Plant Archives Vol. 19, Supplement 2, 2019 pp. 365-369 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

INSECTICIDAL ACTIVITY OF THE TOXIN DIKETOPIPERAZINES COMPARING WITH ITS NANO COMPOSITION ON *CERATITIS CAPITATA* UNDER LABORATORY AND FIELD CONDITIONS

Sabbour M.M.[1] and Nayera Y. Solieman[2]

[1.]Department of Pests and Plant Protection, National research center 33rd ElBohouth St. - Dokki, Giza, Egypt
[2.]Agriculture Economic Department National research center 33rd ElBohouth St. - Dokki, Giza, Egypt
Corresponding author. Prof. Magda Sabbour. E-mail: sabbourm@yahoo.com
Tel.: +202/01223305136 Fax: +202/33370931

Abstract

Diketopiperazines (DKPs) is one of the most effective bio pesticide. The olive plant is a very important crop in Egypt. *Ceratitis capitata* is one of the most olive fruit harmful pest which causes decrease in the yield. DKPs used isoplated from the fungus *Nomuraea rileyi* the nano DKPs Six concentrations were prepared 10, 15, 20 30, 50 and 70ppm were used in bioassays. Results showed that the LC_{50} of Diketopiperazines recoded 74 PPm when *C. capitata* treated with different concentrations Diketopiperazines. When the nano Diketopiperazines applied on the target pests the LC50 recorded was 32 PPm. Under field conditions the infestations of *C. capitata* were significantly decreased to 25±4.2 and 27±2.7 individuals after treated with Diketopiperazines in Ebn Malek (Nobaryia), during 2016, 2017 respectively in Ebn Malek (Nobaryia). In the same last places the nano Diketopiperazines application showed a significantly decrease in the pests infestations reached to 10±2.3 and 10±1.1 individuals as compared to 95±5.2and 96±6.9 individuals in the control. The yields weight in both two regions were significantly increased in the two areas after the nano Diketopiperazines treatments. The usage of nano DKPs give a promising results under laboratory and field conditions against *C. capitata*.

***Key words*:** *Ceratitis capitata,* diketopiperazines, nano, olive.

Introduction

Olive (*Olea europaea* L.) has become one of the important economical crops in Egypt. Its cultivated area expanded largely year by year especially in the last ten years. Olive trees planted in the decade lands, particularly in new reclaimed arid areas (Espin *et al.*, 1989). Olive trees attack by many harmful insect that affect on the fruits and oil. Between the most harmful pest species *Ceratitis capitata* (Konstantopoulou and Mazomenos 2005). *C. capitata* is the key pest damaging olive in the world as well as in Egypt Saafan (1986), it was a native to Mediterranean countries which has 98% of the world's cultivated olive trees. *Ceratitis capitata* is one of the most important insect pests of olives in Egypt and other Mediterranean countries. The insect pests develops three generations per year Saafan (1986).The Mediterranean fruit fly *C. capitata* (Wiedermann) among the serious insect pests which attack the olive fruits and cause an economical destruction to the olive trees. These pests were controlled by chemical insecticides which pollute the environment and causes cancer diseases, where bio-insecticides could control these pests safely. The fungi were the most pathogenic to *C. capitata* causing 97.4 and 85.6% mortality Saafan *et al*. (2000).

Diketopiperazines (DKPs) considered among the cyclic dipeptides which have been showmen in natural products. (DKPs) have antimicrobial, antiviral, antitumor and Immune suppressive activities Prasad (1955). lately, there is an increasing to the importance of these natural materials because of their bioactivity. Most of the literatures of This (DKPs) focuse on the biosynthetic pathways , chemical structures, and biological activities Sathya *et al.* (2016).

The present study aims to evaluate the pathogenicity of the Diketopiperazines and nano Diketopiperazines (DKPs), on *Ceratitis capitata* under laboratory and field conditions in olive trees.

