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The Singular and Combined Effects of Entomopathogenic Fungi *Beauveriabrongniartii* and the Insecticide Imidaclorprid against Corn Pests under Laboratory and Field Conditions in Egypt

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Abstract: ***Under laboratory conditions the LC_{50} obtained was 132 x10^4, 144 x10^4, 170 x10^4 conidia/ml after Ostrinia nubilalis, Sesamia cretica and Chilo agamemnontreated with different concentrations of Beauveria brongniartii respectivelyWhen the two tested pathogens combined at the sublethal does the LC50 for the corresponding insect pests recoded were significantly decreased to 46, 69 and 75 g/L for O. nubilalis, S. cretica and C. agamemnon ., respectivelyAt the harvest time the corn weight obtained 3922±54.6 and 3110±60.4 kg/Feddan among the harvested plots treated with B. brongniartii and imidaclorprid plots as compared to 2710±40.9 and 2511±73.2 kg/Feddan during seasons 2012 1nd 2013, respectivelymortality ranged between 69 and 78% after bioinsecticides treatments.***

Keywords: *Beauveria brongniartii, Ostrinia nubilalis, Chilo agamemnon, Sesamia cretica.Imidaclorprid*

1. Introduction

Maize (Zea mays.) 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: *Ostrinia nubilalis*, *Sesamia cretica* and *Chilo agamemnon*. *O. nubilalis* is the one of key pest damaging corn in Egypt (Eid, 2003). *O. nubilalis* is native to Mediterranean countries which has 98% of the world's cultivated corn (Montiel and Jones, 2002). *Beauveriabrongniartii* (B.b) proved highly pathogenic to aphids and whiteflies (Espinel et al., 2008). The fungus (*B.brongniartii*) exhibit host preferential infections in lepidopterous larvae, the fungi penetrate the integuments, spread rapidly blocked the respiratory pores lead to pest death (Ignoffo et al 1976).

Imidacloprid is a chloronicotinoid insecticide. Chemical name: 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimineImidacloprid is a systemic, chloronicotinoid insecticide, which kills insects via ingestionor contact. It is effective by disrupting the nervous system of an insect pest. It is usedfor controlling sucking insects, soil insects, termites, and some chewing insects. It isapplied as a seed and soil treatment, crop and structural treatment, and a topical fleacontrol treatment on domestic pets (Imidaclorprid 1998) .

Imidaclorpridis a broad-spectrum, organic insecticide. It is, however, relatively non-toxic to mammals and beneficial insects. If used carefully only against insects that actually eat something that has been treated, such as a leaf, are affected. This is different than a lot of other broad-spectrum insecticides that are toxic if the insect merely comes in contact with dry insecticide residues Sabbour et al. (2012). The entomopathogenic *P. carneus* is found on a wide range of material, and especially in soil. It is sometimes isolated from insects, though it appears to be a weak insect pathogen. Some isolates produce several metabolites of the antibiotic group cephalosporins. *P. farinosus* is also commonly isolated from soil. It is a well-known insect pathogen, and there has been interest in its use as an agent of biological control. Sabbourand Abdel-Rahman,2013, Sabbour et al,2011, Sabbour, 2002, Sahab et al, 2014 control the corn borers by different entomopathogenic fungi under laboratory and field conditions. Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops (Sabbour and Sahab, 2007). Commercial products have been developed with entomopathogenic fungi (Alves and Pereira, 1998). Quintela and McCoy (1998) reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* and *B.brongniartii*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. Success of a pest control program using *B. bassiana* however depends on conidia survival in the field environment (Benz, 1987). Conidia survival may be affected either by environmental factors (Furlong and Pell, 1997) or chemical products used to protect plants (Sabbour and Abd-El-Rahman ,. (2007) controlled the cereal aphids with the fungus *B. bassiana* and found that the infestation was reduced after fungal applications under laboratory and field conditions. Sabbour,(2013 a &b) found that the seed oils control many insect pests in the laboratory and semi fieldconditions.Sabbour et al 2014 used the Jatropha seeds and leaves oil against the corn insect pests.

