CHAPTER 2

LITERATURE REVIEW

2.1 Entomopathogenic fungi (EPFs) as biocontrol agents

Parasitoids, predators, pathogens and nematodes have all been used for control of arthropods. Previously, arthropod pathogens and entomopathogenic nematodes have not been used as much as insect parasitoids and predators (Hajek *et al.,* 2007a). Fungal pathogens of invertebrates are increasingly being considered as commercial biological control agents against insects (McCoy *et al*., 1988; Roberts and Hajek, 1992). EPFs possess distinct advantages over other microbial control agents since they are capable of attacking all developmental stages of insects including pupal stages (Ferron, 1978).

They can be exploited for pest management in a number of ways. Fungal entomopathogens have been widely investigated as biological control agents of insect pests in attempts to improve the sustainability of crop protection (Roy *et al*., 2010). EPFs are important naturally occurring pathogens of many insects and arthropods and frequently cause epizootics that can significantly reduce pest populations all over the world.

Biological control strategies include classical, augmentation and conservation approaches. Some classical biological control programs with fungal entomopathogens have been considered successful, causing drastic reductions in pest populations and the pathogen was subsequently released elsewhere. The concern for the development of hyphomycete fungi as suitable BCAs of insect pests leads to the isolation of various insect pathogenic fungi (Dhar and Kaur, 2010). Hajek and Delalibera (2010) examined the use of fungal entomopathogens in classical biological control and concluded that they have been used more frequently than other types of pathogens and provide a sustainable avenue for controlling arthropod pests, especially the increasing numbers of invasive species. Fungal pathogens infect an insect host by penetrating and proliferating inside the haemocoel. Some of the most widely studied and actually commercially available EPF are the species *B. bassiana* (Bals.) Vuill, *Metarhizium anisoplae* (Metsch.) Sorokin, *Paecilomyces fumosoroseus,* and *Verticillium lecanii* (Wize). Isolates of *V. lecanii* have been available commercially for many years in Europe for control of thrips and other glasshouse pests (Van der Schaaf *et al*., 1991; Helyer *et al.,* 1992).

Moreover, in Brazil and China the use of microbial control methods have been successful in the management of several agricultural and forest insects. In both countries, entomopathogenic fungi (EPFs) have been used for pest management since the 1970s. However, production and commercialization of microbial control agents started from 1990s (Li *et al.,* 2010).

2.2 Pathogenic activity of entomopathogenic fungi

Fungi not associated with arthropod hosts in nature can be tested for their pathogenic activity (Hall and Papierok, 1982). The hypocrealean species *B. bassiana* and *M. anisopliae* have broad host ranges in agroecosystems (Meyling, 2008). Some insect-pathogenic fungi have restricted host ranges, while others have a wide host range, with individual isolates being more specific (Maia *et al*., 2001). However, that host range can vary considerably depending on the type of control agent some species of insect pathogenic fungi are able to infect hosts across a range of taxonomic orders (Tanada and Kaya, 1993). Under natural conditions, these pathogens are a frequent and often cause natural mortalities of insect populations. Asexually produced fungal spores or conidia are generally responsible for infection and are dispersed throughout the environment in which the insect hosts are present. Isolates of *Zoophthora radicans* (Pell *et al*., 1993), *M. anisopliae*, *Fusarium* sp., and *B. bassiana* can infect different insect species in screen house or field conditions (Ibrahim and Low, 1993; Vandenberg and Ramos, 1997; Shelton *et al*., 1998; Vandenberg *et al*., 1998).

EPFs, in common with other insect natural enemies, can be employed under three broad biological control strategies, namely classical biological control, augmentation or conservation. Generally, classical biological control is the use of natural enemies against a host which is exotic in an area and has established without its full guild of natural enemies. In general, successful classical biological control programmes provide long-term sustainable and economical control of insect pests (Shah and Pell, 2003). Augmentation usually involves adding *in vitro*-produced mycelia or conidia in aqueous suspensions to a field or glasshouse crop, often in combination with synthetic materials, which are formulation components to enhance persistence and/or infectivity. Biological control through conservation seeks to identify effective indigenous natural enemies and adopt management practices which conserve and promote them in the field. Management practices which favour EPFs may include provision of increased moisture, e.g. by irrigation, reduction in pesticide use and provision of overwintering sites of alternative hosts.

