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*Original Article*

# Eco-friendly Control of *Bactrocera Zonata* through a Mycoprotein-based Fungal Biopesticide Integrated with Synthetic Attractants

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**ABSTRACT:** *A destructive species of fruit fly known as Bactrocera zonata (Tephritidae: Diptera) seriously harms fruit crops economically. Mycoproteins found in biological control agents like Beauveria bassiana have insecticidal effects on B. zonata. The most effective management method for B. zonata is attract and kill. The current study examined, in a laboratory experiment, the fatality rate of B. zonata larvae and adults using various concentrations of crude mycoproteins from B. bassiana (3, 6, 9, 12, and 15 µL/mL). The same experiment was conducted again in a mango orchard against adult B. zonata to examine the activity of crude mycoproteins at higher concentrations (6, 12, 18, 24 and 30µL/mL), which were mixed with artificial attractant baits to entice adult B. zonata towards the traps hung from mango trees. The maximum mortality rate of larvae (90.01±4.81%) and adults (88.89±2.78%) was found at a concentration of 15 L/mL, while the minimum mortality rate of larvae (44.47±2.77%) and adults (36.10±2.78%) was found at a concentration of 3µL/mL, followed by control. According to the field study's findings, adults of B. zonata had a maximum mortality rate of 84.23±2.54% when exposed to crude mycoproteins at a concentration of 30 µL/mL. In conclusion, a pragmatic approach to improving B. zonata management in mango orchards may be to apply crude mycoproteins at a concentration of 30 L/mL in combination with artificial attractant baits.*

**KEYWORDS:** *Fungal toxic proteins, B. bassiana, B. zonata, Synthetic baits, Mortality.*

## 1. INTRODUCTION

Over 50 cultivated and wild species of fruits and vegetables, particularly fleshy fruits like guavas, mangoes, peaches, apricots, figs, and citrus, are affected by the dominant polyphagous pest Bactrocera zonata. The majority of Southeast Asian countries, including India, Sri Lanka, Bangladesh, Thailand, and Mauritius, are home to this species, which is native to Asia. Numerous fruits and vegetables are its main sources of food (El-Minshawy et al., 2018). The species has developed into a major quarantine and economic pest. It can result in losses of up to 89.50 percent in Pakistan and 10–20 percent in the northwest Himalayan region, respectively (Hossain et al., 2017). B. zonata has been reported to result in fruit losses of 3–100% across a wide variety of countries, seasons, and types of fruits or vegetables (Ahmad and Begum, 2017).

The genus Bactrocera zonata (Saunders) poses a significant threat to horticultural crops because of the substantial harm that some species may cause and the vast range of hosts that these organisms can infest. These tephritids mostly cause yield losses by causing fruit degradation, inedibility, and premature dropping brought on by larvae eating on the fruit's flesh. To stop admission and establishment, strict quarantine regulations are put in place for trade (Zhang et al., 2022).

B. zonata management is difficult in many countries due to the multiple life phases' ability to adapt in terms of behavior, eating, and biological characteristics, as well as the ability to eradicate B. zonata through effective broad-spectrum insecticides (Dias et al., 2018). Traditional suppression and eradication operations in areas where B. zonata is present include the use of protein bait sprays with broad-spectrum chemical pesticides (Kheder, 2021). A number of fungal isolates have been examined in the lab for the release of fungal pesticide proteins to find new compounds for insect pest management (Lovett et al., 2018). Food lures attract both male and female fruit flies. Food baits can draw in a wide variety of insects, including helpful ones. Both synthetic and liquid types of prey bait are available. The main attractant created by food baits is ammonia. Commercially, a wide range of food baits is offered. Today, using insect attractants and repellents is one of the most crucial prophylactic measures. An alternative method for attracting and mass-capturing B. zonata has been developed and is more effective, economical, and environmentally friendly. Early food lures had attractants such as fermented sugar, yeast, molasses, and protein hydrolysate. Liquid proteinaceous mixtures with ammonium baits have been used to successfully attract many species of fruit flies (Vázquez et al., 2022). The fruit fly species Anastrepha susupensa, Ceratitis capitata, B. zonata, and B. oleae were successfully attracted by ammonium compounds. Artificial food lures and plant semi-chemicals significantly shorten the lives of tephritidae fruit flies (Brito et al., 2016). Males of the genus B. zonata are attracted to the phenylpropanoid ammonium present in food-based scents to effectively control population dynamics (Ekesi, 2016). Building a robust monitoring system for

