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Effects of *Bacillus thuringiensis* Cry Toxin, Propolis Extracts and Silver Nanoparticles Synthesized by Soil Fungus (*Fusarium oxysporum*) Against Two Species of *Tetranychus* spp. (Acari:Tetranychidae)

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ABSTRACT

Development of new biostrategies for pest control represents a major eco-friendly achievement. The use of micoorganisms represents one of these biostratigies. *Bacillus thuringiensis* d-endotoxins are recognized safe and economic biological insecticidal proteins. Green nanotechnology is one of the most promisg approches in this field. Our study, demonstrated that *B. thuringiensis* (4QSTR1) produced cry proteins, which appeared as stained crystals. The Coomassie Brilliant Blue stain allowed a quick and high productivity evaluation of *B. thuringiensis* (4QSTR1). Synthesized silver nanoparticles by using *Fusarium oxysporum (Fo*-AgNPs) were used. Also, the protein crystals and endospores of *Bacillus thuringiensis* were separated. The bioinsecticidal effect of synthesized *Fo*-AgNPs, *Bacillus thuringiensis* (*Bt*) and propolis extracts (ethanolic extract (EEP) and water extract (WEP)) against *Tetranychus urticae* Koch and *Tetranychus cinnabarinus (Boisduval*) was evaluated. Bioassays results showed that the insecticidal efficacy (LC_{50} and LC_{90}) of *Fo*-AgNPs were more effective than propolis extracts and Bt against the two species of *Tetranychus*. The LC_{50} value for *Fo*-AgNPs, WEP, EEP and Bt against *T. urticae* were 45, 10234, 12755 and 14972, respectively and against *T. cinnabarinus* were 37, 13579, 15881 and 17003, respectively. More than 100% toxicity index was recorded by *Fo*-AgNPs with two species of *Tetranychus*. The total mortality percentage of two species of *Tetranychus* was more affected by *Fo*- Ag-NPs at the lowest dose (10ppm). Whereas, the propolis *extracts and Bt* were more effective at the highest dose (20×10^3 ppm). It is clear that silver nanoparticles synsthized by soil fungus (*Fusarium oxysporum*) have a strong insecticidal effect.

Keywords: Biological control –*Fusarium oxysporum* – Silver nanoparticles – Crystal toxins – Propolis – red spider mites.

INTRODUCTION

The red spider mites, *Tetranychus* spp. (Acari: Tetranychidae) is one of pests that has a major impact on cultivated strawberries around the world. *Tetranychus* spp. is considered a major pest decreasing plant growth and yield on different agricultural crops, including bean plants (Farouk & Osman 2009). The mites has been reported to attack about 1200 species of plants and over150 host plants are of economic value (Zhang, 2003)

Developing countries as in Africa, South America and Asia are suffering from the hazardous routinely utilization of agro-chemicals such as chemical insecticides which have an unexpectedly high ecological cost, contributing to global pollution, unfavorable climate change and loss of microbial biodiversity (Vance, 1998). Pesticides are classified as being extremely or highly hazardous by FAO and WHO. The most promising strategy for sustainable agriculture reaches to substitute hazardous agrochemicals (mineral fertilizers, pesticides) with eco-friendly preparations which could increase the protection from biotic such as pathogens and pests (Yang *et al*., 2009).

Propolis is the dark-brown or black sticky plant derived 'glue' found around wounds and buds of plants. It is used by bees for sealing, lining, strengthening of their hives (Banskota *et al.*, 2001). Various studies demonstrated the antibacterial and antifungal of propolis due to its high content of total phenols and flavonoids. Few studies had been examined the insecticidal properties of propolis. Zewda and Legessa (2016) Evaluate the insecticidal effect of propolis against larvae of lesser wax moth *Achroia grisell* concluded that ethanol extract of propolis at higher concentrations is a powerful contact toxicant against young wax moth larvae.

The microorganisms have taken an excellent position among the options that used to control pests with high specific toxicity and without the use of chemicals applied in agroecosystems (Schünemann *et al*. 2014).

