

EFFICIENCY OF THE FUNGUS, *Beauveria bassiana* (BALS.) AS A BIOLOGICAL CONTROL AGENT AGAINST *Ostrinia nubilalis* (HUB.) AND *Chilo agamemnon* (BLES.) IN MAIZE AND RICE FIELDS AT KAFR EL-SHEIKH REGION

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ABSTRACT

The present investigation was achieved at Sakha Agricultural Research Station during 2008 and 2009 seasons to evaluate efficiency of the fungus *Beauveria bassiana* (Bals.) as a biological control agent in field applications against the European corn borer, *Ostrinia nubilalis* (Hub.) in corn fields (2008 season), the lowest concentration (2.5×10^7) induced 26.87% reduction, while 5×10^7 resulted 44.64% larval reduction, the highest concentration 1×10^8 conidia/ml resulted 58.23 % larval reduction.

Infested internodes were reduced in values ranging between 23.78% and 66.53 % at different *B. bassiana* concentrations. The highest concentration (1×10^8) reduced number of holes and number of broken tassels by 60.03 and 73.28 %, respectively.

In 2009 season, reductions were 60.88, 62.63, 47.80 and 64.85 % for larvae, infested internodes, holes and broken tassels, respectively when the highest concentration (1×10^8 conidia/ml) was applied.

Treated rice plots (2008 season) by *B. bassiana* against the rice stem borer, *Chilo agamemnon* (Bles.) reduced dead hearts by 20.29, 43.59 and 55.0 % fifteen days post treatment. More reduction in dead hearts were obtained 30 days after treatment; 24.23, 50.17 and 71.88 % at 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively. Also, white heads were reduced, 30 days post treatment, by 34.87, 56.23 and 70.88 at 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively.

In 2009 season, 30 days after applications, dead hearts were reduced by 26.48, 51.68 and 73.30, while white heads were reduced by 35.34, 62.26 and 72.04 % for 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively. Thus, two sprays by the fungus at two concentrations could be applied to get sufficient reduction in reducing the infestation by *O. nubilalis* and *C. agamemnon* in maize and rice fields.

INTRODUCTION

The idea of insect pest control using micro-organisms, is so called microbial control, dates back to the middle of the last century (Steinhaus, 1949). Recently, studies on applied biological control of insects by entomogenous; bacteria, viruses and fungi have received a considerable attention (Lacey; 1985, Metwally; 2000, Ambethgar; 2009 and Stefan; 2010).

As an alternative to chemical pesticides, the entomopathogenic fungus; *Beauveria bassiana* (*Cordyceps bassiana* (Bals.) is currently under intensive study as a promising biocontrol agent for insects and other arthropoda pests (Leathers *et al.*, 1993 and Kirkland *et al.*, 2004a).

Different infectious *B. bassiana* propagules can be isolated and selected for host targeting (Alves *et al.*, 2002). Thus, in addition to mycelial

and hyphal growth *B. bassiana* produces a number of mono-nucleated single-cell types, including aerial conidia, blastospores and submerged conidia, which can be isolated from agar plates, rice-broth submerged cultures and nutrients-limited submerged cultures, respectively (Sewify, 1997) referred to the possibility of using *B. bassiana* against *Sesamia cretica* (Led.). This fungus has been used to suppress populations of European corn borer *Ostrinia nubilalis* (Hub.) on maize plants (Bartlett and Lefebvre, 1934) and they stated that, the succulent tissues of corn stalk were an ideal environment for growth of *B. bassiana*. The relationship between *B. bassiana* and corn plants (colonization of the plant by the entomopathogen) was studied by Vakili, 1990 & Lewis and Bing, 1991). They suggested that the fungus can persist on the surface of the corn plant and/or within it.

The European corn borer, *O. nubilalis* is a serious insect pest causing considerable damage to maize in Egypt and other countries particularly, when maize is sown during late July (Metwally 2000). The insect passes the winter as full grown larvae mainly inside their tunnels in maize stalks (Abd El-Rahman *et al.*, 1983 and Metwally 2000).

Many studies were carried out dealing with natural incidence laboratory and field trials for controlling eggs and larvae of *O. nubilalis* by *B. bassiana* (Martel *et al.*, 1980, Bruck and Lewis, 1999; Wagnear and Lewis, 2000 and Metwally, 2010).