B. zonata field investigations can be aided by the attraction elicited by compounds in food baits. The primary goal of a recent study was to develop a system that effectively attracts and kills B. zonata. The transfer of technologies to farmers so they can control these active feeding insects on their fields can be aided by the success of field trials.

### 1.1. OBJECTIVES OF THE STUDY

- ✓ Production of mycoproteins from indigenous isolates of B. bassiana and preparation of synthetic attractant baits
- ✓ To observe the activity of mycoproteins integrated with synthetic attractant baits against B. zonata.

## 2. MATERIALS AND METHODS

### 2.1. PREPARATION OF FUNGAL CULTURE

B. bassiana was cultured from the stock culture of the Insect Pathology laboratory, Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan. The conidial culture was purified on disinfected media plates. The media consists of Potato dextrose agar (Merck KGaA, Darmstadt, Germany). 39g PDA was dissolved in 1000 mL of sterilised water. After that, the solution was placed on the hot plate at 100°C for 3-5 minutes until the media dissolved fully. To ensure its purity, the prepared media was autoclaved at 121°C under 15 pressure for 15 minutes. In order to harden agar media for inoculation in the laminar airflow chamber, hot agar media were put into 90mm glass petri plates. A sterile inoculation pin was used to inoculate the B. bassiana conidia after the media had solidified. The plates were covered with Parafilm tape and kept at 25°C for 5-7 days to cultivate fungi.

### 2.2. PREPARATION OF LIQUID MEDIUM FOR PROTEIN PRODUCTION

A liquid medium's main purpose is to speed up the production of large-scale sporulation. Mycological Peptone, fructose, and glucose make up the majority of the components. Both glucose peptone and fructose peptone were carefully combined with the other components in a ratio of 10:50 each. In a 250 mL Erlenmeyer flask, fresh conidial suspensions were accurately measured by hemocytometer and inoculated into both liquid media. For 3–4 days, the flask was spun on a rotary shaker at 110 rpm. Cell-free culture was produced using Whatman filter paper. Then, 95% ammonium sulphate was used to precipitate the obtained crude secreted proteins for proteins (Table 1).

**TABLE 1 Chemicals and their quantity used for the extraction of mycoproteins from *B. bassiana***

| Sr. No. | Chemicals | Quantity | Sr. No. | Chemicals | Quantity |
|---|---|---|---|---|---|
| 01 | Mycological Peptone | 9g | 06 | Calcium chloride | 4.13 g |
| 02 | Fructose | 11g | 07 | Sodium phosphate | 3.45 g |
| 03 | Glucose | 8g | 08 | Magnesium sulfate | 6.12 g |
| 04 | NaCl | 3.15 g | 09 | Olive oil | 5.00 mL |
| 05 | Tris HCL | 1.75 g | | | |

### 2.3. PREPARATION OF SYNTHETIC BAITS

The Institute of Plant Protection, MNS-University of Agriculture Multan's insect rearing laboratory provided the prepared baits. Protein hydrolysate, Jaggery, KOH, papaya, Kachri powder, and guava pulp were the main components utilized to make the baits. The attractant of traps was boosted by combining three local ammonium compounds with base baits. Ammonium acetate, trimethylamine, and putrescine combined with base baits to form a triplet. Male and female B. zonata were attracted in greater numbers by each tested bait formulation (Hasnain et al. 2022) (Table 2).

