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Evaluation the insecticidal activity of *Purpureocillium lilacinum and Cuminum cyminum* and study their infection impact on some biochemical content in the haemolymph of the cotton leaf worm *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctudiae)

Yasmein A El-Sayed[1], Heba Yousef [1, 2]
[1] Plant Protection Research Institute Agriculture Research Center Dokki, Giza, Egypt
[2] Department of Chemistry, Faculty of sciences and Arts, University of Jeddah, Khulais, Jeddah, Saudi Arabia
[*] Corresponding Author: Yasmein A El-Sayed

Abstract
The current study was designed to examine the toxic effect *of Purpureocillium lilacinum* (*Paecilomyces lilacinus*) and *Cuminum cyminum* on the cotton leaf worm *Spodoptera littoralis* (Boisd.). The toxicity experiment was conducted by applying different concentrations of both tested fungal isolate and essential oil on the 2nd and 4th instar larvae under laboratory condition. The *C. cyminum* showed 96% larval mortality after ten days for the 2nd instar larvae when applied at concentrations 0.25, 5 and 10% and the same larval mortality was achieved by applying 5 and 10% concentrations against the 4th instar larvae. While *P. lilacinum* showed lower mortality rate which was 66 and 62% after ten days of treating the 2nd and the 4th instar larvae, respectively with the highest fungal concentration used (10^{10} Spore/ml). The concentration responsible for killing 50% of the tested larvae (LC_{50}) were calculated, it was 0.001 and 0.21% for *C. cyminum* and 3.7 x 10^6 and 5.7 x 10^7 Spore/ml for *P. lilacinum* after 10 days post treatment of the 2nd and the 4th instar larvae respectively. The total lipids, proteins and carbohydrates contents were also evaluated in the haemolymph of *S. littoralis* 4th instar larvae treated with the two tested biocides. The result data clarified that both of them induced a significant reduction of total protein and the two key enzymes responsible for protein synthesis, GOT (Glutamic oxaloacetic transaminase) and GPT (glutamine pyruvic transaminase) and a significant increase of total carbohydrates compared to control. The total lipid was increased by fungal isolate and decreased by essential oil treatment. Our overall data revealed that there is correlation between insect infection and changes of the insect haemolymph constituents which causes physiological and biochemical disturbance of whole insect's body affect their growth and development and finally lead to insect death.

Keywords: *Purpureocillium lilacinum*, *Cuminum cyminum*, *Spodoptera littoralis*, toxicity, total lipid, total protein, total carbohydrates, GOT and GPT

Introduction
Spodoptera littoralis (Boisd) (Lepidoptera: Noctuidae) is the most common devastative agriculture pest all over the world. It can infest wide range of agriculture crops of economic importance during the year and causes severe damage and economic losses of them (Mohamed, *et al*., 2019) [38], hence there was great request to abolish this wasteful pest. Presently, the most effective control of many subversive pests has been achieved by application of chemical insecticides, but it's difficult to control *S. littorlais* because it developed resistance against them (Ghulam, *et al*., 2017) [24]. Beside the pest resistance, continuous application of these chemical pesticides lead to several hazard like environmental pollution, harmful effect on the beneficial insects and toxic influence to humans, plants and animals. To avert the chemical pesticides riskiness there is a great attention to encourage the use of safer insecticides like plant extracts and bio-control agents as alternatives. For combating the insect pests several biological control agents have been used like predators and entomopathogens (virus, bacteria and fungi) (El-Gaied, *et al*., 2020) [17]. Among these biological insecticides are entomopathogenic fungi which considered the most effective bio-control agent against numerous insect pests. Till now above seven hundred species of fungi are recognized to infect insects (Wraight, *et al*., 2007) [64]. Entomopathogenic fungi are unique in their mechanism of action as they infect their host via the integument (Sevim, *et al*., 2015) [53] and do not have to be ingested like bacteria and virus, therefor they are able to infect stages of non-feeding such as eggs and pupae. They can penetrate the pest body enzymatically by utilization the cuticle hydrolyzing enzymes like lipase, protease and chitenase. The mechanism of action of the entomopathogenic fungi begin when the spore bind to the insect integument then germinate and enter the exoskeleton by forming appressorium. The hyphae develop and reproduce in the pest body and haemolymph and finally lead to death of pest. Secretion of the toxins is a distinguishing feature of some insect pathogenic fungi like Leucinostatins toxin secret by *P. lilacinum* (*Purpureocillium lilacinum* is a new name of *Paecilomyces lilacinus* as it has been changed previously by its discoverer Robert A. Samson (Luangsa-Ard J, *et al.*, 2011)) [33], once penetration the insect host by the fungal propagules these toxic substances can cause insect death even before spread and formation of the spores (Charnley, 2003) [13]. Using the insect pathogenic fungi for controlling the insect pest have numerous advantages summarized in: 1- they are significant natural enemies of arthropod (Chandler, *et al*., 2000) [12], able to infect them via the cuticle. 2- Easily and cheaply cultivation of them and

production of their infective spores (Roberts & Hajek, 1992) [51]. 3- They can be exist under various environmental condition (Ferron, 1978) [22].

