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Effect of Application Frequency of the Entomopathogenic Fungus *Hirsutellathompsonii* (Fisher) on Damage Levels of Coconut Mite *Aceriaguerreronis* (Keifer)

L.C.P. Fernando[1]*, D.C.L. Hapuarachchi[1], and K.P. Waidyarathne[1]

Abstract

The effect of application interval of *H. thompsonii* (Fisher) on damage caused by the coconut mite, *Aceriaguerreronis* (Keifer) (Acari: Eriophyidae) in two coconut plantations in Sri Lanka was evaluated. In each plantation, coconut palms infested by coconut mite were treated with *H. thompsonii* isolate IMI 391722 at 2- and 3-monthly intervals and the fruits with *H. thompsonii*-infected coconut mite cadavers and percentage of harvested fruits in different damage categories were assessed. Infected coconut mite cadavers were found on up to 60% of the fruits treated with the fungus in both plantations at treatment frequencies. The effect of the treatments was site-specific. The treatments reduced the percentage of damaged-small size harvested fruits to half that of the untreated control in both plantations irrespective of the frequency of treatment. At one plantation, the percentages of undamaged fruits and fruits with discontinued damage were nearly doubled to that of untreated fruits.

Key words: *Aceriaguerreronis*, biological control, coconut mite, interval of application, *Hirsutellathompsonii*

[1]Coconut Research Institute, Lunuwila, Sri Lanka.
*Corresponding author. Tel.: +94 31 2255300; Fax: +94 31 2257391, E-mail address: head_cpd@cri.lk (L.C.P. Fernando)

Introduction

Until 1998, the coconut mite *Aceriaguerreronis* (Keifer) (Acari: Eriophyidae), one of the most destructive pests of coconut palm was confined to the American and African continents, but its invasion to India and Sri Lanka in late 1990's (Fernando, 1998; Sathiamma *et al*., 1999), the major coconut growing countries of the world has posed a serious threat to coconut industry of the world. The damage resulting from feeding of coconut mite on the meristematic tissue beneath the bracts (tepals) of developing fruits causes scarring, reduced size and distortion of the fruits and immature fruit fall leading to reduced yield and market value of the fruits. Scarring directly affects the quality of the husk (mesocarp) of the fruits making its removal difficult and extending the time taken. Reduction in the size and deformation of the fruit affects the copra (dried kernel) yield and fibre content (husk weight) of coconut. Damaged-smaller size fresh fruits are generally sold at half of the normal price due to its reduced amount of kernel, whereas deformed nuts are rejected by the buyers. Yield losses in the form of fresh fruits and copra (dried kernel) vary widely across the countries infested by coconut mite, ranging from 10 – 16% in West Africa (Mariau and Julia, 1970) and up to70% in Venezuela (Dórense, 1968). In Sri Lanka, a mean annual loss of 15.9% fruits, 13.4% reduction in husk weight and a loss of about 195kg of desiccated coconut (processed kernel) per 10,000 fruits in the infested areas have been estimated (Wickramananda *et al*., 2007).

Due to the hidden habitat of the coconut mite underneath the bracts of coconut fruit, the massive size of the coconut palm and its continuous fruit production, effective management of coconut mite by continuous application of chemicals have been impractical and uneconomical. Therefore, development of biological control methods was considered a high priority. Apart from the recent investigations in the use of predatory mite, *Neoseiulusbaraki* Athios-Henriot (Acari: Phytoseiidae) in Sri Lanka (Fernando and Aratchige, 2010; Fernando *et al*., 2010), the entomopathogenic fungus, *Hirsutellathompsonii* (Fisher) has been long considered as a potential biological control option for coconut mite. Several attempts to control *A. guerreronis* with the fungus have been only partially successful (Espinosa-Becerril and Carrillo-Sanchez, 1986; Suarez *et al*.,1989; Cabrera, 2002), probably due to the differences in the isolates and formulations and the effect of prevailed macro- or micro-climatic conditions, which are common problems in the use of entomopathogens (Jaronski, 2010).

