Biological management of *Bactrocera zonata* through an effective delivery system of mycoproteins of *Beauveria bassiana* integrated with synthetic attractant baits

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Abstract

A destructive species of fruit fly known as *Bactrocera zonata* (Tephritidae: Diptera), seriously harms fruit crops. Mycoproteins found in biological control agents such as the fungus *Beauveria bassiana* Balsamo, have insecticidal effects against *B. zonata*. The *B. bassiana* is found to be the most successful biological control agent against *B. zonata* through attract and kill technique. The current study was carried out to exam *in vitro* 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^{-1}). 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 30 µL mL^{-1}), which were mixed with artificial attractant baits to attract adult *B. zonata* towards the traps hung from mango trees. The maximum mortality of larvae (90%) and adults (89%) was found at a concentration of 15 µL mL^{-1}, while the minimum mortality of larvae (44%) and adults (36%) was found at a concentration of 3 µL mL^{-1}, as compared to control. According to the field study findings, adults of *B. zonata* had a maximum mortality of 84% when exposed to crude mycoproteins at a concentration of 30 µL mL^{-1}. It is concluded that *B. zonata* can be successfully managed in mango orchards using mycoproteins at a concentration of 30 uL mL^{-1} in combination with synthetic attractant baits.

Keywords: *Bactrocera zonata*, *Beauveria bassiana*, Fungal toxic proteins, Insects, Synthetic baits.

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 a dominant polyphagous pest *B. 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% in Pakistan and 10–20% 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).

B. zonata Saunders poses a significant threat to horticultural crops because of the substantial harm that some species may do 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 through effective broad-spectrum insecticides (Dias *et al.,* 2018). Moreover, chemical control of insects also results in health and environmental risks (Chittrakul *et al.,* 2021). Therefore, presently scientists are in search of alternate environment friendly strategies for the control of insects (Ahmad *et al.,* 2022; Zafar *et al.,* 2022; Maqsood *et al.,* 2023). The application of protein bait sprays with broad-spectrum chemical insecticides is a conventional technique for *B. zonata* suppression and eradication operations (Kheder, 2021). To develop novel chemicals for insect pest management, a variety of fungal isolates have been tested in the lab for the release of fungal pesticide proteins

(Lovett *et al.*, 2018). Both male and female fruit flies are attracted by food lures. Food baits can draw in a broad 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 are 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 it is more effective, more economical, and more environmentally friendly. Early food lures had attractants such fermented sugar, yeast, molasses, and protein hydrolysate. Ammonium baits with proteinaceous liquid were successfully used for the management of various 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, which is present in food-based scents, in order to effectively control the population dynamics (Ekesi, 2016). Building a good monitoring system for *B. zonata* field investigations can be aided by the attraction brought on by compounds included in food baits. The major goal of a recent study was to create a system that would effectively attract and kill *B. zonata*. The transfer of technologies to farmers so they can control this actively feeding insect on their fields can be aided by the success of field trials. The objectives of the present study were the production of mycoproteins from indigenous isolates of *B. bassiana* to assess their activity against different life stages of *B. zonata* in combination with synthetic attractant baits.

Materials and Methods

Preparation of fungal culture

The culture of *B. bassiana* was prepared in 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 used was potato dextrose agar (PDA) from Merck KGaA, Darmstadt Germany. An amount of 39 g PDA was dissolved in 1 L sterilized water. After that solution was placed on the hot plate at 100 °C for 5 min to fully dissolve the media. The prepared media was autoclaved at 121 °C under 103.4 kPa pressure for 15 min. The hot media was poured into 9-cm glass Petri plates. A sterile inoculation pin was used to inoculate the *B. bassiana* conidia after solidification of the media. The plates were covered with Para-film tape and kept at 25 °C for 7 days to cultivate the fungus.

Preparation of liquid medium for protein production

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. The flask was spun on a rotary shaker at 110 rpm for 3 days. Cell-free culture was produced using Whatman filter paper. Then, 95% ammonium sulphate was used to precipitate the obtained crude secreted proteins for proteins (Shen *et al.*, 2016).

