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ACAROPATHOGENIC AND ENTOMOPATHOGENIC FUNGUS *HIRSUTELLA* -A REVIEW

by

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Introduction

Among the entomopathogens, fungi are important as they are virulent, cause infection by contact, persist in environment for long time and are mostly mass-producible (Kalyansundaram, 2010). Since they are considered as natural mortality bioagents and environmentally safe, they received worldwide interest in the utilization and manipulation for biological control of insects and other arthropod pests. More than 750 spp of fungi, mostly deuteromycetes and entomophthorales from about 100 genera are pathogenic on insects. Among these, the *Hirsutella thompsonii* relatively less known is gaining importance because of their target specificity and effectiveness in controlling many microscopic to sub-microscopic pests which are difficult to control by conventional management practices. Beside this, it also attacks many polyphagous and economically important noctuid pests and therefore it is a potential candidate for development in to a mycoinsecticide. Here, we attempt to review and present all round progress that took place centring on the entomopathogenic fungus *Hirsutella* covering its discovery, description, physiology, pleomorphism, insecticidal protein and antibiotics, genetic variability, effectiveness against mite and other pests, mass production and products and merits and demerits.

The genus *Hirsutella*:

1. Discovery : *Hirsutella* is an anamorphic genus name that was originally described by French mycologist Narcisse Théophile Patouillard in 1892. The teleomorphs of *Hirsutella* belong to the genera *Cordyceps* and *Torrubiella*. The fungal genus *Hirsutella* Pat. contains about 94 species (Mycobank, 2007). *Cordyceps sinensis* was identified as the teleomorph of *Hirsutella* sinensis based on molecular studies on 5.8S rDNA and ITS region of rDNA (Chen *et al*., 2001; Liu *et al*., 2001).

2. Species under Hirsutella : This genus *Hirsutella* includes species that are pathogens of insects, mites and nematodes. More than fifty species of *Hirsutella* are reported to be entomopathogenic (McCoy *et al*., 1988). Some of the species are *H. nodulosa*, *H. rhossiliensis*, *H. versicolor*, *H. citriformis*, *H. cryptosclerotium* sp. Nov, *H. thompsonii* var. *synnematosa*, *H. sphaerospora*, *H. eleutheratorum*, *H. verticillioides*, *H. formicarum*, *H. homalodiscae* nom. Prov, *H. minnesotensis* etc.

3. Specificity : The *Hirsutella* species like *H. nodulosus*, *H. citriformis* and *H. gigantean*, infect lepidopterans, hemipteran and dipterans insects. *H. rhossiliensis* and *H. minnesotensis* infect various plant-parasitic nematodes. Of all the species, *Hirsutella thompsonii*, which is mite-specific, is the most widely studied one from this genus (McCoy, 1981). It is a mononematous species that has been separated into three morphologically distinct groups, var. *synnematosa* (tropical), var. *vinacea* (subtropical) and var. *thompsonii* (temperate) (Chandler *et al*., 2000).

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4. Systematic position:

Kingdom-Fungi
Division-Ascomycota
Subdivision-Pezizomycotina
Class-Sordariomycetes
Order-Hypocreales
Family-Clavicipitaceae
Genus-*Hirsutella* Pat.

5. Fungus description: *Hirsutella* produces synnemata which is simple or with numerous branches arising nearly at right angles (some species lack synnemata). Conidiogenous cells or phialides arise laterally on synnema or from mycelium on host. Phialides are hyaline, inflated below and narrow abruptly or gradually to long slender sterigmata. Conidia are hyaline, single-celled, oblong to cylindrical and covered with slime (Barnett and Hunter, 1998).

Parasitic activity

According to Roberts (1981) and Samson *et al*. (1988) most of the entomopathogenic fungal disease development including that of *Hirsutella* involves the following nine steps

i) Attachment of the infective units like conidia or zoospore to the insect epicuticle.
ii) Germination of the infection unit on the cuticle.
iii) Penetration of the cuticle either directly by germ tubes or by infection pegs from appresoria.
iv) Multiplication of the yeast phase-hyphal bodies in the haemocoel.
v) Death of the host.
vi) Growth in the mycelial phase with invasion of virtually all host organs.
vii) Penetration of the hyphae from the interior through the cuticle to the exterior of the insect.
viii) Production of infective units of the exterior of the insect.
ix) Omission or failure of these fungi at first four steps will produce low virulent fungi through they might have high toxin biosynthetic capability.

