CHAPTER - 6

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Pesticides use in tea increases the risk of pesticide residue build up in made tea above the maximum residue limits. In view of the awareness of health consciousness, decreasing susceptibility of the mite to the conventional pesticides and contamination of made tea, a search for better alternative is felt necessary to reduce dependence on chemicals. The concept of plant protection in tea has recently been changing to the adoption of an integrated approach, which includes biocontrol measures also. In biological control system a natural biotic force helps to regulate populations of insects. Conservation of native entomopathogen, augmentation, manipulation and if possible, introduction of exotic biotic agents help the tea crop to reduce the pesticidal load. Considering the need for an alternative eco-friendly approach to control the red spider mite of tea, it was believed to be worthwhile to screen the indigenous entomopathogenic fungal pathogens.

The potentiality of biocontrol agents against the red spider mite has not been fully explored till date. The present investigation based on the following objectives, was aimed at evaluating the entomopathogenic properties of few fungal strains on red spider mite.

- Survey of native fungal pathogens associated with mortality of red spider mite (*Oligonychus coffeae*).
• Isolation and characterization of the fungal pathogens from dead red spider mite and red spider infested tea leaves.

• Prospects of utilization of fungal bio-control agents and its effect on controlling red spider mite.

• Study of pathogenicity \( (LC_{50} \text{ and } LT_{50}) \) of the fungal bio-agents in controlling red spider mite.

6.1: Consumption, per cent leaf area damaged, fecundity and per cent hatching of \textit{O. coffeae} on different TV clones

The data revealed that consumption per female per day differed significantly \( (P<0.05) \) on different TV clones, the highest \((0.16 \text{ cm}^2)\) being recorded on TV1 and the lowest \((0.07 \text{ cm}^2)\) on TV6. The per cent leaf area damaged on different tea clones were also varied significantly and the maximum damage of leaf \((70\%)\) being recorded on TV1 and the minimum \((33.06\%)\) on TV6. The fecundity on different clones were also varied significantly \( (P<0.005) \) and the highest \((78.21)\) eggs being recorded on TV1 and the lowest \((58.3)\) eggs on TV6. The percentage of egg hatching were also significant \( (P<0.05) \) and maximum hatching \((90\%)\) was recorded on TV1 and the minimum \((76.66\%)\) on TV6.

6.2: Duration of egg to adult of red spider mite

There are several generations red spider mite in all stages of development can be found almost any time throughout the year. The duration of the life cycle is shorter in summer months, ranging from 7.20 to 11.40 days in May and July, while it may take as much as 20.80 days in the cold weather in laboratory condition.
In comparison to laboratory treatments in the field condition the duration of egg to adult takes more time and ranging from 10.20 to 12.00 days in May and July however, it was recorded as much as 20.20 days in the month of January.

6.3 : Duration of immature stages from larva to adult

Duration of larval stage of *O. coffeae* was recorded to be lowest in the month of June (1.20 days) and during the month of November it was recorded to be highest (3.00 days). Total duration of larvae and nymphal stages were also found to be lowest in the month of June (7.40 days) while highest duration recorded in the month of February (12.00 days). From the present investigation it was established that amongst the factors which influence the incidence of red spider mite and the intensity of its attack, weather conditions play an important role.

6.4 : Developmental periods of different stages of *O. coffeae* under laboratory condition at different months

Incubation period was found to be highest in the month of February-March (7.22 days) and lowest in the month of May-June (5.05 days).

The larval period of red spider mite was observed to be highest in the month of February – March (1.71 days) and lowest during May-June (1.13 days) only.

The duration of immature stages was observed in three different months were recorded as 9.05 days during February – March, 7.09 days during April. Shortest duration of immature stages observed in the month of May-June and recorded as 5.48 days.
Duration of life cycle of red spider mite was recorded as 16.27 days during February-March, 12.97 days during the month of April and 10.33 days during May-June.

6.5: Numerical ratio of male to female of red spider mite (laboratory reared)

It was observed that the female constitute the great majority of the red spider mite population. During February-March male : female ratio was recorded as 1:2.95. During the month of April male : female ratio was of 1:1.37 while during May it was recorded as 1:1.31. In the month of July and August the sex ratio of male and female varies from 1:1.10 to 1:1.108.

6.6: Size of different stages of *O. coffeae* and adult longevity

The larvae measures 0.16 mm in length and 0.12 mm in breadth while the adults measures on an average 0.31mm length and 0.17 mm in width in case of male and 0.41 mm in length and 0.22 mm in breadth in case of female.

