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Review Article

ENTOMOPATHOGENIC FUNGI A NEED OF HOUR IN MANAGEMENT OF *BACTROCERA ZONATA;* CHALLENGES AND OPPORTUNITIES

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Abstract *The fruit flies (Diptera: Tephritidae) are polyphagous insect pests. Different methods are used to control this destructive pest including physical, chemical, genetic, and biological control. Biological control comprises predators, parasitoids, and pathogens. Now a day's use of Entomopathogens is increasing very rapidly. Entomopathogenic fungi are one of the potential eco-friendly pest management alternatives to conventional pesticides. The most important Entomopathogenic fungi include Beauveria bassiana and Metarhizium anisopliae. These fungi have a specific set of characteristics including cuticle-degrading enzymes, toxins, and proteins which make them an excellent alternative to chemical control in insect pest management. Fungi generate a range of degrading enzymes to assist break down the insect cuticle and then emit poisonous secondary metabolites that aid the fungal invasion of the hemolymph. These degrading enzymes and other metabolites can be extracted and used as biopesticides against fruit flies. These biomolecules have great potential to be an alternative to synthetic chemical pesticides.*

Keywords: *Fruit fly; Entomopathogenic fungi; Management; Enzymes; Proteins*

Introduction

Fruit flies (Tephritidae; Diptera) are phytophagous insects that eat a wide range of fruits and vegetables. Their young larvae preferred to feed on the fruit's fresh flesh or pulpy skin of the fruits for complete development, resulting in early fruit drop, damaged or rotting fruits, and decreased fruit production. However, the most common host plants of *Bactrocera zonata* include peach (Mahmoud *et al*., 2020), Guava (Sharma and Gupta, 2018), mango (Bagheri *et al*., 2017), apricot (EFSA, 2021), Citrus (Ashfaq *et al*., 2020), fig (Choudhary *et al*., 2020), Pear (Bajaj and Singh, 2017), eggplant (Khidr *et al*., 2019), tomato (Yesmin *et al*., 2019), apple (Das *et al*., 2017), loquat (Halawa *et al*., 2019), and potato (Darwish *et al*., 2014). In Pakistan, the yearly loss is believed to be as high as 21% (Khan and Naveed, 2017). Guava orchards have losses of up to 50% of their output potential, while mangoes have seen losses of 10-14 percent of their yield potential (Delrio and Cocco, 2012). *B. zonata* is responsible for 40-80% of mango damage in Southeast Asian nations under extreme circumstances (Binyameen *et al*., 2021). Fruit fly infestations in mango orchards are exclusively evident on ripe as well as unripe mangoes, making the damage patterns considerably more obvious. To lay her eggs, the adult mature female penetrated the fruits with their little sharp ovipositor. Ripe or semi-ripe fruits are chosen, and eggs are implanted slightly beneath the fruit's hard skin. The scarred, discolored, and rotting fruits show the larvae's obvious exit entrance holes (Darwish, 2016). This interior disintegration of the fruits has been shown to provide a breeding ground for fruit flies and a point of entrance for various fungal diseases, such as Anthracnose, and stem end rot of mangoes. *B. zonata* adults are anautogenous, having emerged as young from soil-dwelling pupa. It is 5-6 mm in size and reddish brown in appearance. The dorsal and lateral sides of the thorax have yellow spots. There are faint black marks on the abdomen. A full coastal band is missing (as in the wings of *B. dorsalis*), and the apical brown spot is merely diminished (FAO/IAEA, 2021). Phenylpropanoids (Synthetic Methyl Eugenol) are naturally secreted by *B. zonata* males, and they play an important role in mating. The eggs are placed under the fruit's skin. The eggs hatch into larvae that are pale in color but eventually turn creamy and are legless, thus the name maggots. Maggots are a major internal feeder, feeding on the fleshy section of the fruits, resulting in both aesthetic and economic loss. Mature maggots usually pupate in the soil after leaving the fruits. Pupae are yellowish to brownish in hue, eventually turning dark brown. A maggot prefers to pupate in

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the top 4-6 cm of soil. Within 1-2 weeks, adults emerge from the pupa. Adults reproduce by mating by mating and repeating their life cycle. *B. zonata* has a long adult life span. During her lifetime, a mature female may lay over 500 eggs. *B. zonata* is found all year in Pakistan, except January and February (Khalil *et al*., 2010)

Management

Pest management in crop production is very important because of its negative impact on horticultural yield. Integrated Pest Management is a long-term pest control technique that has largely focused on ecology and evolution in pest management in recent years (Peterson *et al*., 2018). Pest control tactics are solely determined by several aspects, including pest knowledge, agricultural economics, environmental concerns, oral values and laws awareness, sensible policies, extension services, consumer acceptability, and marketing potential (Rezaei *et al*., 2019). Another strategy for reducing pest pressure is habitat manipulation. If natural enemies are exploited, this can be accomplished by lowering fitness costs (Gurr *et al*., 2017). Male annihilation tactics, cultural control, physical control, and synthetic pesticides were all utilized in the past to manage *B. zonata* in mango orchards (Al-Eryan et al., 2018). According to Dias *et al.* (2018), several monitoring and management strategies have been reported, including 29% biological control, 20% chemical control, 18% behavioral control, 17 percent biological insecticides, 13 percent natural insecticides, 7% mechanical control, and only 6% genetic control.

