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Novel Systemic Insecticides, Spirotetramat and Flonicamid: Field Performance against *Bemisia tabaci* (Gennadius) and Potential Safety for Indigenous Bioindicators

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#### **ABSTRACT**

One of the most destructive plant pests is the whitefly, Bemisia tabaci (Gennadius). Spray and soil-drenching applications of the novel insecticides, spirotetramat 10% SC and flonicamid 50% WG were evaluated against the adults and nymphs of the whitefly along two seasons of the cotton crop. Sprays of both insecticides had potent protection against adults for the first 6 days after treatment (DAT), but were shifted from 3 to 6 DAT in soil-drenching. All the applications controlled the nymphs along the 12 DAT. The Beneficial Arthropod Index expressed unfavorable balances for these insecticides between the whitefly and its parasitic wasps. Both insecticide sprays had potent reductions on Eretmocerus mundus (Mercet) over 3-12 DAT. Spirotetramat 10% SC affected Encarsia artenopae (Foerster) over 3-6 DAT, contrary to the unsteady effects of flonicamid 50% WG. Flonicamid's soil-drenching showed unsteady declines on these wasps compared to spirotetramat 10% SC that lasted from 3 to 6 DAT. Based on the International Organization for Biological Control's guidelines, both insecticides were moderately harmful to the overall mean population of soil micro-arthropods along 12 DAT.

#### INTRODUCTION

The whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is one of the most devastating crop pests for a wide range of plant families worldwide, especially in tropical climates (Ferreira *et al.*, 2022). It threatens formidably the yield of the cotton crop (*Gossypium hisutum* L.) via the feeding attitude of sap-sucking, high performance of viral transmission in several plant hosts, and rapid resistance to insecticides (Aslam *et al.*, 2022). Therefore, Schuster *et al.*, (2004) estimated the protection level against the whitefly population not to exceed the threshold limit at  $\leq 5$  adults or nymph individuals per crop leaf.Recently, novel systemic insecticides have been introduced in the controlling program, which revealed great efficiency on the developmental stages of whitefly, *B. tabaci* (Genn.), besides their safety limits on igenous non-target arthropods (Colomer *et al.*, 2011; El-Sherbeni *et al.*, 2018; Shi *et al.*, 2021; Abbas *et al.*, 2022). One of these novel insecticides is spirotetramat (Movento®), which belongs to the derivatives of the tetramic acid class and possesses potent inhibiting action on the fatty acids' biosynthesis (Maus 2008). It is one of

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the few distinguished insecticides that possesses bidirectional systemic action via phloem and xylem (Maus 2008; Mohapatra *et al.*, 2012; Chen *et al.*, 2018). In addition, flonicamid (N-Cyanomethyl-4-trifluromethylnicotinamide), which belongs to the chemical class of pyridine-carboxamides, is a novel antifeedant insecticide against sap-sucking insects (Shi *et al.*, 2021).

Several studies emphasized the high field performance of the foliar application of spirotetramat (Movento®) (Chen et al., 2018; Abbas et al., 2022) and flonicamid (Abbas et al., 2022) as vigor systemic insecticides against all developmental stages of Bemisia tabaci (Genn.). The efficacy of soil treatment with systemic insecticides that undergo the "xylem transport model" performed by Ford et al., (2010) may be associated with the insecticide concentrations exhibited in the volume and velocity of sap in the xylem. Based on the Freundlich isotherm co-efficient, spirotetramat fluently desorbs from assorted profiles of soil textures, and the adsorption capacity has been improved by increases in soil temperature (Chen et al., 2018). Notwithstanding, soil application of flonicamid via drenching or drip irrigation system to the plants' rhizosphere could be implemented at a minimal dosage of 12.5 gm per 1000 plants in terms not to exceed the holding capacity of the soil (Environmental Protection Agency (EPA) 2020). In spite of the high persistence of flonicamid in irrigated water and water from soil sediments, the LC<sub>50</sub> value exceeds 1000 mg kg<sup>-1</sup> of dry weight of soil (Australian Pesticides and Veterinary Medicines Authority 2014). Unfortunately, spirotetramat could cause reductions in both adult and pupae populations of the parasitic wasp, Encarsia Formosa (Gahan) (Hymenoptera: Aphelinidae), and declinations in adult reproduction in both stages (Francesena et al., 2017; Marcic and Drobnjakovic 2021). Regarding the safety studies on the parasitic wasps in agricultural areas, flonicamid was harmless in both adult and mummy stages of Eretmocerus mundus (Mercet) (Hymenoptera: Aphelinidae), except for a few malformations that may appear in its developmental stages (Fernández et al., 2015). Counterwise, applied dosages of spirotetramat's active ingredient and its moieties at 96 gm per hectare or even over-dosages by 10 fold could display a favorable ecotoxicological profile for ecosystems and non-target organisms such as earthworms, soil microorganisms, and soil mites. Even several repetitions of application during the crop season could leftover soil residues that pose a chronic risk to most soil organisms (Maus 2008). Flonicamid had a distinctively minimal impact on beneficial and non-target insects (Colomer et al., 2011). The sub-lethal concentrations of flonicamid fulfill the highest safety terms for earthworms (Zhao et al., 2021) and soil microarthropods (El-Sherbeni et al., 2018).

In this regard, this study focused on the evaluation of the long-term efficacies of spirotetramat 10% SC and flonicamid 50% WG against the adult and nymph stages of *B. tabaci* (Genn.) under field conditions. The tested insecticides were subjected to prescribed international classification and advisory systems to state their potential safety on Indigenous bioindicators throughout their stances on ecosystem balance. Therefore, adverse effects of the tested insecticides were carried out on varied indigenous groups of soil micro-arthropods in the case of soil-drenching as well as on some whitefly-parasitic wasps in the case of spray application.