Recently, plant essential oil received great interest as natural insecticides and considered among the most promising alternatives to chemical insecticides. They extracted from different part of plants and their insecticidal potential were investigated by many authors (Elumalai, *et al*., 2010) [18]. *C. cyminum* is essential oil belonging to Apiaceae family which considered the most known and used families for their richness of essential oils (N.E. BEN-KHALIFA, 2018) [42]. The main components of *C.cyminum* are monoterpenes which have high toxic effect against insect pest (Abdelgaleil, *et al*., 2009) [2]. Generally plant extracted essential oils have repellent, attract and antifeeding activities against insect pests and can also disturb the insect growth and development and make inhibition to eggs oviposition (Tripathi, *et al*., 2003) [60].

Insect haemolymph is a fluid resembles the blood of vertebrates circulate in the arthropod body, consists of mixture of carbohydrates, proteins, lipids, salts, water, hormones, etc. the insect haemolymph constituents have various function responsible for physiological activities of the insects body. The changes in physical and biochemical parameters of haemolymph reflect physiological and biochemical disturbance of the insect tissues, and these are predicting the pathogenic effect of the insects (Emad M. S. Barakat and Mohamed O. Abokersh., 2016) [19], so the present work aimed to test the efficacy of *Purpureocillium lilacinum* (Entomopathogenic fungal local isolate) and *Cuminum cyminum* (Plant essential oil) against 2nd and 4th instar larvae of *S. littoralis* and evaluate the influence of both on some biochemical changes in haemolymph components of the tested insect.

Material and Method

Insect rearing

Spodoptera littoralis larvae were received from Insect Pathogen Unit-Plant Protection Research Institute-Agriculture Research Center, reared on the synthetic diet described by Shory and Hale (1965) at 26°C, 75% RH and natural photoperiod El-Defrawi, *et al.*, (1964) [15] with extremely controlled condition to avert any contamination.

Plant material and extraction method

The dry seeds of *Cuminum cyminum* were obtained from supermarket in Jeddah, Kingdom of Saudi Arabia, in September 2019. The dry seeds (150g) were grounded and then macerated in 500ml methylene chloride. After leaving the solution 7 days, it was filtrated through what man No 40 filter paper. The solvent was removed under reducing pressure using rotary evaporator to obtain oily dark extract. Five concentrations (0.625, 1.25, 2.50, 5 and 10%) were prepared from the stock solution to be used in bioassay experiment to test the plant extract virulence.

The Microorganism

Purpureocillium lilacinum isolate was isolated from soil sample collected from Elqalubia gavarnorate (Shimaa M. Desoky, *et al.*, (2020) [55]. Soil sample (1g) was dissolved in 10ml distilled sterilized water, and then serial dilution till 10^{-5} was made to prevent over-crowding of the fungal colonies. One ml of this dilution was injected on prepared Czapek's Dox media plates. Streptomycin (1%) has been added to the medium before casting in the petri dishes to stop the growth of bacteria. The dishes were incubated at 27^{oC} for 72 hour. After the growth of the fungal colonies purification steps were repeated till the appearance of visible and clear growth of fungi. Preliminary identification based on diameter of the hyphae, conidiophore branching, arrangement, and shape of conidia occurred by spreading small part from fungal mycelium on glass slide contain one drop of sterilized water then covered with cover slip and visualized under light microscope. For confirming light microscope identification the isolate was molecular identified by making amplification of one of the most frequently gene used in fungal phylogentic studies 18s ribosomal RNA and registered in Gene Bank data base with code no MT 102250.

Propagation of *P. lilacinum*

P. lilacinum was inoculated on petri dishes contain Czapek's Dox medium and incubated at 27ºC for fifteen days. After incubation period the spores were reaped by robbing the surface of the cultures in sterile distilled water contain 0.01% tween 80 using sterilized spatula. The concentration of the produced mother suspension was evaluated by Neubauer hemocytometer (Alves & Moraes, 1998), and five concentrations (2.8 x 10^6, 2.8 x 10^7, 2.8 x 10^8, 2.8 x 10^9 and 2.8 x 10^{10} spore/ml) were prepared by serial dilution in distilled water to evaluate the virulence of the tested fungal isolate.

Toxicity test for plant extract and fungal isolate

Concentrations that have been prepared from both plant extract and fungal isolate were tested separately against 2nd and 4th instar larvae. For the tested fungal isolate the larvae were treated by direct spraying of the fungal concentrations using good sprayer and untreated larvae serves as control sprayed only with distilled sterilized water contains a 0.01% tween 80. For plant extract, the diet surface treatment procedure was applied according to Addy N.D. (1969) [5], in which the larvae allowed to feed on contaminated artificial diet with plant extract concentrations for 2 days then transferred to clean cups contain untreated diet and observed daily, the diet which served to control larvae treated only with distilled water. Thirty larvae for each concentration and thirty larvae for control were triplicate**.** The mortality rate was recorded every 2 days till 10 days post treatment.