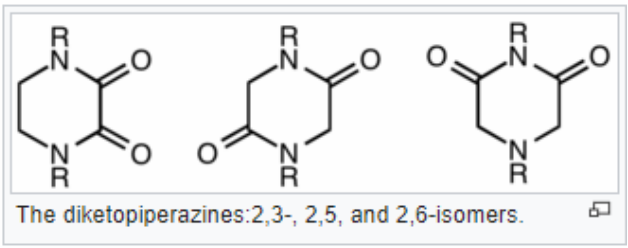

Fig. 1: Diketopiperazines, chemical structure.

Diketopiperazines chemical structure shown in figure 1, which consists of The diketopiperazines:2,3-, 2,5, and 2,6-isomers

Materials and Methods

Laboratory tests: *Ceratitis capitata* adults used in the present work were obtained from laboratory colonies maintained in our laboratory at 25±2°C and 60–65% relative humidity (RH) and 12:12 (L:D) photoperiod. Adults were provided with water and a solid diet consisting of 40% sugar, 10% hydrolyzed yeast, 5% egg yolk. Adults reared in cylinder glass cages (15cm diameter x 22cm height), covered with muslin, and fed on 10% sucrose solution.

Diketopiperazines isolated from the fungus *Nomuraea rileyi* according to Karenina *et al.* (2017). The isolated Diketopiperazines were prepared by the microbiological team in National Research Centre. Six concentrations were prepared 10, 15, 20 30, 50 and 70ppm were used in bioassays. The prepared leaves were dipped in concentrations for 10 sec and allowed to dry for about one hour. The treated leaves were placed in Petri dishes and held in incubator conditions (27±1°C). The bioassays were replicated four times for any isolate and dilution and the control was containing only distilled water. Mortality was recorded at 1, 2, 3 and 4 days after treatment.

The probit and T-test options of SPSS software were used for analyzing time-mortality and comprising means of mortality, respectively. The percentages of mortality were calculated after seven days and corrected according to Abbott (1925), while LC_{50} s was calculated through probit analysis according to Finney (1971).

Field experiments: The experiments were carried in Ebn Malek (Nobaryia) and El-Kanater, during the two successive seasons 2016 & 2017 starting from the first of July till the end of August to evaluate the efficacy of the tested fungi against the target insect pests under field conditions. Three random patches of Olive trees were selected, each comprised 12 trees (12 trees for Diketopiperazines applications and 12 for nano Diketopiperazines, and 12 trees for control) to carry out the field experiment. Diketopiperazines was applied, each as a single treatment at the rate of 60 ppm nano–Diketopiperazines at 10ppm. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten liter sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of the application. Each treatment was replicated four times. Four plots were treated with water as control. Random samples of leaves and fruits olives plants were weekly collected from each treatment and transferred to laboratory for examination. The infestation of, *C. capitata* were estimated in each case. After harvest, yield of each treatment was weighted as Kg/Feddan.

Results and Discussions

Under laboratory conditions

Results showed that the LC50 of Diketopiperazines recoded 74 PPm when *Ceratitis capitata* treated with different concentrations *Diketopiperazines*. When the nano *Diketopiperazines* applied on the target pests the LC50 recorded was 32 PPm. Under field conditions the infestations of *C. capitata* were significantly decreased to 25±4.2 and 27±2.7 individuals after treated with *Diketopiperazines* in Ebn Malek (Nobaryia), during2016, 2017 respectively in Ebn Malek (Nobaryia). In the same last places the nano Diketopiperazines application showed a significantly decrease in the pests infestations reached to 10±2.3 and 10±1.1 individuals as compared to 95±5.2and 96±6.9 individuals in the control. After the harvest period the weight of the olive fruit were significantly increased to 5104± 76.57 and 5116± 82.84kg/feddan in trees treated with Nano-Diketopiperazines in Ebn Malek (Nobaryia)during season 2015 and 2016 respectively. In El-Kanater the weight of olive fruits significantly 5128±69.44 and 5148± 73.77kg/feddan in trees treated with the corresponding pathogen**.**