# **MATERIALS AND METHODS**

# **Tested Insecticides:**

Spirotetramat (Movento®) 10% SC; spray dosage rate 75 mL 100 L<sup>-1</sup>; soil-drenching rate 6 ml 100<sup>-1</sup> infested cotton plants in 42 m<sup>2</sup>) was obtained from Bayer Crop Science, Egypt. Flonicamid (Bryto 50% WG; applied field rate 20 gm 100 L<sup>-1</sup>; soil-drenching rate 2.5 gm 42 m<sup>-2</sup> plot contains 50 infested plants) was obtained from Kafer El-

Zayat of Pesticides & Chemical Co., Egypt. All the spray dosage rates of the mentioned insecticides comply with the behests of the Agriculture Pesticides Committee of the Egyptian Agriculture Ministry. Soil-drenching rates were calculated for flonicamid 50% WG according to the recommendation of EPA (2020) and for spirotetramat 10% SC according to EPA (2010).

# **Field Trials:**

**Infestation Limit of Whitefly on Cotton Plants:** The infestation limit of whiteflies during the field trials was above the threshold limit at  $\leq$  125 for adults or nymph individuals 25<sup>-1</sup> leaves samples Plot<sup>-1</sup> (Schuster *et al.*, 2004). Therefore, the aim of the protection level is to keep the whitefly population below the threshold limit.

Schedule Time and Site of Field Experiments: Foliar spray and soil-drenching treatments against adult and nymph stages of whitefly, *Bemisia tabaci* (Genn.) were carried out on cotton plants (variety, Giza 86) aged by  $\geq$  45 days in both consecutive seasons in 2022 and 2023. The plantation practices adhered to the guidelines of cotton management procedures (Gibbs *et al.*, 2005; The National Cotton Council and the Cotton Foundation 2007). The site of the field experiment was conducted in El-Behira governorate, Egypt (31°05'16.9''N:  $30^{\circ}17'26.6''E$ ). Each type of application was accomplished in a separate area. During the same time, the adverse effects of spray and soil-drenching treatments were evaluated on available indigenous parasitic insects and soil micro-arthropods, respectively.

**A-Foliar Spray Treatment Design:** This application was applied to the vegetative part of the cotton plant. The trials were sectioned into uniform plots (area = 42 m³; contained 50 plants). Each treatment of spirotetramat 10% SC and flonicamid 50% WG contained four plots (replicates) that were randomly partitioned in the whole area of the field experiment based on "Complete Block Design." The recommended spray dosage rates for each insecticide treatment were conducted in a fixed total volume of water (6 L plot<sup>-1</sup>) using CP3 sprayer equipment "Knapsack." Plots of control treatment were sprayed with water only. Sampling and counts on the whitefly population were achieved in the forenoon where a minimal flight performance of the adult individuals occurred (Shah *et al.*, 2021). Pretreatment counts of nymphs of whitefly at zero days before treatments were inspected in the laboratory on adequate samples (25 leaves plot<sup>-1</sup>), whereas post-treatment counts coincided with 0.13 (3 hrs), 3, 6, 9, and 12 days after treatment (DAT). Likewise, pre-and post-treatment counts of the adult population were accomplished in the field location at the same forgoing intervals.

On the other hand, adverse effects of the tested insecticides were estimated by the predator: prey ratio based on the Beneficial Arthropod Index (BIx) (Naranjo *et al.*, 2004; McCravy 2018) to stand on the prospective biological control of the abundant parasitic wasps against whitefly. These indices included the following safety limits:

- (< 2) indicates a whitefly outbreak or unfavorable balance for its indigenous parasitic wasps.
- (2-0) indicates equilibrium status between the whitefly and its indigenous parasitic wasps.
- (>10) indicates the efficiency of indigenous parasitic wasps in defeating the whitefly invasion.

**B-Soil-Drenching Treatment Design:** This application was applied in clay soil adjacent to the feeding root system of the cotton plant. The trials underwent the same foregoing protocol of "Randomized Complete Block Design." Firstly, the water holding capacity (WHC) test was conducted in the laboratory according to the percolation method performed by Slater and Byers (1931) on the treated soil (top layer, 0-25 cm depth) to calculate the total volume of water that is sufficient for soil-drenching application. Water-drenching portions were equally dispensed on the plants in each plot during irrigation time by a medium-volume spray applicator. Thus, the applied soil-drenching dosages of spirotetramat 10% SC and

flonicamid 50% WG were 4.5 ml and 1.25 gm, respectively, in a fixed total volume of water (300 L plot<sup>-1</sup>). Plots of control treatment were drenched with water only. In addition, sampling and counts on the whitefly population followed the same previously mentioned protocol (Shah *et al.*, 2021).

# **Trapping Soil Micro-Arthropods In The Laboratory:**

**Sampling:**Soil samples of the insecticides and control plots were loaded in polyethylene bags from the top layer (0-25 cm depth) adjacent to the feeding root system at intervals of 0.13 (3hrs), 3, 6, 9, and 12 DAT. All samples were delivered to the laboratory to start the tapping process for soil micro-arthropods.

**Trapping Process:**Berlese-Tullgren Funnel extractor constructed by (Bano and Roy 2016) was installed in the laboratory for the trapping processes of soil micro-arthropods. Each treatment had three replicates of independent sets. Equal volumes of top layer samples of soil (538.78 cm<sup>3</sup>) were loaded in sieve trays (diameter, 14 cm; edge height, 3.5 cm) with feasible holes (inner diameter, 0.21 mm) for the movement of micro-arthropods individuals. A constant time of 48 hrs was set for the trapping duration of all treatments. The extracted micro-arthropods were trapped in a small depository containing a small amount of ethanol solution (70%).

# **Sorting and Identification of Extracted Micro-Arthropods:**

The extracted microarthropods were inspected under a stereoscopic microscope and sorted into groups of individual species using a disposable plastic pipette dropper. Finally, each sorted group was preserved in separated vials containing 70% ethanol solution, ready to be loaded on slides for identification and counting under a dissecting microscope according to the key of Gill and McSorley (2012) and Palacios-Vargas (2007). Thereafter, the adverse effects of these insecticides on non-target soil micro-arthropods were categorized based on a field framework rendered by the "International Organization for Biological Control" (IOBC) (Hassan 1992). These categories comprised the following classes:

- Class 1: mortality percentages of < 25%; fulfill the effect of "harmless."
- Class 2: mortality percentages of 25-50%; fulfill the effect of "slightly harmful."
- Class 3: mortality percentages of 51-75%; fulfill the effect of "moderately harmful."
- Class 4: mortality percentages of > 75%; fulfill the effect of "harmful."