Statistical analysis

Concentrations of the tested fungal isolate and the plant extract with mortality rate were computed to be analyzed and to determine the fifty percent lethal concentration (LC_{50}) by using Ldp Line software (Bakr, 2000) [10].

Biochemical assays

Preparation of homogenate samples

after five days post treating the fourth instar larvae with LC_{50} of both fungal isolate and essential oil individually, the homogenate samples were collected and homogenizing in physiological saline then collected in cold tubes (on ice) previously coated with crystals of phenylthiourea to prevent melanization. The samples were centrifuged at 2500rpm for 5 minutes under cooling (4^{oC}) to remove the tissues. After centrifugation the supernatant fluid was divided into small 3 aliquots (0.5ml) and stored at -20^{oC} until analysis.

Estimation of the total lipid

The total lipid content of the haemolyph was determined by the phosphovanillin method of Baronos and Blackstock (1973) [11], and the developed color was measured spectrophotometrically at 540 nm against the blank.

Estimation of the total protein

The protein content of the haemolymph was determined using folin phenol reagent according to the method of Lowry, *et al.*, (1951) [32], and the absorbance was measured spectrophotometrically at 750 nm against the blank.

Estimation of the total carbohydrate:-

The total carbohydrate content of the haemoly mph was determined according to Singh and Sinha (1977) [57], the absorbance was measured spectrophotometrically at 620 nm against the blank.

Estimation of transaminases activity

The level of both glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) was determined colorimetrically according to Reitman and Frankel (1957) [49], the absorbance was measured specrophotometrically at 505 nm.

Statistical analysis

All experiments data were evaluated statistically by ANOVA and means were compared using Duncan's Multiple Range Test at ($P<0.05$). All statistical analyses were done by using the software package Costat.

Results

Toxicological studies

The results illustrated in table (1&2) revealed the effectiveness of various concentrations of *P. lilacinum* and *C. cyminum* on the second and the fourth instar larvae of *S. littoralis* and all data were recorded till 10 days post treatment. The results data indicated that *C. cyminum* has highly toxic effect at all used concentrations and give better results than *P. lilacinum*. The mortality percentage achieved by *C. cyminum* ranged from 83 to 96% and 63 to 96% for the 2nd and the 4th instar larvae while it ranged from 46 to 66% and 36 to 62% by *P. lilacinum* for the same instar larvae, respectively after 10 days post treatment. Toxicity regression lines Fig (1, 2, 3 & 4) which explain the median lethal concentration (LC_{50}) value was made by linear relationship between tested fungal isolate and tested essential oil individually against mortality percentage after 4, 6, 8 and 10 days post treatment. The value of LC_{50} (0.001and 0.21%) and (3.7×10^6 and 5.7×10^7 spore/ml) have been obtained by *C. cyminum* and *P. lilacinum* for the two tested instar respectively after 10 days post treatment, the lower LC_{50} value indicated higher pathogenicity. There are some morphological changes of *S. littoralis* induced by *P. lilacinum* treatment were noticed (Fig 5), can summarized in dark, crumpled and frizzled larvae, pupae – adult intermediate (pupae failed to be moth) and malformed moth which appear shrinking with crumpled wings.

Table 1: Toxic effect of *Purpureocillium lilacinum* against the second and the fourth instar larvae of *Spodoptera littorlais*.

Concentrations (Spore/ml)	Mortality Percentage (%)							
	The 2nd instar larvae				The 4th instar larvae			
	4 days	6 days	8 days	10 days	4 days	6 days	8 days	10 days
10^6	20	33	43	46	10	16	26	36
10^7	20	36	46	53	16	23	33	46
10^8	23	40	50	56	20	30	40	51
10^9	30	43	56	60	23	36	43	57
10^{10}	36	46	60	66	33	43	50	62
LC_{50}	7.4×10^{12}	8.8×10^{10}	4.9×10^7	3.7×10^6	2.5×10^{12}	5.7×10^{10}	9.1×10^9	5.7×10^7

Table 2: Toxic effect of *Cuminum cyminum* against the second and the fourth instar larvae of *Spodoptera littorlais*.

