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Entomopathogenic fungi for the control of economically important whiteflies

B. VASANTHARAJ DAVID and P. SELVARAJ
Sun Agro Biotech Research Centre, 3/340, Main Road, Madanandapuram, Porur, Chennai - 600 116, Tamil Nadu, India
E-mail: sabrcchennai@yahoo.co.in

ABSTRACT: **This paper focuses attention on possible entomopathogenic fungi for the biocontrol of important whitefly pests, namely, *Bemisia tabaci* (Gennadius), *Singhiella cardamomi* (David and Subramanium), *Trialeurodes vaporariorum* (Westwood), *Aleurocanthus woglumi* (Ashby) and *Aleurolobus barodensis* (Maskell). A few success stories of fungal pathogens of whiteflies have been indicated. The scope of entomopathogenic fungi, namely, *Verticilium lecanii, Aschersonia* spp., *Paecilomyces fumosoroseus, Beauveria bassiana* and *Zoophthora* has been brought out. The aspects of different strains, mode of action, available mass multiplication technologies, field application and commercial formulations have been explained. The scope of entomopathogenic fungi and aspects requiring attention are discussed.**

KEY WORDS: Entomopathogenic fungi, field application, mass culture, mode of action, whiteflies

Whiteflies are some of the toughest pests damaging greenhouse plants and cultivated crops. In India, the most important whitefly pests of economic importance are: the cotton or tobacco whitefly, *Bemisia tabaci* (Gennadius); cardamom whitefly, *Singhiella cardamomi* (David and Subramaniam); greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood); citrus whitefly, *Aleurocanthus woglumi,* (Ashby) sugarcane whitefly, *Aleurolobus barodensis* (Maskell) and spiraling whitefly, *Aleurodicus dispersus* Russell. Because of their broad host range, resistance to insecticides, and potential ability to vector a variety of plant virus diseases, they account for heavy crop losses. Therefore the biocontrol agents are being considered as a viable alternative. Owing to the feeding behaviour of whiteflies, only pathogens capable of penetrating the cuticle have been considered as potential biocontrol agents. So far the pathogens reported from whiteflies have only been fungi, since they are able to penetrate through the cuticle.

Till the crisis caused by *Bemisia* during 1990s, the role of entamopathogenic fungi was more or less ignored. Experience gained subsequently from IPM approach and large scale field surveys focused the effort on the utilization of fungal pathogens. Laboratory studies have been conducted to determine the efficacy of different fungal pathogens against various instars of the whitefly *Bemisia tabaci* on tomato and verbena foliage (Cuthbertson *et al.*, 2004). Similarly, the pathogenicity of Japanese strains of *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Aschersonia aleyrodis* against the nymphs of the silverleaf whitefly, *Bemisia argentifolii*, was compared with that of strains from foreign commercial products, and determined that native strains as potential microbial control agents against this whitefly (Sainto *et al.*, 2003; Wraight *et al.*, 1998). This paper emphasizes the need for the exploration of native entomopathogens for whitefly control and commercial production of viable agents.

Entomopathogenic fungi of whiteflies

1. *Verticilium lecanii*

a. Strains: It is the common white halo fungus infesting soft bodied insects like aphids, scales and whiteflies. Initial studies of the efficacy of this fungus and its potential as a practical microbial agent were conducted in early 1970s at the Glasshouse Crop Research Institute (UK) on aphids. Evans and Samson (1986) referred it as a complex species with several distinct morphs. Different strains of *V. lecanii* were isolated worldwide including some isolates from whiteflies of tropics, especially from *T. vaporariorum*. With the spread of *Bemisia*, the incidence of *V. lecanii* on this pest has been on the increase. The biocidal activity of *V. lecanii* against whiteflies and its production on artificial media stimulated interest in commercial development of whitefly-active strains as a microbial insecticide.

b. Mode of action: The second to fourth instar nymphs of *T. vaporariorum* are more susceptible to *V. lecanii*. The aerial conidia protrude from infected hosts or from non-living substrates. The conidia are aggregated in slimy false heads in groups of 6-72. After germination of the conidia, the hyphae grow over the surface of the insect and penetrate through the integument. The death of the are infected insect occurs after 6 days of infection.