Bacillus thuringiensis (*Bt*) is a unique bacterium in that it widely spread in soils throughout the world (Huang *et al*., 2001). *Bacillus thuringiensis* is spore-forming bacterium that produces insecticidal crystal protein toxins during sporulation. *Bacillus thuringiensis* d-endotoxins are recognized safe biological insecticidal proteins Thomas *et al*.,(2000). This bacterium represents an economic source and safe for human health (Siegel, 2001), in addition, the use of *Bacillus thuringiensis* for the production of biopesticides, is likely to be a promising achievement associated with eco-friendly aspects (Frankenhuyzen, 2009).

Nanotechnology has become a promising new strategy for environmental clean-up technologies that could provide economic solutions for some of the most challenging environmental problems (Chinnamuthu and Murugesa Boopathi, 2009). Nanoparticles can be used to produce new insecticides (Owolade *et al*., 2008). The insecticidal effect of the biosynthesized silver nanoparticles against the larvae and adult of *Callosobruchus maculates* on cowpea seed were examined (Rouhani *et al.* 2012) and also, against *Anopheles stephansi, Culex quinquefaciatus* and *Aedes aegypti* (larvae and pupae) (Soni and Prakash 2013 and Banu *et al*., 2014).

The use of microorganisms in the synthesis of nanoparticles is called a green nanobiotechnology which does not produce toxic wastes during the synthesis process. Some microorganisms, including bacteria, yeast and filamentous fungi could be used as nanofactories (Fortin and Beveridge 2000). Fungi have several advantages in the production of nanoparticles including, high growth rate, high production of specific enzymes and easy handling in large scale (Vahabi *et al*., 2011). *Fusarium oxysporum* represents an interesting example for biosynthesis of silver

nanoparticles (Ahmad *et al*., 2003) *Fusarium acuminatum* (Ingle *et al*., 2008) and *Penicillium fellutanum* (Kathiresan *et al*., 2009).

The aim of this study is to evaluate the bioinsecticidal effect of silver nanoparticles synsthized by soil fungus (*Fusarium oxysporum*) (*Fo* AgNPs), *Bacillus thuringiensis* (*Bt*) and propolis extracts against *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* (Boisduval).

MATERIALS AND METHODS

Microbial Strains

Bacillus thuringiensis (4QSTR1) was obtained from *Bacillus* Genetics Stock Center, Biochem. Dept., Ohio Univ., Columbus, USA. *Bacillus thuringiensis* was maintained on Luria-Bertani (LB) medium, containing: 1% tryptone, 0.5% yeast extract and 0.5% NaCl and pH 7.0 (Sambrook *et al*., 1989).

Separation of Crystals and Endospores

Bacillus thuringiensis was grown in petri dishes. The spores were collected from L.B agar plates then washed thrice with ice-cold distilled water. Pellets (spores and crystals) were resuspended in small volumes of distilled water. The bacterial crystals and endospores were prepared according to the method previously described by Karamanlidou *et al*. (1991).

Staining crystal toxin of *Bacillus thuringiensis*

Samples were prepared for microscopic examination according to (Wu and Chang, 1985).

Silver nanoparticles synthesized using *F. oxysporum*

Silver nanoparticles synthesized by *F. oxysporum* (*Fo*- AgNPs) were used in this investigation. The *Fo*-AgNPs were obtained in a previous study by Sabrien and Dawood, (2016). These silver nanoparticles appeared as polydisperesed spherical particles characterized by being stable in solutions and ranging in size from 9-24 nm.

HPLC separation of flavonoids and phenolic compounds

HPLC analysis was conducted in the laboratories of Food Science and Technology institue, Giza, Egypt. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used to identify and quantify the flavonoids and phenols of propolis (Shuai *et al.,* 2014).

Preparation of propolis extracts.

Propolis used in this work was collected and then kept in the dark until processing. The procedure described by. Alencar *et al.*, (2007) with some modification. Twenty grams of fine ground propolis was added to different solvents (ethanol 95% and water) to a final volume 100 mL. The mixtures were protected from light, with moderate shaking during 24 h, at room temperature and left at rest overnight, these mixtures were filtered through Whatman filter paper No.1. These mixtures were ethanolic extract of propolis (EEP) and water extract of propolis (WEP).

Target mites: Adult female of carmine spider mite, *Tetranychus cinnabarinus* (Boisduva) and the two spotted spider mite, *Tetranychus urticae* Koch. (Acari: *Tetranychidae*.) were collected from unsprayed castor bean plants and reared at 25± 2° C and 60± 5% RH in laboratory of Plant Protection Research Institute, Agriculture Research Center.