Table 4, indicates that treating olive trees by using Nano-Diketopiperazines and Diketopiperazines increase acre productivity in the area of Nobaryia by about 13%, 35.9%, 19%, 39.2% while the increase estimated almost 24%, 42%, 28%, 42% in the area of Kanater in 2015 and 2016. The study of abovementioned treatments effect on the invested pound return per produced ton entails the study of produced ton revenue and produced ton return by relying on the rate of feddan productivity increase average as shown in Table 4. Table 4 shows olive trees treatment in the Nobaryia area by using Nano-Diketopiperazines led to the return rise of the invested pound estimated about L.E. 1.343 by increase rates of almost 15.5% and 19.8% and the return rise of the invested pound in the area of Kanater estimated about L.E. 1.343 by almost 7% and 20.6% compared to treatments using Diketopiperazines and Control respectively as shown in the Table. Therefore, the study recommends the necessity of the state adoption of generalizing these treatments at the olive farms level all over the republic through well-trained agricultural guides to ensure the return rise of the invested pound. This will lead to attracting investors to invest in olive agriculture as the investor income will increase because of exporting the production increase rate. Table 4: Return of the invested pound per produced ton of different vital treatments.

Karenina *et al*., 2017, reported that There is little information on the insecticidal activity of DKPs. Only Cycloechinulin, was reported to be effective in the control of coleoptera and lepidoptera such as *Heliothis zea* and *Carpophilus hemipterus*. Three DKPs isolated from *Eurotium cristatum* with the isopentyl group substituted by indole residues, showed cytotoxic activity against *Artemia salina*.

Recent studies revealed that Cycle (Trp-Phe) exhibited dose-dependent anti-alimentary, larvicidal and pupicidal activity against *H. armigera*. In addition, the purified compound prolonged the larval and pupal period when compared to the untreated control Sathya *et al* (2016).

DKPs corresponding to cyclic dipeptides have been isolated from micro-organisms, from sponges and from a variety of tissues and body fluids Rudi *et al*., 1994, Strom *et al*., 2002 and Rosa *et al*., 2003). DKPs are more than simple curiosities Sabbour (2015a) and are well known for their economically beneficial biological activities Prasad (1995). DKPs are relatively simple compounds and therefore are among the most common peptide derivatives found in nature Prasad (1955).

The presence of the diketopiperazine 127 as a co-metabolite in BCC 1449 indicated that replacement of the a-H-atoms with S-atoms should take place with retention of configuration Rudi *et al*. (1994). resources contain a number of DKPs that could act as lead structures for further researches on other biological tests Karenina *et al.* 2017, Sathya *et al.* (2016), Rosa *et al.* (2003), Strom *et al*. (2002) and Rudi *et al.* (1994). Owing to their chiral, rigid and functionalized structures, they bind to a large variety of receptors with high affinity, giving a broad range of biological activities Martins and Carvalho (2007). Therefore, DKPs are attractive structures for the discovery of new lead compounds for the rational development of new therapeutic agents. These results agree with Sabbour (2015a), reported that the nano biopesticide imidacloprid decrease the infestations of olive pests in the field and reduce the infestations under laboratory conditions. Sabbour (2012), proved that the application with bioinsecticides decrease the pollution in the field and reduce the infestations with the olive insects. The infections of insects decreased and the yield increased by the treatments of spinosad in the field and laboratory conditions Sabbour (2013a). The yield significantly increased, the same obtain by Sabbour (2015b) who reported that *Isaria fumosorosea* and *Metarhizium flavoviride* increased the yield and decreased the infestation with insect pests in potato field. Also, results were in accordance Sabbour (2013b) who use the entomopathogenic fungi *I. fumosorosea* against olive pests. The