Hajek and Delalibera (2010) examined the use of fungal entomopathogens in classical biological control and concluded that they have been used more frequently than other types of pathogens and provide a sustainable avenue for controlling arthropod pests, especially the increasing numbers of invasive species. Some traditional fungal species are known to cause epizootics resulting in drastic reductions of arthropod pest populations, while other species, such as the microsporidia, often cause sublethal effects throughout the life cycles of their hosts (Hajek and Delalibera, 2010).

Members of the Hyphomycetes are generally associated with insect in their toxin production overwhelming host defense responses (Roberts, 1981; Samson *et al*., 1988). Fungi invade insects by penetrating their cuticle or "skin." They can infect non- feeding stages such as eggs and pupae. The site of invasion is often between the mouthparts, at intersegmental folds or through spiracles, where locally high humidity promotes germination and the cuticle is non- sclerotised and more easily penetrated (Hajek and St. Leger, 1994; Clarkson and Charnley, 1996). Death is caused by tissue destruction and, occasionally, by toxins produced by the fungus (http://www.nysaes. cornell.edu/ent/ biocontrol/pathogens/fungi.html).

EPFs are a rich source of natural bioactive compounds (Lee *et al.*, 2005). Many insect pathogens release metabolites that increase the chance of insect death. A wide range of pathogenicity factors are known and they come from different metabolic pathways (www.bugs.bio.usyd.edu.au). Ali *et al.* (2010) explained that extracellular proteases of EPFs have been implicated as components of the insect infection process. The chitinase production in *Isaria fumosorosea* gave new avenues for the study of the role of this enzyme in virulence against different insect pests

Table 2.1 New and bioactive compounds from Thai invertebrate pathogenic fungi

Fungus	**New compound**	**Activity**	**Reference**
Cordyceps unilateralis	Nephthoquinine	-	Kittakoop *et al., 1999*
Cordyceps pseudomilitaris	Cordyanhydrides A and B	-	Isaka *et al., 2000*
Paecilomyces tenuipes	Cyclodepsipeptides	Anti-mycobacterial and anti-plasmodial	Nilanonta *et al.*, 2000
Cordyceps nipponica	N-hydroxy- N- methoxy- 2-pyridones: Cordypyridones A-D	Anti-malarial	Isaka *et al.*, 2001
Cordyceps pseudomilitaris	Eleven Bioxanthracenes	Evaluated for their anti-malarial activity	Juturapat *et al.*, 2001
Hirsutella kobayasii	Hirsutellide A	Anti-mycobacterial	Vongvanich *et al.*,2003
Verticillium hemipterigenum	Enniatins H and I	Anti-malarial; Anti *T. tuberculosis*; Weakly cytotoxic	Nilanonta *et al.*, 2003
Verticillium hemipterigenum	Bisdethiodi (methylthio)-demethylhyalodendrin1-demethlhyal odendrin tetrasulfide	Inactive Anti-malarial Both cytotoxic	Nilanonta *et al.*, 2003
Hypocrella discoidea *H. tamurai* *Aschersonia samoensis* *A.badia* *A. tamurai*	Various novel compounds(+) rugulsin: skyrin	All are cytotoxic towards Sf (insect cell lines	Watts *et al.*, 2003
Verticillium hemipterigenum	Novel asochlorin glycoside	Cytotoxic	Seephonkai *et al.*,2004