Entomopathogens

The use of entomopathogens or biopesticides based on entomopathogens is becoming more common in sustainable agricultural production (Dara, 2019). Because of their specificity in action, their efficacy is a little alarming when compared to pesticide application. The use of these entomopathogen-based biopesticides has been useful because they are significantly safer to employ, highly specific in action, and a popular alternative to chemical insecticides (Bahadur, 2018). In the past, the market for biopesticides was projected to be worth 3 billion dollars, accounting for only 5% of the total crop protection market. In the future years, it is predicted to climb by as much as 4.5 US dollars (Marrone, 2014; Olson, 2015; Kumar *et al*., 2019). Biopesticides control insects using natural disease epizootics over large populations. The biopesticide market is substantially dependent on a variety of goods, but it is mostly dependent on the discovery of Bt, with Bt-based products accounting for up to 80% of the market, followed by fungal-based products. These solutions are expected to replace around 20% of pesticides worth $7 billion soon (Ruiu, 2018). By the end of 2050, the massive annual increase in biopesticides' proportion of the market, estimated to be over 15%, may have equaled the production of synthetic chemicals (Dalmas and Koutroubas, 2018).

Entomopathogenic fungi

Entomopathogenic fungi are biological organisms that use the exoskeleton to infect insect hosts. EPFs are well-known biocontrol agents; they are known to kill insects in around 90 genera and 700 species (Maina *et al.*, 2018). EPF aided in the transmission of illnesses in insects to control pest populations (Jaronski, 2015). Over 800 spore-forming species have been identified to induce infection in insects and mites up till now (Dar *et al.,* 2017). *Beauveria bassiana* Balsamo, described by Vuillemin in 1912, *Metarhizium anisopliae* Metchnikoff, described by Sorokin in 1883, and *Isaria fumosorosea* Wize (Ascomycota: Hypocreales) are the most frequent and commonly utilized EPF species (Aguilar-Marcelino *et al*., 2020). EPF have complex methods for producing reproductive entities, like as conidia, which are adhered to the host cuticle and then enzymatically processed. Appressoria grows for post-penetration and eventually kills the insect host by consuming nutrients from the insect body (Brunner-Mendoza *et al.,* 2019).

Mode of action

Fungal spores must undergo some mode of action to cause illness in hosts, and different factors such as biological and physicochemical (genetics and molecular biology) are involved in disease development (Ayudya *et al.*, 2019). However, early scientists compiled all of the information on diverse components of the illness infection process to study the method of action of fungus (Steinhaus, 1949; Muller-Kogler, 1965; Wang *et al*., 2016). Fungi must go through the following processes to cause illness in insects. Conidial attachment is influenced by the biochemical characteristics of conidia and the insects' cuticle. Conidia and cuticle hydrophobicity should be required for adhesion to the host epicuticle. Germination will take place under appropriate conditions once the conidia attach to the surface of the cuticle (Valero-Jiménez *et al.*, 2016). Following germination, peg development begins as the conidial germ tube grows. This will cause the cuticle to multiply and infiltrate the host. Appressoria is generated as a result of this successful infection. This might necessitate or not necessitate a successful infection procedure (Gange *et al.*, 2019). The initial role after the creation of appressoria is to breach the cuticle to increase the period of action and hence boost the infection process (Petrisor and Stoian, 2017). Many fungi cause pathogenesis by producing chemicals such as secondary metabolites, which function as cuticle-destroying enzymes and actively grow into the host body, causing the host to die (Ortiz-Urquiza *et al.*, 2015; Zibaee and Ramzi, 2018). The production of several enzymatic

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metabolites that are considered metabolic poisons resulted in a variety of consequences on host insects, including immune system suppression and death (Vey *et al*., 2001; Anggraini *et al.*, 2021). Toxins formed during enzymatic degradation might include destruxins, bassianolids, bassiacridins, and oxalic acids, among others (Anggraini *et al.*, 2021). The insect host is paralyzed and the midgut epithelium is ruptured as a result of these poisons (Ortiz-Urquiza and Kehyani, 2013). The hyphal bodies split into blastospores when they enter the hemolymph. Blastospores can quickly divide and use resources, eventually colonizing host tissues (Biedermann and Vega, 2020). Hemocytes in the insect's hemolymph are responsible for cell responses that lead to invasion, cell engulfment, and host death (Zhong *et al.*, 2017; De Lerma *et al.*, 2019).

