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Review on Biological Management of *Bactrocera zonata* through Pathogenic Activity of *Beauveria bassiana*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors Sanaullah and HMSA wrote the section of mango production and international trade of mango in the manuscript. Authors SHKN, MZ, US and FA wrote the section of host plant of fruit fly and management through B. bassiana. Authors SS and MSF reviewed this article and gave fruitful suggestions. Authors MSS, HA and IM formatted the manuscript according to the journal. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Bactrocera zonata is the destructive pest of peach, mango and banana by causing Significant econon losses

It reduces fruit quality, which directly causes decline in export of these economical fruits. Many management strategies were carried to suppress the population of this invasive pest. The use of cher insecticides in the orchard for control of *B. zonata* is restricted because insecticides causes several complication in fruit health and makes fruits unfit for human consumption.

The EPF (*Beauveria bassiana*) act as potent biocontrol agent. *B. bassiana* enriched with three cuticle degrading enzymes including protease, lipases and chitinase. These enzymes have ability to hydrolyz degrade the epicuticle and break the antifungal proteins linkage present in that cuticle for facilitating th spore's penetration. The current study emphasizes the activity of cuticle degrading enzymes (CDE) of *Beauveria bassiana* (protease, chitinase, lipases) for the management of *B. zonata*.

Keywords: B. bassiana; B. zonata; biological control; CDE; management.

1. INTRODUCTION

1.1 Mango

Mango (*Mangifera indica*) is the most important and delicious fruit grown worldwide [1]. After citrus, mango is the second-largest fruit grown in Pakistan, which comprises 14% of the total area under fruit cultivation. The cultivation of mango was started in India 4000 to 6000 years ago. In Pakistan, mango was grown on an area of 214415 ha with production of 2344647 tons. The production of mango is facing several issues particularly the insect pests. The attack of several species of fruit flies is a major problem in the international trade of mango fruits [2].

1.2 Fruit Fly, a Threat to International Trade

Bactrocera zonata Saunders (Tephritidae: Diptera) is the major damaging species, causing fruit decline in Asian countries such as Pakistan. As the global trade in agricultural goods grows, the chances of introduction of exotic insect species into new areas where they become pests. *B. zonata* is one of the most serious global dangers to fresh fruits and this species cause damage to 250 to 400 host fruits with US$242 million in economic losses per year in Brazil (Trombik et al, 2023).

Table 1. List of host fruit plants of *B. zonata*

Sr. No.	Common name	Scientific name	Family	References
01	Apple	*Malus domestica*	Rosaceae	EFSA (2019a), (CABI, 2020), [4].
02	Peach	*Prunus persica*		
03	Apricot	*Prunus armeniaca*		
04	Mango	*Mangifera indica*	Anacardiaceae	
05	Fig	*Ficus carica*	Moraceae	
06	Date	*Phoenix dactylifera*	Arecaceae	
07	Phalsa	*Grewia asiatica*	Malvaceae	
08	Guava	*Psidium guajava*	Myrtaceae	
09	Citrus (orange)	*Citrus sinensis*	Rutaceae	

1.3 Management

The major losses in horticultural crops are due to fruit flies that leads to a decline in the production of fruits [4]. The management of fruit flies can be done by several management techniques. The management methods are physical, behavioral, cultural, mechanical, chemical control, and biological control. The male annihilation technique (MAT) and sterile insect technique (SIT) are revolutionary methods used for controlling fruit flies [5].

1.4 Entomopathogenic Fungi

EPF are divided into 12 classes, which are divided into six phyla in the kingdom of fungi. Ascomycota, Zygomycota, and Deuteromycota are the divisions of fungi that are harmful to arthropods [6]. Many of the EPFs that have been discovered so far belong to the Zygomycota class Entomophthorales or the Deuteromycota class. EPF are pathogenic to insects because of their specialized pathogenicity mechanism, extensive host range, and capacity to control sucking pests, aphids, and pests with biting mouthparts, EPF has played a key role among all biological control agents [7].

