CHAPTER 2

REVIEW OF LITERATURE

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2.1. The Economic Importance of Mealybugs

The mealy bugs are sap-feeding insect pests that inflict losses to their host-plants in several ways (**Osborne *et al*., 1994; Gullan and Kosztarab, 1997; Oetting, 2004; Williams, 2004; Watson and Kubiriba, 2005**). They:

1- Suck sap from the host-plant phloem tissue, removing biomass and water.
2- Egest sugary honeydew that fouls plant surfaces, blocking stomata, soimpeding gas exchange, respiration and photosynthesis, and hence yield.
3- The honeydew forms a medium for the growth of sooty mold, blocking light from the leaves, so impeding photosynthesis.
4- Some mealybug species transmit plant virus diseases while feeding.
5- The feeding punctures facilitate infection by secondary diseases.
6- Mealybugs on live plant material in trade present a quarantine threat that may prevent the export, or cause the rejection of fresh produce.
7- Waxy mealybugs impair the aesthetic value of ornamental plants, presenting a serious threat to interior landscaping and greenhouse crops.

Garcia (2006) described the situation of insects and mites in Spanish citriculture. Among the mites, the most important are *Panonychus citri* and *Tetranychus urticae*. The spider mite *T. urticae* is considered to be the most important pest of clementine. The diaspididae, particularly California red scale (*Aonidiella aurantii*) are the pests which require a major number of spray applications of pesticides. Citrus mealybug (*Planococcus citri*), the woolly whitefly (*Aleurothrixus floccosus*) and the cottony cushion scale (*Icerya purchasi*) are frequently observed but these insects rarely reached the pest injury level because of biological control. *Aphis spiraecola* and *A. gossypii* were found during the spring and the citrus leafminer (*Phyllocnistis citrella*) during the summer and autumn seasons and they damage sprouts and leaves in young plants and developing trees. Lemon flowers are damaged by *Prays citri* and early ripened fruits are damaged by the Mediterranean fruit fly (*Ceratitis capitata*). The importance of planning measures to avoid the introduction of new pests from other countries is pointed out mentioning the pests introduced during the last years or those that can be introduced in the future.

Esfandiari *et al.* (2007) stated that the cottony cushion scale *Icerya purchasi* is a polyphagous cosmopolitan and destructive pest that infests more than 200 plant species apart from citrus. Laboratory studies were carried out on orange at three temperatures (17, 27 and 40 ±1º C), 65±5% RH and 14:10 h

(light: dark). The duration of nymphal stages, female longevity and the life cycle at 17±1ºC were 85.8±3.83, 74.9±3.53 and 173.6±7.63 days; and at 27±1ºC were 62.6±4.40, 72.6±4.59 and 144.1±9.26 days, respectively. The duration of the pupal stage and the life cycle of male at 27±1ºC were 16.1±0.78 and 59.4±1.30 days, respectively. Field studies on the pest were conducted from July 2003 to September 2004 at Sharafabad region of Dezful in Iran. At 10-day intervals, five randomly selected orange trees in 3-ha citrus orchards were sampled by taking 75 twigs (15 cm in length) at random. The number of eggs, nymphal stages and adults were recorded. *I. purchasi* was found to have three generations in a year that were recorded as spring, summer and autumn-winter generations. The autumn-winter generation developed in 6 months. *I. purchasi* overwintered as different developmental stages on different host plants. Apart from different citrus cultivars altogether, 41 species of 22 different families of plants were recorded as the host plants of this scale insect in Khuzestan province, Iran.

2.1.1. The Mealy bug *Icerya seychellarum seychellarum* (Hemiptera: Monophlebidae) as an important insect pests infecting fruit trees in Egypt

Westwood (1855) assumed that the insect had been imported along with the plant, and thus was of Seychelles origin.

Ezzat and Nada (1986) reported that the mealy bugs (Pseudococcidae : Margarodidae : Coccoidea : Hemiptera) comprised some of the worst pests of fruit and shade trees in many parts of the world especially in tropical and sub tropical countries. Mealy bugs include two families Pseudococcidae (pseudo mealy bugs) and Margarodidae (true mealy bugs). In Egypt, these families comprise 43 species included in 29 genera.

Williams and Watson (1988) reported that mealy bugs cause damage for plants by inserting their threadlike mouth parts into any part of the plants and sucking out sap. The insect (mealy bug) can excrete sweet honey dew as a sticky liquid. Sooty mould often grows in the honey dew causing infesting plants to turn back. Some species suck juices from its host plants and inject toxic saliva as it feeds. This process leads to the malformation of leaves and fruits as well as stunted leaves and terminal growth which is commonly called bunchy top. The feeding of mealybugs can also lead directly to the death of its host.

Nada (1990) recorded 3 margarodids and 5 pseudococcids attacking citrus trees. Also, **Nada *et al*. (1990)** recorded 3 margarodids and 4

pseudococcids attacking mango trees. The range of host plants of the margarodid *Icerya seychellarum* (Westwood) includes 44 host plant species.