during the infection process (Ali *et al.,* 2010). Furthermore, it was demonstrated that EPFs such as *B. bassiana, B. brongniqrtii, M. anisopliae, Cordyceps militaris,Aspergillus flavus, and Entomophthora muscae* have lipolytic enzymes (Gabriel, 1968; Leopold *et al.,* 1973; Samsinakova and Misikova, 1973; Paris and Segretain, 1975). In Thailand, some 2,200 isolates of invertebrate pathogenic fungi have been made and screened for their bioactivity in a series of assays (Jones, 2004). The selected of the new compounds characterized from member of the *Hypocreales* from Thailand is listed in Table 2.1. *Cordyceps* species are medical fungi well known for their pharmacological actions such as immunomodulatory (Koh *et al*., 2002; Yu *et al*., 2003), anti-inflammatory (Yu *et al.,* 2004a, b), antitumor (Nakamura *et al.,* 1999), antifungal (Kneifel *et al.,* 1977) and antibacterial (Ahn *et al.,* 2000) activities, and contain biologically active components such as nucleosides (cordycepin; 3′-deoxyadenosine, and adenosine), polysaccharides and ergosterol (Li *et al*., 2006).

2.3 Production of entomopathogenic fungi and their cytotoxic effect

A considerable level of interest and involvement in mycoinsecticide has been done in the crop protection (Charnley and Collins, 2007). Microbial insecticides offer effective alternatives for the control of many insect pests. Their greatest strength is their safety, as they are essentially nontoxic and nonpathogenic to animals and humans (Weinzierl *et al*., 2009). Products are based on a restricted number of species, primarily *M. anisopliae, B. bassiana, B. brongniartii, P. fumosoroseus, Lecanicillium longisporum, L. muscarium.* As an example, *M. anisopliae* is registered in the U.S. for control of household cockroaches. *Beauveria bassiana* strain GHA (trade names Mycotrol GH-OF and Mycotrol GH-ES) is registered to control grasshoppers, locusts, and Mormon crickets on rangeland, improved pastures, alfalfa, corn cotton potatoes, rapeseed, safflower, small grain crops, soybeans, sugarbeets, and sunflowers. *Paecilomyces fumosoroseus* Apopka strain 97 has been approved for use on ornamentals, non-food crops in greenhouses, and interiorscapes to manage whiteflies, aphids, thrips, and spider mites and is sold by Thermo Trilogy Corp. In Thailand, Asia AppliedChem make Metazan (*M. anisopliae*) and Buverin (*B. bassiana*) (Charnley and Collins, 2007).

Sosa-Gomez and Moscardi (1994) reported that the use of microbial control is a potentially valuable alternative in comparison with chemical insecticides because of its benefits concerning human safety, selectivity towards target organisms, and environmental preservation. However, existing research suggests that there are minimal effects of EPFs on non-targets, and they offer a safer alternative for use in IPM than chemical insecticides (Goettel and Hajek, 2000; Pell *et al*., 2001). The microorganisms selected for use as microbial control agents do not naturally infect vertebrates, and so are considered safe to humans, livestock and vertebrate wildlife. They produce little or no toxic residue, and development and registration costs are significantly lower than those of synthetic chemical pesticides (Hajek, 2004). Vey *et al*. (2001) proved that consumer concerns regarding mycotoxins entering the food chain has prompted closer scrutiny of the secondary metabolites of all fungal biocontrol agents (BCAs). Zimmerman (1993) summarized existing safety data for warm-blooded animals, reported that no toxicological or pathological symptoms were observed when spores of the fungus were applied by different methods to birds, mice, rats, guinea pigs, or rabbits. Because their residues present no hazard to humans or other animals, microbial insecticides can be applied even when a crop is almost ready for harvest. In some cases, the pathogenic microorganisms can become established in a pest population or its habitat and provide control during subsequent pest generations or seasons (Wang and Chen, 2009).

However, they require many steps in their development as mycoinsecticides, including isolation, identification, strain selection, mass production and formulation, field trial, registration and commercialization (Zimmerman, 1986). Soper and Ward (1981) stated that strain selection is considered an essential starting point in their successful development. Gelernter and Lomer (2000) concluded that for any microbial control agent to be successful, technical efficacy was essential but had to be combined with at least two other criteria from among the following: practical efficacy (easy and cheap uptake), commercial viability (profitability), sustainability (long term control) and/or public benefit (safety).

