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Elicit effects of potassium phosphite versus to emamectin benzoate on the defensive response of cotton seedlings against *Spodoptera littoralis* (Lepidoptera: : Noctuidae)

Samah, M. Hassan; Wael, M. Khamis and Sahar, E. Eldesouky

Plant Protection Research Institute, Agricultural Research Center, Sabahia, Alexandria, Egypt.

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Abstract:

This study was investigated on a novel rule of potassium phosphite as promising elicitors comparing to emamectin benzoate against *Spodoptera littoralis* (Boisduval) **(** Lepidoptera: **:** Noctuidae) . The toxicity on the 2nd instar larvae showed that LC_{25}, LC_{50} and LC_{90} values of emamectin benzoate (0.005, 0.012 and 0.248 mg L^{-1}, respectively) had more toxic than potassium phosphite (1326.2, 5302.4 and 73757.2 mg L^{-1}, respectively) after 96 hrs post-treatment. Gas chromatography–mass spectrometry **(**GC-MS) analysis identified the induced VOCs from untreated cotton seedling compared to those induced by potassium phosphite and emamectin benzoate. Induce Volatile organic compounds (VOCs) by potassium phosphite were featured by dibutyl phthalate, ß-caryophyllene, ethyl palmitate, ethyl linoleate and methyl linolenate, docosane and benzaldehyde, 3-phenoxy-. Major VOCs induced by emamectin benzoate were dibutyl phthalate, bis (2-ethylhexyl) phthalate, squalene and methylprednisolone. Biological tests at LC_{25} and LC_{50} values of emamectin benzoate showed pupal weights (150.4 and 95.2 mg, respectively) < potassium phosphite (262.3 and 224.3 mg, respectively) compared to 309.2 mg in the control. Adult emergence percentages of emamectin benzoate at LC_{25} and LC_{50}s were 31.7 and 18.3%, respectively < potassium phosphite were 72.3 and 53.8%, respectively compared to 86.8% in the control. Emamectin benzoate significantly prolonged the larval durations (16.4 and 17.2 days, respectively) > potassium phosphite (14.3 and 15.9 days, respectively) compared to the control (13.8 days). While no significant changes in pupal durations in both treatments. Significant decreases in pupation percentage revealed at LC_{50}s of emamectin benzoate (21.5%) and potassium phosphite (57.3%) compared to 90.3% in the control. Olfactometer dual choice tests on 2nd instar larvae showed preferable response to untreated cotton seedling versus to each of the two treatments at LC_{90} and LC_{50}s. Choice tests between the two treatments showed surpasses of potassium phosphite in orienting responses of larvae. Finally, these olfactory and biological assessments could enroll potassium phosphite as a novel elicitor against *S. littoralis*.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a destructive and polyphagous insect pest causing great losses in quantity and quality for most of the injured crops (Matthews and Tunstall, 1994). The extensive uses of synthetic insecticides lead to harmful effects to environment and beneficial organisms (Pavela *et al*., 2008). Emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopteran pests. It modulates specific glutamate-gated anion channels in synapse and muscle cells thereby increasing the influx of chloride ions. Furthermore, emamectin benzoate has a lack of cross-resistance compare to other synthetic insecticides (White *et al*., 1997 and Dunbar *et al.,* 1998). Therefore, more safe compounds should be employed alternatively and complementarily with the synthetic pesticides to realize safer pest management strategy for the environment (Bahlai *et al*., 2010).

In these respects, the last evaluations of potassium phosphite applications drew more attention towards strengthening plant vigor, health and tolerance against a variety of pathogens and environmental stress (Costa *et al*., 2018). Potassium phosphite applications provide efficient phosphorus for plant that enhances assimilation to activate its defense (Ogoshi *et al*., 2013). Foliar spray of potassium phosphite on potatoes plants after 48 hours brought out tubers with high significant contents of phytoalexins, phenols and some enzymatic activities that defend the crop against late blight injuries (Mohammadi *et al*., 2019). Moreover, a specific role had been discussed for phytoalexins in plant defense against herbivores. This discussion, reviewed that phytoalexins may include isoflavonoids, terpenoids, alkaloids glucosinolates and benzoxazinoids, which mediate the release of various biocidal aglycone metabolites to motivate the defensive responses of plant against insects attack (Morant *et al*., 2008 and War *et al*., 2012).

The defensive mechanisms could be exploited as an important tool for minimizing insecticides quantities for pest control and to predict the herbivores behavior affected by the induced responses (War *et al*., 2012). The defense compounds like allelochemicals in the form of secondary metabolites and volatile organic compounds (VOCs) possessed defense mechanisms through repellency, reduce digestibility or even toxic against the insect herbivores injuries (Dicke and Baldwin, 2010 and Dong *et al*., 2016). Many evidences showed that feeding behavior of herbivores could elicit the injured plant-defense through induction of repellant VOCs signals (Alborn *et al*., 1997; War *et al*., 2011; Zhou *et al*., 2013 and Krempl *et al*., 2016) and vice versa these signals may attract the natural enemies of herbivores (Turlings *et al*., 1990; D'Alessandro and Turlings, 2005 and Erb *et al*., 2009). Recently, many techniques of olfactometer choice tests were investigated to study insect response to different odors and volatile compounds (Avila *et al*., 2017; Papenberg *et al*., 2019 and Dory *et al*., 2019).