# **Corrected Efficacy Formula and Statistical Analysis:**

Based on Henderson and Tilton's (1955) formula, the reduction percentages of the adult and nymph populations of whitefly in both spray and soil applications were calculated, along with the estimations of the initial and last actions of the tested insecticides and their long-term efficacy. Meanwhile, the survival percentages of abundant indigenous parasitic wasps and soil micro-arthropod populations in treated and control plots were estimated according to the equation of Sun and Shepard (1947). All the obtained data were subjected to an analysis of variance (ANOVA). The means were determined to be significant at 0.05 using the LSD test (SAS Statistical Software 2002).

#### **RESULTS**

### Field Efficacy and Protection Period Against Whitefly:

Data on reduction percentages of soil and spray applications of flonicamid 50% WG and spriotetramat 10% SC against adults and nymphs' populations of *B. tabaci* (Genn.) were achieved at 48 hrs of exposure along the 12 DATs in the seasons of 2022 and 2023. The protection time (days) of these insecticides was expressed by their capabilities to reduce the whitefly populations below the economic threshold limit of injury (Tables 1 and 2). In season 2022, the results of reduction percentages of the soil application of flonicamid 50% WG reached 57.87 and 77.18% in adults' populations, showed excel over spirotetramat 10% SC

that reached 20.33 and 70.20% at 0 and 3 DAT, respectively. Thereafter, both insecticides at 6 DAT had the same reducing effects. Contrariwise, the reduction percentages in adults for spirotetramat 10% SC transcended flonicamid 50% WG at 9 and 12 DAT. Whilst, the reductions in the nymphs' population of flonicamid 50% WG attained 79.11 and 73.74%, they surpassed 43.86 and 60.64% in spirotetramat 10% SC at 0 and 3 DAT, respectively. Both insecticides had no significant variance between their reduction percentages in nymphs from 6 to 12 DAT. Eventually, the protection time of the soil-drenching of these insecticides against adults was initiated from 3 up to 6 DAT. Meanwhile, the protection time of the soil application of flonicamid 50% WG against nymphs extended from 0 to 12 DAT, as well as spirotetramat 10% SC maintained the nymphs' population below the threshold level (Table 1). On the other hand, the results of spray application showed transcend of flonicamid 50% WG with reduction percentages of 74.48, 86.71, and 93.43% in adults population than 43.11, 80.45, and 81.39% in spirotetramat 10% SC at 0, 3, and 6 DAT, respectively. Both insecticides had equivalent reductions in adults at 9 and 12 DAT. Meantime, the reduction in nymphs' population of 85.40, 86.26, and 89.60% in spray application of flonicamid 50% WG was more significant than reductions of 32.39, 76.68, and 74.74% in spirotetramat 10% SC at 0, 3, and 6 DAT, respectively. However, equality in the reduction percentages in nymphs appeared for both insecticides at 9 DAT, spirotetramat 10% SC exceeded flonicamid 50% WG at 12 DAT. Ultimately, the protection time of the spray application of these insecticides against adults covered the period from 3 to 6 DAT. Meanwhile, the protection time of spirotetramat's spray against nymphs extended from 0 to 12 DAT, while flonicamid 50% WG maintained the nymphs' population below the threshold level (Table 1).

**Table 1:** Efficacy and protection period of soil and spray applications of the selected insecticides against adult and nymph stages of *Bemisia tabaci*, season of 2022.

Treatment	Initial population	(Survi	Protection period (day) <sup>2</sup>					
		0.13 (1 hr)	3	6	9	12	Initial action	Last action
			Soil tre	atment on adults				
Spirotetramat	(319)	(275.25 ±4.39)	(101 ±4.71)	(72.75 ±7.26)	(152.50 ±5.24)	(210 ±10.27)	3	6
10% SC	-	20.33b ±5.99	70.20b ±2.97	77.59ª ±3.45	54.70 <sup>a</sup> ±2.96	37.28a ±6.78		
Flonicamid	(337.60)	(156.75 ±29.37)	(84.25 ±5.02)	(63.00 ±5.79)	(254.50 ±19.96)	(328.75 ±19.56)	3	6
50% WG	-	57.87ª ±8.93	77.18ª ±1.57	82.42ª ±1.34	29.45b ±8.15	10.83b ±0.72		
~	(342)	(377)	(371)	(358)	(366)	(368)	-	-
Control	-	00.00° ±00.00	00.00c ±00.00	00.00b ±00.00	00.00° ±00.00	00.00° ±00.00		
			Soil trea	tment on nymph	S			
Spirotetramat	(115.75)	(73.50 ±17.87)	(48.25 ±2.32)	(32.75 ±3.61)	72.00 ±6.49	(90.25 ±3.33)		
10% SC	-	43.86b ±13.93	60.64b ±1.90	71.28a ±2.40	29.13° ±9.54	22.32ª ±5.95	< the thre	shold limit
Flonicamid	(150.60)	(35.75 ±3.28)	(42.75 ±2.18)	(39.00 ±3.28)	(84.50 ±12.82)	(101.50 ±11.09)	0 (1hr)	12
50% WG	-	79.11ª ±4.03	73.74ª ±6.42	69.89ª ±5.20	31.39ª ±8.75	26.10ª ±8.86		
	(124)	(141)	(132)	(122)	(111)	(126)	-	-
Control	-	00.00° ±00.00	00.00° ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00		
			Spray tr	eatment on adult	S			
Spirotetramat	(344.75)	(205.50 ±26.47)	(69.75 ±4.11)	(66.25 ±5.28)	(248.00 ±22.92)	(305.25 ±11.88)	3 (1 hr)	6
10% SC	-	43.11b ±7.42	80.45b ±1.93	81.39b ±1.18	28.90ª ±5.40	8.49ª ±2.50	` /	
Flonicamid	(342.75)	(90.25 ±7.45)	(49.00 ±9.06)	(23.25 ±9.81)	(254.00 ±16.77)	310.75±15.73)	0 (1hr)	6
50% WG	-	74.48ª ±3.50	86.71 <sup>a</sup> ±1.77	93.43ª ±2.71	25.91ª ±6.80	6.32ª ±3.26	` ′	
	(351)	(371)	(369)	(363)	(355)	(341)	-	-
Control	-	00.00° ±00.00	00.00° ±00.00	00.00° ±00.00	00.00b ±00.00	00.00b ±00.00		
			Spray tre	atment on nymp	hs			
Spirotetramat	(137.75)	(105.25 ±13.32)	(34.00 ±2.73)	(34.25±1.45)	(114.50 ±14.26)	(118.00 ±3.28)	0 (1hr)	12
10% SC	- 1	32.39b ±8.04	76.68b ±2.22	74.74b ±1.07	6.83ª ±8.73	15.68a ±1.28	, ,	
Flonicamid	(103.50)	(17.50 ±1.55)	(15.75 ±2.14)	(10.75 ±4.70)	(90.00 ±4.64)	(100.50 ±5.58)	d d	.11.1 1::4
50% WG	-	85.40ª ±1.73	86.26ª ±1.56	89.60ª ±4.30	8.85ª ±1.36	5.22b ±2.52	< tne thre	shold limit
~	(128)	(150)	(141)	(127)	(122)	(131)	-	-
Control		00.00° ±00.00	00.00° ±00.00	00.00° ±00.00	00.00ª ±00.00	00.00° ±00.00		

