A Review :

Progress in Work of *Hirsutella thompsonii* Fisher in India

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International Journal of Plant Protection, Vol. 2 No. 2 : 287-290 (April to September, 2009)

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Key words : Progress, Development, *Hirsutella thompsonii*

***Accepted :* August, 2009**

Hirsutella thompsonii Fisher is considered as a potent natural biocontrol agent of eriophyid mite, *Aceria guerreronis* Keifer infesting coconut. Three separate species of the fungus have been known based on conidiogeneous structure. Temperature has a significant effect on the growth of the fungus. Temperature at 37^0C and above did not record mycelial growth and temperature shifted to 25^0C recovered the mycelial growth. However, temperature did not have any significant effect on the micromorphology of *H. thompsonii*. Simulated sunlight has slightly detrimental effect on conidia production and best conidiation observed under alternating light-dark conditions. A simple magnetic stirrer technique has been developed for mass production of *H. thompsonii*. Earlier, indigenous isolation of this fungus was used to suppress the coconut mite, *A. guerreronis*. Later, its commercial formulation (mycoacaricide) named "Mycohit" has been developed by PDBC, Bangalore and tested against coconut mite under field conditions in Kerala, Karnataka and Andhra Pradesh and obtained satisfactory results. Two new formulations based on *H. thompsonii viz.*, Mycohit LG-20 and Mycohit OS proved equally effective to that of Mycohit against *A. guerreronis*. The adjuvants *viz.*, Glycerol, Yeast Extract Powder (YEP) and Malt Extract Broth (MEB) found to increase the pathogenicity of *H. thompsonii*. The glycerol proved better than YEP and MEB.

The first pathogen found to cause disease in insects was the fungus. There are over 750 species of fungi which are known to attack various arthopods like insects and mites. The fungi which attack on insect called entomopathogenic fungi (EPF). Most informations of EPF infesting insects are available in literature. However, the information on fungi associated with a small creature like mite is scattered and meagre. The fungus, *Hirsutella thompsonii* Fisher has been widely used to manage the havoc caused by coconut mite, *Aceria guerreronis* Keifer in coconut plantations. An attempt has been made to review the informations on coconut the progress and development of this important microbial agent in India.

A lot of research has been done with the fungus, *H. thompsonii* right from its isolation, mass multiplication, genetic improvement and formulation for the control of citrus rust mite (McCoy and Kanavel, 1969; McCoy *et al.*, 1972, McCoy *et al.*, 1975 and McCoy *et al.*, 1984). The efficacy of *H. thompsonii* to control citrus mite in the fields in USA, Surinam, Isarel and China has been recorded to be very promising (McCoy, 1978, Van Brussel, 1975 and Gerson *et al.*, 1979). In China, a single application of laboratory produced *H. thompsonii* mycelia at a dose of 0.5 to 1.0 g/l to citrus caused 90 % reduction in the population of mites in 3 days (Chiang and Huffaker, 1976).

Among *H. thompsonii*, three separate varieties, *viz., H. thompsonii* var. *thompsonii*, *H. thompsonii vinacea* and *H. thompsonii* var *synnematosa* based on the ultra structured analysis of the conidiogenous structures have been identified (Samson *et al.*, 1980). Recently, comparison of different isolates of *H. thompsonii* made by PDBC, Bangalore revealed that Kerala isolate (MF(Ag)b6) produced more number of conidia as compared with the Tamil Nadu isolate (Anonymous, 2008). The percentage of germination, however was better in Tamil Nadu isolates.

Field trials with *H. thompsonii* have given mixed results in the past. The field application of the fungus against coconut mite, *A. guerreronis* in Mexico resulted in mortality up to 75 % (Espinosa and Carrillo, 1986) whereas in certain other fields in West Africa (Anonymous, 1989) and West Indies (Moore *et al.*, 1989) and this pathogen was not effective. It has been suggested that *H. thompsonii* isolated from *A. guerreronis* found to be more effective, bringing about 88 % mortality compared to 35% mortality inflicted

by the isolate from citrus rust mite, *Phyllocoptruta oleivorus* (Sampedro and Rosas, 1989), thus elucidated the need for isolating a pest-specific and pest associated pathogen.