entomopathogenic fungi *Nomuraea rileyi* destroy the olive pests in the field and the toxin DKPs affect on the decreasing the olive pests number under laboratory and field conditions Sabbour Sabbour (2017b). The usage of the bioinsecticides toxin cases a loss and significantly decrease in the number of insect infestations especially when used in the nano pictures Sabbour MM, Singer (2015) found that the toxin Destruxin reduce the infestations number s of olive pests in the field. Sabbour (2017a) controlled the olive harmful pests, *Zeuzera Pyrina* by Destruxin in the olive fields.). The toxin of the bio insecticide imidacloprid cases a significant decrease to *Prays oleae* under field conditions and leads to increase to the yield weight (Sabbour, 2017 a,b, Sabbour, 2016; Sabbour and Nayera, 2016), reduce the infestations by *Schistocerca gregaria* in the corn field. Sabbour (2015), Sabbour and Hussein (2016) reported that the usage of the nano bioinsecticides cause a decrease in many insect pests and control many insects under field conditions

Acknowledgment

This research was supported by Agric. Division, National Research Centre, Cairo, Egypt.

Conclusion

The our results are the first account of insecticidal activity of dikepiperazines (DKPS) against olive pest under laboratory and field conditions. nano-formulation of *Diketopiperazines* , was more effective than *Diketopiperazines,* in controlling *C. capitata.* These results encourage the extension in the use of the nanotechnology for insect pest control. These results would be promising for the development of biocontrol agents form any agriculture harmful pests.

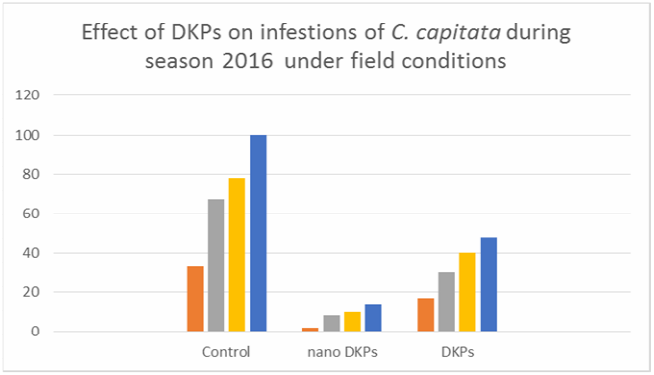

Fig. 2 : The infestations of the target pests under field conditions during season 2016.

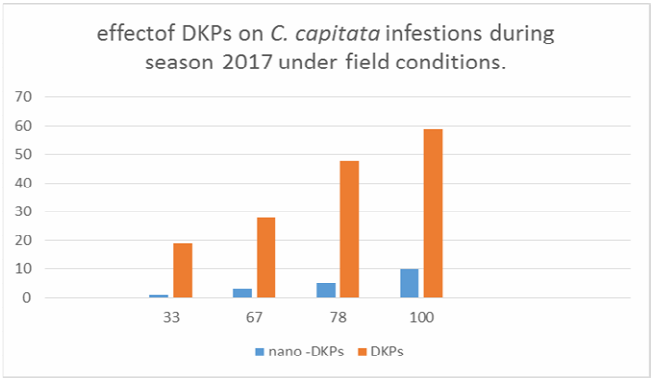

Fig. 3 : The infestations of the target pests under field conditions during season 2017.

Table 1: Effect of tested materials on the target insect pests under laboratory conditions

Pathogens	LC_{50}	Slope	Variance	95% Confidence limits
Diketopiperazines	74 PPm	0.01	0.02	106-59
Nano- Diketopiperazines	32 PPm	0.01	0.02	100-23

Table 2: Infested plants with target insect pests after treatment with the tested pathogens under field conditions through out the two areas during 2015-2016.