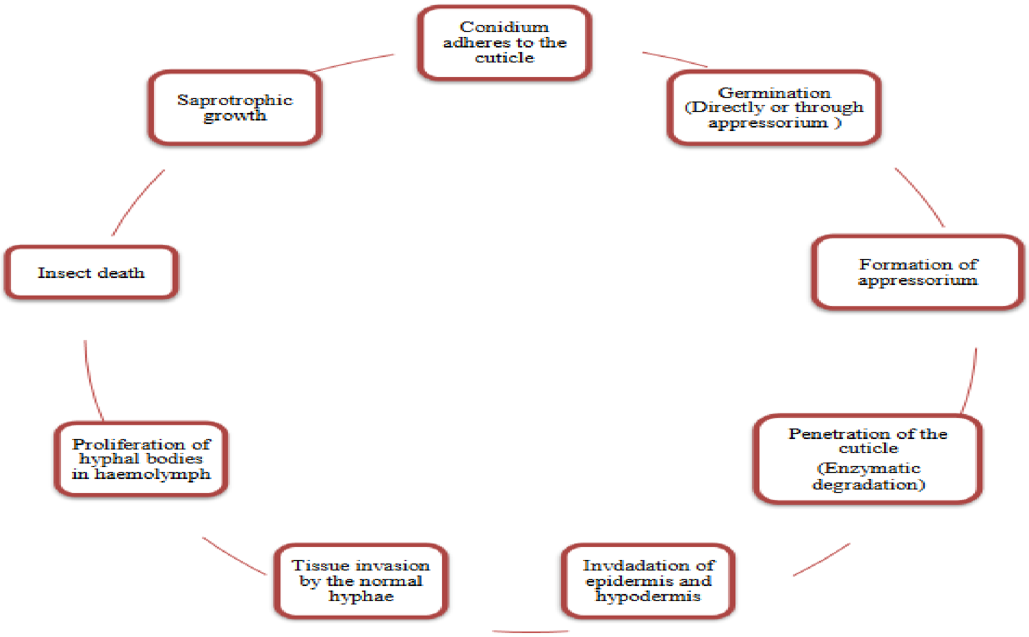

FIGURE 1.1 Diagrammatic representations of infection steps

Fungi species

Beauveria bassiana (Balsamo) Vuillemin.

B. bassiana has many close isolates which were described as a species complex due to its morphological similarities. Rehner and Buckley (2005) classified the evolutionary lineages and designated Beauveria as a separate species. *B. bassiana* was later characterized as a full species with several isolates harmful to insect belongings and as a useful biological agent in pest management (Rehner *et al.*, 2011). *B. bassiana* is a teleomorph that reproduces sexually, yet it is sometimes regarded as an anamorph (Li *et al*., 2001). *B. bassiana* is presently being used to combat a variety of pest species all over the world. Over 750 pest species have been identified as possible hosts for *B. bassiana* isolates, which might have a global influence on arthropod microbial pest control (Mwamburi, 2020). Several laboratory investigations revealed that *B. bassiana* was efficient in killing a variety of insect pests. In field research, however, there was a lot less success (Fernandes *et al*., 2011).

Metarhizium anisopliae (Metchnikoff) Sorokin

Metarhizium anisopliae, a widely distributed isolate typically found in soil, was described by Driver *et al.* (2000). Bischoff *et al*. (2009) later investigated its phylogenetic lineages. *M. anisopliae*, often known as green muscardine disease, produces anamorph spores. Conidia produced by *M. anisopliae* infect the insect cuticle following contact. Insect mortalities began during hyphal development, with the creation of many peptides, including destruxins (Golo *et al.*, 2014). *M. anisopliae* has been identified as infecting over 300 insect species. Phialides are hyphae with septate, simple/branched hyphae that form a cluster on the mycelium (Cito *et al.*, 2014). *M. anisopliae* conidia are single-celled oval in form. The difference was noted as species *anisopliae* has short conidia (3-9), whereas var. *anisopliae* has big conidia (9-18) (Mathulwe *et al.*, 2021).

Factors affecting pathogenicity of EPF

Multiple conditions influence EPF pathogenicity, including sunshine, temperature, humidity, rainfall, and the cuticle composition of the insect host (Jaronski, 2010). The infectious process normally begins within 24 hours of the first entrance and is followed by spore activation, cuticular disintegration, and the production of appressoria, making the insect host more vulnerable regardless of the conditions present (Ortiz-Urquiza and Keyhani, 2016). The practical application of EPF has been extensively researched; however, pathogenesis and virulence factors remain unknown. The existence of certain EPF varies depending on the parameters listed above. *M. anisopliae* is commonly utilized as a biocontrol agent for agricultural pests, but Isaria spp. infects forest pests. *B. bassiana* is recognized to have a wide variety of environmental preferences (Medo and Cagá, 2011). Namasivayam *et al.* (2015) reported their findings, concluding that pH, humidity, temperature, and chemical pesticides all have a substantial influence on *M. anisopliae* colony counts. The most fungal colonies were found under settings with a pH of 7.5, 80 percent humidity, and a temperature of 30°C. This research suggests that these isolates might be useful in biopesticide-based IPM programs. The effect of high temperature and relative humidity in the EPF infection cycle is crucial. RH of up to 50% was proven to be quite effective (Cui *et al*., 2012). Conidial attachment is boosted by high humidity to overcome insect cuticles (Bugti *et al.*, 2020). Temperature extremes do not affect the persistence and effectiveness of fungal isolates (Quiroz *et al.*, 2019). Jaronski (2009) discovered that *B. bassiana* conidia were more viable on the underside of the leaf than on the upper side. The effectiveness of EPF is influenced by solar radiation and UV light (Hajek and Meyling, 2017). UV tolerance in *B. bassiana* and *M. anisopliae*, on the other hand, is well understood (Fernandes *et al.*, 2015). Wu *et al.*, (2020) tested three *B. bassiana*, *M. brunneum*, and *Cordyceps fumosorosea* strains against various temperatures and UV rays. The findings demonstrated that these strains can survive in moderate-temperature environments with high UV intensity. These glycoproteins have a role in spore binding to the insect cuticle (Idris *et al*., 2020). The epicuticle is the major body where the germ tube penetrates, followed by the creation of appressoria, which then breaches insect bodily tissues, resulting in death (Sharma and Sharma, 2021).

