CHAPTER- 2

REVIEW OF LITERATURES
CHAPTER- 2

REVIEW OF LITERATURES

The severity of damage and the loss occurred due to the attack by red spider mite has drawn the attention of a large number of scientists throughout the world. Meanwhile a good number of works have been conducted on the biology, morphology, ecology and chemical control but practically no significant work has been done on the control of this pest by non-chemical method especially through microbial pathogens. Here, an attempt has been made to cite some of those findings that are considered to be important in the light of present investigations.

The literatures pertaining to the present studies on Oligonychus coffeae Nietner, infecting tea Camellia sinensis are very limited. Therefore, the relevant works on different mites, botanicals and some entomopathogenic fungal bioagents on some pests have been reviewed.

2.1: Red spider mite (Oligonychus coffeae Nietner)

The red spider mite O. coffeae (Nietner) has been known as a pest of tea (Camellia sinensis) from the very early days of tea cultivation in North-East India. It was discovered in Assam in 1868. Watt and Mann (1903) gave a comprehensive account of the early history of the pest in N.E. India. Wood-Mason (1884) described it as a new species, Tetranychus bioculatus. Green (1890) however, identified it as the same species that occurred on coffee and described under the name Acarus coffeae Nietner. Baker and Pritchard (1953) referred it to the genus Paratetranychus.
2.2: Distribution and alternative food plants

The red spider mite was first recorded on coffee (Nietner 1861) and later on tea in Ceylon (Green 1890). It is a widely distributed pest of tea in North-East India and occurs in all tea districts under diverse climatic conditions. It occurs also as a minor pest of tea in southern India (Rau 1941). It attacks jute, cotton, castor, mulberry, orange, indigo etc. as alternative hosts in India (Das 1959b). Andrews (1928) found it on *Tephrosia candida* and *Derris robusta*, as well as on many jungle plants in tea growing areas of N.E. India. Jeppson *et al.* (1975) considered the tea red spider mite (*Oligonychus coffeae*) as a serious pest of tea and recorded from Avocado in northern New South Wales.

This species has also been recorded from Transvaal, South Africa, Brisbane, Australia and Florida (Pritchard and Baker 1955). In South Africa a red spider mite was found in tea which was described as a new species under the name *Oligonychus merwei* and was found to be identical with *Oligonychus coffeae* by Pritchard and Baker (1955).

2.3: Biological Parameters

Van de Vrie *et al.* (1972) reported that the life cycle of red spider mite is a typical epimorphosis. The stages are being egg, larva, protonymph, deutonymph and adult. Each of three immature stages is followed by a quiescent stage; nymphochrysalis, deutochrysalis and teleiochrysalis, respectively. There is a marked difference between males and females in the rate of development. The early maturing males locate and remain near the female teleiochrysalis until the female emerges.
Copulation takes place almost immediately after emerging of the young female. Unfertilized eggs produce only males, whereas fertilized ones produce only females. But a mated female produces both sexes, because every egg may not receive a spermatozoan.

2.3.1: Egg

The eggs are spherical, flattened on the lower surface where it is attached to the leaf surface and on an average eggs are 0.11 mm in diameter (Das 1959b). A filamentous process arises from the upper pole and then bands in the form of a hook. The colours of the eggs are blood red to chrome red, but before hatching it changes to light orange.

Prior to hatching, the chorion splits along the side of attachment to the leaf surface and the young larvae come out through the slit. The egg shell remains more or less intact unless mutilated by the mites or otherwise damaged.

The duration of egg stage was reported 4 to 5 days during the month of May to September, but it may be as long as 13 days during the cold weather (Das 1954). Incubation period is dependent upon the temperature at which eggs are reared. The mean incubation period of eggs varied from 3.9 days at 32°C to 10.8 days at 20°C (Das and Das 1967, Gogoi 2000).

2.3.2: Larva

Das (1959b) reported that the larvae are 0.16 mm in length and 0.12 mm in breadth, spherical, yellowish orange in colour at hatching, which become elongated
later on and subsequently change to pale orange. A greenish tinge with dark specks develops dorsolaterally and develops three pairs of legs. The larval period was also reported to be varied at the rearing temperature as it was found 1.3 days, 1.2 days and 1.0 days at a constant temperature of 20°C, 25°C and 30°C respectively (Das and Das 1967, Saikia 1999). They also reported that the larval period was followed by the first quiescent stage which was 1.4 days, 0.8 days and 0.7 days at a constant temperature of 20°C, 25°C and 30°C respectively.

2.3.3: Protonymph

The protonymphs were reported to be 0.21 mm long and 0.13 mm broad, carries four pairs of legs instead of three pairs present in the larvae (Das 1959b, Gogoi 2000). The form of the body was somewhat oval. The legs and the anterior part of the body were pale crimson and abdomen deep reddish brown.

The protonymphal period was 1.3 days, 1.1 days, and 0.8 days at a constant temperature of 20°C, 25°C and 30°C, respectively (Das and Das 1967). The active phase of protonymph is followed by the second quiescent stage, which is more or less similar with first quiescent stage. They reported the second quiescent stage as 1.3 days, 0.9 days and 0.8 days a constant temperature of 20°C, 25°C and 30°C respectively.

2.3.4: Deutonymph

The deutonymph stage is similar to protonymph except for being slightly bigger in size. Towards the end of this stage, the sexes are distinguishable (Das 1959b). The female is slightly bigger and has the abdomen rounded at the posterior
end while the abdomen of the male is narrow. He reported that the average length of deutonymph male was 0.23 mm and average breadth 0.26 mm while deutonymph female was 0.26 mm long and 0.15 mm broad.

The deutonymph period was 1.8 days, 1.1 days and 0.9 days at a constant temperature of 20°C, 25°C and 30°C, respectively (Das and Das 1967). They reported that this period is followed by a short quiescent stage, which is 1.6 days, 0.9 days and 0.7 days at a constant temperature of 20°C, 25°C and 30°C respectively.

