CHAPTER 3
MATERIALS
AND METHODS
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Rapid roving surveys were carried out periodically in different tea gardens of Assam, India to reveal the status of fungal biocontrol agents towards red spider mite from January, 2003 to January, 2004. Surveys revealed the presence of a number of entomopathogenous fungi which may be used for the control of red spider mite.

3.1: Red spider mite (*Oligonychus coffeae* Nietner)

Class - Protostigmata.

Order - Acarina

Family - Tetranychidae

Genus - *Oligonychus*

Species - *coffeae*

The red spider mite is an example of ‘warm season’ mites. This pest has been reported from over 300 host plants including field crops, ornamental plants, house plants and weeds. It is one of the destructive tea pests of North East India. Spider mites are not insects but are more closely related to spider. These arachnids have four pairs of legs, no antennae and a single oval body region. Most spider mites have the ability to produce fine silk webbing. Spider mites are very tiny, being less than 1/50 inch (0.4mm) long when matured to adults.
Plate 1. Life cycle of red spider mite (Oligonychus coffeae)

(a) Red spider mite - an egg (40X)
(b) Red spider mite - a larva (10X)
(c) Red spider mite - a protonymph (10X)
(d) Red spider mite - a deutonymph (10X)
(e) Red spider mite - a male (10X)
(f) Red spider mite - a female (10X)
All spider mites go through the same stages of development. Adult females usually lay eggs on their host plants. The round, pearly white eggs are too small to be seen with the unaided eye. The newly hatched mites go through a larval and two nymphal stages before becoming adults. The time from egg to adult is dependent mainly on temperature.

The eggs hatch in a day to weeks into the first stage, called larva. Larvae are round bodied and have only three pairs of legs. The larvae feed for a few days, seek a sheltered spot to rest and then molt into the first nymphal stage. The first nymph has four pairs of legs. The first nymphs feed a few days, rest and molt into the second nymph. The second nymphs feed, rest and molt into the adult stage. The males are usually the size of the second nymph and have pointed abdomens. The females have rounded abdomens and are the largest mite in size (Plate 1). Their numbers grow up quickly under hot and dry conditions at the temperatures of 25° to 35°C.

In order to study the biological parameters of the pest in laboratory condition the following procedures were adopted.

Red spider mite infested leaf samples were collected from the different tea estates of Assam. The surveyed tea estates are listed below (Table 1, Plate- 2 and 3).
Plate 2. Locations of Rani Organic Tea Estates (Assam, INDIA)
### Table 1: Name of few Tea Gardens under survey and sample collection

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Tea Estates</th>
<th>Total Tea Area</th>
<th>Longitude</th>
<th>Latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Puranimati Tea Estate, Jorhat</td>
<td>300 ac</td>
<td>94.16E</td>
<td>26.46N</td>
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<tr>
<td>2</td>
<td>Rani Organic Tea Estate, Kamrup</td>
<td>900 ac</td>
<td>91.47E</td>
<td>26.11N</td>
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<tr>
<td>3</td>
<td>Parowa Tea Estate, Sonitpur</td>
<td>920 ha</td>
<td>92.50E</td>
<td>26.37N</td>
</tr>
<tr>
<td>4</td>
<td>Bogapani Tea Estate</td>
<td>757.53 ha</td>
<td>94.58E</td>
<td>27.29N</td>
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<tr>
<td>5</td>
<td>Ledo Tea Estate, Tinsukia</td>
<td>398.85 ha</td>
<td>94.60E</td>
<td>27.31N</td>
</tr>
<tr>
<td>6</td>
<td>Dehing Tea Estate, Tinsukia</td>
<td>454.37 ha</td>
<td>94.60E</td>
<td>27.31N</td>
</tr>
<tr>
<td>7</td>
<td>Namdang Tea Estate, Margherita, Tinsukia</td>
<td>703.30 ha</td>
<td>94.60E</td>
<td>27.30N</td>
</tr>
<tr>
<td>8</td>
<td>Powai Tea Estate, Digboi, Dibrugarh</td>
<td>920 ha</td>
<td>94.58E</td>
<td>27.27N</td>
</tr>
<tr>
<td>9</td>
<td>Konapathar Tea Estate, Tinsukia</td>
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<td>94.60E</td>
<td>27.28N</td>
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<tr>
<td>10</td>
<td>Udyan Tea Estate, Golaghat</td>
<td>160 ha</td>
<td>94.00E</td>
<td>26.30N</td>
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<tr>
<td>11</td>
<td>Sakalatinga Tea Estate, Jorhat</td>
<td>456 ha</td>
<td>94.17E</td>
<td>26.48N</td>
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<tr>
<td>12</td>
<td>Dihing Dishang Tea Estate, Digboi</td>
<td>283.45 ha</td>
<td>95.40E</td>
<td>27.33N</td>
</tr>
</tbody>
</table>
Plate 3. Different tea estates of Assam
(a) Rani Organic Tea Estates, Kamrup
(b) Puranimati Tea Estate, Jorhat
Plate 3. Different tea estates of Assam