The present study aims to evaluate the pathogenicity of the isolates of entomopathogenic fungus, *B. broganitii* and imidaclorprid against corn insect pests under laboratory and field conditions. It is necessary to find alternative safe insecticides to reduce the heavy doses of chemical insecticides which is using for the control of corn pests Sabbour and Abel-Rahman (2007).

2. Materials and Methods

2.1 Tested Insects

*Sesamia cretica**;*** *Ostrinia* nubilalis*; Chilo* agamemnon rearedon corn leaves under laboratory conditions 26 ± 2o C and 60± 5 RH. leaves changed every two days.

2.2 Entomopathogenic Fungi

The fungus, *Beauveria brongniartii*isolated from the Egyptian soil from Ismailia governorate. They were reproduced on potato dextrose agar (PDA) plus 0.4% yeast extracts (PDAY) and poured onto sterilized Petri-dishes (Alves *et al.*, 1998). Plating was performed according to the full dish method. The conidia were transferred from the Eppendrof 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 (200 ml) containing 200 ml 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 obtained.

2.3 Efficacy of Entomopathogenic Fungi against Pests Larvae

Spores of the entomopathogenic fungi; *Beauveria brongniartii*, collected from the surface of mycelium growth and spore suspensions with 2 drops of tween 80 were prepared and adjusted at $1x10^7$ conidia/ ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hrs, using light microscope at 400 x. 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/dish). For each concentration (4 replicates/ each), ten L3 larvae of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated castor leaves. Percentages of mortality were calculated according to Abbot, while LC_{50} was calculated throughout probit analysis. The experiment was carried out under laboratory conditions at 26°C± 2 and 60-70 % RH. Physiological and metabolic characteristics of *Beauveria brongniartii.*

2.4 Efficacy of imidaclorprid against the target insect pests

The insecticide imidaclorprid were tested at the 6 concentrations: 6 g, 5g ,4g,3g, 2g,1*g*.The insecticide, prepared 6 concentrations (prepared according Samehe*t al.*, 2009) Percentages of mortality were calculated according to Abbott's formula (Abbott, 1925), while the LC_{50} values was calculated throughout probit analysis (Finney, 1971). The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH.

2.5 Effect of the combined Imidaclorprid+*B. broganitii* at the sub-lethal doses

The sub-lethal dose for the fungi*B. broganitii*at 16 spores/ml *+ 2%* Imidaclorpridwere tested against the three corn borers under laboratory conditions.

2.6 Field Trials

Field trials were carried out at Nobaria region (Behera Governorate), Egypt during the two successive corn seasons 2012 and 2013 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. Fungi were applied at the concentrations of $16.5X10^4$ conidia/ml . Fungi were applied as single treatments in randomize plots. The combined effects aof the fungi at $4.25X10^4$ conidia/ml + 5g for imidaclorprid. 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. Samples from each treatment were collected weekly and transferred to the laboratory for investigation. Percentages of infection were estimated.

2.7 Yield Assessment

Yield data in treated and untreated plots in the corn harvest seasons (2012and 2013), represented by weight in kgs were determined.

Yield loss was estimated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield} - \text{Actual yield}}{\text{Potential yield}} \times 100$$

Potential yield is Imidaclorprid+*B. broganitii*treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones (Actual yield).