Preparation of synthetic baits

The synthetic attractant baits were taken from stock culture of Insect Pathology Laboratory of Institute of Plant Protection Muhammad Nawaz Shareef University of Agriculture Multan. The synthetic attractant baits were prepared by combining various quantities of protein hydrolysate, KOH, Jaggery, Kachri powder and, papaya, and guava pulp. 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)

Laboratory bioassay

The rearing of *B. zonata* was done in insect rearing laboratory at the Institute of Plant Protection, MNS-University of Agriculture, Multan. Infected mango, guava, and other fruits were added each year to maintain and grow the colony. The rearing of *B. zonata* was done under 25 °C temperature and 60% relative humidity with 16/8 light and dark hours.

Crude mycoproteins were taken, and their concentrations (3, 6, 9, 12 and 15 µL mL^{-1}) were put into 9-cm diameter Petri plates that had been sterilized. Along with an untreated control, the experiment included six treatments, each treatment replicated thrice. Adult *B. zonata* were fed the mycoproteins as food. The 2^{nd} instar larvae of *B. zonata* were subjected to sterilized petri plates that contain required concentration of mycoproteins. The experiment was conducted using CRD (completely randomize design) and 12 larvae were exposed in one petri plate at concentrations of 3, 6, 9, 12, and 15 mL mL^{-1}, along with an untreated control. The experiment was carried under controlled conditions having 25°C temperature, 65% relative humidity and 16/8 light/dark photoperiods. Data on larval mortality were recorded after 1, 2, 3, and 4 days of application. The same bioassay was done against adult stage of *B. zonata* with 216 insects (male & 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 4 days, the

mortality data were taken.

Field trial

The experiment was carried out at site area of Mango Research Institute Multan Pakistan. The experiment was done under randomized completely block design with eighteen mango trees loaded with ripened mangoes and massive population of *B. zonata* was observed. Eighteen traps (6 × 9 cm) were set up, one trap for each tree at a height of 2m above the ground. A concentration of 6, 12, 18, 24, and 30 µL mL^{-1} of mycoproteins and attractant baits were 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.

Statistical analysis

The recorded data were subjected to ANOVA under completely randomized design for lab and randomized completely block design under 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 $P \leq 0.05$ (Beris, 2021).

Results

In vitro* 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. The highest mortality of 64% was recorded at 15 µL of mycoproteins, while the lowest mortality of 36% was found at 3 µL mL^{-1}, followed by a group of insects that weren't given any treatment. After 2 days, the significant mortality was 69%, while the control group came in second with 44% having the lowest mortality. Following 3 days and 4 days, the highest mortality rates were 78 % and 92 % at 15 µL mL^{-1}, respectively, whereas the lowest mortality rates were 56% and 69% respectively followed by untreated insects (Fig. 1).

In vitro* insecticidal activity of mycoproteins against adults of *B. zonata

Mycoproteins from *B. bassiana* were tested against *B. zonata* adults that were three days old to determine their pathogenicity. The highest mortality, 58%, was reported at 15 µL of mycoproteins, while the lowest mortality, 36%, was noted at 3 µL mL^{-1}, followed by a batch of insects that had not been treated. The lowest mortality after 2 days was 53%. After and 4 days, the highest mortality was 89% at 15 µL mL^{-1}, while the lowest mortality was 35% followed by untreated insects (Fig. 2).

The activity of mycoproteins and baits against adult of *B. zonata* in a mango orchard

Mycoproteins from *B. bassiana* were tested against adults of *B. zonata* to determine their pathogenicity. The highest mortality was recorded at 30 µL of mycoproteins (84%), while the lowest mortality was found at 6 µL mL^{-1} (36%), followed by untreated traps. The significant mortality after 2 days was 69%, whereas the lowest mortality was 44%, which was followed by control (Fig. 3).

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 research (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 began 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 of 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 pathogenic entity in *B. zonata* (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 fungal 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 and environmentally friendly management, the mycoproteins can be employed to combat 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 (Mehrotra *et al.*, 2017).

Conclusion

B. zonata is an invasive fruit pest that deteriorates fruit quality and has a major impact on the import and export of fruits. The biological control agent *B. bassiana* has mycologically toxic proteins that act as biopesticides against different stages of *B. zonata*. The mycoproteins present in *B. bassiana* can rapture the epithelial layer of the gut of *B. zonata* due to its toxicological effect. The field and laboratory observations showed that larvae were more susceptible than the adults. The combined application of synthetic attractant baits and mycoproteins proved a more effective, ecofriendly, and safer approach for the management of *B. zonata* in an orchard.