Dreistadt *et al.* (1994) reported that the mealybug, *Icerya seychellarum* (Westwood) (Hemiptera: Margarodidae) infests different parts (leaves, branches and fruits) of mango trees (*Mangifera indica* L.). The female lays a large number of eggs (600-800 eggs/female) in an ovisac made of wax secreted from wax gland lays on the lower side of *I. seychellarum*. The mealybug is usually found in clusters on branches and leaves; it also feeds on the sap sucked from the host plant tissues. As this sap contains only a very low concentration of protein, the insect sucks a great amount of sap from which it obtains the amount of protein sufficient for its growth and egg development. The high number of insects, attacking leaves, branches and fruits of the tree results in a great loss of sap, thus leading to defoliation, dryness and reduction of the tree vitality. In addition, the mealybugs secrete honeydew, which offers a suitable medium for the growth of fungus

Mangoud (2000) studied the distribution of the margarodid *I. seychellarum* on apple trees where 61.9% of the margarodid were concentrated on old branches, 31.7% on new branches, 4.2% on old leaves and 2.2% on new leaves (an average of two seasons studies).

In recent year, *I. seychellarum* become established and increased in Egypt. **Tawfik and Mohammad (2001)** recorded *I. seychellarum* on *Morus alba* in Giza Governorate and it was found to have two peaks. **El-Serafi *et al*. (2004)** found *I. seychellarum* on guava trees at Mansoura district. **Hassan and Radwan (2008)** detected that, the incandescent population of all stages of *I. sychellarum* on persimmon (*Diospyros kaki*) through August and summer is convenient season for *I. seychellarum* activity. It has four overlapping generations on kaki trees.

Unruh and Gullan (2008) reported that the mealy bug *Icerya seychellarum* is one member of a group including five species belong to this group: *I. crocea, I. formicarum, I. hanoiensis, I. menoni and I. seychellarum.* Until recently, *I. crocea* was considered a synonym of *I. seychellarum*, but the two were separated based on the shape and distribution of the open-centre pores and genetic differences.

Justin Gerlach (2010) recorded that one species of Margarodidae (Coccoidea; Hemiptera; Insecta) from the Seychelles islands: *Icerya seychellarum*. This is a widely introduced species and its original distribution

is not known. The earliest records of the species are reviewed and it is concluded that this is probably a Western Indian Ocean species native to Seychelles, the Mascarenes and Madagascar. A second Margarodidae species is described from the Seychelles island of Silhouette. *Gigantococcus dilleniae* is an endemic species with an obligate association with the endemic tree *Dillenia ferruginea*.

Reda *et al*. (2012) inspected that the mealybug *Icerya seychellarum* on guava trees during the two studied years, from August 2005 to July 2007, as a monthly visit to the three areas (EL-Khanka, Shebeen ELQanater and Benha orchards). at Qaluobiya Governorate**.**

Salman and Bakry (2012) found that there was a relationship between the rate of infestation by *Icerya seychellarum* during three peaks of insect activity in October, May and August and the yield loss of seedy Balady mango trees at Esna district, Luxor Governorate through the two convective seasons of 2010/2011 and 2011/2012. The obtained results revealed that the increase in population density in three peaks of insect population decreased the yield slightly (inverted relation) by 3.6, 6.5 and 4.3 kg/tree and 2.5, 4.1 and 2.3 kg/tree during two successive, respectively and increased the percentage of the yield loss by 1.47, 2.64 and 1.77 % seasons and 1.47, 1.97 and 1.08 % , when the yield data were correlated with the peaks of insect population in October, May and August through the two successive seasons of 2010-2011 and 2011-2012, respectively. The early infestation during May was more effective causing the greatest loss in mango yield during the two seasons. The reduction in mango yield was a summation of many factors including level and time of infestation and the ability of variety to infestation.

Abd-Rabou *et al*. (2012) stated that the mealybug *Icerya seychellarum* infesting apple trees in Egypt during July, 2009**.**

Mesbah *et al*. (2012) showed that the common white mealybug *Icerya seychellarum seychellarum* (West.) (Hemiptera: Monophlebidae) infests *Dodonia viscose* Jacq in Montazah garden during two successive seasons (2005-2006 and 2006-2007). The results showed that the weak significant positive relationship between daily mean temperature, relative humidity and dew point and estimated population density of *I. seychellarum seychellarum* individuals. But on the other hand, this relationship was significantly negative with wind speed.

2.2. Biological control means for controlling mealy bugs

Biological control is a fundamental tactic for pest suppression with an effective integrated pest management (IPM) program. Biological control refers to the use of natural enemies against a pest population to reduce the pest's density and damage to level lower than would occur in their absence **(Lee and Landis, 2001).** Sustainable agriculture will rely increasingly on alternatives to conventional chemical insecticides for pest management that are environmentally friendly and reduce the amount of human contact with pesticides. Microbial control agents *i.e.* insect pathogens can provide effective control conserved the biodiversity and serve as alternatives to chemical insecticides under several conditions. Due to their specificity for insects, the entomopathogens (including viruses, bacteria and fungi) and beneficial nematodes are ideal candidates for incorporation into integrated pest management strategies in orchards where their effects on other natural enemies will be minimal. There is also an excellent potential for combining microbial control agents with other soft technologies such as mating disruption (pheromones via sex traps). Increased use of microbial control will depend on a variety of factors including improvements of the pathogens (virulence, formulation, delivery, etc…) and an increased awareness of their attributes by growers and the general public **(Lawrence and Shapiro, 2003).**

Evans (1974) mentioned that biological control is the use of living natural enemies to control noxious organisms (pests). By that definition, it therefore involves the manipulation of biological system (in one way or another) by man in an attempt to achieve control and should not be confused with natural control which occurs with mans deliberate intervention.