Factors affecting EPF

Pathogen

Epizootics caused by a fungus are mostly affected by the compatibility of the host and the pathogen, but they can also be caused by other variables such as spore density, dispersion, persistence, virulence, genetics, host range, and latency (Qayyum *et al.*, 2021). To effectively infect and kill insects, fungus spores need to be present in a certain concentration (Cafarchia *et al*., 2022). The likelihood that insects may encounter sufficient infectious propagules that cause disease chances to increase by high spore density (Mantzoukas *et al.*, 2022). Based on the capacity of pathogen to recognize and overcome the defense system of host, virulent strains of fungi must be attached with the insect host (Shang *et al*., 2022). A pathogen's capacity to cause disease is influenced by the effect of environmental conditions on host-pathogen interactions (Escudero-Pérez *et al.*, 2023). The strains that perform excellent in field are capable of effective host penetration and are environmentally suited (Oke *et al.*, 2022). Higher environmental persistence also enhances the likelihood that a vulnerable insect may come into touch with enough inoculum to cause disease (Cecílio *et al*., 2022).

The Host

A wide range of physiological, behavioral, and morphological factors, including insect population, food, ecdysis, developmental stage, preening, basking, and damage caused by mechanical, chemical, or microbiological agents have a significant impact on the susceptibility EPF against the insect pest (Cours *et al.*, 2023). It has been observed that stress may increase insects' susceptibility to entomopathogens. Chemical pesticides, diet and environment are stressors (Xu *et al.*, 2023). They concluded that inadequate food increases the insect's susceptibility to the fungus (Teder and Kaasik, 2023). The study evaluated the effects of synthetic and natural insecticides on spore production and mycelial development, finding that the effects of insecticides ranged from -169 to 94.1 percent and -25.6 to 87.6 percent, respectively (Sain *et al.*, 2019). In contrast, sub-lethal fipronil insecticidal stress on the bait weakens termites and lowers their defense allowing for fungal invasion (Zhu *et al.*, 2016). Srei *et al.* (2019) discovered a substantial relationship between sex and the fungus transmission that occurs through *Agrilus planipennis*. The male *A. planipennis* insects are a prospective source of fungus for mating-related transmission to the females. The interaction between infected and uninfected populations is enhanced by higher host density, which also makes infection easier and increases the pathogen's availability of nutrients and substrates for growth. As a result, there will be more inoculum accessible to aid in transmitting disease across the ecosystem (Van Allen *et al.*, 2023). When EPF is used, mortality in older larvae can reach 71.6%, whereas mortality in immature larvae can reach 79.8% (Dannon *et al.*, 2020). However, *Glossina morsitans* younger than one day old had higher resistance to *M. anisopliae* infection than those between 20 and 40 days old (Ayaovi *et al.*, 2021). It has also been shown that the insect's developmental stage has a significant impact on the epizootics of EPF. In comparison to the older third instar larvae, the 1st and 2nd instar larvae were more susceptible to infection (Mohamed and Shairra, 2023). When pests are given the EPF, some insects seem to behave differently. By raising their temperature of thoracic to 30–32.6°C, which is higher than *B. bassiana* optimal temperature of 25°C, and by basking in hotter environments, infected organisms exhibit certain behavioral responses to the pathogenic fungus (Zembrzuski *et*

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al., 2023). Carpenter ants (Camponotus spp.) with parasitic fungal infections have also been seen to have a dangerous death grip (Will *et al.*, 2023). In honeybees, ants, and termites, the smell of *Metarhizium* spores will result in behavioral resistance to infection (Jiang *et al.*, 2023). Some pest cuticles have physicochemical characteristics that affect the way a disease spreads, and some insects coat their cuticles in gland secretions that carry antimicrobial agents (Lu *et al.*, 2016). Once the fungus penetrates the hemolymph, numerous immunological responses are triggered as a defensive strategy (Wang *et al.*, 2021). For instance, in one research of the 26 species of ants examined secreted fluid into their body, which was produced by thoracic metapleural glands (Yek *et al.*, 2011). Some insects' black cuticles are naturally resistant to EPF (Grizanova *et al.*, 2019). According to research on *Musca domestica*, cuticular blackness is correlated with immune competence and phenoloxidase titers in the blood (Kariithi *et al.*, 2017). For instance, cuticular extracts from larval *Helicoverpa zea* hindered the germination of *B. bassiana* conidial cells (Nicoletti *et al.*, 2023). Some Insects hindered the germination of conidia of *M. anisopliae*, which has been connected to the influenced of the aldehyde (E) 2-decenal (Kumari *et al.*, 2023).

The Host Plant

Plant alters survival of spore, chemistry of surface, and morphology of leaf that influence the phenomenon of fungal spore enlistment by the insect pest, and plant soluble materials that impact the conidia are just a few examples of the plant-mediated effects on EPF (Brandt, 2022). Hountondji *et al.* (2005) investigated how three isolates of the acaropathogenic fungus Neozygites tanajoae were induced to produce spores by volatiles coming from green mite-infested cassava plants (*Mononychellus tanajoa*). Plant epicuticular waxes, which contain fungistatic chemicals, promote faster and more extensive fungal germination (Butt *et al.*, 2016). Surface leachates and soluble leaf extracts increased the germination of conidia and pathogenicity of *M. anisopliae* against *Phaedon cochleariaea* (Björkman *et al.*, 2011). Insect immunity and disease resistance are influenced by plant elements such allelochemicals and nutrients, whereas plant shape affects the frequency of spore encounters during insect activity (Cory and Ericsson, 2010). Phenylethyl-3-butenyl isothiocyanates are present in the Phaedon cochleariae plant, and when *M. anisopliae*-inoculated insects are exposed to these vapours, the pathogenicity is reduced (Westerdahl *et al.*, 2023). When compared to those eating a diet high in protein, the insects that fed on the carbohydrate-rich and protein-rich caterpillars were somewhat less (Graham *et al.*, 2014).