Technical efficacy, and to a lesser extent practical efficacy, is essential for success and major advances have been made in the production, formulation and application of hyphomycete fungi as mycoinsecticides (Shah and Pell, 2003). Survival strategies for prolonged periods in the absence of hosts are very important for long-term establishment of classical biological control agents. Production processes for fungal biopesticides must be low-cost and high yield concentration of viable, virulent, and persistent spores (Jackson, 1997). Methods for commercial production of conidia are usually done on solid substrates that consist of cereal grains, rice or other starch-based substrate (Goettel and Roberts, 1992; Calderon *et al*., 1995; Burtet *et al*., 1997; Soccol *et al*., 1997, and Dalla Santa *et al*., 2005). Solid state fermentation is advantageous because it is easy to carry out and raw material is cheap (Deshpande, 1999).

There is a high potential for the use of Hyphomycetes such as *Metarhizium* or *Beauveria* for biological control because such fungi can be cost-effectively mass-produced locally, and many strains are already commercially available. The mycelial growth and spore yield on artificial media depends upon the fungal isolates and the components used in the culture media. Although saprophytic fungi are able to utilize a wide range of nutrient sources (Liu and Chen, 2002), but simple and less expensive media are needed to permit their mass-production and commercialization (Shah *et al*., 2005). The comparison of biopesticides with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost (Kaya and Lacey, 2007). However, taking into account environmental benefits including: (1) safety for humans and other non-target organisms, (2) reduction of pesticide residues in the aquatic and terrestrial environments, (3) increased activity of most other natural enemies, and (4) increased biodiversity in aquatic ecosystems, their advantages are numerous (Lacey *et al*., 2001). Besides these four advantages of microbial control agents (Tanada and Kaya, 1993),

1. Specificity to the target organism or to a limited number of host species,
2. Little or no development of resistance by the target organism (however, resistance has developed in some target insects to *Bacillus thuringiensis* (*Bt*), *B. sphaericus* and baculoviruses),
3. No secondary pest outbreak,
4. Compatibility with other biological control agents,
5. Possibility of long-term control,
6. Ease of application,
7. No pre-harvest interval, and

8. Adaptable to genetic modification through biotechnology.

Although the advantages of MCAs are many, some of them are also disadvantages. If more than one pest species occurs in the ecosystem, additional control measures are needed.

1. Specificity only to target organism,

2. Strict timing of application for maximal effect,

3. Long period of lethal infection (*i.e.*, little or no “knock-down” effect),

4. Inactivation by environmental factors (*e.g.*, ultraviolet light, desiccation, temperature extremes, etc.) and therefore, short field persistence,

5. Difficult to produce obligate pathogens, and/or difficult to formulate,

6. Short shelf life,

7. Development of resistance by target organisms, especially to *Bt* and *B. sphaericus*,

8. Uneconomical except for niche markets, and

9. Risks or non-acceptability by consumers associated with genetically-modified organisms.

Formulation plays an important role in delivering the fungal entomopathogen to the target environment. Formulated fungal entomopathogens are typically prepared as technical concentrates, wettable powders or oil dispersions (Faria and Wraight 2007).

2.4 Economic Importance of Fruit Fly (*Bactrocera* spp.)

Tephritidae fruit flies are among the major pests of fruits throughout the world and represent the most economically important group of phytophagous Diptera (White and Elson-Harris, 1994). Fruit flies belonging to the family Tephritidae (Order: Diptera) are considered a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers (Diamantidis, 2008). Fruit flies in the *Bactrocera* group infest more diverse host plants (Allwood *et al*., 1999). Fruit flies are attracted to host plants when fruit is developing. Different fruit fly species have different host ranges. Fruit flies feed and breed around their host plants and lay eggs in the ripening fruit (Drew and Romig, 1997). All countries in Southeast Asia and the Pacific Islands suffer major economic losses from infestations of tropical fruit flies. The pest is seriously undermining fruit and vegetable production and livelihood sustainability at the smallholder farm level especially in the Mekong River Basin countries.

