

Interactions between Entomopathogenic Fungi and Entomophagous Insects

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Abstract

Direct treatment of predators with entomopathogenic fungi (EPF) at different concentrations revealed that some species, like *Beauveria bassiana* and *Metarhizium anisopliae*, were found to have detrimental effects on all developmental stages of some predator species. Other species, like *Numoraea rileyi* and *Paecilomyces fumosorosius*, showed little or no effect on treated predators. Adult predators were found to avoid feeding on fungi-infected prey. EPF could be isolated from hibernating or field-collected coccinellids. Some studies indicated that combining a fungus with a predator could increase the rate of insect control in the field. As for parasitoids, this review indicates that the developmental stages of the egg parasitoids can develop successfully in host eggs treated with fungi. However, fungus-infected hosts negatively impact the development of larval parasitoids inside such hosts and this impact depends on the species and concentration of the fungus as well as the time between infection and parasitism. Some parasitoid females can discriminate between fungus-infected and uninfected hosts and do not parasitize the infected hosts. With appropriate timing, the parasitoids or predators could be combined with fungi for the biological control of insects in the field.

Keywords

Entomopathogenic Fungi, Predators, Parasitoids, Natural Enemies, Intraguild Predation

1. Introduction

The interactions between insect pathogens, parasitoids and predators have been termed “Intraguild predation (IGP)” [1]. It occurs when two species share a host or prey (and therefore may compete), or engage in a trophic interaction with each other (parasitism or predation). This interaction may impact the popula-

tion dynamics of biological control agents and target pests. Common forms of IGP include pathogens that infect both herbivores and parasitoids of the herbivores; facultative hyperparasitoids which can parasitize either a herbivore or a primary parasitoid of the herbivore; predators that attack herbivores which harbor a developing parasitoid, and predators that attack each other. Thus, IGP can be intense resulting in high levels of mortality for one or both of natural enemies, while the total mortality imposed on the target pest populations is minimal. Despite such negative interactions demonstrated in the laboratory, it is less often documented in the field [2].

The interactions between entomopathogens and other bio-control agents can be synergistic, additive or antagonistic depending on the specific biological control agents as well as their concentrations, timing of application and host species [3]. Synergistic interactions result in a higher mortality than the combined individual mortalities of the pest population. Additive interactions occur if the natural enemies do not interact and, thus, the total level of mortality is equivalent to the combined individual mortalities caused by each agent. The antagonistic interactions occur if the total mortality is less than when either natural enemy acts alone [4].

2. Entomopathogenic Fungi (EPF)

EPF are associated with insects, in almost all orders, living in diverse habitats. They are one of the most common pathogens that cause diseases in insects infesting vegetables, field crops, orchards and ornamentals. Insect adults as well as the newly molted larvae and the newly formed pupae are more susceptible to infection than those in which the cuticle has fully hardened. The egg stage is rarely infected. Over 700 EPF species have been recorded, however, very few commercial products of fungi was proved to be successful insecticides [5]. This fact is attributed to that the virulence of EPF depends on limits of environmental conditions including 20°C - 30°C and 90% or above relative humidity (R.H.).

3. Mode of Infection of EPF

EPF can infect insects through body cuticle, spiracles mouth parts and other external openings of the insect. In general, when the environmental conditions are suitable (mainly temperature of 20°C - 30°C) and 90% or above relative humidity as mentioned earlier) are suitable, the infective spore of the fungus, which is attached to host cuticle, germinates forming a germ tube that penetrates the insect epicuticular layer. Enzymes activity as well as mechanical force is involved in cuticle penetration and hyphal bodies are generally produced and apparently multiply within the hemocoel of the host. Some fungal species and strains produce sufficient toxins at this time causing death of the host although no vital organs have been invaded. The fat body is generally the preferred site for hyphal invasion. After death of the insect, the fungus grows saprophytically in the hemocoel to form a mycelial mass that turns into a hard sclerotium, and the re-

productive spores are produced within the sclerotium or on sporophores. The sporophores and the hyphae emerge from the cadavers to form the characteristic mycelial growth on the host's integument and sporulation occurs on the cadavers [6].