2.3.5: Adult

According to Das (1959b) the adult females are 0.32-0.44 mm in length and 0.15-0.23 mm in breadth. Body segmentation is distinct with four transverse lines on dorsum, posterior three lines being distinct only along with their margins. The female differs from the male in size and also in the form of the body. The female is nearly elliptical, being the abdomen broadly rounded at its posterior end. The legs and front portion of the body (propodosoma) are bright crimson in colour, while the abdomen is dark purplish brown.

The length and breadth of the adult males are 0.27-0.31 mm and 0.14-0.16 mm respectively (Das 1954). The male is smaller and has a slimmer body, the abdomen being much narrower and almost tapering to a point. The legs particularly the first pair is longer than those of the female. It has the same body colour as the female except that the tip of the abdomen is crimson.

The female usually dies soon after the last egg is laid, but occasionally they may live a day or two more. Das (1959b) found that the maximum life of a female
was 29 days with an average of 24.4 days during the month of April. The males usually die within four or five days under field conditions. If however, they remain unmated they may live longer and the maximum length of life of one of a batch of unmated male was found to be 12 days in the month of April (Das 1959b).

The total life cycle of this mite is shorter in summer months and may be completed in 9.4-12.0 days in May-June, while it may take as much as 28 days in the cold weather (Das 1959b). It was reported that the male emerges 2-6 hours earlier in summer, but in the winter its life cycle may be shorter by a day or two than that of the female.

2.3.6: Mating

According to Das (1959b) both males and females of *O. coffeae* are sexually mature on emergence. The males emerge earlier than the females and are found wondering in search of female deutonymphs. Quite often, several males congregate around a single female deutonymph and there is a tussle after the emergence of the female to impregnate it. A male could mate with several females but a female usually mate with a single male only.

2.3.7: Pre-oviposition period

According to Das (1959b) the female usually starts egg laying within 24 hours of emergence from March to June, but the pre-oviposition periods may be prolonged in the cold weather and may last for 2 or 3 days. Das and Das (1967) also recorded the pre-oviposition period as 2.3 days, 1.2 days and 0.9 day at a constant temperature of 20°C, 25°C and 30°C, respectively.
2.3.8: Oviposition period

The oviposition period of *O. coffeae* lasted for 18.2-18.6 days during January-April and 20.9 days in May and 9.6-10.2 days during July-August (Das 1959b, Gogoi 2000). It was also reported that the oviposition period of this mite lasted for 30.6 days, 17.8 days and 11.4 days at a constant temperature of 20°C, 25°C and 30°C respectively (Das and Das 1967).

2.3.9: Post-oviposition period

The post-oviposition period of *O. coffeae* was 2.0, 1.6 and 1.5 days at a constant temperature of 20°C, 25°C and 30°C respectively (Das and Das 1967).

2.3.10: Fecundity

According to Das (1959b) the maximum number of eggs laid by a female during her lifetime was 137, the average being 91 during May. He reported seasonal variation in egg laying capacity of the mite. Das and Das (1967) also reported the average fecundity as 107.3, 99.7 and 59.3 days at a constant temperature of 20°C, 25°C and 30°C respectively. Mukherjee (1977) also recorded the fecundity as 127 eggs at 32°C and 107 eggs at 20°C. Saikia (1999) reported that the fecundity of fertilized female was maximum (77.60-68.00) eggs/female) during March-April and was 60.61 eggs per female during May-June with further increase in temperature and humidity, Saha *et al.* (1999) also reported higher fecundity both in the case of fertilized (75.50 eggs) and unfertilized female (64.17 eggs) at 33.20°C with 79.85% RH and lowest fecundity both in the case of fertilized (68.50 eggs) and unfertilized female (58.50 eggs) at 26°C with 71.60% RH.
2.3.11: Sex ratio

Boudreaux (1963) reported that the sex ratio in the offspring, produced by the females depends upon the amount of spermatozoa released during mating and this feature further depends upon the length of mating time. However, Overmeer (1972) stated that a ratio of 1 male to approximately 3 females was often found in *Tetranychid* mites and it may be considered as normal. Pillai and Jolly (1986) found no specific proportion of the sex ratio, but recorded irregular combination of two sexes. They further stated that unfertilized eggs gave rise to male only, while fertilized ones gave rise to female Das (1959b) reported the sex ratio of *O. coffeae* as 1:2.83 during March, 1:1.34 during April and 1:1.09 in the month of June.

2.4: Nature of damage to tea plants

According to Das (1959b) the damage to tea plant is caused by the larval, nymphal and adult mites, which feed on the sap from the upper surface of the leaves and occasionally on the petioles. As a result of feeding a yellowish spot is first formed on the surface at the point of feeding, which subsequently turns brown. Repeated feeding in the vicinity increases the number of spots, which coalesce and form bigger spots or patches and in course of time the injury extends to the whole of the surface. The damaged leaves later show a coppery brown discoloration, ultimately turning bronze and may dry up and eventually fall off.

Harrison (1938) suggested that old leaves in which cells are not turgid and the flow of the sap is slow are preferred by the red spider mite, while young leaves in a
turgid condition with sap flowing freely are less attacked. He reported that it causes 43 per cent reduction in yield of tea.

2.5: Preference of oviposition

Finney (1953) observed the association of leaf depression with mite population and found that weak webbers like *Panonychus ulmi* and *P. citri* tend to colonize along the midribs and veins under harsh situation which provide protection. Das (1959b) reported that mites prefer to lay eggs usually on depressions, mid-rib, veins and deflected tip and the attack is also confined along these sites.

Van de Vrie *et al.* (1972) studied the leaf texture and contours (ridges, depressions) on the population build up of *Tetranychids*. He found that these features are important for reproductive potential of *Tetranychids* that provides substrates for feeding and attachment of webbing or as direct protection from disturbing or harmful micro weather on the leaf surface. They reported that for strong webbers like *Tetranychus urticae, Tetranychus pacificus* and *Eotetranychus sexmaculatus*, the development is mostly restricted to the depressions and rough surfaces where suitable anchorage for webbing is necessary.

