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Microbial control of sucking pests using entomopathogenic fungi

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Abstract: Sucking pests cause serious damage to several agricultural, horticultural and plantation crops either by direct feeding or by transmitting plant viral diseases. Since sucking pests like plant and leaf hoppers, aphids, whiteflies, scale insects, thrips and mites to have developed resistance to insecticides, biological control using microbial pathogens, particularly fungal pathogens like *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii*, has been explored for a number of pests. Several commercial formulations based on entomopathogenic fungi were developed for the control of sucking pests in different countries. Mycotrol and Botanigard based on *B. bassiana*, Mycotal based on *V. lecanii* and PFR-97 and Pae-Sin based on *Paecilomyces fumosoroseus* were developed for the control of whiteflies, aphids and thrips in USA, Europe and Brazil. In India, *Fusarium pallidoroseum* was found effective in controlling cowpea aphid (*Aphis craccivora*) in Kerala. *Hirsutella thompsonii* has been tried against the coconut eriophyid mite, but not found very promising. The wetland ecosystems like the rice fields as well as the sugarcane ecosystem have been found ideal for the use of *M. anisopliae* against sucking pests. *M. anisopliae* and *B. bassiana* were found to infect the sugarcane woolly aphid (*Ceratovacuna lanigera*) but these were also pathogenic to potential predators like *Dipha aphidivora* and *Micromus* and hence cannot be recommended. Apart from these fungi, entomophthoralean fungi like *Erynia neoaphidis*, *Neozygites fresenii* and *Zoophthora radicans* are reported to cause epizootics in several aphid species in nature. Fungal pathogens occur very widely in nature and there is a wide scope for isolating strains of fungal pathogens with enhanced virulence as well as desired cultural characteristics. Fungal formulations developed as microbial insecticides should possess good fluid stability and shelf life. Oil formulations have been found to be more effective against target pests even under low RH and also possess a good shelf life. Since the muscardine fungi are pathogenic to beneficial insects like silk worms, honey bees, pollinators, as well as predators like coccinellids, utmost care should be exercised. Other pathogens like entomopathogenic nematodes (EPN) have been considered for the management of sucking pests but not with much success. The chance of acquisition of bacterial and viral pathogens into the gut of the sucking pests is limited and hence these pathogens have not been considered for the management of sucking pests. Infectivity of protozoans to sucking pests has been rarely reported. The current status of microbial control of sucking pests and the future prospects are reviewed in this paper.

KEY WORDS: Entomopathogenic fungi, microbial control, sucking pests

Fungi as microbial control of sucking pests

More than 750 species of fungi, mostly deuteromycetes and entomophthorales are pathogenic to insects, many of them offer great potential for the management of sucking pests. Species that have been most intensively investigated for mycoinsecticides in the sucking pest control include *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Hirsutella thompsonii*, *Entomophthora* sp., and *Fusarium* spp. Among these, *V. lecanii*, has been used in large scale for the control of several sucking pests over a number of years, while others have been attempted in recent times. Fungal pathogens have certain advantages in pest control programmes over other insect pathogens. Mass production techniques of fungi are much simpler, easier and cheaper than those used for other microbial agents.

Fungi unlike bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection and hence for sucking insects, entomopathogenic fungi are the most preferred microbial agents. Environmental conditions, particularly humidity and temperature, play an important role in the infection and sporulation of entomopathogenic fungi. High humidity (70-90%RH) and cooler temperatures (20-30°C) are required for occurrence of epizootics and for effective control of pests with mycoinsecticide formulations.