The main targets of our study were directed towards: (1) Investigation of the toxicity and sub-lethal effects of potassium phosphite on some biological aspects compared to emamectin benzoate against *S. littoralis* larvae. (2) Simulation method for extracting induced VOCs

from elicited plant by the tested compounds preluding to be identified by Gas chromatography–mass spectrometry (GC-MS). (3) Evaluation of olfactometer dual choice test for the responses towards the induced VOCs by the tested compounds. (4) Reviewing discussion on the capabilities of the tested compounds to regulate *S. littoralis* larvae behavior.

Materials and methods

1. Insect rearing:

A susceptible strain of *S. littoralis* was reared on fresh castor leaves (*Ricinus communis*), under controlled conditions according to the method of Eldefrawi *et al*. (1964).

2. Tested compounds:

Two compounds were submitted in this study as follows:

2.1. An inducer compound for plant defense response known by potassium phosphite (Quelagrow Iberica –Spain; applied dosage rate of 170 ml/ 100 L).

2.2. Semi-synthetic insecticides known by emamectin benzoate (El-Helb pesticides & chemical Co – Egypt; applied dosage rate of 40 ml/ 100 L).

3. Larvicidal bioassay technique:

Toxicity of emamectin benzoate and potassium phosphite was determined by using the leaf dipping method. Six sequential concentrations of each tested compound were freshly prepared in distilled water. Treated castor leaf pieces with each concentration were dried at room temperature before being placed to newly ecdysed 2nd instar larvae. Untreated larvae were fed on castor leaf pieces immersed in distilled water only. Each treatment was replicated four times with 10 larvae per replicate. Mortality percentages were recorded after 96 hrs post-treatment and subjected to probit analysis according to (Finney, 1971). Sub-lethal concentrations of the tested compounds were calculated with their 95% confidence limits.

4. Extracting and sampling of emitted volatile organic compounds (VOCs) in static headspace:

VOCs emissions were trapped and extracted by static headspace method from cotton seedlings (3 weeks old) in pots (dia. 25 cm) under laboratory conditions (Figure, 1). These emitted VOCs induced by foliar spray treatments of emamectin benzoate and potassium phosphite at concentrations equivalent to their applied doses as well as distilled water in control. The treated cotton seedlings were enclosed under an inverted glass tube (dia. 10 cm X L. 25 cm) immersed in freshly agar layer poured as isolated barrier above the soil surface and sealed the emitted volatiles against leakage. The emitted VOCs were trapped in darkness overnight and then extracted by injecting ethanolic solvent in the static headspace through a lateral opening in the glass tube. Then the obtained ethanolic solvent samples were stored in a sealed glass bottle below 0ºC preluding for GC-MS analysis (Rohloff and Bones, 2005 and Tholl *et al.*, 2006). The emitted VOCs in head state were classified by World Health Organization (WHO) according to their evaporation activity based on initial boiling point into three class; very volatile organic compounds (VVOCS), volatile organic compounds (VOCs) less or equal 260º and Semi- volatile organic compounds (SVOCs) ranged from 260 up to 400 ºC (WHO, 1989).

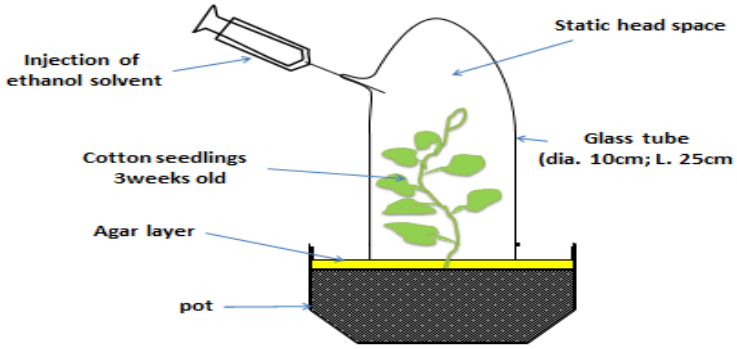


Figure (1): Extracting and sampling of emitted volatile organic compounds in static headspace.

5. Gas chromatography–mass spectrometry (GC-MS) analysis:

The chemical composition of the obtained ethanolic solvent samples eluted VOCs of treated seedlings was performed using Trace GC-ISQ Q mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C / min to 200 °C, hold for 2 min followed by increasing to the final temperature 300°C by 30°C / min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 ml/ min. The solvent delay 3 min and diluted samples of 1 µl was injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–550 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with mass spectral database of WILEY 09 and NIST 11.