Concentrations (%)	Mortality Percentage (%)							
	The 2nd instar larvae				The 4th instar larvae			
	4 days	6 days	8 days	10 days	4 days	6 days	8 days	10 days
0.625	43	63	76	83	30	43	53	63
1.25	53	73	86	93	43	60	70	83
2.5	56	80	93	96	50	66	76	90
5	73	83	93	96	60	73	86	96
10	86	93	96	96	83	90	96	96
LC_{50}	1.1	0.25	0.01	0.001	2.04	0.86	0.84	0.21

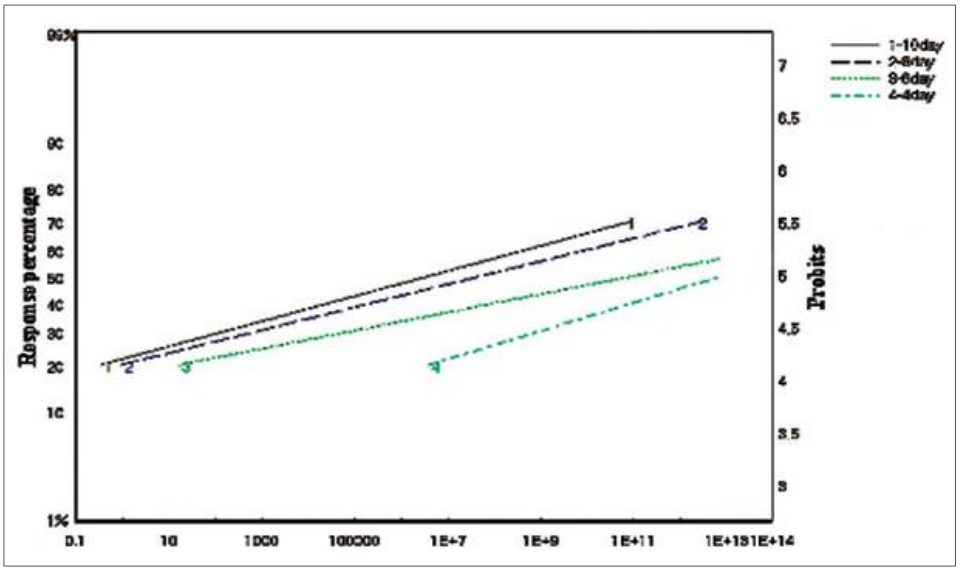

Fig 1: Toxicity regression lines for the second instar larvae of *S. littoralis* treated with *P. lilacinum* after 4, 6, 8 and 10 days.

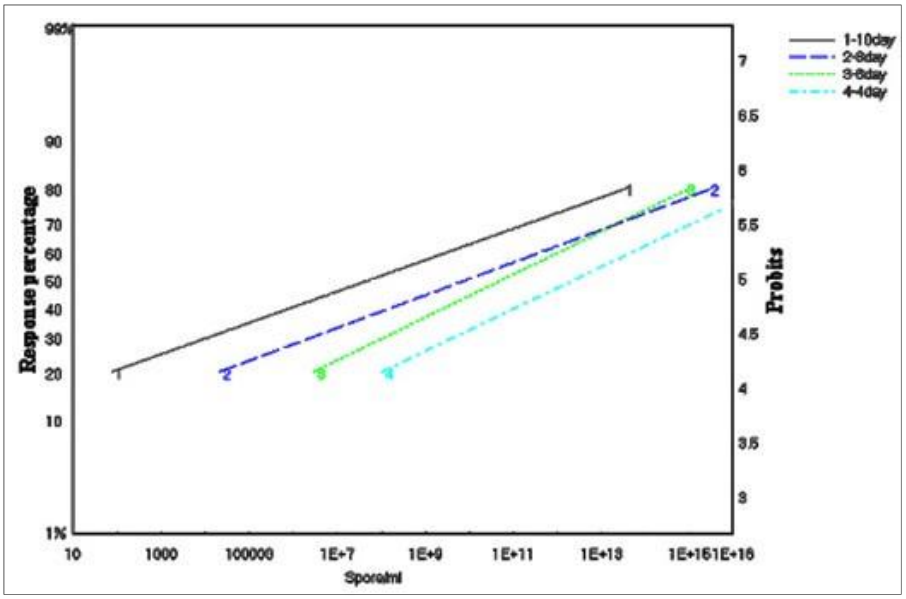

Fig 2: Toxicity regression lines for the fourth instar larvae of *S. littoralis* treated with *P. lilacinum* after 4, 6, 8 and 10 days.