(c) Parowa Tea Estate, Sonitpur
(d) Bogapani Tea Estate, Tinsukia
Plate 3, Different tea estates of Assam

(e) Ledo Tea Estate, Tinsukia
(f) Dihing Tea Estate, Tinsukia
Plate 3. Different tea estates of Assam

(g) Namdang Tea Estate, Margherita (Tinsukia)
(h) Powai Tea Estate, Digboi, Dibrugarh
Plate 3. Different tea estates of Assam

(i) Konapathar Tea Estate, Tinsukia
(j) Udyan Tea Estate, Golaghat
Plate 3. Different tea estates of Assam

(k) Sakalatinga Tea Estate, Jorhat

(l) Dihing Dishang Tea Estate, Digboi, Dibrugarh
3.2: Rearing of the mite

Red spider mite was cultured on detached mid staged leaves of various tea clones (TVI, TV2, TV4, TV6, TV9, TV10, TV14, TV19 and China variety) in petridishes (Hazari et al. 1995). The petiole was wrapped in a plug of moist cotton to keep the leaf afresh for a longer period and the leaf was placed by keeping the upper surface up on a wad of cotton wool in a petridish (15 cm diameter). The cotton wool was kept wet by adding distilled water periodically as and when necessary. This is termed as leaf culture (Plate 4).

The mite infested leaves were collected from the field in a UV sterilized polythene bag and brought to the laboratory. The mites were then released on the leaf culture with the help of a fine camel hair brush (size zero) and subsequently when the leaf became crowded, the mites were then maintained through fresh detached leaf culture.

To each fresh detached leaf, 2-3 freshly emerged males and a female quiescent deutonymph were released. Five such sets of mites were used for each of the study. They were observed daily at morning and evening under stereo-binocular microscope (Model-CXRII, LABOME; 10 X magnifications) for determining various biological parameters like the fecundity of the mated female and the larval period. Protonymphal period, deutonymphal period, pre oviposition period, post oviposition period, oviposition period and incubation period were also recorded.

For determining percentage of hatching, five mated females were released in five fresh detached leaf of each clone for egg laying and the eggs laid after 24 h were
Plate 4. Laboratory maintenance of red spider mites (leaf culture)

(a) Release of red spider mites on detached young tea leaves
(b) Highly infested tea leaves
counted and allowed to hatch for 3-5 days based on which hatching percentage was calculated.

On the other hand 'Leaf disc culture' (instead of the whole leaf a part of a leaf of size 2.5 cm² was used) was also tried for studying the biological parameters (Helle and Sabellis 1985). However, in the leaf disc culture method, the mites were unable to maintain for a longer period of time in comparison to the leaf culture method. In leaf disc culture method as the leaves were cut into reasonable size, after two to three days the sides were found to be dried up or damaged due to various physiological changes on the trimmed ridges of the leaf. So the modified method of leaf culture have been adopted for further experiments where the pests can be maintained up to 7 days without any reasonable damage in the cultured leaf.

3.3: Fungal bio-control agents

The wild type of strains Beauveria bassiana, Metarhizium anisopliae, Penicillium purpurogenum, Verticillium lecanii, Trichoderma harzanum were isolated from dead red spider mite collected from different tea gardens of Assam.

The Verticillium lecanii and Paecilomyces lilacinus were also obtained from the fungal culture collection of the biocontrol lab of Tocklai Tea Research Centre, Jorhat. Conidospores from these mother cultures served as inocula for the further experiments and were maintained at 26±1°C on PDA media, Special peptone, Rose bengal agar media, Czapek-dox agar, Sabarauds dextrose agar media.
Inoculums of mould were allowed to grow on PDA media for culturing by single spore isolation method (Hildebrand 1938, 1950). Isolated fungi were sub cultured in culture tubes for identification and maintaining for further study.