The characteristics associated with the virulence of this entomopathogenic fungi are fast germination, high sporulation, absence of extracellular amylase activity and high extracellular chitinase activity. The most important limiting factors of these fungi are elevated humidity, narrow range of optimal temperature, stage of whitefly and strain of fungi.

c. Mass production: The pathogen can be cultured on crushed moist sorghum / rice / maize grains or carrot broth. The pure culture of the fungus is maintained on potato dextrose agar medium / standard maltose agar + 1 % yeast slants in tubes.

Different methods are followed for its mass production. They are:

i. Crushed grains method

ii. Coconut water method for small scale production, and

iii. Fermentation method for large scale production.

i. Crushed grains method

After 10 days of suitable growth and sporulation at 26±1°C, the fungus is suspended in sterile water + 0.1 % Tween – 80 under aseptic conditions and the conidial load is counted in the suspension (using modified Neauberg's chamber) and adjusted to 10^6/ ml by adding sterile distilled water.

Grains are crushed to make small pieces, which can pass through 12 mesh sieves. A quantity of 200 g crushed grains is placed in 0.2 mm thick 250 g capacity high density polythene bags and 190 ml water added. Then the bags are heat sealed and autoclaved at 120° C for 45 minutes. After cooling, 5 ml fungal spore suspension is added by cutting open one corner of the bag under aseptic conditions (in a laminar flow chamber). The opened area is resealed. The bags are incubated at 26±1° C for 20 days. The fungal mass along with grain carrier is harvested and dried at 40° C for 24 h and ground in a mixer to get a fine powder.

ii. Coconut water method

Coconut water (40 ml) obtained in 375 ml side-wise flat bottles plugged with cotton wool is sterilized in batches of 9-10 bottles in 12 litre pressure cooker for 15 minutes. The bottles are inoculated with 1 ml spore suspension with the help of a previously boiled (for 30 min) injection syringe. Before inserting the needle within the sterile bottles for drawing spore suspension for inoculation, the needle of the syringe and the collar region of the bottles are flamed (over candle or glass burner or any lamp). The bottles are incubated resting on flat surface for 20 days or till the surface of the medium is fully covered by the olive green sporulated fungus. The whole culture is crushed and used in the field. From a single average sized coconut, 5 to 6 bottles of culture can be made. The fungus is applied in water suspension and mixed with 0.05 % Sandovit.

iii. Fermentation method

The standard method used for large scale production of microorganisms is the process of fermentation. There are many types of fermentation; two most common are submerged fermentation and semi solid fermentation.

a. Submerged fermentation

In this method growth of the microorganisms takes place fully under liquid system. The advantages of this

method include the ability to hold constant temperature and pH, and pump large quantities of air into the system and disperse by means of stirring impellers, creating reasonably homogeneous conditions to maximize the growth of microorganism. This method is suitable for large scale mass production of *V. lecanii*.

b. Semi-solid fermentation

In this method, the fungal growth is primarily on the wet surface of the solid medium. Often processed cereal grain with additional nutritional adjuvants is used as growth medium. Sometimes, waste materials or low value media such as straw are used. It allows are the fungi to grow in conditions similar to the natural condition of *V. lecanii*.

2. *Aschersonia* spp.

Mode of action: Twenty five strains of *Aschersonia aleyrodis* have been reported from whiteflies (Fransen, 1987). These strains produce their spores which germinate and penetrate the cuticle of whitefly nymphs and the hyphal bodies grow in the haemolymph. At this point, the insect changes from a transparent green colour to opaque white. The colour of the nymph changes to either opaque or transparent orange when *A. aleyrodis* colonizes the host's organs. The fungus may emerge from the insect under favourable conditions and form a fringe of mycelium extending from the marginal area of the body before covering the whole insect, which is initially white but can eventually take a yellow to orange appearance. The conidia protrude in slimy masses with conspicuous colour from soft yellow to orange and brown.