2.4.1 Fruit fly damage symptom

Fruit flies damage fruits by puncturing and laying eggs under the soft skin in both mature and green fruits (Hollingsworth and Allwood, 2000). The eggs hatch and feed inside the fruit causing the fruits to rot (Dhillon *et al*., 2005) resulting in unmarketable fruits. Due to the larva’s three instars the fruits can be totally destroyed (Ye and Liu, 2005). The larvae feed inside the fruits making them unmarketable and unfit for human consumption. As they can easily spread and becoming invasive, fruit flies are rigorously controlled quarantine pests in all tropical and subtropical countries.

2.4.2 Biology (Life Cycle) of fruit fly

Fruit flies breed in fruits but also in other living plant tissues as leaves, buds, stems and flowers. The host ranges of fruit flies can vary from monophagous (e.g. Mediterranean fruit flies) to highly polyphagous (e.g. Melon flies and Oriental fruit flies). Fruit flies go through four development stages; eggs, larvae (three larval instars), pupae and adults. The life cycle from egg to adult takes between 14 and 27 days.

Egg- Curved (banana-shaped), 1 mm long, shining white, milky when ready to hatch.

Larva- Fruit fly eggs hatch into larvae within 12 to 24 hours. Elongated and pointed at head. Length from 1 mm just after hatching to 7 - 8 mm just before pupation. The colour is white or the same color as the fruit pulp. During the larvae stage (third instar) fruit flies can move long distances by jumping (White *et al*. 1992).

Pupa- Fruit flies typically spend eight days between the egg and larval stages and remain inside the pupal stage for approximately six more days. Most fruit flies are producing mature adults within two weeks. Cylindrical, about 4 mm long, dark reddish-brown, resembling a swollen grain of unhusked rice.

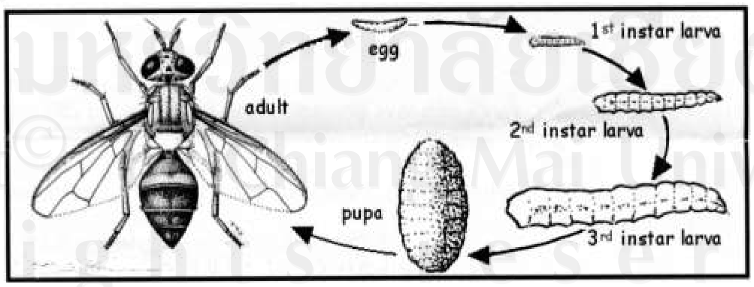

Figure 2.1 Life cycle of fruit fly

http://www.bugsforbugs.com.au/images/large/FruitFlyLifeCycle2.jpg

2.4.3 Management strategies

An ecologically sound and highly effective solution in controlling fruit fly reside in Integrated Pest Management (IPM) that aims to reduce the use of pesticides to address human health and environmental issues, and to limit the development of resistance in the pest populations.

2.4.3.1 Trapping

Trapping is a useful tool that offers a lot of possibilities to the study and control of fruit flies (Allwood, 1997). The attractants and lures can be of either synthetic or natural origin and are used to catch the male insects. The attractants are several; methyl eugenol, cuelure and trimedlure are the most commonly used. To trap females, food baits based on fermented sugar are used. Methyl eugenol was used already in early 1900 (Howlett, 1912) and its effectiveness in attracting e.g. *B. dorsalis* has been well proven. It can attract nearly 100 different species of the genus *Bactrocera* (IAEA, 2003). However, the response to lures is low during periods when adults are sexually inactive as in winter but also age and time of day can be influencing factors.

2.4.3.2 Cultural control

2.4.3.2.1 Sanitation measures

The infested fruits should be removed; in particular, the fruit on the tree that present signs of attack should be removed instead of removing fallen fruits on the ground were the larvae have already left the fruit. In fields where sanitation measures are practice the level of fruit flies decreases significantly (Verghese *et al*., 2004).