Author's contributions

Sanaullah and MAQ conceived idea, SS conducted experiments, SA ANP and Burhanullah analyzed data and conducted statistical analyses, and US and Sanaullah wrote the paper.

Conflict of interests

Authors declare that there is no conflict of interest.

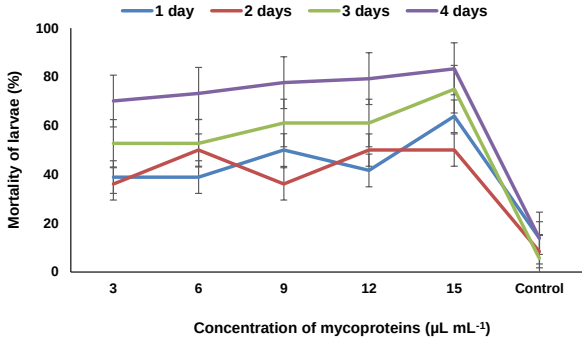

Fig. 1: *In vitro* mortality of third instar larvae of *B. zonata* due to different concentrations of mycoproteins.

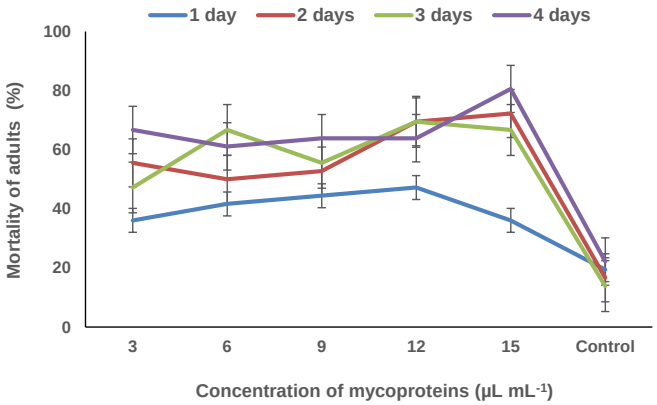

Fig. 2: *In vitro* mortality of the adult of *B. zonata* due to different concentrations of mycoproteins.

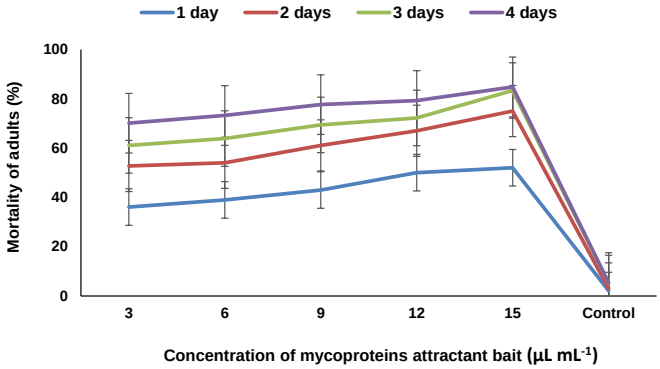

Fig. 3: Mortality of adult of *B. zonata* using mycoproteins and synthetic attractant baits in an orchard.

References

Ahmad S, Zafar R, Khan IH, Javaid A, Intisar A, 2022. Assessment of toxicity of *Parthenium hysterophorus* L. extract against larvae of *Trogoderma granarium*. *Plant Prot.*, **6**: 239-245.

Ahmad U, Begum U, 2017. A weekly study on *Bactrocera zonata* S. and *Bactrocera dorsalis* H. (Diptera: Tephritidae) against methyl eugenol, raspberry essence and GF-120 in persimmon orchards from Kohat, Pakistan. *Int. J. Agric. Res.*, **51**: 176-179.

Beris E, 2021. Evaluation and environmental testing of entomopathogenic fungi for their effectiveness as bio-control agents of major vineyard pests. *Plant Prot.*, **5**: 13-23.

Brito NF, Moreira MF, Melo AC, 2016. A look inside odorant-binding proteins in insect chemoreception. *J. Insect Physiol.*, **95**: 51-65.

Boulahia-Kheder S, 2021. Advancements in management of major fruit flies (Diptera: Tephritidae) in North Africa and future challenges: A review. *J. Appl. Entomol.*, **145**: 939-957.

Chittrakul J, Sapbamrer R, Sirikul W, 2021. Insecticide exposure and risk of asthmatic symptoms: A systematic review and meta-analysis. *Toxics*, **9**: 228.