<sup>&</sup>lt;sup>1</sup>Standard error.

Means of reduction percentage signed by the same letters are not significantly different according to the LSD<sub>0.05</sub>

<sup>&</sup>lt;sup>2</sup>Estimations based on maintaining the whitefly population at threshold limit of  $\leq 125$  adults or nymph individuals  $25^{-1}$  leaves samples Plot<sup>-1</sup> (Schuster *et al.*, 2004).

In season 2023, the results of reduction percentages in the adults' population in the soil application of flonicamid 50% WG (41.22%) excelled spirotetramat 10% SC (20.33%) at 0 DAT. Both insecticides had the same reducing effects at 3, 6, and 12 DAT. On the contrary, in the reduction percentages in adults for spirotetramat, 10% SC (21.13%) transcended flunicanid 50% WG (3.01%) at 9 DAT. While reductions in the nymphs with flonicamid 50% WG (70.02%) surpassed spirotetramat 10% SC (51.94%) at 0 DAT. Both insecticides had equal reductions in nymphs from 3 to 9 DAT. Eventually, the protection time of the soil application of both insecticides on adults was initiated from 3 to 6 DAT. Meanwhile, the protection time of the soil applications of both insecticides on the nymphs' population extended from 0 to 12 DAT (Table 2). On the other hand, the results of spray application showed equality for both insecticides in their reductions in adult populations. Meantime, the reduction in nymph's population in spray application of flonicamid 50% WG (60.07%) was higher than spirotetramat 10% SC (23.82%) at 0 DAT. However, reductions of both insecticides in nymphs were insignificant from 3 to 12 DAT, except for the 9<sup>th</sup> DAT, where spirotetramat 10% SC surpassed flonicamid 50% WG. Ultimately, the protection times of flunicamid's spraying against the adults extended from 3 to 6 DAT, while spirotetramat 10% SC extended from 0 to 6 DAT. Meanwhile, the protection time of the spray application of both insecticides against the nymphs' population extended from 0 to 12 DAT (Table 2).

**Table 2:** Efficacy and protection period of soil and spray applications of the selected insecticides against adult and nymph stages of *Bemisia tabaci*, season of 2023.

Treatment	Initial	(Su	OATs .	Protection period (day) <sup>2</sup>				
Теаншен	population	0 (1 hr)	3	6	9	12	Initial action	Last action
			Soil t	reatment on adults				
Spirotetramat	(348.75)	(300.50 ±39.46)	(106.25 ±5.30)	(111.75 ±4.64)	(247.75 ±48.98)	(307.50 ±14.37)	3	6
10% SC	-	20.33b ±5.99	64.39ª ±2.06	64.67ª ±3.65	21.13ª ±15.38	4.62ª ±1.70		
Flonicamid	(364.75)	(209.00 ±52.93)	(111.00 ±11.37)	(92.25 ±17.77)	(323.75 ±11.69)	(331.50 ±11.02)	3	6
50% WG	-	41.22ª ±16.53	64.95ª ±2.73	72.70ª ±5.06	3.01 <sup>b</sup> ±0.37	1.99ª ±0.44		
	(400)	(400)	(345)	(370)	(366)	(371)	-	-
Control	-	00.00° ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00	00.00ª ±00.00		
		·	Soil tr	eatment on nymph	S			
Spirotetramat	(125.50)	(59.75 ±7.42)	(49.25 ±3.82)	(34.75 ±3.04)	(67.25 ±7.74)	(119.00 ±7.01)	0 (41 )	40
10% SC	-	51.94b ±4.83	58.75ª ±2.33	69.61ª ±2.59	36.35ª ±8.41	00.35b ±1.63	0 (1hr)	12
Flonicamid	(137.60)	(41.00 ±3.28)	(46.25 ±1.44)	(39.25 ±3.69)	(84.00 ±14.01)	(98.75 ±13.25)	0 (1hr)	12
50% WG	-	70.02ª ±7.20	65.25ª ±11.37	67.12ª ±5.27	26.31ª ±9.28	22.04ª ±8.52		
	(140)	(138)	(133)	(128)	(119)	(133)	_	-
Control	- '	00.00° ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00		
			Spray	treatment on adult	S			
Spirotetramat	(335.50)	(124 ±25.86)	(64.75 ±10.59)	(36.00 ±14.28)	(266.00 ±10.46)	(308.00 ±17.96)	0 (1 hr)	6
10% SC	-	60.90ª ±11.03	77.99ª ±2.95	88.56 <sup>a</sup> ±4.49	14.59ª ±4.65	1.57ª ±0.90	` '	
Flonicamid	(344.75)	(205.50 ±26.47)	(69.75 ±4.11)	(66.25 ±5.28)	(248.00 ±22.92)	(305.25±11.88)	3	6
50% WG	-	39.56ª ±7.88	76.20° ±1.29	79.67ª ±1.29	23.23ª ±5.83	4.83ª ±2.60		
	(395)	(393)	(341)	(374)	(370)	(369)	-	-
Control	-	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00		
			Spray t	reatment on nympl	hs			-
Spirotetramat	(135.25)	(104.50 ±15.11)	(71.75 ±4.33)	(48.75±10.92)	(74.50 ±4.73)	(109.25 ±2.87)	0 (1hr)	12
10% SC	-	23.82b ±10.98	46.71 <sup>2</sup> ±4.23	61.67ª ±8.25	28.49a ±3.83	1.86ª ±1.03		
Flonicamid	(134.00)	(54.50 ±0.76)	(50.00 ±0.76)	(46.50 ±7.65)	(89.75 ±16.21)	(108.75 ±5.77)	0 (11-)	10
50% WG	-	60.07a ±10.88	62.83ª ±10.50	62.36a ±6.90	12.56b ±12.50	00.28ª ±3.74	0 (1hr)	12
	(130)	(132)	(130)	(122)	(100)	(107)	-	-
Control	-	00.00b ±00.00	00.00b ±00.00	00.00b ±00.00	00.00° ±00.00	00.00a ±00.00		