6. Effect of tested compounds on some biological aspects of *Spodoptera littoralis*:

The sub-lethal effects of potassium phosphite and emamectin benzoate at their equivalent concentrations of LC_{25} and LC_{50} on some biological aspects of *S. littoralis* were evaluated. Each treatment was replicated four times. Each replicate had one hundred newly ecdysed 2nd instar larvae. These larvae were fed on treated and untreated castor leaves with the tested compounds and distilled water in control, respectively. Surviving larvae were transferred to jars containing sufficient portions of untreated fresh leaves after 96 hrs of exposure and observed daily for larval and pupal development durations (days), larval and pupal weights (mg), pupation and adult emergence percentages.

7. Olfactometer dual choice test:

A simulated still-air olfactometer illustrated by (Weeks *et al*., 2011) made from a tube container (dia. ٥ cm; L. ١٠ cm) to insert the 2nd instar 24 hrs pre-starved larvae of *S. littoralis*, which exposed over 3 hrs to VOCs emitted by each of untreated and treated cotton seedlings that previously incubated in darkness over 24 hrs under glass tube (dia. 10 cm; L. 25 cm). The VOCs passed via short junctions from one lateral opening of the inverted glass tube upon cotton seedlings to the lateral hole of the tube container (dia. 2.5cm). A tube

container was sealed to prevent larvae escape and external foreign odors that contaminate the test environment. Thus, the exposed larvae were allowed to express their preference for the VOCs emitted from each treatment at intervals of times 1, 2 and 3 hrs. Each treatment was replicated 3 times with 10 larvae per replicate. The dual choice tests were design to evaluate the preferable ability of larvae to choose separately between each of treated and untreated cotton seedling as well as choice between each of the two treated cotton seedling (Figure, 2). The determination of olfactometer responses by equation mentioned by Del Socorro *et al*. (2010):

$$\%\, Total\ response = 100 \times (T + C)/N$$

Where; T= number of larvae entering the test chamber, C= number of larvae entering the control chamber and N= total number of larvae in the olfactometer.

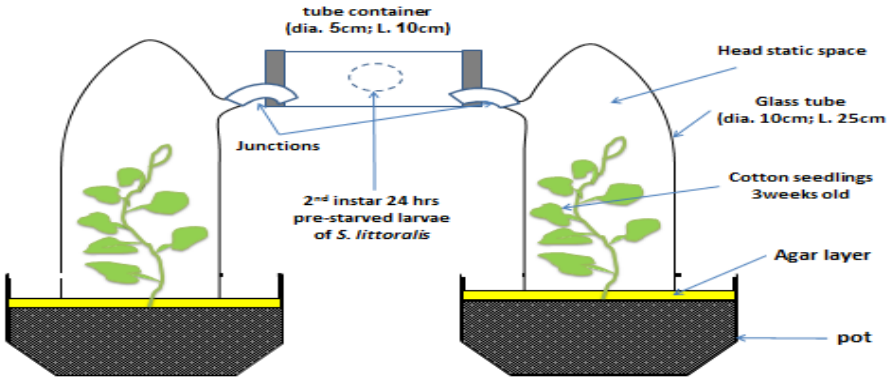


Figure (2): Olfactometer dual choice test.

8. Statistical analysis:

The data were analyzed using one-way analysis of variance (ANOVA). Means were determined for significant differences using SAS software (2002) (LSD at P < 0.05). Olfactometer responses were determined by using Paired T-test.

Results and discussion

1. Toxicity of emamectin benzoate and potassium phosphite against of *Spodoptera littoralis*:

Toxicity of tested compounds against 2nd instar larvae of *S. littoralis* were presented in Table (1). Emamectin benzoate was more toxic than potassium phosphite. LC_{25}, LC_{50} and LC_{90} values were recorded after 96 hrs post-treatment for emamectin benzoate (0.005, 0.012 and 0.248 mg L^{-1}) and potassium phosphite (1326.2, 5302.4 and 73757.2 mg L^{-1}), respectively.

Table (1): Toxicity of emamectin benzoate and potassium phosphite against 2nd instar larvae of *Spodoptera littoralis* after 96 hrs post-treatment.

Compounds	Conc. (mg L^{-1})		Confidence limits (mg L^{-1})	Slope ± SE*
Emamectin benzoate	LC_{25}	0.005	0.004-0.006	1.78 ± 0.13
	LC_{50}	0.012	0.011-0.014	
	LC_{90}	0.248	0.16-0.38	
Potassium phosphite	LC_{25}	1326.2	962-1824	1.12 ± 0.18
	LC_{50}	5302.4	3560-7285	
	LC_{90}	73757.2	30630.9-38304-E	

***SE means Standard Error**

2. Gas chromatography–mass spectrometry analysis:

GC-MS analysis of the ethanolic extract of the VOCs emitted by untreated cotton seedling identified main compounds of 1,2 benzenedicarboxylic acid (25.1%), Linoelaidic acid (16.2%), Phthalic acid, butyl hex-3-yl ester (5.32%) and 1,2-benzenedicarboxylic acid, dibutyl ester (4.24%) out of ten identified compounds (Table, 2). On the other hand, VOCs emitted from elicited cotton seedling by potassium phosphite at equivalent concentration to dosage rate of 170ml/100L revealed presences of seven identified compounds of docosane (7.43%), Dibutyl phthalate (3.68%), 12, 15-Octadecadienoic acid, methyl ester (2.24%), Benzaldehyde, 3-phenoxy- (1.27%), Hexadecanoic acid, ethyl ester (1.21%), ethyl (9z, 12z)-9, 12-octadecadienoate (0.86%) and ß Caryophyllene (0.84%) (Table, 3). Eventually, induced VOCs from cotton seedling by emamectin benzoate at equivalent concentrations to 40ml/100L were distinguished by eight compounds representing majorities of squalen (9.6%), Bisn (2-ethylhexyl) phthalate (9.2%), Methylprednisolone (5.65%) and Dibutyl phthalate (3.48%) (Table, 4).