The adult female lived longer and longevity ranged from 23 to 31 days with an average of 27.00 days while male was found short lived and longevity ranged from 10 to 17 days with an average of 13.50 days.

6.7: Site for persistance of red spider mite in natural condition

The upper surface of tea leaf was found to be superior in comparison to lower surface. The variation of persistence may be due to various bio-physical character of different clones exist in the tea blocks and some other morphological characters of tea leaves in natural habitat.
6.8: Seasonal incidence of red spider mite

The attack of red spider mite is started in early March for pruned tea and even earlier on unpruned or skiffed tea. With the rise in temperature, the rate of multiplication is increased and the generations also follow in quick succession, resulting in a heavy build-up of population in late March or in the month of April. The newly build red spider mite population cause severe injury in May and June until the monsoon rains set in. From the month of July onwards the red spider mite population declined. Even during cold weather also it persist and the rate of multiplication is considerably reduced during this period.

6.9: Preference for site of oviposition by O. coffeae on different tea clones

The red spider mite preferred to lay eggs near the midribs, depressions, angle formed by the veins and curved area of leaf tips. The highest number of egg laying per female red spider mite per day was recorded 1.98 on the sides of main vein in China variety which is followed by TV1 clone (1.92).

The number of egg lay varied in different tea clones and the variation of oviposition may be due to various bio-physical and morphological characters of tea leaves of different clones.
6.10: Some biological parameters of \textit{O. coffeae} under laboratory conditions at different months during 2003-2006

The oviposition period during February-March was found on an average to be 18.30 days and during April it was recorded as 16.66 days while in the month of May-June, oviposition period reduced to 14.44 days.

The rate of deposition of eggs per female per day was recorded as 4.33, 4.07 and 4.18 eggs during the months of February-March, April and May-June respectively.

6.11: Some biological parameters of \textit{O. coffeae} on different tea clones under laboratory conditions during 2004-2006

The incubation period 7.89 days, larval period 1.82 days while the protonymphal period 2.06 days, and deutonymphal period 2.06 days were recorded to be comparatively higher on TV6. In China variety larval period 1.09 and protonymph period 1.46 days was recorded to be lowest in comparison to other tea clone studied. However, on analysis it was revealed that there was no significant difference in duration taken by developmental stages of \textit{O. coffeae} on different tea clones.

6.12: Persistence of red spider mite on leaves of tea plants during cold weather

The red spider mite persists in all stages of its development on some of the old leaves of tea bushes during cold weather. This population is primarily responsible for the build-up of the red spider mite population in the spring. So, clean pruning removes a great portion of the red spider mite population persisting on the tea bushes in the
early part of the winter. However, in unpruned tea the reduction of the red spider mite population is slight or negligible.

6.13: Relationship between different groups of predatory mite and red spider mite based on their seasonal incidence on tea bush

Mainly two groups of predatory mites (Amblyseius sp. and Preneuma sp) were observed in the experimental plot. The persistence of predatory mite per tea leaf found to be highest during August to November. It was also established that with the increase in the number of predatory mite the persistence of red spider mite declined. It may also mention that as a whole the number of different predatory mite increases in the organic conversion period.

6.14: Mycelial growth and sporulation of various fungal strains on different culture media

The radial growth of Pencillium purpurogenum was maximal (10.00 cm) in Special Peptone media which was followed by PDA (9.50 cm), Sabarauls Dextrose media (8.33 cm) and Rose Bengal media (7.00 cm). In case of Beauveria bassiana the radial growth was also recorded to be higher in Special peptone media (9.30 cm) while in case of Verticillium lecanii it was found to be higher in Rose Bengal media (8.78 cm). Similarly, Trachoderma harzianum shows higher radial growth in Rose Bengal media (8.60 cm). The radial growth of Metarhizium anisopliae was recorded to be highest in Special Peptone medium (7.90 cm) and in case of Paecilomyces lilacinus it was recorded maximal (8.00 cm) in PDA media.
Sporulation (x $10^6$/ml) of *Penicillium purpurogenum* was recorded to be maximal (49.30) in Special Peptone media. Higher sporulation of *Beauveria bassiana* was recorded (40.56) in PDA media while moderate sporulation was observed in *Verticillium lecanii* (29.30) in Rose Bengal media and *Trichoderma harzianum* (23.13) in Special peptone media. In case of *Paecilomyces lilacinus* the sporulation was found to be poor and recorded maximum of 2.233 numbers of spores per ml.