2.4.3.2.2 Resistant crops

The production of crop varieties that are less attractive for fruit flies has shown good effects.

2.4.3.2.3 Bagging

A single fruit or a cluster or even a whole tree can be covered by a bag. The bags prevent fruit flies from infesting the fruits.

2.4.3.2.4 Early harvest

Fruit flies prefer to attack fruits and vegetables depending on the stage of maturity. In some crops there is the possibility to harvest fruits early to avoid fruit fly infesting.

2.4.3.3 Chemical control

The application of insecticides is done by spray cover on the entire crop or trees. Insecticides can also be used in a mix with attractants like cuelure and methyl eugenol. Insecticides can even be used together with protein baits. This method is very important in the control of both female and male fruit flies in distinction when using insecticide and attractants who is specified for male fruit flies.

2.4.3.4. Biological control

The microbial control of fruit flies can be a process that can partially replace other methods of control in integrated management programs for these insects, especially the use of agrochemicals, presenting economic and environmental advantages for tropical fruit.

Introduction of parasitoids to infested fields has given good results in management of fruit flies. *Psyttalia fletcheri*, Silvestri (Hymenoptera: Braconidae) is one of the parasitoids that had showed a high parasitism degree in *B. cucurbitae* (Coquillett). *Fopius arisanus* (Sonan) is other promising parasitoid tested in Hawai to control *B. latifrons,* Hendel (Bokonon-Gatan *et al*., 2007). Apart from regular parasitoids even birds that feed on infested fruits in the field are very important for the reduction of fruit fly populations. Sometimes that kind of predation has been more successful than the control of fruit flies by parasitoids.

EPFs offer greater opportunity for biological control of adult fruit flies compared to bacteria and viruses (Sookar *et al*., 2008). However, among the studied EPFs, for the control of fruit flies are *P. fumosoroseus, B. bassiana* and *M. anisopliae* (de Oliveira, 2010). Moreover, Aemprapa (2007) mentioned that *Beauveria* sp. isolate 6241 killed 50% of fruit flies in Thailand. Sookar *et al*., 2008 demonstrated that all locally isolated EPFs were pathogenic to two fruit fly species, *B. zonata* (White & Evenhuis) and *B. cucurbitae* (Coquillett).

2.5 Economic importance of two-spotted spider mite (*Tetranychus urticae* Koch)

Two-spotted spider mite (TSSM), *T. urticae* Koch is considered to be the most polyphagous species of the Tetranychidae. It is a major pest of vegetables and ornamentals in greenhouses and of several other agricultural outdoor crops. This species is adapted to various environmental conditions and is distributed worldwide, causing loss of quality and yield or the death of the plants by sucking out the contents from the leaf cells (Granham, 1985 and Vrie, 1985).

The mite feeds on the underside of leaves causing speckling, or in severe cases, premature leaf drop that result in yield loss or plant death (Bugeme *et al*., 2009). Large colonies of *T. urticae* Koch produce a very fine webbing around the leaves where they feed and toward the tops of plants where they tend to congregate. Populations of TSSM tend to explode during periods of low humidity and high temperatures (http://www.benemite.com/twospot.html).

2.5.1 Two-spotted spider mite damage symptom

TSSMs will feed on an extremely wide variety of host plants, including many grassy and broadleaf weeds, fruit crops, vegetable crops, and field crops such as soybean and field corn (Olechowski, 1989). Spider mite infestations build up quickly in warm temperatures and can destroy plants when infestations are not detected early enough. Thin-leafed plants are more susceptible to spider mites than plants with thick or waxy leaves (www.planthealth.info/pdf_docs/mites_MN.pdf). TSSMs damage plants by piercing and sucking the contents of cells, which results in speckling on leaves as the cells turn yellow and die. As damage increases, the whole leaf may turn yellow and wither (www.growercentral.com/PDFS). The first sign of infestation is usually a chlorotic, stippled appearance on the leaves, although this may not be as apparent on thick-leaved plants. Mite damage to the open flower causes a browning and withering of the petals that resembles spray burn. Animals, humans and farm equipment can also spread mites from field to field and within fields (Steinkraus *et al*., 2001).

