aphis gossypii*				*Bemisia tabaci*			
	LC_{50}[a] (mg L^{-1}) (95% CL)	LC_{90}[b] (mg L^{-1}) (95% CL)[c]	Slope ± SE[d]	(χ^2)[e]	LC_{50} (mg L^{-1}) (95% CL)	LC_{90} (mg L^{-1}) (95% CL)	Slope ± SE	χ^2
Nan-silica	> 1000	-	-	-	> 1000	-	-	-
Spirotetramat	8.17 (6.26-11.03)	95.31 (53.48-145.88)	1.20 ± 0.13	0.09	10.30 (7.90-14.17)	113.25 (62.58-187.09)	1.23 ± 0.14	0.17
Spirotetramat + Nano-silica (250 mg L^{-1})	7.26 (5.58-9.63)	80.29 (46.63-126.25)	1.23±0.14	0.45	9.18 (7.07-12.36)	96.42 (55.06-159.73)	1.25 ± 0.14	0.19
Spirotetramat + Nano-silica (500 mg L^{-1})	4.28 (3.16-5.63)	54.32 (32.48-82.53)	1.16 ± 0.13	0.51	7.32 (5.50-9.62)	84.28 (48.12-126.34)	1.20±0.13	0.35
Spirotetramat + Nano-silica (1000 mg L^{-1})	3.15 (2.30-4.11)	34.16 (21.94-56.38)	1.24 ± 0.15	0.20	4.56 (3.48-5.87)	45.46 (28.79-70.49)	1.28±0.16	0.22

[a] The concentration causing 50% mortality
[b] The concentration causing 90% mortality
[c] Confidence limits
[d] Slope of the concentration-mortality regression line ± standard error
[e] Chi square value

Table 2. Effect of spirotetramat alone and its mixtures with nanosilica on the earthworm, *Eisenia fetida* after 7 days of treatment by using the artificial soil test

Treatments	LC_{50}[a] (mg kg^{-1} dry soil) (95% CL)	LC_{90}[b] (mg kg^{-1} dry soil) (95% CL)[c]	Slope ± SE[d]	(χ^2)[e]
Nano-silica	> 1000	-	-	-
Spirotetramat	217.11 (177.54-265.14)	945.26 (672.96-1290.84)	2.06 ± 0.28	0.32
Spirotetramat + Nano-silica (250 mg kg^{-1} dry soil)	206.70 (165.84-256.32)	902.35 (641.92-1178.43)	1.89 ± 0.27	0.70
Spirotetramat + Nano-silica (500 mg kg^{-1} dry soil)	185.52 (158.86-224.80)	788.13 (638.67-978.12)	2.03 ± 0.26	0.11
Spirotetramat + Nano-silica (1000 mg kg^{-1} dry soil)	148.36 (113.27-183.48)	744.82 (579.32-937.49)	1.82 ± 0.25	0.15

[a] The concentration causing 50% mortality
[b] The concentration causing 90% mortality
[c] Confidence limits
[d] Slope of the concentration-mortality regression line ± standard error
[e] Chi square value

E. fetida compared with their toxicity on *A. gossypii* and *B. tabaci*.

Detoxification enzymes activities of *A. gossypii*, *B. tabaci* and *E. fetida*

The effect of spirotetramat and its mixtures with nanosilica on carboxylesterase and glutathione *S*-transferase enzymes activities are illustrated in Figures 1 and 2. Spirotetramat and nanosilica at 1000 mg L^{-1} mixture gave the highest significantly increased in CarE