2.6: Bio-physical basis of resistance

Plant-insect relationship is an important phenomenon in nature, where some plants can tolerate and recover, whereas others suffer and perish from the infestation of a particular insect pest. This may be due to one or more characteristics in one variety or to entirely different characteristics in other varieties of the same crop.
In crop plants, three principal mechanisms, viz., non-preference, antibiosis and tolerance are responsible for imparting resistance to insect pests (Painter 1951). Literature reveals a number of cases where responses of the plant varieties to the insect attack differed and that the plant types exhibited varying degrees of resistance or susceptibility to insects and mites, due to presence of morphological and biochemical characters. Various physical characters like leaf depression, thickness, leaf size etc. were found to influence the incidence of mite pests.

Leaf structure has been shown to be associated with damage caused by the mite. Kuenen (1949) reported less damage on pear and plum varieties from *Panonychus ulmi* that had a thin cuticle. He reported that when the thicker upper cuticle is softening by higher temperature, mites can pierce the leaf more easily.

Blair (1951) observed the association between damage and number of layers of mesophyll in apple varieties. Das (1959b) reported that mites preferred to lay eggs usually on depressions and deflected tip and attack was also confined along these sites. It has been reported that the development of red spider mite is mostly restricted to the depressions and rough surfaces of leaf (Van de Vrie et al. 1972).

According to Banerjee (1975) the age specific survivorship and fecundity are higher on the China variety than on the Assam variety. He reported that the morphological and biochemical nature of leaves of the two varieties influence the growth rate of *O. coffeae*. 
The different morphological characters like glands, hairs, leaf shapes and thickness of epidermis, some of which seems to be effective against spider mite population on cotton (Bailey et al. 1978, Schuster and Kent 1978).

2.7: Bio-chemical basis of resistance

Various biochemical components were found to impart resistance against mite pests. Generally phenols, tannin and amino acids are found to either act as stimulants for a certain species or as an inhibitor to others.

Fernando (1967) correlated the abundance of *O. coffeae* to the rhodoxanthin content of leaves of tea emphasizing its property as a phagostimulant or reproductive stimulant. Likewise, Kaneko (1980) found that the population of spider mite, *Tetranychus kanzawai* increased with increase in the level of L-arginine in tea leaves. Kaneko (1981) also reported that L-arginine increased fecundity of *T. kanzawai* while catechin gallates present in young leaves acted as feeding inhibitor.

Perring et al. (1982) evaluated several grain sorghum characteristics for resistance to the Bank grass mite, *O. pretensis* (Banks) and concluded that no resistance was attributable to leaf tannin, leaf bloom and midrib juiciness.

Carmine spider mite *Tetranychus cinnabarinus* resistance in cotton appeared to be positively correlated with speed of leaf growth, quantities of waxy substances and several amino acids (Wang et al. 1991).

Lane and Schuster (1981) reported that resistant variety contains a relatively high content of condensed tannins. Similarly, Lege et al. (1992) reported that
condensed tannin acted as feeding deterrents to bollworm, *Helicoverpa zea* (Boddie), tobacco bud worm, *Heliothis virescens* (F) and two spotted spider mite *T. urticae* in cotton, *Gossypium hirsutum*. Wilson (1994) working on effect of plant quality on life history parameters of two spotted spider mite on cotton mentioned that mite strongly preferred to feed an oviposit on younger leaf tissue compared with older leaf tissue. He also reported that tannin content increased progressively with plant development and was higher in older leaves than in younger leaves.

Xu *et al.* (1996) revealed that the leaves of tea varieties resistant to pink mite, *Acaphylla theae* had denser pubescence, stronger cuticularization on the lower surface and lower stomatal density, high content of total amino acid, threonine amino acid and caffeine and lower content of reducing sugar and water soluble sugar. Glutamate dehydrogenase and phenylalanine ammonia lyase (PAL) activity were stronger in the resistant varieties. PAL was also observed to be positively correlated in resistance with strawberry (Inoue *et al.* 1985). Leaf morphology inhibits mite feeding, whereas biochemical parameters contribute to antibiosis.

On the other hand, Chen *et al.* (1996) attributed causes of resistance to *A. theae* to morphological as well as biochemical characteristics of the leaf like pubescence with lower stomatal density, having high amino acid content and caffeine but lower reducing sugar content. Likewise, Saikia (1999) reported that morphological characters like leaf depressions, leaf thickness serrations contributed resistance to tea against *O. coffeae*.

Earlier it was reported (Banerjee 1975) that the age specific survivorship and fecundity were higher on the China variety than on Assam variety. He reported that
the morphological and biochemical nature of the leaves of the two varieties influenced the growth rate of *O. coffeae*.

Nangia *et al.* (1999) working on biochemical changes in different varieties of mulberry infested by *Eutetraminonychus suginamentis* revealed that feeding by the mite resulted in reduction in transpiration rate, total chlorophyll, proteins, total sugars and amino acids content of leaf. Similar results were also reported by Wu Kongming (1989) in case of cotton for the spider mite. He found that the content of amino acid and soluble protein in cotton leaf were reduced as the damage of spider mite became heavier. The content of most of the 17 amino acids especially the glutamic acid, histidine and tyrosine were decreased, while the content of cysteine was increased. Ahmed (1994) reported that resistant varieties of cucumber against *T. urticae* contained lower amount of total protein and amino acid as compared to the susceptible one.

Borah *et al.* (1978) working on biochemical changes in tea leaves after infection with red rust, *Cephaleuros parasiticus* reported that variegated leaf tissues contain more amino acids, and soluble sugar and lower content of polyphenols, chlorophyll, and dry matter.

2.8: Parasite and Predators

The review across the world reveals that at least 120 species of major pest insects and 27 species of weeds have been controlled by introducing parasites / predators from different regions, including control of cottony cushion scale by Australian lady beetle, the coconut moth in Fiji by the techinid fly from Java, the
coffee mealy bug in Kenya by the Enmyrtid parasite from India and prickly pear weed in Australia (Speight et al. 1993). The nymhal and adult parasite of sugarcane *Pyrilla, Epircania melanoleuca* has been very successfully established in the states of Karnataka, Rajasthan, West Bengal, Maharastra and Gujarat (Powar 1986). The parasite has adaptability to a wide range of ecological conditions.

