

TOXICITY AND BIOCHEMICAL EFFECT OF ORGANOPHOSPHATES AND BIOPESTICIDES AGAINST ROOT-KNOT NEMATODE, *Meloidogyne incognita*

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ABSTRACT

This study was carried out to investigate the toxicity and biochemical effects of two (biopesticides) biofly, abamectin and two organophosphates pesticides cadusafos and fenitrothion against root-knot nematode, *Meloidogyne incognita* egg filtrates and second stage juveniles (J2) as well as on laboratory experiment, also the inhibitory effect of the tested pesticides to acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) were determined. Results indicated that the tested pesticides have toxic action against *Meloidogyne incognita* second-stage juveniles (J2) and egg filtrates after 24 hrs from application to 72 hrs and the toxicity increased with the time. Abamectin was the most toxic followed by fenitrothion cadusafos and biofly was least in its toxicity to *M. incognita* second stage juveniles (J2) and egg filtrates while the toxicity was depending on dose. The tested pesticides have inhibitory effect on AChE and ATPase activity and the inhibitory effect increased with AChE in case of abamectin and cadusafos while the potency of the organophosphate pesticides to inhibit ATPase was limited refers to its mode of action.

Keywords: Toxicity, organophosphate, biopesticides, *Meloidogyne incognita*, enzymes

INTRODUCTION

Plant-parasitic nematodes are recognized as the causes of serious yield losses on a wide range of crops (Javad *et al.*, 2006). The most destructive species is *Meloidogyne incognita* which cause serious problems in various agricultural crops. Root-knot nematode, *Meloidogyne incognita* Kofoid and White Chitwood is a major plant parasitic nematodes affecting quantity and quality of the crop production in many annual and perennial crops. Infected plants shows typical symptoms including root galling stunting and nutrient deficiency, particularly nitrogen deficiency (Siddiqui *et al.*, 2001). *M. incognita* causing an estimated yearly crop loss of \$100 billion worldwide (Oka *et al.*, 2000). Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs/female (Natarajan *et al.*, 2006). Chemical control is expensive and is economically viable only for high value crops and create a potential hazard to the environment and human health (Tsay *et al.*, 2004). Biopesticides currently are integrated into many diverse agricultural production schemes. These materials can be effective and safe, but their use requires more sophistication than chemical pesticides on the part of the user. Many of these products have specific requirements for storage and application, and to treat them like a chemical pesticide often results in failure. As biological organisms they require appropriate biotic as well as abiotic

conditions for success. Users would benefit from learning how to maximize efficacy before or after the organisms have been applied. Among microorganisms regulating nematode densities in soil, fungi hold an important position due to their parasitic, antagonistic or predatory behaviours. Some species have potentials in biocontrol and exhibit a range of antagonistic activities, including production of nematotoxic compounds (Siddiqui and Mahmood 1996; Kerry 2000; Lopez-Llorca and Jansson 2006). Nematophagous fungi directly parasitize nematodes or secrete nematicidal metabolites affecting viability of one or more stages. The search for nematotoxic or antagonistic compounds in culture filtrates has greatly intensified in recent years, due to the number of toxins, enzymes or compounds derivable from their metabolites (Ciancio, 1995; Liu *et al.*, 2008; Lopez-Llorca *et al.*, 2008). Inhibition of ChE activity is one of the best characterized biomarkers and has been intensively used in environmental studies, showing a specific response to organophosphate (OP) and carbamate pesticides (Gruber and Munn, 1998; Thompson, 1999). Abamectin is a macrocyclic lactone derived from the soil bacterium *Streptomyces avermitilis* that has been shown to have nematicidal properties (Putter *et al.*, 1981), and a different mode of action than the other currently available nematicides (Turner and Schaeffer, 1989). It is suitable compound for seed treatment since it can be stored for several months while maintaining its nematicidal properties. It can be applied to seeds at high concentrations, does not bio-accumulate and is not taken up by plants (Dybas *et al.*, 1989). Cadusafos is an organophosphorus nematicide under the trade name "Rugby, Cadusafos controls a wide range of plant parasitic nematodes, such as *Tylenchulus semipenetrans* (McCutchen and Flexner, 1994). ChE stands out as an ideal biomarker to evaluate agriculture-related pollution effects in the area. Further, it would be useful to trace a link between pesticide use, detection of residues in the environment and their toxic effects. AChE has been widely studied in many different species because it can be inhibited by OPs and CAs. ATPase is a group of enzymes that play an important role in intracellular functions and that are considered to be a sensitive indicator of toxicity (Yadwad *et al.*, 1990). It can be target for many groups of pesticides. The aim of this study is to investigate the effect of Organophosphates (cadusafos, fenitrothion) and the biopesticides (abamectin, biofly) against root knot nematode *Meloidogyne incognita*. The potency of these pesticides to inhibit acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activity of such pathogenic nematode, was also investigated.

MATERIALS AND METHODS

Nematode Cultures:

The root-knot nematode *M. incognita* was isolated from infected roots of eggplant (*Solanum melongena* L.) obtained from El-Nubaria region, Behera Governorate, Egypt. Eggs and second-stage juveniles (J2) were

extracted from infected roots by the sodium hypochlorite method (Khan *et al.*, 2004).