2. EPF AS AN ECO-FRIENDLY TOOL

The use of EPF is one of the most used biological control strategies. Biological crop protection using EPF is now an important component of initiatives aimed at long-term insect pest management. EPF provides several benefits over traditional pesticides, including cost-effectiveness, no negative side effects for beneficial insects, and no chemical residues in the environment. EPF may colonize plants as endophytes, functioning not only as pest and disease control agents but also as plant growth promoters, in addition to direct application as contact bioinsecticides [8].

3. MODE OF ACTION OF EPF

When fungal spores come into touch with an insect's cuticle (skin), they germinate and enter the cuticle through germ tubes or infection pegs from the appressoria, growing directly through the cuticle to the host's inner body. The fungus spreads throughout the body of the insect, creating poison and depleting the insect's nutrition before killing it. Tissue breakdown and, on rare occasions, poisons may cause death. Once the fungus has killed its host, it returns through the softer part of the cuticle, enveloping the insect in fungal growth. When fungal spores come into touch with an insect's cuticle, they germinate and develop appressoria, which pierce the cuticle and grow right through it. The fungus spreads throughout the body of the insect, creating poisons and depleting the insect nutrition, finally killing it. Tissue damage and fungus-produced poison induce death. EPF penetrates directly into insect cuticles to infect insect pests [9].

3.1 *Beauveria bassiana* (Balsamo)

White muscardine fungus is a common name of *B. bassiana*, which is a historically important fungus. Muscardine was named after the discovery of muscardine disease in domestic silkworms. *B. bassiana* belongs to entomopathogenic fungi that are naturally present in soil and can cause disease in different arthropods. *B. bassiana* has mycoinsecticidal action against agricultural, veterinary, and medical insect pests with an extensive and diversified host range of various isolates. *B. bassiana* is a generalist fungus, which has a wide host range and was observed on different insect pests. It is used as a biopesticide due to its broad spectrum against many insect pests (Sharma *et al.,* 2023)

3.2 Role of Enzymes in the Pathogenicity of *B. bassiana*

When a disease or insect infection occurs, a combination of biochemical, physiological, and genetic events is referred to as fungal pathogenesis. The ability of an entomopathogenic fungus to induce death is described as virulence, which is a process involved in the mortality of insects. The enzymes of the EPF start penetration in the cuticle of the host insect during the infection process. EPF has extracellular protease enzymes that are the main component of fungi during the infection process on any insect. The characterization of enzymes is essential to find out the role of enzymes in the infection process [10]. The study was conducted to regulate and synthesize extracellular protease enzymes including Pr1 and Pr2 from various isolates of *Isaria fumosoroseus* . The cuticle of insects is made up of protein, chitin, lipids, wax, and phenolic chemicals, all of which act as a barrier against the invading fungus. In response to various insects, the entomopathogenic fungus can create a variety of enzymes as virulence factors. To better understand the host-pathogen interaction. Most studies have focused on the cuticle-degrading enzymes and extracellular activities of entomopathogenic fungus (Wang *et al.,* 2023).

The pathogenicity of EPF depends on the activity of different enzymes including proteases and chitinases that helps in breaking the cuticle of insects. These enzymes help in breaking long chains of alkenes and fatty acids of insects in the integument. The CDE including proteases, lipases, and chitinases are important for the pathogenicity of these organisms on insects because they tear down the insect cuticle, allowing the fungal germ tube to pass into the insect body. To allow the entomopathogenic fungus to permeate the insect cuticle, mechanical pressure and enzymatic degradation are used [11].

3.3 Cuticle-Degrading Enzymes

The cuticle of the insect contains an interlinked network system of protein, insoluble polysaccharides, and chitin. Through the action of enzymes like chitinase, proteases, and hydrolysis, EPF, particularly *B. bassiana*, causes direct toxicity in the host insect's cuticle *Pichia pastoris* produces hybrid proteases such as CDEP-1 and BmhBD, which boost the insecticidal activity of *B. bassiana*. The variety of enzymes produced by EPF like lipases, chitinases, and proteases have a huge proteolytic ability to degrade insect cuticles. EPFs primarily enter the insect's body through its integument. Various EPF, including *B. brogniarti, B. bassiana,* and *M. anisopliae* have cuticle-degrading enzymes that can be controlled depending on nutritional circumstances. Insects become infected by EPF by penetrating the cuticle with the aid of CDE that is secreted by the fungus. EPF introduces a number of poisons and damaging enzymes that aid in the infectious process and cause the insect to exhibit a variety of symptoms. The extracellular enzymes like proteases, lipases, and chitinases perform a vital role in showing pathogenicity for degrading insects during conidia adhesion to the host cuticle, germination, and colonization following penetration [12].