4. Parasitoids and Predators

Parasitoids and predators are among the biological control agents that play an important role, naturally, against agricultural pests infesting field crops, vegetables, orchards and ornamentals. They constitute a considerable part within IPM programs which include suitable timing of plantation, suitable agricultural practices, using biological control agents (parasitoids, predators and entomopathogens) and finally selective chemical insecticides if needed.

5. Interactions between EPF and Predators

5.1. Laboratory Experiments

5.1.1. Effect of EPF on Treated Predators

1) The eggs

Metarhizium anisopliae (Ascomycota, Hypocreales) and *Hesperomyces virescens* (Ascomycota, Laboulbeniales) caused 14.66% and 11.53% mortality, respectively, in the egg stage of the coccinellid, *Harmonia axyridis* (Pallas) [7]. However, the fungus, *Lecanicillium muscarium* (previously known as *Verticillium lecanii*) did not affect the eggs of the coccinellid, *Eriopis connexa* (Germar) when treated at concentrations of 1.6×10^3 or 1×10^7 conidia/ml [8].

2) The larvae

50% of the coccinellid, *Cryptolaemus montrouzieri* Muls larvae died when they fed on mealybugs sprayed with 1% Boverin (a commercial product of *Beauveria bassiana* (Balsamo), (Ascomycota, Hypocreales) [9]. At 5 concentrations of 4 fungal species (ranging from 10^4 to 10^8 conidia/ml), *B. bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* caused up to 95, 97 and 56% mortalities, respectively, in 1st and 2nd instar larvae of the coccinellid, *Hypodamia convergens* Guerin-Meneville whereas *Numoraea rileyi* did not cause mortality in such larvae [10]. At a concentration of 3×10^7 conidia/ml, *B. bassiana* caused 35.7% mortality in 2nd instar larvae of *Chrysoperla carnea* [11]. Also, different strains of *M. anisopliae* caused up to 67.50% mortality in different larval instars of the coccinellid predator, *Menochilus sexmaculatus* (F.) [12].

The fungus, *Verticillium lecanii* was found to be highly pathogenic to the 3rd instar larvae of *C. carnea* and decreased the emergence rate of adults as well as the fecundity of females [13]. Similarly, infection of the aphid, *Rhopalosiphum padi* (L.) with *V. lecanii*, 72 h before exposing to larvae of its predator, *H. axyridis* reduced the consumption rate of the aphid by such larvae [14]. *Lecanicillium longisporum*, did not cause detrimental effects on survival or prey consumption of the dipteran aphid predator, *Aphidoletes aphidimyza* (Rondani) when sprayed directly on the 4-day old larvae [15]. In contrast, at a concentration of 1×10^7

conidia/ml, *L. muscarium* caused 45% and 30% mortality in the 1st and 2nd instar larvae, respectively, of the coccinellid, predator, *Eriopis connexa* (Germar) [8].

3) The pupae

At a concentration of 1×10^7 conidia/ml, *L. muscarium* did not cause mortality in the pupae of *E. connexa* (8). However, *B. bassiana* proved to be pathogenic to the pupae of the same predator [16].

4) The adults

N. rileyi did not infect the treated adults of the predators, *H. convergens*, *C. carnea* and the pentatomid, *Podisus maculiventris* (Say) [17]. In contrast, up to 95.8% mortality in adults of *Teretrius nigrescens* Lewis (Col.: Hesteridae) a predator of the larger grain borer, *Prostephanus truncates* (Horn) (Col.: Bostrichidae) occurred after direct exposure to 1×10^9 conidia/ml of *B. bassiana* [18]. Percentage of mortality in the adults of the coccinellids, *Coccinella septempunctata* (L.), *Harmonia axyridis* (Pallas) (from Japan and Britain) and *Adalia bipunctata* (L.) was assessed when treated with *B. bassiana* [19]. Mortality of both *C. septempunctata* and *A. bipunctata* was high relative to *H. axyridis*. The impact of *B. bassiana* on *H. axyridis* Britain strain was detected via reduced fecundity at all tested doses (10^5 , 10^7 and 10^9 conidia/ml) [19].