Table (2): Gas chromatography–mass spectrometry chemical profile of volatile organic compounds emitted by untreated cotton seedling.

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
1,2-benzenedicarboxylic acid, dibutyl ester	-	15.95	4.24	278	SVOC
Phthalic acid, butyl hex-3-yl ester	-	16.87	5.32	306	SVOC
Linoelaidic acid	Monoterpens	17.80	16.2	280	VOC
4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-d ihydroxy-7-methoxy	Aroma	18.27	1.02	344	SVOC
Isochiapin B	-	19.37	1.13	346	VOC
N-butylboronate of methyl 9,10-dihydroxy-stearate	-	19.56	1.17	396	VOC
1H-cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a (1ah)-pentol, 3-[(acetyloxy)methyl] -1b,4,5,7a,8,9-h exahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate	-	19.73	1	534	VOC
Flavone 4'-oh,5-oh,7-di-o-glucoside	-	19.84	2.11	594	VOC
1,2 benzenedicarboxylic acid	-	20.07	25.1	390	VOC
4H-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-d i-á-d-glucopyranosyl-5,7-dihydroxy	-	21.32	1.30	610	SVOC

*__The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = volatile organic compounds and VOCs = semi volatile organic compounds.__

Table (3): Gas chromatography–mass spectrometry chemical profile of induced volatile organic compounds from cotton seedlings by potassium phosphite.

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
ß Caryophyllene	Sesquiterpene	12.95	0.84	204	VOC
Benzaldehyde, 3-phenoxy-	-	20.2	1.27	198	SVOC
Hexadecanoic acid, ethyl ester	Ethyl palmitate fatty acid	20.66	1.21	284	SVOC
Dibutyl phthalate	Phthalic acid	22.44	3.68	278	SVOC
Ethyl(9Z,12Z)-9,12-Octadecadienoate	Ethyl linoleate	22.86	0.86	308	SVOC
12,15-Octadecadienoic acid, methyl ester	Methyl linolenate	23.59	2.24	294	SVOC
Docosane	Higher alkane	27.04	7.43	310	SVOC

***The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = Volatile organic compounds and VOCs = Semi volatile organic compounds.**

Table (4): GC-MS chemical profile of VOCs from elicited cotton seedlings by emamectin benzoate

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
Dibutyl phthalate	Phthalic acid	16.85	3.48	278	SVOC
Cyclopropane	-	18.62	3.54	302	VOC
Methylprednisolone	Corticosteroid	18.85	5.65	374	VOC
1,3-Dioxolan-2-one,5-methyl-4-(4,4-dimethyl-2,3- di methyl enecyclohexyl)	Alkyl-amides	19.52	2.73	236	VOC
2-[1-(adamantan-1-ylamino)-2,2,2-tri fluoro-ethylidene]-malononitrile	-	19.69	2.5	295	SVOC
1-Heptatriacotanol	Alcoholic compound	19.52	1.45	536	SVOC
Bis(2-ethylhexyl) phthalate	Phthalates	20.06	9.20	390	SVOC
Squalene	Triterpene	20.75	9.6	410	VOC

***The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = volatile organic compounds and VOCs = semi volatile organic compounds.**

3. Effect of the tested compounds on some biological aspects of *Spodoptera littoralis*:

Sub-lethal effects of emamectin benzoate and potassium phosphite were manifested on some biological aspects of 2nd instar larvae of *S. littoralis* in Tables (5 and 6). Treatments at LC_{50}s affected the average weights of 2nd instar larvae as emamectin benzoate had the lowest values of 96.4, 125.3, 56.2 and 28.4 mg while potassium phosphite significantly surpassed with values of 118.2, 173.4, 248.2 and 358.2 mg compared to the highest values of 167.5, 347.2, 889.7 and 1247.3 mg in the control at 3, 6, 9 and 12 days post-treatment, respectively. Symmetrically, treatments at LC_{25}s had lower significant effects on the larval average weights. Longest average durations were recorded for treated larvae with LC_{25} and LC_{50} values of emamectin benzoate (16.4 and 17.2 days, respectively) and significantly decreased in those treated with potassium phosphite (14.3 and 15.9 days, respectively) whereas control treatment had the shortest duration time of 13.8 days (Table , 5).

Table (5): Sub-lethal effects of tested compounds on the 2nd instar larvae of *Spodoptera littoralis* via larval mean weight after sequent days of treatment and larval duration.

