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ChemTech

International Journal of ChemTech Research

CODEN (USA): IJCRGG ISSN: 0974-4290

Vol.8, No.9, pp 167-173, 2015

Usage of the nano phosphorous fertilizers in enhancing the corn crop and its effect on corn borers infestations after fungi treatments

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Abstract: The effect of the fertilizer phosphorous and the bio-insecticide fungi, were studied under laboratory and field conditions. Results, showed that the LC50 of the target insect pests after treatment with nano phosphorous mixed with the entomopathogenic fungi under laboratory conditions. The obtained data show that LC50 of *O. nubilalis* recorded 101 and 116 conidia/ ml after treated with *B. bassiana* (B.b) + Nano-Phosphorous fertilization and *M. a*nisopliae (*M.a*)+ Nano-Phosphorous fertilization respectively. When *S. cretica* were treated with corresponding treatments the LC50 cached 106 and 119X 10^4 conidia/ml. The applications of the nano bioinsecticides *M. anisopliae* and *B. bassiana* after the corn plants fertilize by the nano phosphorous fertilizer which detected that in the control plots the infestation of *Ostrinia nubilalis* were 78±2.3and 86±2.1 individuals during season 2013 and 2014., respectively after 100 days of applications. The infestations of *Sesamia cretica* reached to 88±5.1 and 93±2.9 individuals after 100 days of applications. *Chilo* agamemnon individual recorded after100 days during 2013 and 2014, 99±3.9 and 99±2.3 individuals., respectively. When the corresponding insect pests treated with *M.a* only during season 2014, the individual mean number obtained, 28±2.8, 26±2.1 and29±9.3 individuals for the three pests., respectively. The infestation were highly significant decreased among the *B.b*+ Nano-Phosphorous fertilization. The inflation means number for the three pest 8±2.9, 9±2.9 and 12±9.3individuals during 2014. During season 2013, the field applications in maize field , show that the nano phosphorus have a positive effect for increasing the corn weight which reached to 5563±32.92 kg/ feddan after nano phosphorous fertilizations plots. In the plots treated with *B.b*+ Nano-Phosphorous fertilization the weight of the corn recorded 6667±89.76 kg/ feddan Kg/ feddan as compared to 2310±79.02kg/ feddan in the control . During season 2014 the B.b+ Nano-Phosphorous fertilization recoded the highest weight amount reached to 7866 ±89.76kg/ feddan as compared to 2002 ±34.12kg/ feddan in the control plots.

Key Words: *Beauveria bassiana, Metarhizium anisopliae, Ostrinia nubilalis, Chilo agamemnon, Sesamia cretica.*

Introduction

Maize (*Zea mays* L.) is an important crop all over the world and also in Egypt. Its demand continuously increases. Corn is subjected to attack by many insect pests that affect the yield quality and quantity. Among the most common pest species surveyed in Egypt are: the European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae), *Sesamia cretica* (Lepidoptera : Noctuidae). *Chilo agamemnon*. *O. nubilalis* is the one of key pest damaging corn fruit in the world as well as in Egypt [1]. *O. nubilalis* is native to Mediterranean countries which has 98% of the world's cultivated corn plants [2]. *C. agamemnon* is also one of the most important insect pests of corns in Egypt and other Mediterranean countries. The moth develops three generations per year [2]. In Egypt the first generation of moths appears in April and female lays its eggs on the flower buds after that newly hatched larvae feed on the buds and flowers of corn plants [2]. When adults of corn pests were exposed to conidia of *Mucorhiemalis, Penicillium aurantiogriseum, P. chrysogenum* and *Beauveria bassiana* isolates through contact and oral bioassays showed moderate to high mortality [3] .*Nomuraea rileyi* and *Paecilomyces fumosoroseus* proved highly pathogenic to aphids and whiteflies [3]. The fungus exhibit host preferential infections in lepidopterous larvae [4, 5]. The entomopathogenic fungi, are of particular research interest because of their potential as commercial bioinsecticides. Some studies had focused on identifying nutrient substrates that *B. bassiana* can utilize with application to industrial production, while others focused on the pathogenic processes of *B. bassiana*, *M. anisopliae* interactions with insect cuticle [6]. It is necessary to find alternative safe insecticides to reduce the heavy doses of chemical insecticides which are used for the control of corn pests [5,6].Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops [7,8]. Commercial products have been developed with entomopathogenic fungi [9] [9, 10, 11,12,13,14] reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* and *N. rileyi* affected the larval development, movement and mobility of corn borers larvae during the seedlings and vegetative stages of corn plant under laboratory; greenhouse and field conditions. However, the success of a pest control program using *fungi* depends on conidia survival in the field environment [15]. Conidia survival may be affected either by environmental factors or chemical products used to protect plants. [16] controlled the cereal pests with the fungus *B. bassiana* and found that the infestation was reduced after fungal applications under laboratory and field conditions.