Most of the entomopathogenic fungi infect the host through the cuticle. The process of pathogenesis begins with adhesion of fungal spore on cuticle followed by germination, penetration and development of fungus inside the host leading to the death of the host. Fungi usually cause insect mortality by nutritional deficiency, destruction of tissues and releasing of toxins. After the penetration of germinating hyphae into the haemocoel, the fungus produces hyphal bodies, hyphal strands and protoplasts that fill the haemocoel completely. Several mycotoxins like, Beauvericin, Beauverolides Bassianolide (by *B.bassiana, V.lecanii, Paecilomyces* spp.) and Destruxins A, B, C, D, E, F (by *M.anisopliae*) are produced during pathogenesis and these act like poisons for the insects. After the death of the insects the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers. At early stages of fungal infection, the insects show little or no symptoms except for a few necrotic spots that may develop at the invasion sites. At late stages of infection, the insects become restless, less active, loose appetite and coordination. The internal tissues show disintegration prior to the insect death. External fungal growth on the cadavers is another diagnostic feature of the fungal infection in insects. An insect covered with powdery white spores would be suspected of having *B.bassiana/ V.lecanii* infection, powdery green spores would suggest an infection with *M.anisopliae* The fungal infected insects are usually hardened and not soft like in bacterial infections.

Most of the entomopathogenic fungi can be grown on a variety of solid substrates or in liquid media. Sorghum grain, rice, rice flakes, puffed rice, wheat, wheat flakes, maize, cowpea grains, millets, rice bran, wheat bran, groundnut hull meal, bengal gram husk, coffee husk, potato, carrot etc. are some of the solid substrates used for mass production of *B. bassiana, M. anisopliae, V.lecanii.* These solid substrates are moistened, sterilized and inoculated with the fungus in bottles/polypropylene bags and incubated at optimum temperature and humidity for a period of 10- 20 days. Then the viable propagules are harvested directly in water containing wetting agent and vegetable oil by repeated washing or centrifugation and used as foliar sprays. Alternatively, talc based formulations can be prepared by homogenizing the substrate along with fungal growth into a fine powder and mixing with inert materials like, talc (1:1 to 1:2 proportion) and used for foliar sprays.

In liquid cultures, these fungi can be grown in static liquid cultures or shake cultures or in fermentors using cheap and inexpensive liquid media like molasses, carrot extract, potato extract or synthetic media. The infective propagules can be harvested by filtration/centrifugation and mixed with water containing wetting agent and vegetable oil to get spore suspension with required spore concentration. In case of certain fungi like *B.bassiana* and *M.anisopliae*, submerged cultures give rise to blastospores or a mixture of blastospores and conidia. The blastospores are highly sensitive to high temperatures and other environmental stresses and their viability is lost quickly. To overcome this difficulty, diphasic fermentation technique is used for maximum production of aerial conidia. In this method, the fungus is allowed to grow in fermentor upto the end up the log phase for maximum production of mycelial biomass, then it is subsequently transferred to nutritious (grains) or inert substrates (talc, clay granules) for production of aerial conidia in the form of natural inocula.

Aphid control with fungal pathogens

The pathogenicity of four isolates each of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* to apterous adult *A. craccivora* was evaluated in the laboratory at 4 concentrations of conidia (Ekesi *et al.*, 2000). All fungi isolates tested were found to be pathogenic to the insect but their virulence varied among species and isolates within species. Three isolates, *B. bassiana* CPD 11 and *M. anisopliae* CPD 4 and 5 caused significantly higher mortality than the other isolates at the various concentrations tested causing mortality of between 58-91%, 64 to 93% and 66-100%, respectively, at 7 days post treatment. The results indicate that these isolates are promising candidates for the control of the cowpea aphid but their pathogenicity to various aphid non-target beneficial organisms within the cowpea agroecosystem warrant further investigation before initiating field control (Ekesi *et al.*, 2000). Laboratory and field experiments were conducted to examine the development, pathogenicity and incidence of *Neozygites* *fresenii* in *A. craccivora* infesting some legumes in Philippines (Mejia *et al*., 2000). Based on squashed mounts of *N. fresenii*-inoculated aphids, it took only 54 h post-inoculation for the aphids to succumb to mycosis. The most susceptible aphid was *Aphis citricola* followed by *Brevicoryne brassicae* (L.) with an average mortality of 53% and 41% (Mejia *et al*., 2000). The presence of two species of *Neozygites* in the Philippines is documented (Villacarlos, 2000). Epizootics due to *Neozygites fresenii* were observed on *Aphis craccivora* populations on *Gliricidia sepium* and string beans, *Vigna sesquipedalis* and on *A. citricola* infesting the weed *Mikania cordata*. Epizootics of both *N. fresenii* and *N. fumosa* resulted in drastic reduction in the aphid and mealy bug populations. Enhancing the occurrence of these fungi may have potential in the biological control of these pests. *N. fresenii* was found in populations of *Aphis craccivora* on faba bean in Egypt during November-December 1998 (Sewify, 2000). This fungus is epizootic in high populations of *A. craccivora* on faba bean plants. The study suggested that *N. fresenii* may be a promising biocontrol agent against *A. craccivora* in Egypt (Sewify, 2000).