Treatments	Conc. (mgL^{-1})	Larval mean weight (mg) ± SE				Larval duration (days) ± SE
		3-days	6-days	9-days	12-days	
Control	-	167.5^{a} ± 2.4	347.2^{a} ± 3.5	889.7^{a} ± 3.2	1247.3^{a} ± 5.2	13.8^{c} ± 1.6
Emamectin benzoate	**0.005**	124.6^{c} ± 1.3	154.5^{c} ± 2.6	84.8^{d} ± 2.4	64.2^{d} ± 3.8	16.4^{b} ± 1.2
	0.012	96.4^{d} ± 1.2	125.3^{d} ± 2.2	56.2^{e} ± 2.1	28.4^{e} ± 2.4	17.2^{a} ± 1.3
Potassium phosphite	**1326.2**	148.2^{b} ± 1.9	265.7^{b} ± 2.4	574.3^{b} ± 2.8	893.8^{b} ± 4.6	14.3^{c} ± 1.8
	5302.4	118.2^{c} ± 1.6	173.4^{c} ± 2.8	248.2^{c} ± 2.3	358.2^{c} ± 3.2	15.9^{b} ± 1.3

***Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$.**

Data showed highest declinations in the mean weights of pupae treated with LC_{25} and LC_{50} values of emamectin benzoate (150.4 and 95.2 mg, respectively) followed by potassium phosphite (262.3 and 224.3 mg, respectively) compared to 309.2 mg in the control treatment. However, pupal duration did not change significantly in all treatments compared to the control. Significant decreases in pupation percentage revealed in the LC_{50}s of emamectin benzoate (21.5%) followed by potassium phosphite (57.3%) compared to 90.3% in the control treatment. Reduction in the adult emergence percentage was significantly exhibited in all treatments. Adult emergence percentages treated with LC_{25} and LC_{50} values were 31.7 and 18.3% for emamectin benzoate besides 72.3 and 53.8% for potassium phosphite, respective compared to 86.8% in the control treatment (Table, 6).

Table (6): Sub-lethal effects of tested compounds on 2nd instar larvae of *Spodoptera littoralis* via mean weight and duration of pupa, pupation and adult emergence percentages:

Treatments	Conc. (mgL^{-1})	Pupal mean weight (mg) ± SE	Pupal duration (days) ± SE	Pupation (%) ± SE	Adult emergence (%) ± SE
Control	-	309.2^{a} ± 2.8	10.3^{a} ± 0.6	90.3^{a} ± 2.5	86.8^{a} ± 2.1
Emamectin benzoate	**0.005**	150.4^{d} ± 3.4	10.6^{a} ± 0.3	36.4^{d} ± 1.2	31.7^{d} ± 1.3
	0.012	95.2^{e} ± 2.6	10.8^{a} ± 0.2	21.5^{e} ± 2.3	18.3^{e} ± 1.8
Potassium phosphite	**1326.2**	262.3^{b} ± 3.2	10.4^{a} ± 0.3	78.6^{b} ± 1.7	72.3^{b} ± 1.6
	5302.4	224.3^{c} ± 2.4	10.6^{a} ± 0.5	57.3^{c} ± 1.4	53.8^{c} ± 2.2

***Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$.**

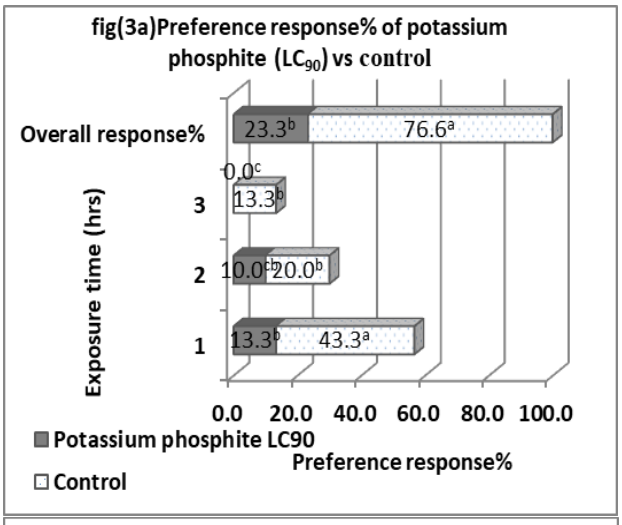
4. Olfactory response (%) choice tests:

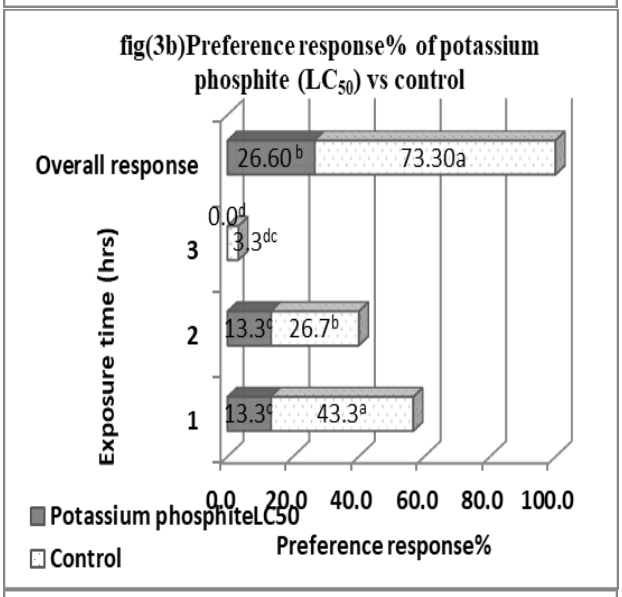
Data of the overall preference response percentage of the 2nd instar 24 hrs pre-starved larvae of *S. littoralis* that exposed over 3 hrs to treated and untreated cotton seedlings after incubating in darkness over 24 hrs (Figures, 3a, 3b, 4a, 4b, 5a and 5b).

