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Combining effect of Enthomopathogenic fungus *Beauveria bassiana*, and insecticides against *Chilo suppressalis* in field conditions

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Abstract

One of the most devastating pests of rice is the lepidopteran insect striped stem borer (SSB) (*Chilo suppressalis*) (Lepidoptera: Pyralidae). Present investigation was carried out to evaluate the relative efficacy of ecofriendly formulation of Beauveria bassiana (1×108 conidia/ml, Diazinon granule 15 kg/ha. and Fipronil granule 20 kg/ha.) for sustainable management of Chilo suppressalis. The fungus Beauveria bassiana is one of natural enemies of the striped stem borer, Chilo suppressalis in the rice fields. During the treatments, precent mortality, infestation of Dead heart and White heads both in vegetative and reproductive stages was observed after spray application at different times intervals i.e., 2 DAT, 7 DAT, 14 DAT and 21 DAT. All the treatments significantly reduced population of Chilo suppressalis larvae compared to control due to their mode of action. It was concluded that maximum mortality (59.29%) and (74.56%) of Chilo suppressalis were achieved with combined application fo B. bassiana both Diazinon insecticide after 21th day of application both fisrt and second generation of insect pest, respectively. In this study, The lowest infestation Dead heart and White heads 0.57 and 1% observed in treatments Beauveria bassian and Diazinon granular after 21th day, respectively. In this research, the highest infection is created dead hearts and white heads rice was observed in the control treatment 10 and 11.67%. Therfore, the above findings showed the these combinations can be successfully can be used in rice IPM programs for Chilo suppressalis.

Key words : *Chilo suppressalis*, Dead haerts, Fungus, Infection, Mortality, Rice,White heads.

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Introduction

Rice (*Oryza sativa* L.) the grain of life and staple food is one of the most important crops in the world, providing food for nearly half of the global population (FAO, 2004). Rice is the most important source of nutrition in the world after wheat and the worldwide rice cultivation area is estimated about 163.3 million hectares with total paddy production of 749.7 million tons (FAO, 2016). In Iran, yearly paddy production is estimated to be about 3.5 million tons and of which about two million tons of white rice is produced. However, the significant portion of the country's rice requirement is imported every year. Most of the area under rice cultivation belongs to Guilan and Mazandaran provinces producing about 75 percent of Iran's rice crop (Alizadeh *et al.*, 2006).

There are number of factors which lead to the reduction in rice yield, among these the losses caused by insect pests varies from 20-35% annually (Inayatulla *et al.,* 1989; Salim *et al.,* 2001). Different studies have been reported that about 128 different species of insect pests infest the rice crop but 20 among these are of major economic importance (Ahmed, 1981). In Iran, Rice striped stem borer, *Chilo suppressalis* Walker, Green similooper, *Naranga aenascence* (Moore), common armyworm, *Cirphis unipuncta* (Haworth) are the most significant pests and cause major damage to rice crop which may fluctuate from 5-30%, The striped stem borer is the most serious insect pest of rice fields in Iran, This pest was introduced to Iran in 1973 and has been widely distributed in all rice fields of Iran (Expect of Khuzestan Province) and has caused economic damage for four past decades (Majidi-Shilsar, 2017). Yield losses are reported to 33% in Iran due to SSB, (Khosrowshahi *et al*., 1979). This insect attacks the crop from the seedling stage to the harvest time and thus causes complete loss of affected tillers (Salim & Saxena, 1992). Dead hearts are produced when the insect attacks at vegetative stage, while white heads occur when the stem borer attack at time of heading (Mahmood-ur-Rehman *et al.*, 2007; Pathak, 1975).

Neonate larvae move to the tip of leaf and feed on Parenchyma. The second instar larvae pierce the rice stems and enter the stems and feed them. This borer has been implicated as the major constraint to rice production worldwide. Current strategies for controlling this pest rely on the use of chemical insecticides, which cause public concern about food safety and environmental pollution. The losses can reach 50% if the pest is not controlled (Ghose, 1960). Furthermore, ***C. suppressalis*** control is difficult using chemical or biological insecticide sprays because the larvae enter the stem shortly after hatching, feed voraciously inside the stem and develop through several larval stages to become pupa (Sallaud *et al.*, 2003). Chemical compounds are the most effective tools for controlling the pests and are always required to suppress rapidly expanding insect pest populations (Ramazan-Asl *et al.*, 2010). Traditionally, chemical pesticides have been used for the protection of agricultural products from arthropod pests, but the indiscriminate use of these compounds can cause serious problems such as pest outbreaks (Metcalf, 1986). *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreal) is a fignificant natural pathogen of insects and infect more than 700 species of arthoropods (Moraes, 2005). These fungi infect their hosts by penetrating through the cucicle, gaining access to the haemolymph, produting toxins and grow by utilizing nutrients present in haemocoel to avoid insect immune responses (Hajeck, 1994).