The strains were isolated in pure form and preliminary identifications were done under stereo-binocular microscope in the laboratory itself and finally identified in the Institute of Microbial Technology (IMTECH), Chandigarh.

3.4: Inoculum preparation and maintenance

Leaf samples which were collected from different tea estates were incubated (12 h day light) in moist petridishes over filter paper to encourage the visible growth of entomogenous and other associated fungus on egg, nymph and adults of red spider mite. The inoculums of mould were isolated from the pests were again plated into petridishes containing potato-dextrose agar and streptomycin sulfate (antibiotic to avoid bacterial contamination) and incubated in a BOD chamber at 26±1°C and a 12 hour photophase maintained for seven days. Next, they were reinvigorated on nymph or adult red spider mites in order to maintain the pathogenic virulence. The single spore isolation method (Hildebrand 1938, 1950) was applied to isolate the fungi and was subcultured in culture tubes containing PDA culture medium with streptomycin for identification and maintaining for further experiments (Plate 5).

These strains were further multiplied in plates containing complete culture medium (CM) consisting of yeast extract, glucose, minerals, agar and distilled water (Alves et al. 1998).
Conidial viability was verified under the optical microscope, by means of the percentage of germinated and non-germinated conidia after 24 hours of plating on PDA medium containing antibiotics.

The potentiality of biocontrol agents against the red spider mite has not been fully explored till date. The main objective of the present study is to evaluate the potentiality of local isolates collected from dead insect hosts prevailing in different geographic areas to control red spider mite.

3.5: Experimental plot of tea

The field site was located in the Rani Organic Tea Estate (Plate 2) Kamrup, Assam and soil is a fine sandy loam. The area was planted in 1972 with TV1 clone at a spacing of 120cm x 90cm and was shaded by *Albizia chinensis*. The rectangular 0.2 ha field site was designed to allow for efficient maintenance and the detection of fungal pests control on red spider mites.

Questing adult of red spider mites were collected from Rani Tea Estate, Guwahati, Assam. These red spider mites were then treated with different fungal spore suspensions at concentrations ranging from $1 \times 10^3$ to $1 \times 10^8$ spores per milliliter.

Transfer of the fungus by mechanical means was minimized by using a field test design and field test protocol that included the buffer zone and tools and footwear disinfestations.
The plot was treated with organic manure. Both mechanical and manual weeding was done when necessary. Standard plucking was done at weekly interval to monitor the yield and quality of tea.

3.6: Application of fungal biocontrol agents

In each trial, ten numbers of red spider adult mites were placed on detached tender tea leaves (leaf culture) and sprayed with 2.8 ml (approx) of spore suspension using a hand sprayer. The spore suspensions were prepared by adding 15 ml of sterilized distilled water. The biosurfactant Tween 20 (0.005%) was also added for proper dispersion of the conidia. The spore counts were made in a Neubauer chamber and the suspensions were standardized at $10^5$ to $10^8$ conidia / ml. The same volume of sterile water with Tween 20 (0.005%) (When biosurfactant was used for conidial dispersion) was sprayed on control leaf. The tea leaves were then placed on petridishes containing two layers of filter paper circles, moistened with sterile distilled water and left to dry for twenty minutes in a laminar flow chamber.

Adult mites aged 0-24 hours after emergence from the stock colony were transferred to the leaf with brush; five males and five females per leaf were placed on the upper surface of the leaf. The petridishes contained 30ml sterile distilled water, which served as a physical barrier for the mites and also to maintain the leaf turgidity, the water level was maintained daily. Trials at each concentration, including the control, were repeated five times.

Immediately after treatment, these red spider mites were incubated for 1 week in a humid chamber at 90% RH and 26±1°C. It was reported by various workers that
the high relative humidity is required for optimal survival and growth of fungal inoculum (Majchrowicz et al. 1990, Puzari and Hazarika 1992). Red spider mites were examined daily and mortality was recorded.

Evaluation of relative pathogenicity of the fungal isolates by classical bioassays is beset with problems. In view of the difficulty to rear the pest on artificial diet and foster mating stages, the present study has been attempt to overcome by smooth shifting of the pest from leaf to leaf so as to cause least disturbance to their natural habitat at the same time facilitating collection of mortality data after treatment with various isolates.