2.5.2 Biology of two-spotted spider mite

In both male and female TSSM, development proceeds through the following stages: egg, larva, protonymph, deutonymph, and adult (Cagle, 1949). The length of time from egg to adult varies greatly depending on temperature. Under optimum conditions (approximately 80˚F), spider mites complete their development in five to twenty days. There are many overlapping generations per year. The adult female lives two to four weeks and is capable of laying several hundred eggs during her life. The life span of the adult female is divided into the preovipositional period and the ovipositional period, the former being the time between emergences from the teliochrysalis to the deposition of the first egg. The preovipositional period can last less than 0.5 day and as long as 3 day depending on temperature. The period during which eggs are deposited (ovipositional period) can last from 10 day at 35˚C to 40 day at 15˚C (Sabelis, 1981) and from 5 day of adulthood.

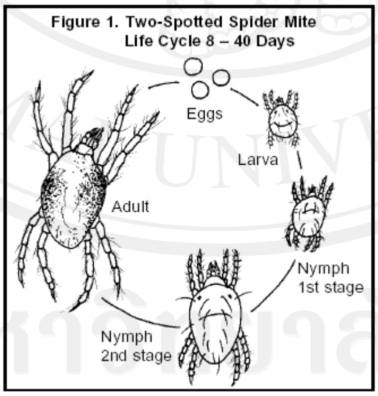

Figure 2.2 Life cycle of two-spotted spider mite (*T. urticae* Koch)

http://www.agf.gov.bc.ca/cropprot/images/mites_fig1.gif

Females generally lay an average of 38 eggs in total, but it is possible for a single female to lay over one hundred eggs during the oviposition period (Smith-

Meyer, 1981). Eggs are deposited on leaf undersides. They are spherical, clear and about 0.14 mm in diameter. Eggs are round and translucent, turn orange and larvae hatch in about 5 d under optimum conditions of 25-30˚C and 45-55% RH. The eggs are attached to fine silk webbing and hatch in approximately three days. The egg becomes opaque as it develops and two red eyespots appear just before hatching. The larva has 3 pairs of legs, while the protonymph and deutonymph have 4 pairs.

The TSSM exhibits arrhenotokous parthenogenesis (Boudreaux, 1963). Fertilized eggs will result in female offspring, whereas unfertilized eggs will produce male offspring.

2.5.3 Management of two spotted spider mite

The management of *T. urticae* Koch has mainly relied on the use of synthetic acaricides (Jeppson *et al*., 1975). The TSSM develops a resistance to most acaricides after prolonged use and environmental pollution. Most miticides are not effective on eggs (http://www.entnemdept.ufl.edu/creatures/orn/twospotted_mite.htm) Therefore, it is necessary to use the alternatives of chemicals to minimize the pollution hazards.

Especially in greenhouses, TSSM can be a difficult pest to control. Once their feeding damage or webbing becomes apparent, populations are already well-established and more difficult to control. On houseplants spider mites often become a problem in November when the heat comes on and the air is drier. These are often coupled with high winds which disperse the mite great distances. Although a heavy rainfall will wash many mites off leaves and drown them, a fungus disease that develops in the humid conditions following a rainfall is more important in the decline of mite populations (Olechowski and Schaafsma, 1989).

2.5.3.1 Cultural control

Cultural practices can have a significant impact on spider mites. Water-stressed trees and plants are less tolerant of spider mite damage. In gardens and on small fruit trees, regular, forceful spraying of plants with water will often reduce spider mite numbers. Adequate watering of plants during dry conditions can limit the importance of drought stress on spider mite outbreaks. Periodic hosing of plants with a forceful jet of water can physically remove and kill many mites, as well as remove the dust that collects on foliage and interferes with mite predators. Disruption of the webbing also may delay egg laying until new webbing is produced. Sometimes, small changes where mite-susceptible plants are located or how they are watered can greatly influence their susceptibility to spider mite damage (Cranshaw and Sclar, 2006).