Synthetic Insecticides:

A) Abamectin

Group name: Avermectin (Biopesticide) **Common name:** Abamectin **Trade name:** Vabcomic **Empirical formula:** C₄₈H₇₂O₁₄ (80% avermectin B1a); C₄₇H₇₀O₁₄ (20% avermectin B1b) **Molecular weight:** 873.1 (avermectin B1a); 860.1 (avermectin B1b) **Formulation:** 1.8 % E.C. **Source:** Hebei Veyong Bio-Chemical Co, ltd, China)..

B) Biofly:

Group name: Biological insecticide **Common name:** *Beauveria bassiana* **Used and applied rate:** Entomopathogenic fungus used for control of a wide range of coleopteran, homopteran and heteropteran pests. Fungus applied at rate of 100 cm³ / 100 liter water **Trade name:** Biofly **Source:** E1-Nasr Bio insecticides and Fertilizers Company, E1-Sadaat, Egypt.

C). Cadusafos

Group name: Organophosphorus, **Common name.** cadusafos **Trade name:** Rugby. **Chemical name (IUPAC):** S,S-di-sec-butyl O-ethyl phosphorodithioate **Empirical formula:** C₁₀H₂₃O₂PS₂ **Molecular weight:** 270.4 **Formulation:** 20% E.C **Use:** Agricultural Nematicide / Insecticide **Source:** FMC Australasia Pty Ltd.

D) Fenitrothion

Group name: Organophosphorus **Common name:** Fenitrothion **Trade name:** Fentro **Chemical name (IUPAC):** O,O-dimethyl O-4-nitro-*m*-tolyl phosphorothioate **Empirical formula:** C₉H₁₂NO₅PS **Molecular weight:** 277.2 **Formulation:** 50% E.C applied at rate of 250 cm³/100 liter water **Use:** insecticides **Source:** Agrochem, Alwatneia Company, Alex., Egypt

Nematicidal Assay on Eggs:-

M. incognita was cultured in the greenhouse on tomato plants (New L-402, nematode susceptible) inoculated with a single nematode egg mass (Khan *et al.*, 2004). After 55 days, egg masses were hand picked from galls of tomato roots and surface sterilized in 0.5% sodium hypochlorite for 3 min and washed with sterile water 3 Times. J2 were hatched from the egg masses, collected daily and stored at 4°C. 2ml of egg masses of nearly uniform size were transferred to a 6 cm-diameter autoclaved Petri dishes containing 2 ml filtrate of different dilutions of (1.8% abamectins, 100% biofly 20% cadusafos and 50% fenitrothion) the series of concentrations were around recommended dose of each pesticide, eggs maintained at the same volume of distilled water served as control; three replicates of each treatment and control were included. Plate lids were sealed with parafilm and the plates were kept at 25°C. After 7 days hatched J2 were counted with the use of an inverted microscope and egg hatch reduction for a treatment was calculated as: average % of non hatched J2 in the filtrate/average % non hatch in control with sterilized water × 100

Nematicidal Assay of Second stage juveniles (J2):-

2 ml of the above solutions as previously described from each concentration was added to 2 ml of nematode suspensions *M. incognita* second stage juveniles in 50 ml glass capsule. A control treatment was made by adding 2 ml of distilled water plus 2 ml of the nematode suspension. Each treatment was replicated three times. The number oviabile and dead nematodes was counted with the aid of a light microscope after 24, 48 and 72 hrs at 25±1C⁰ and the nematode mortality was calculated for each treatment. Only the nematodes which did not regain motility were considered "dead". The mortality percentage was calculated according to (Abbott's, 1925). formula:

$$\text{Mortality (\%)} = \frac{m - n}{100 - n} \times 100$$

Whereas: m is percentages of mortality in treated sample
n percentages of mortality in control.

Enzyme preparation

One ml, of nematode suspension or egg filtrate of each pesticides mixture stoked for 48 hrs from bioassay test were homogenized with ice cold buffer for 30 s and a small amount of glass beads (<106 lm, Sigma) were added during homogenization with cooling to obtain at least 95% breakage. Homogenate was centrifuged. The clear supernatant was collected and kept frozen at -20 C⁰ until assaying.

Enzyme determination

Acetylcholinesterse (AChE) activity.

The AChE was determined by the colorimetric method of (Ellman *et al.*, 1961). The suspension was homogenized in 0.1 M phosphate buffer (pH 7.0). The homogenates were then centrifuged at 5,000 rpm for 20 min at 0C⁰. The supernatants were used as enzyme source for assay of AChE activity. Enzyme (150 uL), 100 ul DTNB (0.01 M), and 30 uL ATChI (0.075 M) were added to 2.8 mL 0.1 M phosphate buffer (pH 8.0). The mixture was incubated at 37C⁰ for 15 min. The absorbance was measured at 412 nm using Unico 1200 spectrophotometer. All of the treatments were done in triplicates. The specific activity of AChE was expressed as nmoles of acetylthiocholine iodide hydrolyzed/ mg protein/min. Inhibition percentages of the activities against control were estimated in the enzymatic assay.

Total Protein Assay

Total protein was determined according to the method of Lowry *et al.*, (1951). This method was used to determine the protein content in nematode extract Protein extract (100 uL) was added to 2mL alkaline copper reagent [48 of 2% (w/v) sodium carbonate in 0.1 N sodium hydroxide + 1 mL 1% (w/v) sodium-potassium tartrate +1 mL 0.5% (w/v) copper sulfate] and immediately mixed. After 10 min, 0.2 mL Folin-Ciocalteu phenol reagent was added and the samples were thoroughly mixed, then the absorbance of the developed blue color was measured at 600 nm using an Unico 1200 spectrophotometer.