The field efficacy of different formulations and concentrations of *Fusarium pallidoroseum* against *Aphis craccivora* was investigated in a cowpea crop in India. The water suspension and diatomaceous earth wettable powder formulations at $7X10^6$ spores/ml achieved 100% mortality of *A. craccivora* at 12 and 16 days after treatment, respectively (Sunitha *et al*., 1999). Wheat bran and rice bran were found to be suitable substrates for the mass production of *F. pallidoroseum*, an entomogenous fungus attacking *A. craccivora*, as the maximum number of spores with a higher virulence was produced within the shortest period on these substrates. Eight days after inoculation appeared to be the best time to harvest spores (Faizal *et al*.,1996). Addition of jaggery (3%) to rice bran significantly increased spore production (12.6×10^5 / ml) compared to rice bran without jaggery (0.8×10^5 / ml) (Susamma *et al*., 2002). *Fusarium semitectum* was associated with dead aphids of *Myzus persicae* in cole crops in Tamil Nadu and the fungus was found pathogenic to *M. persicae* in laboratory bioassays (Nagalingam and Jayaraj, 1996). Incidence of *Entomophthora* sp. on *M. persicae* on mustard crop was reported in West Bengal, Haryana and Himachal pradesh (Nath and Bandhopadhyay, 1973, Rohilla *et al*., 1996 and Desh raj *et al*., 1998). Nirmala *et al*. (2006) studied the pathogenicity of twelve fungal isolates belonging to *B.bassiana*, *M. anisopliae* and *V. lecanii* against *A. craccivora*, *Aphis gossypii* and *Rhopalosiphum maidis* using detached leaf bioassay technique. All twelve isolates of the three fungi were found to be pathogenic to *A. craccivora* and *A. gossypii* at a concentration of 1.0×10^7 spores/ml. The mortality ranged from 2 to 74 percent in *A. craccivora*, 14 to 80.8 percent in *A. gossypii* and 6 to 50 percent in *R. maidis*. Bb5a isolate of *B.bassiana* caused highest percent mortality in *A. gossypii* (80.8%) and *R. maidis* (50%) indicating its broad spectrum action. Vl-1 isolate of *V. lecanii* recorded maximum mortality of 80.8% of *A. craccivora*. *R. maidis* was relatively less susceptible to the three fungi than *A. craccivora* and *A. gossypii*. Four isolates in each of *B. bassiana*, *M. anisopliae* and *V. lecanii* were tested for their pathogenicity to the sugarcane woolly aphid, *Ceratovacuna lanigera* in oil emulsion formulations under field conditions at Arabhavi, Karnataka. Mycosis was observed with six isolates *viz*., *B. bassiana*, Bb4 (10%), Bb5a (19.8%), Bb6 (8.3%) and *M. anisopliae*, Ma2 (4.7%), Ma3 (16.2%) and Ma4 (42.3%). Pathogenicity was confirmed by re-isolation of the respective fungal isolate from the mycosed aphids. None of the *V. lecanii* isolates showed mycosis on *C. lanigera*. The spores of *M. anisopliae* (Ma4) prepared in oil emulsion formulation showed higher percent of mycosis (31.8%) than with the spore suspensions prepared in 0.5% Tween-80 (42.3%). In the laboratory bioassay studies, *M. anisopliae* (Ma-4) and *B. bassiana* (Bb-5a) were found pathogenic to the predator of sugarcane woolly aphid, *Dipha aphidivora* causing 29.3 and 10.4% mycosis. *M. anisopliae* (Ma-4) isolate was also found pathogenic to another predator of SWA, *Micromus* sp. causing 29.14% mycosis. The virulence of twenty-five isolates of entomopathogenic fungi belonging to *B. bassiana*, *V. lecanii*, *M. anisopliae* and *P. fumosoroseus* originating from a wide range of insect species was investigated in laboratory bioassays on cabbage aphid, *Brevicoryne brassicae* (Linnaeus), at different regimes of temperature (20, 25 and 30°C) and relative humidity (75, 85, 90 and 95 %). All fungal isolates except *N. rileyi* isolates were pathogenic to the aphid, but in varying degrees. Among three levels of temperature tested, aphid mortality was significantly higher at 25°C than 20 and 30 °C. Aphid mortality decreased with decreasing relative humidity. Among all isolates in all combinations of temperature and relative humidity, four isolates of *V. lecanii*, V.l-1, V.l-2, V.l-6, and V.l-7 showed higher virulence to *B. brassicae*. In multiple dose bioassays, lowest LT_{50} was obtained from V.l-7 isolate. The highest virulence of V.l-7 isolate of *V. lecanii* to *B. brassicae* suggests that the isolate would be a potential candidate as a microbial control agent for the cabbage aphid.