However, recent studies in India and Sri Lanka have shown promise with this candidate. In India, *H. thompsonii* isolate MF(Ag)5 (IMI 385470) has given over 80% mortality of coconut mite (Sreerama Kumar, 2002) and the powder formulation of Mycohit and its liquid variants Mycohit-LG20 and Mycohit-OS produced by the Project Directorate of Biological Control, Bangalore, India have reduced coconut mite populations up to 90% (Sreerama Kumar, 2010). The ability of mycelia of *H. thompsonii* along with adjuvants to bring down coconut mite populations and its damage has been demonstrated (Sreerama Kumar and Singh, 2008). The Sri Lankan isolate, IMI 391722 was superior to other isolates collected from Sri Lanka in reducing the coconut mite population (Fernando *et al*., 2007). Only less than 10% of the fruits receiving this isolate had high levels of coconut mites (>100 live mites) at 4 weeks after the treatment. However, the efficacy declined with progress of time, but mycosis of dead coconut mites due to infection of *H. thompsonii* was evident up to 18 weeks after the treatment. Therefore, it has been necessary to quantify the effect of *H. thompsonii* isolate IMI 391722 on damage levels of harvested fruits for its use in the field. The preliminary study reported in this paper envisaged to assess the effect of *H. thompsonii* on damage levels of the harvested mature fruits of coconut and determine the suitable frequency of application.

Materials and Methods

A field study was conducted in two coconut plantations at Madurankuliya (> 800mm rainfall) and Ariyagama (>1100mm rainfall) in the North-western Province of Sri Lanka. At Madurankuliya, 3 blocks, each comprising of 60 palms of 30 years old with coconut mite infestation were selected. Since, the coconut mite infestation in the plantation at Ariyagama was not uniform only 60,25 years old coconut mite infested palms were selected and randomly assigned to 3 groups of 20 palms. In both estates, two groups of palms received the fungus either at 2-monthly or 3-monthly intervals, while the remaining group of palms was used as the untreated control.

H. thompsonii isolate IMI 391722 isolated from coconut mite on coconut fruits in Sri Lanka was cultured on rice ("samba" BG variety) as described by Fernando *et al.* (2007) and extracted by washing in water. The suspension was strained through a wire mesh with a mesh size of 11 squares/cm and a suspension of 10^6-10^7/ml colony forming units (CFU) which included spores and mycelia was prepared. In the first application, two groups of palms in each estate were treated with the suspension. The treatments were applied from the ground using a knapsack sprayer in which the delivery tube was extended to a length of about 10m and the lance fixed to the distal end of the tube. The lance of the sprayer was attached to a long pole to reach the fruits of the palms. In each palm, fruit bunches of 1 - 6 month old (from the most recently opened inflorescence) was sprayed with approximately 1 litre of the suspension. The group of control palms were treated with the same volume of water, but without the fungus. Treatments were applied in late afternoon and each block received treatments at 2- or 3-monthly intervals up to one year. The palms in the control group were sprayed with water at 2-monthly intervals during the same period.

To assess the infection of coconut mite by *H. thompsonii,* 10 and 15 randomly selected palms from each group at Ariyagama and Madurankuliya respectively were marked. Before the first application one 4-month old coconut mite infested fruit was picked from each marked palm, 20 dead mites were collected from each fruit and placed on glass microscope slides. The slides were placed on moistened tissue paper inside sealed petri dishes and incubated at $27\pm1^{o}C$ for 3 days. The coconut mites were then examined for *H. thompsonii* infection using a phase contrast microscope. Hyphae growing out from dead coconut mites and the presence of characteristic fruiting bodies of *H. thompsonii* were used to confirm mycosis of coconut mites due to *H. thompsonii*. Sampling was repeated every month, up to 9 months.

Coconut fruits are mature enough for harvesting in 12 - 13 months after opening of the inflorescence. Therefore, assessment of damage levels on harvested fruits were commenced 12 months after the first application of the fungus and continued up to 9 months at 1.5 month intervals. At Madurankuliya harvested fruits were collected from 30 palms including 15 marked palms in each block and at Ariyagama they were collected from all 20 marked palms in each block. Each harvested fruit was categorized into one of the following groups according to the damage level; undamaged- normal size (A), discontinued damage-normal size (B1), damaged-normal size (B2), damaged-small size (C1), damaged-deformed (C2) and undamaged-small size and undamaged-barren fruits (D). In the damaged-normal size fruits the damage scar on the fruit surface was continued to the level of the bracts whereas in discontinued damage-normal size fruits the damage scar on the fruits do not extend up to the bracts. Generally A, B1 and B2 fruits are sold at a normal price, C1 fruits at half of the normal price and C2 and D are rejected. If the husk of the fruits is used for coir production the damaged fruits (B1, B2 and C1) may not fetch a higher price due to the difficulty in removing the husk, high wastage of fibre and high labour cost.

