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Potential and Bio-Chemical Effects Of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) An Entomopathogenic Nematode Against *Bactrocera zonata* and *Ceratitis capitata* (Diptera: Tephritidae)

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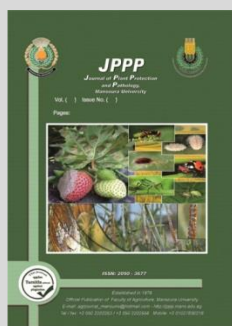

ABSTRACT

The potential and biochemical effects of the *Steinernema carpocapsae*, an entomopathogenic nematode, against two species of fruit flies in two types of soil texture (sand and sandy-loam) were examined under laboratory conditions. Data obtained revealed that the type of soil altered the potential effect of *S. carpocapsae* on *Bactrocera zonata* and *Ceratitis capitata*. The mortality percentages for *B. zonata* ranged between 30.00 and 94.43% in sand and between 33.33 and 96.67% in sandy-loam, whereas, for *C. capitata*, it ranged from 54.43 to 90.00% in sand and from 55.57 to 97.67% in sandy-loam. Generally, the mortality increased with the increase of nematode concentrations. According to LC_{50} values, *S. carpocapsae* was more efficient against *B. zonata* in sand, but the opposite was obtained in sandy-loam against *C. capitata*. In addition, *S. carpocapsae* caused variable biochemical changes in activity of certain enzymes of the two fruit fly species, since it exhibited only a significant increase in the acid phosphatase in *B. zonata*, whereas the other tested enzymes significantly declined. The activity of amylase and acid phosphatase enzymes significantly induced in *C. capitata*, whereas the activity of trehalase, acetylcholine esterase and alkaline phosphatase significantly decreased. Non-significant effects were obtained in activity of invertase and chitinase within the infected individuals of *C. capitata*. Thus, the entomopathogenic nematodes could be used as a sustainable bio-control agent in Integrated Pest Management (IPM) programs for soil-inhabitant insects, such as fruit flies.

Keywords: Biological control, enzymes, Integrated Pest Management, LC_{50}, soil texture.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tiphritidae) are serious pests of several fruit species. The most tephritid species are polyphagous pests infesting more than 260 fruit and vegetable species (White and Elson-Harris, 1992). These deleterious pests are distributed all over the world and causing direct and indirect effects in both quality and quantity of fruits. The most preferred hosts for *C. capitata* are apricot, peach and citrus fruits, while in case of *B. zonata*, are guava, peach and mangoes.

The use of chemical insecticides is widespread among large number of insect species and causing many problems as insect-resistance strains; prevent exporting and negative impact on humans, animals and natural enemies. Therefore, the modern world approaches depend on using the biocontrol agents. Among them, the entomopathogenic nematodes, from family Steinernematidae, are consider as effective and safe alternative agents against a wide range of agricultural pests in fruits and vegetables plants (Gaugler and Kaya, 1990; Abd-El Wahed and Elhadidy, 2018). The infective juveniles (IJs) of entomopathogenic nematodes penetrate their host bodies through natural openings and rarely through host cuticle (Shapiro and Lewis 1999) and those belonging to Steinernematidae kill their hosts through the association with the mutualistic bacteria, i.e. *Xenorhabdus* spp. These mutualistic bacteria release toxins that finally kill the host within 1-3 days. The nematodes then reproduce within the cadavers.

The aim of this work therefore is to estimate the potential activity of *S. carpocapsae* against the late instar larvae of both *B. zonata* and *C. capitata* in two types of soil texture (sand and sandy-loam) under laboratory conditions. Further, to determine the biochemical changes in host enzymes after infection with *S. carpocapsae*.

MATERIALS AND METHODS

Insect cultures

Larvae of the greater wax moth, *Galleria mellonella* L., were used as suitable host for reproducing the entomopathogenic nematodes. Larvae used in the present study were obtained from bee hives heavily infested with *G. mellonella* which was collected from the apiary of Plant Protection Research Institute, Dokki, Giza, Egypt. These larvae were reared according to method of Poinar (1975). The nematode, *Steinernema carpocapsae*, was reared and multiplied on *G. mellonella* larvae based on method of Kaya and Stock (1997). Nematoda *Steinrnema carpocapsae* (EGAZ 9) which used in these experiments was isolated from Belbeis, Sharkia, Egypt and reproducted in Pest

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Physiology Department, Plant Protection Research Institute according to Meligy *et al* (2017).