<sup>&</sup>lt;sup>1</sup>Standard error.

Means of reduction percentage signed by the same letters are not significantly different according to the LSD<sub>0.05</sub>

#### **Adverse on Parasitic Wasps:**

Data on the long-lasting adverse effect of foliar and soil application of spirotetramat 10% SC and flonicamid 50% WG on the existent parasitic wasps, *Eretmocerus mundus* (Mercet) and *Encarsia artenopae* (Foerster) (Hymenoptera: Aphelinidae) were

<sup>&</sup>lt;sup>2</sup>Estimations based on maintaining the whitefly population at threshold limit of  $\leq$ 125 adults or nymph individuals 25<sup>-1</sup> leaves samples Plot<sup>-1</sup> (Schuster *et al.*, 2004).

accomplished in field trials in the seasons of 2022 and 2023 (Tables 3 and 4).

The obtained results of reduction percentages of the foliar spray application in parasitic wasps in the season, 2022 in Table (3), showed superiorities of the tested insecticides in reducing E. mundus (Mercet) over the control plots from 3 to 12 DAT. The highest reductions of 50.29, 48.91, and 34.78% were recorded in spirotetramat 10% SC on E.mundus (Mercet) over 3, 6, and 9 DAT, respectively. In the second rank, flonicamid 50% WG followed spirotetramat 10% SC with its high reduction percentages, which transcended the control from 3 to 12 DAT. Meantime, spirotetramat 10% SC significantly transcended the control plots with potent reductions of 36.84, 47.29, and 48.66% in E. artenopae (Foerster) at 0, 3, and 6 DAT, respectively. Whereas, flonicamid 50% WG had no significant reductions compared to the control. On the other hand, the soil application of flonicamid 50% WG was significantly higher than the control with percentages of 22.06 and 21.25% at the 6<sup>th</sup> and 12<sup>th</sup> DAT, respectively, as well as spirotetramat 10% SC surpassed all treatments from 0 to 6 DAT. Furthermore, flonicamid 50% WG exceeded the control plots with potent reductions of 29.02 and 27.05% in E. artenopae (Foerster) at 6 and 9, respectively. While, the reductions in E. artenopae (Foerster) in spirotetramat 10% SC exceeded the control from 0 to 9 DAT and recorded the most vigor reduction of 34.89% that surpassed flonicamid 50% WG at the 6<sup>th</sup> DAT. In addition, BIx values of both insecticides on indigenous parasitic wasps did not exceed the value of 2, which may point to unfavorable balances towards the target pest of whitefly.

**Table 3:** Long-lasting impacts at 48 hrs of exposure to the tested insecticides on parasitic wasps and their balance with *Bemisia tabaci* in field trials of season, 2022.