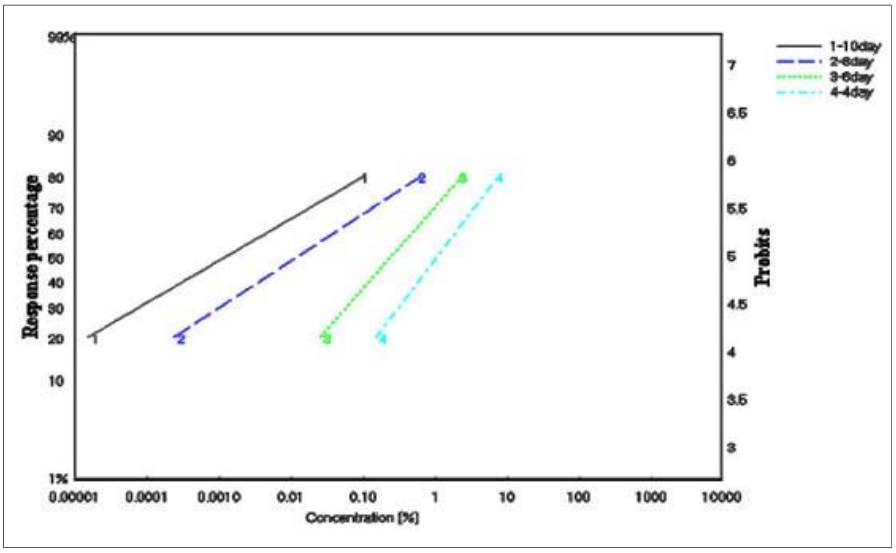

Fig 3: Toxicity regression lines for the second instar larvae of *S. littoralis* treated with *C.cyminum* after 4, 6, 8 and 10 days.

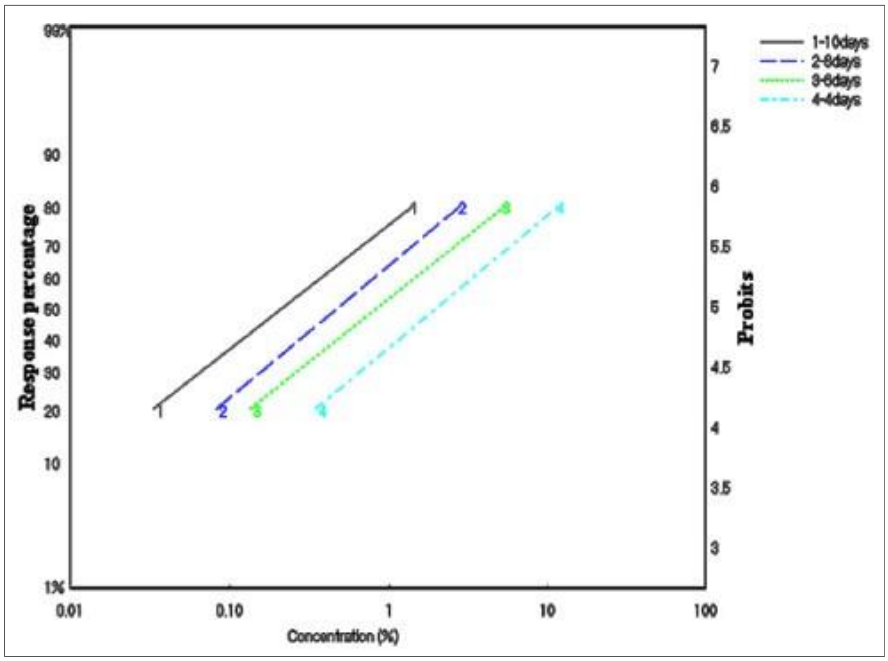

Fig 4: Toxicity regression lines for the fourth instar larvae of *S. littoralis* treated with *C. cyminum* after 4, 6, 8 and 10 days.

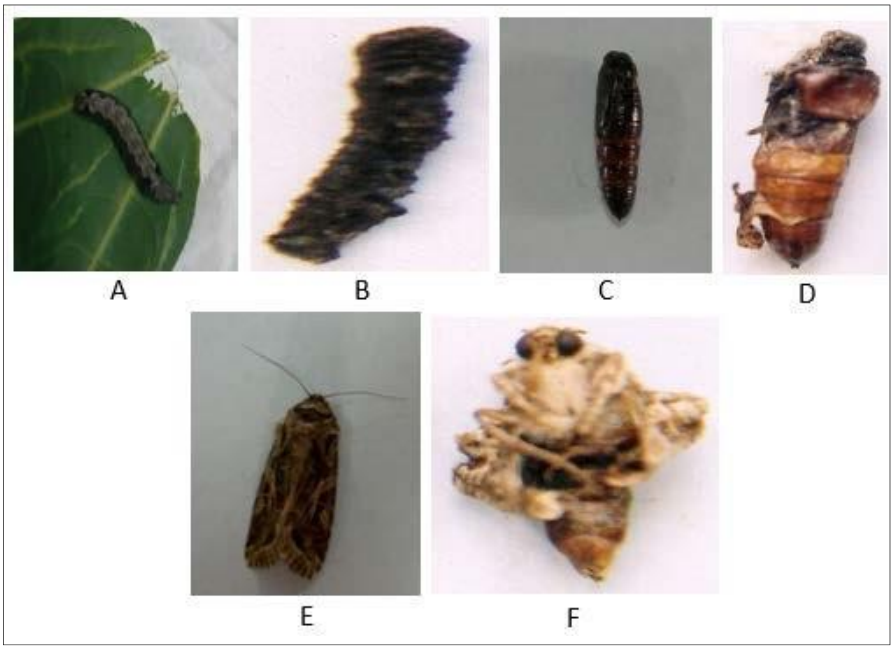

Fig 5: Malformation of *S. littoralis* treated with *P. lilacinum*, (A) normal larvae (B) dark and crumpled larvae (C) Normal pupae (D) intermediate stage of pupae and adult (E) Normal moth (F) malformed moth with shrinking wings