3.4 Activity of Proteases

Proteolytic enzymes break down peptide connections in insect cuticles, exposing chitin fibrils, a key component of EPF. The Pr1 is a protease that destroys cuticle proteins allowing spores to adhere more easily found in *M. anisopliae, O. sinensis,* and *B. bassiana*. According to certain research, EPF proteases perform the primary role of hydrolysis of cuticular proteins; proteases can effectively inactivate antifungal proteins present in the insect's epidermis [13]. EPF comprises a biochemical and mechanical process that aids to overcome the integument of insect pests. The role of enzymes like proteases is essential for causing fungal pathogenic infection and genetic modifications against insect pests. The cuticle degrading enzymes including Pr1 and Pr2 proteases secreted after penetration of fungus causes direct toxicity in insect cuticle. The past experiments revealed that the molecular mass of proteases is 19kDa with a 7-12 optimum pH range and 35-45°C temperature. The secretion of CDE and activity of proteases vary with isolates and strains of *B. bassiana*. The overexpression of various isolates of proteases was developed by genetic engineering techniques. The virulence and pathogenicity of proteases depend on isolates and host insects. Protease helps to cause direct toxicity in target insects [14].

3.5 Activity of Proteinases

B. bassiana cultured on various carbon and nitrogen sources it produces two chymotrypsin-like serine proteinases. After inoculating fungus on *Apis mellifera* larvae, the enzymes were extracted from the culture filtrate using acetone precipitation and gel filtration. The purified molecular weight of enzymes was 32KDa [15].

3.6 Activity of Lipases

Lipases are enzymes that break down the ester bonds in the lipids, waxes, and lipoproteins found in the integument of insect. Lipases play a critical role in the insect's tegument penetration, breaking down, and defensive mechanisms. The earliest enzymes made were lipases, which hydrolyze lipids and lipoproteins in the insect epicuticle. Lipases hydrolyze the triacylglycerols' ester bonds to release free fatty acids, triacylglycerols, monoacylglycerols, and glycerol and enhance hydrophobic contacts between the fungus and the cuticle surface, assisting germination spores in adhering to insect cuticles. The fungus *B. bassiana* creates phospholipase C, which dissolves phospholipids in insect cell membranes and hydrolyzes phosphodiester linkages, allowing of the fungus to pass through the insect's hemocel and infect its tissues [16].

3.7 Activity of Chitinase

Chitinases are the last category of enzymes, and they are divided into two groups endochitinases and exochitinases based on where they operate on the chitin molecule. The virulence of *B. bassiana* mutants is defective in chitinase synthesis against *Melolontha melolonta* and *Dysdercus peruvianus*, respectively. EPF also makes several enzymes that are not directly connected to acid trehalase, the enzyme that breaks down the cuticle. It converts trehalose, the primary disaccharide in insect hemolymph, into two molecules of glucose. *B. bassiana* is an entomopathogenic Deuteromycota with a wide host range that infects insects and ticks. Even though all strains studied so far produce considerable levels of chitinases. There are roughly six different chitinases found in *B. bassiana* [17].

3.8 Role of EPF in Pest Management under Field Conditions

The most damaging pest of the bitter gourd is *B. cucurbitae*. Chemical pesticides have always been used to manage it, despite their dangers. EPFs are a chemical-free, environmentally friendly solution. The researchers were interested in how successful *M. anisopliae* and *B. bassiana* were at combating *B. cucurbitae* on bitter gourd. *B. bassiana* and *M. anisopliae* were found to be efficient against *B. cucurbitae* in both laboratory and field settings. However, contact applications (73.43 and 59.72% respectively) had the highest efficacies. The pathogenicity of both fungi enhanced as concentration and time intervals increased. When both fungi were sprayed with 108 CFU/ml concentrations in the field, considerably lower fruit infestations and a much higher population decrease of *B. cucurbitae* were detected at 30 DAT. EPF had the potential to be utilized against *B. cucurbitae*, but the former proved to be more effective [18].