3. Results and Discussion

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

Data in table (1), show that under laboratory conditions the LC_{50} obtained was 132 $x10^4$, 144 $x10^4$, 170 $x10^4$ conidia/ml after *O. nubilalis*, S. *cretica* and C. *agamemnon* treated with different concentrations of *Beauveriabrongniartii* respectively. When the corresponding pests treated with imidaclorpridthe corresponding LC_{50} 176, 189 and 195 g/l;

respectively (Table2). When the two tested pathogens combined at the sub-lethal does the LC50 for the corresponding insect pests recoded were significantly decreased to 46, 69 and 75 g/L for *O. nubilalis*, S. *cretica* and C. *agamemnon* ., respectively (Tabe3). The same results obtained by Sabbour and Abdel-Rahman (2007) reported that under laboratory conditions results showed that the LC_{50} of*Phyllotretacruciferaem, Pegomyiahyoscami* and*Cassidavittata*of the tested fungi *Verticillium lecanii (V.l), Beauveriabrongniartiii (B.r)* and*Paecilomyces fumosoroseus* (*P.f*), respectively against the three pests ranged between $5.4x10^6$ and $1.43x10^7$ spores/ml. Satisfactory results with the entomopathogenic fungi were reported by Sharaf El-Din (1999) and Sabbour and Ismail (2001). Sabbour and Abd El-Aziz (2002) as they found that the fungi; *B. bassiana* and *M. anisopliae* reduced the LC_{50} of *S. littoralis* under laboratory conditions.Data in Table 4 ,show that the application of the bioinsecticides which affected on decreasing the infestation. The number of infestations of *O. nubilalis*significantly decreased to 23±2.2 and 26±2.1individuals after treatment with *B.brongniartii after* 50 day as compared to 69±4.3 and 72±3.1 individual in the control during both two seasons 2012 and 2013. In all treatments the number of corn pests were significantly decreased. *Chilo*agamemnoninfestation decreased to 48±1.3and 36±3.3 individuals after 90 days as compared to96±3.3and 98±1.3 in the control plots in both two seasons. When combined the two pathogens tested against the target pests the results showed that, after 90 days theinfestations recorded24±6.2, 23±2.4 and 27±5.3 individuals for *O. nubilalis*, S. *cretica* and C. *agamemnon*., respectively during season 2012. During season 2013 the corresponding infestations for the these pets recorded 18±1.2, 24±2.5, and 27±4.5 individuals., respectively (Table 4). In all treatments *B.brongniartii* + imidaclorprid gave the pest results for controlling the target pests.The same results obtained by Sabbour 2003,(20014a&b), 2013.Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014, Magda Sabbour, 2001, Sabbour (2002 a &b), Magda Sabbour and Ismail2002, Sabbour and Sahab 2005 &2007, 20011.The obtained results are similar to other studies carried out by Castillo *et al.* (2000) and Espin*et al.* (1989) on their work on*C. Capitata*and increased the yield. These results agree with Sabbour &ShadiaAbd El-Aziz, (2002 and 2010) who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with Castillo *et al.* (2000) who reported that the virulence of *B. bassiana* against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. Espin*et al.* (1989) recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments. The same findings obtained by Sabbour (2002 a &b), Magda Sabbour and Ismail2002, Sabbour and Sahab 2005 &2007, 20011.The same results obtained Sabbour 2006, Sabbour and Abd el Aziz 2007, Sabbour, 2007, Sabbour and Abbas, 2007. Sabbour and Hany, 2007, Sabbour,2008. Asmaa et al 2009. Sabbour 2014 control *Tuta absoluta* by three microbial control agents *Bacillus thuringiensis(B.t)var kurstaki; Beauveria bassiana(B.b*)which increase the yied. Sabbour 2014 control *T.absoluta* by fungi under laboratory and field conditions. The same obtained by Sabbour & Singer2014, Sabbour & Soliman 2014, Sabbour and Moursy2014, Sabbour and Abdel-Raheem 2014. The same findings obtained by Sabbour, 2013(a,b,c,d,e,f,g,h,I,j).