The nematode individuals were multiplied in the seventh instar larvae of *G. mellonella* at 26±1 °C and > 80 RH% based on method of Woodring and Kaya (1988). After 12 days, the IJs were collected in cell culture bottles (40 mL) every other days using Pasteur pipettes and then stored at 16±1 °C and > 80 RH % for one week before bioassay tests.

Larvae of *B. zonata* and *C. capitata* which used in these experiments were obtained from a laboratory strain that maintained on an artificial dietconsisting of sugar (82.5g), yeast (82.5g), sodium benzoate (4g), citric acid (4g), wheat bran (330g) and water (500ml). Adults were introduced in a wooden cage (50 ×50 × 50 cm) covered with screen mesh and provided with distilled water and sugar mixed with protein hydrolozate at 10:1. The insects were reared at 27 ± 5 ºC, 65±5% RH% and 12: 12 h photoperiod. Fruitfly populations were observed daily and only the third instar larvae (or full grown larvae) were used in experiments.

Bioassay procedure

Virulence of the nematode species against *B. zonata* and *C. capitata* larvae were tested under seven juvenile concentrations (200, 400, 600, 1000, 1500, 2000 and 2500 IJs/ ml). Plastic cups (250 ml) filled with about 20 g of either sterile sandy or sandy-loam soils at 10% w/v moisture were used. In each cup, 30 larvae of each species of fruit flies were added. Each juvenile concentration replicated three times. The check treatment consisted of soil mixed with water only at the same time in parallel to treatment. The cups were closed with pieces of muslin and rubber bands. They were placed in an incubator at 28±2 °C, 70±10% RH% and 12 h photoperiod for ten days till emergence of all adults according to Fetoh and El-Gendi (2006).

Mortality estimation

Insect mortality was recorded after adult emergence, and was corrected against natural mortality that was obtained from check treatment using Abbott's formula (Abbott, 1925). All individuals failed to emerge successfully considered dead. Dead insects were kept in a Petri-dish having moist filter paper, under sterilized conditions, at 25°C. To confirm the existence of IJs, the dead larvae and pupae were dissected under the microscope (Poinar, 1976).

Biochemical analysis

About 200 full grown larvae of each fruit fly species were treated in the sand soil with LC_{50} value of *S. carpocapsae*. After 24 hours of treatment, all treated individuals (larvae and pupae) as well as untreated pupae of each species were collected, isolated in plastic tubes and kept under freezing point till the biochemical analysis.

Preparation of homogenate samples of insects for enzyme activities

Insects (5 g) were homogenized in distilled water and collected in cold tubes (on ice), then centrifuged at 6000 rpm for 10 min at 5 °C using Beckman GS-6R Centrifuge. Afterwards, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at –20 °C to analyze enzyme activities. Each enzyme activity was replicated three times.

Determination of enzyme activities

Activity of Acid phosphatase (AcP) and alkaline phosphatase (AlkP) activities was determined based on the method characterized by Powell and Smith (1954). In this method, the phenol, released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the brown color is appeared.

Carbohydrate hydrolyzing enzyme activities were determined, depending on digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively, based on method of Ishaaya and Swiriski (1976). The free aldehyde groups of glucose formed after trehalose, starch and/or sucrose digestion were determined using 3,5-dinitrosalicylic acid reagent, using standard curve of glucose.

Chitinase activity was assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

Aacetylcholine esterase (AchE) activity was estimated based on method of Simpson *et al.* (1964), using acetylcholine bromide (AchBr) as substrate. The reaction mixture contained 0.5 ml 0.067 M phosphate buffer (pH7), 200 µl enzyme solution, and 0.5 ml AchBr (3 mM). The test tubes were incubated at 37ºC for exactly 30 minutes. One ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH was added to the test tubes. Then 0.5 ml of Hcl (1 part of conc. HCl and 2 parts of ΔH_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min. Then, 0.5 ml of ferric chloride solution (0.9 M $FeCl_3$ in 0.1M HCl) was added and mixed well. The decrease in AchBr by AchE hydrolysis was read at 515 nm.