Biochemical studies

Insect haemolymph nutrients should be affected when treated with insecticide so difference in total haemolymph protein, lipids and carbohydrates Table (3) plus the two main enzymes of protein synthesis glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) Table (4) of *S.littoralis* 4th instar larvae treated individually with median lethal time (LC_{50}) of the *P. lilacinum* and *C. cyminum* after five day post treatment was studied. The results demonstrated that, both *P. lilacinum* and *C. cyminum* caused reduction in haemolymph protein content (308±1.6 and 332±1.7 mg/ml) compared to that of the control (430±1.6 mg/ml), and stimulation in the total carbohydrates content (256±1.4 and 265±0.8 mg/ml) as compared to that of the control treatment (252±1.4 mg/ml) respectively. Our results also clarified that total haemolymph lipids of the untreated larvae is 372± 2.4 mg/ml, in the *P. lilacinum* treated larvae it showed significant increase, recorded 390 ±1.8 mg/ml while it decrease in the larvae treated with *C. cyminum* (384± 1.5 mg/ml). The mean level of GOT and GPT enzyme respectively in the untreated larvae is 44 ± 1.3 and 53 ± 0.9 mg/ml while great inhibition noticed in both enzyme level in the larvae treated with *P. lilacinum* and *C. cyminum* recorded a 28 ± 0.6 and 39 ± 0.3 mg/ml and 43 ± 0.4 and 43 ± 1.1 mg/ml respectively.

Table 3: Effect of *Purpureocillium lilacinum* and *Cuminum cyminum* on the total lipid, protein and carbohydrates content (mg/ml) in the haemolymph of the 4th instar larvae of *Spodoptera littorlais*.

Treatment	Total Lipid (Mean ± S.D)	Total Protein (Mean ± S.D)	Total Carbohydrate (Mean ± S.D)
Control	372 ± 2.4	430 ±1.6	252 ±1.4
P. lilacinum	390±1.8	308 ±1.6	256 ±1.4
C. cyminum	284 ±1.5	332 ±1.7	265 ± 0.8

Table 4: Effect of *Purpureocillium lilacinum* and *Cuminum cyminum* on the Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) (mg/ml) in haemolymph of the 4th instar larvae of *Spodoptera littorlais.*

Treatment	GOT (Mean ± S.D)	GPT (Mean ± S.D)
Control	44 ± 1.3	53 ± 0.9
P. lilacinum	28 ± 0.6	43 ± 0.4
C. cyminum	39 ± 0.3	43 ± 1.1

Discussion

Insect pathogenic fungi are the most common organisms that infect several orders of the insect pests (Majeed, *et al*., 2017) [34], there is a great interest for using them for the bio control of insect pests, as they play significant role in suppression of the insect population (Smith, *et al*., 1999) [59]. Entomopathogenic fungi have a unique place among other entomopathogens (Richard, *et al*., 2010) [50] in which they should not be swallowing like virus and bacteria and can attack their hosts directly by binding to their integument. Among the insect pathogenic fungi, *Purpureocillium* which considered essential natural bio-control agents and source of mycopesticides for pests management all over the world (Sanjaya, *et al*., 2016) [52].

Plants produce several secondary chemical compounds to protect themselves from any pathogens attack, these chemical compounds can be used in the pest control, (Pavela, 2016) [46] so recently, researchers focused on these plant natural products to be used as safe insecticides, *C. cyminum* is an essential oil belonging to Apiaceae Family which considered the most family that well known of their insecticidal activity (Ebadollahi, 2013) [14]. In the current study the tested fungal isolate *P. lilacinum* and tested essential oil *C. cyminum* were tested against the second and the fourth instar larvae of *S. littoralis* and the results data proved the toxic effect of both against the two tested larval instar. The results clarified that there is positive relationship between the concentration and the mortality rate and also the younger instar larvae is much more sensitive than the older one Purwar and Sachan (2005) [47]. Obtained data in the present study are agree with several authors examined

the potential use of *P. lilacinum* and *C. cyminum* as biological control agents against several insects. Kepenekci, *et al*., (2015) [29] reported a potential infectivity of *P. lilacinum* against the last larvae instar of *Phthorimaea operculella* (Zeller) and *Leptinotarsa decemlineata* (Say) and recorded mortality percentage 43.3% and 33.2% respectively at the 10th day of treatment with the fungal concentration of 10^8 spore/ml, also Lopez, *et al*., (2014) [31] proved the potential pathogenicity of *P. lilacinum* against herbivores and aphid of cotton under greenhouse and field conditions. In parallel Hoang Chinh NGUYEN, *et al*., 2017 [28] isolated six *Purpureocillium* species and evaluated their pathogenicity against *Plutella xylostella* and *Spodoptera litura* and confirmed the strong virulence of the six isolate while *P. lilacinum* isolate showed the highest infectivity against the two tested insects. In addition, *P. lilacinum* has been used as an effective nematocide against nematodes (Meloidogyne spp.) (Sharma, *et al*., 2014) [54].