The rice stem borer, *C. agamemnon* causes yield losses. Field losses due to stem borer are estimated as 5-8 % depending on rice cultivar (Sherif, 1996). Managing rice insects, to minimize yield losses, depends on the integration among different tactics of insect pest control; host plant resistance, cultural practices, biological control, and eventually, if needed, chemical control. The combination among these tactics is crucial to avoid the excessive application of insecticides that are commonly misused among the farmers (Heong *et al.* 1994).

According to Israel and Abraham (1967), 1 % increase in stem borer infestation reduced grain yield by 0.28 % in young plants, and by 0.62 % in plants of reproductive stage. Pathak (1968) reported that 1% white heads caused 1 to 3 % yield loss.

In Egypt, Abdallah *et al.*, (1989) estimated the damage of *C. agamemnon* by detillering. At 40 days after transplanting, 10 % simulated dead hearts resulted in 6-8 % yield losses by the progress of the season, 2% damage occurring 75 days after transplanting, resulted in about 5% yield reduction. Isa (1989) estimated losses due to the borer as 5-6 %. Sherif *et al.* (1991) reported that for every 1 % increase in dead hearts rice yield was reduced by 0.4 %, while 1 % increase in white heads resulted in about 1 % yield loss. The rice stem borer, *C. agamemnon* has been considered, since several decades, the most important insect pest of rice. Besides rice, it attacks maize, sugar-cane and shift to weeds (Metwally, 2010). Dead hearts (DH) and white heads (WH) are the two symptoms of infestation during the vegetative stage and reproductive stage, respectively. However, the dead hearts are less responsible for yield loss than white heads, because rice plants can compensate for most of DH, especially those occurring before the maximum tillering stage.

Therefore, the present work was carried out at Sakha Agric. Res. Station during 2008 and 2009 seasons in maize and rice fields to determine the effect of the fungus *B. bassiana* on population levels of *O. nubilalis* and *C. agamemnon* under field conditions. So as to identify safe alternatives to insecticides.

MATERIALS AND METHODS

Fungal production for field application:

Beauveria bassiana used in this study is an isolate collected from *Sesamia cretica* (Led.) larvae in Kafr El-Sheikh region (Sewify, 1997). Fungal conidia were produced on barley substrate (Arreger, 1992) which contained 50 g of shelled barley, 35 ml distilled water and 2 ml pressed sunflower oil. The barley mixed with water and oil was autoclaved in Erlenmeyer flasks (300 ml) for 20 min at 121 °C. Immediately after the lumps of grain were destroyed by shaking the flasks vigorously, the flasks were cooled at room temperature and inoculated with 1 ml of conidia suspension (10^6 spores/ml), then incubated for 2-3 weeks in the dark at 25 ± 1 °C. The conidia were harvested by suspending them in 50 ml of 0.5 % Tween. The suspension was filtered through a double layers of muslin and the desired concentration for field application was adjusted by addition of sterile distilled water. The fungus suspension was used at final concentration of 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml formulated with sunflowers oil.

Locations and experimental design:

The European corn borer, *Ostrinia nubilalis*:

This experiment was conducted for two successive seasons, 2008 and 2009 at Sakha Agricultural Research Station to study the efficiency of the fungus *B. bassiana* to control the European corn borer, *O. nubilalis*.

The most susceptible maize variety (Giza 2) to *O. nubilalis* infestation was sown on 24 July to ensure a high level of the insect infestation El-Naggar (1991) and Metwally (2000).

The experimental area was divided into 12 plots (21 m² each) and the completely randomized block design was followed. Three treatments each of 3 plots received the following fungus concentrations 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml. Each plot received 2 L of the fungus suspension using an atomizer. Three plots were sprayed with tap water containing 0.5 % Tween 80 and used as a control. First application was conducted 45 days after sowing and the second application was conducted after 20 days from the first application. Three weeks before harvest, 10 maize plants/plot were cut and dissected and the following parameters were recorded; alive larvae, infested internodes, numbers of holes and broken tassels.

The rice stem borer, *Chilo agamemnon* (Bles.):

This experiment was conducted on rice for two successive seasons 2008 and 2009. The susceptible variety Giza 178 was sown on 30 May and transplanted one month later. The experimental field was divided into 12 plots (21 m² each). Three treatments X 3 replicates and 3 plots as a control. The

plots were arranged in a completely randomized block design. The fungus treatments were applied 45 days after transplanting by spraying one liter of the suspension for each plot (a rate of 200 L/feddan).