Burges (1981) indicated the recent development in the microbial control of insect pests. He indicated that the entomopathogenic fungi (as microbial agents) have great potential for the control of a variety of insect-pests. Those microbial agents are registered by EPA (Environmental Protection Agency, USA) **(Starnes *et al.*, 1993).**

Ferron (**1985**) reported that the entomopathogenic hyphomycete fungi have great potential as biological control agents against insects and can play a role as one component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance and can be exploited as biological agents for the management of agriculture pests **(Evans, 1999).**

Microbial control has been considered an important tool in integrated pest management (IPM) and is an ecologically favorable strategy compared to conventional chemical control (Barranco‑Florido *et al*., 2002). In this approach, entomopathogenic fungi are employed as biocontrol agents reducing pest populations and, consequently, their damages in different agro‑ecosystems **(Inglis *et al*., 2001).**

Bekheit (2005) reported that the biological control has the advantage of being self perpetuating once established and usually does not harm non- target organisms found in the environment. In addition, it is not polluting or disrupting the environment as chemical pesticides nor does it leave residues on food. However, the use of the biological control does require detailed knowledge of the pest's biology and population dynamics as well as the natural enemies associated with the pest and their impact. Control is usually not complete with this IPM method since a residual population of the pest is often necessary for the natural enemies to remain in the environment, so some non – economic population levels of pests must be acceptable or tolerated.

2.3.1. The Usage of the Entomopathogens as Bio-pesticides

Burgess and Hussey (1971) named eight attributes that are desirable if an entomopathogens is to be suitable for use as a microbial insecticide, namely virulence, predictability of control, ease of application, ease of production, low cost, good storage properties, safe and aesthetically acceptable and able to reduce pest population to sub-economic levels. These are all attributes that are characteristic of the chemical insecticides with which microbial insecticides have to compete.

Work by **Ferron (1977)** and also **Ignoffo *et al.* (1977)** have demonstrated that fungi can infect insects regardless of the environmental humidity and this has led to the supposition that the microclimate at the boundary layer of air on the insect cuticle is the important factor controlling germination. They also found that humidity is also important for sporulation, which completes the cycle of infestation after the death of the host.

Roberts (1981) mentioned that there are many problems associated with the use of fungi as microbial insecticides. From these, the production and use of toxins produced by some fungi is particularly important since these toxins may have insecticidal properties if they can be isolated, identified and synthesized. He also added that the presence of those fungal isolates with a

high toxin production is important for their use in pest control since a highly toxigenic fungal isolate will kill its host more quickly than those isolates without toxin. Few toxins isolated from the entomopathogens, *Beauvaria bassiana* have been investigated and have been identified as depsipeptides such as "Destruxin" and "Beauverin" which appear to disrupt the sites of energy production within the mitochondria and endoplasmic reticulum of the host.

Hall and Papierok (1982) mentioned that the individual species of fungi: such as *Metarhizium anisopliae* have diverse insect hosts including species of Coleoptera, Lepidoptera, Orthoptera, Hemiptera and Diptera. They also reported that a major problem with the use of fungi as microbial insecticides is their requirement for a high humidity for germination and spore valiability. Saturated or near saturated air, or water film, is necessary for spore germination of the vast majority of fungi.

Carruthers and Soper (1987) found that some microbial pathogens infect their host primarily through ingestion and the gut wall, whereas fungi usually infect the insect by penetration of the wall. As a consequence, fungi are able to infect phytophagous insects with sucking mouth-parts which are susceptible to few other infectious diseases. Also, they added that unlike other pathogens, all stages of the life cycle, the eggs, larvae, pupae and imagines are susceptible to mycoses.

Fuxa (1987) cleared that quick use of the fungal pathogens is required if fungi are to be used as microbial pesticides as they usually take at least a week to kill their host or even so to stop its feeding. He also added that when an infection has been established, the fungi can be lost if the insect moults which makes such an approach ineffective for those insects that having a short intermoult period.

Prior *et al.* (1988) stated that the pathogens can be dissolved and so must be uniformly distributed as suspension if the formulation is prepared as a powder. A formulation may contain many additives to enhance the effectiveness of the pathogen. They added that wetting agents are often added to improve the coverage on the sprayed surface. Also, protectants are usually added to microbial formulations to protect the pathogen from the effect of sunlight and UV radiation. The formulations of microbial insecticides have not received the attention, they deserve. It has been found that the use of oil-based fungal pathogen formulation showed much greater promise than water-

based formulations that evaporate very rapidly under dry conditions. The authors cleared that in dose-response studies on a weevil pest, the fungus *B. bassiana* was over 30 times more effective by topical application when it was formulated in vegetative oil.

Dent (1991) mentioned that there are over 500 fungi known to be associated with insect diseases from five classes of fungi, the Deuteromycetes, Zygomycetes, Oomycetes, Chytridiomycetes and Trichomycetes. He said that the largest number of pathogenic fungi are belonging to the class Zygomycetes, but to date the potentially and most useful fungi have come from the Deuteromycetes (imperfect fungi), namely species of *Beauvaria*, *Metarhizium*, *Nomuraea*, *Verticillu*m and *Hirsutella.* He added that the entompathogenic fungi as a whole have a remarkable host range but just within the Deuteromycetes, fungi attack virtually all species of insects and arachnids.