2.9: Microbial control of pests

The first attempts to use bacteria for microbial control occurred in the early 20th century. Felix d’Herelle isolated a bacterium from diseased locusts which he considered to be the casual agent of the epizootic and subsequently named by Steinhaus (1949) as ‘*Cocoobacillus acridorum*’. The findings of *Bacillus thuringiensis* were effective against the corn borer led to further studies against other insect pests (Metalnikov and Chorine 1929). However, the full potential of *Bacillus thuringiensis* was not developed until Steinhaus (1951) brought this bacterium to the attention of insect pathologists.

The development of the first insect pathogen as a microbial control agent had its beginnings in 1916 when the Japanese beetle was accidentally introduced into a nursery in New Jersey from Japan (Fleming 1968). By 1926, studies on diseases of the beetle were initiated and bacteria, fungi, nematodes and protozoa were isolated from the larvae. The nematode *Neoaplectana glaseri (Steinernema glaseri)* was isolated (Glaser and Fox 1930) and applied with some success in controlling the Japanese beetle larvae (Glaser and Farrell 1935).
In 1948, *B. popilliae* was the first insect pathogen to be approved by the United States Federal Government for use against an insect pest. The following year, *B. lentimorbus* was approved by the federal government. Both bacteria are still being produced commercially in Japanese beetle larvae. A significant landmark in the history of insect pathology and microbial control was achieved in 1945 and made numerous contributions to the fundamental and applied aspects of insect pathology (Knipling 1979, Tanada 1985).

Microbial control programs are actively pursued in many countries from Far East to Europe and from South America to Australia and Africa. Pathogens not registered in the United States are registered in some of the other countries. In England, the fungus *Verticillium lecanii* is registered for use against aphids and whiteflies and another fungus *Trichoderma viridae* has been registered and used as antagonist against *Stereum purpureum*, a fungal pathogen of wounded plum trees. In Japan a cytoplasmic polyhedrosis virus is used to control the pine caterpillar, in Brazil *Metarhizium anisopliae* is used against spittlebugs, and in Russia *Beauveria bassiana* is used against the Colorado potato beetle (Burges 1981 and Kurstak 1982).

2.9.1: Viruses

A large variety of entomopathogenic viruses representing at least 13 virus families have been reported from insects in addition to numerous as yet unclassified viruses (Hunter-Fujita et al. 1998). Two of the most commonly observed groups are Cypovirus (CPV: Reoviridae) and the baculoviruses (Baculoviridae). Most research and development of viruses as microbial control agents has been devoted to this latter group and over 600 insect species, including Lepidoptera, Hymenoptera, Coleoptera,
and Dioptera have been reportedly infected by baculoviruses. With a few exceptions, most baculoviruses are quite specific, infecting a single species of insect or a few species within the same genus (Cory 2003).

Viruses in the family Baculoviridae are very common in some of the most important insect pests. Approximately 60 per cent of the 1200 known insect viruses belong to this family and it has been reported that such viruses could be used against nearly 30 per cent of all the major pests of food and fibre crops (Payne 1989). As many as 35 viruses have been reported from different insects (Pawar and Thombre 1991).

However, NPVs (Nuclear polyhedrosis virus) are the only viruses that have shown promise as microbial insecticide. The results of the field trials on chickpea with the Heliothis NPV at Gujarat (Mistry et al. 1984), at Tamil Nadu (Jayaraj 1988) and Maharashtra (Pawar et al. 1990) have been very promising. NPV application was reported to be effective in cotton (Dhandapani et al. 1987, Anon 1993), Sunflower (Rabindra et al. 1985), Pigeonpea (Pawar and Thombre 1991) and tomato (Mistry et al. 1984). Similar field application of NPV of Spodoptera litura has been successful in crops like cotton, tobacco, and cauliflower (Jayaraj 1988, Ramakrishanan et al. 1981 and Chaudhari and Ramakrishnan 1980).

Spectacular results were also reported for the control of Oryctes rhinoceros, a noxious pest of coconut by introducing the Oryctes baculovirus. It has been used for the pest control in South-East Asia and the Pacific (Dhaliwal and Arora 2003). There are several baculoviruses that are currently under development by a number of industry and other institutions across the world. These include NPVs, Autographa
California (Speyer), *Choristoneura fumiferana* (Clemens), *Euproctis chrysorrhæa* (Linnaeus), *Galleria mellonella* (Linnaeus), *Gilpinia hercyniae* (Hartig), *Spodoptera frugiperda* (JE Smith) and *Trichothesia ni* (Hubner) and GV (granulosis virus) of *C. fumiferana*, *Pieris brassicae* (Linnaeus) and *Plodia interpunctella* (Hubner) (Lisansky 1989).

In India, the efficacy of baculoviruses against agricultural insect pest has been extensively studied by various workers (Dhaliwal and Arora 2003). The studies conducted at the International Crop Research institute for Semi Arid Tropics (ICRISAT) have revealed that spraying of chickpea with suspension of Coimbatore strain of NPV resulted upto 60 per cent mortality of *Helicovera armigera* (Hubner). Similarly, the effectiveness of NPV against *H. armigera* has been reported on pigeonpea, lablab and redgram. Field trials with NPV of *Spodoptera litura* on cauliflower, banana, tabacco, black gram and cotton have also given encouraging results (Battu et al. 1993).

Kodomari (1993) while working on biological control of tea insect pests in Japan, reported that tortricids are attacked by granulosis virus (GV) and nuclear polyhedrosis virus (NPV). Such viruses may also occur in tea pests of North-East India.

2.9.2: Bacteria

Non-spore forming bacteria are important mortality factors for a number of insects. The bacterium, *Serratia entomophila*, a pathogen of the New Zealand grass grub, *Costelytra zealandica* (White) which has shown promise for development as a
biological control agent (Maddox 1994). The spore forming bacteria also reported to be promising organisms for microbial control. Although more than 100 bacterial species have been identified as insect pathogens, only certain *Bacillus* species have gain the commercial status (Dhaliwal and Arora 2003).