Environment

The most significant abiotic factors that affect the effectiveness of EPF against insects include temperature, sunshine, humidity, wind, and rainfall. Since these variables are in line with other environmental elements (such as soil and crops). They can be handled interactively to alter entomopathogens (Yang *et al.*, 2022).

Temperature

It is the factor that has the most impact on how well EPF works. It affects the progression of the disease and the time of mortality (Xia *et al.*, 2023). According to Allsup *et al.* (2023), Strains of EPF from hotter areas operate best at greater temperatures whereas those from colder locations function best at low temperatures. For six strains of *M. anisopliae* that infect three species of fruit flies. The optimum temperature is 30°C although temperatures between 25 and 30°C are suitable for diverse fungal isolates (Onsongo *et al.*, 2022). *B. bassiana* and *M. anisopliae* isolates which were investigated developed more favorably in the 25 to 30°C range of temperature (Sani *el al.*, 2023). *B. bassiana* can withstand temperatures as low as 8°C and as high as 37°C (Kryukov *et al.*, 2012). *B. bassiana* and *M. anisopliae* strains grow at temperatures as low as 5°C and as great as 35°C and higher, it has been repeatedly discovered that 20°C to 30°C is the ideal temperature range for germination (Borisade and Magan, 2014). However, compared to 20°C or 30°C, several isolates germinate more quickly at 25°C (Mwamburi *el al.*, 2015). Except TP-GHA isolate of the *B. bassiana*, that germinated better at 30°C. The maximum germination rate (80–100%) was discovered for isolates of *M. anisopliae* and *B. bassiana*. *B. bassiana* F-263 exhibited approximately 100% germination in 20 hours of incubation at 25 °C to 33 °C. 14 of the 14 spores showed>90% germination in a temperature range of 25-35°C, while only 9 of the 14 spores still exhibited comparable germination rates at a temperature of 38°C (Wongwanich *el al.*, 2017).

Solar radiation

Solar radiation has an impact on the field survival capacity of EPF (Cafarchia *el al.*, 2022). Solar UVA and UVB decrease conidia viability, potentially because of fatal and mutagenic DNA damage (Sutanto *et al.*, 2022). Significant conidial UV penetration reduces germination and sometimes complete inhibition of conidia, which reduces the effectiveness of bio-pesticides (Sharma *et al.*, 2020). This damage includes degradation of cytoplasmic membranes and organelles as well as direct and indirect DNA damage. Living fungal conidia must undergo extensive regeneration and restart germination after being exposed to UV light (Braga *el al.*, 2015). Infectious conidia of the *Metarhizium* are purposefully rendered inactive by even brief exposure to sunlight, particularly the UV-B region of

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the spectrum (285–315 nm) (Brancini *et al.*, 2022). The genetic susceptibility of EPF species to UV radiation varies (Acheampong *et al.*, 2020). Indirect sun radiation can have negative consequences on conidia, such as desiccation and heat stress (Nascimento *et al.*, 2010). The time and intensity of conidia's exposure to sun radiation dictate its effects (Gonzales *et al.*, 2017). Fungal sun radiation tolerance exhibits both inter- and intra-specific variations regularly (Harrouard *et al.*, 2023). When the relative humidity is close to saturation (>95.5% RH), spore germination and germ-tube elongation are at their optimum (González-Mas *et al.*, 2019). For most fungi, moisture levels >96% are required for spore germination and mycelial growth (Wan *et al.*, 2023). *B. bassiana* and *M. anisopliae* can infect their hosts under dry conditions, possibly due to adequate humidity within the microhabitats (Jaronski *et al.*, 2010). Low moisture content conditions are less harmful shortly after the application of fungal bodies (Marciano *el al.*, 2021).

Rainfall

Rain may quickly remove conidia from plant cuticles and insect pest cuticles and spread those conidia (Behle and Birthisel, 2023). Rainfall caused many conidia of *B. bassiana* and *M. anisopliae* to be washed from plant surfaces (Rossouw *et al.*, 2023). On fungal epizootics, however, precipitation has positive effects (Ulloa-Avellán *et al.*, 2023). Conidia are dispersed from substrates by precipitation, although there is considerable evidence that spores and conidia may be removed in significant numbers from the cuticle during precipitation, despite the hydrophobic processes of conidial adherence (Quesada-Moraga *et al.*, 2023). Oil carriers appear to have higher rain strength than watery carriers (McGuire *et al.*, 2023).