6.15: Extension rates of different entomopathogenic fungal isolates at different temperatures

Increase of temperature above the optimal level adversely effected the growth and resulted in declining the rate of extension. All the fungal isolates studied were able to grow at 20° and 30° C while with the increase in temperature only four fungal isolates were survived and able to grow at 35° C. These are isolates of *Penicillium purpurogenum*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii*. Of the different temperature studied, the extension rates of various fungal isolates were found to be superior at 25° C.

6.16: Effect of Temperature on disease development of red spider mite caused by various fungal strains after 7 days inoculation

From the present investigation it was established that temperature was also found to be one of the important factors affecting the development of fungal infection in red spider mite. In most of the isolates disease development was recorded in a suitable temperature ranged from 20° C to 25° C. Among the fungal isolates studied *Penicillium purpurogenum* showed highest per cent (100%) of disease development
and mortality of red spider mite recorded at 25° C which was followed by *Metarhizium anisopliae* and recorded (87%) response for disease development and mortality. Temperature above the optimal level (25° C), growth and development of fungal bio agents retarded which results in the declination of disease development and mortality of red spider mite.

6.17: Effect of Temperature on spore germination of various fungal strains tested for Red spider mite infection after 24 hours of incubation

Temperature was also found to be one of the important factors affecting the spore germination of various fungal strains. Temperature regime of 25° C was found to be optimal for most of the fungi tested with highest spore germination of 88.5% in *Penicillium purpurogenum* after 24 hours of incubation. At low temperature the germination percentage was recorded to be low in all the tested fungal strains. However, with the increase in temperature above the optimal level *i.e.* at 30° C and 35° C the spore germination was affected. Considering the percentage of spore germination, the temperature level between 20° C and 25° C was optimal for the tested fungi. Spore germination of the *Penicillium purpurogenum* was followed by *Beauveria bassiana* (82%), *Metarhizium anisopliae* (80%), *Trichoderma harzannum* (76%), *Paecilomyces lilacinus* (70%) and *Verticillium lecanii* (50%) at 25° C after 24 hours of incubation.
6.18: Effect of various fungal strains on egg laying female red spider mites

From the present investigation it was established that all the fungal isolates studied at all tested concentration could inhibit the rate of oviposition of red spider mite.

From the observations it was revealed that the survival rate found to be poor in the treatment of fungal spore *Penicillium purpurogenum* (10^8 conidia/ml) where only 10 numbers of adult female were survived. It was followed by *Metarhizium anisopliae* (14.4 numbers of female survived), *Beauveria bassiana* (15.6 number of female survived) and *Verticillium lecanii* (17.0 numbers of female survived).

The highest inhibition (2.0 No/female) was recorded in the *Penicillium purpurogenum* at 10^8 conidia/ml which was as per with *Beauveria bassiana* and *Metarhizium anisopliae*. These strains were found to be superior to all other treatments.

It was also observed that with the application of fungal spore of *Penicillium purpurogenum* the production of egg reduced to 0.20 numbers per female.

6.19: Effect of *Verticillium lecanii* fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

From the observations it was revealed that the survival rate of red spider mite was found to be poor in the treatment of fungal spore *Verticillium lecanii* (10^8 conidia/ml) with 0.005% Tween 20. Fungal infection also reduced the production of eggs by red spider mite females in comparison to treatments that having only Tween 20.
20 or water. With the application of wetting agent Tween 20 along with fungal spore further influenced on the mortality of the pest and reduced the numbers of egg per female and recorded 0.26 numbers per female. These results support the possibility of exploiting the entomopathogenic fungal strain *Verticillium lecanii* as a biological acaricide against red spider mite.

6.20: Effect of *Trichoderma harzianum* fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

The survival rate of the red spider mite was recorded to be declined with the application of fungal spore *Trichoderma harzianum* (10⁸ conidia/ml) along with wetting agent (0.005% Tween20) and recorded only 16 numbers of female pest alive after treatment with an average of 0.62 eggs produced per female in this treatment. Thus the fungal infection of female red spider mite results in the reduction of the production of eggs in comparison to treatments that having only Tween 20 or water. Though the results support the possibility of exploiting the entomopathogenic fungal strain *Trichoderma harzianum* as a biological acaricide but there is necessity of further investigation for the exploitation of this strain against red spider mite.