*Bacillus thuringiensis* (*Bt*) is a Gram positive, spore-forming bacterium that can produce an array of insecticidal toxins and is the leading microbial control agent worldwide (Charles *et al.* 2000, Glare and O'Callaghan 2000). The utilization of *B. thuringiensis* δ-endotoxin gene constructs in transgenic crops in recent years (Shelton *et al.* 2002) has significantly changed the use patterns of this microbial insecticide in some of the major crop systems. Borthakur (1984) established the possibility of utilizing *Bacillus thuringiensis* var. *thuringiensis* (HB III), an isolate from *Hilothis obsoleta*, for the management of Looper caterpillar, *Buzura suppressaria* (Mazumdar *et al.* 1955).

2.9.3: Fungal bio-control agents

Naturally occurring entomopathogenic fungi are one of the important components of integrated pest management which regulate pest population in nature (Gillespie and Claydon 1989, Carruthers and Hural 1990). In Integrated Pest Management (IPM), biological control with entomopathogens should be considered as an important reduction factor in pest population density. So, the conservation of naturally occurring entomopathogens is needed and which can be applied or introduced with the objective of controlling pests. However, the use of incompatible pesticides may inhibit the development and reproduction of these pathogens affecting the IPM strategy (Malo 1993, Duarte *et al.* 1992, Anderson and Roberts 1983). More
than 1000 species of fungi are reported from insect's body all over the world and a few are registered for commercial utilization as biocontrol agent. Entomopathogenic fungi occur on tea pests (Baruah 1983) and are responsible for natural mortality of insects and mites. Many species of fungi are reported on tea red spider mites in North East India (Debnath 2002, 2004 a, b).

During the late 19th and early 20th centuries, there was a proliferation of attempts to use fungi against insect pests in many countries. Attempts were made with various fungal species against such insects as the nun month, grasshoppers, cockchafers, whiteflies, scales and chinch bugs in different parts of the world.

It has been reported that over 750 fungal species (100 genera) are known to attack terrestrial and aquatic arthropods (Tanada and Kaya 1993). The most commonly encountered fungi pathogenic to insects belong to three subdivisions of the Eumycotina. The majority are found in the Deuteromycotina, others in the Mastigomycotina and Ascomycotina. The important genera are Coelomomycyces, Entomophthora, Massospora, Cordyceps, Podonectria, Torrubiella Aschersonia, Aspergillus, Beauveria, Fusarium, Hirsutella, Metarhizium, Nomuraea, Paecilomyces etc. (Maddox 1994). Insect-pathogenic fungi usually need moisture to enable infection and natural epizootics are most common during wet or humid conditions. The effectiveness of these fungi against pest insects depends on the efficient fungal species and strain with the susceptible insect life stage at the appropriate humidity and soil texture (Weinzierl and Henn 1989).

Agustino Basii (1935) for the first time reported a fungal disease of silk worm caused by Beauveria bassiana and put forward the concepts of controlling insects by
infectious fungus. The first record of a *Neozygites* species infecting spider mites was made by Fisher (1951) who observed adult mortality ranging from 32-95% in population of the citrus red mite, *Panonychus citri*. Weiser and Muma (1966) reported *Neozygites floridana* as a pathogen of the Texas citrus mite, *Eotetranychus banksii*.

Narasimhan (1970) made pioneering studies of entomogenous fungi in India. Agarwal (1990) studied entomogenous fungi and made a comprehensive review on species of insect pathogenic fungi reported from India. The potentiality of naturally occurring entomopathogenic fungi for the control of scale insect pests of tea in North East India was reported by Debnath (1990). Borthakur (1984) reported the current status and biocontrol prospects of tea pest of North East India. Kodomari (1993) reviewed the microbial pest control in Japan with particular emphasis on *Bacillus thuringiensis*. Hazarika et al. (1994) reviewed the current status and future prospects of microbial control of tea pests in North East India.

Debnath (1996) reported *Paecilomyces lilacinus* and *Acrimonium strictum* on tea pest, jelly grub pest (*Cromettia apicata*) and lymentrid caterpillar (*Lymentria albunata*) respectively. Debnath (1997) also studied the pathogenicity of *Paecilomyces temipes* and reported to be effective for the control of flush worm larvae of tea. Poinar and Poinar (1998) also review on the parasites and pathogens of mites. Omoto and McCoy (1998) studied the toxicity of fungal toxin isolated from *Hirsutella* and evaluated against citrus rust mite (*Phyllocoptruta oleivora*). Tamai et al. (1998) tested 152 different isolates of the fungi *Beauveria bassiana*, *B. brongniarti*, *Beauveria* sp., *Metarhizium* sp., *Paecilomyces lilacinus* and *P. farinosus*.
against *Tetranychus urticae* and reported *Beauveria* sp caused mortality at the range of 35–95%.


Although fungi have great potential for development as microbial control agents, only a few have been used on an operational scale. Modern exploitation of fungi as inundative insecticides began in the 1960's and several products based on *Beauveria bassiana* were used for control of numerous pests in the People's Republic of China (Feng *et al.* 1994) and the Colorado potato beetle in the former USSR (Ferron *et al.* 1991).

The white muscardine fungus, *Beauveria bassiana* has been detected from over 700 species of insects. *B. bassiana* is one of the most ubiquitous and extensively studied entomopathogenic fungi (Feng *et al.* 1994, Hajek and St. Leger 1994, Reithingar *et al.* 1997, Arcas *et al.* 1999). It is an insect-pathogenic fungus found naturally on some plants and in the soil (Mahr 1997). Epizootics are favored by warm and humid weather. The host of *B. bassiana* is mainly Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera (Tanada and Kaya 1993, Adane *et al.* 1996,
Varela and Morales 1996). Moreover, this fungus has the potential to control over 70 insect pests of various crops, and appears to be innocuous to most non-target organisms (Feng et al. 1994). It is reported to be effective against a number of important pests including the whiteflies, aphids, grasshoppers, termites, Mexican bean beetle, boll weevil, cereal leaf beetle, bark beetles, lygus bugs, chinch bug, fire ants, European corn borer, Ostrinia nubilalis (Hubner), the codling moth, Douglas fir tussock moth, Cydia pomonella (Linnaeus), the Japanese beetle, Popillia japonica (Newman), the Colorado potato beetle, Leptinotarsa decemlineata (Say), the Chinch bug, Blissus leucopterus (Say), cabbage caterpillar, Pieris brassicae (Linnaeus), Cotton whitefly, Bemista tabaci (Gennadius), Lygus lineolaris, (Palisot de Beauvois), Sunn pest, Eurygaster integriceps (Bartlett and Lefebre, 1934, Ferron 1981, Hussey and Tinsely 1981, Lewis and Bing 1991, Riba 1984, York 1958, Weinzierl and Henn 1989, Edgington et al. 2007).