Foliar application									Soil application							
Treatments	DATs	Eretmo	Eretmocerus mundus Encarsia artenopae													
	2.115	Reduction% ±SE <sup>1</sup> BLX		BLX <sup>2</sup>	Reduction% ±SE		BLX	Reduction% ±SE		BLX	BLX Reduction		BLX			
	0	15.86ecd	±8.86	0.003	36.84 <sup>ba</sup>	±13.65	0.003	24.17 <sup>ba</sup>	±9.09	0.001	23.40 <sup>bac</sup>	±9.38	0.002			
	3	50.29a	±3.96	0.002	47.29a	±17.42	0.001	35.48a	±13.46	0.002	34.06 <sup>ba</sup>	±11.56	0.003			
Spirotetrama t 10% SC	6	48.91ª	±2.50	0.004	48.66ª	±16.47	0.001	33.21a	±6.29	0.003	34.89a	±11.68	0.004			
11070 SC	9	34.78 <sup>ba</sup>	±14.67	0.004	26.81 <sup>bac</sup>	±15.51	0.004	23.57ba	±12.38	0.002	25.94 <sup>bac</sup>	±9.09	0.001			
	12	22.09 <sup>bed</sup>	±8.37	0.017	26.37 <sup>bac</sup>	±10.92	0.018	2.08 <sup>dc</sup>	±2.08	0.002	13.50 <sup>dc</sup>	±5.78	0.001			
	0	7.96 <sup>ed</sup>	±4.23	0.002	14.60 <sup>bc</sup>	±8.01	0.001	9.94 <sup>bdc</sup>	±5.91	0.002	8.44 <sup>dc</sup>	±1.99	0.002			
	3	23.95 <sup>bed</sup>	±3.68	0.003	24.55bac	±9.23	0.002	16.72 <sup>bdac</sup>	±4.95	0.003	17.61 <sup>bdac</sup>	±8.31	0.002			
Flonicamid 50% WG	6	22.15 <sup>bed</sup>	±1.98	0.003	25.86 <sup>bac</sup>	±9.00	0.003	22.06 <sup>ba</sup>	±6.48	0.004	29.02 <sup>bac</sup>	±10.60	0.003			
3070 11 0	9	25.37 <sup>bed</sup>	±3.19	0.005	7.95°	±7.53	0.004	16.93 <sup>bdac</sup>	±7.54	0.001	27.05 <sup>bac</sup>	±10.41	0.001			
	12	26.30bc	±3.83	0.019	22.95bac	±7.69	0.014	21.25bac	±7.48	0.001	11.36 <sup>dc</sup>	±3.92	0.001			
	0	0.00e	±0.00	-	0.00°	±0.00	-	0.00 <sup>d</sup>	±0.00	-	0.00 <sup>d</sup>	±0.00	-			
	3	0.00e	±0.00	-	0.00°	±0.00	-	0.00 <sup>d</sup>	±0.00	-	0.00 <sup>d</sup>	±0.00	-			
Control	6	0.00e	±0.00	-	0.00°	±0.00	-	0.00 <sup>d</sup>	±0.00	-	0.00 <sup>d</sup>	±0.00	-			
	9	0.00e	±0.00	-	0.00°	±0.00	-	0.00 <sup>d</sup>	±0.00	-	0.00 <sup>d</sup>	±0.00	-			
	12	0.00e	±0.00	-	0.00°	±0.00	-	0.00 <sup>d</sup>	±0.00	-	0.00 <sup>d</sup>	±0.00	-			

<sup>&</sup>lt;sup>1</sup>Standard error.

Means of reduction percentages for each column signed by the same letters are not significantly different according to the LSD<sub>0.05</sub>.

Results on the reduction percentages of the second field trial in the season of 2023 (Table 4) indicated that the foliar applications of spirotetramat 10% SC and flonicamid 50% WG almost surpassed the control with high reductions in *E. mundus* (Mercet) along all the given DATs and from 3 to 12 DAT, respectively. Meantime, potent reductions in *E. artenopae* (Foerster) for spirotetramat 10% SC were 34.92 and 27.23%, as well as flonicamid 50% WG were 29.47 and 34.54% at 3 and 6 DAT, respectively, compared to the control. On the other hand, the soil application of spirotetramat 10% SC attained potent reductions of 40.59, 40.55, and 25.92% in *E. mundus* (Mercet) extended over 0, 3, and 9

<sup>&</sup>lt;sup>2</sup>Beneficial Arthropod Index (Naranjo et al., 2004; McCravy 2018).

DAT, respectively. Whereas, the potent reductions of flonicamid 50% WG were 27.12, 26.95, and 27.38% extended over 3, 9, and 12 DAT, respectively. Moreover, spirotetramat 10% SC possessed the highest reductions of 31.53, 27.41, and 25.91% in *E. artenopae* (Foerster) extended over 3, 6, and 12 DAT. Meanwhile, flonicamid 50% WG had the most vigor reductions of 33.12 and 30.94% along the 6<sup>th</sup> and 12<sup>th</sup> DAT, respectively. Additionally, BIx values of both insecticides on these parasitic wasps were less than the value of 2, which may have accentuated unfavorable balance toward the whitefly population.

**Table 4:** Long-lasting impacts at 48 hrs of exposure to the tested insecticides on parasitic wasps and their balance with *Bemisia tabaci* in field trials of season 2023.

	DATs		F	oliar app	olication		Soil application						
Treatments		Eretmo	cerus mun	dus	Encarsia artenopae			Eretmo	cerus mun	dus	Encarsia artenopae		
		Reduction	% ±SE <sup>1</sup>	BLX <sup>2</sup>	Reductio	n% ±SE	BLX	Reduction% ±SE		BLX	Reduction% ±SE		BLX
	0	30.81 <sup>ba</sup>	±12.64	0.003	21.72 <sup>ba</sup>	±13.08	0.002	40.59ª	±14.49	0.001	22.37ba	±11.27	0.001
	3	40.56a	±8.45	0.003	34.92ª	±11.83	0.002	40.55ª	±14.52	0.002	31.53a	±10.55	0.002
Spirotetramat 10% SC	6	39.83ª	±13.30	0.002	27.23a	±14.81	0.002	20.49 <sup>bac</sup>	±10.54	0.004	27.41a	±10.80	0.002
107000	9	24.19 <sup>ba</sup>	±10.63	0.002	12.04 <sup>ba</sup>	±12.04	0.002	25.92 <sup>ba</sup>	±11.67	0.003	14.90 <sup>ba</sup>	±12.90	0.002
	12	21.99 <sup>ba</sup>	±7.89	0.001	21.64 <sup>ba</sup>	±11.92	0.003	6.87 <sup>bc</sup>	±6.87	0.003	25.91ª	±8.90	0.001
	0	16.30bc	±6.45	0.003	24.18 <sup>ba</sup>	±7.61	0.002	16.45 <sup>bac</sup>	±11.47	0.002	18.50 <sup>ba</sup>	±4.86	0.002
	3	29.17 <sup>ba</sup>	±2.63	0.003	29.47ª	±8.69	0.003	27.12 <sup>ba</sup>	±10.03	0.003	24.98 <sup>ba</sup>	±7.09	0.002
Flonicamid 50% WG	6	29.26 <sup>ba</sup>	±3.39	0.003	34.54ª	±10.70	0.002	24.67 <sup>bac</sup>	±9.10	0.004	33.12ª	±9.32	0.002
3070 113	9	26.28 <sup>ba</sup>	±4.88	0.004	9.09 <sup>ba</sup>	±8.60	0.004	26.95 <sup>ba</sup>	±7.95	0.001	20.70 <sup>ba</sup>	±8.84	0.001
	12	33.49 <sup>ba</sup>	±1.23	0.018	29.44a	±9.18	0.001	27.38 <sup>ba</sup>	±7.41	0.001	30.94ª	±8.46	0.001
	0	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-
	3	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-
Control	6	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-
	9	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-
	12	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-	0.00°	±0.00	-	0.00 <sup>b</sup>	±0.00	-

<sup>&</sup>lt;sup>1</sup>Standard error.