Data Analysis

Out of the dead coconut mites incubated to determine the infection by *H. thompsonii*, if at least one *H. thompsonii*-infected coconut mite cadaver per fruit was present that fruit was considered as infected by the fungus and the fraction of fruits with infected coconut mites was calculated.

The analysis on damage levels were not carried out on fruits of D category because the numbers of those fruits were negligible in both estates at all harvesting occasions. However, for calculation of the percentages of fruits in each damage category the total number of fruits harvested from palms of each treatment was considered. Analysis of variance was carried out on the percentage damage fruits under each category at each harvesting occasion in the two estates separately. The comparison among2- and 3-monthly applications and control was done by Duncan's Multiple Range Test.

Results

Prior to the treatment with the fungus, there were no *H. thompsonii*-infected coconut mite cadavers found on the fruits (Figs. 1 & 2). During the period of treatments, they were prevalent on treated fruit sat Ariyagama, irrespective of the frequency of application of the fungus (Fig. 1). At Madurankuliya, infected cadavers were present up to 5 month son fruits treated either 2- or 3-monthly intervals but thereafter the presence of infected cadavers were erratic(Fig. 2). Although there were significant differences ($p<0.001$) among the percentages of fruits with *H. thompsonii*-infected coconut mite cadavers among all 3 treatments in both estates, there was no such difference between the two application intervals. Dead mites sampled from the untreated fruits were not infected with the fungus at any occasion in both estates. The percentage of fruits with *H. thompsonii*-infected coconut mite cadavers ranged from 10% to 60% and 6.6% - 60% at Ariyagama and Madurankuliya respectively.

The two estates did not show a consistent pattern in the effect of the treatments on damage levels with respect to the percentage of harvested fruits in each damage category between treatments and among harvesting occasions. At Ariyagama, percentage of harvested fruits in A, B1, B2 and C1 categories were significantly different ($p<0.01$) among the treatments and among harvesting occasions ($p<0.05$), but the differences showed a similar trend in all occasions. At Madurankuliya, the treatments had significantly affected only the percentage of C1 fruits ($p<0.05$), but significant differences ($p<0.05$) were observed among the harvesting occasions in all categories. At Ariyagama, the percentages of A and B1 category fruits were significantly higher and B1 and B2 category fruits were significantly lower on the treated palms than on the untreated palms, irrespective of the application interval (Table 1)indicating the positive effect of the treatments. Further, the percentage of C1category fruits was significantly lower and almost half in the treatments than in the control (Table 1). At Madurankuliya the percentage of C1category fruits in the two treatments was nearly half of the untreated control (Table 1).

Discussion

Application of *H. thompsonii*, at 2- and 3-monthly intervals caused mycosis in coconut mites due to the fungus at Ariyagama and Madurankuliya. The application of the fungus showed a positive impact on the damage levels improving market value of the fruits at harvest as well as the husk, although it varied in the two estates. At both estates, fruits that are sold at half of the normal price has halved by the treatments. The increase in percentage of undamaged and damaged-discontinued fruits by nearly 2-fold at Ariyagama favourably contributes to the coir industry as the husk quality of the fruits has improved by the treatment. The results indicated that the effect of both 2- and 3-monthly application of the fungus was similar in causing mortality of coconut mites and reducing damage on fruits.