Bioassay test: To assess the activity of bioniscticides (*Bacillus thuringiensis*, silver nanoparticles and propolis) against adult females of *T. cinnabarinus* and *T. urticae*. Thirty newly emerged adult females were transferred to the lower surface of castor leave discs (2.5 cm diameter) placed separately on moist cotton wool in petri dishes. Each petri dish contains three replicates, ten individuals in each replicate. Each acaricide had four concentrations which were sprayed on the individuals.Mortality was examined 1, 3, 5 and 7 days after treatment. The mortality percentage was estimated and corrected according to the Abbott's formula (1925). LC_{50} values were determined using probit analysis statistical method of Finney (1971).

Equation: Sun (1950) (to determine LC_{50} index)

$$\text{Toxicity index for LC50} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the least effective compound}} \times 100$$

RESULTS AND DISCUSSION

To confirm that *B. thuringiensis* (4QSTR1) produced cry proteins, light microscopy and Coomassie staining of spore-crystal mixtures were used. Cry protein appeared as stained crystals (Fig. 1). Coomassie Brilliant Blue stain allowed a rapid and high productivity assessment of *B. thuringiensis* (4QSTR1). There is a relationship between the insecticide activity and the crystal morphology of *Bt* (Maeda *et al*., 2000). Therefore, the strain was examined for crystal morphology. A few of the crystals did not staining well, however the differences between spores and crystals were clear enough to easily differentiate the two, even when crystal morphology mimicked that of spores (Fig. 1). The spores were appeared as dark-staining body.

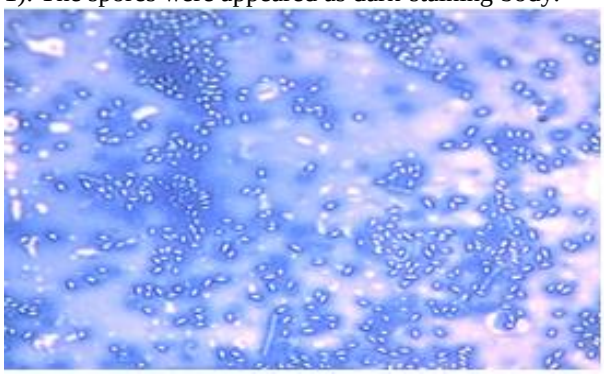

Fig. 1. Light Microscopic examination of stained sporulating culture of *Bacillus thuringiensis* using 100 x lens

HPLC separation of flavonoids and phenolic compounds

High-performance liquid chromatography equipped with a diode array detector was used to separate flavonoids and phenolic compound from samples with varied matrixes. It was used to identify and quantify the flavonoids and phenolic compounds in propolis. Table 1 shows the amount (µg/100 g of dry matter) of fifteen phenolic acids and fifteen flavonoids. The resultant appeared that chlorogenic was the most identified phenolic compound (120.62 µg/100 g dry weight) and the acacetin was the major identified flavonoid component in propolis

(647.53 µg/100 g dry weights) (Shuai *et al*. 2014). These results can state that propolis has general biological activities as insecticides activity

Table 1. Polyphenol and flavonoid contents of propolis

Polyphenols	µg /100 g*	Flavonoids	µg /100 g*
Pyrogallol	11.05	Acacetin	647.53
Gallic	5.12	Apigenin -7- glucose	96.98
Ellagic	13.58	Quercetrin	64.19
Protocatechuic	23.91	Rutin	94.66
Catechol	3.51	Hisperidin	561.09
Chlorogenic	120.62	Narenigin	69.86
p.coumaric	7.05	Quercetin	45.64
Coumarin	8.21	Hesperitin	41.68
Salycillic	3.63	Apigenin	57.31
Cinnamic	4.85	Kaempferol	8.54
Epicatechin	8.13	Rhamnetin	11.54
Caffeic	14.32	Naringenin	13.23
Caffeine	19.53	Catechin	53.00
Vanillic	13.58	Luteolin	109.42
Ferulic	7.01	Luteolin- 7 - glucose	11.25

*** µg/100 g of dry matter**

Toxicity of certain bioinsecticides against *Tetranychus urticae* Koch adult after seven days at 25±2 ◦C and 65±5% RH.