2.5.3.2 Biological control

Biological control strategies for tetranychid mite pests include various techniques, like use of predators/parasites, mite pathogens, resistant varieties of host plants etc (Price, 1997). Several species of naturally occurring insects and mite preys on spider mite, a lady beetle the spider mite destroyer (*Stethorus* sp.); predaceous thrips, six spotted thrips (*Scolothrips sexmaculatus*) Perg. and minute pirate bugs (*Orious* sp.) are important (Parvin *et al*., 2010).

The predatory mite *Phytoseiulus persimilis* Athias- Henriot has been studied extensively for biological control of tetranychid mites on vegetables and ornamentals in greenhouses (McMurtry, 1982; Hussey and Scopes, 1985; Kostiainen and Hoy, 1996 and McMurtry and Craft, 1997). It is the most common predator and preys on all stages of mites (Osborne *et al*., 1999). It can consume 20 eggs or five adults daily. Both larvae and adult of *Stethorus punctillum* Weise beetles feed on all stages of spider mites. Adults can eat 50 mite eggs per day or 10 adults and can consume 240 spider mites during their developmental period. Thrips of various species are considered some of the most important predators of spider mites and mite eggs. The six spotted thrips *Scolothrips sexmaculatus* Pergande is only the thrips predator of spider mite. An adult *S. sexmaculatus* Pergande consumes 1000- 3000 *T. urticae* Koch (Hoddle, 2004).

The most promising microbial control agents of *T. urticae* Koch are EPFs, which invade their hosts by growing through the cuticle. They are able to infect sap-feeding pests, such as *T. urticae* (Koch), which are unlikely to acquire a pathogen *per os*. The entomopathogenic fungus, *Neozygites floridana* (Weiser and Muma) (Zygomycetes: Entomophthorales) has been reported infecting naturally at least 18 species of tetranychids worldwide (Ribeiro *et al*., 2009). It is an obligate pathogen with a restricted host range, pathogenic only to species of spider mites (Keller, 1997). Mites infected with this fungus only die at late stage of infection as opposed to other EPFs (Deuteromycetes) which produce toxins that cause host death before the fungus completes colonization of the host. At the late stage of *Neozygites* infection, the dead mite is filled with fungal hyphal bodies and becomes mummified (Duarte *et al*., 2009).

2.5.3.3 Chemical controls

Chemical control of spider mites generally involves pesticides that are specifically developed for spider mite control (*miticides* or *acaricides*). Few insecticides are effective for spider mites and many even aggravate problems. Furthermore, spider mites resistant to pesticides frequently develop that make control difficulty. Because most miticides do not affect eggs, a repeat application at an approximately 10-14 days interval is usually needed for control (http://www.ext.colostate.edu/pubs/insect/05507.html).

Table 2.2 Some pesticides that may be useful for managing spider mites in yards and gardens (Cranshaw and Sclar, 2006).

Active Ingredient	Trade Name (s)	Comments
Acephate	Orthene, certain Isotox formulations	Insecticide with some effectiveness against spider mites. Systemic.
Abamectin	Avid	For commercial use only on ornamental plants. Primarily effective against twospotted spider mite; less effective against mites on conifers. Limited systemic movement.
Bifenthrin	Talstar, others	Insecticide with good miticide activity.
Hexythiazox	Hexygon	For commercial use only on ornamental plants. Selective miticide that affects developing stages and eggs only. One application per season label restriction.
horticultural oils	Sunspray, others	Used at the "summer oil" rate (2 percent), oils are perhaps the most effective miticide available for home use.
insecticidal soap	Several	Marginally effective against twospotted spider mite and where webbing prevents penetration. Broadly labeled.
Spiromesifan	Forbid	For commercial use only on ornamental plants. Selective against mites and conserves natural enemies.
Sulfur	Various	Generally sold in dust formulation for control of various fungal diseases and some mites on some ornamental and vegetable crops.