There had been no natural incidence of *H. thompsonii* in coconut mite populations at Ariyagama and Madurankuliya prior to commencement of the treatments. From the first month onwards, the mite populations were infected by the fungus at both estates indicating that the effect of treatment in causing mycosis in coconut mites. However, the percentage of fruits with infected mite cadavers had not increased with the continuation of treatments, probably because the sampled fruit (4-month old) had always received the treatment either once or twice. It was not possible to collect fruits, which received the treatment more than twice, as the mite populations on fruits over 6-month old was very low (Fernando *et al*., 2003). In general, the percentage of fruits with *H. thompsonii*-infected coconut mite cadavers were low as 40% or less in many occasions and varied in the two estates. One reason could be the low number of dead coconut mites sampled per fruit (20 mites), which had lowered the chances of recording infected mites. We were unable to examine

Figure 1. Mean percentage of fruits with *Hirsutellathompsonii*-infected coconut mite cadavers at monthly intervals after commencement of 2- and 3-monthly application of *H. thompsonii* and in untreated control at Ariyagama

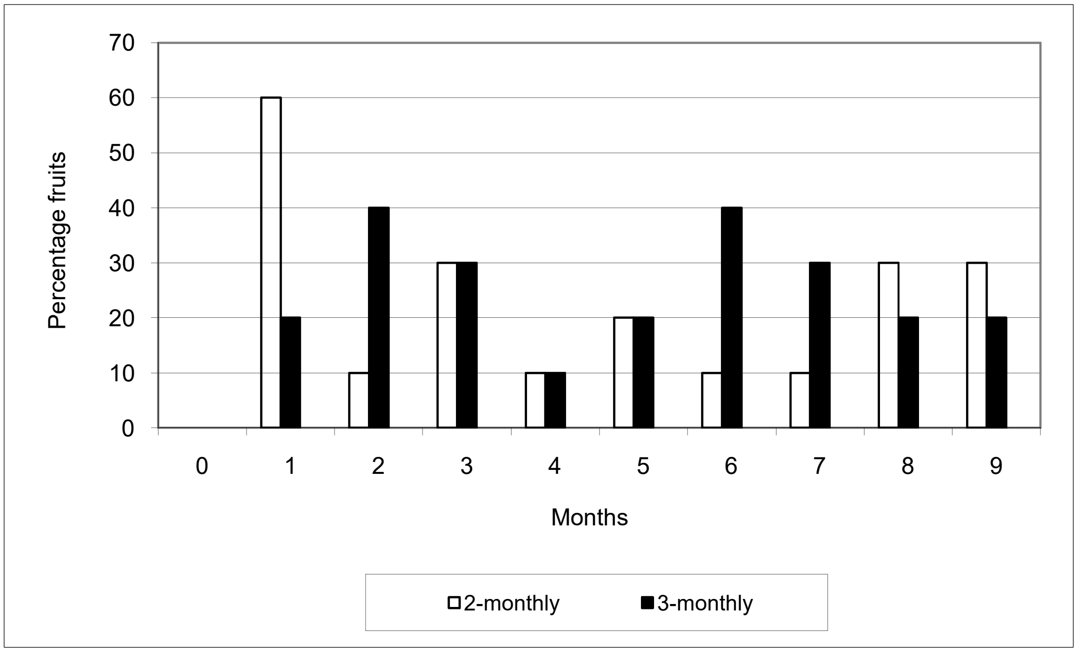

Figure 2. Mean percentage of fruits with *Hirsutellathompsonii*-infected coconut mite cadavers at monthly intervals after commencement of 2- and 3-monthly application of *H. thompsonii* and in untreated control at Madurankuliya