Kadir and Barlow (1992) suggested that the term "mycochemical pesticide" can be used as a convenient means of distinguishing pesticides that are based on micro-organism, derived metabolites from either the biopesticides or the synthetic chemical pesticides which all fall within the general description of agricultural pesticides.

Van Emden (1996) reported that the early work cocentrated on the genus *Beauveria*, especially *B. bassiana* which can be used particularly against cabbage caterpillars. In general, insect control with sprays of fungi spores proved to be unreliable, since the spores require moisture to sporulate and really quite high humidity are required for success. He added that an obvious use of fungi is therefore against soil pests, and the fungus *Metarhizium anisoplae* has shown considerable promise for soil application. The entomopathogenic fungi have also been used in glasshouses, where high humidity are available and can be maintained. He showed that the presence of the required level of relative humidity is a limiting factor in the success of the fungi isolates under fixed conditions.

Olga Malsam *et al*. (2002) evaluated the efficacy of *Metarhizium anisopliae* in combination with sublethal concentrations of oils and potassium-oleate for biological control of whiteflies under controlled conditions. Three commercial products (Biola®, Naturen® and Neudosan®) and five experimental formulations of plant oils were tested. The efficacy of *M. anisopliae* against *Trialeurodes vaporariorum* and *Bemisia tabaci* without additives was about 50%. At 1/20 of their recommended dosages, all the tested compounds significantly increased the efficacy of *M. anisopliae* for the

control of *T. vaporariorum* with the formulated sunflower oil. Biola® giving the highest synergistic effect reaching nearly 100% control. Not only was the level of control increased but also the speed of action was improved resulting in a higher reliability of control. Three of seven additives showed no effects on the viability of conidia on the leaf surface whereas the formulations of the other oils and oleates reduced the longevity of spores. The synergistic effect of Biola® resulted from the more even distribution of *M. anisopliae* conidia on leaves and insects. Other positive effects of oils on the efficacy of *M. anisopliae* were discussed in relation to an extended spectrum of environmental conditions and pests to be controlled.

Marcos and Wraight (2007) reported that a substantial number of mycoinsecticides and mycoacaricides have been developed worldwide since the 1960s. They presented an updated comprehensive list of these products. At least 12 species or subspecies (varieties) of fungi have been employed as active ingredients of mycoinsecticides and mycoacaricides for inundative and inoculative applications although some are no longer in use. Products based on *Beauveria bassiana* (33.9%), *Metarhizium anisopliae* (33.9%), *Isaria fumosorosea* (5.8%) and *B. brongniartii* (4.1%) are the most common among the 171 products described in their list. Approximately 75% of all listed products are currently registered, undergoing registration or commercially available (in some cases without registration), whereas 15% are no longer available. Insects of the orders Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, and Orthoptera comprise most of the targets distributed among at least 48 families. A total of 28 products are claimed to control acarines (mites and ticks) in at least 4 families although only three products (all based on *Hirsutella thompsonii*) were exclusively developed as acaricides. Fungi formulation types have been identified with technical concentrates (fungus-colonized substrates) (26.3%), wettable powders (20.5%) and oil dispersions (15.2%) and those are being most common.

2.3.2. Fungi as Bio-control Agents

Steinhaus (1949) mentioned that entomopathogenous fungi in nature cause a regular and tremendous mortality of many pests in many parts of the world and do in fact constitute an efficient and extremely important natural control factors.

McCoy *et al*. (1988) reported that the use of the Entomophthoraceae as microbial control agents has been hindered by the poor survival of conidia. This group of fungi has been tested for microbial control of aphids with some

degree of success in the former U.S.S.R. and Australia.

McCoy (1990) reported that several species of phytophagous insects are attacked by entomopathogenic fungi. Most of the entomopathogenic fungi are belonging to Deuteromycetes (imperfect gungi) (Family: Moniliacea). About 30 genera have been reported to contain one or more species that infect insects. Imperfect fungi are mycelial fungi that reproduce by means of conidia that are generally produced on free aggregated conidophores on the substrate surface. Since these fungi apparently lack asexual or perfect stage, they are known as imperfect fungi. Mycologists believe that many of these fungi have lost the ability to reproduce sexually. They have developed para sexually reproduction in which nuclear fusion occurs but not meiosis proper. The para-sexual process provides mechanism for genetic exchange among imperfect fungi that produce conidia on more or less loose cottony hyphae are oftentermed hyphomycetes .

Van Emden (1996) used the hyphomycetous fungi (as biocontrol agents) in a bioassay test against the Russian wheat aphid *Diurabhis noxia* (Kurdgumov). Dose–response assay were done using 5-7 concentrations of 2 isolats of *Beauveria bassani* (Palsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith.

Goettel *et al*. (2000) reported that fungal diseases in insects are common and widespread. There are more than 700 species of entomopathogenic fungi currently known.