Among entomopathogenic fungi *B. bassiana* fungi with a long history (more than a century and a half) to control harmful insects and mites agricultural products have specific position compared to other large studies on pathogens and pathogen infected host the influence environmental factors such as ultraviolet solar radiation, formulas, etc. (Gotel, 1996). Also, reasons for using this fungus, having contact and digestive effects, host range, the establishment of target insects (Gupta *et al.*, 1994), no adverse effects on non-target organisms, the possibility of mass production *in vivo* with many strains and as well as commercial production is (Jackson, 2000).

Laboratory studies N,Doye (1977) in France showed that the fungus could be used as a biological agent for the control of rice striped stem borer is considered, as stem borer larvae and egg masses were infected with the fungus. Rambach studies (1994) showed that nine species of insects harmful to rice fields in South East Asia, including green leafhopper, brown plantopper, rice black bug, maize stem borer and used. In Iran *B. bassiana* was reported for the first time from the larva and pupae of *Chilo suppressalis* Walker in Guilan and Mazandaran provinces by (Rezvani & Shah-Hosseini, 1976). The most important factor in the insect contamination by pathogenic fungi is the high relative humidity. Majidi-Shilsar *et al*., (2011) in the evaluation of fungicidal activity of *B. bassiana* on the overwintering larvae suggested that the larvae of stem borer were infected after the rice harvest in the paddy field. Majidi-Shilsar *et al.*, (2007) reported that this fungus more activity in the fall and winter months compared to July and August in Guilan province. **D**jafari *et al*., (1996) reported that infection of *B. bassiana* on the rice leaf green worm (*Naranga aenescens*). Majidi-Shilsar *etal.*, (2007) two species of pathogenic fungi *B. bassiana* and *Metarhizium anisopliae* on rice stem borer larvae identifying, introduction and their pathogenicity *in vitro* activity examined. Hence, the use of alternate eco-friendly strategies like microbial insecticides and use of micro nutrients to induce host plant resistance, is a more appropriate approach to suppress pest population (Usman Shakir, 2015). Majidi-Shilsar (2017) suggested that *B. bassiana* isolate MCB18 was more pathogenic than the *B. bassiana* other isolate*s* and *M. anisopliae* of the entomopathogenie fungus on *Chilo suppressalis*. The purpose of this study was to investigate the combined effect of *Beauveria bassiana*, Diazinon and Fipronil granule against *Chilo suppressalis* Walk. under paddy field conditions.

Materials and methods

Field preparation and Experimentation

There were five treatmenes, T1- *Beauveria bassiana*; T2- *Beauveria bassiana*+Diazinon; T3- *Beauveria bassiana*+Fipronil; T3- Diazino alone T4- Fipronil alone and T5- Untreated check). Agronomic practices were followed uniformly in all the plots. The experiment was laid down in Randomized Complete Block Design (RCBD) with factorial arrangement having three replications. The incidence of Rice Striped Stem Borer was observed at regular intervals in each experimental unit and per-treatment data was recorded, when population density of rice striped stem borer reached up to peack of moths, after 7 day 1th application of treatments was carried out. Treatments was applied with the help of hand sprayer according to the field raets in order to suppress the test insect pest population. *Beauveria bassiana* treatment was applied immediately After the appearance of larvae in rice field. The post treatment data regarding stem infestation caused by *C. suppressalis* was ercorded 2, 7, 14 and 21 days of treatments application by using standards procedures. Second application of treatments was applied after 35th day of 1th application of treatments and data was recorded after the same interval of time as in the first application. To do this, the ground plowed and then proceed to the seedling nursery production, seedling growth after two to three leaf stage (22-25 day) was used for transplanting. **The experimental plots** with dimensions 5×3 m were considered. The plot of the rice variety of Hashemi with distance 20×20 cm was grown. The nitrogen fertilizer with 46% nitrogen, 120 kg/ha. (to be split in three stages: a third before planting, one-third of the maximum tillering stage and a third stage of the Pregnancy stage), 80 kg phosphorus per hectare of triple super phosphate and with 100 kg of potassium sulfate a week later transplanting they were consumed in the soil. In addition to the control rice blast disease the amount 0.5 kg/ha. Tricylazol fungicide in two stages (leaves and clusters) and for control of weeds the amount 3 lit/ha. the common herbicides (butachlor against barnyard grass) were used in the field.