Statistical analysis

The corrected mortality was subjected to Ldp-line" log-Probit analysis software that developed by Dr. Ehab Bakr (Plant Protection Research Institute http://www.Ehabsoft.com) and based on Finney (1971) to obtain LC_{50}, LC_{90} and slope values. In addition, data were analyzed using one-way ANOVA and means were separated using Tukey's honestly significant difference at $P < 0.05$. all tests were conducted using CoStat R software (Costat, 2007).

RESULTS AND DISCUSSION

Mortality estimation

The mean number of dead individuals and mortality percentages in *B. zonata* and *C. capitata* in two types of soil texture (sand and sandy-loam) that treated with *S. carpocapsae* at different concentrations of infective juveniles under laboratory conditions are presented in Table (1). The mean number of dead individuals of *B. zonata* gradually increased with the increase in infective juvenile concentrations in both sandy and sandy-loam soils. The same trend was occurred in case of *C. capitata*, except with sandy soil at 1500 infective juveniles/ ml that recorded 76.67% mortality. Statistical analysis proved that the highest concentrations in the two tested soil types for the two fruit fly species gave the highest mortalities. In general, the increase of infective juveniles led to an increase in mortality percentages. The mortality percentages in *B. zonata* populations ranged between 30.00 and 94.43% in sand sill and between 33.33 and 96.67% in sandy-loam soil, whereas,

it ranged between 54.43 and 90.00% in sand soil and between 55.57 and 97.67% in *C. capitata* populations that inhibiting sandy-loam soil.

The values of LC_{50} and LC_{90} estimated for *B. zonata* in sandy and sandy-loam soils were 37.90, 2043.90 in sandy soil and 406.70, 1979.10 IJs in sandy-loam soil, respectively. But, that recorded for *C. capitata* were 192.30, 3494.80 in sandy soil and 161.50, 2129.30 IJs in sandy-loam soil, respectively. The slope of LC-p line for the two species of fruit flies in case of sandy-loam slightly steeper than that occurred in case of sand (Table 2).

Table 1. Mean number of dead individuals and mortality percentages of two species of fruit flies in two types of soil texture treated with different concentrations of *S. carpocapsae* after emergence under laboratory conditions

Concen.	*B. zonata*				*C. capitata*			
IJ/ml	Sand	% Mortality	Loamy sand	% Mortality	Sand	% Mortality	Loamy sand	% Mortality
200	9.00 c	30.00	10.00 e	33.33	16.33 d	54.43	16.67 b	55.57
400	19.33 b	64.43	12.00 de	40.00	17.67 cd	58.90	17.67 b	58.90
600	21.00 b	70.00	14.00 d	46.67	21.00 bc	70.00	20.67 b	68.90
1000	21.67 b	72.23	25.00 c	83.33	24.00 ab	80.00	27.00 a	90.00
1500	23.30 ab	77.67	26.33 bc	87.77	23.00 ab	76.67	27.00 a	90.00
2000	24.67 ab	82.23	28.00 ab	93.33	25.00 ab	83.33	28.30 a	94.33
2500	28.33 a	94.43	29.00 a	96.67	27.00 a	90.00	29.30 a	97.67
Control	1.33 d	4.43	1.67 f	5.57	1.67 e	5.57	1.33 c	4.43
Average	18.58		18.25		19.46		20.99	
"F" test	16.55		157.95		33.74		45.61	
L.S.D	6.59		2.42		4.15		4.15	

Table 2. Efficiency of *S. carpocapsae* against two species of fruit flies in two types of soil texture under laboratory conditions

Species	Soil type	LC_{50}	LC_{90}	Slope
B. zonata	Sand	37.90	2043.90	1.7318±0.1518
	Sandy-loam	406.70	1979.10	1.8651±0.1560
C. capitata	Sand	192.30	3494.80	1.0176±0.1299
	Sandy-loam	161.50	2129.30	1.1441±0.1586