Balakrishnan et al. (1994, 1995) reported the effectiveness of Beauveria bassiana on certain coffeae pests in India. Soybean farmers in some areas of Asia have also successfully used biopesticides, deploying Beauveria bassiana to control the soybean pod borer, Leguminivora glycinivorella.

Metarhizium anisopliae is an imperfect, green muscardine, naturally occurring entomopathogenic fungus found in soils throughout the world and infects over 200 insect species, including termites (Kramm et al. 1982, Vey et al. 1992, Tanada and Kaya 1993, Milner et al. 1998). This fungus has received much attention for its potential use in the control of a variety of insect pests (Meyling et al. 2007). It was first recognized as a biocontrol agent in the 1880’s. It has a very broad host range and
is extensively used as a biocontrol agent in Brazil against spittle bug, *Mahanarva posticata* in sugar cane and alfalfa (Wraight and Roberts 1987, Moscardi 1989, Hoffmann and Frodsham 1993). In Australia, *Metarhizium anisopliae* had been commercialized for the control of subterranean pasture scarab, *Adoryphorus couloni*. Goettel (1994) reported the effectiveness of this bioagent and recorded that one time application proved effective against the pasture scarab for over four years. *Metarhizium anisopliae* has recently received registration in the United States for control of various ticks, beetles, flies, gnats, thrips and termites.

It has also been widely investigated for the control of a number of economically important insects by various workers (Kramm *et al.* 1982, Milner *et al.* 1998, Delate *et al.* 1995, Zoberi *et al.* 1995, Krutmuang and Mekchay 2005). It is especially active against chrysomelid, curculionid and Scarabaeid beetles (Zimmermann 1993). *Metarhizium anisopliae* is applied as spores or mycelia in various formulations. Control reported to be achieved through the induction of a fungal epizootic where new spores and vegetative cells produced in infected insects are spread to healthy members of the population. It is a promising agent for the biological control because the conidia are visible in the soil (Zimmerman 1982) they naturally adhere to insect cuticle, and are easily transferred to others (Kramm *et al.* 1982).

The ubiquitous fungal pathogen *Metarhizium anisopliae* kills a wide range of insects (Wang and Leger 2007). The entomogenous fungus *Metarhizium anisopliae* was reported to be more efficacious than chemical insecticides (imidacloprid, fipronil) in killing pupae of the western flower thrips (70–90% versus 20–50%) in a range of
horticultural growing media (Ansaria et al. 2007). *Metarhizium anisopliae* also attacks sugarcane *Pyrilla*, white grubs and was reported to be capable of causing natural epizootic under favourable condition. The spore dust when disseminated through *Pyrilla* adults' causes 98.4% mortality in *Pyrilla* nymphs. The fungus was also used for the control of scarabaeid grubs like rhinoceros beetle of coconut, *Oryctes rhinoceros* and *Hirsutella consanguinea* (Anon 1988). Lingappa (2003) evaluated some entomopathogenic fungi against the aphid and found *Metarhizium anisopliae* to be effective in preliminary studies. Mass production methods of these fungal pathogen have also been developed (Anon 1988). Joshi and Viraktamath (2004) reviewed the pest status and bio control prospect of sugarcane woolly aphid (*Ceratovacuna lanigera*). Moreover, *Metarhizium anisopliae* produces several toxic secondary metabolites whose precise role in pathogenesis and virulence remains unclear (Gillespie and Claydon 1989).

*Metarhizium anisopliae* strain was found to be highly infectious against termites and reported 100% mortality of *Reticulitermes flavipes* workers with a concentration of $3 \times 10^6$ conidia/cm$^3$ (Wang and Powell 2004). *Metarhizium anisopliae* was reported to be efficacious against black vine weevil when used alone or in combination with sublethal doses of imidacloprid or fipronil (Shaha et al. 2007).

Soaking conidiospores of *Metarhizium anisopliae* in distilled water accelerated and synchronized germination was reported (Dillon and Charnley 1985). Soaked conidiospores of *Metarhizium anisopliae* were also recorded to be more virulent towards the tobacco hornworm, *Manducta sexta*, than were non-soaked conidiospores (Hassan and Charnley 1983).
Recent field trials conducted against sugarcane woolly aphid at Arabhavi, Karnataka with oil-in-water emulsion of *Beauveria bassiana* and *Metarhizium anisopliae* caused 19.84 and 42.26% mycosis respectively (Nirmala 2003). Zimmermann (1993) summarized the safety data of *Metarhizium anisopliae* and reported that when the fungus was applied by different methods to birds, fish, mice, rats, guinea pigs or rabbits, no toxicological or pathological symptoms were observed. It has been developed into commercial products for use in several countries. Bidochka *et al.* (1998, 2000, 2001) reported the occurrence of genetic grouping in *Metarhizium anisopliae* and *Beauveria bassiana* which are influenced by habitat type. These kinds of factors were expected to strongly influence the evaluation and ecology of entomopathogenic fungi (Destefano *et al.* 2004).

*Nomuraea rileyi* is another pathogenic fungus which was found to be a majority of noctuids including *Helicovera armigera*, *Spodoptera litura*, *Tetranychus* *sp.* and *Plusia* *sp.* It can be effectively used under conditions of high humidity (RH>70%) and moderate temperature (20-30°C) on economically important crop like cotton, groundnut, soybean and vegetables (Vimala Devi and Prasad 1997). The genetic alterations of microbes using modern technology are found to be beneficial by increase host range, virulence and persistence. (Arora *et al.* 2000).

*Entomophthora muscae* is another entomopathogen which infects flies include the adults of the onion maggot, cabbage maggot, and seedcorn maggot. The fungus multiplies within the body of the adult fly which becomes enlarged, yellowish bands of fungal spores stripe the abdomen (Tanada and Kaya 1993). Related species include *Pandora neoaphidis*, a common, naturally occurring pathogen of aphids that can be
particularly effective during moist periods. *Zoophthora radicans* is another common fungus having a broad host range (Weinzierl and Henn 1989). Joshi and Viraktamath (2004) reviewed the pest status and biocontrol prospects of sugarcane woolly aphids.