The protein content of the sample was determined by comparing to the standard curve of BSA.

Adenosine Triphosphatase (ATPase) Activity Assay

ATPase activity was determined according to (Koch, 1969). After 48 h of bioassay test on the tested concentration, the nematode suspension was homogenized in Tris-HCl buffer (pH7.4). The homogenates were centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant was then centrifuged at 17,000 rpm for 30 min at 4°C. The pellets were resuspended in the same buffer. This suspension was used as enzyme source for the assay of ATPase activity. Enzyme suspension was added to the reaction mixture that contained 100 mM NaCl, 20 mM KCl, 5 mM Mg₂Cl, and 5 mM ATP and the volume was adjusted to 850 µL with Tris-HCl buffer (pH 7.4). This mixture was incubated at 37°C for 15 min and then stopped with 150 µL TCA. Four milliliters of fresh color reagent (5 g ferrous sulfate in 10 mL ammonium molybdate solution prepared in 10 N sulfuric acid) was added and absorbance was measured at 740 nm by using Unico 1200 spectrophotometer. The enzyme activity was represented as micromoles inorganic phosphorus (Pi/mg protein/h). Inhibition percentages of the activities compared with control were considered in the enzymatic assay.

Statistical analysis:

Data obtained were statistically analyzed according to SAS software program (SAS Institute, 1998) Statistical analysis was performed using the SPSS 12.0 software program (Statistical Package for Social Sciences, USA). The log dose-response curves allowed determination of the LC₅₀ values for the nematode bioassay according to probit analysis (Finney, 1971). The 95% confidence limits for the range of LC₅₀ were determined by least-square regression analysis of the relative growth rate (percentage of control) against the logarithm of the compound concentration. The data for AChE and ATPase activities were analyzed by one-way analysis of variance (ANOVA).

RESULTS

Toxicity of the tested pesticides to *M. incognita* :

Results in (Tables 1a, 1b, 1c, 2) indicated that all tested pesticides have toxic effect against *M. incognita* second-stage juveniles (J2) after 24 hrs of application where abamectin was the most toxic one with LC₅₀ value 2.94 mg/L followed by 147.44 mg/L for fenitrothion, 976.77 mg/L for cadusafos while biofly was the least toxic one with LC₅₀ value of 3190.18 mg/L. The toxicity increased after 48 hrs of treatment and the LC₅₀ values were amounted to (1.68 & 34.69 & 163.13 and 877.98 mg/L) respectively for abamectin, fenitrothion, cadusafos and biofly. The same trend of toxicity observed after 72 hrs of treatment abamectin was the most toxic one followed by, fenitrothion & cadusafos and biofly was the least toxic with LC₅₀ value (1.07, 20.64, 47.6 and 386.48) mg/L respectively. On the other hand the effect of the same tested solutions on percentage of egg hatch reduction was calculated as: average % of non hatched J2 in the filtrate /

average % non hatch in control with sterilized water×100. Result in(Table (2). indicated that abamectin was the most effective in hatch reduction of *M. incognita* followed by cadusafos , fenitrothion and biofly was the least effective one with the LC50 values (1.102. 308.04, 577.69, 620.62) mg /L, respectively.

Table (1a):Acute toxicity of abamectin ,biofly cadusafos and fenitrothion to To *M. incognita* second stage juveniles (J2).

Compounds	Concentration (ppm)	Mean of Death	Mortality (%)	LC ₅₀ (ppm) ^a	Slope ^b ± SE	(x2) ^c
After 24 h						
Abamectin	0.225	5.67	4.72 ±2.37	2.94 (2.11-3.71)	1.72 ±0.16	7.3
	0.45	12.33	10.28 ±3.61			
	1.125	24.67	20.56 ±5.94			
	2.25	31.00	25.83 ±3.16			
	4.50	91.00	75.83 ±1.44			
Biofly	100	24.33	20.28 ±5.98	386.48 (210.12-501.12)	0.9 ±0.13	1.5
	200	29.00	24.17 ±4.59			
	500	35.33	29.44 ±2.22			
	1000	46.33	38.61 ±1.00			
	2000	56.00	46.67 ±3.00			
cadosafos	300	20.33	17.76 ±1.54	3190.18 (1613.43-14849.36)	0.58 ±0.13	0.96
	600	43.00	37.66 ±6.57			
	1500	80.67	70.65 ±4.03			
	3000	88.00	77.29 ±2.29			
	6000	94.67	83.23 ±3.85			
Fentrothion	30	23.67	19.72 ±5.10	147.44 (122.37-176-32)	1.45 ±0.11	1.2
	60	26.67	22.22 ±2.00			
	125	56.00	46.67 ±2.55			
	312	86.00	71.67 ±3.00			
	612	92.00	76.67 ±1.27			
	1250	112.33	93.61 ±6.39			
control	0.00	0.00 ±0.00				

Table (1b): Acute toxicity abamectin ,biofly cadusafos and fenitrothion to To *M. incognita* second stage juveniles (J2).