Dias NP, Zotti MJ, Montoya P, Carvalho IR, Nava DE, 2018. Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Prot.*, **112**: 187-200.

El-Minshawy AM, Abdelgaleil SA, Gadelhak GG, Al-Eryan MA, Rabab RA, 2018. Effects of monoterpenes on mortality, growth, fecundity, and ovarian development of *Bactrocera zonata* (Saunders)(Diptera: Tephritidae. *Environ. Sci. Poll. Res.*, **25**: 15671-15679.

Ekesi S, 2016. Baiting and male annihilation techniques for fruit fly suppression in Africa. In: *Fruit fly research and development in Africa - Towards a sustainable management strategy to improve horticulture.* Springer. pp. 275-292.

Gul HT, Saeed S, Khan FA, 2014. Entomopathogenic fungi as effective insect pest management tactic: A review. *Appl. Sci. Busin. Econ.*, **1**: 10-18.

Hossain MA, Momen M, Uddin MS, Khan SA, Howlader AJ, 2017. Abundance of peach fruit fly, *Bactrocera zonata* (Saunders) in mango orchard. *Bangladesh. J. Entomol.*, **27**: 25-34.

Hasnain M, Saeed S, Naeem-Ullah U, Ullah S, 2022. Development of synthetic food baits for mass trapping of *Bactrocera zonata* S. (Diptera: Tephritidae). *J. King Saud Univ.-Sci.*, **34**: 101-667.

Hasan MU, Malik AU, Khan AS, Anwar R, Latif M, Amjad A, Amin M, 2020. Impact of postharvest hot water treatment on two commercial mango cultivars of Pakistan under simulated air freight conditions for China. *Pak. J. Agric. Sci.*, **57**: 1381-1391.

Irsad PQR, Haq E, 2019. Fruit Fly (Bactrocera spp.): A Major Threat to Guava Production and Its Integrated Management. In: *Recent trends in insect pest management.* AkiNik Publisher.

Karimi A, Khadem-Erfan MB, Nosratabadi M, Riazipour M, Kachuei R, 2022. Direct detection of *Phanerochate chrysosporium*, *Engyodontium album*, and *Fusarium venenatum* in soil samples collected from different regions of Iran by PCR method. *J. Appl. Biotechnol. Rep.*, **9**: 651-656.

Panahi HKS, Dehhaghi M, Jouzani SG, Zare R, Aghbashlo M, Tabatabaei M, 2020. Fungal biocontrol agents as a new source for bioethanol production. In: Jouzani SG, Tabatabaei M, Aghbashlo M. (eds), *Fungi in Fuel Biotechnology*. Springer, Cham. pp. 69-104.

Maqsood S, Shafi MU, Javaid A, Khan IH, Ali M, Ferdosi MFH 2023. Control of insect pests and yield improvement in brinjal by plant extracts. *Int. J. Biol. Biotechnol.*, **20**: 329-335.

Mehrotra S, Kumar S, Zahid M, Garg M, 2017. Biopesticides. In: Singh R (ed.), Principles

and applications of environmental biotechnology for a sustainable future. Springer Link. pp. 273-292.

Piñero JC, Souder SK, Smith TR, Fox AJ, Vargas RI, 2015. Ammonium acetate enhances the attractiveness of a variety of protein-based baits to female *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entomol.,* **108**: 694-700.

Shen T, Wang C, Yang H, Deng Z, Wang S, Shen B, Shen Q, 2016. Identification, solid-state fermentation and biocontrol effects of *Streptomyces hygroscopicus* B04 on strawberry root rot. *Appl. Soil Ecol.,* **103**: 36-43.

Vázquez A, Cloonan KR, Rohde BB, Gill MA, Mosser LK, Crane JH, Kendra PE, 2022. Attraction and longevity of 2-and 3-component food cone lures for the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *J. Econ. Entomol*., **115**: 1231-1239.

Zafar R, Ahmad S, Javaid A, Khan IH, Iqbal M, Ferdosi MFH, 2022. Insecticidal effect of ethanolic leaf extract of *Conocarpus lancifolius* Engl. against khapra beetle. *Int. J. Biol. Biotechnol.,* **19**: 511-515.

Zhang Y, Hughes AC, Zhao Z, Li Z, Qin Y, 2022. Including climate change to predict the global suitable area of an invasive pest: *Bactrocera correcta* (Diptera: Tephritidae). *Glob. Ecol. Conserv*., **34**: 20-21.