Means of reduction percentages for each column signed by the same letters are not significantly different according to the LSD<sub>0.05</sub>.

# **Identified Taxonomic Groups of Soil Micro-Arthropods:**

The identified taxonomic groups of total micro-arthropods extracted from the soil layer (25 cm depth) in the assigned plots included Collembola, Symphylla, Psocopetra, Pauropoda, Oribatida, Actinedida, and Gamasida during the two successive seasons of 2022 and 2023.

# **Long-Lasting Adverse Effects On Soil Micro-Arthropods:**

Data on the long-lasting adverse effects of soil application of the selected insecticides against total populations of soil micro-arthropods at 48 hrs of exposure along the 12 DATs were accomplished in the seasons of 2022 and 2023 (Table 5). Results of the overall mean of survival percentages of the soil micro-arthropods in both seasons showed insignificant differences between the tested insecticides compared to the completeness of their survival rates in the control along the given DATs. Regarding the data calculated by IOBC on the survival micro-arthropods, spirotetramat 10% SC and flonicamid 50% WG were slightly and moderately harmful, respectively at 0 DAT. Then, harmful adverse were occurred in flonicamid 50% WG from 3 to 6 DAT in both seasons, except spirotetramat 10% SC was harmful and moderately harmful at 3 and 6 DAT, respectively, in the season of 2023. In both seasons, spirotetramat 10% SC and flonicamid 50% WG had harmless and slightly harmful effects, respectively, at the 12<sup>th</sup> DAT. Ultimately, both insecticides revealed moderately harmful adverse effects on the overall mean survival of micro-arthropods in both seasons.

<sup>&</sup>lt;sup>2</sup>Beneficial Arthropod Index (Naranjo et al., 2004; McCravy 2018).

un	ais of sea	sons, 2	2022 6	anu 20	123.									
Treatments	Initial Mean of	Surviv	al% ±SE	and classification of IOBC <sup>3</sup> along the interval days after treatments (DAT)									Overall mean of survival%	
	Populations <sup>1</sup>	0		3		6		9		12		$\pm SE$		
Season 2022														
Spirotetramat 10% SC	66.00	64.76 <sup>b</sup>	±4.30	8.85gf	±1.54	24.52e	±2.17	66.81°	±3.48	79.83 <sup>b</sup>	±2.99	48.95 <sup>b</sup>	±2.71	
		Slight l	narmful	Harmful		Harmful		Slight harmful		Harn	ıless	Mod. harmful		
Flonicamid 50% WG	68.33	49.68°	±3.33	3.78g	±2.93	12.92 <sup>f</sup>	±2.33	68.03 <sup>cb</sup>	±4.98	66.24°	±1.22	40.13 <sup>b</sup>	±2.76	
		Mod. harmful		Harmful		Harmful		Slight harmful		Slight harmful		Mod. harmful		
Control	63.33	100.00a	±00.00	100.00a	±00.00	100.00 <sup>a</sup>	±00.00	100.00a	±00.00	100.00a	±00.00	100.00a	±00.00	
					Sea	son 2023								
Spirotetramat 10% SC	68.33	65.69 <sup>b</sup>	±4.15	5.50gf	±1.93	26.80e	±2.49	64.60°	±1.36	79.03 <sup>b</sup>	±2.51	48.36 <sup>b</sup>	±2.49	
		Slight harmful		Harmful		Mod. harmful		Slight harmful		Harmless		Mod. harmful		
Flonicamid 50% WG	76.99	40.37°	±8.64	0.00g	±0.00	13.44 <sup>f</sup>	±3.45	71.62 <sup>cb</sup>	±3.94	66.38°	±1.35	38.32 <sup>b</sup>	±3.48	
		Mod. harmful		Harmful		Harmful		Slight harmful		Slight harmful		Mod. harmful		
Control	65.00	100.00 <sup>a</sup>	±00.00	100.00 <sup>a</sup>	±00.00	100.00 <sup>a</sup>	±00.00	100.00 <sup>a</sup>	±00.00	100.00 <sup>a</sup>	±00.00	100.00 <sup>a</sup>	±00.00	

**Table 5**: Long-lasting impacts and safety limits of soil application of the tested insecticides against total population of the soil micro-arthropods at 48 hrs of exposure in field trials of seasons, 2022 and 2023.

#### **DISCUSSION**

So far known, spirotetramat and flonicamid are novel systematic insecticides that could realize efficient control of the developmental stages of the whitefly, *B. tabaci* (Genn.) via their spray and drenching applications (Chen *et al.*, 2018; EPA 2020; Abbas *et al.*, 2022). Furthermore, this research attempted to find out the potential safety limits of these insecticides for the abundant bioindicators in their agro-environment sites, based on some international safety classifications (Hassan 1992; McCravy 2018).