**TABLE 2 Ingredients used for the preparation of synthetic attractant baits**

| Sr. No. | Ingredients | Quantity | Sr. No. | Ingredients | Quantity |
|---|---|---|---|---|---|
| 01 | Ammonium hydrolysate | 8g | 05 | Kachri Powder | 12g |
| 02 | Jaggery | 15g | 06 | Guava pulp | 10mL |
| 03 | KOH | 3g | 07 | Ammonium acetate | 13.6g |
| 04 | Papaya | 12mL | 08 | Trimethylamine | 11mL |

### 2.4. LABORATORY BIOASSAY

#### 2.4.1. INSECT CULTURE

*B. zonata* culture was obtained from the lab stock colony of the insect rearing lab at the Institute of Plant Protection, MNS-University of Agriculture, Multan. Mango, guava, and other fruits that were infected were added each year to maintain and grow the colony. The raising circumstances were set at a temperature of 25°C, a relative humidity of 60°C, and a photoperiod of 16:8 L.D. respectively.

#### *2.4.2. LARVAL BIOASSAY*

Crude mycoproteins were collected, and their concentrations (3, 6, 9, 12, and 15µL/mL) were put into Petri plates that had been sterilized (9cm). Along with an untreated control, the experiment included six treatments, each of which was replicated three times. Adult B. zonata were fed the mycoproteins as food. B. bassiana crude mycoproteins. Used diet tests to test against larvae. Larvae's feeding was tested in standardized square Petri dishes. Second instar larvae that had just been mounted were exposed to a protein-rich artificial diet. Under the randomized complete design, twelve larvae were exposed in one Petri plate at concentrations of 3, 6, 9, 12, and 15 mL/mL per plate, along with an untreated control. The treatment settings included a temperature of 25°C and a relative humidity of 65°C at 16:8 light and dark photoperiods. Data on larval mortality were collected 1, 2, 3, and 4 days after application. The adult bioassay was also repeated with 216 insects (male and female). Six treatments were used in three replicates throughout the trial. Petri plates were used to hold the three-day-old adults. After 1, 2, 3, and 4D, the mortality data were taken.

## 3. FIELD TRIAL

### *3.1. RESEARCH SITE*

The experiment was carried out at the site area of Mango Research Institute (MRI), Multan, Pakistan. The experiment was done under Randomized Completely block design (RCBD) with eighteen mango trees loaded with ripened mangoes, and a massive population of B. zonata was observed. 18 traps (6×9) were set up, one trap for each tree at a height of 2 metres above the ground. A concentration of 6, 12, 18, 24, and 30µL/ml of mycoproteins and attractant baits is present in each trap. The liquid medium was applied to cotton swabs before they were placed in the trap. In the center of each tree canopy, treated traps were strung. After 1, 2, 3, and 4 days of treatment, the percent mortality was noted.

### *3.2. STATISTICAL ANALYSIS*

The recorded data were subjected to ANOVA under a Completely Randomized Design (CRD) for the lab and randomized Completely Block Design (RCBD) in field conditions. Minitab 10.0 was used to analyze the data. As a post-ANOVA method, the means were separated using Tukey's HSD test with a 5% probability (Beris, 2021).

## 4. RESULTS

### *4.1. LABORATORY TRIAL*

#### *4.1.1. INSECTICIDAL ACTIVITY OF MYCOPROTEINS AGAINST THIRD-INSTAR LARVAE OF B. ZONATA*

The pathogenicity of mycoproteins from *B. bassiana* was investigated in relation to *B. zonata* third-instar larvae. Significant mortality was noted after 1D of treatment ($F_{5, 12}$ =63.84, P=0.0023). The highest mortality of 63.89±2.54% was recorded at 15µL of mycoproteins, while the lowest mortality of 36.11±1.35% was found at 3µL/5mL, followed by a group of insects that weren't given any treatment. After 2D, the significant mortality was 69.45± 2.99%, while the control group came in second with 44.47±1.55% having the lowest mortality. Following 3D and 4D, the highest mortality rates were 77.78 ±3.40% and 91.67 ±3.20% at 15 µL/mL, respectively, whereas the lowest mortality rates were 55.55±1.22% and 69.47±2.23% after untreated insects, respectively (Figure 1).