Evaluation: Fransen (1987) evaluated 44 isolates of *Aschersonia* spp. for their ability to sporulate and germinate on semi-artificial media and to infect insect hosts. After a selection based on spore production and infection, virulence of 31 isolates was evaluated on third instar nymphs of *B. argentifolii* and *T. vaporariorum*. Infection levels varied between 2 and 70%. Unidentified species of *Aschersonia* originating from Thailand and Malaysia, *A. aleyrodis* from Colombia, and *A. placenta* from India showed high spore production on semi-artificial medium. High infection levels on *B. argentifolii* and *T. vaporariorum* were exhibited by *A. aleyrodis* (Lacey *et al*, 1996). Therefore, the entomopathogenic fungi of the genus *Aschersonia* have hope as biocontrol agents.

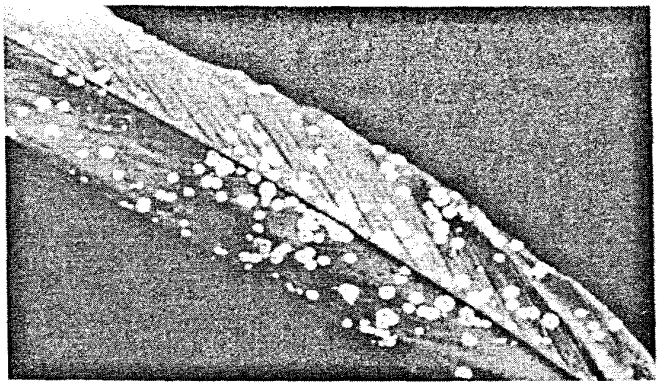


Figure 1. Cardamom leaf with puparia of *Dialeurodes cardamomi* showing signs of fungal attack

In India, the small cardamom (*Elettaria cardamomum* Maton) is cultivated over an area of 91,000 ha, distributed in the forest regions of Kerala, Karnataka and Tamil Nadu. During 1980s, *Singhiella* (=*Dialeurodes*) *cardamomi* became a serious pest of cardamom in Kerala, especially in the Idukki District and Nelliampathy area, resulting in yellowing and drying of leaves. Examination of infested cardamom leaves showed 95% of the whitefly puparia were infected by a fungal pathogen (Muraleedharan, 1985). Laboratory studies with this isolate recorded 90 per cent pupal mortality and it was identified as *A. placenta*. The same species was recorded in the USSR which showed 97% control of *T. vaporariorum* and 90% mortality of citrus whitefly, *Dialeurodes citri*. Similarly, *A. wogluni* was also infected by endemic *A. aleyrodis*. In 1950s, *D. citri* was first observed as a pest of citrus in Georgia. It was successfully controlled by exotic *Aschersonia* spp. from different parts of the world. *Aschersonia* spp. was reported successfully reducing whitefly population in citrus in Florida and China. As a result, *Dialeurodes citri* and *D. citrifolii* were kept under control (Lacey *et al.*, 2003).

Mass multiplication technology

A. aleyrodis was cultured on coarse corn flour and was sub-cultured five times. By rinsing with sterilized distilled water, spores were harvested from a 3-week-old culture grown at 25±1° C. The spore suspension ($4x10^6$ spores /ml) was sprayed on the underside of the leaves using power sprayer. The results of the experiment showed that the infection declined with increasing age and the adults did not get the infection.

The viability of the spores was tested by spraying the suspension on agar plates and germination of spores

Table 1. Spore production and infection of *Bemisia* by *Aschersonia* isolates from different origins

Species / isolate/ Source	Host	Origin	Sporulation on media			Capacity to infect *Bemisia*
			PDA	SDA	Millet	
Aschersonia sp.						
94021L. Lacey	*D. citri*	Brazil	L	L	L	+
94024L. Lacey	whitefly	Thailand	R	L	H	+
94026L. Lacey	whitefly	Malaysia	H	VH	VH	+
94027L. Lacey	whitefly	Malaysia	L	L	R	+
A. aleyrodis						
ARSEF 992 N. Oho	*D. citri*	Japan	H	VH	H	+
RS1088 R. Samson	Whitefly	Colombia	H	VH	VH	+
A. placenta						
Ap1 S. Selvakumaran	*D. cardamomi*	India	H	VH	VH	+

PDA – Potato Dextrose Agar medium, SDA – Synthetic Defined Medium.

was checked 24 h after spraying; it appeared to be about 99 per cent throughout the experimental period (Fransen, 1987). The results showed that the spores remain viable for at least 7 days on the abaxial leaf surface at 20° C.