3.9 Success Stories of Using EPF to Manage Fruit Flies

Several agricultural and forest insects have been successfully managed using microbial control approaches in Brazil and China. EPF has been employed for pest management in both nations since the 1970s. However, neither country's EPF production nor marketing has been consistent. From 1970s to 1990s, several businesses and cooperatives ceased operations or went out of business. Brazilian farmers' lack of trust in current mycoinsecticides and China reduced engagement and government subsidies for biological control. *B. bassiana* was used on an annual basis in 0.81.3 million hectares in China until the 1980s. The two most researched fungal species for the management of stored-product insect species are *B. bassiana* and *M. anisopliae*. Both species have a wide range of hosts and have been tested against a wide variety of stored-product insect pests. This paper covers the use of these agents in conjunction with other pest control methods as well as the influence of biotic and abiotic factors on the virulence and efficacy of entomopathogenic fungi in storage pest management. Entomopathogenic fungi's effectiveness is influenced by temperature and relative humidity, with varying results; however, utilizing EPF in combination with diatomaceous earth has a synergistic or additive impact in many situations. Two *B. bassiana* isolates and 12 *M. anisopliae* isolates were evaluated for pathogenicity against adult fruit flies *C. capitata* and *B. zonata* under laboratory conditions. Fruit fly suppression with EPF may be achievable by utilizing an autoinoculation device, according to these studies [19].

4. CONCLUSION AND RECOMMENDATIONS

The purpose of this work was to express the capacity of *B. bassiana* cuticle degrading enzymes to have good effects on larval mortality and induced deformity in different stages of *B. zonata*. The ultimate penetration of *B. bassiana* in insects happens mostly through the intact cuticles, rather than wounds or natural orifices, and involves a mix of mechanical and enzymatic mechanisms. The death of the insect host occurs because of tissue penetration, widespread development in the hemolymph, and the synthesis of toxins after penetration. *B. bassiana* and *M. anisopliae* are anamorphic fungi that reproduce predominantly by blastospores rather than hyphal development [20]. Hydrolytic enzymes including proteases, chitinases, and lipases are generated and secreted during fungal penetration into the host cuticle and are thought to be critical for the commencement of the infection process, which leads to cuticle transposition. *B. bassiana* infect the insect's juvenile stages and found concentration-dependent patterns. Infected larvae's biochemical study revealed an increase and reduction in total proteins, carbohydrates, and lipids [21-23].

5. SUMMARY

The current study revealed that *Bactrocera zonata* (S) is a highly damaging pest of fruit crops. This specie is extremely difficult to control due to its small, polyphagous, multivoltine, and a lot of mobility in the adults. The use of biological control agent against *B. zonata* is most convenient and ecofriendly. The entomopathogenic fungi (EPF) can manage the population of *B. zonata.* Among the most promising biocontrol agents are entomopathogenic fungi. They have seen a lot of application in agroecosystems and have proven to be effective at preventing pest outbreaks. These microbes are one of a kind in that they infect their hosts through the cuticle, relying largely on a set of hydrolyzing enzymes to do so. To get beyond the initial defense barrier in insects, the integument, and effective entomopathogenic fungus uses a mix of mechanical and biochemical processes. *Beauveria bassiana* is an amorphous fungus capable of controlling *B. zonata. B. bassiana* is pathogenic to *B. zonata* because it contains CDE and Mycoproteins. When mixed with *B. bassiana* mycelial media, the isolated cuticle degrading enzymes including proteases, lipases, and chitinases with varying molecular masses demonstrated pathogenicity against adult, larvae, and pupae of *B. zonata*. These enzymes cause direct toxicity in the cuticle of *B. zonata* larvae, pupae, and adults, resulting in severe mortality.

MAIN HIGHLIGHTS

- Entomopathogenic fungi *Beauveria bassiana*
- Biological control through Cuticle degrading enzymes
- Biological Management of *Bactrocera zonata*

NOVELTY STATEMENT

B. bassiana is enriched with cuticle degrading enzymes including protease, lipase, chitinase that helps in degradation of insect cuticle directly. These enzymes destroy the antifungal proteins present in insect cuticle.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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