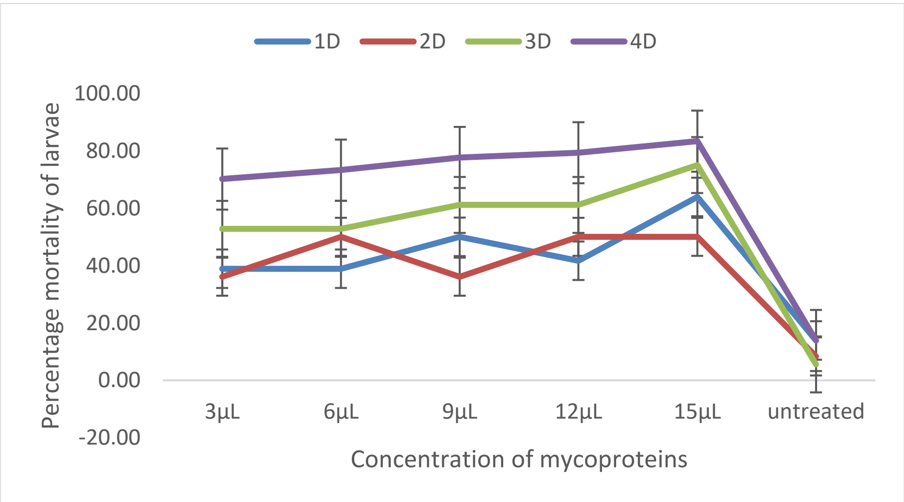


**FIGURE 1 Percent mortality of third larval instar *B. zonata* at various time intervals with different concentrations of mycoproteins under laboratory conditions**

#### *4.1.2. THE ACTIVITY OF MYCOPROTEINS AGAINST 3-DAY-OLD ADULTS OF B. ZONATA*

Mycoproteins from B. bassiana were tested against B. zonata adults that were three days old to determine their pathogenicity. Significant mortality was observed after 1 day of administration ($F_{5, 12}$= 58.33, P=0.0032). The highest mortality, 58.33±4.81%, was reported at 15 µL of mycoproteins, while the lowest mortality, 36.11±2.33%, was noted at 3 µL /mL, followed by a batch of insects that had not been treated. The lowest mortality after 2D was 52.77±1.23%, and control was followed by the substantial mortality of 69.47±1.22% after 2D. Following 3D and 4D, the highest mortality was 88.89±2.22% at 15 µL /mL, while the lowest mortality was followed, respectively, by untreated insects (Figure 2).