Fungal pathogens in the control of Thrips

Thrips tabaci

In laboratory tests, *T. tabaci* proved susceptible to isolates of *B. bassiana, M. anisopliae, V. lecanii* and *P. fumosoroseus* (Gillespie, 1986). The most pathogenic isolates were *M. anisopliae* (ME2) and *B. bassiana* (31) that killed all treated insects within 4 days, while *V. lecanii* isolates killed a maximum of 85% in the same time. A glasshouse experiment indicated that *V. lecanii* was able to reduce thrips populations on cucumbers (Gillespie, 1986). In Israel, sixteen isolates of entomogenous fungi, belonging to 4 species, were tested as potential biocontrol agents against *T. tabaci* and *Frankliniella occidentalis* (Gindin *et al*., 1996). In general, *T. tabaci* was more susceptible to all the fungi tested than *F. occidentalis*. *P. fumosoroseus*, *B. bassiana* and one isolate of *M. anisopliae* showed the highest virulence towards both thrips species. In Kenya, an isolate of *M. anisopliae* was tested in field for three seasons as a potential alternative for control of *T. tabaci* in onion. The fungal pathogen was applied at the rate of 1 x 10^{11} conidia ha-1 at weekly/ bi-weekly intervals and compared with the bi-weekly spray of the chemical insecticide dimethoate (Rogor, 17.5 g a.i. ha-1). Thrips density and damage were significantly lower in the fungal and chemical insecticide treatments compared with the untreated control. Onion bulb yield did not differ significantly among the treatments during the first season trial. However, in the second season trial, dimethoate-treated plots provided the greatest bulb yield (17 metric tons ha-1) and in the third season trial, *M. anisopliae* applied weekly recorded the highest yield (24 metric tons ha-1). With the exception of spiders, densities of nontarget organisms were higher in plots treated with *M. anisopliae* than in dimethoate-treated plots. The results indicate the potential of using *M. anisopliae* for the control of *T. tabaci* while protecting biodiversity in the onion agroecosystem (Maniania *et al*., 2003). In India, research on biocontrol of onion thrips using fungal pathogens has not been carried out so far.

Western flower thrips, *Frankliniella occidentalis* (Pergande)

The efficacy of the entomopathogenic fungi *M. anisopliae, V. lecanii* and *B. bassiana* was investigated in laboratory, pot, greenhouse experiments and fields against the western flower thrips (*F. occidentalis*). Field trials with *V. lecanii* indicated its efficacy against *F. occidentalis* on bush beans (*Phaseolus vulgaris*). Glasshouse trials with *B. bassiana* in chrysanthemums (*Dendranthema grandiflora*) indicated reduction of population of *F. occidentalis* (Ludwig and Oetting, 2002). In Brazil, *M. anisopliae* in combination with methiocarb was the best strategy for thrips control (Lopes *et al*., 2002). *M. anisopliae* reduced both the adult and larval populations of *F. occidentalis* significantly on chrysanthemum in greenhouse experiments, although the level of control of larval populations was much lower than for adults (Maniania *et al*., 2002). Combined application of *M. anisopliae* and Methomyl (Lannate), however, resulted in a significant reduction of both the larval and adult stages. The use of both control agents might be helpful in reducing the selection pressure for resistance to chemical insecticides, thereby delaying or preventing the build-up of resistant populations in greenhouses. The M. a-7 of strain *M. anisopliae* was found to be effective in reducing the population growth of *F. occidentalis* under greenhouse conditions on Cucumber (cv. BA), particularly when the initial thrips population was low to moderate (Azaizeh *et al*., 2002). Liquid and powder formulations of the entomopathogenic fungus *B. bassiana* strain GHA (as BotaniGard R) were evaluated for management of *F. occidentalis* in ornamental and vegetable crops in greenhouses in California and Maryland, USA and Morocco. Weekly applications provided control of thrip population comparable to chemical insecticides and considerably better than releases of predator mites. (Bradley *et al*., 1998).