The susceptibility of adults of 6 coccinellid species to *B. bassiana* was estimated at a concentration of 2.5×10^5 conidia/ml [20]. It was found that *Olla v-nigrum* (Mulsant), *Cycloneda munda* (Say) and *H. convergens* were susceptible to infection; 63%, 60% and 35% mortality, respectively. In contrast, *Coleomegilla maculata* (Degeer), *C. septempunctata*, and *H. axyridis* were resistant to infection. The fertility, pre-oviposition, oviposition and post-oviposition periods as well as life span of the coccinellid, *E. connexa* were negatively affected by *B. bassiana* [21]. Similarly, adults of *E. connexa* were found to be susceptible to *B. bassiana* infection and the daily fecundity of female was significantly reduced compared to the control [16].

The impact of *B. bassiana* and *Metarhizium brunneum* on the adults of *Dalotia coriaria* (Kratz) (Col.: Staphylinidae) and the two mites, *Strateolaelaps scimitus* (Womer) and *Gaeolaelaps gillespiei* Beaulieu (Acarina: Acaridae) was studied at 2 concentrations (1×10^5 and 1×10^7 conidia/ml) [22]. The results showed that mortality in *D. coriaria* was not significantly higher than the control at both concentrations of *B. bassiana* and the concentration of 1×10^5 conidia/ml of *M. brunneum*. However, the 1×10^7 concentration of *M. brunneum* caused significant higher mortality in the predator (41%). The treated *S. scimitus* was not significantly affected by both fungi species while *G. gillespiei* was significantly adversely affected by both fungi; 36% and 31% mortality by *M. brunneum* and 61% and 51% by *B. bassiana* at the concentrations of 1×10^5 and 1×10^7 conidia/ml, respectively.

V. lecanii was found to have no significant effect on fecundity and longevity of the coccinellid, *Delphastus catalinae* (Horn), a predator of whiteflies [23]. When the mirid predator, *Dicyphus Hesperus* Knight (Hemiptera) females were ex-

posed to leaf discs treated with the fungus, *Paecilomyces fumosoroseus* at a concentration of 100 fold of LC₅₀ of the nymphs of the whitefly, *Trialeurodes vaporariorum* (Westwood) nymphs, 38% of the females died. In addition, the survived females showed 38% feeding reduction [24].

5.1.2. Discrimination between Fungi-Infected and Uninfected Prey

In non-choice experiments, adults of *C.septempunctata* and the carabid, *Pterostichus madidus* (F.) were shown to consume aphids at a late stage of infection by the fungus, *Erynia neoaphidis* [25] [26]. However, larvae of the syrphid predator, *Episyrphus balteatus* De Geer and the chrysopid, *C. carnea* never consumed *E. neoaphidis*-infected aphids [26]. Similarly, *H. convergens* avoided feeding on the wheat aphid, *Diuraphis noxia* (Mordvilko) infected with *P. fumosoroseus* [27]. The females of the mirid predator, *Dicyphus hesperus*, were able to discriminate between healthy and *P. fumosorosius*-infected nymphs of the whitefly, *Trialeurodes vaporariorum* when the latter nymphs were offered to them 5 days post treatment [24]. Similarly, both male and female of *C. septempunctata* avoided *B. bassiana* spores through contact with leaf surfaces, soil-inoculated, and mycosed cadavers [28].

5.1.3. Isolation of Fungi from Predators

B. bassiana could be isolated from hibernating and/or collected adults of the coccinellids, *C.septempunctata* [29]; *Adalia bipunctata* [30]; *Cycloneda sanguinea* (L.) [31]; *Olla v-nigrum* (Mulsant) [32].