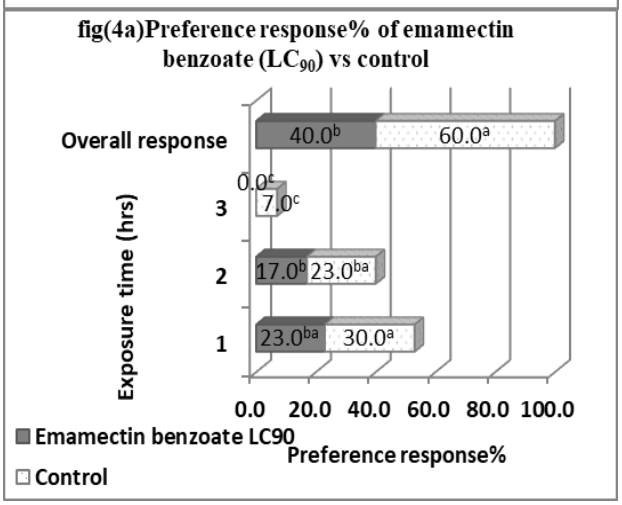
In the first dual choice test, high significant overall preference response percentage to control treatment (76.6%) were arisen compared to potassium phosphite treatment at LC_{90} (23.3%). Precisely, the exposed larvae via 1st, 2nd and 3rd hrs of exposure showed high significant preference response percentage to control (43.3, 20.0 and 13.3%, respectively) versus to potassium phosphite at LC_{90} (13.3, 10.0 and 0.0%, respectively) (Figure, 3a). Moreover,

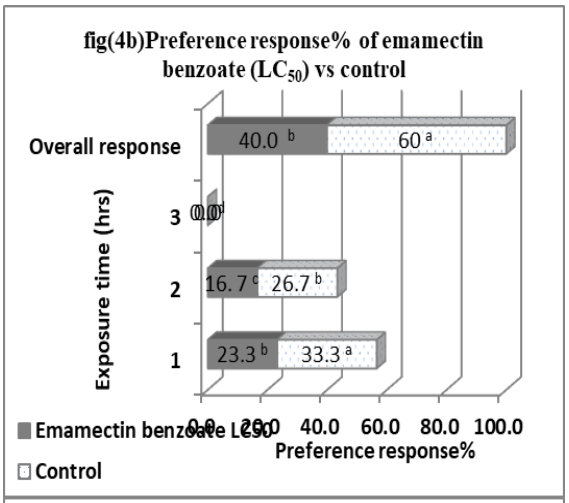
high significant of the overall preference response percentage in potassium phosphite treatment at LC_{50} (26.60%) was occurred versus to the control treatment (73.30%). Particularly, high significant preference response percentage was revealed in the control treatment over potassium phosphite at LC_{50} via 1st, 2nd and 3rd hrs of exposure (Figure, 3b).

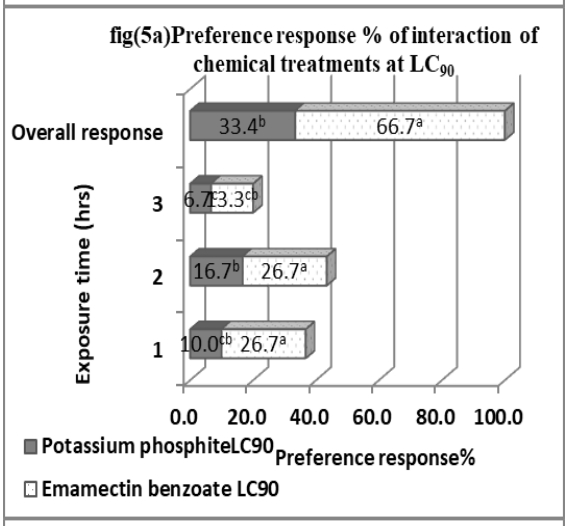
The second dual choice test showed that emamectin benzoate at both of LC_{90} and LC_{50} caused the same lower overall preference response percentages of 40.0 % compared to control treatments (60.0 %). Particularly, the exposed larvae after 1st, 2nd and 3rd hrs showed high significant preference response percentage to control (7.0, 23.0 and 30.0 %, respectively) versus to emamectin benzoate at LC_{90} (0.0, 17.0 and 23.0 %, respectively) (Figure, 4a). Otherwise, preference response percentage was revealed in the control treatment (26.7 and 33.3 %) over emamectin benzoate at LC_{50} (16.7 and 23.3 %) via 1st, 2nd hrs of exposure, respectively (Figure, 4b). The results of the third dual choice test between the two tested compounds at LC_{90} and LC_{50} showed significant overall preference response percentages to emamectin benzoate (66.6 and 66.7 %, respectively) more than potassium phosphite (33.4 and 33.3 %, respectively). Predominately, the exposed larvae to sub-lethal concentrations of LC_{90} and LC_{50} throughout the three hours of exposure showed significant preference response percentages to emamectin benzoate more than potassium phosphite treatments (Figures, 5a and 5b).