Treatment	Days after treatment	Ebn Malek (Nobaryia) El-Kanater			
		2016	2017	2016	2017
Control	20	29.0±2.1	30.2±10	29.4±2.9	29.2±9.4
	50	60±2.3	70±.2	67±3.4	72±3.4
	90	68±3.4	86±2.4	88±3.7	89±4.6
	120	95±5.2	96±6.9	99±3.3	96±3.4
Diketopiperazines	20	0±0.0	1.1±1.8	1.7±2.7	5.4±5.3
	50	13±2.2	13±3.1	19±4.5	17±4.4
	90	20±4.1	21±3.1	23±3.4	27±3.4
	120	25±4.2	27±2.7	27±8.7	28±5.9
Nano- Diketopiperazines	20	0±0.0	0±0.0	1±1.1	3 ±2.8
	50	1±1.5	1±5.1	5±3.4	6±2.8
	90	5±2.5	5±1.2	6±3.4	6±1.7
	120	10±2.3	10±1.1	11±3.5	11±7.2

Table 3: Weight of harvested olive fruits after treatment with the pathogens against target insect pests during two successive seasons in the planted areas.

Treatment	Wt. kg/feddan			
	Ebn Malek (Nobaryia)		El-Kanater	
	2016	2017	2016	2017
Control	3781± 19.81	3762± 62.12	3611±80.13	3640± 36.36
Diketopiperazines	2473± 69.32	4477± 79.38	4478±65.61	4640± 62.38
Nano-Diketopiperazines	5104± 76.57	5116± 82.84	5128±69.44	5148± 73.77
F-value	23.2		33.1	
LSD 5%	73		83	

Table 4: Effects of olive trees vital treatments on the invested pound return per produced ton

Treatment	Ebn Malek (Nobaryia)				El-Kanater			
	Productivity increase %	Ton production cost	Ton return	Return/ invested pound	Productivity increase %	Ton production cost	Ton return	Return/ invested pound
Diketopiperazines	16	4301	5000	1.163	26	4129	5000	1.211
Nano-Diketopiperazines	37.6	3723	5000	1.343	42	3858	5000	1.296
Control	3.772	4460	5000	1.121	3.618	4650	5000	1.075

Source: computed from Table 4, Ministry of Agriculture and Lands Reclamation- Annual Newsletters of costs in 2015.

References

Abbott, W.W. (1925). A method of computing the effectiveness of an insecticide, J. Economic Entomol. 18: 265-267.

Espin, G.A.; Laghi, T.; De Messias, C.L. and Pie-Drabuena, A.E. (1989). Pathogenic dad de *Metarhizium anisopliae nas* diferentesfases de desenv olvimen to de *Ceratitis capitate* (Wied.) (Diptera: Tephritidae), Revista Brasileria de Entomologia, 33: 17-23.

Finney, D.J. (1971). Probit Analysis, Cambridge, Cambridge University Press.

Guzman, F.S.; Dowd, P.F.; Gloer, J.B. and Wicklow, D.T. (1993). *Cycloechinulin antiinsectan* metabolite, US5196420 A.

Karenina, M.; Analía, S.; Alicia, B.; Elena, C.; Mario, A. and Nancy, V. (2017). Insecticidal activities of diketopiperazines of *Nomuraea rileyi* entomopathogenic fungus, 2(4): 1586- 1596.

Konstantopoulou, M.A. and Mazomenos, B.E. (2005). Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. BioControl, 50(2): 293-305.

Krejcarek, G.E.; Dominy, B.H. and Lawton, R.G. (1968). The interaction of reactive functional groups along peptide chains. A model for alkaloid biosynthesis, Chem Commun, 22: 1450–1452.

Martins, M.B. and Carvalho, I. (2007). Diketopiperazines: biological activity and synthesis, Tetrahedron 63: 9923–9932.

Prasad, C. (1995). Bioactive Cyclic Dipeptides. Peptides, 16: 151-164.

Rosa, S.D.; Mitova, M. and Tommonaro, G. (2003). Marine bacteria associated with sponge as source of cyclicpeptides, Biomol Eng., 20: 311–316.