Raymond and Shams-Pirzadehi (2000) reported that *Aspergillus* spp. cause disease in a broad range of organisms but it is unknown if strains are specialized for particular hosts. Therefore, they evaluated isolates of *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus nidulans* for their ability to infect bean leaves, corn kernels and insects (*Galleria mellonella*) (the wax moth). Strains of *A. flavus* did not affect nonwounded bean leaves, corn kernels or insects at 22°C but they killed insects following hemocoelic challenge and caused symptoms ranging from moderate to severe in corn kernels and bean leaves injured during inoculation. The pectinase P2c implicated in aggressive colonization of cotton bolls is produced by most *A. flavus* isolates but its absence did not prevent colonization of bean leaves. Proteases have been implicated in colonization of animal hosts. All *A. flavus* strains produced very similar patterns of protease isozymes when cultured on horse lung polymers. Quantitative differences in protease levels did not correlate with the ability to colonize insects. In contrast to *A. flavus* strains *A.*

nidulans and *A. fumigatus* could not invade living insect or plant tissues or resist digestion by insect hemocytes. The results indicated that *A. flavus has* parasitic attributes that are lacking in *A. fumigatus* and *A. nidulans* but that individual strain of *A. flavus* are not specialized to particular hosts.

Wraight *et al.* (2000) showed the possibility of using the entomopathogenic fungi as a biological control agent for greenhouse insects. The species *Beauveria bassiana* and *Paecilomyces fumosoroseus* have been reported to be effective against many aleyrodid pests including the whitefly of field and greenhouse crops.

Fumio *et al*. (2001) reported that entomopathogenic fungi with high pathogenicity against the brown-winged green bug adult (*Plautia stali*) were selected from 711 isolates of different entomopathogenic fungi in laboratory culture collections including *Beauveria*, *Metarhizium* and *Paecilomyces* genera. For the first screening, a plate assay method in which stink bugs contacted with conidia on their developing, plates was used and 31 isolates of *Beauveria bassiana* and 20 isolates of *Metarhizium anisopliae* were selected. For the second selection, stink bugs were dipped into a conidial suspension and LC_{50} values were estimated from the data of 7-days bioassay. The results showed that *Metarhizium* isolates were relatively more virulent than *Beauveria* isolates. The minimum LC_{50} value was obtained from *M. anisopliae* isolate FRM515 being $6X10^4$ conidia/ml. When the suspension of $13X10^7$ conidia /ml was used, LT_{50} value of the isolate was 4.9 days. The highest pathogenicity of the isolate FRM515 suggested that the isolate would be a potential candidate as a microbial control agent for the stink bug.

Mitsuaki (2001) investigated the cultural and morphological characters of certain isolates belonging to an entomogenous *Paecilomyces* species commonly found in Japan and compared with the literature. This species has not been recorded in major monographs of this genus but identified as *Paecilomyces cateniannulatus* Liang which has been previously described in 1981 from China. Mycelia on insects and media were pure white and sometimes produce loose synnemata up to 10 mm. Growth rate of the fungus is moderate. The reverse side of colonies on SDY or MEA media is colorless to light yellow. Phialides are flask shaped with a narrow neck (3.0–16.1 and 1.3–3.2 mm) often forming a whorl. Conidia are oval to short cylindrical (2.1–4.6 and 1.3–2.4mm) shapes arranged in basipital chains. Conidial chains are often irregularly curved and sometimes form a loop. Early stage of the conidiogenous structure of this fungus resembles that of *Beauveria* but is distinguished by formation of conidial chains.

Mitsuaki *et al.* (2002) measured the monthly changes in densities of *Beauveria bassiana* in the air and soil of forest stands using selective media to investigate the density dynamics of this fungus. In a windbreak forest of *Pinus thunbergii* along the coast where *B. bassiana* has been introduced for the experimental control of *Monochamus alternatus;* fungal density was higher than in a neighboring untreated forest. Wild *B. bassiana* was also isolated from inland forests of *Quercus serrata* and *Chamaecyparis obtusa*; in particular the density of *B. bassiana* in the soil of the *Q. serrata* forest was extremely high. Utilizing the same selective medium, conidial dispersal from a non-woven fabric fungus carrier was also investigated. The fungal conidia were dispersed by the air; however the density of the fungus in the air at more than 50 m from the source did not differ from the natural density of the fungus. This result was compared with the lethal density of the fungus on mulberry leaves for the silkworm and the risk of infection is thought to be very rare.

Fumio *et al*. (2003) isolated 65 entomopathogenic fungi with pathogenicity from soil samples and tested against the chestnut weevil larvae. *Metarhizium anisopliae* strain HF293 showed the highest pathogenicity. Under laboratory conditions, the LD_{50} value of strain HF293 on the newly emerged chestnut weevil larvae was 9.93103 conidia/larva at the 10^{th} day after treatment and the mortality rate of larvae reached 56% at five weeks post-treatment with a conidial dose of 2.03102/larva. Susceptibility of the larvae that had been stored at 6°C was gradually decreased over the first three months of storage but stabilized by the fourth month. The results of a field study of larvae in flowerpots indicated that the survival rate of larvae decreased with increasing doses of conidia of *M. anisopliae* strain HF293.