3.7: Microscopical observations of infected red spider mite and in vitro culture of fungal biocontrol agents

3.7.1 Incubation: Leaf samples were incubated (12 h day light) in moist petriplate over filter paper (Moslim et al. 1999) to encourage visible growth of entomogenous and other associated fungus on egg, nymph and adults of red spider mites.

3.7.2 Observation: Leaves bearing red spider mites were examined under simple microscope (X20) after 72 h of incubation. The population of healthy and fungus infected red spider mites (nymphs and adults) were counted and recorded. Population of red spider mites were in various stages of parasitization due to fungal growth later examined under high power x600 light microscope and confirmed to be the targeted strains.

It is usually possible to identify a fungal pathogen directly from the insect by mounting in lactophenol or lactic acid with some aniline blue.
Immature specimens without sporulation should be placed again in moist chambers for further growth. Old or over mature specimens, on which no conidial structures can be recognized, must be reisolated in pure culture. For this Potato-Dextrose agars with antibiotics were used. Some times, Sabarauds Dextrose Agar (Samson 1974) was used for species that grows or sporulate poorly on PDA media.

Mass culture of various potent fungal isolates was done on sterilized rice husk (Plate- 6).

When strains are used for infection or physiological experiments, the cultures were freeze-dried since regular subculturing may reduces physiological and biochemical properties. Cultivation of pathogenic fungi on artificial media is important for detailed study of the development of the fungi.

3.7.3: Methodology in details

The fungus was isolated as per McCoy and Kanavel (1969) and pure culture was obtained through single spore isolation method in five basal media namely Potato Dextrose Agar, Czapek Dox Agar, Rose Bengal Agar, Special Peptone Agar and Sabarauds Dextrose Agar medium. Out of which Potato Dextrose Agar medium amended with 1% malt extract was mainly used.

Infected mites may be stuck inside a petridish lies with soft agar. If the fungus has not yet emerged from the mite the body, should be surface sterilized in 0.5% sodium hypochlorite solution for 10 seconds and washed in sterile distilled water. The surface sterilized mites are then placed on the medium where the fungus can grow.
Pure stock culture of *Beauveria bassiana*, *Metarhizium anisopliae*, *Penicillium purpurogenum*, *Trichoderma harzianum*, *Verticillium lecanii*, *Paecilomyces lilacinus* were maintained in the laboratory in PDA medium. The pathogens were cultured in more than 30 petridishes to obtain sufficient quantity of the inoculas.

Three weeks old cultures were used in all experiments by which time, the fungus had sporulated abundantly. Fully sporulated fungal mat was taken out, filtered and used at the required spore concentration. Spore concentration was determined using a double ruled Neubauer Haemocytometer.

Adult red spider mites were collected from the field. They were surface sterilized with 0.5% sodium hypochlorite solution for 10 seconds and rinsed with sterile distilled water before treatment. Excess moisture was removed by blotting them on filter paper.

3.8: Selection of the most pathogenic isolates

Experiments were designed for the fungus in a completely randomized design, consisting of the isolates with 5 replicates and one control for each fungus. Leaves were replaced on the fifth day after treating with various fungal isolates and again maintained for another three days. The dead mites were placed in a wet chamber to confirm the causal agents and to determine confirmed mortality. The daily mortality values were accumulated during the experiments for further statistical analysis.
Plate 5. Various fungal strains isolated from dead red spider mite collected from different tea estates.

(a) *Verticillium lecanii* isolates  
(c) *Trichoderma harzanum* isolates  
(e) *Beauveria bassiana* isolates  
(g) *Penicillium purpurogenum*

(b) *Verticillium lecanii* isolates  
(d) *Beauveria bassiana* isolates  
(f) *Metarhizium anisopliae* isolates  
(h) *Paecilomyces lilacinus* isolates

Plate 6. Mass culture of *Penicillium purpurogenum* on rice husk (for field application)
3.8.1: Leaf bioassay technique

Two common methods to test the efficacy of fungal isolates against insect pests are direct application of fungal spores on to the insect or allowing insects to feed on leaves treated with fungal spores. In the present study five adult mites were released in each fresh detached leaves. Then keep it ideal for 15 to 30 minute for adjustment. The mite contained tea leaves were then treated with various concentration of the spore suspension on the dorsal side, prepared from 7-day old cultures of different fungal strains. The biosurfactant Tween 20 (0.005%) also used for the preparation of spore suspension. The treated mite containing leaves were kept on moist filter paper in Petridishes (90 x 15mm) and incubated at 26±1°C (Ali and Varma 1994). During the course of the experiment, the numbers and time of final mortality were observed and recorded. Concentration - mortality data were subjected to probit analysis (Finney 1977).