Physiology of *Hirsutella* spp.

H. thompsonii grows moderately on agar-based (solid) artificial media (McCoy and Kanavel, 1969). It prefers dextrose and sucrose as organic carbon source; and yeast extract and peptone as source of organic nitrogen. Growth of the fungus is influenced more by amino acids than vitamins. *H. thompsonii* grows optimally at pH of 7.5 though growth occurs in the pH range of 6.0-9.2. *H. thompsonii* and *H. gigantean* have been reported to have similar nutritional requirements. *H. thompsonii* does not sporulate in submerged condition. Proper aeration is essential for its growth (McCoy *et al*., 1972). Microcyclic conidiation has been observed in fermenter in case of some isolates of the species which produce primary conidia in submerged culture (Latge *et al*., 1988).

Over 700 fungal species belonging to about 90 genera are reported to be pathogenic to insects. Of these, only a few members of Entomophthorales and Hyphomycetes have been studied well and their potential for insect pest management at field level has seriously been viewed only in the recent past. Molecular biology and biochemistry have become the new area of research in entomopathogens (Khachatourians and Qazi, 2008).

In vitro study on physiological requirements of another acaropathogenic species *Hirsutella kirchneri* (Rostrup) Minter, Brady and Hall revealed that its mycelial growth was maximum on media containing yeast extract and dextrose. However, maximum number of conidia was produced in potato dextrose agar (PDA) medium. Like most other fungi it grows best at 25°C and conidia can germinate well in the temperature range of 10 - 35ºC under dark conditions. Alternating light and dark promotes better colony growth and conidia production. The fungus produces synnemata (asexual fruiting body) when grown under continuous light. Within the host body, the fungus sporulates best and cause maximum mortality at 25°C (Sztejnberg *et al*., 1997).

H. kirchneri (Rostrup), *H. necatrix* (Minter) and *H. gregis* (Minter) could be grown successfully on solid media like malt agar, PDA, and Sabouraud dextrose agar and even liquid media with aeration (Minter *et al*., 1983).

Initial studies made by McCoy and Selhime (1977) revealed that the fungal hyphae usually develop in the haemocoel and form spherical chlamydospores as the hyphal cells break apart. On germination the chlamydospores form hyphae again which may then penetrate the body of host and carry out asexual reproduction on the leaf surface. Hyphae present in the dead host body can emerge out through any of the natural openings or even directly through body wall (McCoy, 1981).

Pleomorphism as a basis for taxonomic differentiation in *Hirsutella*:

In vitro culture of *H. thompsonii* shows pleomorphism or phenotypic plasticity (McCoy, 1996; Maimala, 2004) which makes the identification of isolates a difficult task based on morphology (Aghajanzadeh *et al*., 2006). Some isolates of *H. thompsonii* can exist in multiple conidial states. Laboratory grown cultures of different isolates of *H. thompsonii* show considerable variation especially with respect to conidiogenous structure (phialide). Phialide shows two distinct types *viz*., solitary or sometimes proliferating and polyblastic. The mycelium from infected mites bears only the former type which is also seen more commonly in culture media. Ultrastructural analysis of the phialides also formed the basis for differentiation into three morphological groups or varieties. While one group *H. thompsonii synnematosa* prefers tropical habitat, the other two *H. thompsonii thompsonii* and *H. thompsonii vinacea* prefer temperate or subtropical habitat. Isozyme pattern study of *H. thompsonii* isolates at subcellular level closely corroborate with the above morphological grouping into varieties (Boucias *et al*., 1982).

Genetic diversity of *H. thompsonii*

RAPD profile was used to characterize various isolates of *H. thompsonii*, *H. necatrix* and *H. kirchneri* (Mozes-Koch *et al*., 1995). RAPD analysis also revealed that almost all of the isolates from the citrus rust mite, *Phyllocoptruta oleivora* Ashmead, clustered together. The isolate of *H. thompsonii* from *Aceria guerreronis* Keifer clustered separately (Aghajanzadeh *et al*., 2007). High intraspecific polymorphism was observed in isolates of *H. thompsonii* and there was no monophyletic group within the species (Tigano *et al*., 2006). AFLP study among isolates of *H. thompsonii* revealed the existence of 3 different clades. However, based on â-tubulin sequences two clades were identified. Such grouping had relation neither with geographic origin nor with fungus-mite specificity.