Rice plants were examined twice at 15 days intervals after treatments to count number of dead hearts and white heads. Each plot was represented by 500 tillers percentages of reduction in rice stem borer damage due to applications were calculated according to Abbot Formula (1925).

RESULTS AND DISCUSSION

The European corn borer, *Ostrinia nubilalis*:

Results in Tables (1&2) show the efficiency of treating maize plants by *Beauveria bassiana* conidia at different concentrations on damage caused by *O. nubilalis* in two successive seasons.

In 2008 season, application of 2.5×10^7 conidia/ml resulted in a reduction of *O. nubilalis* larval density by 26.87 % increased to 44.64 and 58.23 % as the concentration increased to 5×10^7 and 1×10^8 conidia/ml, respectively.

The number of infested maize internodes was reduced from 51.55 to 39.29/10 plants due to spraying the plants with 2.5×10^7 conidia/ml which measures 23.78 % reduction. The later increased to 41.37 and to 66.53 % due to application of 5×10^7 and 1×10^8 conidia/ml, respectively. Similar trends were found when holes and broken tassels are considered. The holes were reduced by 13.34, 26.64 and 60.03 % and the broken tassels by 23.82, 38.50 and 73.28 % due to applications of 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively.

Table (2) indicates that the results of season 2009 are close to those of season 2008 although the population density of larvae was lower in 2009 than that in 2008 season. The highest concentration of the fungus (1×10^8 conidia/ml) resulted in higher reductions in all parameters compared with 2.5×10^7 or 5×10^7 conidia/ml. Thus 1×10^8 concentration could be recommended.

Many authors used the entomopathogenic fungus, *B. bassiana* to suppress the larval populations of the European corn borer, *O. nubilalis* in maize fields (York, 1958; Reba, 1984; Marchanier and Reba, 1986 and Fing *et al.*, 1988).

Table (1): Effect of different concentrations of *Beauveria bassiana* conidia on *Ostrinia nubilalis* in season 2008.

| Concentration (conidia/ml) | Number/10 maize plants | | | | | | | |
|-------------------------------|------------------------|------------|---------------------|------------|---------|------------|----------------|-----------|
| | larvae | | Infested internodes | | holes | | Broken tassels | |
| | No. | Reduction% | No. | Reduction% | No. | Reduction% | No. | Reduction |
| Control | 69.22 a | - | 51.55 a | - | 59.22 a | - | 8.31 a | - |
| 2.5×10^7 | 50.62 b | 26.87 | 39.29 b | 23.78 | 51.32 b | 13.34 | 6.33 b | 23.82 |
| 5×10^7 | 38.32 c | 44.64 | 30.22 c | 41.37 | 43.44 c | 26.64 | 5.11 ab | 38.50 |
| 1×10^8 | 28.91 d | 58.23 | 17.25 d | 66.53 | 23.67 d | 60.03 | 2.22 b | 73.28 |

In a column means followed common letter are not significantly different at 5% level by DMRT.

Table (2): Effect of different concentrations of *Beauveria bassiana* conidia on *Ostrinia nubilalis* in season 2009.

| Concentration (conidia/ml) | Number/10 maize plants | | | | | | | |
|-------------------------------|------------------------|-------------|---------------------|-------------|---------|-------------|----------------|-------------|
| | larvae | | Infested internodes | | holes | | Broken tassels | |
| | No. | Reduction % | No. | Reduction % | No. | Reduction % | No. | Reduction % |
| Control | 46.63 a | - | 30.27 a | - | 40.23 a | - | 5.69 a | - |
| 2.5×10 ⁷ | 37.22 b | 20.18 | 25.31 b | 16.38 | 33.31 b | 17.20 | 4.23 ab | 25.65 |
| 5×10 ⁷ | 25.22 c | 45.93 | 18.04 c | 40.40 | 23.67 c | 41.16 | 3.21 ab | 43.58 |
| 1×10 ⁸ | 18.24 d | 60.88 | 11.31 d | 62.63 | 21.00 d | 47.80 | 2.00 b | 64.85 |

In a column means followed common letter are not significantly different at 5% level by DMRT.