The lethal effect of synthesized Fo Ag-NPs, *B. thuringiensis* and propolis extracts was investigated against adult mites *Tetranychus urticae* Koch *in vitro* (Table 2) and Fig. 2. Bioassays data appeared that the efficacy of synthesized *Fo*-AgNPs (LC_{50}, 45 and LC_{90} was 14) were more effective than water extract of propolis (WEP), ethanolic extract of propolis (EEP) and *Bt* (LC_{50}, 10234and LC_{90}, 110465) (LC_{50}, 12755 and LC_{90}, 205649), *and* (LC_{50},14972 and LC_{90}, 67820), respectively. More than 100% toxicity index was recorded by synthesized *Fo* Ag-NPs. The LC values of *Bt* are affected by crystal protein and geographical variation of the parental *Bt* strain. These results agreed with Subarani *et al*. (2013) who found that the maximum efficacy was achieved by using synthesized AgNPs against the fourth instar larvae of *Anopheles stephensi* (LC_{50}, 12.47 and 16.84 mg/mL; and LC_{90}, 36.33 and 68.62 mg/mL) in 48 and 72 h of exposure and against *Culex quinquefasciatus* (LC_{50}, 43.80 mg/ mL; and LC_{90}, 120.54mg/mL) in 72-h exposure. Soni and Prakash (2013) who examined the insecticidal effect of AgNPs synthesized by *Aspergillus niger* against larvae and pupae of *Aedes aegypti* and found that the efficacy LC_{50} 4 and LC_{90} 12 ppm. Sinha *et al*. (2009) reported that the silver nanoparticles synthesized by biological methods have many advantages over chemical and physical methods. Recently, Salunkhe *et al*. (2011) studied the larvicidal potential of AgNPs synthesized using fungus, *Cochliobolus lunatus*, against *Aedes aegypti* and *Anopheles stephensi*. On the other hands, (Aronson *et al*. 1991) showed the effect of different *B. thuringiensis* strains on *Spodoptera littoralis* and found great variability in toxicities depending on the insects that were fed crystals, solubilized crystals or *in vitro* activated crystals).

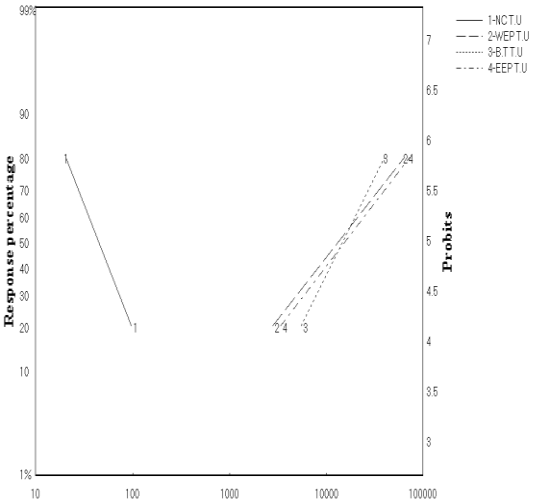

Fig. 2. log concentration probit lines showing Lethal cocncentration of bioinsecticides – against adult female of *Tetranychus urticae* Koch.

Table 2. Efficiency of Lethal concentration of bioinsecticides against two spotted spider mite, *Tetranychus urticae* Koch after seven days at 25±2 ◦C and 65±5% RH.

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/ LC_{50}	R	P
WEP	5000	36.66	10234	110465	1.2405±0.519	0.44	10.79	0.98	0.88
	10000	46.66							
	15000	56.66							
	20000	66.66							
EEP	5000	33.33	12755	205649	1.0614±0.518	0.35	16.12	0.98	0.92
	10000	46.66							
	15000	50							
	20000	60							
Fo- AgNPs	10	96.66	45	14	-2.4870±0.426	100	0.31	-0.99	0.78
	20	76.66							
	40	56.66							
	80	26.66							
Bt	5000	23.33	14972	67820	1.9534±0.5585	0.30	4.53	0.88	0.15
	10000	26.66							
	15000	43.33							
	20000	70							

R: Regression P: Propability--Water extract of proplis (WEP)-ethanolic extract of proplis (EEP)-- *Fusarium oxysporum* silver nanoparticles (*Fo*-AgNPs)-- *Bacillus. thuringiensis* (*Bt*)

The effect of bioinsecticides against two spotted spider mite, *Tetranychus urticae* Koch. at 25±2 ◦C and 65±5% RH.