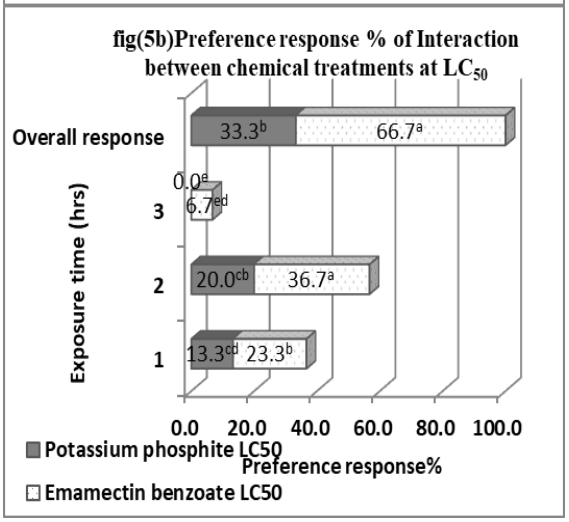












- **Means of preference response % based on treatments and time (hrs) interactions with the same letter are not significantly different.**
- **Means of overall response % over 3 hrs of exposure with the same letter are not significantly different.**

Many studies have been investigated the role of potassium phosphite to induce the synthesis of plant defense and resistance against pathogens and environmental stress (Babu *et al.*, 2003; Rios *et al.*, 2014; Araujo *et al.*, 2015 and Nascimento *et al*., 2016). Plant defensive mechanisms to curb herbivores attacks are still limited. Needs for more acquaintance about these mechanisms could develop pest control management and regulation of herbivores responses (War *et al*., 2012). Therefore, these evidences motivate our study to explore new defensive and biological activity for potassium phosphite against *S. littoralis* versus to emamectin benzoate one of the most common and environmentally safe semi-synthetic insecticide (Dunbar *et al*., 1998).

The obtained results of leaf-dip bioassay on the sub-lethal concentrations of emamectin benzoate was more toxic than potassium phosphite at 96 hrs post-treatment against 2nd instar larvae of *S. littoralis*. These results came in accordance to the toxicity tests of emamectin benzoate which seemed to be more fit and sensitive with leaf-dip bioassay against different larval stages of *S. Iitura* due to its stomach poison and contact mode of action (Birah *et al*., 2008). Meanwhile, the toxic effect of sub-lethal concentrations of potassium phosphite on cotton seedlings may be related to the phenolic derivative of benzaldehyde, 3-phenoxy- that could produce direct toxins that deter the insect's feeding (Chen *et al.*, 2009 and War *et al*., 2012).

The obtained data by GC-MS analysis for induced VOCs by untreated cotton seedling (control) were distinguished by majorities of phthalic acid, butyl hex-3-yl ester, linoelaidic acid (monoterpen), 1,2 benzenedicarboxylic acid and 1,2-

benzenedicarboxylic acid dibutyl ester out of ten identified compounds. Exclusively, the induced VOCs by potassium phosphite were featured by dibutyl phthalate, ß-caryophyllene (sesquiterpene), fatty acid derivatives (ethyl palmitate fatty acid, ethyl linoleate and methyl linolenate), docosane and benzaldehyde, 3-phenoxy-. Whereas, the identified VOCs by emamectin benzoate were differentiated by major components of dibutyl phthalate, bis (2-ethylhexyl) phthalate, squalene (triterpene) and methylprednisolone besides other minor groups. From the previous results, the differences in the emission patterns of the green leaf volatiles in the entire pathway in plant results of treatment modification of existing pathways via up-/ down-regulation of biochemical steps or by blocking the competing pathways. The concurrent temporal changes in activities of enzymes responsible for the final steps of VOC formation, enzyme protein content, and the expression of corresponding structural genes suggest that the developmental biosynthesis of volatiles is regulated largely at the level of gene expression (Dudareva *et al*., 2000; McConkey *et al*., 2000 and Muhlemann *et al*., 2012).