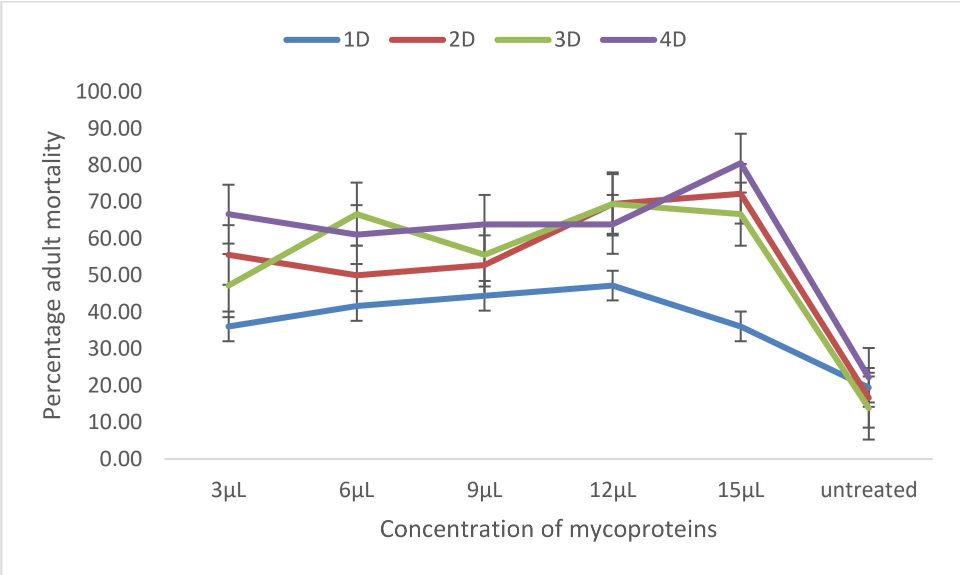


**FIGURE 2 Percent mortality of the adult of *B. zonata* at various intervals with different concentrations of mycoproteins under laboratory conditions**

### *4.2. FIELD TRIAL*

#### *4.2.1. THE ACTIVITY OF MYCOPROTEINS AND BAITS AFTER 1, 2, 3, AND 4 DAYS OF FIELD APPLICATION AGAINST THE ADULTS OF B. ZONATA IN A MANGO ORCHARD*

Mycoproteins from *B. bassiana* were tested against adults of *B. zonata* to determine their pathogenicity. Significant mortality was noted one day after treatment ($F_{5, 12}$ =63.84, P=0.0000). The highest mortality was recorded at 30 µL of mycoproteins (84.23±2.54%), while the lowest mortality was found at 6 µL /mL (36.11±1.35%), followed by untreated traps. The significant mortality at 2 days was 69.45±2.99%, whereas the lowest mortality was 44.47±1.55%, which was followed by the control. The maximum mortality was 35.78±2.40% and 32.67±1.20% after 3 and 4 days, respectively, with untreated insects coming in second and third (Figure 3).

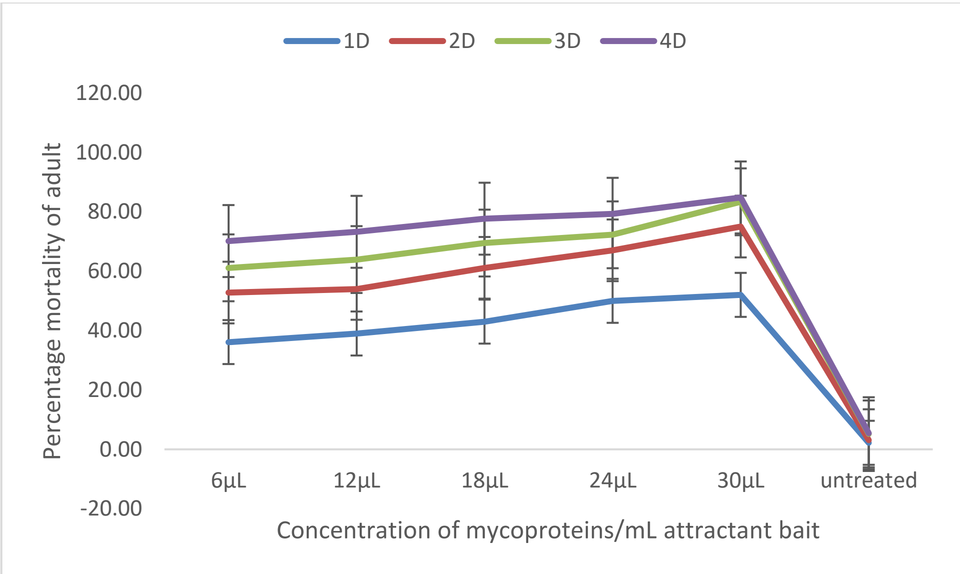


**FIGURE 3 Percent mortality of the adult of *B. zonata* at various time intervals with different concentrations of mycoproteins per mL of attractant baits under field conditions**

## 5. DISCUSSION

The most harmful insect pests are Tephritid fruit flies, which have a negative influence on global agricultural production, yield losses, and the value and marketability of horticulture crops. Fruit flies are a serious pest in Pakistan that harms farmers, as well as dealers, retailers, and exporters. Estimated uncontrolled fruit and vegetable losses in Pakistan are 21% and 24%, respectively (Hasan *et al.,* 2020).