Insecticidal protein from *H. thompsonii*

Crude broth extract of *H. thompsonii* var. *thompsonii* was reported to be insecticidal by Vey *et al*. (1993). Two proteins hirsutellin A and B were isolated from the species and found to be toxic to *Galleria mellonella* larvae and *Plutella xylostella in-vitro* (Mazet *et al*., 1992). Toxicity of *Hirsutella* extracts to aphids and mites are also reported (McCoy *et al*., 1992). A toxic protein with unusual properties named hirsutellin A has been characterized from *H. thompsonii*. The toxic protein which has a molecular mass of 15 kDa is antigenic and resistant to proteolytic and thermal degradation. It is neither glycosylated nor does it show proteolytic activity (Mazet and Vey, 1995). Different isolates of *H. thompsonii* from various eriophyid mites differed in their ability to produce exotoxin hirsutellin A (Maimala *et al*., 2002).

The insecticidal protein hirsutellin A was further characterized (Herrero *et al*., 2008) and was found to be a polypeptide consisting of 130 amino acid residues. Structurally, though it is smaller than other known ribotoxins, functionally it behaves as a ribotoxin as it inactivates ribosomes by producing alpha fragment. It also interacts with phospholipids membrane thus exhibiting cytotoxic property, the property that is usually lacking in small sized ribonucleases available in fungi. Thus hirsutellin A is a unique ribonuclease with unusual properties.

Antibiotic from *Hirsutella*

The first report of the antibiotic phomalactone production from *Hirsutella* was reported by Krasnoff and Gupta (1994). Phomalactone was toxic to apple maggot *Rhagoletis pomonella* and inhibitory to conidial germination of *Beauveria bassiana*. *Hirsutella* is also a source of antimycobacterial cyclohexadepsipeptide Hirsutellide A, and antimycobacterial alkaloids Hirsutellones A - E (Liu *et al*., 1995; Vongvanich *et al*., 2002; Isaka *et al*., 2005). *Hirsutella* can produce exopolysaccharide (EPS) which show antibacterial activity especially against gram positive bacteria. However, optimization of fermentation condition and medium for mycelial growth and EPS production was done recently by Li *et al*. (2010).

Effectiveness of Hirsutella species against mites and other plant pests

1. *H. thompsonii* as a mycoacaricide

There are about 50 entomopathogenic species under the genus *Hirsutella* (McCoy *et al*., 1988), whose potential to be developed as mite-controlling bioagent has been exploited. Five species of *Hirsutella* have been reported from eriophyoid mites; the very well-known being *H. thompsonii* Fisher naturally affecting citrus rust mite *Phyllocoptruta oleivora* Ashmead (Muma, 1955; McCoy, 1981) and blue berry bud mite *Acalitus vaccinii* Keifer. The first report on association of *H. thompsonii* with citrus rust mite dates back to 1924 by Spears and Yothers. However the fungus was described by Fisher much later in 1950 and its pathogenicity for citrus rust mite was proved in late 1960s (McCoy and Kanavel, 1969). The potential of three species of *Hirsutella*, *H. kirchneri* (Rostrup), *H. necatrix* (Minter) and *H. gregis* (Minter) were identified as controlling agent of eriophyoid mite vector of rye grass mosaic virus, *Abacarus hystrix* (Nalepa). The above three species were also reported to cause about 16% mortality of *A. hystrix* (Nalepa) (Minter *et al*., 1983). *P. oleivora* was found to be infected by *H. nodulosa* and *H. kirchneri*. *H. nodulosa* also infected *Aceria guerreronis* (Keifer). Besides, eriophyoid mites, many new species of *Hirsutella* have been reported from other leaf-inhabiting mites (Minter and Brady, 1980) and other insect species.

Of all the species of *Hirsutella*, probably *H. thompsonii* has received the most attention and studied most widely for its potential to be developed as mycoacaricide in various countries and against various phytophagous mites for example *P. oleivora* on citrus in Florida, USA (McCoy *et al*., 1971; McCoy and Selhime, 1977; McCoy and Couch, 1982), China, Surinam, Cuba and Brazil; for control of the citrus bud mite *Aceria sheldoni* (Ewing), in Argentina, the Bermuda grass stunt mite, *A. cynodoniensis* (Sayed) on turf grass in the USA (McCoy, 1981), the coconut flower mite, *A. guerreronis*, on coconut in Mexico and *Retracrus elaeis* Keifer on hybrid oil palm in Colombia. Much of the field research in this area has been summarized by McCoy (1981) and McCoy *et al*. (1988).