Studies for the isolation of indigenous *H. thompsonii* from coconut mite, *A. guerreronis* were seriously pursued in India. The first report Ramarethinam *et al.* (2000) was isolation of *H. thompsonii* from coconut eriophyid mite. The application of *H. thompsonii* alone @ 10 g per tree brings about 23 to 25% reduction in mite damage. However, when combined with *Verticillum lecanii*, *Paecilomyces* sp. and nimbecidine (an Azadirachtin containing neem derivatives), suppression was affected to the tune of 30 to 40 %. Subsequently, a formulation of *H. thompsonii* named "Mycohit" has been developed by the Project Directorate of Biological Control (PDBC), Bangalore. Kerala Agricultural University, Thrissur has isolated *H. thompsonii* var. *synnematosa* which is specific to eriophyids, especially *A. guerreronis*.

"Mycohit", the mycoacaricide formulation of *H. thompsonii* developed at PDBC, Bangalore has been evaluated against coconut eriophyid mite, *A. guerreronis* under field conditions (Anonymous, 2001). The experimental results revealed that maximum (60.47%) dead colonies of mites were noticed at 49 days after treatment (DAT) in trees sprayed twice at 14 days interval with 'Mycohit' and thereafter there was a decline in mortality. Highest mortality (75%) was realized at 63 DAT. In contrast, there was a steady increase in the percentage of dead colonies in trees treated thrice with 'Mycohit' indicating the usefulness of an additional spray to sustain the mortality for more than two months. Comparative efficacy of Mycohit with other miticides against coconut mite, *A. guerreronis* tested at Kerala Agricultural University, Thrissur revealed that per cent reduction of mite population over control was 28.59 in Mycohit treated trees as against 25.12 in azadirachtin and 35.04 in sulphur treated trees (Anonymous, 2002). Results concluded that all the three treatments (Mycohit, Azadirachtin and sulphur) evaluated in the study were found to be statistically at par.

A multilocation field study was conducted by PDBC, Bangalore to evaluate two new formulations of *H. thompsonii i.e.* Mycohit LG-20 and Mycohit OS along with 'Mycohit' (already available formulation) against the coconut mite, *A. guerreronis* and concluded that all the three formulations of Mycohit significantly reduced the mite population on the nut surface of coconut fruits (AnonAnonymous, 2003). Mycohit OS showed a reduction of 10.87 % mite population over the treatment of control. However, there was no significant reduction in nut damage in both the tagged bunches. Similarly, both the new formulations of Mycohit (LG- 20 and OS) along with Azadirachtin and Mycohit evaluated against the mite at Pallukulangara (Alappuzha district) and Valiakulangara (Kollam district) of Kerala revealed that there was no significant difference among the treatments evaluated (Anonymous, 2003). The mean infestation index of nuts at maturity did not differ significantly among the treatments including control. Both the new formulations of Mycohit based on *H. thompsonii* along with triazophos 40 EC have also been evaluated against coconut mite at two different locations in East Godavari district of Andhra Pradesh (Anonymous, 2003) and concluded that there was no mycelial fragments on the mite colonies in any of the observations recorded at 45 days after spray application. As a result, no significant reduction in the population of the mite was observed.