In agreement with the previous reports, larvae of the fruit flies are highly susceptible to entomopathogenic nematodes (Gazit *et al*., 2000). The nematode, *S. carpocapsae* achieved higher cumulative mortality than control treatment with particularly preference to the loamy-sand soil. The obtained results agreed with those found by GiuliaTorrini *et al*. (2017) who reported that *B. olea* pupae were susceptible to *S. carpocapsae* with 62.5% mortality. The most noteworthy result is that *S. carpocapsae* is able to infect 21.90% of the emerged adults. Also, Abd-El Wahed and Elhadidy (2018) reported that *S. carpocapsae* caused 100% mortality in the fifth nymphal instar of *Locusta migratoria* three days post treatment with three concentrations (10, 20 and 40 IJs/g in sand). The nematode, *S. carpocapsae* caused high mortality rate in locust populations, which significantly increased with the increase in its concentrations. This effect might be because of enzyme system confusion. The strong potential of *S. carpocapsae* against *C. capitata* and *B. zonata* larvae came in the same line with those of Yee and Lacey (2003) who found that *S. carpocapsae* and *S. feltiae* exhibited high efficiency against *Rhagolites indifferens* larvae. The obtained results are also consistent with other reports concerning with the efficiency of entomopathogenic nematodes against *Anastrepha ludens* (Lezama-Gutierrez *et al*., 2006) and *C. capitata* (Karagoz *et al*., 2009). In contrast, Sirjani *et al*. (2009) found that *Bactrocera oleae* larvae were more susceptible to infection with *S. feltiae* than to *S. carpocapsae, S. glaseri, S. riobrave, Heterorhabditis bacteriophora* and *H. marelatus*. Variations in potential of entomopathogenic nematode species may be because the foraging strategy of IJs and the behaviour of third instar larvae of *C. capitata* and *B. zonata*. This is in the same direction with Gaugler and Kaya (1990) who reported that entomopathogenic nematodes of genus *Steinernema* consider as an effective insect-biocontrol agent. Further, it agrees with other reports concerning the virulence of entomopathogenic nematodes on *B. zonata* (Attala and Eweis, 2002), the cucurbit fly, *Dacus ciliatus* (Fetoh and El-Gendi, 2006) and also against *B. zonata* and *C. capitata* (Soliman, 2007 a & b).

Biochemical changes in enzyme activity under infection with *S. carpocapsae*

As shown in Table (3), *S. carpocapsae* differently affected the tested enzymatic systems in both fruit fly species.

Digestive enzymes

Significant decrease and increase in activity of the amylase enzyme by *S. carpocapsae* recording activity ratio of 0.675 and 1.146 of the untreated insects of both *B. zonata* and *C. capitata*, respectively. Further, *S. carpocapsae* significantly decreased the activity of the other two digestive enzymes, invertase and trehalase, in *B. zonata* by 0.768 and 0.483 of the untreated individuals. But in case of *C. capitata*, non-significant and significant reductions in activity of invertase and trehalase by 0.970 and 0.602 of the untreated individuals, respectively were obtained. The exploitation of hemolymph glucose by nematodes could support the production of more glucose through increasing the trehalase activity of the host. Comparing amylase activity in the untreated individuals of *B. zonata* the enzyme was more twice that recorded in *C. capitata* (Fig. 1). The entomopathogenic nematodes of *S. carpocapsae* achieved more decrease and slight increase in activity of amylase in *B. zonata* and *C. capitata*, respectively. But, in case of invertase (Fig. 2), the opposite direction was observed in both untreated and infected individuals, where the activity of invertase in *C. capitata* was higher than that of *B. zonata* in the untreated individuals. *Steinernema carpocapsae*-infected individuals showed higher decrease and lower increase in invertase activity in *B. zonata* and *C. capitata*, respectively. On the other hand, the activity of trehalase in the untreated individuals of *B. zonata* was higher than that recorded in *C. capitata*. The larval infection with *S.*

carpocapsae comparatively suppressed the activity of trehalase in the two species of fruit flies at nearly level (Fig. 3). This activity, therefore, could increase host fat body glycogenolysis and/or decline glycogenesis to keep adequate concentrations of trehalose in the hemolymph (Condon and Gordon, 1977). These findings may interpret the increase in the level of glucose in larvae of *C. capitata* and *B. zonata* after infection by *S. carpocapsae*.