Preparation of fungus

For this study, the isolate Mcb18 the fungus *B. bassiana* (separated from the striped stem borer, Rice Research Institute, Rasht fungus on medium (40 gr. dextrose, 10 gr. peptone, 15 gr. of agar, 10 gr. of yeast extract, 0.3 gr. streptomycin and 1000 cubic centimeters of distilled water) and were cultured *in vitro*. After complete sporalation (14-21days) ,spores from the culture medium were scratched by the metal loop and by water and two hundredths of a percent Tween solution 80 separate was poured into the flask. After this step, the existing suspension was liquidated by Tiffany cloth only be prepared spore. The number of spores available the stock solution using heamacytometre cell were counted. Suspension available, concentration of 108 conidia per milliliter provided and then get ready in the refrigerator 4 C0 until tested in glass McCarthy kept black. In order to maintain and durable spores from ultraviolet sun, and other factors such as the agar material 0.1 percent, Tween 80 two-hundredths of a percent and 0.1 percent glycerin were added (Fig. 1).

Statistical analysis

Standard procedures were followed to record the dead larvae Dead hearts and White heads in vegetative and reproductive stages of rice plant, The data collected was analyzed statistically by using SAS, 9.1. (2004) The treatment means were compared by Duncan,s Multiple range Test (DMRT) for their significante at 5% probability level (Gomez & Gomez, 1985).

Results and Discussion

Effects on Mortality

The results revealed that all the treatments have significant insecticidal activity against *C. suppressalis*, both when applied alone or in combination. The results were more protruding, when applied in combination as compared to alone. Mortality in first generation of SSB was highest (59.29%) (Fig. 2), when *B. bassiana*+Diazinon were applied in combination, followed by *B. bassiana*+Fipronil (54.29%) after 21th day of treatment application of 1th spray. Mortality was (49.12%) in case of Diazinon alone, followed by (45.78%) by Fipronil alone, *B. bassiana* and untreated check plots with mean mortality values of 42.37 and 6.75%, respectively.

Similarly, after the application of 2th spray, highest mortality was 74.56% (Fig.2) in case of *B. bassiana*+Diazonon treated plots, followed by *B. bassiana*+Fipronil (69.56%), Diazinon alone (64.96%), Fipronil alone (60.96%), *B. bassiana* alone (59.29%) and mortality was lowest when water alone (Control) (10.18%) was applied.

Furthermore, results regarding mortality the first application (Fig. 1, 2) showed that effect of time interval was also statistically significant. The mortality values in case of *B. bassiana* alone treated plots were 5.09, 6.75, 10.18 and 42.37.29 after 2, 7, 14 and 21 day of treatment application. While in case of diazinon alone treated plots was in ascending order as 2nd day (5.09%)<7th day (10.18%)<14th day (33.95.79%)<21th day (45.19%). Mortality values in Fipronil alone treated plots were 5.09, 10.17, 30.61 and 45.78%, after 2, 7, 14 and 21day, respectively. Similar trend was observed after 2nd spray application (Fig.4).

Almost the same results were also explored by Anderson *et al*., (1989). According to his finding, higher insect mortality was observed when combined application of *B. bassiana* and sub lethal concentrations of insecticides was applied against Colorado potato beetle (*Leptinotarsa decemlineata*), and also resulting into higher rates of synergism between two agents. Patel (2005) concluded that effect of Bifenthrin, Oxydemetomethyl and *B.bassiana* insecticides each alone less than effect of these pesticides mixed with spores has been *B.bassiana*. Hassan and Charnely (1989) also studied the combined applications of entomopathogens and sublethal dosages of synethic insecticides synergistic and additive effects leading to increased mortality were observed by the combined application of imidacloprid and Metarhizium .anisopliae. Usman Shakie *et al*., (2015) showed that combined applications of Potassium silicate + *B. bassiana* + imidacloprid leading to increased mortality when was applied against *Cnaphalorcosis medinalis* Guenee. Maniania studies (1991) showed that egg masses of maize stem borer (*Chilo partellus*) by using isolates ICPEP4 of *B.bassiana* at a concentration of 108 conidia per milliliter mortality have up to 100%. Also, Maniania (1993) in the study of efficacy *B.bassiana* in the field on maize stem borer (*C. partellus*) showed that the formulations contain spores sprayed at a concentration of 1013 conidia per milliliter and fungal granules 1013 conidia per ha. in comparison with Trichlrofen insecticide, granular formulations fungal better than the other two formulations are recommended to control this pest.