The present study aims to evaluate the pathogenicity of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* (bio-insecticide) against corn insect pests *Sesamia cretica, Ostrinia nubilalis* and *Chilo agamemnon* under laboratory and field conditions.

Materials and Methods

Tested Insects:

Sesamia cretica, Ostrinia nubilalis and *Chilo agamemnon* were eared on corn leaves under laboratory conditions 26±2°C and 60±5% RH. Leaves changed every second day.

Entomopathogenic Fungi:

The fungi, *B. bassiana*, *M. anisopliae* were obtained from Florida Univ., USA. They were reproduced on potato dextrose agar (PDA) plus 0.4% yeast extracts (PDAY) and poured onto sterilized Petri-dishes. Plating was performed according to the full dish method. The conidia were transferred from the appends of vial to dish containing medium by platinum loop and then streaked. Plates were incubated at 25°C with 12 hours photo-phase for fungus growth and sporulation. After ten days, conidia were scraped and transferred to conical flasks (250 ml) containing 200ml sterilized distilled water with 0.02% the speeder sticker (Tween-80). Conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemacytometer. Then the suspensions were standardized until the direct concentration $1x10^7$ conidia/ml was achieved.

Efficacy of Entomopathogenic Fungi against pests larvae:

Spores of the entomopathogenic fungi; *B. bassiana, M. anisopliae*, collected from the surface of mycelium growth and spore suspensions with 2 drops of Tween-80 were prepared and adjusted at 1x107 conidia/ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hours, using light microscope at 400X. Conidial viability was 95-100%. The surface of cultures was gently brushed in the presence of 20ml of sterilized water in order to free the spores and the suspension was filtered through muslin. Six concentrations of spore suspensions were prepared *i.e.*, 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 conidia/ml. Piece of corn leaves were dipped in the prepared suspensions and left for drying under laboratory conditions then placed in Petri-dishes (one concentration/dish). For each concentration (4 replicates/each), ten of 3rd instar larvae (L3) of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated corn leaves. Percentages of mortality were calculated according to [17], while LC_{50} was calculated throughout probit analysis. The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH.

Field Trials:

Field trials were carried out at Nobaria region (Behera Governorate), Egypt during the two successive corn seasons 2013 and 2014 to study the effectiveness of the tested fungi on corn borers. Corn (variety Giza-2) was cultivated by end of May during the two seasons in an area of about half feddan (500 meter). Fungi were applied as single treatments in randomize plots and replicated four times. Regular agricultural practices were performed and no chemical control was used during the study period. Weeds were removed by hand. Five plots were sprayed with water as control the experiments were replicated 4 times. Twenty Samples from each treatment were collected weekly and transferred to the laboratory for investigation. Percentages of infection were estimated.

Yield Assessment:

Yield data in treated and untreated plots in the corn harvest seasons (2013and 2014), represented by weight in Kg were determined. The Yield loss was estimated according to the following equation:

Potential yield – Actual yield Potential yield
Yield loss = --
Potential yield

Potential yield is the yield which gave the pest amount among all treatments *B. bassiana* treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones.

Results and Discussion

***In-vitro* effect of Entomopathogenic fungi on the target insects**

Data in Table 1 and 2 show that the LC50 of the target insect pests after treatment with nano phosphorous mixed with the entomopathogenic fungi under laboratory conditions. The obtained data show that Lc50 of *O. nubilalis* recorded 101 and 116 conidia/ ml after treated with B.b+ Nano-Phosphorous fertilization and M.a+ Nano-Phosphorous fertilization respectively . When S. cretica were treated with corresponding treatments the LC50 cached 106 and 119X 10^4 conidia / ml (Table1&2).

Table (1): Determinations of entomopathogenic fungi, *B.b*+ Nano-Phosphorous fertilization against the target insect pests larvae under laboratory conditions

Insects	LC_{50}	Slope	variance	95% confidence limits
Ostrinia nubilalis	$101x10^4$	0.1	1.01	94-189
Sesamia cretica	$106x10^4$	0.2	1.00	118-170
Chilo agamemnon	$106x10^4$	0.1	1.03	119-177

Table 2): Determinations of M.a+ Nano-Phosphorous fertilization against target insect pests under laboratory conditions

Insects	LC_{50}	slope	variance	95% confidence limits
Ostrinia nubilalis	116	1.01	0.02	110-166
Sesamia cretica	119	0.10	1.01	101-167
Chilo agamemnon	115	0.10	1.01	100-169