Compounds	Concentration (ppm)	Mean of Death	Mortality (%)	LC ₅₀ (ppm) ^a	Slope ^b ± SE	(x2) ^c
After 48 h						
Abamectin	0.225	11.67	9.72 ±2.22	1.68 (1.02-3.36)	1.76 ±0.15	1.4
	0.45	20.00	16.67 ±3.63			
	1.125	33.00	27.50 ±5.85			
	2.25	66.33	55.28 ±6.74			
	4.50	102.00	85.00 ±3.00			
Biofly	100	33.00	27.50 ±5.02	877.98 (610.61-1467.41)	0.73 ±0.12	1.02
	200	33.33	27.78 ±3.38			
	500	49.00	40.83 ±6.79			
	1000	66.67	55.56 ±2.42			
	2000	71.33	59.44 ±3.28			
cadosafos	300	64.00	56.46 ±6.48	163.13 (51.96-296.81)	0.76 ±0.13	0.91
	600	77.33	68.16 ±6.07			
	1500	89.33	78.43 ±1.31			
	3000	92.67	81.39 ±1.97			
	6000	100.67	88.55 ±5.84			
Fentrothion	30	59.67	49.72 ±1.00	34.69 (17.93-52.16)	0.83 ±0.13	1.4
	60	67.33	56.11 ±4.82			
	125	80.67	67.22 ±0.73			
	312	93.33	77.78 ±1.00			
	612	103.33	86.11 ±2.78			
	1250	120.00	100.00 ±0.00			
control	0.00	0.00 ±0.00				

Table (1c): Acute toxicity of abamectin ,biofly cadusafos and fenitrothion to To *M. incognita* second stage juveniles (J2).

Compounds	Concentration (ppm)	Mean of Death	Mortality (%)	LC ₅₀ (ppm) ^a	Slope ^b ± SE	(x2) ^c
After 72 h						
Abamectin	0.225	21.33	17.78 ±0.56	1.07 (0.56-2.08)	1.39 ±0.13	2.31
	0.45	41.00	34.17 ±3.47			
	1.125	45.67	38.89 ±1.47			
	2.25	89.00	74.17 ±0.83			
	4.50	97.00	80.83 ±0.96			
Biofly	100	35.67	29.72 ±2.27	386.48 (210.12-501.12)	0.9 ±0.13	1.5
	200	45.00	37.50 ±4.64			
	500	61.33	51.11 ±10.83			
	1000	82.67	68.89 ±2.27			
	2000	89.67	74.72 ±0.56			
cadosafos	300	88.67	78.16 ±7.48	47.6 (35.36-66.2)	0.41 ±0.15	1.24
	600	90.67	79.70 ±3.21			
	1500	94.67	83.20 ±3.10			
	3000	99.33	87.33 ±3.97			
	6000	103.00	90.60 ±5.09			
Fentrothion	30	73.00	60.83 ±2.55	20.64 (8.84-33.65)	0.86 ±0.14	2.3
	60	73.33	61.11 ±4.72			
	125	88.67	73.89 ±3.86			
	312	96.00	80.00 ±0.83			
	612	113.33	94.44 ±5.56			
	1250	120.00	100.00 ±0.00			
	control	0.00	0.00 ±0.00			

^a Lethal concentration causing 50% mortality after 24 and 48 h with 95% confidence limits.

^b Slope ± Standard Error of the concentration-mortality regression line.

^c Chi square.

Table (2): Acute toxicity of abamectin ,biofly cadusafos and fenitrothion to To *M. incognita* egg hatching of nematode .

Compounds	Concentration (ppm)	Mean of Death	Egg hatching reduction (%)	LC ₅₀ (ppm) ^a	Slope ^b ± SE	(x2) ^c
Abamectin	0.225	8.67	10.20 ±0.39	1.102 (0.9-1.62)	1.98 ±0.15	2.09
	0.45	25.67	30.20 ±8.22			
	1.125	26.00	30.59 ±3.59			
	2.25	62.67	73.73 ±10.98			
Biofly	100	13.67	16.08 ±2.39	620.62 (512.16-690.12)	1.17 ±0.13	0.36
	200	31.33	36.86 ±1.96			
	500	36.00	42.35 ±10.59			
	1000	36.33	42.75 ±7.07			
	2000	72.00	84.71 ±4.71			
cadusafos	300	38.33	45.10 ±1.96	308.04 (133.22-495.19)	0.74±0.13	0.85
	600	52.67	61.96 ±4.37			
	1500	62.00	72.94 ±2.04			
	3000	66.00	77.65 ±1.80			
	6000	68.00	80.00 ±3.11			
Fentrothion	30	8.33	9.80 ±3.98	577.69 (339.60-2311.06)	0.87 ±0.1	0.46
	60	12.33	14.51 ±2.39			
	125	29.33	34.51 ±11.57			
	312	43.33	50.98 ±3.06			
	612	44.33	52.16 ±2.83			
	1250	44.67	52.55 ±5.10			
	control	0.00	0.00 ±0.00			

^a Lethal concentration causing 50% mortality after 24 and 48 h with 95% confidence limits.

^b Slope ± Standard Error of the concentration-mortality regression line.

^c Chi square.

The *in vivo* Inhibitory Effect of abamectin, biofly .cadusafos and fenitrothion to *M. incognita* Acetylcholinesterase (AChE) Activity.