Two separate field experiments were laid out for the management of coconut mite in the coconut plantation of college of Horticulture, Vallanikkara district Thrissur, Kerala during the year 2002. Mycohit LG-20 and Mycohit OS were evaluated along with Mycohit and wettable sulphur. Post treatment count of live mites/mm^2 area was significantly lower in all the three Mycohit formulations as well as wettable sulphur than in control (Anonymous, 2003). Wettable sulphur treated tagged branches recorded lowest live mite population. Of the two field trials, damage grading on nuts did not show a significant reduction due to the application of Mycohit formulations in first trial. However, in second trial the nut damage score in wettable sulphur treatment was significantly lower followed by Mycohit LG-20 treated nuts. Similar treatments were also evaluated in field conditions at Dharwad, Karnataka and found that there was no significant difference among the treatments. However, all the three formulations of *H. thomsonii* were found to be at par with wettable sulphur, but significantly superior over untreated check. Mycohit LG-20 recorded the least number of mites (Anonymous, 2003). Similarly field trials conducted at Huskura, Bangalore (Karnataka) showed that the formulation of *H. thompsonii* (1 %) + adjuvants (0.5 %) reduced the coconut mite, *A. guerreronis* population significantly over the control. This treatment was as effective as triazophos (Anonymous, 2008).

Effect of temperature on *H. thompsonii* and *H. thompsonii* var. *synnematosa* studied at PDBC, Bangalore (Anonymous, 2005) revealed that temperature had a significant role on the growth of both the fungi. It did not record mycelial growth at 37^0C and above, however, when shifted to 25^0C, the mycelia recovered excellent growth compared to the growth at other

temperatures (27, 35 and 36^{0}C). Results also revealed that temperature did not have any significant effect on the micromorphology of the two fungi. Simulated sunlight found to be slightly detrimental to conidia production by *H. thompsonii*. Best conidiation was observed under alternating light dark conditions (Anon., 2008).

Studies on effect of different inoculum loads on *H. thompsonii* in liquid culture (Sabourad Dextrose Broth-SDB) showed that in continuous shake culture when Oat Meal Agar (OMA) discs were used, the colony forming units (CFU), pellet number and wet as well as dry weights increased proportionately with the number of mycelial discs per flask (Anonymous, 2005). The size of the pellet, however decreased with increasing number of discs. The CFU obtained with 6 discs was 6.4 times more than that produced by one discs. Similar trend was recorded with Potato Dextrose Agar (PDA) discs. In alternating shake-stationary culture, the number of colonies, pellet number, wet and dry weights increased with increase in the inoculum load. However, the pellet size was maximum at an inoculum level of 4 discs of *H. thompsonii* indicating that pellet formulation was affected by inoculum load. Storage of *H. thompsonii* and *H. thompsonii* var. *synnematosa* pellets in sterile water was found to be better under refrigerated conditions. Although the pellets after 10 and 11 months of storage under room conditions showed growth, dark coloration and reduction in fluffiness in the culture (Anonymous, 2005).

A simple magnetic stirrer technique for faster mass production of *H. thompsonii* has been developed with two media (Potato Dextrose Broth and Sabourad Dextrose Agar) and two different mycelial plugs (Potato Dextrose Broth and Oat Meal Agar). A combination of OMA plugs and SDB medium recorded maximum CFU numbers and wet as well as dry weights in both the magnetic stirrer technique and shake cultures (Anonymous, 2005). The adjuvants have been found to increase the pathogenicity of the fungi. *H. thompsonii* (1%) in combination with three adjuvants (glycerol, yeast extract powder (YEP) and Malt extract broth (MEB) @ 0.5 % evaluated against coconut mite at Huskuru, Bangalore (Anonymous, 2007). It was revealed that mite population was significantly reduced in all the treatments. The fungus was able to cause disease in mite as evidenced during the post-sampling. Maximum (97.2%) reduction in coconut mites was brought by the fungus in combination with MEB. However, the results of similar trial conducted at CPCRI farm, Kasargod indicated that there was no significant difference between the treatments or period of application (Anonymous, 2007). In a study on effect of different adjuvants on the growth of *H. thompsonii*, it was found that higher number of colonies emerged from the biomass treated with glycerol which was followed by MEB and YEP (Anonymous, 2008). Among the 3 adjuvants, glycerol was found to be the best adjuvant than YEP and MEB. Latter both the adjuvants proved to be equally effective (Anonymous, 2008).

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