Table 3. Biochemical effects of *S. carpocapsae* on certain enzyme activities in two species of fruit flies inhibiting sandy soil

Enzyme		*B. zonata*					*C. capitata*				
		Infected	Control	Activity ratio	F. test	LSD	Infected	Control	Activity ratio	F. test	LSD
Amylase	µg glucose/min/g.b.wt.	893.73 b	1323.48 a	0.675	113.0	112.22	781.36 a	681.65 b	1.146	7.83	98.92
Invertase		409.00 b	532.88 a	0.768	21.48	68.99	721.40 a	743.73 a	0.970	0.12	-
Trehalase		943.09 b	1951.74 a	0.483	124.40	251.08	317.67 b	527.92 a	0.602	36.40	96.71
Acetylcho-line Esterase	µg AchBr/min/g.b.wt.	3531.23 b	7354.31 a	0.480	106.50	1028.57	2859.10 b	3091.10 a	0.925	49.80	91.26
Chitinase	gµ NAGA/min/g.b.wt.	25.19 b	52.81 a	0.477	136.90	6.55	21.06 a	20.20 a	1.043	2.89	-
Acid Phosphatase	µg phenol/min/g.b.wt	73.53 a	47.70 b	1.542	17.36	17.36	116.87 a	51.63 b	2.264	872.5	6.10
Alkaline Phosphatase		4.39 b	15.76 a	0.276	454.70	1.48	1.29 b	4.33 a	0.289	126.5	0.75

Activity ratio = infected/ control

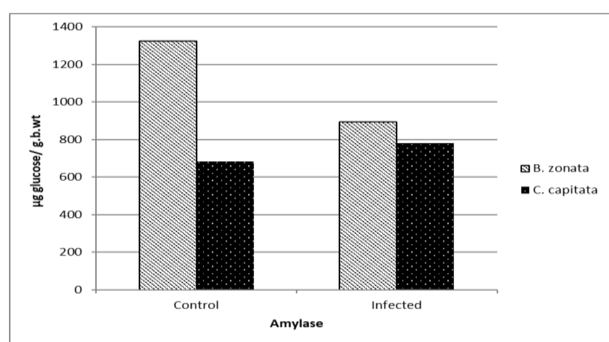

Figure 1. Biochemical activity of Amylase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitate*

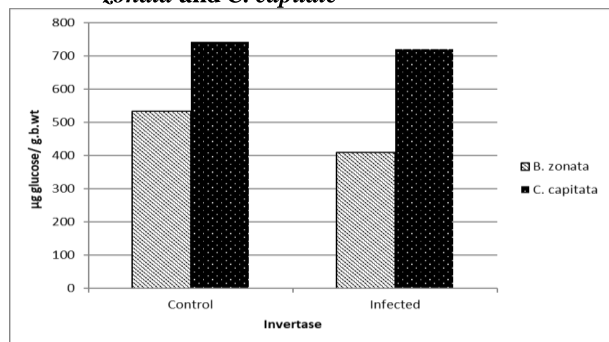

Figure 2. Biochemical activity of Invertase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

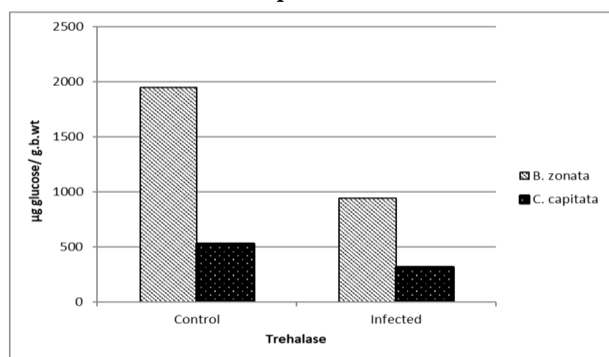

Figure 3. Biochemical activity of Trehalase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

Acetylcholine esterase

The activity of acetylcholine esterase differently and significantly decreased in the infected individuals of both *B. zonata* and *C. capitata* showing activity ratio of 0.480 and 0.925 of the untreated individuals of the first and second species of fruit flies, respectively. As shown in Figure (4), the activity of acetylcholine esterase was comparatively more twice in *B. zonata* untreated-individuals than that investigated in the corresponding individuals of *C. capitata*. The infection of tephritid larvae with *S. carpocapsae* highly and lowly decreased the activity of acetylcholine esterase in individuals of *B. zonata* and *C. capitata*, respectively. In closely relation with insect resistance to organic pesticides, acetylcholine esterase (AChE) degrades, through its hydrolytic activity, the neurotransmitter acetylcholine, producing choline and an acetate group (Soreq and Seidman, 2001). Acetylcholine esterase (AChE) is a main enzyme of the cholinergic system due to its regulation to the level of acetylcholine (ACh) and termination nerve impulses by catalyzing the hydrolysis of acetylcholine. Its inhibition causes immoderate accumulation of acetylcholine in the synapses which in turn leaves the acetylcholine receptors permanently open, leading to hyperactivity and consequently paralysis and death (Fournier, 2005). *S. carpocapsae* infection induced a significant increase in AChE activity of *C. capitata* and *B. zonata* larvae, respectively. The significant production was observed 24 h, which correspond to early infection and development of EPNs and the mass production of the symbiotic bacteria, respectively. In addition, the decline in AChE activity may be correlated with secretions that produced by the nematode and bacterium complex, or with the virulence of the nematode against host insects, although the mechanism of destruction is unknown. This corresponds with the report in which the changes in AChE activity was correlated with resistance in *S. exigua* caterpillars in response to consumption of particular allelochemicals (Zhang *et al.,* 2011). The decreased production of AChE by *C. capitata* and *B. zonata* larvae during the infection represents a central role in the degradation toxic substances and also may contribute to cell death in host.