Legume thrips (*Megalurothrips sjostedti*)

The susceptibility of immature stages of the legume flower thrips, *M. sjostedti*, to *M. anisopliae*, was investigated under laboratory conditions. The adult stage was found to be more susceptible to infection than the larval and pupal stages. Mortality at all stages was concentration-dependent, with the highest concentration of 1X108 conidia ml-1 producing the highest mortality (26, 46 and 100% for larvae, pupae and adults, respectively) at 8 days post-inoculation. Fecundity, egg fertility and longevity in adults surviving infection as larvae were significantly reduced compared to the control (Ekesi and Maniania, 2000). Field experiments were conducted in western Kenya for two seasons to evaluate the potential of *M. anisopliae*, for biological control of the legume flower thrips, *M. sjostedti*, on cowpea. An ultra-low-volume (ULV) oil/aqueous formulation and a high-volume (HV) aqueous formulation of conidia were applied three times each at two concentrations of 1 X 10^{11} and 1 X 10^{13} conidia ha $^{-1}$. Flower and pod production was significantly higher in treated plots compared to the control plots. HV formulation was superior to ULV formulation in reducing thrips population and plant damage, and in increasing flower and pod production. No significant difference in grain yield was found between the fungal-treated plots and the synthetic insecticidal treatment (lambda-cyhalothrin) during the second season. It is suggested that *M. anisopliae* is a potential candidate for the management of *M. sjostedti* on cowpea (Ekesi *et al.*, 1998).

Pear thrips (*Thrips inconsequens*)

The natural prevalence of entomopathogenic fungal infections at different phases in the life cycle of *T. inconsequens* was determined in sugar maple (*Acer saccharum*) stands at 4 different sites in Vermont in 1992. The species of entomopathogenic fungi most commonly isolated from *T. inconsequens* during the course of the study were *B. bassiana, P. farinosus, M. anisopliae, V. lecanii, V. fusisporum* and *Hirsutella* sp. The developmental stages associated with the forest soil had the highest levels of infection compared to life stages found predominantly on foliage. The highest rates of infection were seen in larvae recovered from soil samples (11.9%), followed by adults collected from emergence traps and buds (5.9%) and larvae collected from the forest floor (3.9%). In contrast, 1.9% of the individuals sampled from the understorey and 1.9% of the thrips collected from the upper canopy were infected. It is concluded that further studies are needed to characterize the fungal strains recovered and assess their potential for use as biological control agents of *T. inconsequens* (Brownbridge *et al.*, 1999).

Thrips palmi

A field study was carried out during 1994 in Taiwan on entomopathogenic fungi in vegetable fields infested with *T. palmi*. A total of 136 entomopathogens was identified (out of 400 isolates), including 6 genera and 10 species. *M. anisopliae* var. *anisopliae* and *Fusarium* spp. were the most abundant (32.4 and 31.4% of all isolates, respectively). In another study on the management of *Taeniothrips inconsequens* in *Acer saccharum* forest in Vermont, USA, the persistence and vertical movement of *Beauveria bassiana* in the soil were investigated, after application as an emulsifiable concentrate (EC) or a nutrient-based granular formulation (NBG). The number of colony-forming units (CFU) recovered from different soil levels over time was used as an evaluation parameter. Elevated CFU-levels at a depth of 0-2 cm were still found in NBG-plots after 16 weeks, but not beyond 8 weeks in the EC-plots. Vertical movement of *B. bassiana* was mainly restricted to the upper 2 cm (Parker *et al.*, 1996).