Hesperomyces virescens could be isolated from *Harmonia axyridis* [33] [34]; *Chilocorus stigma* (Say), *C. bipustulatus* (L.), *Adalia bipunctata* (L.) and *H. convergens* [35], as well as *E.connexa* [34]. The fungus, *Isaria farinosa* (Ascomycota: Hypocreales) could be isolated from *Harmonia axyridis* [36].

5.2. Field Experiments

5.2.1. Compatibility between Predators and Fungi as Bio-Control Agents

Field application by *B. bassiana* against alfalfa pests revealed that the fungus caused 75% - 93% mortality in *H. convergens* when applied early in the season but the application late in the season had little impact [37]. The authors related this little impact late in the season to the prevailing temperature which exceeded 25°C. In a greenhouse experiment, enhanced control was recorded against the thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on pepper when the adult of the predatory mite, *Neoseiulus* (= *Amblyseius*) *cucumeris* (Oudemans) was combined with *B. bassiana* with no significant effects of the fungus on the predator [38]. Similarly, in a greenhouse trial on tomato, the simultaneous use of the predator, *Dicyphus Hesperus* and the fungus, *P. fumosoroseus* resulted in additive whitefly mortality; they reduced the whitefly densities by 62% relative to the control plants in 6 weeks [24]

The combined effect of the dipteran predator, *Aphidoletes aphidimyza* (Ron-dani) (Fam.: Cicedomyiidae) and the fungus, *L. longisporum* on the aphid, *Myzus persicae* was estimated in cages in a greenhouse [15]. The results showed an

additive effect of both natural enemies as a higher reduction of aphid population was observed in treated cages compared to the treatment with the predator or the fungus alone. Similarly, the rove beetle, *D. coriaria*, was found to be compatible with *M. anisopliae* in greenhouse experiments as the fungus did not inhibit the ability of the predator to consume its prey [39]. Also, the compatibility between the fungus, *M. anisopliae* and the coccinellid, *Cheilomenes lunata* (Fab.) could provide a sustainable strategy for effective management of aphids on crucifers [40]. *A. aphidimyza* and the fungus, *M. brunneum* could be combined against the aphid, *Rhopalosiphum padi* infesting sweet corn in the greenhouse. *A. aphidimyza* applied alone suppressed the aphid population more effectively than *M. brunneum* applied alone. However, the aphid population was most suppressed when both agents were combined [41].

5.2.2. Transmission of Fungi by Predators

The potential of coccinellid adults to transmit the fungus, *Erynia neoaphidis* in aphid populations in the field (in caged plants) was demonstrated [42]. It was found that the contaminated coccinellids caused 5% infection in the aphid population. However, despite this low percentage of infection, an epizootic was initiated by such a level of infection [26]. *C. septempunctata* adults which foraged on plants with different densities of sporulating aphid cadavers became contaminated with the fungus, *E. neoaphidis* conidia. Such adults carried the conidia to uninfected aphid populations and initiated infection [43].

5.2.3. Discrimination between Healthy and Fungus-Infected Prey

From field observations, it was found that no visibly *E. neoaphidis*-infected aphids were consumed by either larvae or adults of *C. septempunctata* [42].

6. Interactions between EPF and Parasitoids

There is a fungistatic substance secreted by the parasitoids into the hemolymph of the host insect that impedes the development of mycosis and enable normal parasitoid development and emergence. This fungistatic substance is linked to the time interval between parasitism and contamination of the host by the fungus [44].