Rudi, A.; Kashman, Y.; Benayahu, Y. and Schleyer, M. (1994). Amino acid derivatives from the marine sponge Jaspis, Digonoxea. J Nat Prod. 57: 829.

Saafan, M.H. (1986). Studies on the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) with emphasis on sterile insect technique (SIT), Ph.D. Thesis, Faculty of Agriculture, Cairo Univ.PP166.

Saafan, M.H.; Tadros, A.W. and Foda, S.M. (2000). Certain pests of edible fig trees 1-ecological studies on fruit flies in fig orchards at the north-western coast of Egypt, Egyptian J. Agric. Res., 78(5):1967-1977.

Sabbour, M.M. (2015a). Nano-Imidaclorprid against three olive pests under laboratory and field conditions, J. Biosci. Bioengin. 2: 45-49.

Sabbour, M.M.; Abd-El Rahman, M.A. (2012). Efficacy of some microbial control agents against olive insect pests in Egypt. Journal of Applied Sciences Research, 8(7): 3448-3452.

Sabbour, M.M. (2013a). Efficacy of *Nomuraea rileyi* and Spinosad against olive pests under laboratory and field conditions in Egypt, Global J. Biodiv. Sci. Manag. 3: 228-232.

Sabbour, M.M. (2015). The Toxicity effect of nano- fungi *Isaria fumosorosea* and *Metarhizium flavoviride* against the potato tuber moth, *Phthorimaea operculella* (Zeller)" Am. J. Biol. Life Sci., 3: 155-160.

Sabbour, M.M. (2015 b). Efficacy of *Isaria fumosorosea* against olive pests under laboratory and field conditions in Egypt, I. J of development, (1): 55-61.

Sabbour, M.M. (2013b). Efficacy of *Nomuraea rileyi* and Spinosad against olive pests under laboratory and field conditions in Egypt, Global Journal of Biodiversity Science and Management, 3(2): 228-232.

Sabbour, M.M. and Singer, S.M. (2015). The entomotoxicity of Destruxin and Nano-Destruxin against three olive pests under laboratory and field conditions, International Journal of Scientific & Engineering Research, 6(8): 1954-1961.

Sabbour, M.M. (2017a). Probing the Effects Destruxins on Leopard *Zeuzera Pyrina* (L.) (Lepidoptera: Cossidae), in olive Trees, Agricultural Studies, *Agricultural Studies,* 1(1): 41-49.

Sabbour, M.M. (2017 b). Pathogenicity effect of bio pesticides and its nano against *Prays oleae* (Lepidoptera: Plutellidae), in Press.

Sabbour, M.M. (2016). Observations of the effect of Chitosan and its nano compositions against the locust *Schistocerca gregaria* (Orthoptera: Acrididae), International Journal of Chem Tech Research, 9(6): 270-276.

Sabbour, M.M. and Hussein, M. (2015). Usage of the nano phosphorous fertilizers in enhancing the corn crop and its effect on corn borers infestations after fungi treatments, International Journal of Chem Tech Research 8(9): 167-173.

Sabbour, M.M.; Nayera, Y. and Solieman, (2016). Control of grasshopper *Hetiracris littoralis* (Orthoptera: Acrididae) by using nano-imidaclorprid in corn fields,

International Journal of ChemTech Research, 9(01): 259-266.

Sathya, A.; Vijayabharathi, R.; Kumari, B.R.; Srinivas, V. and Sharma, H.C. (2016). Palanisamy Sathyadevi P, Gopalakrishnan S, "Assessment of a diketopiperazine, cyclo(Trp-Phe) from *treptomyces griseoplanus* SAI-25 against cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae)", Appl Entomol Zool. 51, 11.

Strom, K.; Sjogren, J.; Broberg, A. and Schnurer, J. (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-pro) and 3-phenyllactic acid, Appl Environ Microbiol., 68: 4322–4327.