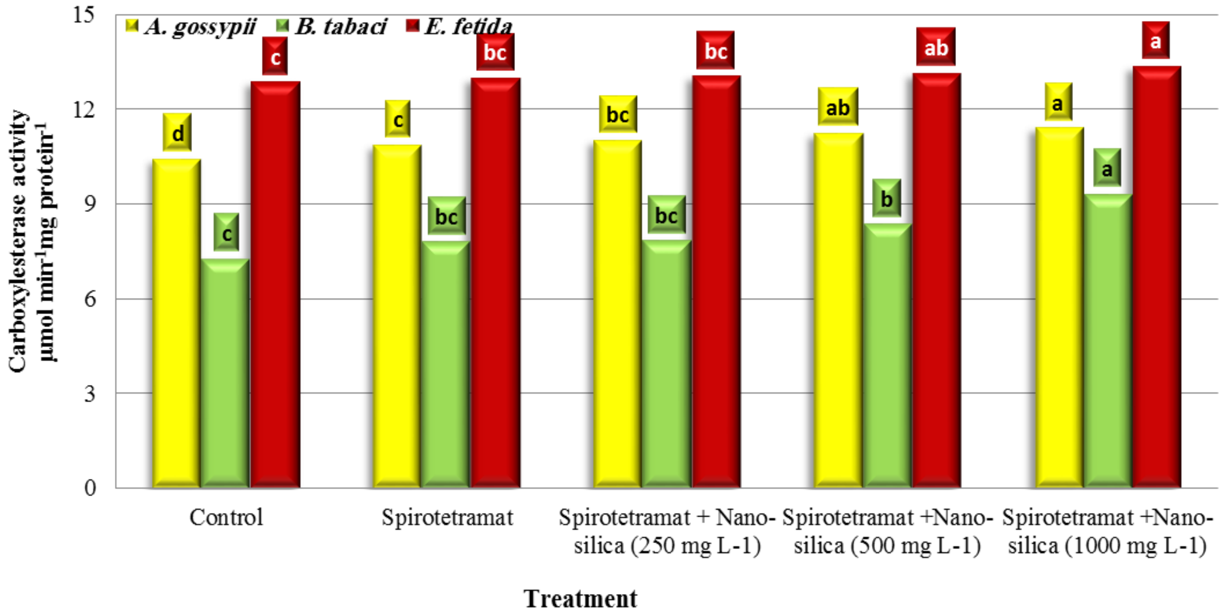

Fig. 1. Effects of spirotetramat alone and its mixtures with nanosilica in the carboxylesterase activity of *Aphis gossypii*, *Bemisia tabaci* and *Eisenia fetida*

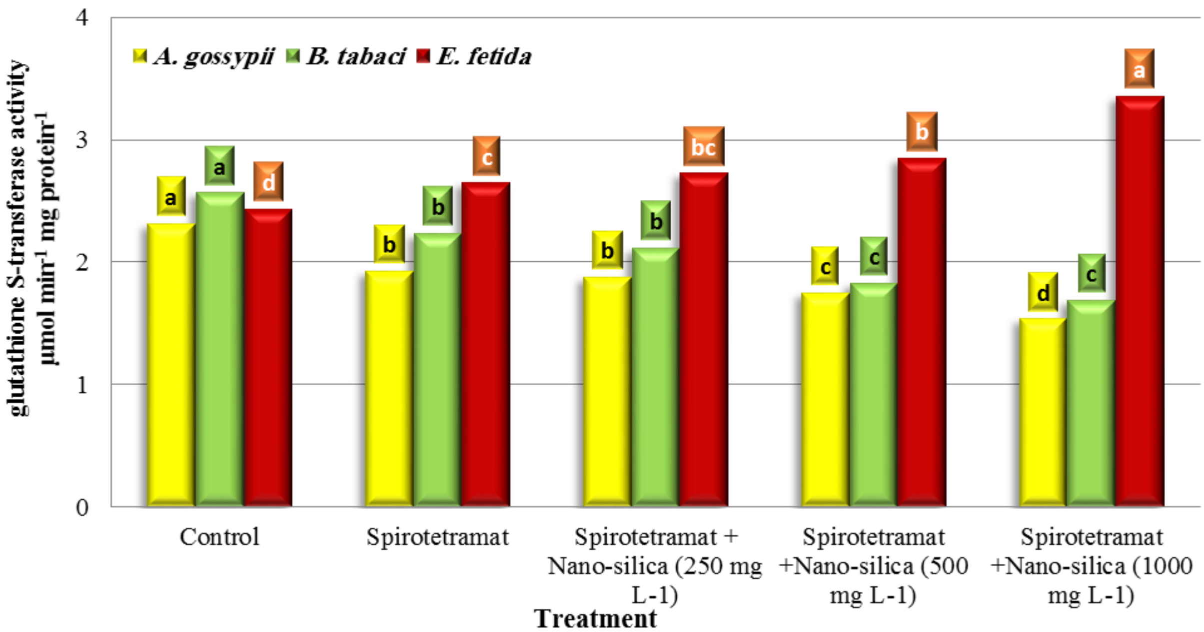

Fig. 2. Effects of spirotetramat alone and its mixtures with nanosilica in the glutathione *S*-transferase activity of *Aphis gossypii*, *Bemisia tabaci* and *Eisenia fetida*

activity of *A. gossypii* and *B. tabaci* (11.42 and 9.33 µmol min^{-1} mg $protein^{-1}$) compared with10.44 and 7.28 µmol min^{-1} mg $protein^{-1}$ for control, the same mixture significantly decreased GST activity (1.54 and 1.69 µmol min^{-1} mg $protein^{-1}$), respectively, compared with 2.32 and 2.57 µmol min^{-1} mg $protein^{-1}$ in the control. While, when the mixture, spirotetramat and nanosilica at 1000 mg L^{-1}, was used on *E. fetida*, it increased the enzyme activities of CarE and GST with values 13.37 and 3.36 µmol min^{-1} mg $protein^{-1}$, respectively, compared with 12.78 and 2.44 µmol min^{-1} mg $protein^{-1}$ in the control.