The fungus *Hirsutella thompsonii* has been reported as an effective biocontrol agent against *Aceria guerreronis*, the coconut mite in India (Beevi *et al*., 1999; Kumar and Singh, 2000; Gopal and Gupta, 2001; Kumar *et al*., 2002). *Hirsutella* sp. has potential to be developed as myco-acaricide taking care of the aspects of formulation, application and ecology in addition to the characteristics of the fungus (Moore, 2000). *H. thompsonii* var. *synnematosa* was reported for the first time from coconut mite by Beevi *et al*., (2000) from Tamil Nadu state of India. Rabindra and Ramanujam (2007) observed that *H. thompsonii* was not found very promising for controlling coconut eriophyid mite. Method to test pathogenicity of *H. thompsonii* against eriophyid coconut mite was made simpler by Kumar and Anuroop (2004). Several other reports on effectiveness of *Hirsutella* species against various mites are summarized in table 1.

9.2. *Hirsutella* species against nematodes:

Hirsutella rhossiliensis was found to be pathogenic to juveniles of *Meloidogyne hapla* (Viaenne and Abawi, 1998). The root knot nematode *M. hapla* in lettuce crop was checked and yield of lettuce increased when *H. rhossiliensis* was applied along with chitin as soil amendment (Chen *et al.* 1999). *H. rhossiliensis* was found to infect second stage juvenile of *M. hapla* (Viaene and Abawi 2000). Egg hatching of *M. incognita* was significantly reduced by *H. rhossiliensis* (Shahda *et al*., 1998). There was 47% reduction in number of root galls, and egg

Table 1: Reports of *Hirsutella* sp. on various mites that affect agricultural crops

***Hirsutella* species**	**Mite**	**Crop**	**Reference**
H. thompsonii	Citrus rust mite (*Phyllocoptruta oleivora)*	Citrus	McCoy *et al.* 1995; Bergh and McCoy, 1997; Aghajanzadeh *et al.*, 2006; Geest, 2004
H. thompsonii	Cassava mite (*Mononychellus tanajo a*)	Cassava	Bartkowski *et al.* 1988; Odongo *et al.*, 1998; Yaninek *et al.*, 1996
H. thompsonii	*Dolichotetranychus floridanus*	Pineapple orchards in Puntarenas, Costa Rica	Zoebisch *et al.*, 1992
H. thompsonii	*Aceria guerreronis*	Coconut in St. Lucia	Moore *et al.*, 1989
H. nodulosa	*Aceria guerreronis*	Coconut	Cabrera and Dominguez, 1987
H. thompsonii	*Aceria guerreronis*	Coconut in Sri Lanka	Fernando *et al.*, 2007; Edgington *et al.*, 2008; Wijesinghe and Rubasinghe, 2003
H. thompsonii	*Aceria kenyae* and *Spinacus pagonis*	Mango in Cuba	Cabrera *et al.*, 2008
H. thompsonii	*Tetranychus neocaledonicus*, red spider mite in okra	Karnataka, India	Rachana *et al.*, 2009a; Rachana *et al.*, 2009b
H. thompsoni	Three mites, *Calacarus heveae*, *Phyllocoptruta seringueirae* and *Tenuipalpus heveae*	Rubber tree in Cerrado	Demite and Feres, 2008; Bellini *et al.*, 2005
H. thompsonii	*Tetranychus urticae*		Kumar and Singh, 2007; Ghosh *et al.*, 2007; Aghajanzadeh *et al.*, 2006; Chandler *et al.*, 2005
H. thompsonii	Pink mite, *Acaphylla theae*		Selvasundaram *et al.*, 2001

Table 2: Incidence of parasitism by *Hirsutella* spp. on various agricultural pests