Tounou *et al*. (2003) evaluated the efficacy of *Metarhizium anisopliae* strain Ma43 and *Paecilomyces fumosoroseus* strain Pfr12 (both Deuteromycotina: Hyphomycetes) against adults of the jassid (leafhopper) *Empoasca decipiens* (Homoptera: Cicadellidae). Also, the potential side effects on the egg parasitoid *Anagrus atomus* (Hymenoptera: Mymaridae) were investigated in greenhouse cage and laboratory experiments. Treating leafhopper-infested faba bean plants at a dose rate of $1X10^7$ conidia/ ml resulted in up to 97% mortality 7 days after application and a 100% infection rate. Experiments on the residual effects revealed a significant decrease in adult *E. decipiens* mortality with increasing time from application to insect release. The decrease in mortality over time corresponded well with data from conidia germination tests. The germination of conidia on agar medium after

washing them from the surafce of sprayed plants declined significantly from 95 and 96% immediately after application for *M. anisopliae* Ma43 and *P. fumosoroseus* Pfr12, respectively to 29 and 27% five days later. Experiments on potential side effects of the entomopathogenic fungi on *A. atomus* showed that the tested isolates had no influence on adult emergence and longevity; however the rates of parasitism were significantly reduced.

Susumu and Yamaji (2003) reported that termites *Reticulitermes speratus* Kolbe that have been reared individually were highly susceptible to the entomopathogenic fungus *Metarhizium anisopliae.* In contrast, termites reared in groups were highly resistant to *M. anisopliae.* When reared in groups, the termites treated with *M. anisopliae* conidia on the body surface were groomed by their nestmates and more than 90% of the conidia were removed from the cuticle within 3 hrs. However, the conidia affiliated with the termites reared individually did not show a marked reduction. Within 3 hrs, almost all of the termites held in groups contained the conidia in their foreguts but no conidia were detected in the foreguts of the termites reared individually. These data suggest that one of the functions of grooming by nestmates is the removal of foreign bodies such as fungal conidia from the cuticle and the mutual grooming behavior is very effective in protecting them from *M. anisopliae* infection.

Mitsuaki (2004) developed a novel technique to measure the virulence of an entomopathogenic fungus *Beauveria bassiana* by exposing the tarsi (part of the leg) of adults to dry conidia to evaluate the effectiveness of this fungus for controlling adults of the Japanese pine sawyer *Monochamus alternatus*. To regulate inoculum density without suspending conidia in water, conidia were killed by heating at 100°C for 1 h and a step dilution series of conidia was prepared by mixing dead conidia with live conidia at different ratios. The conidial mixtures were attached to tarsi of CO_2- anesthetized adults with a fine hairbrush. The 50% lethal doses determined by this method on 14 days were $5.5X10^6$ conidia/ individual for aged adults and $1.9X10^6$ conidia/individual for young adults and on 30 days they were $2.8X10^5$ conidia/ individual for aged adults and $2.4X10^4$ conidia /individual for young adults. The number of conidia produced on a non-woven fabric strip formulation of the fungus was $3.5X10^8$ conidia/cm^2 and $8.5X10^5$ conidia/ individual were attached to adult beetles when they walked on that treated fabric strip. Based on these results, the validity of a biological control method for *M. alternatus* to prevent vectoring of the pine wilt disease was discussed.

Ali *et al*. (2005) stated that several entomopathogenic fungi produce toxins and they could be used as bioinsecticides in integrated pest management programs. *Paecilomyces fumosoroseus* is currently used for the biological control of the whiteflies *Bemisia tabaci* and *B. argentifolii*. Supernatants from submerged batch culture where the fungus produced abundant dispersed mycelium conidia and blastospores were found to be toxic to the whitefly nymphs. The most abundant metabolite was purified by HPLC and identified by mass spectrometry and NMR as dipicolinic acid. Both the dipicolinic acid produced by the fungus and the chemically synthesized compound had insecticidal activity against third-instar nymphs of the insect. Dipicolinic acid was toxic to the whitefly nymphs in bioassays involving topical applications. In submerged culture, the specific growth rate of *P. fumosoroseus* was 0.054/ h, the specific glucose consumption rate was 0.1195 g /g/h and the specific dipicolinic acid production rate was 0.00012 g /g/h. Dipicolinic acid was detected after 24 hrs when the fungus started growing; and dipicolinic acid production was directly correlated with fungal growth. Nevertheless, the yield was low and the maximal concentration was only 0.041 g /l. The maximal concentrations of conidia and blastospores (per milliliter) were 1.4×10^8 and 7×10^7, respectively.

Makoto and Takafumi (2005) investigated the susceptibilities of five thrips species *Frankliniella intonsa, F. occidentalis, Thrips coloratus, T. hawaiiensis* and *T. tabaci* to three isolates of an entomopathogenic fungus *Beauveria bassiana* (isolates AZA38, GOM03 and KOG02) under laboratory conditions. Among the three fungal isolates the five thrips species were the most susceptible to isolate KOG02 when inoculated with conidial suspensions at a concentration of $1X10^7$ conidia/ ml. Females of *F. intonsa* were more susceptible to the fungi than males, while males of *F. occidentalis* and *T. coloratus* were more susceptible than females. Both males and females of *T. hawaiiensis* were highly susceptible to the isolate KOG02. *T. tabaci* was highly susceptible to isolate KOG02 even by inoculation of the conidial suspension at a concentration of $1X10^6$ conidia/ ml. Although, isolates AZA38 and GOM03 exhibited weaker pathogenicity to the five thrips species than did isolate KOG02, the gross mortality increased significantly with the inoculation of these two isolates as compared with the control.