Insect pathogenic fungi enter the insect body directly through the integument (Sevim, *et al*., 2015) [53], by physical and enzymatic process. Many factors combined in the action mechanism of entomopathogenic fungi, firstly, the fungus spores attach on the insect cuticle, then germinate and enter the cuticle by forming appressorium. Hyphae grow and reproduce continuously in the insect body and haemolymph and finally lead to the insect death. The most fundamental factor in entomopathogenic mechanism is toxin secretion by fungi, for example paecilotoxin (Leucinostatins) sectrated from *P. lilacinum* Abbas H. Burhan and Mohammed R. Annon., 2019. These toxic substances can cause insect death even before spread of spores in tissue of parasitic fungus (Charnley, 2003) [13].

N.E. BEN-KHALIFA, *et al*., 2018 [42] tested the efficacy of six Apiaceae extracted oils on *S. littoralis* and showed that *C. cyminum* produced 100% larval mortality against the tested third instar larvae. Mukesh Kumar Chaubey, 2017 [41] evaluated the effectiveness of *C. cyminum* and *black piper* oils on greater grain weevil and revealed that both oil repelled the weevil adults, induced high mortality and exhibit median lethal time (LC_{50}) 0.2 and 0.1 µl/cm2 and 0.2 and 0.1 µl/cm^2 for the two essential oil respectively, also they inhibit both AchE activity and oviposition of *S. zeamais* adults. Toxic effect of *C. cyminum* related to monoterpense (main constituents) which have effective insecticidal toxicity (Abdelgaleil, *et al*., 2009) [2], penetrate very rapidly in the body of the insects and intervene with the insect physiological function (Haouas, *et al*., 2012). Another main components of *C. cyminum* is Cuminaldehyde that effectively inhibit actylcholinestearse enzyme activity. Abdelgaleil, *et al*., (2009) [2]. Yeom, *et al*., (2012) [65] tested some Apiaceae family extracted oils including *C. cyminum* and reported that at a concentration of 5 mg/filter paper they have approximatly 90 % fumigant toxicity against German cockroaches adult male. (Tunc¸ *et al*., 2000) [61] mentioned that *C. cyminum* oil produce 100 % mortality on *T. confusum* and *Ephestia kuehniella* Zeller eggs.

The present study indicated the influence of insect haemolymph nutrients due to pathogen attack. Proven results in this study showed difference in total lipid, protein, carbohydrates, GOT and GPT of treated *S. littoralis* 4th instar larvae with both *P. lilacinum* and *C.cyminum* when compare to control and these data are agree with El-Badawy, S. S, *et al*., 2018 [16] who investigated the effect of some entomopathogenic fungi including *P. lilacinum* on biochemical content in haemolymph of *S. littoralis* and found that *P. lilacinum* isolate caused significant increase in total carbohydrates and total lipids and reduction in total protein content compared to control at fourth day post treatment. Nirupama, (2015) [44] detected that total protein content of silkworm, *Bombyx mori* was reduced gradually at the end of 4th and 5th day due to the fungi infection. Also our results were proved by Vidhya, *et al.*, (2016) [62], they mentioned that infection of the army worm *S.litura* (Fabricius) by three fungal pathogens *B. bassiana, M.anisopliae* and *Verticillum lecanii* showed significant decreased of total protein content at fourth day post treatment as compared to control. In parallel, Meshrif, *et al*., (2010) [36] revealed that, there was significant increase in plasma carbohydrates of *S. littoralis* 5th instar larvae when injected by fungi (*Nomuraea rileyi* and *B. bassiana*). Gabarty, (2011) [23] showed significant increase in the total content of lipids in the greasy cut-worm *Agrotis ipsilon* (Huf.) larvae treated with *B. bassiana* and *M. anisopliae* as compared with untreated one. In contrast to our results the obtained data of Nada, (2015) [43] showed that total lipids decreased significantly when adults of *N. virdula* treated with *M. anisopliae*. In the same line Marei, *et al*., 2009 [35] evaluated the influence of *Sesame oil* on the biochemical aspects of *S.littoralis* and found great reduction in total lipids. Abou El-Ghar, *et al*., 1996 [3] observed high inhibition of both total lipid and total protein of *Agarotis ipsilon* treated with ethanol extract of *Melia azedarach.* Also, some results of Amal S. Sobhi, *et al*., 2020 [9] are agree with our results as they recorded inhibition of total lipid and total protein of *S.littoralis* treated with essential camphor oil while reduction in total carbohydrates they observed are in contrast to our findings as we reported stimulation of total carbohydrates after treatment with essential *C. cyminum* oil.