Species of *Hirsutella*	Target pest	Remarks	Reference
H. nodulosa	Sugarcane internode borer (*Chilo sacchariphagus indicus)*	Coimbatore area of Tamil Nadu, India	Easwaramoorthy *et al.*, 1998
H. nodulosa	*Dioryctria zimmermanni, Neodiprion sertifer* and *Chionaspis pinifoliae*		Eliason and McCullough 1997
H. thompsonii var. *synnematosa*	*Zeuzera pyrina*		Campadelli, 1996
H. versicolor	*Idioscopus nitidulus* in mango	Malaysia	Lim and Chung, 1995
Hirsutella sp.	*Thrips palmi*	Aubergine crop in Trinidad and Tobago	Hall, 1992
H. citriformis	*Pyrilla perpusilla*	Sugarcane foliage from Uttar Pradesh, India	Varma *et al.* 1990
H. cryptosclerotium sp. *nov*	*Rastrococcus invadens*	Togo	Fernandez *et al.*, 1990
H. sphaerospora	*Rastrococcus invadens*	Togo	Garcia and Moore, 1988
H. eleutheratorum	*Rhyzobius lophanthae*, predators of diaspidids		Ricci, 1986
Hirsutella sp.	*Toumeyella cubensis*	On citrus in Cuba	Cabrera *et al.* 2007
H. verticillioides	Cassava lace wing bug (*Vatiga illudens*)	Cause 68% mortality	Junqueira *et al.*, 2005
H. homalodiscae nom. Prov	Glassy-winged sharp shooter *Homalodisca coagulate*		Boucias *et al.*, 2007
H. citriformis, H.formicarum	*Thrips tabaci*	Thailand	Thungrabeab *et al.*, 2006
Hirsutella sp.	*Pseudacysta perseae*	Cuba	Morales *et al.*, 2002
Hirsutella sp.	*Nephotettix virescens*	West Bengal, India	Satpathi, 2000
H. citriformis	*Diaphorina citri*	Cuba	Aragon and Ravelo, 2000
H. subulata	Codling moth (*Laspeyresia pomonella* and *Cydia pomonella)*	Poland	Machowicz, 1986

masses of another nematode species *M. incognita* in brinjal plant when treated with *H. rhossiliensis* (Ibrahim *et al.* 1999). Alginate pellet containing *H. rhossiliensis* suppressed *M. javanica* (Jaffee and Muldoon, 1997). Lackey *et al.*, (1994) reported that *H. rhossiliensis* suppressed *H. schachtii* in cyst-infested loamy sand and loam soil when applied as pelletized hyphae. They reported that the fungus was more effective against juvenile stages. However, in contradiction, mycelial formulation of *H. rhossiliensis* failed to suppress cyst nematode *Heterodera schachtii* (Jaffee *et al.*, 1996). In another study in Italy, more than 90% samples of second stage juveniles of the cyst nematode *Heterodera daverti* was found to be parasitized by *H. rhossiliensis* (Sorbo *et al.*, 2003). Parasitic nature of the fungal species is reported to be decreased with addition of wheat straw or composted cow manure to a loamy sand naturally infested with *H. rhossiliensis* (Jaffe *et al.*, 1994). Soybean cyst nematode was reported to be controlled by two species of *Hirsutella*, *H. rhossiliensis* and *H. minnesotensis* (Chen and Liu, 2005; Zhang *et al.*, 2008). Another species of *Hirsutella i.e., H. minnesotensis* was found to be effective against the endoparasitic nematode, *M. hapla* and the overall reduction of nematode density was 31-83% (Mennan *et al.*, 2006, 2007)

9.3. *Hirsutella* species against other crop pests

Other then phytophagous mite and nematode there were also many instances where *Hirsutella* was used in managing many crop pests. No other insect pathogenic fungi, but *H. citriformis* was found to effectively check the population of Asian citrus psyllid *Diaphorina citri* (Etienne *et al.* 2001). The fungus was also reported as natural enemy of *D. citri* in Cuba (Rivero and Grillo, 2000). Yet another species *Hirsutella verticillioides* was found effective against lace bug (*Leptopharsa heveae*) of rubber tree in Brazil (Junqueira *et al.*, 1999). Sudoi (1999) reported that *Hirsutella* sp. isolated from soil of tea garden, Timbilil Estate Kericho, Kenya caused 63% mortality of tea weevil (*Entypotrachelus meyeri micans*). Singaravelu *et al.* (2003) documented from Uttar Pradesh, India that *H. citriformis* was able to control significantly the sugar cane leaf hopper, *Pyrilla perpusilla* under the field condition.

The table below (Table 2) provides a glimse of important crop pests parasitized or infected by various *Hirsutella* species under natural or artificial conditions.

10. Mass Production:

Mass production methods both at laboratory and industrial scale have been developed for the fungus *H. thompsonii*. McCoy *et al*. (1975) devised large-scale production technique of mycelial mass under submerged condition. Semi-solid agar medium was not suitable for mass production of *Hirsutella*. Soybean agar and malt extract agar media produced significantly higher number of colony forming units of *H. thompsonii* var. *synnematosa* whereas spore production was not observed in broth (Maimala *et al*., 1999). Synthetic medium Sabouraud Maltose Broth (SMB) was reported to be better than other liquid media for mass production of mycelia (Aghajanzadeh *et al*., 2006). When commercial preparation of *H. thompsonii* (potency $1x10^9$ cfu/g) was sprayed at optimum weather conditions at a dose of 2-4 pounds per acre gave effective result in citrus with no significant difference in per cent fruit injury when compared with standard chemical pesticide treatment.