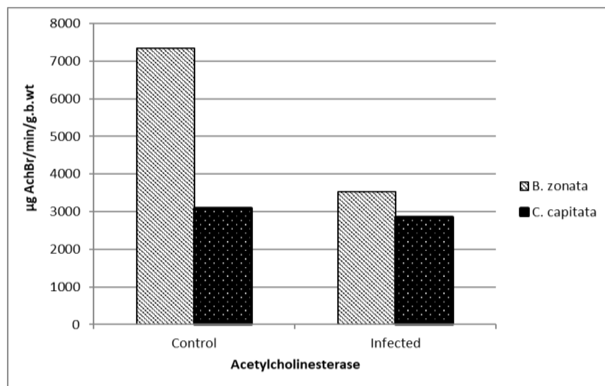

Figure 4. Biochemical activity of Acetylcholine esterasein untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

Chitinase

Stienernema carpocapsae significantly decreased the activity of enzyme by 0.477 of the untreated insects of *B. zonata*, whereas in case of *C. capitata* the reverse took place where the activity of chitinase slightly and insignificantly increased in the treated individuals by 1.043 of control. Data in Figure (5) indicate that the activity of chitinase in normal or uninfected individuals if *B. zonata* slightly more twice than that existed in individuals of *C. capitata*. Larval infection of the two species of fruit flies with *S. carpocapsae* caused sharply decrease in activity of chitinase in case of the peach fruit fly, whereas slightly increase in case of the Mediterranean fruit fly. There are several chitinase enzymes exist in many living organisms from bacteria to human, with functions in cell wall modification, carbon source degradation and defense against pathogens (Fuhrman, 1995).

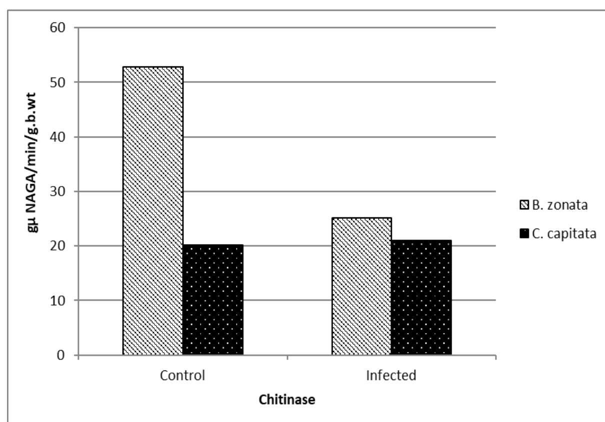

Figure 5. Biochemical activity of Chitinase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

Chitinases support a variety of functions in nature including nutrition, pathogenesis, and morphogenesis (Gooday, 1994). Chen *et al*. (1996) reported that *X. bovienii* (one strain), *X. nematophilus* (three strains), and *Photorhabdus luminescens* (one strain) exhibited both exo- and endo-chitinase on PAGE gel. Variation in exo-and endo-chitinase activities among varying species and strains was observed with the strongest activity in *X. nematophilus* and the weakest one in *P. luminescens*. The increase in chitinase activity after infection with *S. carpocapsae* may be attributed to release the chitinase from the symbiotic bacteria *X. nematophilus* to hydrolyze chitinous cell wall in insect or to digest the chitinous nutrient (Forst *et al*., 1997).