A. aleyrodis grows on (semi) artificial media and the sporulation occurs only on solid media. This may complicate the scaling-up of a mass culturing system. Therefore, research may be directed in two ways:

i. Up-scaling mass production on solid media.

ii. Investigating the possibilities of producing spores in liquid fermentation.

The treatment of greenhouse whitefly belonging to different development stages results in differences in infection. Eggs are not infected by *A. aleyrodis* spores, but the spores stay viable on the leaf surface and newly hatched larvae become infected.

i. The outcome of an application of *A. aleyrodis* can be evaluated by the grower, as infected whitefly larvae become orange coloured and can clearly be distinguished from healthy whitefly larvae and pupae (transparent or opaque white) and parasitized pupae (black).

ii. The application of the spore suspension can be carried out during any time of the day. Successful infection was achieved after spraying *A. aleyrodis* during mid-day when the temperature was about 30°- 35°C and the relative humidity was 50 per cent.

iii. *A. aleyrodis* is able to infect whitefly larvae also when temperatures are constantly high (30°C). Thus, the fungus shows prospects for use in environments where temperatures can be high.

iv. *A. aleyrodis* being a selective pathogen on whitefly larvae does not cause any detrimental effects to other natural enemies used in the glass house environment.

3. *Paecilomyces fumosoroseus*

Paecilomyces fumosoroseus is a geographically widespread and common entomopathogen of several insect species frequently isolated from soil. Among whiteflies, it attacks *B. tabaci* and *T. vaporariorum*. Large epizootics caused by *P. fumosoroseus* in *Bemisia* spp. have been observed in several locations in the Indian subcontinent (Table 2). All stages of the host including eggs are susceptible to this fungus. But in field condition infected adults outnumber the nymphs. The conidia germinate on the dorsum of the insect and penetrate the host within 24 h. The mycelia grow in the haemocoel and the aerial hyphae emerge from the host within 48 h. From

the controlled field trial experiment, it is known that *P. fumosoroseus* can infect whitefly nymphs even under relatively dry ambient environmental conditions. Spore production on the other hand seems to require high atmospheric moisture, thus dry condition may restrict secondary infection by the inoculum. *P. fumosoroseus* may remain viable in dried whitefly cadavers for some time and sporulate when adequate moisture becomes available. However, it is not known how long desiccated fungi can survive in the canopy and desiccated cadavers may drop to the soil. The spore dispersal may occur through the whitefly adult dispersal and also by aerial movement of conidia.

Lacey *et al.* (1996) investigated the effect of media on blastospore production and infectivity. The Murashige-Skoog (MS) medium was one of the most effective in producing the maximum number of propagules (5 x 10^8 blastospores / ml) in the relatively short time of 72 hours. The MS medium contains a complex mineral and vitamin mixture and a carbon to nitrogen ratio of approximately 36: 1 (casamino acids and glucose).

The majority of field studies on entomopathogenic fungi for control of silver whitefly have been conducted on *P. fumosoroseus* and *B. bassiana.* Both species of fungi have provided effective control of silver whitefly in cotton, cucurbits, and greenhouse crops. The most detailed field tests for the control of silver whitefly to date have been conducted by Wraight *et al.* (1994 & 1998) on various cucurbit crops in the lower Rio Grande valley in Texas. Multiple (five to seven) applications of 5 x 10^{13} conidia per hectare of P. *fumosoroseus* and *B. bassiana* at 4 to 5-day intervals provided better than 90 per cent control of large nymphs. A single application of 5 X 10^4 conidia per cm^2 of *P. fumosoroseus* (*Pfr* 97) to greenhouse tomatoes and cucumbers resulted in 82 to 88 per cent control of silver whitefly at 14 days post treatment. Nearly 100 per cent of fungus-killed nymphs sporulated within 14 days post-treatment, providing an important source of secondary inoculum.