Zhang *et al.* (1990) stated that one application of *B. bassiana* at 2.5×10⁹ conidia/ml in the whorl stage reduced the population of the Asian corn borer, *Ostrinia furnacalis* by 90.5-91.6 %. Bing and Lewis (1991) found that the foliar application by the fungus *B. bassiana* on maize plants provided 98.3 % reduction in *O. nubilalis* infestation. They indicated that this entomopathogenic fungus colonized the corn plant at whorl stage, moved within the plant, and persisted to provide season long suppression of *O. nubilalis*.

The rice stem borer, *Chilo agamemnon*:

Results presented in Table (3) show the effect of controlling *C. agamemnon* using different concentrations of *B. bassiana* conidia/ml in rice field, in 2008 season.

In the untreated plots, number of dead hearts were 59.23/500 rice tillers. When the fungus was applied at a concentration of 2.5×10⁷ conidia/ml, dead hearts were reduced to 47.21/500 tillers, 15 days after treatments which means 20.29 % reduction. More reductions in dead hearts occurred due to application of higher concentrations. The effect of the fungus was more obvious 30 days after treatments, since the reductions in dead hearts were 24.23, 50.17 and 71.88 for the concentrations 2.5×10⁷, 5×10⁷ and 1×10⁸ conidia/ml, respectively.

Table (3): Effect of different concentrations of *Beauveria bassiana* on *Chilo agamemnon* infestation in season 2008.

| Concentration (conidia/ml) | Dead hearts/500 tillers | | | | White heads/500 tillers | |
|-------------------------------|-------------------------|-------------|---------|-------------|-------------------------|-------------|
| | 15 days | | 30 days | | 30 days | |
| | No. | Reduction % | No. | Reduction % | No. | Reduction % |
| Control | 59.23 a | - | 64.66 a | - | 28.13 a | - |
| 2.5×10 ⁷ | 47.21 b | 20.29 | 48.99 b | 24.23 | 18.32 b | 34.87 |
| 5×10 ⁷ | 33.41 c | 43.59 | 32.22 c | 50.17 | 12.31 c | 56.23 |
| 1×10 ⁸ | 26.65 d | 55.00 | 18.18 | 71.88 | 8.19 d | 70.88 |

The most important symptom of rice stem borer infestation to rice plants is the white head which results in empty panicles (Sherif, 1996) and thus rice yields is reduced. In case of control plants, 28.13 white heads per 500 tillers were counted. These white heads were reduced to 18.32, 12.31 and 8.19 per 500 rice tillers for the concentrations 2.5×10⁷, 5×10⁷ and 1×10⁸

conidia/ml, respectively. The corresponding values of reductions were 34.87, 56.23 and 70.88, respectively.

Statistical analysis revealed significant differences among numbers of dead hearts and number of white heads as well.

Data in Table (4) show the effect of the fungus in reducing damage of rice stem borer, *C. agamemnon* in 2009 season.

Reductions in dead hearts took a trend similar to that of the previous season. Fifteen days after fungus application, the reduction in dead hearts were 17.80, 29.56 and 57.91 for 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively. The corresponding values for reductions, 30 days after application were 26.48, 51.68 and 73.30, respectively.

Table (4): Effect of different concentrations of *Beauveria bassiana* on *Chilo agamemnon* infestation in season 2009.

| Concentration (conidia/ml) | Dead hearts/500 tillers | | | | White heads/500 tillers | |
|-------------------------------|-------------------------|-------------|---------|-------------|-------------------------|-------------|
| | 15 days | | 30 days | | 30 days | |
| | No. | Reduction % | No. | Reduction % | No. | Reduction % |
| Control | 56.29 a | - | 52.14 a | - | 24.22 a | - |
| 2.5×10^7 | 46.27 b | 17.80 | 38.33 b | 26.48 | 15.66 b | 35.34 |
| 5×10^7 | 39.65 c | 29.56 | 25.19 c | 51.68 | 9.14 c | 62.26 |
| 1×10^8 | 23.69 d | 57.91 | 13.92 d | 73.30 | 6.77 d | 72.04 |

On the other hand, reductions in rice tillers having white heads were 35.34, 62.26, 72.04 due to *B. bassiana* application of the considered concentrations; 2.5×10^7 , 5×10^7 and 1×10^8 conidia/ml, respectively. Also, statistical analysis revealed significant differences among all tested concentrations for both dead hearts and white heads.