Effect on Infestation of Plant

Results regarding infestation of rice stem showed that all the tratments have significant reduction in dead hearts and more promising when combined application of *B. bassiana* Diazino or Fipronil insecticides was carried out. Infestation of Dead hearts (Fig. 5), after 21th day of 1st spraryapplication was lowest (0.57%) in *B. bassiana*+Diazinon treatment which was statistically similar with *B. bassiana*+Fipronil (0.9%). Control treatment, infectation of dead hearts was highest (10%). Dead hearts values ranged from 0.57- 10 % after 21th day of treatment application.

Similarly, after 2th spray application (Fig. 6), infestation of rice stems, White heads caused of *Chilo suppressalis* was minimum(1%), in *B.bassiana*+Diazinon treated plots, followed by *B. bassiana*+Fipronil (2%) and that was at Diazino alone and Fipronil alone (2%) and(2.33%), respectively. In control treated plots (only Water) in fested to white heads was highest (11.67%) after 21th day of application. That's it, a field trial by (Rameash *et al.*, 2012) in assessing the impact of herbal insecticides, contains tobacco extracts, Neem and chemical pesticides of showed that the least of dead hearts in the first generation and the least of white heads in the second generation at treatment of Karate insecticide was used, was observed.

The results of this study showed that *B.bassiana* spores sprayed and sprayed with diazinon granules once in the first generation and the second generation of once a pest stem borer larvae have the greatest impact on mortality and afterwards the lowest dead hearts and white heads were compared to the check. Rao (1975) studies showed that among the fungal pathogen *B .bassiana* fungus has created the greatest impact on rice stem boreres under laboratory conditions. Riba (1984) reported that granular formulations fungus *B.bassiana* more effective than liquid formulations of fungus and granules trichlorofon poison against European corn stem borer (*Ostrinia nubilalis* Hbn) have been under field conditions. Ferron (1971) suggested almost similar findings about the combined application when he used the mycoinsecticide" Boverin" based on *B. bassiana* with the reduced doses of Trichlorophon against the second generation outbreaks of *Cydia pomonella* L. He observed the significant results as compared to inividual applications. The current study results showed that when *B. bassiana* with Dazinon or Fipronil were used in combination, They significantly reduced the compatibility of entomopathogenic fungi with granules insecticides. The same results were also found or entomopathogenic fungi with different pesticides

(Milner *et al.*, 2003; Sabbahi *et al.*, 2008; Gatarayiha *et al*., 2010; Jiang *et al.*, 2012). Mishra, (2006) showed that the efficiency of variouschemical insecticides has been restricted because C. medinalis have developed resistance against synthetic chemicals, therefore, it was useless to apply synthetic chemicals.

Investigations of Dhuyo & Soomro (2008) in evaluating two isolates of *B. bassiana* on the yellow rice stem borer, *Scirpophaga incertulas* Walker, Rice Research Institute in Pakistan showed that from among two isolates 274 and 373 of the fungus two concentrations 105 and 109 spores per ml., isolated 274 at a concentration of 109 spores per ml. most mortality the egg masses, larvae, pupae and even the insect pest were observed. In this connection they stated that 274 isolates also causes reducing to hatch of eggs this is a pest. The farmers highly rely on synthetic insecticide as a tool of choice in the battle against this noxious pest because of broad-spectrum activity, relatively low cost and rapid killing attributes. However, like all tools, insecticides have limitations. The excessive and indiscriminate use of insecticides resulted in severe adverse effect on agro-ecosystem, human health and wild life. Now-a-days, to overcome this crisis emphasize has been given on less disruptive control measures with judicial use of pesticides as a last resort (Chatterjee & Mondal, 2014). Exhaustive attemps have been made to discover alternate methods of insect pests control (Nathan & Kalaivani, 2005). Over reliance and indiscriminate use of highly toxic and hazard pesticides has resulted in pest resistance ,resurgence and higher magnitude of enviromental pollution leading to imbalance in natural ecosystem. The obvious way to combine the maximum possible effect of chemical and biological control agents on rice insect pests is to use selective insecticides, and to use them only when action thresholds of pest population levels have been exceeded (Heinrichs, 1994). According to studies of Nishida *et al.*, (1967) reported *Beauveria bassiana* from the rice striped stem borer at the rice fields of the Philippines. Our studies, however, confirmed that fungus *B. bassiana* alone or in combination with treated insecticides have high potential against *C. suppressalis.*