Phosphatases

The nematodes significantly increased the activity of acid phosphatase in both *B. zonata* and *C. capitata*. The increment in case of infected individuals was 1.542 and 2.264 of the untreated ones of the first and second tested species of fruit flies, respectively. On the other hand, the activity of alkaline phosphatase in the infected individuals of both thepeach and Mediterranean fruit flies was significantly decreased recording approximately the same values of activity ratio of 0.279 and 0.298 of control, respectively.Activity of acid phosphatase (Fig., 6) in the untreated individuals of *B. zonata* was slightly lower than that recorded for *C. capitata*. The infected individuals of both *B. zonata* and *C. capitata* differently induced the activity of acid phosphatase showing high level of increment in case of the Mediterranean fruit fly. But, in case of activity of alkaline phosphatase (Fig., 7) the reverse took place, where the activity of enzyme in untreated individuals of *B. zonata* was more three times than that of *C. capitata*. The 3rd larval infection of the two species of fruit flies with *S. carpocapsae* reduced the enzyme activity approximately at the same level.The first step in achieving a control strategy based on bioinsecticides involves characterizing gut enzyme from host after treatment.The non-feeding, third-instar IJs is the only instar that survives outside of the host. Once the IJ finds the suitable host, it invades and penetrates the host's heamocel from the natural openings and then releases the bacteria inside the host that kill the host during 48 hours. These bacteria produce antibodies that inhibit other microorganisms from colonizing the cadavers of host. In addition, it serving as a food source for nematodes, the bacteria digest the host tissues, thereby providing suitable nutrients for growth and development of nematodes (Ehlers, 2001).This fact is consistent with that given by Serebrov *et al.* (2006) who found that acid phosphatase increased in *G. mellonella* larvae after treatment with *Metarhizium anisopliae.*

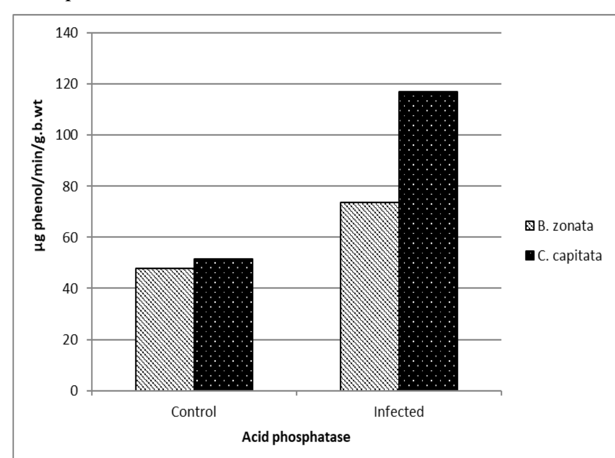

Figure 6. Biochemical activity of Acid phosphatase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

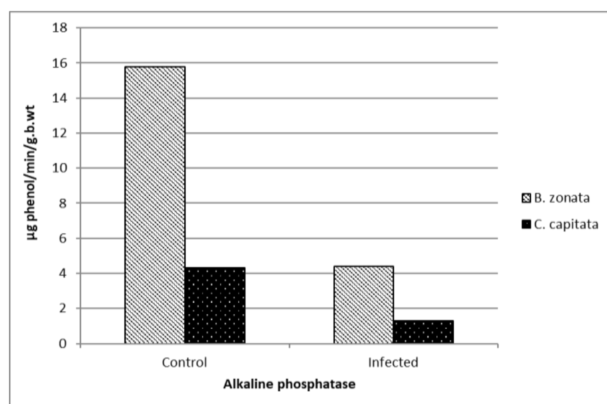

Figure 7. Biochemical activity of Alkaline phosphatase in untreated and *S. carpocapsae*-infected individuals of *B. zonata* and *C. capitata*