In the present study, the presented data in Table 3 appeared that the highest mortility proportion (96.66%) was obtained at the lowest dose (10 ppm) of *Fo*-AgNPs after 7 days, Meanwhile, at the highest dose (20 x 10^3 ppm) of *Bt* –bioinsectcide the highest mortility proportion was (70%) after 7 days. On the other side, by using WEP and EEP at dose (20 x 10^3 ppm), the highest mortility proportion was (66.66, 60 %), respectively after 7 day. The total mortality percentage was more affected by *Fo*- AgNPs at the lowest dose (10 ppm). Whereas, the propolis extracts and *Bt* were more effective at the highest dose (20 x 10^3 ppm). The obtained results supported that the biosynthesized *Fo*-AgNPs had a strong insecticidal effect. The results agreed with Jalalizand *et al*. (2013) who found that significant mortality effect of silver nano particles at different concentrations of 2.5, 5, 10, 50, 100, 200, 500, 1000, 2000 and 3000 ppm on adult mites. Whereas, Chapman and Marjorie (2009) found that different concentrations of *Bacillus thuringiensis* var. *tenebrionis* caused less mortality on adult females of *T. urticae* but had more toxicity to *Metaseiulus occidentalis* females at 48 h. Female *T. urticae* exhibited 90.0 ± 14.2 % survival vs. 26.0 ± 23.4 % survival for *Metaseiulus occidentalis* Nesbittis at the field rate. The significantly increased mortality was obtained when the female predators were starved for 24 h prior to treatment with *Btte* wettable powder.

Table 3. Corrected mortality % of bioinsecticides against two spotted spider mite, *Tetranychus urticae* Koch under laboratory conditions 25±2 ◦C and 65±5% RH.

No.	Treatments	Conc. (ppm)	Mortality after treatments % One day	Three days	Five days	Seven days
1	WEP	5000	----	16.67	30	36.66
		10000	13.33	23.33	36.33	46.66
		15000	20	26.67	40	56.66
		20000	26.67	33.33	53.33	66.66
2	EEP	5000	----	16.67	20	33.33
		10000	3.33	13.33	30	46.66
		15000	13.33	20	33.33	50
		20000	20	23.33	43.33	60
3	*Fo*- AgNPs	10	3.33	53.33	93.33	96.66
		20	33.33	40	53.33	76.66
		40	36.66	39.66	46.33	56.66
		80	----	13.33	23.33	26.66
4	*Bt*	5000	---	----	3.33	23.33
		10000	----	-----	10	26.66
		15000	20	33.33	36.66	43.33
		20000	10	10.0	43.33	70
5	Control (Distilled water)		0	0	0	0

Water extract of proplis (WEP)-ethanolic extract of proplis (EEP)-- *Fusarium oxysporum* silver nanoparticles (*Fo*-AgNPs)-- *Bacillus. thuringiensis* (*Bt*)

The lethal effect of synthesized Fo-AgNPs, *B. thuringiensis* and propolis was investigated against adult mites, *Tetranychus cinnabarinus (Boisduval) in vitro* (Table 4 and Fig. 3). Bioassays results showed that the efficacy (LC_{50} and LC_{90}) of *Fo*-AgNPs (37 and 6.554) was more effective than WEP, EEP and *Bt* (13579 and 146880.6), (15881and 164125) *and* (17003 and 81996.7), respectively.

Table 4. Efficiency of certain bioinsecticides against carmin spider mite, *Tetranychus cinnabarinus* (Boisduval). after seven days at 25±2 ◦C and 65±5% RH.

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/ LC_{50}	R	P
WEP	5000	30	13579	146880.6	1.2394±0.5235	0.270	10.81	0.9920	0.9531
	10000	43.33							
	15000	50							
	20000	60							
EEP	5000	26.66	15881	164125	1.264±0.5292	0.230	10.334	0.9931	0.9576
	10000	40							
	15000	46.66							
	20000	56.66							
Fo- AgNPs	10	80	37	6.554	-1.7157±0.3786	100	0.179	-0.9766	0.5802
	20	70							
	40	53.33							
	80	23.33							
Bt	5000	20	17003	81996.7	1.876± 0.5654	0.215	4.8225	0.9181	0.3371
	10000	26.66							
	15000	40							
	20000	63.33							