Conclussion

It was concluded that maximum mortality (59.29%) and (74.56%), (54.29%) and (69.56%) of *Chilo suppressalis* were achieved with combined application fo *B. bassiana* both Diazinon and Fipronil insecticide after 21th day of application both fisrt and second generation of insect pest, respectively. Similarly, infestation level of Dead hearts and White heads of rice plant were lowest after application of *B. bassiana*+Diazinon after 21th days interval. Therefore, our findings showed that application combinations fungus *B. bassiana* spores with diazinon granules have more effect on *Chilo suppressalis*, and can be used in integerated pest management program in the rice field.

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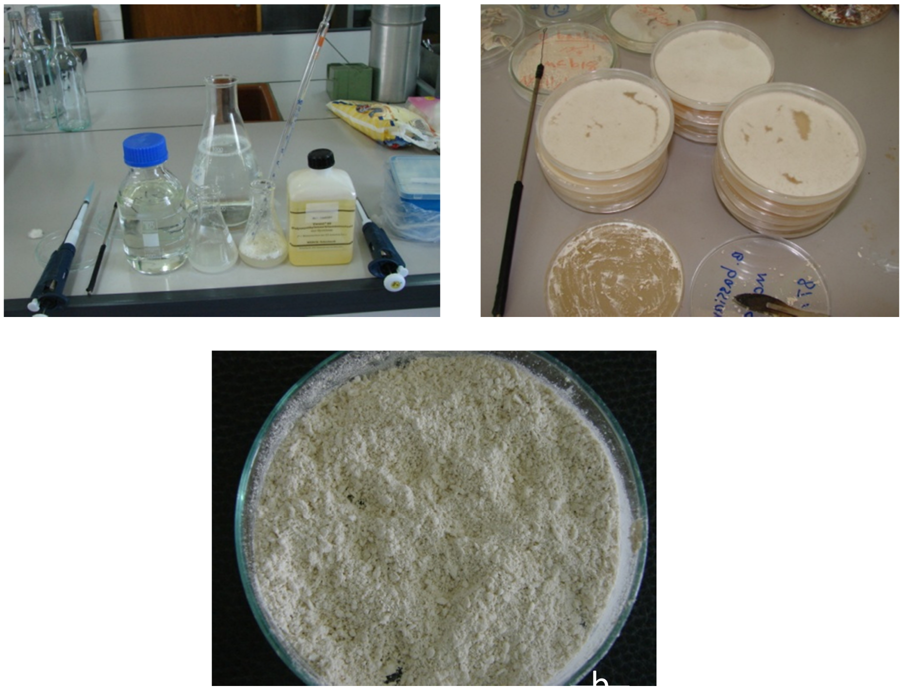

Fig. 1. Culture of *Beauveria bassiana* native isolate Mcb8 grown on Sabouraud Dextrose Agar yeast (SDAY) medium (a, b and c)