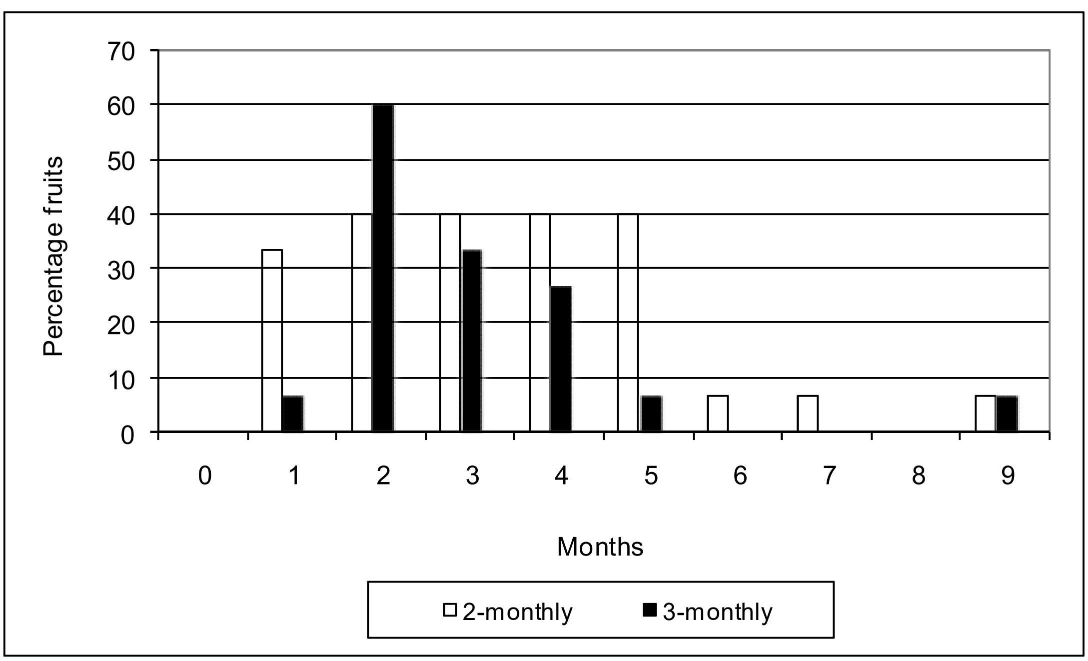

Table 1. Evaluation of *Hirsutellathompsonii* at Ariyagamaand Madurankuliya

Fruit damage category	**Mean percentage of harvested fruits (S.E)**		
	in *H. thompsonii* treatments at different intervals		**in Control***
	2-monthly	**3-monthly**	
Ariyagama			
Undamaged (A)	23.05±0.01A	22.60±0.01A	12.14±0.02B
Damaged-discontinued (B1)	34.38±0.03A	36.96±0.03A	15.96±0.9B
Damaged-continued (B2)	18.65±0.03B	22.57±0.03B	41.52±0.04A
Damaged-small size (C1)	15.33±0.12B	12.30±0.18B	24.48±0.03A
Damaged-deformed (C2)	5.86±0.01A	2.62±0.01A	5.23±0.01A
Madurankuliya			
Undamaged (A)	13.83±0.01A	18.37±0.01A	16.12±0.01A
Damaged-discontinued (B1)	22.27±0.03A	19.82±0.03A	19.31±0.03A
Damaged-continued (B2)	50.45±0.03A	46.11±0.03A	48.18±0.03A
Damaged-small size (C1)	6.76±0.01B	6.71±0.01B	11.12±0.01A
Damaged-deformed (C2)	3.29±0.01A	4.42±0.01A	3.40±0.01A

Mean in the same column followed by a common letters are not significantly different at $p<0.05$.

*Water spray at 2-monthly interval

more number of dead coconut mites per fruit due to time consuming nature of picking mites from the fruits. Also, it seems that the infection rate on fruits is site-specific. In a previous study, the same isolate of *H. thompsonii* caused mycosis of coconut mites in about 90% fruits (Fernando *et al*., 2007). It is well known that many abiotic and biotic factors affect the efficacy of mycoinsecticides in both foliar and soil applications. Sunlight, humidity, temperature, and phylloplane-associated factors can affect both immediate efficacy and persistence of the fungus on plants (see Jaronski, 2010).

Ariyagama, which is situated in the intermediate zone experiences an annual rainfall of >1100 mm, whereas Madurankuliya, which is in the dry zone, only receives >800 mm rainfall annually. Since, infection rate and persistence of *H. thompsonii*is enhanced at high relative humidity (>90%)the higher annual rainfall received at Ariyagama may have contributed to the presence of *H. thompsonii*-infected coconut mite cadavers on fruits at all times during the sampling period compared to Madurankuliya, resulting in lower damage levels. This factor needs to be considered in future studies and recommendation of *H. thompsonii* for the management of coconut mite.

Since application interval showed no difference with respect to damage levels, 3-monthly application is suggested. However, several laboratory and field studies are required to improve the formulation of the fungus to enhance mycosis in mites and its persistence, identify areas with suitable climatic conditions for the application of the fungus. Also, costs in the production of the fungus and field costs involved in treating palms and the income gained by the coconut grower by the treatment need to be assessed in future pilot trials.

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