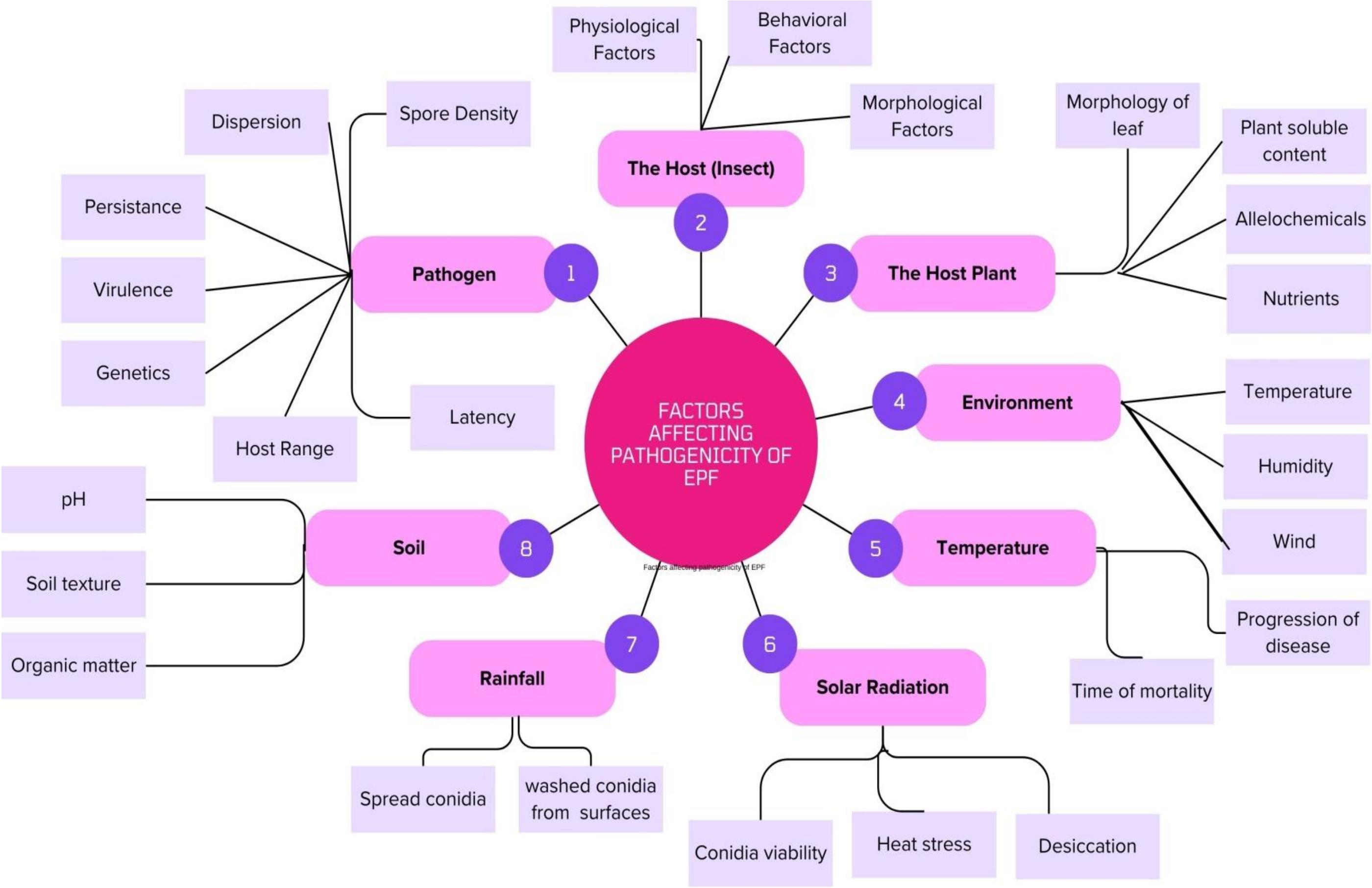

Figure 1.2 Factors affecting pathogenicity of Entomopathogenic fungi

Soil

The frequency and distribution of EPF in the soil are significantly influenced by a variety of soil factors (Bueno-Pallero *et al.*, 2020). Greater percentages of EPF harboring have been linked to acidic soils, a thick soil texture, and a high organic matter concentration (Litwin *et al.*, 2020). The soils of hedgerows were where *I. fumosorosea* was most frequently found, but *I. farinosa* was only found in forest environments. In addition to being in natural environments, *M. anisopliae* is not adversely impacted by intensive agriculture (Medo and Cagá, 2011). They investigated EPF prevalence across several botanical soil ecoregions. *B. bassiana*, *Fusarium oxysporum*, and *M. anisopliae* were the most typical EPF isolates (34%, 14%, and 2% respectively). Insect harmful fungi showed 96% variability in canonical correspondence analysis of soil abiotic factors (Bueno-Pallero *et al.*, 2020).