6.1. Laboratory Experiments

6.1.1. Effect of Fungi on the Development of the Parasitoids

The fungus *N. rileyi* inhibited the development of the braconid parasitoid, *Microplitis croceipes* (Cresson) if larvae of *Heliothis zea* (Boddie) were infected one day after parasitism [45]. Similarly, when the aphids were infected with the fungus, *Pandora neoaphidis*, less than 4 days after being parasitized by the parasitoid, *Aphidius rhopalosiphii*, the parasitoid did not complete its development [46]. However, when infection occurred more than 4 days after parasitism, development of the parasitoid was significantly impaired but no histological evidence that the fungus invaded the tissues of the parasitoid. Spraying the fungus, *Paeci-*

lomyces fumosoroseus against the whitefly, *Trialeurodes vaporariorum* (Westwood) on seedlings of beans at the recommended rate for the greenhouses did not affect the development of the parasitoid, *Encarsia formosa* Gahan inside its host nymph [47].

The interaction between the aphid parasitoid, *Aphidius nigripes* (Ashmead) and the fungus, *V. lecanii* was found to be dependent on the relative timing between parasitism and infection. A high proportion of the parasitoid developed successfully when aphids were exposed to the fungus 4 days after parasitism [48]. The effects of *V. lecanii* on cereal aphids, *Rhopalosiphum padi* (L.) and *Sitibion avenae* (Fab.) and whether the fungus affects the performance of the parasitoid, *Aphidius colemani* (Viereck) were studied [14]. The study revealed that percent parasitism by *A. colemani* in both insects was not affected by the fungus when aphids were first treated with *V. lecanii* and then exposed to the parasitoid. However, the emergence % of adults from the mummies was lower in infected hosts than in uninfected ones when the aphids were first exposed to the parasitoid and then treated with the fungus. The female ratio in the emerging adults was lower in fungus-treated aphids in both species than in untreated ones.

Similarly, the impact of *B. bassiana* on the biological characters of *Aphidius matricariae* (Haliday), a braconid parasitoid of the peach aphid, *Myzus persicae* (Sulzer) was evaluated [49]. Aphids were first infected with *B. bassiana* at a concentration of 2×10^8 conidia/ml and then exposed to parasitoid females for one day at 1, 2, 3 and 4 days post infection. The number of mummies produced per female parasitoid varied from 11.8 to 24.8 and was significantly different according to intervals between infection and exposure to the parasitoid (1 - 4 days). The possibility of combined use of the same fungus and the same parasitoid against the same aphid was investigated [50]. The mummies of parasitized aphids were treated with the fungus suspension on the same day of their formation at the recommended application rate (5.5×10^7 conidia/ml). The results showed that percent of adult emergence, females ratio and longevity of adults from treated mummies did not differ significantly from those from untreated ones. However, when parasitized aphids were treated with the fungus, at the same concentration, immediately or 2-day post parasitism, the numbers of formed mummies were 38 and 39, respectively, compared to 62 mummies in the control aphids.

In another experiment, the interactions between *B. bassiana* and the aphid braconid parasitoid, *Diaeretiella rapae* (MacIntosh) were studied [51]. Nymphs of *M. persicae* were first exposed to parasitoid females for 24 h and then sprayed with *B. bassiana* (1×10^{10} conidia/ml) at 0, 24 and 48 h after parasitism. Likewise, aphids were sprayed with the fungus and exposed to the parasitoid at 0, 24 and 48 h post infection. The results showed that % parasitism varied from 13% to 66.5% and it was lower, significantly, in both treatments within 0 - 24 h compared to the control. Percentage of parasitoid emergence was negatively affected when fungus-treatment occurred before exposure to the parasitoid. In addition, a decrease of longevity of female parasitoid F1 generation was observed when

the parasitized aphids were treated with the fungus.