SK Mirhaghparast, *et al*., 2013 [58] tested the effect of two entomopathogenic fungi on the metabolic enzyme of *S.littoralis* and found high activity of all tested enzymes including GOT and GPT enzyme, there is also noticeable elevation in the two enzyme of *S.littoralis* treated with four plant essential oils Mona, K. Elhadek, *et al*., 2015 [39], these results disagree with what has been accessed in the present study as there is reduction in the two enzyme observed after *P.lilacinus* an *C.cyminum* treatment.

Many studied revealed the ability of *Neemazal* (a neem preparation) and *N. sativa* extracts (Hamadah, 2009) [26] in disruption of GPT activity of *S. gregaria*.

Proteins are the most important compounds found in every cells of all living organisms, including many substances like enzymes and hormones that necessary for the main function of the living organisms (Fagan, *et al*., 2002) [21]. Carbohydrates are contributed to the structure and function of the insect tissues and organs, they providing the energy wanted for the growth and development of the cell (Lee, *et al*., 2002) [30]. Lipids are significant source of energy compared to carbohydrates, they can supply as much as eight time more energy per unit weight (Ali, 2011) [7]. Several species store lipids which are used during starvation Panizzi and Hirose 1995 [45]. They occupy a central place in insect physiology. They are considered to be a fundamental source of metabolic energy for the cell protection and proliferation.

In the present study change in the level of some biochemical parameters of *S. littoralis* larvae haemolymph may be due to

physiological disturbance which are induced by the presence of a the physiological confrontation in the body like microorganism infections, tissues impairment or being a toxic material (Giboney, 2005) [25].

The significant reduction in the total protein may be due to binding with foreign substances, as any insecticides. This was revealed by Ahmed, *et al*., (1985) [6] when investigated dieldrin insecticides against *Periplaneta americana*, Or maybe due to the repression of DNA and RNA synthesis, as proposed by Mitlin, *et al*., (1977) [37] for boll weevils treated with chitin synthesis inhibitors and by Qadri and Narsaih (1978) for *P. americana* last nymphal instar injected with the plant extract, *azadirachtin*. In addition, the reducing in total protein may be due to the cracking of the protein into amino acids. GOT and GPT are the major enzymes in the protein metabolism (Mordue & Goldworthy, 1973). So, the reduction in total content of protein may be related to the reduction in both GOT and GPT that were resulted in the present study, and vice versa, the reduction in those enzymes may be due to the reduction of total protein as the enzymes are protein in nature (Mitlin, *et al*., 1977) [37]. In other way the disturbance in these two enzymes is closely related to utilization of protein and amino acids hence damage many physiological functions and eventually lead to insect death (Ezz and Fahmy, 2009) [20]. Lipids are important structural constituents of the cell membrane and cuticle. Inhibition of total lipid can be attributed to the toxic stress induced by the tested insecticides stimulate the utilization of lipid and rapid conversion of total lipid contents to protein occurred to produce supplementary energy required by the insect body (Abuldahab, *et al*., 2011) [4]. Also, carbohydrates are important compounds as they are utilized by the insect body for the production of energy or conversion to lipids or proteins. Utilization of carbohydrates is controlled by amylase, trehalase, and invertase enzymes which play the main role in the digestion and utilization of carbohydrates by insects (Wigglesworth, 1972) [63]. Stimulation in total carbohydrates due to two tested insecticides noticed in this study may be due to disruption in the enzyme responsible for utilization of them. Generally biochemical changes in *S.littorlais* haemolymph nutrient content (Protein, Lipid and carbohydrates) recorded in this study either by increasing or by decreasing may be due to alteration or mutation of the genes responsible for biosynthesis of polypeptide chains constructing the enzymes regulating the metabolism of them and this affect these enzymes synthesis and function.

Conclusion

Results of the current study clarified the capability of the two tested biocides *P. lilacinum* and *C.cyminum* to induce high mortality rate to the two tested instar larvae of *S.littoralis* and caused sharp disturbance in lipid, carbohydrates, proteins and both GOT and GPT enzymes. Toxicity and biochemical changes of *S.littoralis* treated individually by the two biocides used are prompted as a result of the physical invasion of *P. lilacinum* vegetative growth, sporulation, enzymatic degradation and toxin productions, also due to the main active constituents of *C.cyminum* (Cuminaldehyde, β-pinene, γ-terpinene and p-cymene) which characterized by high insecticidal potential including toxicity, repellent, antifeeding and disturbance ability of insect growth and development. Consequently these two biocides agents can be used as a promising eco-friendly alternatives of the chemical pesticides against this detrimental, harmful and injurious pest *S. littoralis.*

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