Pathogenicity of *B. bassiana* and *M. anisopliae* to *B. zonata*

EPF's effectiveness against tephritid fruit flies is widely known, and it has been tested on a variety of fruit fly species (Beris *et al.*, 2013; Shapiro-ilan *et al.*, 2017). These EPF isolates were tested against a variety of fruit fly species, including *B. zonata* (Sookar *et al.*, 2014a) *B. dorsalis* (Ugwu *et al.*, 2020), *Anastepha ludens* (Montoya *et al.*, 2020), *A. oblique* (Dias *et al.*, 2020), *Ceratitis capitata* (Chergui *et al.*, 2020), *Rhagoletis indifferens* (Yee, 2020), and *R. cerasi* (Drobnjaković *et al.*, 2019). Mahmoud *et al.* (2009) demonstrated the potential effect of *B. bassiana* and *M. anisopliae* against larval and pupal stages of *B. zonata*, demonstrating the dangerous effect of pesticides and increased cases of resistance due to insecticide use. *B. zonata* showed sensitivity to these possible isolates in laboratory bioassays. Contact and oral bioassays were used to test both sexes. *M. anisopliae* was shown to be substantially more harmful to male populations than female populations, followed by *B. bassiana*. At 2.5×10^5 conidial suspension, the combined action of *B. bassiana* and *M. anisopliae* demonstrated mortality of up to 80%. Sookar *et al.* (2014a) investigated the impact of *M. anisopliae* on *B. zonata* fertility and fecundity. Adult fruit fly populations treated with *M. anisopliae* solution were tested to see if they affected *B. zonata* courting behaviour. Both the treated donor and the untreated recipient populations were kept. To infect females, dry conidia weighing 0.3 g were distributed on a velvet piece and placed in glass jars. The results were favorable since the recipient females were determined to be vulnerable and were killed. The death rate in *B. zonata* females increased to 83 percent after 14 days after inoculation. Males of *B. zonata* were also shown to be susceptible to fungal spores, with mortality rates of up to 88 percent. Abdel-Galil *et al*. (2019) compared *B. bassiana* and *B. zonata* in laboratory research. All adult population of *B. zonata* was killed by the spore suspension @ 3×10^7. After 72 hours, there was a 100 percent mortality rate. When concentrations @ 3×10^6 were used, 100 percent mortality was found. Spore suspensions were found to be fetal after 120 hours following application. When *B. bassiana* was used @ 3×10^7 the lethal time (LT50) to kill 50% of the population was much longer (46.06 h).

Role of enzymes in fungal pathogenesis

EPF produces a wide range of extracellular enzymes including as lipase, protease, catalase, phospholipase, and chitinase, which have cuticle destroying characteristics and have a negative role in virulence (Butt *et al.*, 2016). The existence of a cuticle; an exterior skeleton performed a variety of tasks in sustaining insects and anchoring insect muscles (Wang *et al.*, 2017). The active involvement of the cuticle, yet fungi are the only entomopathogens that can overcome this rigid exocuticle (Mannino *et al.*, 2019). EPF pathogenesis usually begins with the fungal body adhering to the insect cuticle. Appressoria grows after successful germination, surpassing the insect cuticle with quick breach (Pedrini, 2018). As a result, these potential EPF isolates can be infected by both cuticle and oral routes. However, the insect has a tougher time optimizing and resisting the effects of EPF (Rafaluk-Mohr *et al.*, 2018). Cuticle's hydrophobic qualities assist *B. bassiana's* function and subsequent enzyme release (Mazutti *et al.*, 2020). Extracellular enzymes such as chitinase, lipase, and protease are secreted by *B. bassiana*, indicating their function in physiological processes in the host. The pathogenic capacity of the EPF in insect mortalities is due to these enzymes (Moharram *et al.*, 2021). Following spore adhesion to the insect exocuticle, enzymatic activity begins with the creation of typical protease enzymes, which promote the action of appressoria, which breaks the insect body and causes invasion, resulting in death due to nutritional shortage. Elkhateeb *et al*. (2021) looked at EPF's activity profile, which includes the release of certain enzymes and possible poisons that allow the EPF to kill the prospective host. The mechanisms of penetration, fast multiplication of hyphal structures inside the insect's body, and rapid physiological processes leading to death are all well understood. Lipase is the most significant extracellularly released fungal enzyme (Geoffry and Achur, 2018), and it is present in a wide range of fungal species (Mehta *et al.*, 2017). Pascoal *et al.* (2018) investigated the involvement of lipase enzymes in the fungus infection process. Lipase, a cuticle disintegrating enzyme, is significantly related to virulence in EPF such as *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* (Cristina and Gheorghe, 2017). Splitting esters and long chain fatty acids found in insect cuticles is the job of this lipase (Meghwanshi and Vashishtha, 2018; Verma *et al.*, 2021). The release of lipase is completely responsible for the hydrolysis of all proteins, chitins, and lipids in insect cuticles (Mazutti *et al.*, 2019). *B. bassiana* (Dhawan and Joshi, 2017) and *M. anisopliae* have been shown to secrete lipases regularly (Dunaevsky *et al.*, 2020). Enzymatic activities and metabolite productions are increasingly being studied and frequently employed in pest management strategies (Litwin *et al.*, 2020). Chitinase is designed to break down the cuticle, which is protected by many proteins. These chitinases are unable to function alone; instead, they

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have been observed in combination with other enzymes (Dionisio *et al.*, 2016). The chitinase enzyme aids in the breakdown of chitin-rich insect cuticle. The midgut epithelium, also known as the peritrophic membrane, will be targeted, resulting in lower host insect survival and growth (Gupta and Jindal, 2014). This has stopped an abrasive attack on the insect's stomach wall, resulting in insect death. This enzyme is the most prevalent in the manufacture of chitin. Chitinases are classified according to the domains and numbers they encode (Doucet and Retnakaran, 2012). Parray and Shameem (2019) investigated the devastating impact of the Chitinase enzyme in insect and antagonistic plant infections, as well as the classification of pathogen strains and domains. Chitinase synthesis in EPF is regulated by chitin fibres, which in turn are regulated by the insect host and diet (Niassy *et al.*, 2013: Anwar *et al.*, 2019). EPF has a variety of chitin-degrading enzymes, including 10-20 distinct chitinases that scavenge resources and perform a variety of aggressive functions (Hamid *et al.*, 2013; Agrawal *et al.*, 2015). Chitinases have a variety of genes and structures that aid with EPF pathogenicity (Bhanu *et al.*, 2012). Different hydrolytic enzymes from *B. bassiana* and *M. anisopliae* were examined and described by Hayakawa *et al.* (2017). Chitinase's chitinolytic activity was found to be harmful in both vitro and vivo studies. *B. bassiana* and *M. anisopliae* both manufacture chitinases with cuticle-damaging properties. In addition to protease, they work together to hydrolyze insects (Amobonye *et al.*, 2020). Dhawan and Joshi (2017) studied the differences in enzyme release and variability across *B. bassiana* isolates. The data indicate that chitinase is the most abundant enzyme, followed by protease and lipase. The study discovered that these extracellular enzymes may play a role in *B. bassiana's* pathogenicity against pests.