Three isolates of *B. bassiana* and 2 isolates of *M. anisopliae* were tested against larvae and pupae of the ecto-larval parasitoid, *Habrobracon hebetor* (Say) [52]. The results showed that the isolates EUT105 of *B. bassiana* and M396 of *M. anisopliae* did not cause mortality in larvae of *H. hebetor* at 6 tested concentrations (10^6 - 10^{11} conidia/ml). The EUT116 and IRAN187C isolates of *B. bassiana* at the concentration of 10^{10} conidia/ml caused 6.67% and 51.1% mortality, respectively, to the parasitoid larvae. All tested isolates of both fungi did not affect the treated pupae of the parasitoid. The life table parameters of *H. hebetor* were studied when reared on 3rd instar larvae of *Helicoverpa armigera* (Hubner) previously infected at 0, 24, 48, and 72 h with a sub-lethal concentration (LC_{30}) of *M. anisopliae* [53]. It was found that fungal infection adversely affected life table parameters of the parasitoid depending on the time between infection and parasitism. Statistically, different variations for parasitoid infection increased when the time between infection and parasitism was longer than 24 h.

Rate of parasitism of the eulophid ecto-nymphal parasitoid, *Tamarixia* sp. on nymphs of *Bactericera cockerelli* (Sulk) (Hemiptera: Triozidae) that were previously infected with three isolates of *B. bassiana* was evaluated [54]. The results showed that rates of parasitism in fungus-treated nymphs did not differ significantly from those of the untreated ones. Laboratory experiments were carried out to investigate the effects of application of two *B. bassiana* isolates on *Tamarixia* sp. during its development on its host, *B. cockerelli* [55]. Three concentrations (LC_{20} , LC_{50} and LC_{90} of the fungus were applied to the 4th nymphal instars parasitized with *Tamarixia* sp. 3, 5, 8 or 12 days earlier at 25°C. The greatest infection of the parasitoid larvae was observed when LC_{90} was used regardless the isolate, and when parasitism occurred 3 or 5 days before infection. The lowest infection rate of the parasitoid larvae was observed when parasitism occurred 8 or 12-day before infection regardless the isolate or concentration. The highest parasitoid emergence percent was achieved at the lowest *B. bassiana* concentration and applied to *B. cockerelli* at 12-day before infection. Longevity of adult parasitoids emerged from treated hosts was greater when parasitism occurred 12 days before infection.

The interactions between *Tamarixia radiate* (Waterston) and the fungus, *Isaria fumosorosea*, on the psyllid, *Diaphorina citri* Kuwayama (Hem.: Liviidae) were investigated [56]. Three interactions were studied: 1) Parasitoid survival when parasitized nymphs were exposed to the fungus blastospores before or after host mummification; 2) Parasitoid survival when mummies containing larvae or pupae were exposed to the fungus hyphae; 3) Parasitoid oviposition on fungus-infected hosts with or without visible hyphae. It was found that topical application of blastospore formulation (7×10^6 spores/ml) onto the nymphs parasitized with 2nd instar parasitoid larvae (3-day after parasitism) reduced host mummification by 50% and parasitoid emergence by 85%. However, fungus-application on mummies containing 4th instar parasitoid larvae (6-day after parasitism) did not affect parasitoid emergence. Likewise, fungus application on

mummies containing parasitoid pupae (9-day after parasitism) and covered with blastospores formed by the fungus hyphae did not affect parasitoid emergence.

6.1.2. Effect of Fungi on the Treated Adults

The fungus, *Nomuraea rileyi* did not affect the adults of the parasitoids, *Voria ruralis* (Fallen), *Cotesia margiventris* (Cresson), *Campoletis sonorensis* (Cameron) and *Telenomus proditor* Nixon when exposed to high concentrations of conidia (25 times higher than used in field experiments) [17]. Similar results were obtained for *V. lecanii* which did not infect the treated adults of whitefly parasitoid, *Encarsia formosa* [57]. Also, adult of *Cotesia plutellae* (Kurdjumov), a larval parasitoid of *P. xylostella*, was proved to be not susceptible to *Zoophthora radicans* (Zygomycetes: Entomophthorales) [58]. In contrast, 90% mortality in adults of the parasitoid, *Diadegma semiclausum* (Hellen), a larval parasitoid of *P. xylostella* was recorded when sprayed with the fungus, *Z. radicans* [59]. Also, the fungus, *P. neoaphidis* took 8 - 9 days to kill the adults of the aphid parasitoid, *Aphidius rhopalosiphii* De Stefani-Perez [46].