Maniania (1993) experimented the effect of the fungus *B. bassiana* on *Chilo partellus* infestation and found the damage caused to maize plants was reduced due to applications of the fungus whatever the formulation was.

The results illustrated in this investigation show that using the fungus *B. bassiana* to control the two insects whether in maize and rice fields are of great importance and encourage the use of this fungus strain as a microbial insecticide against the former two borers at Kafr El-Sheikh region.

The effectiveness of the fungus against these insects can be attributed to the high virulence of *B. bassiana* strain, the high susceptibility of larvae to disease infection and the suitable weather in addition to ecological factors prevailing in maize and rice fields particularly the high relative humidity. Steinhaus (1949) and Müller-Kögler (1965) considered these factors of great importance for successful infections of pathogenic fungi.

It is concluded that, *B. bassiana* may be a potential biocontrol agent against *O. nubilalis* and *C. agamemnon* on maize and rice fields.

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Metwally, M. M.

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كفاءة فطر البيوفاريا باثيانا في مكافحة ثاقبة الذرة الأوروبية و ثاقبة ساق الأرز في حقول الذرة والأرز في منطقة كفر الشيخ

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أجريت هذه الدراسة الحقلية عامي 2008 و 2009 بمحطة البحوث الزراعية بسخا - كفر الشيخ، بهدف استخدام الفطر كعامل من عوامل مكافحة الحبوبية وذلك للحفاظ على البيئة وتشجيع دور الأعداد الحيوية ولذا تم عزل سلالة من فطر البيوفاريا باثيانا وجدت على يرقات القصب الكبيرة في حقول الذرة الشامية، وتم تنميتها على بيئة صناعية و جهزت للرش للتطبيق الحقلى لمكافحة هاتين الأفتين في حقل للذرة الشامية وآخر للأرز.

وكانت النتائج المتحصل عليها كالآتى:-

- 1- ثاقبة الذرة الأوروبية: فى موسم 2008 انخفض تعداد اليرقات بنسب 26.78، 44.64، 58.23 % وانخفضت نسب العقل المصابة بالآفة بمعدل 23.78، 41.34، 66.53 % عند استخدام التركيزات الآتية 2.5 × 10⁷، 5 × 10⁷، 1 × 10⁸ كونيديا/مل على التوالي وعند أعلى تركيز 1 × 10⁸ كونيديا/مل انخفض عدد الثقوب والعقل المصابة والنورات المذكرة المكسورة بنسبة 60.30، 73.28 % على الترتيب. وفى موسم 2009 كانت النسب 60.81، 62.63، 47.10، 64.85 وذلك فى كل من المحتوى اليرقى، والعقل المصابة وعدد الثقوب فى الحقل المصاب، النورات المذكرة المكسورة على الترتيب وذلك عند استخدام التركيز الأعلى وهو 1 × 10⁸ كونيديا/مل.
- 2- ثاقبة ساق الأرز: فى موسم 2008 انخفضت نسبة القلوب الميتة نتيجة المعاملة بالفطر بمعدل يتراوح بين 2.21، 43.59 % وذلك بعد 15 يوماً من المعاملة، وانخفضت الإصابة بدرجة أكبر بعد مرور 30 يوماً من المعاملة حيث وصلت إلى 71.88 % عند تركيز 1 × 10⁸ كونيديا/مل وانخفضت نسب الإصابة بالسنايل البيضاء بعد 30 يوماً من المعاملة بالفطر بمعدلات 34.87، 56.53، 70.88 % عند تركيزات 2.5 × 10⁷، 5 × 10⁷، 1 × 10⁸ كونيديا/مل على التوالي. وفى موسم 2009 انخفضت نسب القلوب الميتة بعد 15 يوماً من المعاملة إلى 17.15، 29.56، 57.91 % وإلى 26.48، 51.68، 73.30 % بعد 30 يوماً من المعاملة، وانخفضت نسب السنايل البيضاء إلى 35.34، 62.26، 72.04 % عند استخدام التركيزات 2.5 × 10⁷، 5 × 10⁷، 1 × 10⁸ كونيديا/مل على التوالي

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