Role of toxins and proteins in pathogenicity of EPF

Exogenous toxins and proteins are among the numerous elements that contribute to EPF pathogenicity in insect pest management (Leger and Wang, 2010). These fungi create a variety of hazardous metabolites through various reactions, and they have a wide range of chemical characteristics, morphological structures, and final insecticidal toxicity (Xu *et al.*, 2021). These toxins have a lot of promise as biological control agents because of their genotoxic effects. Depending on the synergistic effects of both crude and refined extracts, they have lethal effects on various host insect species. Different approaches are currently being used to identify and purify these poisons and proteins. Dudley *et al.* (2010) used mass spectrometry to detect a variety of poisons known as destruxins. Cyclic peptides are generated by a fungus that infects insect hosts. Successful growth circumstances of these DTXs found in various cultural environments necessitate a variety of optimizations. *B. bassiana's* host pathogenic interaction sheds light on their dynamic interactions with their hosts (Butt *et al.*, 2016). Metabolites from *B. bassiana* have the potential to treat human diseases (Schmid, 2015). Only one species of fungus, Beauveria, generates harmful substances that may increase the pathogenic potential and virulence of other fungi, such as alkaloids, Oosprien, Bassianin, Pyridovericins, Cyclopeptides, and Cyclades Peptides (Patocka, 2016). Xu *et al.* (2009) reported bassianolide, an insecticidal virulence factor for *B. bassiana*. In a comparative study, it was shown that *Helicoverpa zea*, *Spodoptera exigua*, and *Gallaria mellonella* were infectious and had established virulence factors. *M. anisopliae* can produce destruxins, which are physiologically harmful substances, in addition to directly attacking the host insect. The metabolites poisoned several insect species. Utilizing a variety of culture media, the in vitro separation of these metabolites and toxins from conidial suspensions is straightforward (Carollo *et al.*, 2010). In addition to DTXs, Metarhizium spp. also produces myroridins, tyrosine, viridoxin, swainsonine, serinocyclins, and aurovertins (Krasnoff *et al.*, 2006; Krasnoff *et al.*, 2007; Azumi *et al.*, 2008). Liu and Tzeng (2012) reported that *M. anisopliae* displayed the insecticidal and toxic properties of DTXs. These DTXs improve *M. anisopliae's* virulence factor against specific insect species thanks to a variety of biological effects, including immunological suppression, anti-proliferative, cytotoxic, and phytotoxic properties.

Mycotoxins like Beavericins are produced by several genera of the fungus in the family Hypocreales (Xu *et al.*, 2009). The existence and generation of several Beavericins among the Hypocreales fungus are discussed by Urbaniak *et al.* (2019). *B. brongniartii* and *B. bassiana* both generate beauveriolides. This toxin also contributes to the pathogenicity of the fungal species against some host insects (Yin *et al.*, 2020). The effectiveness of *Metarhizium spp*. DTXs against a range of endophytic pests has been demonstrated (Golo *et al.*, 2014). Due to the production of potential lytic enzymes and metabolites, *Metarhizium spp.* possesses DTXs, which have been effective against a wide variety of insect species (Wang *et al.*, 2012). However, the effectiveness of these metabolites is inhibited when pesticides are applied to the soil. The longevity of potential pesticides like Acetamiprid (Neonicotinoids) in soil, plant and animal tissues, and fungal cells limits the capacity of EPF to survive. Acetamiprid's persistence in soil boosted the spores' propensity to infect the soil, but its usage against insects along with

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Metarhizium anisopliae significantly reduced the production of DTX (Litwin *et al*., 2020).

Conclusions and recommendations

This review emphasizes the value of Biological Control Agents (BCAs) in the management of fruit flies. In organic farming or other situations where the use of synthetic pesticides is constrained by pesticide resistance or environmental concerns, Entomopathogenic fungus can replace conventional pesticides.

- Use native EPF strains because, in their native environments, they are frequently more virulent than exotic ones.
- Encouraging academics and funding bodies to set up field research projects to optimize the field eradication of fruit fly species.
- To improve collaboration and connections among the countries concerned with managing fruit flies, regional and interregional cooperation must be formed and expanded. By exchanging experiences, this would improve the spread of important outcomes.
- When compared to chemical pesticides, biological control would likely be more affordable in several situations (Dias *et al*., 2022). Thus, the environmental advantages of adopting BCAs in IPM programs must be included in cost-benefit evaluations.
- There must be more studies related to the increased persistence of EPF fungus under field conditions.
- Extraction and formulation of crude proteins of EPF will be the best option in management.

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

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Authors' Contribution

Umer Sharif wrote the paper and set it according to journal requirements. Mirza Abdul Qayyum outlined the paper flow and reviewed the manuscript. Akhtar Hameed, Muhammad Ishtiaq, Unsar Naeem-Ullah, and Hasan Taha coined the idea and reviewed the manuscript.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.

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