Data in table 3 show the applications of the nano bioinsecticided *M. anisopliae* and *B. bassiana* after the corn plants fertilize by the nano phosphorous fertilizer which detected that in the control plots the infestation of *Ostrinia nubilalis* were 78±2.3and 86±2.1 individuals during season 2013 and 2014, respectively after 100 days of applications. The infestations of *Sesamia cretica* reached to 88±5.1 and 93±2.9 individuals after 100 days of applications. *Chilo* agamemnon individual recorded after100 days during 2013 and 2014 , 99±3.9 and 99±2.3 individuals ., respectively (Table 3). When the corresponding insect pests treated with *M.a* only during season 2014, the individual mean number obtained, 28±2.8, 26±2.1 and 29±9.3 individuals for the three pests., respectively. The infestation were highly significant decreased among the *B.b*+ Nano-Phosphorous fertilization. The inflation means number for the three pest 8±2.9, 9±2.9 and 12±9.3individuals during 2014 (Table 3).

Table (3): Determinations of different treatments on the target insect pests under field conditions

Post 1st application date	Treatments	Number of three pests infestation (Mean±SE) during the two seasons					
		Ostrinia nubilalis		***Sesamia cretica***		***Chilo* agamemnon**	
		2013	**2014**	**2013**	**2014**	**2013**	**2014**
15 45 100	Control (without any treatments)	44±1.2 69±4.1 78±2.3	69±3.1 77±3.2 86±2.1	61±2.1 79±3.4 88±5.1	71±9.1 85±4.9 93±2.9	69±3.4 85±5.4 99±3.9	78±2.8 93±1.9 99±2.3
15 45 100	*M. anisopliae (* M.a) only	10±3.2 19±4.3 28±2.3	10±9.3 19±3.7 28±2.8	11±2.5 12±3.4 25±5.1	10±9.3 13±2.5 26±2.1	11±3.4 11±3.8 28±5.9	11±2.2 11±5.3 29±9.3
15 45 100	*B. bassiana* (B.b)only	9±3.2 12±4.3 22±2.3	9±9.3 12±3.1 22±2.7	6±2.5 17±3.4 18±5.4	7±9.3 18±2.5 19±2.1	7±3.4 14±3.4 18±3.4	7±2.2 15±7.3 19±4.3
15 45 100	Nano-Phosphorous fertilization only	40±3.2 59±4.3 78±2.3	59±9.3 70±3.1 87±2.9	60±2.5 79±3.4 89±5.9	70±9.3 83±4.5 92±7.5	65±3.4 84±4.4 98±5.8	72±2.1 91±6.3 99±5.4
15 45 100	*M.a*+ Nano-Phosphorous fertilization	8±3.2 9±4.3 10±2.3	9±9.8 10±3.1 10±2.1	6±7.5 9±3.4 10±5.1	7±9.7 8±7.5 12±2.1	7±8.4 8±8.4 12±3.9	7±9.2 11±1.3 12±9.3
15 45 100	*B.b*+ Nano-Phosphorous fertilization	4±3.2 5±4.8 8±2.3	6±9.3 7±3.9 8±2.9	6±5.5 7±4.4 8±5.1	7±9.3 8±2.5 9±2.9	8±3.4 9±3.6 11±6.9	9±2.6 9±6.3 12±9.3
F-value LSD at 5%		33.7 112	32.9 111	30.8 100	30.5 101	30.6 102	33.7 121

During season 2013, the field applications in maize field , show that the nano phosphorus have a positive effect for increasing the corn weight which reached to 5563±32.92 kg/ feddan after nano phosphorous fertilizations plots. In the plots treated with *B.b*+ Nano-Phosphorous fertilization the weight of the corn recorded 6667±89.76 kg/ feddan Kg/ feddan as compared to 2310±79.02kg/ feddan in the control (Table4) . During season 2014 the B.b+ Nano-Phosphorous fertilization recoded the highest weight amount reached to 7866 ±89.76kg/ feddan as compared to 2002 ±34.12kg/ feddan in the control plots (Table4).

Table (4): Determinations of the nano- Phosphorous fertilization on yield weight under field conditions

Treatments plots	**Season 2013**	**Season 2014**
	Weight of corn crop (kg/ feddan)	**Weight of corn crop (kg/feddan) yield loss %**
B. bassiana (B.b)only *M. anisopliae (* M.a) only Nano-Phosphorous fertilization B.b+ Nano-Phosphorous fertilization M.a+ Nano-Phosphorous fertilization Control(without any treatments)	5342±31.30 4350±63.32 5563±32.92 6667±89.76 5643±87.23 2310±79.02	5998±43.22 4999±53.17 6560±56.26 7866 ±89.76 5999 ±45.32 2002 ±34.12

F-value LSD at 5%	41.2 112	38.4 133

The same results were obtained by [11], who reported that under laboratory conditions results showed that the LC_{50} of *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* of the tested fungi *Verticillium lecanii*, *Nomuraea rileyi* and *Paecilomyces fumosoroseus*, respectively against the three pests *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* ranged between $5.4x10^6$ and $1.43x10^7$ spores/ml. Satisfactory results with the entomopathogenic fungi were reported by [15,16]. [18] as they found that the fungi; *B. bassiana* and *M. anisopliae* reduced the lethal concentration 50 (LC_{50}) of *Spdoptera littoralis* under laboratory conditions.