Overall, for the two hot seasons, the soil application of the tested insecticides against the adults of whitefly had foremost protection durations from 3 to 6 DAT, with more extension in the spray application from 0 to 6 DAT. All the applications of these insecticides provided mostly protection intervals against the nymphs along the 12 DATs. This finding was consistent with field trials of spirotetramat foliar spray, which resulted in the greatest reduction in the total population of sugarcane whitefly, Aleurolobus barodensis Maskell ((Hemipera: Alyrodidae) in the sugarcane variety of Ratoon US-633 from 14 to 27 DAT (Muhammad et al., 2021). Based on the Freundlich isotherm co-efficient, soil-drenching of spirotetramat could be efficient due to its high desorption and adsorption in different soil textures during high-temperature seasons (Chen et al., 2018). Furthermore, field trials of flonicamid applications by soil-drenching and foliar sprays brought out significant reductions in the populations of all the developmental stages of B. tabaci (Genn.) compared to the control plots along 6 weeks after treatments (Assadi et al., 2022). In addition, semifield trials of foliar spray of flonicamid showed a potent toxic effect against the laboratory strain of cotton aphid, Aphis gossypii Glover (Hemipera: Aphididae), as one of the common piercing-sucking insects within the first 2 DAT. It could also provide full protection against A. gossypii Glover within median periods of 5 to 7 weeks (Khamis et al., 2021).

In both seasons, spray application of the tested insecticides showed extended adverse effects of up to 12 DAT on the parasitic wasps, *E. mundus* (Mercet). Spirotetramat 10% SC had steady potent impacts on *E. artenopae* (Foerster) up to 6 DAT, likewise flonicamid 50% WG only in the 2<sup>nd</sup> season. On the other hand, the soil-drenching of spirotetramat 10% SC steadily surpassed all treatments on *E. mundus* (Mercet) for less than

 $<sup>^{1}</sup>$ Total volume of soil = 1570.80 cm $^{3}$ , at top layer with 25 cm in depth.

<sup>&</sup>lt;sup>2</sup>Standard error.

<sup>&</sup>lt;sup>3</sup>(International Organization for Biological Control) performed classification system for safety in the field (Hassan 1992). Means of survival population percentage of soil micro-arthropod along the 12 DATs that interact with the treatments in the same letter are not significantly different at LSD<sub>0.05</sub>.

a week. In the second rank, flonicamid 50% WG excelled the control on E. mundus (Mercet) and E. artenopae (Foerster) only within a few distant days. Whilst, spirotetramat 10% SC impact on E. artenopae (Foerster) may extend for more than a week. In addition, BIx values of both insecticides on the parasitic wasps expressed unfavorable balances towards the target pest. Further studies corroborated our findings and showed that flonicamid was harmless in both the adult and mummy stages of E. mundus. These parasitic wasps were directly dead after a short time of exposure to spirotetramat, besides the harmful aspects that appeared in the reproduction activity of E. mundus (Mercet) and malformations in one or both of its life stages (Fernández et al., 2015). Along the same lines, treated pupae of E. mundus (Mercet) by spirotetramat did not affect the surviving adult's emergence and reproductive activity, while an apparent reduction in the first progeny's longevity, adult survival, and longevity was manifested. In addition, spirotetramat led to a considerable disturbance in the demographic parameters of the substantive rate of increase, net reproductive rate, and mean generation time. Ultimately, spirotetramat was harmless for the pupal and adult stages of E. mundus (Mercet) (Francesena et al., 2017). Although flonicamid exhibited increases in mortality of the hoverfly Sphaerophria rueppellii Wiedemann (Diptera: Syrphidae) whenever fed on contaminated honeydew, no significant adverse effects had been revealed on the parasitic wasp Anagyrus vladimiri (Hymenoptera: Encyrtidae) in the treated areas (Calvo-Agudo et al., 2020). Spirotetramat caused reductions in both adults (<30%) and pupae (<10%) of *Encarsia formosa* Gahan, as well as its lessened effects on reproductive and demographic parameters in both stages (Marcic and Drobnjakovic 2021).

Data from both seasons in this research accentuated seven identified taxonomic groups of micro-arthropods, comprised of Collembola, Symphylla, Psocopetra, Pauropoda, Oribatida, Actinedida, and Gamasida. There were insignificant differences between spirotetramat 10% SC and flonicamid 50% WG on the survival populations of these abundant soil micro-arthropods, as well as their moderately harmful effect on the overall mean of these populations along the 12 DAT. At zero DAT, spirotetramat 10% SC was slightly harmful, while flonicamid 50% WG was moderately harmful. Both insecticides possessed harmful adverse effects from 3 to 6 DAT. At the 12<sup>th</sup> DAT, spirotetramat 10% SC and flonicamid 50% WG caused harmless and slightly harmful effects, respectively. These data were confirmed by a similar study conducted by El-Sherbeni et al., (2018) that showed the highest degree of safety was exhibited by flonicamid 50% WG for the soil microarthropod communities during two successive seasons. Likewise, the recommended doses of spirotetramat were generally safe enough for the soil communities of microarthropods. It exhibited a limited effect on collembolans and a direct toxic effect on Astigmata and Oribatida mites. The population of collembolans realized a correlated increase with the significant reduction in Astigmata, which could compete directly with collembolans for food (Campos 2020).

#### **Conclusion:**

In general, all the applications of spirotetramat 10% SC and flonicamid 50% WG provided high protection against the nymphs for 12 days. All treatments showed unfavorable balances on the parasitic wasps towards the adult and nymph stages of the whitefly population.

Firstly, spray application of both tested insecticides afforded efficacious protection levels against the adults of whitefly along the first 6 DAT. They showed extended impacts on *E. mundus* (Mercet), over 3-12 DAT. *E. artenopae* (Foerster) was relatively affected by spirotetramat 10% SC over 3-6 DAT and exposed to limited impacts by flonicamid 50% WG.

Secondly, soil-drenching with both insecticides extended against the adults of whitefly from 3 to 6 DAT. They grant safety to soil micro-arthropods after the 6<sup>th</sup> DAT. The

soil-drenching of flonicamid 50% WG showed apparent safety with its unsteady declines within a few distant days on the parasitic wasps compared to spirotetramat 10% SC that could extend from 3 to 6 DAT.

**Declarations:** 

**Ethical Approval:** Not applicable.

**Authors Contributions:** W M K conceived of the presented idea, collected the data, carried out all laboratory and field experimental designs and analyzed the results. W M K and R S interpreted the results, the discussion, and the final manuscript.

**Competing Interests:** No competing interests were found between authors.

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