Field evaluation of spirotetramat alone and its mixtures with nanosilica against *A. gossypii* and *B. tabaci*

The mean reduction percentages of *A. gossypii* and *B. tabaci* during 2020 and 2021 cotton seasons are displayed in Table 3. Data revealed that, the highest reduction percentages in *A. gossypii* (92.5 and 95.5 %) and *B. tabaci* (79.9 and 84.4 %) for 2020 and 2021 cotton seasons, respectively, as affected by the treatment with spirotetramat and nanosilica at 1000 mg L^{-1}. While, the reduction percentages of *A. gossypii* and *B. tabaci* caused by spirotetramat alone were 88.6 and 73.6 %, respectively at 2020 cotton season and 90.7 and 78.9 %, respectively at 2021 cotton season. There are no significant differences in all treatments in two seasons.

DISCUSSION

Nanotechnology shows considerable promise for protection of crops and foodstuffs (Stadler *et al*., 2010), it represents as a new generation of technology that could bring an economic and environmental solution (Ali *et al*., 2014). Through the current study, the toxicity of nanosilica along with spirotetramat against *A. gossypii* and *B. tabaci* was evaluated. The insecticidal activity of silica nanoparticles against aphids has been previously mentioned by Abd El-Wahab *et al.* (2016) who showed that hydrophilic nanosilica at 500 mg kg^{-1} was effective for the control of aphid species *Myzus persicae*, *Acyrthosiphon pisum* and *Aphis craccivora*. Pavitra *et al.* (2018) also recorded that green silica nanoparticle at 2000 mg L^{-1} caused mortality on *A. gossypii* after five days from treatment. Nanoparticles toxicity has been demonstrated against many insects such as *Sitophilus oryzae* (Debnath *et al.*, 2011), mosquitoes, including *Anopheles stephensi* Liston, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say (Barik *et al.*, 2012), cotton leafworm, *Spodoptera littoralis* (El-bendary and El-Helaly, 2013) and the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Rouhani *et al*., 2013 and Arumugam *et al*., 2016). The insecticidal activity of nanosilica was also confirmed by Ziaee and Ganji (2016); Diagne *et al.* (2019); Rouhani *et al.* (2019); Haroun *et al*. (2020) and Salem (2020) against *Rhythopertha dominica* F., *Tribolium confusum* Jacquelin du Val., *Caryedon serratus* (Olivier), *Tribolium castaneum* Herbst., *C. maculatus* F., and *Sitophilus granarius* (L.).

Spirotetramat is environmentally safe and harmless to pollinators and has a broad spectrum activity against many sucking insects with a very long lasting efficacy. Thus, the current study explained the insecticidal and biochemical activities of spirotetramat against *A. gossypii* and *B. tabaci*, which is being in agreement with results observed by Arnaudov and Petkova (2020) whose confirmed that spirotetramat was effective in the control of *M. persicae* and significantly superior in efficacy and persistence than that of the reference neonicotinoids imidacloprid and thiamethoxam. Chen *et al*. (2018) also, showed that spirotetramat was highly toxic to *B. tabaci* nymphs but not adults. Gong *et al.* (2016) and Ramalakshmi *et al.* (2020) revealed that spirotetramat was highly effective on the *A. gossypii* fecundity and increased the total CarE activity dramatically. At the same pace, the insecticidal effect of spirotetramat was confirmed against other insects, cotton mealybug, *Phenacoccus solenopsis* (Rezk *et al.*, 2019 and Sequeira *et al.*, 2020), *Tetranychus urticae* Koch (Marcic *et al.*, 2011 and Saryazdi *et al.*, 2013). Fiaz *et al.* (2018) found that combined application of spirotetramat along with *Isaria fumosorosea* formulation has shown a significant synergistic effect against *Diaphorina citri* infestation. Tang *et al.* (2020) also, affirmed that Thiamethoxam + spirotetramat 40% SC at 60–80 mg/kg was effective for the control of the Asian citrus psyllid, *D. citri* with a control efficacy of 72.92 to 99.29% during 3–30 days. On the other hand, the present results disagree with the results of Behnam-Oskuyee *et al.* (2020) who found that spirotetramat was less effective against sugarcane whitefly, *Neomaskellia andropogonis* Corbett.