Tsutomu (2005) tested the electrostatic application of a mycoinsecticide *Paecilomyces fumosoroseus* for controlling the infestation of the silverleaf whitefly *Bemisia argentifolii* attacking tomato plants in greenhouses. Electrostatic application had no impact on fungal viability measured as colony-forming units (cfu) and resulted in good fungal deposition (cfu/cm^2 of

leaf surface) on both the adaxial and abaxial surfaces of leaves. The adaxial/abaxial surface ratio (1.8) was comparatively low suggesting that the electrostatic application of mycoinsecticides is potentially useful for controlling greenhouse pests. However, experiments for controlling the whitefly produced unsatisfactory results with larval mortalities of 48.1% on day 11 after the first electrostatic application of the mycoinsecticide and 23.9 and 8.1% on day 7 and 19 after the second application, respectively. It is likely that the low larval mortalities were caused by environmental conditions not conducive to fungal infection.

Tsutomu and Keitarou (2005) compared the pathogenicity of Japanese strains of the entomopathogenic fungi *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Aschersonia aleyrodis* (used against the nymphs of the silverleaf whitefly *Bemisia argentifolii*) with those strains of the foreign commercial products (formulations) including *P. fumosoroseus*, *B. bassiana* and *Verticillium lecanii*. With a single dose of $6X10^8$ conidia/ml, the highest mortality was observed for the Japanese strain *P. fumosoroseus* PF3110, although it did not cause significant different mortality than the strains from the foreign commercial products did The LC_{50} values of the native strain were determined on various days after inoculation and the LT_{50} values were determined at different doses. The native strain *P. fumosoroseus* PF3110 has potential as a microbial control agent against this whitefly (*Bemisia argentifolii*).

Dai *et al*. (2006) isolated a strain of *Beauveria brongniartii* PBbr-1 from infected larvae of *Heptophylla picea*. This isolated strain attained 100% mortality of *H. picea* adults at a concentration of 1.0×10^7 conidia/ml with a short median lethal time (LT_{50}) of 8.4 days for females and 7.0 days for males. The number of eggs laid by adults and the percentage of eggs hatching for females inoculated with PBbr-1 were lower than those by healthy females. However when PBbr-1 was inoculated directly to the eggs, hatchability was almost as the same as that in non-inoculated eggs. Since infected females appeared to lay eggs just before death, the eggs from females infected with PBbr-1 were thought to be unfertilized. Furthermore, PBbr-1 showed a high virulence to the first larval stage. PBbr-1 not only had a high virulence to the adults but also suppressed subsequent generations. PBbr-1 is thus thought to be a very effective control agent for *H. picea* in combination with *Beauveria amorpha* HpBa-1.

Peter and Tigano (2006) reported that a phylogenetic analysis of the 5.8S rDNA and internal transcribed spacer (ITS1 and ITS2) sequences from

some entomogenous *Paecilomyces* species supports the polyphyly of the genus and showed the existence of cryptic species. The Eurotiales, *Paecilomyces variotii* and *Paecilomyces leycettanus* were found to be related to the teleomorphs *Talaromyces* and *Thermoascus*. In the Hypocreales, three major ITS subgroups were found, one of which included *Paecilomyces viridis*, *Paecilomyces penicillatus*, *Paecilomyces carneus* and isolates identified as *Paecilomyces lilacinus* and *Paecilomyces marquandii*. However, the majority of the *P. lilacinus* and *P. marquandii* isolates formed a distinct and distantly related subgroup while the other major subgroup contained *Paecilomyces farinosus*, *Paecilomyces amoeneroseus*, *Paecilomyces fumosoroseus* and *Paecilomyces tenuipes*.

El-Sinary and Rizk (2007) studied the relationship between the concentrations of the entomopathogenic fungus *B. bassiana* and their efficacy against the fourth larval instar of the greater wax moth; *G. melonella*. There was a positive correlation between the fungal concentration and its lethality for the treated larvae. The larval mortality percentages increased significantly with 108 spores/ ml as it reached 75.87% after 96 hrs from the beginning of the treatment, while it scored 44.83% with 104 spores/ mlG1 after 96 hrs as compared with 3.33% for the untreated control. When three different doses of gamma irradiation were exposed to the fourth larval instar of *G. melonella* (50, 100 and 150 Gy) combined with the fungal pathogenicity effect, the efficiency of *B. bassiana* increased especially when the gamma irradiation dose was increased. No adults were produced with both fungal concentrations and 150 Gy gamma irradiation dose. Males were more tolerant than females in all examined treatments. Histological studies were carried out by transversal sections in the midgut of the treated larvae of *G. melonella*. Obtained micrographs showed great damage in the epithelial layer with increasing the number and size of vacuoles. Fungal spores, hyphal bodies, chlamydospores and blastospores were clearly marked in all treatments. Gamma irradiation combinations with the fungal concentrations increased the damage in the larval midgut.

Eliana *et al*. (2007) developed a method for the extracellular protease production by *Beauveria bassiana* CG432 in liquid medium containing glucose and yeast extract. *B. Bassiana* presented active growth after lag period of 24 hrs produced 80% of the total of the extracellular protease activity in 48 hrs which was at its maximum level on the 5^{th} culture day. The extracellular protease presented optimum activity at 60^{o}C and it was stable during 15 days at 4^{o}C and -18^{o}C, but was not stable if frozen repeatedly.