Because *Bemisia* nymphs are attached to the underside of leaves, application strategies must be used that deliver the majority of the conidia to this surface. Wraight *et al.* (1994) developed an application system that enables treatment of the undersurface of leaves.

Commercial products of *P. fumosoroseus* are available from Thermo Trilogy Corp. (Columbia, MD) and Agrobiologicos del Noroeste, S.A. (Culiacan, Sinaloa, Mexico).

4. *Beauveria bassiana*

This is an extremely common pathogen of a number of insects, isolated from a number of habitats including soil. Though it is found occasionally in whiteflies, it shows significant potential when applied as a mycoinsecticide against *Bemisia.* The results of field trials conducted with *P. fumosoroseus* strains and *Beauveria bassiana* strains on *B. argentifolii* showed that *B. bassiana* isolates were on par with highly pathogenic *P. fumosoroseus* strains. Although *B. bassiana* is rarely reported from *Bemisia*, laboratory assays and field evaluation of several isolates showed insecticidal potential against silver whiteflies (Fransen, 1990).

Under favourable conditions (25±52° C and 70±5 % RH), usually wet and warm, *Bemisia* populations can be drastically reduced by natural infestations of *B. bassiana* which have been commercialized and are available for field and greenhouse application against *Bemisia.* Two commercial products based on *B. bassiana* that have been used for control of *Bemisia* are available in the United States from Troy Biosciences, Inc. (Phoenix, AZ) and from Mycotech Corp (Butte, MT).

The fungus can be mass multiplied in carrot broth medium as described under green muscardine fungus. This fungus can be mass cultured in nutrient agar

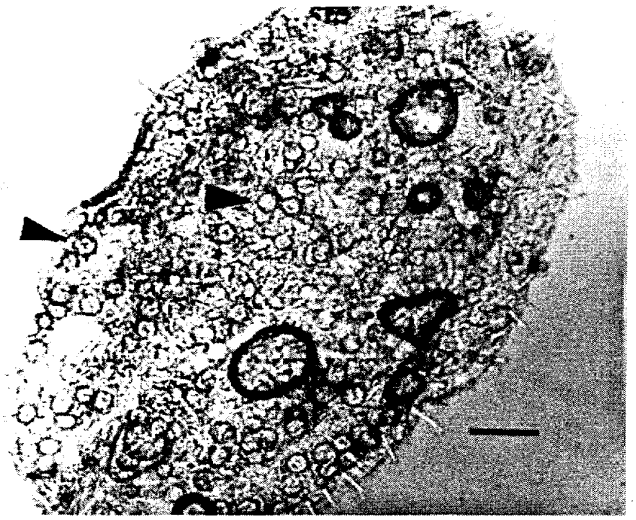
Table 2. Natural occurrence of *Paecilomyces fumosoroseus* and related species in *Bemisia* in India

Host	Location	Reference
Bemisia spp.	Padappai (Tamil Nadu)	Lacey *et al.*, 1993
	Madurai (Tamil Nadu)	
	Andhra Pradesh	
	Parbhani (Maharastra)	

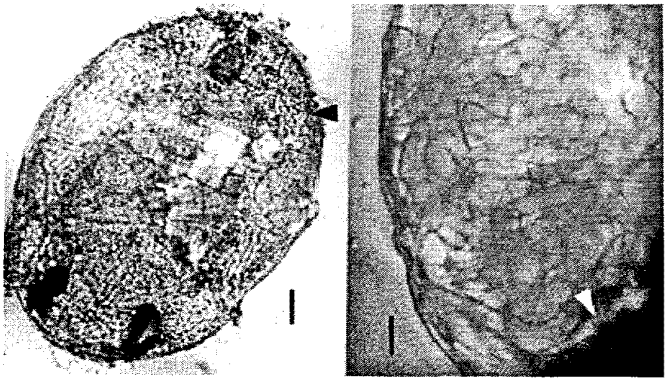
medium, or nutrient broth, potato dextrose agar medium.

5. *Zoophthora radicans*

Natural infections caused by fungi classified in the Entomophthorales have rarely been observed in whiteflies. *Zoophthora* (=*Erynia*) *radicans* (Brefeld) Batko was reported to attack *B. tabaci* in Israel (Ben-Zeev *et al.*, 1988) and Chad (Silvie & Papierok, 1991). Occurrence of this entomopathogen in whiteflies needs to be explored.