Data in table (3) show that the application of the bioinsecticides which affected on decreasing the infestation, the number of three pests infestations of *Ostrinia nubilalis* and *Sesamia cretica* significantly decreased to 20±2.1 and 22±1.1., respectively, after treatment with *M. anisopliae* after 20 day as compared to 69±9.3 and 70±9.1 individual in the control for the corresponding pests, during seasons 2014. In all treatments the number of corn pests were significantly decreased. *Chilo agamemnon* infestation decreased to 20±3.3 and 25±3.1 individuals after 90 days during season, 2014 and 2013 as compared to 98±9.3 and 99±9.3 individuals in the control plots in both two seasons.The obtained results are similar to other studies carried out by [19] and [3] on their work on *Ceratitis. capitata.*

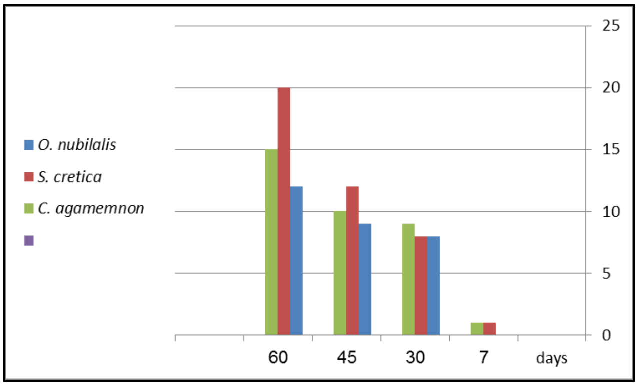

Fig (1) Effect of the fungus B. bassiana under field conditions

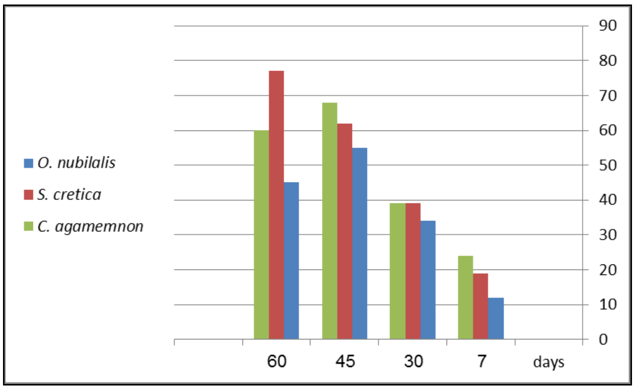

Fig (2) Effect of the fungus *M. anisopliae* under field conditions

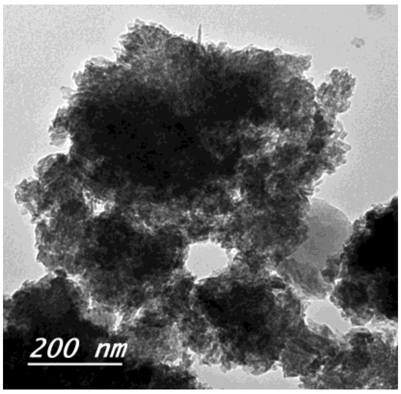

Fig. (2). Scanning electron microscopy of the phosphorous nano- particles.

Figures (1 & 2) show that the percentage of infestations were significantly decreased during both seasons. These results are also agree with [20, 21] Efficacy of some bioinsecticides against *Bruchidius incarnatus* (BOH.) (Coleoptera: Bruchidae) Infestation during storage. The same obtained agree with [19, 22, 23] who Also, results were in accordance with [19] who reported that the virulence of *B .bassiana* against *Ceratitis capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. [3] recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments. The same results were obtained by [20, 21, 22] when they controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. [11, 12, 13,23]. [24] found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. [25, 26, 27, 28, 29, 30, 31, 32, 33] proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests.

Conclusion

The uses of the bioinsecticides is demanded especially the fungi *B. bassiana, M. anisopliae* under corn field conditions for controlling the three harmful corn borers, *Ostrinia nubilalis, Chilo agamemnon* and *Sesamia cretica.*

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