6.21: Effect of *Beauveria bassiana* fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

In comparison to the single effect of Tween 20 or water the fungal infection of *Beauveria bassiana* (10⁸ conidia/ml) tremendously reduced the production of eggs by red spider female mite. The survival rate of the pest was found to be significantly declined in the treatment of fungal spore *Beauveria bassiana* (10⁸ conidia/ml) with
water or 0.005% Tween 20. It has also been observed that fungal spore of *Beauveria bassiana* suspended in water recorded an average of 14.8 numbers of female pests alive after treatment and only 0.35 eggs produced per female. However, the treatment of fungal spore with wetting agent (0.005% Tween 20) significantly reduces the survival percentage of female red spider mite and recorded only 0.25 eggs per female.

6.22: Effect of *Metarhizium anisopliae* fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

Survival percentage of female red spider mite after the treatment of the fungal spore *Metarhizium anisopliae* (10^8 conidia/ml) with wetting agent (0.005% Tween 20) found to be significantly reduced. It was also observed that the fungal infection of *Metarhizium anisopliae* significantly reduced the egg production (0.29 eggs/female) by red spider mites in comparison to treatments that having Tween 20 or water alone. The findings support the possibility of commercial exploitation of the fungal pathogen *Metarhizium anisopliae* as a biological acaricide for the control of red spider mite a major pest of Tea in North East India.

6.23: Effect of *Penicillium purpurogenum* fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

The survival rate of the red spider mite was found to be very poor in the treatment of *Penicillium purpurogenum* spores (10^8 conidia/ml) with wetting agent (0.005% Tween 20) and recorded only 9.0 numbers of female pests alive after treatment. The fungal infection also reduced the production of eggs by red spider mite females and recorded an average of 0.22 eggs per female in this treatment in
comparison to treatments that having only Tween 20 or water. From the present findings it was also established that the fungal strain *Penicillium purpurogenum* was found to be the most effective entomopathogen in comparison to other fungal strains tested and there is enormous scopes for the commercial exploitation of this fungal strain as a biological acaricide against red spider mite.

6.24: Effect of *Paecilomyces lilacinus* (10^8 conidia/ml) fungal spore along with wetting agent (Tween 20) on egg production of female red spider mite

From the observations it was revealed that the survival rate was found to be poor in the treatment of fungal spore *Paecilomyces lilacinus* (10^8 conidia/ml) with wetting agent (0.005% Tween 20). With the application of *Paecilomyces lilacinus* along with wetting agent (0.005% Tween 20) influenced on the mortality of the pest and recorded 10.6 numbers of female survived with the egg production rate of 0.47 numbers per female. From the findings it is revealed that there is a scope for the exploitation of this fungal strain with further investigation to use as a biological acaricide against red spider mite.

6.25: Comparison of *O. coffeae* egg mortalities on exposure to different entomopathogenic fungal conidial concentration

Cent percent (100%) egg mortality was recorded on *Penicillium purpurogenum* which was followed by *Metarhizium anisophae* (90.4%) and *Beauveria bassiana* (86%). There were four strains that had significantly higher egg mortalities at intermediate conidial concentrations than were observed in the controls. At low concentrations only *Penicillium purpurogenum* caused a significantly higher
mortality than the background mortality. Thus, different fungal species varied in ability to infect *O. coffeae* eggs and their impact on the egg mortalities largely depended on the conidial concentrations sprayed.

6.26: Efficiency of *Verticillium lecanii* against red spider mite

The percentage of infections resulting mortality by the isolate *Verticillium lecanii* was recorded to be higher in egg (50.0 %) after 3.6 days, nymph (53.0 %) after 3.04 days and adult (47%) after 4.0 days at the spore load $10^8$ spores per ml with LC$_{50}$ 6.4 x $10^7$ and LT$_{50}$ 4.3 days. With the increase in the concentration of spore of the isolates, pest infestation and percentages of mortality increases. A significant positive correlation (P<0.05) was found between spore density and red spider mite mortality, and a negative correlation between spore density and pest population. This suggests that maintaining viable spores at a higher density will reduce the red spider mite population.