The *in vivo* inhibitory effect of the above pesticides on AChE activity isolated from culture filtrate of *M. incognita* second stage juveniles (J2) and eggs filtrates was examined and the results are presented in (Tables 3&4). Specific activity calculated as (nmoles of acetylthiocholine iodide hydrolyzed/mg protein/min).

Table(3): The *in vivo* inhibitory effect of abamectin, biofly .cadusafos and fenitrothionTo *M. incognita* Acetylcholinesterase (AChE) Activity on second stage juveniles (J2).

Compounds	Concentrations (ppm)	nmoles ATChI hydrolyzed/mg protein/min ± SE	Inhibition (%) ±SE	I ₅₀ (ppm)
Abamectin	0.24	0.019 ±7.36 ^a	66.59 ±1.28 ^b	0.06
	0.45	0.018 ±0.002 ^{ab}	68.33 ±4.01 ^b	
	0.9	0.017 ±0.002 ^{ab}	70.39 ±3.60 ^c	
	2.25	0.015 ±0.001 ^b	73.40 ±1.77 ^{ab}	
	4.5	0.015 ±0.002 ^b	73.49 ±3.98 ^{ab}	
Biofly	100	0.0028 ±5.68 ^{abc}	43.40 ±11.14 ^{cd}	1379
	250	0.002 ±7.92 ^{abcd}	46.043 ±15.53 ^{bcde}	
	500	0.0023 ±1.81 ^{abcde}	54.36 ±3.55 ^{abcde}	
	1000	0.0018 ±6.27 ^{cd}	63.08 ±12.30 ^{abc}	
	1250	0.0017 ±7.45 ^{de}	66.24 ±14.61 ^{ab}	
	1500	0.0016 ±0.00 ^a	66.9 ±0.00 ^a	
cadusafos	2000	0.0016 ±6.51 ^a	68.28 ±12.78 ^a	1158.56
	300	0.004 ±9.87 ^c	22.48 ±19.37 ^c	
	600	0.003 ±7.12 ^{cd}	29.76 ±13.96 ^{bc}	
	1500	0.002 ±3.47 ^{cd}	63.46 ±6.81 ^b	
	3000	0.001 ±3.19 ^{cd}	65.12 ±6.26 ^b	
Fentrothion	6000	0.0007 ±4.04 ^d	86.26 ±7.92 ^a	1238.66
	30	0.0033 ±3.23 ^a	35.15 ±6.34 ^d	
	60	0.0031 ±6.01 ^{ab}	38.056 ±11.79 ^{de}	
	125	0.0030 ±7.53 ^{ab}	40.75 ±14.76 ^{de}	
	321.5	0.0027 ±5.21 ^{abcd}	46.47 ±10.22 ^{bcde}	
	625	0.0027 ±2.37 ^{abcd}	46.11 ±4.66 ^{bcde}	
	1250	0.0025 ±5.20 ^{abcde}	51.17 ±10.21 ^{abcde}	
Control		0.018 ±0.003 ^{ab}	0.00 ±0.00 ^d	

Data are averages ± SE of three replicates. Values within a column bearing the same superscript letters are not significantly different(P B 0.05) according to Student–Newman–Keuls (SNK) test.

percentage of inhibition were calculated .It was observed that pesticides induced decrease in AChE activity compared with the control. Cadusafos was tested at concentration ranged between 300, to 6000 mg/ L. All concentration had inhibitory effect on AChE activity and the high inhibitory effect was found at 6000 mg/ L 94.81 inhibition. Where The inhibition percentages are dose dependant (Table 3). .Abamectin at concentrations ranged from 4.5 to 0,25 mg/ L decreased the activity of nematode second stage juveniles AChE at all tested concentration and the most inhibitory effect was 73.93 at 4.5 mg/ L followed by biofly that decreased the activity of larval second stage enzyme to 68.28 at 2000 mg/ L. It was observed that fenitrothion had the least inhibitory effect on AChE activity and the percentage of inhibition that was

57.19 at 1250 mg/ L. Data a (Table 4), illustrate the inhibition of AChE activity in *M. incognita* egg filtrates abamectin at 4.5 mg/ L induced 73.93 while Cadusafos at 6000 mg /L induced 64.81 inhibition followed by biofly at 2000 /mg L induced 62.73 inhibition fenitrothion induced only 44.34 inhibition at 1250 mg/ L it was the least effective on the *in vivo* effect inhibition of AChE activity in *M. incognita* egg filtrates than second stage juveniles (J2). It was observed that all the tested pesticides has an inhibitory effect on AChE activity and the inhibitory effect increased when the pesticides were applied at the juveniles more than egg filtrates .

Table (4): The *in vivo* inhibitory effect of abamectin, biofly ,cadusafos and fenitrothion To *M. incognita* Acetylcholinesterase (AChE) Activity on egg filtrate after 7 days of treatment.