3.8.2: Artificial inoculation

Koch’s postulates were satisfied by inoculating tea leaves containing active population of red spider mites with various doses of (1 x 10^5 - 1 x 10^8 aqueous mycelial and conidial suspension of different fungal entomopathogens from pure culture inside moist petridishes. The fungus was re-isolated from infected mites for conformity test.

The fungal suspensions at the concentration of 10^5, 10^6, 10^7 and 10^8 spores per ml were sprayed on the eggs, nymphs and adults which were transferred earlier to the detached tea leaf. These were maintained at 26 ± 1°C and 70-80% R.H in petridishes
provided with wet filter paper at the bottom to absorb excess moisture. Each treatment was replicated five times. A suitable control was also maintained.

The culture of red spider mite was maintained on clone TV1 in the laboratory as this clone being susceptible to red spider mite. Mortality of mites after every 24 hours was counted. Data were transformed into angular values and were subjected to statistical analysis using randomized block design. Observations were made daily and mortality due to fungus and other causes were recorded. The mortality percentage due to fungal infection and the time taken for death of red spider mite was also calculated.

The percentage of hatching was recorded soon after hatching of the eggs in the control treatment and counted up to the 9th day after oviposition. Those eggs which did not hatch after this period were regarded as non viable. For the assessment of ovicidal properties of the fungal spores, ten gravid females were released on a leaf of TV1 for 4 hours to oviposit, which were then removed. After 24 hours the eggs laid were sprayed with $10^5$, $10^6$, $10^7$ and $10^8$ of each fungal spores. In all cases distilled water was sprayed as control.
3.9: Media composition

1. Czapek-Dox Agar (amended)

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Quantity (g/l)</th>
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<tbody>
<tr>
<td>NaNO₃</td>
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</tr>
<tr>
<td>K₂HPO₄·7H₂O</td>
<td>1.00</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
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</tr>
<tr>
<td>KCl</td>
<td>0.50</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
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</tr>
<tr>
<td>Sucrose</td>
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</tr>
<tr>
<td>Yeast extract</td>
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</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1lit.</td>
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</tbody>
</table>

(One gram of yeast extract per Czapek’s Sucrose nitrate agar contains only 2g NaNO₃ per lit).

2. Rose- Bengal Agar (Martin 1950)

<table>
<thead>
<tr>
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<tr>
<td>Glucose</td>
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<tr>
<td>Peptone</td>
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<tr>
<td>KH₂PO₄</td>
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</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.50</td>
</tr>
<tr>
<td>Streptomycin</td>
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</tr>
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<td>Rose Bengal</td>
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<td>Agar</td>
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<td>Distilled water</td>
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3. Potato Dextrose Agar

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<tr>
<td>Potatoes, Peeled and diced</td>
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<td>D-Glucose</td>
<td>20g</td>
</tr>
<tr>
<td>Agar</td>
<td>15g</td>
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4. Special Peptone Agar

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<td>Special peptone</td>
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<tr>
<td>Dextrose</td>
<td>20.00</td>
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<tr>
<td>Agar</td>
<td>20.00</td>
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<td>Distilled water</td>
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5. Sabarauds Dextrose Agar

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<td>Sabarauds dextrose</td>
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</tr>
<tr>
<td>Agar</td>
<td>10.00</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1lit.</td>
</tr>
</tbody>
</table>
3.10: \( \text{LC}_{50} \) and \( \text{LT}_{50} \)

The \( \text{LC}_{50} \) (concentration required to kill 50% of red spider mite) and \( \text{LT}_{50} \) (time required to kill 50% of red spider mite) were evaluated after 4 weeks using the inhibition concentration approach (Lewis et al. 1984) and the daily mortality values accumulated.