At the harvest time the corn weight obtained 3922±54.6 and 3110±60.4 kg/Feddan among the harvested plots treated with *B. brongniartii* and imidaclorprid plots as compared to 2710±40.9 and 2511±73.2 kg/Feddan during seasons 2012 1nd 2013, respectively Table 5.When Imidaclorprid+*B. broganitii plots gave the highest yield* 4510 ± 43.9 and 4919 ± 50.9 kg/fedaan during season 2012 and 2013 respectively. The same results controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions(Sabbour &Sahab 2005, 2007, and Sahab and Sabbour 2011) found that the fungi *B. bassiana, M. anisopliae, Pacilomyces fumosoroseus Verticillium lecanii***;**reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. Sabbour and Abdel-Rahman 2013 found that,in all treatments the number of corn pests were significantly decreased. loss of the yield by Sabbour &Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the infestations. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab (2005, 2007 and 2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with Sabbour&Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the the infestation with insect pests.

3.2 Acknowledgements

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Table 1: Effect of the entomopathogenic fungi, *B. broganitii*against the target insect pests larvae under laboratory conditions.

Insects	LC_{50}	slope	variance	95% confidence limits
Ostrinia nubilalis	$132X10^4$	0.1	1.01	99-166
Sesamia cretica	$144X10^4$	0.2	1.00	110-189
Chilo agamemnon	$170. X10^4$	1.03	1.01	135-199

Table 2: Effect of Imidaclorpridagainst target insect pestsunder laboratory conditions**.**

Insects	LC_{50}	slope	variance	95% confidence limits
Ostrinia nubilalis	76	1.01	0.02	111-79
Sesamia cretica	89	0.1	1.01	134-97
Chilo agamemnon	95	0.1	1.01	145-99

Table 3: Effect of Imidaclorprid+*B. broganitii*against target insect pestsunder laboratory conditions**.**

Insects	*LC50*	*slope*	*variance*	*95% confidence limits*
*Ostrinia*nubilalis	46	1.01	0.02	11-89
Sesamia cretica	69	0.1	1.01	34-95
Chilo agamemnon	75	0.1	1.01	45-93

Table 4: Effect of different treatments on the target insect pests under field conditions

Post 1st applicationdate	*Treatments*	*Number of infestation (means)±s.e during both two seasons*		
		Ostrinia nubilalis 2012 2013	*Sesamia cretica 2012 2013*	*Chilo agamemnon 2012 2013*
20	Control	41 ±3.2 49±3.1	60±2.5 71±2.3	66±3.4 72±2.2
50		69±4.3 72±3.1	78±3.4 82±2.5	83±3.4 91±1.3
90		79±2.3 88±2.1	87±5.1 91±2.1	96±3.3 98±1.3
20	*B. brongniartii*	15±3.1 18±2.1	21±4.4 23±5.1	21±4.3 20±1.2
50		23±2.2 26±2.1	23±4.7 25±3.2	24±3.4 26±4.4
90		26±3.2 18±1.2	24±2.4 29±2.3	28±2.3 29±4.2
20	*Imidaclorprid*	44±3.3 38±2.1	44±2.6 39±2.4	41±3.4 37±2.2
50		46±4.2 36±1.2	52±3.5 59±3.2	44±2.3 41±4.2
90		39±4.2 30±1.3	45±4.1 49±2.5	48±1.3 36±3.3
20	Imidaclorprid+ *B. broganitii*	14±3.1 14±2.1	20±4.8 22±5.3	20±5.3 20±1.1
50		20±2.7 20±2.1	21±5.7 22±3.5	23±7.4 24±4.4
90		24±6.2 18±1.2	23±2.4 24±2.5	27±5.3 27±4.5
F value = 27.4 12.1 31.220.131.1 26.4 Lsd5%= 16.415.414.714.215.116.8				

Table 5: Assessments of damage caused in corn field after the fungi treatment

Treatments	Season 2012 Wt of corn crop (kg/ feddan) yield loss%	Season 2013 Wt of corn crop (kg/feddan) yield loss %
*B.brongniartii**Imidaclorpridcontrol*Imidaclorprid+*B. broganitii*	3922± 54.6 33110± 60.7 312710 ± 40.9 394510 ± 43.9 -	4241 ±60.4 133239 ± 84.1 342511± 73.2 484919 ± 50.9 -
F value Lsd5%=	33.6 120.7	31.7 115.5

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