Applying *B. bassiana* and *M. anisopliae* at a concentration of 10^7 conidia/ml killed 48% and 40%, respectively, of the adults of *Cephalonomia stephanoderis* Betrem (Hym.: Bethyridae), the parasitoid of the coffee berry borer, *Hypothenemus hampei* Ferrari (Col.:Curculionidae) [60]. However, exposure of adults of the parasitoids, *Bracon hebetor* and *Anagyrus lopezi* (De Santis) (Hym.: Encyrtidae) to one isolate of *B. bassiana* and 11 isolates of *M. anisopliae* resulted in 100% mortality of both parasitoids [61]. The effect of direct spraying of *B. bassiana* at a concentration of 2×10^8 conidia/ml on longevity and fecundity of *D. semiclausum* was investigated [62]. The results showed that all fungus-treated wasps died within 4 days and all incubated cadavers showed characteristic mycosis. However, when the female was introduced immediately after treatment to healthy *P. xylostella* host larvae, the average number of parasitoid pupae produced/female was 5.1 compared to 18 pupae/untreated female in 4 days. The LC_{50} values for adults of the larval parasitoid, *Trybliographa rapae* Westwood (Hym.: Figitidae) of *Delia radicum* (L.) (Dipt.: Anthomyiidae) were 1.83×10^7 and 1.57×10^7 conidia/ml by *B. bassiana* and *M. brunneum*, respectively [63].

6.2. Field Experiments

6.2.1. Direct Effect of Fungi on Parasitoids

A commercially formulated isolate of *B. bassiana* was found to be highly efficient in greenhouses against whiteflies, thrips and mites but with no impact on beneficial insects [61]. Similarly, in a large scale experiment, it was found that the reproductive success of *Aphidius ervi* (a parasitoid of the pea aphid, *Acyrtosiphon pisum*) was not affected by applying the fungus, *P. neoaphidis* [64].

6.2.2. Combined Effects of EPF and Parasitoids

The fungus, *Hirsutella cryptoscleroium* reduced levels of parasitism by *Gyranusoidea tebya* (Hym.: Encyrtidae) on the mealybug, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) but the overall mortality of the mealybug

was greater than when both natural enemies acted separately [65]. *B. bassiana* and *M. anisopliae* were applied on artificially infested coffee branches by the coffee borer, *Hypothenemus hampei* (Col.: Curculionidae), at a concentration of 7.9×10^9 conidia/tree [60]. Application was carried out 21, 14, 7 and 0 days before releasing the parasitoid, *Cephalonomia stephanoderis* (Hym.: Bethyridae), and 2, 4 and 7 days after release. The results showed that the highest mortality of the parasitoids (24% by *B. bassiana* and 5% by *M. anisopliae*) occurred when fungi were sprayed on the same day of the parasitoid release. In all other treatments, however, the mortalities were less than 7% and did not differ significantly from the untreated control.

In contrast, there was an additive effect with regard to aphid control with no detrimental effects on percentage of parasitism or parasitoid emergence when the fungus, *P. fumosoroseus* and the parasitoid, *Aphelinus asychis* (Walker) (Hym.: Aphelinidae) were used together for the control of wheat aphid, *Diuraphis noxia* Kurdjumov under field conditions [66]. Similarly, in a large scale experiment, it was found that the reproductive success of *Aphidius ervi* Haliday (a parasitoid of the pea aphid, *Acyrtosiphon pisum* (Harris) was not affected by the fungus, *Pandora neoaphidis* [64]. Also, in greenhouses over an extended time experiments, the combined use of the fungus *B. bassiana* (BotaniGard Commercial Product) and the parasitoid *Aphidius matricariae* as bio-control agents for the peach aphid, *M. persicae* had synergistic effects on aphid suppression. In addition, the numbers of parasitoids presented at the end of longer scale experiments were higher in treatments with *B. bassiana* and the parasitoid than in treatments with the parasitoid alone [50].