R: Regression P: Propability--Water extract of proplis (WEP)-ethanolic extract of proplis (EEP)-- *Fusarium oxysporum* silver nanoparticles (*Fo*-AgNPs)-- *Bacillus. thuringiensis* (*Bt*)

As shown from the results presented in Table 5 showed that mortality rates at the concentration of bioinsecticides were ranged between; 20 to 80 % when *Tetranychus cinnabarinus adult* were treated with bioinsecticides. Thus, mortality was gradually increased to a maximum of 70, 80 when used low concentration of bioinsecticide *Fo*- AgNPs at concentrations 20 and 10 ppm, respectively. The *Bt*-bioinsecticide appeared the hight mortality percentage recorded (63%) at high concentration 20 x 10^3 ppm. In addition, the bioinsecticide *Fo*-AgNPs (10 ppm) recorded the highest mortility (80 %) after 7 days, whereas, the

mortality percentage of highest dose of *BT* – bioinsectcide (20 x 10^3 ppm) was recorded (46.66%) after 5 days, and the mortality percentage of WEP and EEP (20 x 10^3 ppm) were recorded (43.33 and 23.32 %), respectively after 5 days. The results in agreed with Stadler *et al*., (2010) described the insecticidal effect of alumina nanoparticles on *Sitophilus oryzae* and *Rhyzopertha dominica* (Fabricius). Whereas, Mohan and Gujar (2003) reported that the differences in sensitivity of two populations of the diamondback moth, *Plutella xylostella* L to *B. thuringiensis* Cry1Ab were not due to midgut proteolytic activity. Further, the proteolytic patterns of Cry1A protoxins were equal in the resistant as well as sensitive populations of *P. xylostella.*

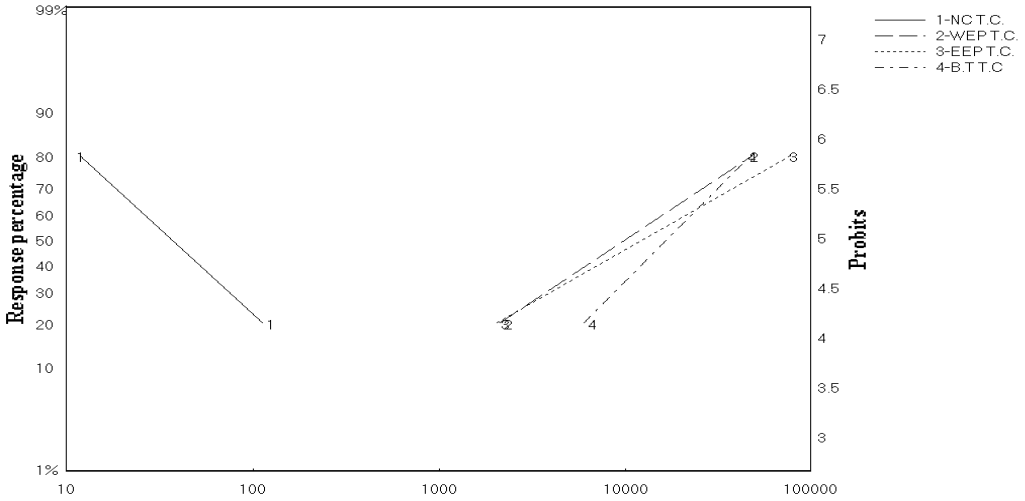

Fig. 4. log concentration probit lines showing Lethal concentration of bioinsecticides against adult female of *T. cinnabarinus* (Boisduval)

Table 5. Mortality rates of different concentrations of bioinsecticides against carmin spider mite, *Tetranychus cinnabarinus* (Boisduval) at 25±2 ◦C and 65±5% RH.