Third-instar *T. vaporariorum* infected by *Z. radicans*



Third-instar *T. vaporariorum* with spores and mycelia of *Z. radicans*

Scope and aspects requiring attention

Fungal pathogens of the immature and/or adult stages of Aleyrodidae include the hyphomycetes *Aschersonia* spp., *Beauveria bassiana* (Balsamo), *Paecilomyces farinosus* (Holm ex. S. F. Gray), *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Verticillium lecanii* (Zimmerman) Viegas, and *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin (Osborne & Landa 1992 ; Lacey *et al.* 1996).

Fungal pathogens of whiteflies, such as *B. bassiana*, have shown promise as microbial insecticides for whitefly suppression and are registered in the USA for use in greenhouses and outdoor crops. Current research shows that other entomopathogenic fungi, *P. fumosoroseus*, *Aschersonia* spp., and *V. lecanii* are effective in controlling whiteflies (Sainto and Sugiyama, 2004). These organisms, however, are either not available commercially or are not labeled for use in greenhouses.

In India, presently commercial formulations of *B. bassiana* and *V. lecanii* are available. It is imperative to identify strains more potent against whitefly pest species. Therefore, the utilities of *P. fumosoroseus* and *A. placenta* in India have to be explored. There is a need to look for the occurrence of entomopathogens in whitefly natural biocontrol.

When applying fungal spores as a microbial insecticide, the following aspects are of importance:

i) Fungal characteristics: Germination, virulence, sporulation, viability, in relation to mass production on artificial media and activity in the field.

ii) Host characteristics: Population dynamics, density, developmental rate, differential susceptibility.

iii) Environmental characteristics: Relative humidity, temperature, canopy, light, soil, wind.

iv) Product characteristics: Shelf-life, standardization, formulation, spraying techniques.

v) Side effects:

a. Effect on beneficial insects
b. Safety of fungicides
c. Toxicological aspects

CONCLUSIONS

As more environmentally responsible agricultural strategies are adopted, natural enemies of whiteflies will play ever increasing roles in their control. Practical interest in using entomopathogenic fungi as microbial

control agents of whiteflies is high for the following reasons.

1. The four species or species groups emphasized in this review (*Aschersonia* spp., *P. fumosoroseus*, *V. lecanii* and *B. bassiana*) are highly virulent to whiteflies.
2. Excluding *B. bassiana*, most of these are known to cause natural epizootics in whiteflies under field and/or greenhouse conditions, which may allow them to spread once inoculated on to a crop.
3. They can all be grown on artificial media and applied with conventional insecticide application equipment.
4. They are well adapted to survive in the canopy environment.
5. Unlike insect natural enemies, commercially produced conidia remain viable in storage and pathogenic for whiteflies several months after production.
6. Under most conditions, they are compatible with or even complementary to other natural enemies of whiteflies.

However, their widespread use will depend not only on efficacy as mycoinsecticides, but ultimately on their cost effectiveness.

One of the major limitations of entomopathogenic fungi is the requirement for high humidity for the germination and infection processes. This obstacle might be overcome with improvements in formulation and application technology that enables infection. This possibility has already been demonstrated with *Metarhizium flavoviride* against desert locust in very low humidities (Bateman *et al.*, 1993).

A greater understanding of the factors that enable epizootics of entomopathogenic fungi will be required in order to optimize their utility. Restrictions placed on the use of exotic strains limit in their exploration. The utility of a diversified fungal germplasm will be severely encumbered until this issue is resolved. Consideration should be given to the fact that even the less selective species, such as *B. bassiana*, are much less harmful to non-target organisms and the environment than are broad spectrum insecticides. Diversified germplasm repositories ultimately will also provide a rich source of genetic material for future genetic manipulation (Heale, 1988; Heale *et al.*, 1989).

Over the next decade, as public pressure and subsequent legislative action result in the non-renewal of registrations of several conventional chemical insecticides, fungi and other whitefly natural enemies will be increasingly used to fill the void left by insecticides for the integrated control of whiteflies.

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