With appropriate timing, each of the parasitoids, *Aphidius matricariae* (Haliday) and *A. colemani* (Dalman) could be combined with *B. bassiana* for the biological control of the aphid, *M. persicae* [44]. Likewise, *B. bassiana* or *M. brunneum* were found to be compatible with *A. colemani* against *M. persicae* without any negative interactions between them [67]. Also, the parasitoid, *A. colemani* and the fungus, *Lecanicillium muscarium* (Ascomycota: Hypocreales) could be used together for the control of the aphid, *M. persicae* [68].

6.2.3. Transmission of EPF by Parasitoids

Adults of the ichneumonid larval parasitoid, *D. semiclausum*, contaminated with the spores of the fungus, *Z. radicans* was not infected and did not transmit the fungus to the populations of its host *P. xylostella* [59]. However, treatment of *D. semiclausum* adult by the same fungus prior to exposure to *P. xylostella* larvae significantly reduced the number of parasitoid cocoons developed from host larvae [58]. In a large scale experiment, enhancing of transmission of *B. bassiana* to the pea aphid, *Acyrtosiphon pisum*, in the presence of its parasitoid, *Aphidius ervi* was observed [64].

6.2.4. Discrimination between Healthy and Fungi-Infected Hosts

The females of the aphid parasitoid, *Aphidius rhopalosiphii* (De Stephani-Perez)

could discriminate between *B. bassiana*-infected and uninfected hosts and deposited lower numbers of eggs in the infected hosts [69]. Similar results were obtained with the egg parasitoid, *Anagrus atomus* (L.) of the green leafhopper, *Empoasca decipines* Paoli treated with *B. bassiana* [70]. Also, the female of the parasitoid, *Encarsia formosa* was able to detect and reject the whitefly *T. vaporariorum* nymphs treated with the fungus, *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes) 7 days post fungus application [71].

In contrast, the females of *Cotesia plutellae* (Kurdjumov) and *D. semiclausum* did not discriminate between *Z. radicans*-treated and untreated *P. xylostella* larvae and no difference in oviposition attempts was noticed [58]. Similarly, adult females of the bethylid ecto-parasitoid, *Cephalonomia tarsalis* (Ashmead) laid eggs on *B. bassiana*-infected larvae of its host, *Oryzaephilus surinamensis* (L.) (Col.: Silvanidae) up to within one day of the host-death [72]. However, *D. semiclausum* could recognize *B. bassiana*-infected *P. xylostella* larvae and did not attempt to oviposit in dead larvae [62]. Also, *Trybliographa rapae* females, (Hym.: Figitidae) could discriminate between healthy and infected *Delia radicum* (L.) (Dipt.: Anthomyiidae) larvae and laid significantly more eggs in healthy larvae than in the *M. brunneum*-infected ones. In contrast, the numbers of eggs laid in healthy larvae and in those infected with *B. bassiana* did not differ significantly [63].

7. Conclusions

Laboratory experiments revealed that fungi-treatment of larvae and adults of predators may cause mortality or detrimental effects on such insects depending on the species of the fungus and its concentration. Mostly, the predators avoid feeding on fungus-infected prey. Different fungi species could be isolated from hibernating and field collected adult predators. Some field studies proved that EPF can be combined with different predator species as bio-control agents of insect pests.

In general, the negative impact of fungi on the development of the parasitoids on or inside the treated hosts depends on the time between infection and parasitism. Fungus-treatment of parasitoids' adults causes different rates of mortality in such adults. The field studies demonstrated the success of the combination between fungi and parasitoids as bio-control agents against insect pests. Despite such results, efforts should be done to obtain EPF products capable of acting at lower R.H. in order to expand the commercial use of fungi as bio-control agents.

Conflicts of Interest

The author declares that he has no competing interests.

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Abbreviations

Entomopathogenic Fungi (EPF), Intraguild Predation (IGP)