Data of biological tests showed that LC_{25} and LC_{50} values of emamectin benzoate had the highest significant declinations on the larval and pupal average weights and pupation percentage of the 2nd instar larvae more than potassium phosphite but normally increased in control treatment. In addition, sub-lethal concentrations of emamectin benzoate significantly prolonged the larval durations more than potassium phosphite and control treatment while no significant changes in pupal durations in both treatments compared to the control. These results were agreed with the data of biological aspect of emamectin benzoate that significantly decreased the consumption index; relative growth rate and efficiency of converting ingested and digested food in body tissue, while significantly did not affect the approximate digestibility of survived larvae of *S. littoralis*. Emamectin benzoate significantly prolonged the larval duration and decreased the pupal duration, pupal and larval means weight, pupation and adult emergence percentages of *S. littoralis* compared with control treatment (El-Dewy, 2017 and El-Sayed *et al*., 2017). However, the obtained data by GC-MS analysis showed that VOCs came out from cotton seedling in response to potassium phosphite particularly contain benzaldehyde, 3-phenoxy- that may probably possessed negative adverse against the larvae of *S. littoralis*. This allegation was supported by many reviews carried out on the direct toxic and deterrent effect of phenoxy derivatives and other oxidative radicals on the insect's feeding by reducing the plant digestibility, increasing nutrient deficiency and reducing growth and development of insects (Zhang *et al*., 2008; Chen *et al*., 2009 and War *et al*., 2012). Eventually, cotton seedlings VOCs induced by potassium phosphite were distinguished by high potent of long carbon chain double bonded-fatty acid derivatives (ethyl palmitate fatty acid ($C_{18:1}$), ethyl linoleate ($C_{20:2}$) and methyl linolenate ($C_{19:2}$)) which may play larvicidal effects on larvae of *S. littorals* compared to only linoelaidic acid ($C_{18:2}$) in control treatment. These data were justified by the observations concerning the defensive and toxic activity of fatty acids that may related by the increase of unsaturated bonds in carbon chain against the 4th instar larvae of *S. littoralis* as well

as their inhibitory action on the growth of some bacteria (US EPA, 2002; Marounek *et al*., 2002; Maia *et al*., 2010 and Abay *et al*., 2013). These toxic activities might be due to the relative abilities of the fatty acids to involve either the site of acetyl cholinesterase or octopaminergic receptors (Perumalsamy *et al*., 2015 and Hikal *et al*., 2017). In the way, phytoalexins originate in cotton plant; family Malvaceae is commonly existed found in the form of Terpenoids, naphthaldehydes and/or gossypol (Sunilkumar *et al*., 2006 and Jeandet *et al*., 2014). Consequently, naphthalene compounds were supposed to mediate in the formation of dibutyl phthalate and bis (2-ethylhexyl) phthalate which has been detected in our study by GC-MS analysis for the treatments of potassium phosphite and emamectin benzoate, respectively. These detected derivatives of phthalate might cause toxic effects. This supposition were supported by many reviews and investigations compiled on the natural formation of phthalic acid (Heudorf *et al*., 2007; Husein *et al*., 2014 and Przybylińska and Wyszkowski, 2016).

The designed dual choice tests in this research showed preferable ability of 2nd instar larvae to untreated cotton seedling versus to each of potassium phosphite and emamectin benzoate at concentrations of LC_{90} and LC_{50}, separately. Preference toward the volatiles blends emitted by untreated plants over the blends emitted by treated plants by tested compounds these preferences could be attributed to amounts of linoelaidic acid in untreated plants. However, choice tests between the two treatments showed surpasses of potassium phosphite in orienting responses of larvae over emamectin benzoate at all concentrations. These preferences could be attributed to amounts of ethyl linoleate and methyl linolenate emitted by cotton seedling treated by potassium phosphite. This result agreed with (Carlsson *et al*., 1999 and Shelton and Badenes-Perez, 2006) which demonstrated that either linalool or geraniol could serve as olfactory attractants to 3rd instar of *S. littoralis*. In addition; ß-caryophyllene emitted by cotton seedling in response to potassium phosphite could probably play a role in regulating the response behavior of *S. littoralis*. This thought was emphasized by the phenomena of attracting nematodes of *Heterorhabditis megidis* by ß-caryophyllene released by maize roots injured by larvae of the beetle *Diabrotica virgifera* in the soil (Rasmann *et al*., 2005 and Kant *et al*., 2015). Furthermore, the presence of high amounts of terpenoids in GC-MS analysis from treated cotton seedlings by emamectin benzoate were identified as squalene (triterpene) while potassium phosphite induced ß-caryophyllene (sesquiterpene). Moreover, many findings of that terpenoids (*β*-myrcene, (*E*)-*β*-ocimene, DMNT and (*E*)-*β*-caryophyllene) induced by cotton plant VOCs were considered as direct repellents for *S. exigua*, *Helicoverpa zea* and *Lygus Hesperus* as well as attractive for predators and parasitoids (Röse *et al*., 1998; Manrique *et al*., 2005 and Huang *et al*., 2015).

Eventually, the olfactometer and biological assessments in this study enrolled potassium phosphite as a novel inducer compound for plant defense against *S. littoralis*. Potassium phosphite treatment on cotton seedlings was distinguished by induced active blends of VOCs included benzaldehyde, 3-phenoxy- and fatty acid derivatives that elucidate the latent toxic, defensive and biological activities besides ß-

caryophyllene and dibutyl phthalate, which had a latent role in regulating larval responses. These observations, may leads to employ potassium phosphite amongst the applications of synthetic insecticides in the control of *S. littoralis*.

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