3.11: Statistical analysis

The recorded data was subjected to statistical analysis. The experimental design was completely randomized. Five observations were taken for each of the characters and the data on all above studies were subjected to Fisher’s method of analysis of variance (Steel and Torrie 1960). In the field study, the proportion of red spider mite that died from various fungal treatment varied from plot to plot. These proportions, or mortality rates, did not follow a normal distribution. Therefore a nonparametric test had carried out to evaluate the differences between mortality rates observed in treatment versus control one.

Data was analyzed using one way Analysis of Variance (ANOVA) and subjected to Duncan’s Multiple Range Test (DMRT) (Duncan 1955).
3.11.1: Analysis of Variance

<table>
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<th>Mean Square MSS=SS/df</th>
<th>Calculated F</th>
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<tbody>
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<td>Treatment</td>
<td>(p-1)</td>
<td>Treatment SS</td>
<td>Treatment MSS</td>
<td>Treatment MSS/ Error MSS</td>
</tr>
<tr>
<td>Period/ Growth factor</td>
<td>(q-1)</td>
<td>Period SS/ Growth factor SS</td>
<td>Period MSS</td>
<td>Period MSS/ Error MSS</td>
</tr>
<tr>
<td>Error</td>
<td>(p-1)(q-1)</td>
<td>Error SS</td>
<td>Error MSS</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>pq-1</td>
<td>Total SS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.11.2: Critical Difference (CD)

The analysis of variance tables gives only a broad indication of performance of the fungal pathogens, duration of treatments on mortality of red spider mite, concentration of fungal spore and the influence of various physical parameters on radial growth and virulancy of the entomopathogenic fungi. But in order to get clear appraisal of the specific phenomenon, calculation of critical difference was considered necessary.
Critical difference (CD) was calculated using the formula -

\[ \text{CD (0.05)} = \sqrt{\frac{\text{Error MSS} \times 2}{n}} \times \text{'}t' \text{ value at 5\% or 1\% level of error degrees of freedom} \]

Where, \( n = \frac{\text{Total units}}{\text{Individual units}} \)

= actual number used in calibrating the means

3.11.3: Test of significance

The method of calculating the probability of obtaining an observed result from some hypothesis and regarding the hypothesis to be rejected or not, is known as test of significance. If the calculated value is greater than some pre selected value, the observed result is said to be statistically significant at a chosen value of probability. If the calculated value is more than the tabulated value at 1\% probability, then it is said to be highly significant and if the calculated value is lower than the tabular value at 1\% but higher than tabular value at 5\% probability it is said to be significant. In biological experiments generally probability of 0.05 is also referred to as 5\% level of significance. From the calculations, the result is compared with the standard values for different probability distribution from statistical tables.
3.12: Probit analysis

An alternative to logistic regression analysis is probit analysis. The term "probit" was coined in the 1930's by Chester Bliss and stands for probability unit. These two analyses, logit and probit, are very similar to one another. Logit analysis is based on log odds while probit uses the cumulative normal probability distribution.

The probit model is defined as

\[ \Pr(y=1|x) = \Phi(xb) \]

Where \( \Phi \) is the standard cumulative normal probability distribution and \( xb \) is called the probit score or index.

Since \( xb \) has a normal distribution, interpreting probit coefficients requires thinking in the Z (normal quantile) metric. The interpretation of a probit coefficient, \( b \), is that a one-unit increase in the predictor leads to increasing the probit score by \( b \) standard deviations. Learning to think and communicate in the Z metric takes practice and can be confusing to others. We will make use of a number of tools developed by Long and Freese to aid in the interpretation of the results.

The log-likelihood function for probit is

\[ \ln L = \sum w_j \ln \Phi(x_j b) + \sum w_j \ln \left( 1 - \Phi(x_j b) \right) \]

Where \( w_j \) denotes optional weights.
Probit

\[ Y' = \Phi^{-1}(p) \]

Where \( Y' \) is the probit transformed value (5 used to be added to avoid negative values in hand calculation), \( p \) is the proportion (\( p = \) responders/total number) and inverse \( F(p) \) is the 100*p% quantile from the standard normal distribution.

Logit

\[ \text{Odds} = \frac{p}{1-p} \]

\[ p = \text{proportional response, i.e. r out of n responded so } p = \frac{r}{n} \]

\[ \text{Logit} = \log \text{odds} = \log \left( \frac{p}{1-p} \right) \]