6.27: Efficiency of *Trichoderma harzanum* against red spider mite

In comparison to other fungal bioagents studied, *Trichoderma harzanum* was found to be less pathogenic for the control of red spider mite in all the stages of development. In case of *Trichoderma harzanum*, the mortality percentage of red spider mites egg (40% after 6 days), nymph (42% after 5 days) and adult (33% after 5.0 days) was recorded at the spore load $10^8$ with the LC$_{50}$ 7.0 x $10^6$ and LT$_{50}$ 6.03 days. Similar results were also recorded on the spore load $10^7$ for the mortality of various stages of red spider mites. However, in comparisons to other strain tested
results are found to be poor and further study may be carried on to make it effective against red spider mite.

6.28: Efficiency of *Beauveria bassiana* against red spider mite

Pathogenicity of *Beauveria bassiana* against the tea pest red spider mite was found to be encouraging. After spraying the spore suspension of *B. bassiana* isolate, the highest percentage of egg (90% after 2.6 days), nymph (90% after 2 days) and adult (84% after 2 days) mortality was recorded at the spore load $10^8$ with the $LC_{50}$ $4.9 \times 10^7$ and $LT_{50}$ 3.9 days. This study also showed increase mortality with increase in spore concentrations. A positive relationship was found between inoculum density from $10^5$ to $10^8$ spores/ml and mortality of the pest.

6.29: Efficiency of *Metarhizium anisopliae* against red spider mite

Pathogenicity of *Metarhizium anisopliae* against red spider mite was found to be positive. At 1.6 to 4.3 days after treatment, all treatments caused significantly higher ($P<0.05$) mortality than the control.

The highest percentage of egg (90% after 2.3 days), nymph (92% after 1.6 days) and adult (90% after 2.0 days) mortality of red spider mite was recorded at the spore load $10^8$ with $LC_{50}$ $3.5 \times 10^6$ and $LT_{50}$ 3.18 days. From the mortality rates the fungal isolate *Metarhizium anisopliae* was found to be virulent.

6.30: Efficiency of *Penicillium purpurogenum* against red spider mite

*Penicillium purpurogenum* was found to be highly effective against the major tea pest red spider mite. There was no distinct difference in the susceptibility of
nymphal and adults to the fungal pathogen. The mortality of egg, nymph and adult increased with the increases in spore concentrations. 100 per cent mortality of red spider mites (all stages) were recorded with the treatment of *Penicillium purpurogenum* at the spore load range $10^7$ to $10^8$ spores/ml. Thus, the efficacy of the sporulation @ $10^7$ spores/ml was found to be similar to the $10^8$ spores/ml with the LC$_{50}$ 3.0 x $10^6$ and LT$_{50}$ 2.24 days. Both these dosages were more effective than the lower dosage rates as they caused highest egg, nymph and adult mortalities. The rate of mortality was found to be directly proportional to the dosage of the fungus applied.

6.31: Efficiency of *Paecilomyces lilacinus* against red spider mite

The response towards red spider mite mortality with the treatment of *Paecilomyces lilacinus* was found to be highest in egg 76% after 4.0 days. Nymph mortality was found to be 77 % after 3.7 days while the adult mortality recorded 70 % after 4 days of treatment at the spore load range $10^7$ to $10^8$. It was followed by the spore load $10^6$ with LC$_{50}$ 5.0 x $10^7$ and LT$_{50}$ 6.03 days. At the lower concentrations of the spore the mortality percentage reduced. However, in comparison to other isolates the virulancy of *Paecilomyces lilacinus* was recorded to be poor.

6.32: Control of red spider mite in field condition

In the field experiment out of different fungal pathogens tested with a spore load of $10^8$/ ml *Penicillium purpureogenum* was found to be superior and recorded 42% reduction of red spider mite population with in 7 days of treatment which was followed by *Metarhizium anisopliae* (38%), and *Beauveria bassiana* (30%). In field treatment, the rate of mortality even increases with the duration of time. After 4th
week it increases to *P. purpureogenum* (86%) and followed by *M. anisopliae* (82%), and *B. bassiana* (70%). It was observed that from 4th week onward the mortality of red spider mite declined probably due to reduction of viable spores in the environment and recorded *P. purpureogenum* (78%) followed by *M. anisopliae* (76%), and *B. bassiana* (50%). The lowest infection and population reduction was recorded in the plot treated with *Trichoderma harzianum* (18%) after 5th weeks of treatment. From the present findings it was established that in field condition the effectiveness of fungi against red spider mite depends on many physical and physiological factors along with duration of treatment which also play important role on the reduction of pest population.