Compounds	Concentrations (ppm)	nmoles ATChI hydrolyzed/mg protein/min ± SE	Inhibition (%) ±SE	I ₅₀ (ppm)
Abamectin	0.24	0.0029 ±9.04 ^D	46.81 ±17.73 ^{bc}	2.16
	0.45	0.0026 ±0.00 ^D	48.04 ±0.00 ^b c	
	0.9	0.0025 ±0.001 ^D	49.14 ±25.43 ^{bc}	
	2.25	0.0025 ±0.00 ^b	51.21 ±0.00 ^b c	
	4.5	0.0026 ±0.002 ^D	83.93 ±9.15 ^a D	
Biofly	100	0.0041 ±5.20 ^a	26.29 ±10.21 ^D	2321
	250	0.0031 ±0.00 ^a	35.73 ±0.00 ^{ab}	
	500	0.0033 ±5.43 ^a	36.03 ±10.66 ^{ab}	
	1000	0.0031 ±8.4 ^a	37.52 ±16.65 ^{ab}	
	1250	0.0032 ±0.001 ^a	39.47 ±24.67 ^{ab}	
	1500	0.0027 ±0.001 ^{ab}	46.57 ±23.12 ^{ab}	
	2000	0.001 ±4.05 ^b	59.97 ±0.04 ^a	
cadusafos	300	0.0031 ±4.47 ^b	38.51 ±8.78 ^c	3548.07
	600	0.0029 ±1.96 ^b	42.12 ±3.85 ^b c	
	1500	0.0026 ±0.002 ^b	46.25 ±39.61 ^b	
	3000	0.0024 ±5.30 ^D	48.92 ±10.40 ^b D	
	6000	0.0004 ±0.005 ^b	94.81 ±21.89 ^a	
Fentrothion	30	0.0031 ±0.001 ^a	35.93 ±20.00 ^{ab}	1580.22
	60	0.0032 ±7.64 ^a	39.85 ±14.98 ^{ab}	
	125	0.0034 ±2.60 ^a	40.89 ±5.11 ^{ab}	
	321.5	0.0023 ±5.62 ^a	42.24 ±11.02 ^{ab}	
	625	0.0035 ±3.86 ^a	43.03 ±7.19 ^{ab}	
	1250	0.0021 ±5.95 ^a	44.34 ±11.68 ^{ab}	
Control		0.027 ±0.001 ^a	0.00 ±0.00 ^D	

Data are averages ± SE of three replicates. Values within a column bearing the same superscript letters are not significantly different(P B 0.05) according to Student–Newman–Keuls (SNK) test.

The *in vivo* effect of abamectin, biofly,cadusafos and fenitrothionTo *M. incognita* ATPase activity .

Results (Tables 5, 6). indicated that abamectin was more effective than other pesticides on ATP ase activity and the inhibition increased in case of egg filtrates than second stage juveniles (J2) ,it was 90.44 with egg filtrates at 4.5 mg/ L of abamectin while it was 81.95 at the same concentration in case of juveniles.

Table (5): The *in vivo* effect of abamectin, biofly .cadusafos and fenitrothionTo *M. incognita* ATPase activity second stage juveniles (J2).

Compounds	Concentrations (ppm)	µmoles Pi/mg protein/h ± SE	Inhibition (%) ±SE	I ₅₀ (ppm)
Abamectin	0.24	3.090 ±0.74 ^d	15.02 ±20.46 ^{cd}	2.28
	0.45	2.37 ±0.45 ^{bc}	34.94 ±12.34 ^{bc}	
	0.9	1.97 ±0.34 ^{bc}	45.64 ±9.41 ^{bc}	
	2.25	1.88 ±0.44 ^{abcd}	48.20 ±12.18 ^{abc}	
	4.5	0.65 ±0.13 ^d	81.95 ±3.67 ^a	
Biofly	100	2.916 ±0.77 ^b	19.80 ±21.17 ^{cd}	1300.15
	250	2.51 ±0.75 ^{bc}	31.05 ±20.60 ^{bcd}	
	500	1.89 ±0.20 ^{bcd}	47.88 ±5.60 ^{abc}	
	1000	1.63 ±0.69 ^{bcd}	55.24 ±19.18 ^{abc}	
	1250	1.57 ±0.72 ^{bcd}	56.58 ±20.03 ^{abc}	
	1500	0.77 ±0.29 ^d	78.77 ±8.01 ^a	
Cadusafos	300	2.74 ±0.87 ^{bc}	24.59 ±24.06 ^{bcd}	2974.04
	600	2.50 ±0.74 ^{bc}	31.04 ±20.60 ^{bcd}	
	1500	1.85 ±1.41 ^{cd}	40.05 ±38.74 ^{abc}	
	3000	1.77 ±0.20 ^{bcd}	41.11 ±5.6 ^{ab}	
Fenitrothion	6000	1.68 ±0.66 ^{cd}	43.82 ±18.12 ^{ab}	2263.20
	30	2.53 ±1.64 ^{bc}	20.29 ±45.05 ^{bcd}	
	60	2.52 ±0.95 ^{bc}	22.74 ±26.16 ^{bcd}	
	125	2.24 ±0 ^{bc}	28.49 ±0.00 ^{bc}	
	321.5	2.18 ±1.14 ^{bc}	30.023 ±31.37 ^{bc}	
	625	1.66 ±0.21 ^{cd}	31.35 ±5.70 ^{bc}	
	1250	1.41 ±0.09 ^{cd}	34.18 ±2.69 ^{ab}	
	Control	7.45 ±0.32 ^a	0.00 ±0.00 ^d	

Data are averages ± SE of three replicates. Values within a column bearing the same superscript letters are not significantly different (P B 0.05) according to Student-Newman-Keuls (SNK) test.

Table (6): The *in vivo* effect of abamectin, biofly .cadusafos and fenitrothionTo *M. incognita* ATPase activity egg filtrates.