No.	Treatments	Conc. (ppm)	Mortality after treatments % One day	Three days	Five days	Seven days
1	WEP	5000	----	13.33	20.33	30
		10000	10	23.33	33.33	43
		15000	6.66	19.99	29.99	50
		20000	10	23.33	43.33	60
2	EEP	5000	---	6.66	13.33	27
		10000	---	3.33	16.66	40
		15000	---	3.33	33.33	46
		20000	---	6.66	23.32	57
3	*Fo*- AgNPs	10	6.66	26.66	43.32	80
		20	6.66	26.66	43.32	70
		40	30	30	43.33	53
		80	---	10	20.0	23
4	*Bt*	5000	---	---	6.66	20
		10000	----	----	13.33	27
		15000	13.33	19.99	23.32	40
		20000	13.33	16.66	46.66	63
5	Control (Distilled water)		0	0	0	0

Water extract of proplis (WEP)-ethanolic extract of proplis (EEP)-- *Fusarium oxysporum* silver nanoparticles (*Fo*-AgNPs)-- *Bacillus. thuringiensis* (*Bt*)

CONCLUSION

In the present investigation, bioinsecticidal effect of *Bacillus thuringiensis* cry toxin, propolis extracts and silver nanoparticles synsthized by soil fungus (*Fusarium oxysporum*)(*Fo*-AgNPs) against *Tetranychus urticae* Koch and *Tetranychus cinnabarinus (Boisduval)* were studied. The results revealed that the biosynsthized silver nanoparticles would be suitable for developing a biological process and can be used successfully in IPM program to control of two *Tetranychus* spp.

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تـأثير البـروتين السـام لبكتريـا *Bacillus thuringiensis* ومستخلصـات البـروبليس وكـذلك دقـائق الفضـة النانومتريـة المخلقـة حيويـا باسـتخدام فطر التربـة *Fusarium oxysporum* ضد نـوعين من Acari) spp (Tetranychidae: *Tetranychus*)

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ان اكتشاف إستراتيجيات حيوية جديدة لمكافحة الأفات الضارة يعد من الإنجازات الصديقة للبيئة. وتمثل الكائنات الحية الدقيقة واحدة من تلك الإستراتيجيات و تعد بكتيريا *Bacillus thuringiensis* والتى تنتج السموم البروتينية الدلتا توكسين من الوسائل الآمنه للبيئة وكذا لصحة الإنسان. كما تعد فى الوقت الراهن تقنية الدقائق متناهية الصغر الخضراء (النانوتكنولوجى) واحدة من أكثر التطبيقات الواعدة فى هذا المجال. ومن هذه الدراسة ظهرت قدرة *Bacillus thuringiensis* على إنتاج البروتين الكريستالى السام باستخدام صبغة Coomassie Brilliant Blue . كما تم استخدام دقائق الفضة النانومترية المخلقة حيويا باستخدام فطر التربة *Fusarium oxysporum* لتقييم تأثيرها كمبيدات حيوية على الأنثى البالغة لنوعين من الاكاروس التابعين لعائلة تترنيكيدى لهما أهمية إقتصادية وهما Koch *Tetranychus urticae* و *Tetranychus cinnabarinus (Boisduval)* . أيضا تم استخلاص مواد حيوية أخرى وتقييم تأثيرها كمبيدات حيوية على كلا النوعين ،وهذه المواد هى التوكسين المنتج من بكتيريا *Bacillus thuringiensis* وكذلك المستخلص المائى والمستخلص الإيثانولى للبروبيليس. وأوضحت النتائج أن دقائق الفضة النانومترية أكثر فعالية من توكسين *Bt* ومن مستخلصات البروبليس. وكانت قيم LC_{50} للنوع Koch *Tetranychus urticae* هى كالآتى: 45 - 10234- 12755 – 14972 لكل من دقائق الفضة النانومترية – المستخلص المائى للبروبليس – المستخلص الإيثانولى للبروبليس- توكسين *Bt* على الترتيب. وبالنسبة للنوع (*Tetranychus cinnabarinus (Boisduval* كانت قيم LC_{50} كالآتى: 37 – 13579 – 15881– 17003 لكل من دقائق الفضة النانومترية – المستخلص المائى للبروبليس – المستخلص الإيثانولى للبروبليس – توكسين *Bt* ، على الترتيب. أظهرت النتائج أيضا، أن أعلى نسبة موت كانت مع أقل تركيز من دقائق الفضة النانومترية (10 جزء فى المليون) لكلا النوعين على الترتيب بينما باقى المواد الحيوية المستخدمة كانت أكثر فعالية عند أعلى تركيز (10×20 جزء فى المليون). وقد أعطت النتائج المتحصل عليها مؤشرا على أن دقائق الفضة النانومترية المخلقة حيويا من فطر التربة *Fusarium oxysporum* مبيدات حيوية أكثر فعالية.