Among the most harmful are *B. zonata*. This polyphagous species hunts its 40 various varieties of fruits and vegetables. Fruit-destroying pest *B. zonata* is primarily managed using baits. Environmentalists and the general public are opposing the use of more insecticides to manage *B. zonata*. In order to manage *B. zonata*, it is therefore necessary to wait before using the biocontrol approach. Crude mycoproteins isolated from *B. bassiana* have recently attracted increased interest due to the search for safer, environmentally acceptable alternatives (Hasnain *et al.,* 2022).

The present study used both biological and behavioral controls. The liquid media based on ammonia that is used to attract fruit flies makes up the synthetic bait, along with ammonium acetate, trimethylamine, and other ingredients. It has been shown that molasses and protein hydrolysate work better together and that molasses is essential for luring fruit flies. In order to improve the attraction of both male and female *B. zonata*, these synthetic baits can be introduced to food (Pinero *et al.,* 2015). Several compounds are being combined with crude mycoproteins from *B. bassiana* to combat insect infestations. The significance of the synthesis of hazardous chemicals by fungi has been shown by numerous studies (Irsad *et al.,* 2019). Toxic proteins found in *B. bassiana* have the potential to infect the host insect. The mycoproteins damage the digestive tract of insects and cause the infection, which begins after consumption. Mycoproteins prevent the digestive system's epithelial layer from performing its function, which causes the gut to deteriorate. Insect death is possible after damage to the epithelial layer. Therefore, the best and most sensible management tactic in both field and laboratory settings is the use of mycoproteins against *B. zonata*. The mycoproteins extracted from *B. bassiana* are a key agent for causing pathogenicity in *B. zonata* (Kazemi Shariat Panahi *et al.,* 2020).

The pathogenic effect of *B. bassiana* on *B. zonata* was discovered by Gul *et al.* (2014). They used oral, contact, and soil inoculation methods to target different life phases. In comparison to other procedures, contact treatments of the fungus resulted in the highest fatality rates. These fungi destroy the host insect, though, by releasing a variety of enzymes and poisons that have a variety of functions in the infection process. However, little is understood regarding post-penetration occurrences, particularly the release of fungus-produced harmful proteins after fungus colonization. The unusual release of poisonous proteins from fungi may be related to conidia's pathogenicity, which is greatly influenced by the media's nutritional composition. Purified *B. bassiana* proteins have been discovered to be efficient against the Peach fruit fly (*B. zonata*). So this study aims to isolate and purify the fungal toxic proteins under laboratory conditions and test out the pathogenesis of different life stages of *B. zonata* (Quesada-Moraga *et al.,* 2010).

The mycoproteins are poisonous and have pathogenic properties that make them harmful to numerous insect pests. A quick-acting biological control technique is hence immune to temperature changes. For more effective, environmentally friendly management, mycoproteins can be used to control adults, larvae, and pupae of *B. zonata* (Karimi *et al.,* 2022). Secondary metabolite release and the production of harmful enzymes are linked to *B. bassiana's* pathogenicity. These dangerous proteins, which are found in fungi, are enzymes with potent insecticidal effects. New management strategies for *B. zonata* will be discovered through the planned study.

## 6. CONCLUSION

The pathogenicity of *B. bassiana* against the larvae and adults of *B. zonata* is significantly influenced by the presence of microbial toxic protein in the plant. For the larval and adult stages of B. zonata, this mycotoxin protein was isolated from *B. bassiana* and combined with synthetic attractant baits in both lab and field settings. After the application of mycoproteins, the mortality in the larval stage was more than that of the adult stage of *B. zonata*, according to the field and laboratory results that have been provided. The larval stage was allegedly more vulnerable than the adult stage.

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