Compounds	Concentrations (ppm)	µmoles Pi/mg protein/h ± SE	Inhibition (%) ±SE	I ₅₀ (ppm)
Abamectin	0.24	2.15 ±0.15 ^{ab}	40.76 ±3.94 ^d	1.01
	0.45	1.86 ±0.40 ^{abc}	48.83 ±11.13 ^b	
	0.9	1.86 ±0.23 ^{ab}	48.84 ±6.395 ^b	
	2.25	1.18 ±0.45 ^{ab}	67.38 ±12.32 ^{ab}	
	4.5	0.35 ±0.31 ^c	90.44 ±8.42 ^a	
Biofly	100	2.15 ±0.14 ^d	40.76 ±3.94 ^d	2545
	250	1.86 ±0.40 ^{bc}	48.83 ±11.12 ^d	
	500	1.55 ±0.48 ^{bcd}	57.37 ±13.31 ^{cd}	
	1000	1.18 ±0.29 ^{cd}	67.39 ±8.07 ^{bc}	
	1250	1.00 ±0.06 ^{cd}	72.4 ±1.83 ^{bc}	
	1500	0.65 ±0.57 ^{ef}	81.95 ±15.70 ^{ab}	
cadusafos	2000	0.66 ±0.57 ^{ef}	81.98 ±15.67 ^{ab}	7615.25
	300	2.40 ±0.28 ^a	33.93 ±7.89 ^d	
	600	2.14 ±0.92 ^{ab}	41.14 ±25.41 ^b	
	1500	2.08 ±0.46 ^{ab}	42.74 ±12.66 ^b	
Fentrothion	3000	2.01 ±0.83 ^{ab}	44.79 ±22.79 ^b	1500.54
	6000	1.85 ±1.41 ^{ab}	49.05 ±38.74 ^b	
	30	2.15 ±0.14 ^d	29.76 ±3.94 ^d	
	60	1.86 ±0.23 ^{bc}	31.84 ±6.39 ^d	
	125	1.52 ±0.11 ^{bcd}	38.15 ±3.29 ^{cd}	
	321.5	1.19 ±0.44 ^{cd}	40.38 ±12.32 ^{bc}	
Fentrothion	625	1.00 ±0.06 ^{cd}	42.4 ±1.83 ^{abc}	1500.54
	1250	0.38 ±0.57 ^c	44.38 ±15.70 ^a	
	Control	1.01 ±0.194 ^{bc}	0.00 ±0.00 ^c	

Data are averages ± SE of three replicates. Values within a column bearing the same superscript letters are not significantly different (P B 0.05) according to Student-Newman-Keuls (SNK) test.

Biofly had the same inhibitory effect on ATPase activity where it was 81.98 at 2000 mg/ L with egg filtrates and 78.80 at the same concentration with juveniles. Cadusafos and fenitrothion had low inhibitory effect on ATPase activity in nematode egg filtrates and second stages juveniles comparing with abamectin and biofly.

DISCUSSION

This study illustrated the possibility of using abamectin and biofly (biological pesticides) in controlling *M. incognita* instead of using the chemical pesticides to decrease the environmental pollutants of organophosphates, carbamates and other group of pesticides where the excessive use of organophosphates in agriculture has originated serious problems in the environment (Singh & Walker, 2006). Although, these pesticides degrade quickly in water, there is always the possibility that residues and byproducts will remain, in relatively harmful levels in the organisms (Ragnarsdottir, 2000). This work indicates that abamectin, the bio-product which based on pathogenic micro-organisms often referred as microbial pesticide; it is host specific and is potential candidate with regard to integrated pest management (Arora *et al.*, 2000). It has to be effective in controlling root knot nematode as cadusafos and more toxic than fenitrothion the organophosphate pesticides in this result was in agreement with (El-Nagdi and Youssef, 2004), who found that abamectin significantly reduced the population density of *M. incognita* with increasing the measured plant growth. Also Nwadinobi *et al.* (1989) reported that Abamectin is a biocides reduced galling and delayed invasion and development of *Meloidogyne* spp. for 20 days when roots of 14 day old tomato seedlings were dipped in a low concentration of abamectin The present study also investigates the toxicity of biofly the (biological pesticides) to nematode it act as the nematicides on its toxicity although it is less toxic than the other pesticides used but it had an inhibitory effect on both tested enzymes, it is a chemical inherently toxic. Therefore, alternative environment friendly measures are needed to be developed, also biopesticides are often considered as one of the lowest impact on many beneficial organisms compared with other pesticides. They have attracted considerable attention recently for their inclusion in integrated pest management (IPM) programs, but their effects are highly variable depending on the species and studied developmental stage (Darvas and Polgar 1998; Schneideret *et al.*, 2003). AChE activity is not due exclusively to organophosphates and carbamates, but also due to other classes of environmental contaminants such as complex mixtures of pollutants, detergents, and they also involved in AChE reduction (Payne *et al.*, 1996; Bendahou *et al.*, 1999; Frasco *et al.*, 2005; Guilhermino *et al.*, 1998). There is general agreement that the toxic action of organophosphate and carbamate pesticides upon nematodes, insects and vertebrates is caused by their ability