The potential of *B. bassiana* (strains BbH and BbHa) and *P. fumosoroseus* (strain 97) as biological control agents of *T. palmi* was studied in greenhouse experiments. Twenty-four per cent mortality was found on thrips larvae treated with *B. bassiana* BbHa but infection only developed when leaves with larvae were incubated after being sprayed with the pathogen. Mortality by *P. fumosoroseus* was very low (0.20%). A reduction of 50% in adult emergence was observed when potting soil with pupae was sprayed with *B. bassiana* BbHa (Castineiras *et al.*, 1996).

Whiteflies

Several commercial formulations based on entmopathogenic fungi were developed for the control whiteflies in different countries. Mycotrol and Botanigard are biopesticides based on *B. bassiana* developed for the control of whiteflies, aphids and thrips in glasshouse conditions in USA. Mycotal, another mycoinsecticide based on *V. lecanii* was developed by Koppart, Netherlands for the control of whiteflies and thrips. PFR-97 and Pae-Sin are the other biopesticides developed based on *P. fumosoroseus* for the control of white flies in USA and Brazil. *M. aniosopliae* was reported to cause 30-92% mortality of *B.tabaci* on eggplants under field conditions in Israel (Bhatta, 2003). Epizootic occurrence of *V. lecanii* on *B.tabaci* was reported from Brazil in soybean fields (Lourencao *et al.*, 2001).

The incidence of fungal pathogens like *Aspergillus* sp., *Paecilomyces* sp. and *Fusarium* sp. on *Bemisia tabaci* in cotton in A.P. were recorded during November, suppressing adults, nymphs and eggs to the extent of 86.6, 19.0 and 39.8% respectively (Rao *et al.*, 1989). The fungus *P. farinosus* was found infecting adults of *B. tabaci* on cotton in the field in Uttar Pradesh in 1971. In the laboratory, the fungus caused 90% mortality of adults of *B. tabaci* (Nene, 1973). *B. bassiana* is a potent bioagent against *B. tabaci*, when it was formulated with 1% coconut oil followed by groundnut, sunflower and castor oils. This could be used in managing the whiteflies and in turn limit the incidence and spread of tomato leaf curl virus, thereby reducing the yield losses in tomato crops (Manjula *et al.*, 2003).

Scale insects

Citrus green scale and coffee green scale (*Coccus viridis*) are the major pests susceptible to *V. lecanii* in India. Field trials were carried out in Tamil Nadu, to determine the effectiveness of *V. lecanii* in controlling the coffee green scale. When applied @ 16 X 10^6 spores/ml twice at 2 weeks interval, the fungus caused 73.1% mortality of the pest. Maximum mortality of 97.6% was obtained when the surfactant Tween 20 was added to the spore suspension. A high-volume spray was more effective than a low-volume one. During the summer, addition of a sub-toxic level of fenthion to a lower dose of spore suspension (4 X 10^6 spores/ml) caused 88.8% mortality after 14 days (Jayaraj, 1989). Application of Bordeaux mixture prior to *V. lecanii* spraying (1,2,3 and 4 weeks before) in coffee did not significantly affect the efficacy of the fungus in the control of coffee green scale (Easwaramoorthy *et al.*, 1977).

Future Thrust Areas of Research

1. Systematic collection from different cropping systems, evaluation and identification of potential isolates for various sucking pests and establishment of a centralized repository are to be taken up with top priority.

2. For large-scale production and commercialization of these fungi, techniques of solid/liquid/ diphasic fementation should be standardized. At the same time, mass production techniques using, cheap and inexpensive agricultural byproducts should be encouraged so that the farmer himself can multiply and use them.

3. Formulation technologies have to be standardized for increasing shelf life and improving the field efficacy and persistence.

4. Studies on compatibility of entomofungal pathogens with pesticides and other bioagents and their biosafety should be carried out to determine their utilization in BIPM.

5. Steps should be taken for registration of formulations intended for commercial production. Quality parameters like, cfu, % moisture, shelf life etc. have to be framed and implemented strictly for commercial formulations.

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