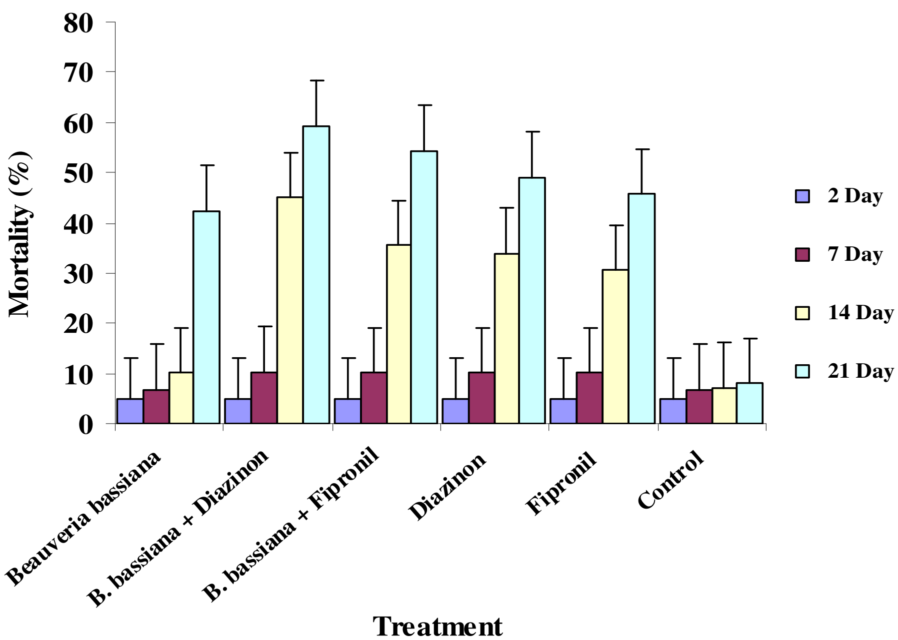

Fig. 2. Effect of different treatments on mortality of *Chilo suppressalis* after first generation at different intervals.

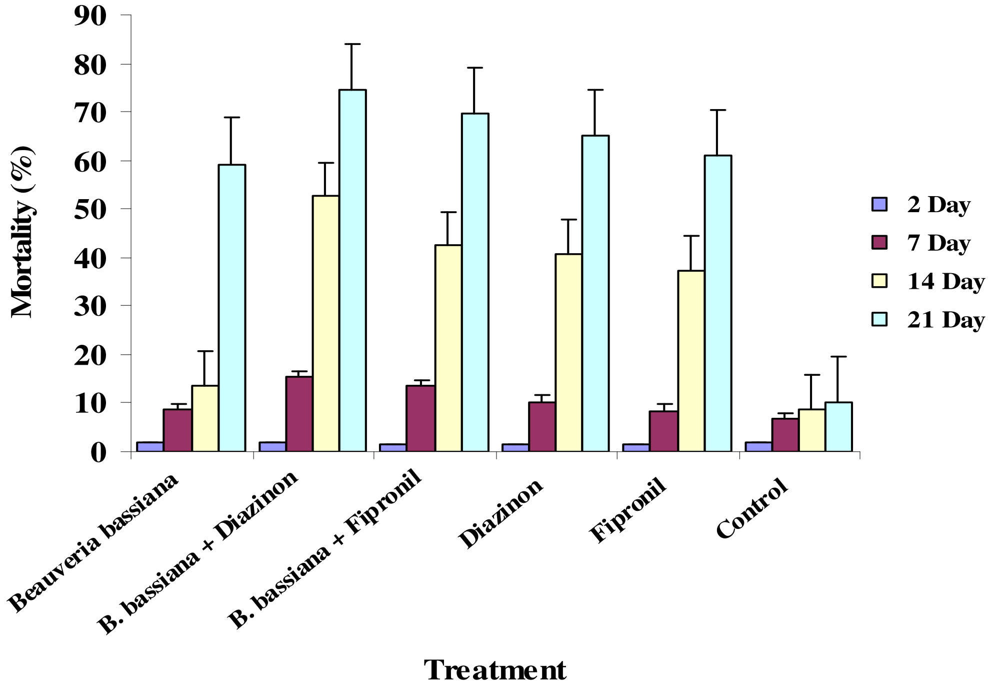

Fig 3. Comparative effects of different treatments on mortality of *Chilo suppressalis* after second generation at different intervals.

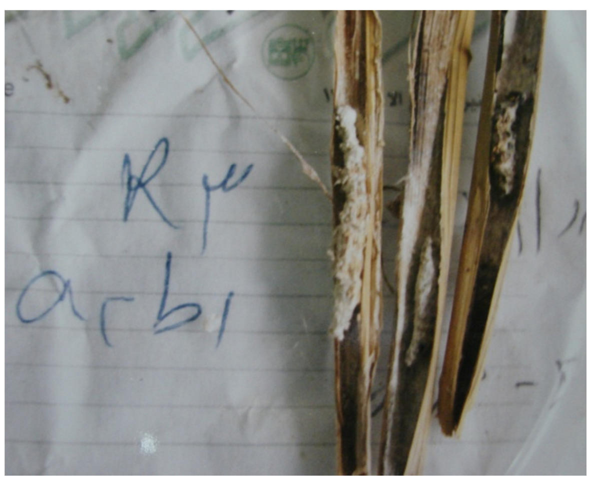

Fig. 4. Mortality stem borer larvae in rice growth stage affected by the impact of the insecticide diazinon and fungi *B. bassiana* at second spray.