Regrettably, earthworms with other beneficial soil microorganisms have become target organisms to pesticides. So, the present study focused on the impact of spirotetramat and nanosilica on *Eisenia fetida* (Savigny). There are no available studies about the effect of spirotetramat against *E. fetida*, but the effect of neonicotinoid insecticides on the other earthworm species has been evaluated in several studies. Capowiez *et al.* (2005) showed that LC_{50} of imidacloprid for *Aporrectodea nocturna* and *Allolobophora icterica* was between 2 and 4 mg kg^{-1} dry soil. Dittbrenner *et al.* (2010) assessed the sub-lethal effects of imidacloprid on two earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*) after 7 days of exposure in contaminated soil, a significant loss of body mass was 0.66 mg kg^{-1} dry soil. De Lima e Silva *et al.* (2017)

Table 3. Field evaluation of spirotetramat alone and its mixtures with nanosilica against *Aphis gossypii* and *Bemisia tabaci* during 2020 and 2021 cotton seasons

Cotton seasons	Treatments	Mean numbers of *A. gossypii* Reduction percentages (%)						Mean numbers of *B. tabaci* Reduction percentages (%)					
		No. before spray	1-day	3-day	7-day	10-day	General mean	No. before spray	1-day	3-day	7-day	10-day	General mean
2020	Control	1250	1300	1360	1480	1532	1418	285	287	292	298	303	295
	Spirotetramat	940	192 80.4	128 87.5	98 91.2	56 95.1	88.6^{a}	316	118 62.9	95 70.7	72 78.2	58 82.7	73.6^{a}
	Spirotetramat + Nano-silica (250 mg L^{-1})	895	174 81.3	115 88.2	76 92.8	48 95.6	89.5^{a}	290	105 64.1	78 73.8	63 79.2	45 85.4	75.6^{a}
	Spirotetramat + Nano-silica (500 mg L^{-1})	780	132 83.7	97 88.6	58 93.7	36 96.2	90.6^{a}	196	65 67.1	52 74.1	39 81.0	28 86.6	77.2^{a}
	Spirotetramat + Nano-silica (1000 mg L^{-1})	648	93 86.2	65 90.8	32 95.8	23 97.1	92.5^{a}	278	83 70.4	64 77.5	48 83.5	35 88.2	79.9^{a}
	$LSD_{0.05}$	-	-	-	-	-	8.9	-	-	-	-	-	13.1
2021	Control	1030	1050	1200	1320	1450	1255	252	260	263	275	282	270
	Spirotetramat	965	152 84.6	123 89.1	94 92.4	45 96.7	90.7^{a}	334	112 67.5	83 76.2	58 84.1	46 87.7	78.9^{a}
	Spirotetramat + Nano-silica (250 mg L^{-1})	820	110 86.8	94 90.2	62 93.5	28 97.6	92.0^{a}	225	73 68.6	48 79.6	36 85.3	29 88.5	80.5^{a}
	Spirotetramat + Nano-silica (500 mg L^{-1})	785	94 88.3	72 92.1	38 96.2	20 98.2	93.7^{a}	186	58 69.8	36 82.3	24 88.2	20 90.4	82.7^{a}
	Spirotetramat + Nano-silica (1000 mg L^{-1})	730	67 91.0	46 94.6	24 97.4	9 99.1	95.5^{a}	162	43 74.3	28 83.4	20 88.7	16 91.2	84.4^{a}
	LSD 0.05	-	-	-	-	-	6.9	-	-	-	-	-	13.3

Means within the same column with the same superscript have no significant differences at $p \leq 0.05$. Spirotetramat was applied at field rate = 75 ml/ 100 L water. Silica nanoparticles at 250, 500 and 1000 mg L^{-1} were mixed with spirotetramat at half field rate. Reduction percentages were calculated according to Henderson and Tilton (1955) equation.