*Verticillium lecanii* is a common pathogen of scale insects in tropical and subtropical environment. It is commonly used in Europe against greenhouse whitefly and thrips and aphids (Filotas et al. 2003), especially in greenhouse crops. *Verticillium lecanii* was also tried against soft green scale of coffee, *Coccus viridis* and found effective. The fungus was also reported to be pathogenic to guava scales and chilli aphids. It has been reported that *V. lecanii* also has the ability to control aphids and white flies (Hall 1981).

Two isolates of *V. lecanii* were introduced into the Netherlands in 1981 and 1982. One controls aphids in chrysanthemum, while another control white flies in cucumber and tomato (Milner 1997). In Korea, eight species of fungi which infest insects, including *Pandora neoaphid* and *Zoophthora radicans*, were found on several species of aphid (Yoon et al. 2000). Yoon et al. (1999) had also reported the virulency of *V. lecanii* at different temperatures. They also recorded the mortality of white fly nearly 100% at 25°C, and 80% at 15°C and 35°C on the 7th day of entomopathogenic treatment.

*Penicillium purpurogenum* is another entomopathogens from which beta-glucosidase was reported to be purified (Hidalgo et al. 1992). *Paecilomyces fumosoroseus* and *Verticillium lecanii* are commercially produced and used for control of whiteflies and aphids in greenhouses in Europe and the USA (Copping 2001). Szajer et al. (1988) derived the lytic enzymes from the culture filtrate of
Penicillium purpurogenum grown on disintegrated mycelium of Penicillium paxilli and reported the release of maximum numbers of protoplasts from 36 h mycelium with lytic enzymes at 30° C.

Neozygites floridana has also been successfully tested against spider mites and Nomuraea rileyi has been found active against green cloverworm, cabbage looper, imported cabbageworm, armyworms, corn earworm and tobacco budworm (Anon 1991). Hirsutella thompsonii is another entomopathogenic fungus which has been reported as highly virulent to the citrus rust mite, Phylocoptruta oleivora (Tanada and Kaya 1993). Hirsutella thompsonii also infects mites and was registered for use in the United States (Weinzierl and Henn 1989). Recently, the potential to control Brevipalpus phoenicis (flat mite) populations by spraying with fungus Hirsutella thompsonii Fisher was suggested (Rosas-Acevedo and Sampedro 2000, Rossi 2002).

Mineiro et al. (2004a, b) had reported the efficacy of Lecanicillium muscarium against mites on coffee plants (Coffea arabica L. cv. Mundo Novo). The pathogenicity of this fungus was reported to be effective against three different developmental stages (eggs, nymphs, and adults) of three species of mites Brevipalpus phoenicis, Oligonychus ilicis and Tetranychus urticae (Mineiro et al. 2004 a, b).

Cassava green mite (CGM), Mononychellus tanajoa, a significant pest of cassava in Brazil and West Africa was reported to be significantly controled by fungal pathogen, Neozygites tanajoae (Delalibera et al. 2004a). The fungal pathogen Neozygites tanajoae (Zygomycetes: Entomophthorales) is used in Africa as a
biological control agent against the introduced cassava green mite (Delalibera et al. 2004b).

Filotas et al. (2004) reported the efficacy of a number of fungal isolates (B. bassiana, M. anisopliae and P. fumosoroseus) against the green peach aphid, Myzus persicae, and the melon aphid, Aphis gossypii; the two most common aphid pests of US greenhouse crops. While both species of aphids were equally susceptible to B. bassiana, A. gossypii tended to be more susceptible to M. anisopliae and P. fumosoroseus. However, nymphal stages were generally not highly susceptible to most isolates, with less than 25% mortality of test insects observed for 38 of 48 isolates.

Ramarethinam et al. (2002) reported the efficiency of four entomopathogenic fungi such as Paecilomyces fumosoroseum, Beauveria bassiana, Metarhizium anisopliae and Verticillium lecanii against the hairy caterpillar of tea. Kima et al. (2005) reported that the parasitoid and fungus may be used together for aphid biocontrol as long as fungus applications are timed to allow late-instar development of the parasitoid.

There are numerous biotic and abiotic constraints on the ability of fungi to infect their target hosts. These include: microbial antagonists; host behaviour, physiological condition and age; pathogen vigor and age; sunlight, desiccation, presence of pesticides; and temperature, humidity and inoculum thresholds (Inglis et al. 2001).
2.9.3.1: Pathogenicity of entomopathogenic fungi

Different investigators have established different experimental procedures for testing the pathogenicity of fungi with the host insect. The various pathogenicity techniques include: exposing insect for a stipulated period of time on the fungal culture (Prasertphon 1993, Yendol and Paschni 1965), topical application of conidial suspension (Roberts and Yendol 1971) or by dipping the insects in conidial suspension (Aguda and Rombach 1987). Pathogenicity depends upon the strain of the fungus, composition of nutrient media in which it is cultured and the insect immune system (Huxham 1989, Fargues et al. 1994, Baruah 2003, Phukan 2003). One of the factors limiting the ability of entomopathogenic fungi to control rapidly developing insects due to their frequent molting can be overcome by the use of fungi in combination with low doses of insect growth regulators, which interfere with molting and thus may provide fungal spores with more time to penetrate the host (Filotas et al., 2005 a, b).

Lerche et al. (2004) studied the dissemination of the entomopathogenic fungus *Verticillium lecanii* (Zimmermann) Viégas in a population of *Frankliniella occidentalis* and reported that the population density influenced the dissemination of the fungal spores in the population. They reported that higher the population density results in higher the insetting control effect. Lerche et al. (2004) also studied the influence of temperature on the speed of fungal effect and reported that higher the temperature influence on earlier the control effect started and results in increase of the natural mortality of the pest. It has also been reported by some workers that pest species display differential susceptibility to the entomopathogenic fungi *B. bassiana* and *M. anisopliae*, and the ability to overcome fungistatic compounds present in the
pest may determine the likelihood of successful infection and virulence (Kirkland et al. 2004).