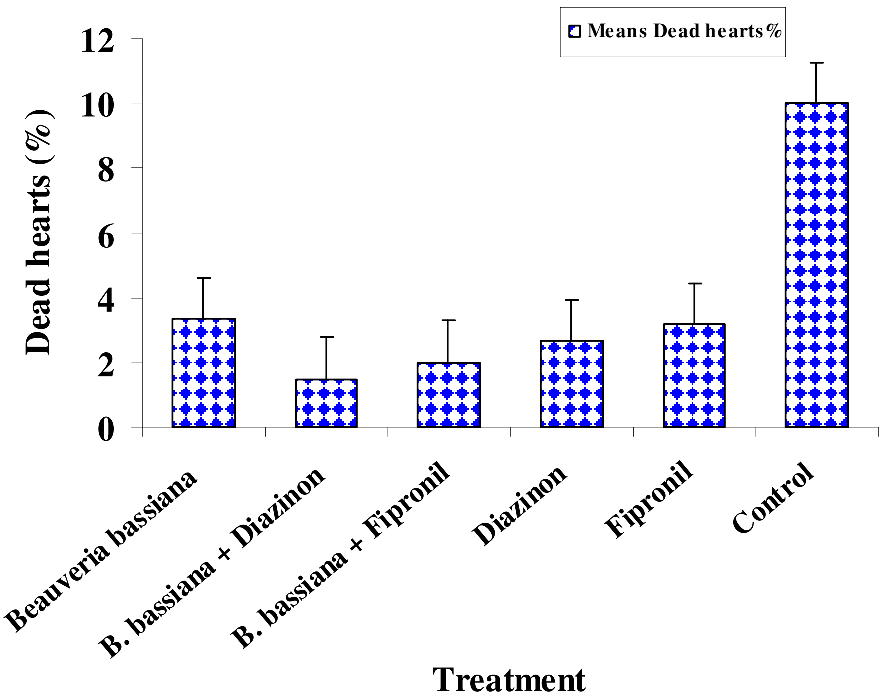

Fig. 5. Effect of different treatments on Dead hearts caused of *Chilo suppressalis* after 1th spray.

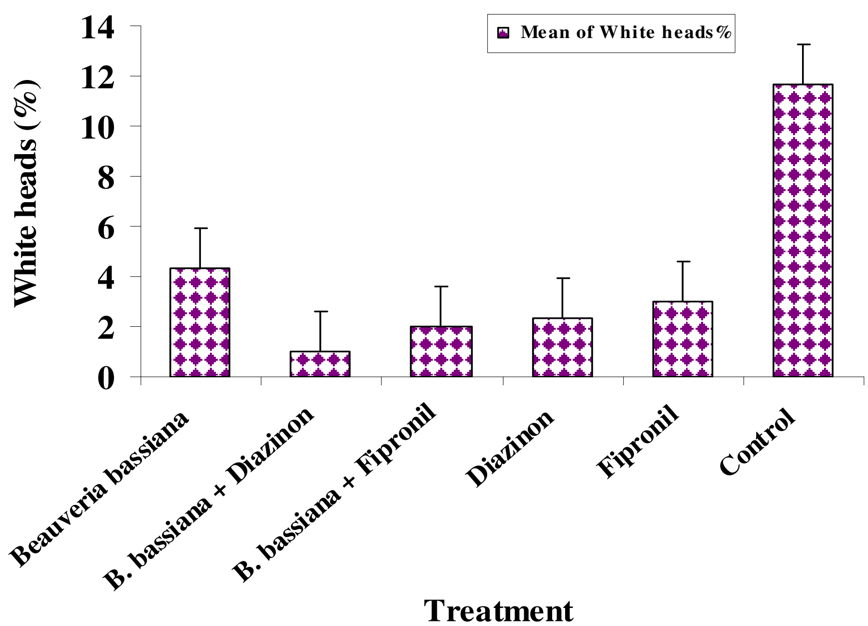

Fig. 6. Effect of different treatments on White heads caused of *Chilo suppressalis* after 2th spray.