to inhibit acetylcholinesterase (AChE) in various parts of the nervous system, and thereby, disrupt nervous transmission at these locations (Corbett, *et al.*, 1984). So this illustrates why cadusafos and fenitrothion had an inhibitory effect on AChE activity and why this enzyme is used as a biomarker for cadusafos and fenitrothion toxicity in nematodes. It was accepted that the mode of action of organophosphate (cadusafos) was reasonably a certain that these compounds acted by the inhibition of acetylcholinesterase (AChE) at cholinergic synapses in the nematode nervous system. Inhibition of AChE was most likely the explanation for the observed effect of organophosphate and carbamate nematicides on the orientation behavior of nematodes (Wright, 1981; Opperman and Chang, 1990). These chemicals perform their action by impairing nematode neuromuscular activity, thereby, reducing their movement, invasion, feeding and consequently the rate of development and reproduction (Nelmes *et al.*, 1973). Bunt (1987) suggested that these chemicals acted against the root-knot nematode by inhibiting egg hatching, their movement and host invasion by infective juveniles and checked further development of second stage juveniles that had penetrated the roots. Using AChE and ATPase as biomarkers for the biofly and abamectin is considered to be a new trail to show whether these pesticides can follow this mode of action in nematode or not while results indicated that abamectin and biofly had an inhibitory effect on both enzymes. This result was in agreement with (Turner and Schaeffer, 1989). They indicated that although abamectin is a macrocyclic lactone derived from the soil bacterium *Streptomyces avermitilis* it has been shown to have nematicidal properties and a different mode of action than the other currently available nematicides. Also Radwan (2001) reported that abamectin studies are needed in more precisely molecular level to strictly detect the mode of action of these pesticides. An explanation of this decrease of AChE activity could be referred to the new mode of action of these biopesticides, which seem to work in a similar manner of other closely related compounds (i.e. metabolites of actinomycetes) our result in agreement. This hyperactivity was different insect control agent which all of them caused either no change or a reduction in AChE activity. It seems as if it works in a reversible manner, producing an extra release of AChE which may prevent principally any message to be sent to the receptor and thus the insect becomes without neural orientation. Although the previously used abamectin was believed to be of non cholinergic role, it seems that in the present study. A hypothesis was offered to explain this decrease in AChE during the use of a closely related actinomycete derived compound Spinosad where (Salgado, 1997) demonstrated that the receptors do so by mimicking the action of ACh at its binding site. The ability of spinosad to prolong the action of AChE indicates that it and ACh can act simultaneously and therefore other studies are needed in more precisely molecular level to strictly detect the mode of action of biopesticides as abamectin and biofly which holds much promise to control insects in a novel mode of action. ATPase is a group of enzymes that play an important role in intracellular functions and that are considered to be a sensitive indicator of toxicity (Yadwad *et al.*, 1990; Ozcan Oruc *et al.*, 2002). In the present study, the statistical tests performed on the data

represent that biofly the biological pesticides and abamectin as biopesticides have modern mode of action on inhibiting such enzyme in nematode while the organophosphates pesticides cadusafos and fenitrothion had low inhibitory effect on ATP ase this related to their mode of action as an organophosphates and their action related to the inhibition acetylcholinesterase (AChE) at cholinergic synapses in the nematode nervous system. (Wright, 1981; Cpperman and Chang, 1990). Inhibition of AChE was most likely explanation for the observed effect of organosphosphate and carbamate nematicides on the orientation behavior of nematodes Finally, it could be concluded that the results from this study indicated that using of both organophosphates and biopesticides achieved high activity against the root-knot nematode, *M. incognita*. Therefore, the results imply that it should focus on using biological agents as a safety method for human and environment to management the root-knot nematode in Egypt.

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التأثير السام و البيوكيميائي للمركبات الفسفورية العضوية و المبيدات البيولوجية ضد نيماتودا تعقد جذور الطماطم.

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تم القيام بدراسة تأثير اثنين من المركبات الفسفورية العضوية هما مركب الكاموسافوس (راكبي) و هو مبيد نيماتودي ومبيد الفينثروثيون وكذلك اثنان من المركبات الحيوية هما مركب الابسامكتين و مركب البيوفلاي ضد نيماتودا تعقد جذور الطماطم تحت الظروف المعملية على العمر اليرقى الثانى وكذلك على مستخلص البيض حيث تم تقدير النسبة المئوية للموت و كذلك حساب التركيز النصفى القاتل ل 50% من النيماتودا المعاملة فى العمر اليرقى و مستخلص البيض تم تقدير نشاط كل من انزيمى (الاسيتايل كولين و انزيم الادينوسين تراهي فوسفاتيز) حيث ثبت من النتائج وجود تأثير كلا من المبيدات المختبرة على نسبة الموت فى كلا من اليرقات و مستخلص البيض. و كان مبيد الابسامكتين اكثرهم تاثيرا يلية مبيد الفينثروثيون ثم الكادوسافوس ثم البيوفلاي كما تم تقدير التأثير التثبيطى على نشاط كلا من الانزيمين فى حين ان مبيد الكادوسافوس و الفينثروثيون لم يثبت لهم تاثير واضح فى تثبيط نشاط انزيم الادينوسين تراهي فوسفاتيز و هذا ما يلقى الضوء على امكانية الفعل السام للمبيدات الحيوية فى تثبيط نشاط كلا من الانزيمين موضع الاختبار على نيماتودا تعقد جذور الطماطم.

قام بتحكيم البحث

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