2.9.3.2: Bioassay of entomopathogenic fungi

For clear understanding of the relative potency of the pathogenic species and strains, Burgers and Thompson (1971) emphasized the need for determining the fungal inoculum load and time required to achieve 50% mortality of the test insects. The relative potency is measured in terms of LC$_{50}$ by conducting bioassay tests with different fungal pathogens. Many such studies are available for example, LD$_{50}$ of Beauveria bassiana against Cnaphalocrosis medinalis was determined as 7.4 x 10$^3$ mg l$^{-1}$ (Aguda and Rombach 1986). Liu et al. (2002) while studying the virulence of Beauveria bassiana against Lygus lineolaris (Hemiptera: miridae) reported the LC$_{50}$ value as 5 x 10$^5$ conidia/ml. Gillespie (1986) found that LC$_{50}$ for different isolate of Metarhizium anisopliae Metschinko was 1.80 x 10$^6$ conidia mg l$^{-1}$ after four days of incubation at 25°C.

LC$_{50}$ value for Verticillium lecanii against aphid mortality was 1.4 x 10$^4$ conidia/ml, LT$_{50}$ values for concentrations 10$^4$, 10$^6$, 10$^7$ and 10$^8$ conidla/ml was 10, 10, 9, 8 and 6 days, respectively (Ashouri et al. 2004).

2.9.3.3: Fungal control in North East India with special reference to Tea.

Biological approach for the control of pests as components of integrated pest management in developing countries is recognized as viable alternative to reduce pesticide use. Pathogens which cause epizootics in the natural population and carry maximum regulatory powers have not been fully explored except identifying a few
entomopathogenic fungi from this region (Baruah 1983). There are many fungi which parasitize and kill insects and thus play an important role in insect pest control without any environmental threat.

The first report of association of fungi with insect in India can be traced back to 1854 when Berkeley recorded two entomogenous fungi viz. *Lordyceps falcate* on dead caterpillar in Khasi Hills of Meghalaya. Since then several entomogenous fungi have been recorded from North-East India (Petch 1921, Sarmah 1960, Butler and Bisby 1931) and majority of them are hyphomycetes. *Aspergillus ferrugeneous* had been reported from Barak Valley of Assam (Butler and Bisby 1931) on the pupa of eri silk worm (moth). Two species of *Fusarium* viz. *F. coccidicola* and *F. coccinellum* have been reported on *Aspidotus* (Petch 1921) from Jorhat.

*Beauveria bassiana* has been recorded on a number of beetles (Roy and Puzari 1979, Roy and Deka 1985, Puzari and Hazarika 1992.) Rice hispa, tiger moth and rice caseworm (Hazarika and Puzari 1990, Puzari and Hazarika 1992, Puzari and Hazarika 1994). Besides *B. bassiana*, the other dominant entomogenous hyphomycetes recorded on rice hispa are *Aspergillus flavipes*, *A. flavus*, *A. parasiticus*, *A. sydowi* and *Fusarium heterosporium* (Puzari and Hazarika 1992, Puzari and Hazarika 1994).

The efficiency of entomogenous fungi *Beauveria bassiana* against different stages of rice hispa (*Dicladispa armigera*) had been reported by various workers (Hazarika and Puzari 1995, 1997, 1998 and Puzari and Hazarika 1994). The entomogenous fungi belonging to this group and occurring on insects other than rice hispa in the ecosystem of rice in Assam are *Arthrinium urticae* on steam borer and *A.*
flavipes on short horned grasshopper, B. bassiana on rice caseworm and black paddy bug.

In India considerable work has been carried out on the natural enemies of tea pests such as parasitoids and predators (Loganathan and Muraleedharan 1992, Muraleedharan et al. 1998, Radhakrishnan and Muraleedharan 1992), however studies relating to the efficacy of entomopathogenic fungi against tea pests are only few (Debnath 1998, Ramarethinam et al. 2000, Selvasundaram and Muraleedharan 2000).

Native entomopathogenic fungi are currently being considered all over the world. The success has been claimed in many developed countries by using entomopathogenic bio agents for various pest control projects. Tea ecosystems in North East India harbour a rich diversity of entomopathogenic fungi (*Verticillium lecanii*, *Paecilomyces tenuipes*, *Fusarium coccophilum*, *Acrimonittm* sp., *Paecilomyces lilacinus*, *Metarhizium anisopliae*, *Aschersonia* sp., *Fusarium* etc.) (Debnath et al. 2005). Pesticide application in tea ecosystem poses considerable risk to the survival of entomopathogenic fungi.

The pathogen has long association with insect complex in Tea ecosystem of North East India with rich diversity of microbial pest control agents and in the past native entomopathogenic fungus *Verticillium lecanii*, *Paecilomyces lilacinus*, *Fusarium oxysporum*, *Hirsutelle thompsonii* (Debnath 1998, Debnath 2005, Debnath et al. 2005, Debnath et al. 2001) have been recorded to infest red spider mite.

Biological control for red spider mites also includes a predatory mite, *Phytoseiulus permilis*. This insect reproduces at twice the speed of red spider mite at
18°C (64°F). The *Phytoseiulus* mite eats only red spider mite and each can eat many hundreds of them.

Few workers have reported the control of red spider mite through plant based pesticides, but till date there is no report recorded on red spider mite control through fungal pesticides. Sarmah *et al.* (1999) reported the control of this pest by petroleum ether and acetone extracts of *Polygonum hydropiper*. The roots and leaf extracts of *Linostoma decandrum* Wall were found to be effective against the red spider mite (Bora *et al.* 1998).

The tea plant is subject to attack by at least 150 insect pests and 380 fungal diseases. In North- East India, where 125 pests and 190 fungi have been detected; losses from pests and diseases were recently estimated at 67,000,000 pounds (30,000,000 kilograms) of tea per annum. More than 100 pests and 40 diseases occur in the tea fields of Japan. Sri Lanka, where estates are closing together or contiguous, have recorded many blights and suffered serious losses. Africa has little trouble with blights; the tea mosquito (*Helopeltis theivora*) is the only serious pest.