The first diphasic fermentation method for the fungus was developed in 1975-76, by Abbott laboratories, USA (McCoy and Heimpel**,** 1980) and *H. thompsonii* was developed as a product under the name Mycar ™ in 1981 for controlling eriophyoids on citrus and turf.

Fragmented mycelial formulation has been used by researchers mainly as prophylactic measure on the newly formed foliage before onset of damaging level of mite population on it. However, mycelia based commercial formulation had low shelf-life in room temperature as the hyphal cells lysed during storage (McCoy, 1981). Though storage under refrigerated condition provides enhances the shelf-life, it only made the product expensive. Sensitivity of conidia to free water also hinders the development of conidial formulation. The above two factors as well as factors associated mainly with the stability of the formulation during storage and transport discouraged the commercial production in USA.

11. Success stories of *Hirsutella* and commercial products

The entomopathogenic fungus *Hirsutella* is a polyphyletic genus parasitizing various damaging pests of agricultural importance *viz*., mites, insects and nematodes. The use of *Hirsutella* species for biological pest control is limited as it is difficult to grow. However, several success stories achieved using this fungus including control of the citrus rust mite (*Phyllocoptruta oleivora*) in Florida suggest its potential to be used as mycoacaricide (Ekesi and Maniania, 2007). Mite complex (*Brevipalpus phoenecis* and *B. obovatus*) of acidic lemon (*Citrus aurantifolia*) could be successfully controlled by application of mycelial (50 g/l) and conidial suspension (1x 10^6 cfu/ml) of *H. thompsonii* which persisted on leaf up to seven months after spraying (Rosas, 2000). Three products based on *Hirsutella thompsonii* are recorded to be developed as acaricides (Faria and Wraight, 2007). 'Mycohit', is regarded as the first mycoacaricide base exclusively on *H. thompsonii*, for suppressing the coconut eriophyid mite in India. The eriophyid mite *Aceria guerreronis* has been a serious pest in Americas and West Africa for many years (Moore, 2002). It appeared in serious proportion in coconut growing areas of peninsular India reducing the yield significantly in recent years. In several places of southern India eryophyid mite was found to be naturally parasitized by the fungal species. The strain MF(Ag)5 was considered to be the most promising biological control agent for the mite and developed in powder formulation giving 80% control of the mite under field conditions (Kumar and Singh, 2002).

12. Merits and demerits of *Hirsutella*

The most important feature of *Hirsutella* is it able to control many microscopic or sub-microscopic pests including phytophagous mite, nematodes and many insect pests which are difficult to control by conventional means. Being self perpetuating they are able to control the target pests for longer period even after single application. So far there is no report of development of resistance, resurgence or secondary pest out break. *Hirsutella* also found compatible with many conventional insecticides like monocrotophos.

One of the major demerits that is associated with the *Hirsutella* is the slow activity. The use of *Hirsutella* species for biological pest control is limited as it is difficult to grow under laboratory conditions. After mixing with water, *Hirsutella* products should be sprayed as soon as possible, as fungal spores die and material loses its viability overnight. Equipment that gets the material to the undersides of the leaves will result in prolonged activity, as spores are inactivated by sunlight. Evening applications are advisable. Non-availability of the commercial product and poor shelf life also restrict its popularity among the farmers.

13. Conclusion

The entomopathogenic fungi *Hirsutella* is effective against wide group of crop pests including mites, nematodes and insects. They offer great scope in microbial control with which the hazards of the chemicals can be avoided or minimized in pest management. However, certain important points should be taken in to consideration while applying in the field *viz*., suitable strain, weather factor, stage of the pests and mode of application. Mass production technology has to be standardized for commercial production. Effective formulation and suitable application technology also have to be developed for its better efficacy. Compatibility with chemical insecticides has to be checked before mixing with it. Biosafety test for natural enemies and pollinators should be conducted rigorously for its wider use. The mite/insect and *Hirsutella* fungus system needs further studies both in basic and applied aspects. Isolation of new pathotype of the fungus and improvement of formulation technology will remain the key area of research in the days to come.

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