Xia *et al*. (2000) suggested that acid phosphates, as a lysosomal enzyme, may have a role in autophagy and defense as well as cell turn over. Therefore, it appears that the enhancement of acid phosphates activity in *C. capitata* and *B. zonata* larvae infected with *S. carpocapase* was an attempt by the insects to defend against the invasion of the pathogens. These authors also added that phogocyteosis is known to induce the production of lysosomal enzymes in which acid phosphates consider a main component. Acid phosphate has been detected in insect haemocytes and found to be released into the plasma (Lai-Fook, 1973; Rowley and Rakcliffe, 1979). Chen (1983) noted hyper synthesis of acid phosphatase by haemocytes of the mollusk, *Biomophalaria globrata* during phagocytosis. The enzyme was subsequently released into the plasma where its role is unknown although a direct role of acid phosphates in cell killing cannot be ruled out. On the other hand, alkaline phosphates of secreting products across cell boundaries. In the present study, acid phosphates activity was higher than alkaline phosphates activity in non-infected nematode larvae. The predominance of acid phosphates activity could be correlated to an active range (Pant and Lacy, 1969). In agreement with the obtained results, Soliman (2002) found alteration of acid and alkaline phosphates activity in last instar larvae of *C. capitata* infected with *S. riobrave* and *H. bacteriophora.* Our data are in agreement with several studies on host species infected with nematodes (Żółtowska *et al*., 2006; Wu *et al*., 2013 and Ahmed *et al*., 2014). The decline in activity of acid phosphatase could be due to the inability of the cell to undergo enzymatically controlled reactions under nematode infection (Soliman, 2002). Further, the reduction in alkaline phosphatase could be attributed either to the reduction in enzyme synthesis or binding of toxicants at the active site of enzyme (Shakoori *et al*., 1994). In general, these enzymes are involved in dephosphorylation and energy transfer. In conclusion, *S. carpocapsae* can affect the late third instar of *C. capitata* and *B. zonata* by targeting various biochemical molecules in different metabolic pathways. The interaction between this nematode species and its host larvae supposed to be primarily nutritional. The nematode absorbs small molecular weight components from host larvae, prohibiting them from nutrients necessary for development. Thus, growth of the nematode proceeds, whereas the nutritional status of the host larvae deteriorates; i.e., the host becomes in a state of physiological starvation.

Finally, because *S. carpocapsae* exhibited high pathogenicity against larvae and pupae of C. *capitata* and *B. zonata* and high stability in soil, it could be nominated as an efficient biocontrol agent in Integrated Pest Management (IPM) programs.

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تأثيرات الفاعلية والبيوكيميائية للنيماتودا الممرضة للحشرات على ذبابة فاكهة البحر الابيض المتوسط وذبابة ثمار الخوخ

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أجريت دراسات معملية مقارنة لتحديد الفعالية والتأثيرات البيوكيميائية للنيماتودا الممرضة للحشرات (من نوع *S. carpocapsae*) على ذبابة فاكهة البحر الابيض المتوسط وذبابة ثمار الخوخ فى كل من التربة الرملية والطمية. وقد أثبتت النتائج اختلافات فى تأثير النيماتودا على نوعى ذباب الثمار. فقد تراوحت نسبة الموت فى ذبابة ثمار الخوخ بين 30.0- 94.43 فى الرمل و 33.33- 96.67 فى الطمى، بينما تراوحت نسب الموت فى حالة ذبابة فاكهة البحر المتوسط بين 54.43- 90.0 (فى الرمل) و 55.57- 97.67 (فى الطمى). وعموما زادت نسبة الموت مع زيادة تركيزات النيماتودا، وطبقا لقيم التركيزات النصفية المميتة كانت النيماتودا اكثر فعالية على ذبابة ثمار الخوخ فى الرمل وكان العس صحيحا مع ذبابة فاكهة البحر المتوسط فى الطمى. ايضا تسببت النيماتودا فى تغيرات متفاوتة فى نشاط بعض الانزيمات فى نوعى ذباب الثمار المختبرة حيث أدت الى زيادة معنوية فى نشاط انزيم الفوسفاتيز الحامضى فى ذبابة الخوخ فقط فى حين تسببت فى انخفاض معنوى لباقى الانزيمات. اما فى حالة ذبابة فاكهة البحر المتوسط فقد أحدثت النيماتودا زيادة معنوية لنشاط انزيمى اميليز والفوسفاتيز الحامضى، بينما أظهرت انخفاضا معنويا فى نشاط انزيمات تريهاليز، اسيتيل كولين استيريز الفوسفاتيز القاعدى. هذا وقد اظهرت النتائج انخفاض وارتفاع غير معنوى فى نشاط انزيمى انفرتيز وكيتينيز فى افراد ذبابة فاكهة البحر المتوسط المصابة بالنيماتودا على التوالى. وعلى هذا يمكن استخدام النيماتودا الممرضة للحشرات كعوامل مكافحة حيوية مستدامة ضمن برامج المكافحة المتكاملة لمكافحة حشرات التربة مثل ذباب ثمار الفاكهة.