proved that imidacloprid was more toxic than thiacloprid on *Eisenia andrei*. Ritchie *et al.* (2019) investigated that exposure to clothianidin resulted in a 56-d LC_{50} of 0.26 mg kg^{-1} dry soil for *E. andrei*. Exposure to thiamethoxam was less toxic, with LC_{50} of 3.0 mg kg^{-1} dry soil for *E. andrei*. Other studies confirmed the toxicity of neonicotinoid insecticides against *Eisenia* species compared with the other insecticides. Wang *et al.* (2012a) tested the toxicities of 24 insecticides against *E. fetida* and found that acetamiprid and imidacloprid were the two most toxic insecticides overall. Wang *et al.* (2012b) found that clothianidin, the neonicotinoid insecticide, was the most toxic pesticide to *E. fetida*. Alves *et al.* (2013) found that imidacloprid was the most toxic for *E. andrei* than the other tested substances at lower concentrations under tropical conditions. Other observations by Shoults-Wilson *et al.* (2011) who found that *E. fetida* consistently avoid soils containing silver nanoparticles. Feng *et al.* (2015) studied the effects of thiacloprid on molecular biomarkers (GST, CarE and DNA damage) of *E. fetida* using the artificial OECD soil for the first time. Lackmann *et al.* (2021) reported significant changes for catalase, carboxylesterase and multixenobiotic activities in *E. andrei* after 48-h exposures to esfenvalerate, thiacloprid and two herbicides.

CONCLUSION

Lastly, the obtained results indicated that spirotetramat alone and its mixtures with nanosilica could be utilized in a safe integrated pest management program for the control of *A. gossypii* and *B. tabaci*. Further studies are also needed to evaluate the performance of nanoparticles in field conditions and their toxicity on non-target organisms in order to select chemicals that cause little harm to the soil ecosystem.

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الملخص العربي

السمية والتأثيرات البيوكيميائية للسبيروتترامات وخلائطه مع النانوسيليكا تجاه منّ القطن *Aphis gossypii* (Glover) وذبابة القطن البيضاء *Bemisia tabaci* (Gennadius) ودودة الأرض *Eisenia fetida*

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تسهم تقنية النانو فى جعل الزراعة صديقة للبيئة من خلال تقليل تركيزات المبيدات الحشرية المستخدمة، وهو أمر هام من منظور السلامة البيئية. لذلك تم تقييم فاعلية الاسبيروتترامات منفرداً وخلائطه مع النانوسيليكا تجاه العمر الحورى الثالث لمنّ القطن *Aphis gossypii* والحشرات الكاملة لذبابة القطن البيضاء *Bemisia tabaci* وذلك معملياً وحقلياً خلال العامين (٢٠٢٠ و٢٠٢١). كذلك تم تقييم التأثير المحتمل للمعاملات على دودة الأرض *Eisenia fetida* كأحد الكائنات غير المستهدفة. وقد تم تقدير النشاط الإنزيمي لكل من الكربوكسيل استيريز (CarE) والجلوتاثيون اس ترانسفيريز (GST) فى الحشرات المختبرة ودودة الأرض. لوحظ تحت الظروف المعملية أن سمية الاسبيروتترامات زادت عند خلطه مع النانوسيليكا بتركيزات ٢٥٠ و ٥٠٠ و ١٠٠٠ ملجم/ لتر، بمعدل يبلغ ١,١٣ و ١,٩١ و ٢,٥٩ ضعف على المنّ كما بلغ المعدل ١,١٢ و ١,٤١ و ٢,٢٦ ضعف على ذبابة القطن البيضاء وبلغ ١,٠٥ و ١,١٧ و ١,٤٦ ضعف على دودة الأرض، على الترتيب. سجل خليط الاسبيروتترامات والنانوسيليكا ١٠٠٠ملجم/لتر أعلى زيادة للنشاط الانزيمي للكربوكسيل استيريز فى *A. gossypii* و *B. tabaci* بمقدار ١١,٤٢ و ٩,٣٣ ميكرومول/دقيقة ملجم بروتين ، على الترتيب. كما تسبب فى نقص النشاط الانزيمي للجلوتاثيون اس ترانسفيريز بمقدار ١,٥٤ و١,٦٩ ميكرومول/دقيقة ملجم بروتين، على الترتيب مقارنة بالكنترول. فى حين أن نفس الخليط أدى إلى زيادة النشاط الانزيمي لكلا الإنزيمين CarE و GST فى دودة الأرض بمقدار ١٣,٣٧ و٣,٣٦ ميكرومول/دقيقة ملجم بروتين، على الترتيب. بالإضافة إلى ذلك، تم تسجيل أكبر نسبة خفض فى عشائر المنّ بمقدار ٩٢,٥ و٩٥,٥% ولعشائر ذبابة القطن البيضاء بمقدار ٧٩,٩ و٨٤,٤% خلال موسمي القطن ٢٠٢٠ و٢٠٢١، على الترتيب، بتأثير المعاملة باستخدام الخليط اسبيروتترامات مع النانوسيليكا ١٠٠٠ملجم/ لتر.

الكلمات المفتاحية: الاسبيروتترامات، النانوسيليكا، منّ القطن، ذبابة القطن البيضاء، دودة الأرض، النشاط الإبادي، التأثيرات البيوكيميائية.