2.4.1. *Verticillium lecanii* (Zimm.) Viegas associated with Hemipteran insects

Verticillium lecanii (formerly known as *Cephalosporum lecanii*) was first described in 1861 and is a cosmopolitan fungus found on insects. It is a common pathogen of scale insects and mealybugs in tropical and subtropical climates. *V. lecanii* is known as a "white-halo" fungus because of the white mycelial growth on the edges of infected scale insects. The conidia (spores) of *V. lecanii* are slimy and attach to the cuticle of insects. The fungus infects insects by producing hyphae from germinating spores that penetrate the insect's integument; the fungus then destroys the internal contents and the insect dies. The fungus eventually grows out through the cuticle and sporulates on the outside of the body. Infected insects appear as white to yellowish cottony particles. Diseased insects usually appear in 7 days. However, due to environmental conditions, there may be some considerable lag time from infection to death of insects. *V. lecanii* works best at temperatures of 15 to 25°C and a relative humidity of 85 to 90%. The fungus needs high humidity for at least 10 to 12 hours. *V. lecanii* spores are damaged by ultra-violet radiation. In greenhouses, heating pipes may reduce the effectiveness of the fungus, because this creates a microclimate where the air is drier and humidity is lower. the fungal mycelium of *V. lecanii* produces a cyclodepsipeptide toxin called bassianolide, which has been shown to kill silkworm. The fungus produces other insecticidal toxins such as dipicolinic acid. The activity of *V. lecanii* depends on the strain of the fungus. *V. lecanii* strains with small spores infect aphids, whereas fungal strains with large spores infect whiteflies. Higher doses of the fungus result in faster kill. Virulence depends on the density of spores and rate of sporulation, which is dependent on environmental conditions. Fungal virulence varies with the method of conidial production. Less virulent conidia are obtained from fermented media as compared to shaken liquid or solid media. Formulated products from conidial production can last up to 1 year. These products are easy to be wet and diluted for spraying. Also, the fungus can stick to the surface of leaves and host insects. *V. lecanii* has been commercially available in Europe for control of aphids (Vertalec)® and whiteflies (Mycotol)®. *V. lecanii* may be readily incorporated into pest management programs that utilize biological control agents. However, it is not currently registered for use in North America. **(Midwest Biological Control News Online, 2014).**

Easwaramoorthy and Jayaraj (1978) isolated *Cephalosporium lecanii* (*Verticillium iecanii*) from the coffee green bug *Coccus viridis* from India and studied the effectiveness of this pathogen against the mealybug.

Limited studies mentioned the infectivity of mealybug by another species of the entomopathogenic fungi include *Hirsutella cryptosclerotium* and *Metarhizium anisoplae* (Sorok.) Metsch.

Pena *et al.* (1987) noticed that the occurrence of *V. lecanii* (the standpoint of entomologists and invertebrate pathologists) associated with the scale insect *Philephedra tuberculosa* Nakahara and Gill in Florida, USA during July-October of 1983.

Boucias and Pendland (1998) reported that the Deuteromycetes *Verticillium lecanii* (Zimmermann) Vegas was first described on coffee scale insects obtained from Java in the late 1800's. It has been reported to infect several other insects most notably aphid, whiteflies, thrips, grasshoppers as well as nematodes and the fungal phytopathogens such as rusts and powdery mildews. Huge number of isolates of this species have been reported especially those associated with aphids around the world. Also, *Beauveria bassiana* (Balsamo) Vuillemin was one of the first insect pathogen to be described; it was observed about 900 AD in silkworms in Japan and it is the organism from which the germ theory of disease was first postulated.

Hatting *et al*. (1999) found for the first time in South Africa, through a survey of aphid entomopathogenic fungi, the two Hyphomycetes *B. bassiana* and *V. lecanii* obtained from the Russian wheat aphid *Diuraphis noxia* (Kurdjumov).

Steenberg and Humber (1999) reported that *Acremonium* sp. has been isolated from *B. tabaci* and *Thrips tabaci* in rearing cages. A fungi monograph includes *Verticillium* sp. and a range of other related fungi was illustrated.

Abdel-Mallek *et al*. (2003) and Hamam (2003) reported that *Beauveria bassiana*, *B. brongniartii*, *B. alba*, *Verticillium lecanii*, *Metarhizium anisoplae*, *Paecilomyces farinosus*, *Basidiobouls* sp., *Entomophthora* sp., *E. planchoniana*, *Neozygites* f*resenii*, *Nomurae rileyi*, *Zoophthora radicans*, *Conidiobouls thromboides*, *C. coronatus*, *C. obscurus*, *Panadora coronatus and P. neoaphidis* were found and recorded in Egypt.

Bhattacharyya *et al.* (2004) mentioned that recently development in pest control research have proved the urgent need for developing biological control methods with the use of microbial pathogens in the control of several pests that cause serious crop degradations year after year. Among microorganism, the entemopathogenic fungi constitute the largest single group

of insect pathogens. Such insect-killing fungi are very fast micro-organisms to be recognized as disease causing agent in insects. Entomogenous fungi are promising as biocontrol agents for a number of crop pests. Several species belonging to order Lepidoptera, Coleoptera, Homoptera, Hymenoptera and Diptera are susceptible to various fungal infections. Fungal pathogens particularly *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Nomuraea rileyi* were found to be promising for the control of several agricultural pests.

Chandrashekharaiah *et al.* (2013) evaluated the bio-efficacy of *Verticillium lecanii* against whitefly on greengram under field conditions. Field experiments were conducted at three locations (GKVK, Rajankuntte and Arakere) during kharif (August-November, 2011). At location 1 (GKVK), 87.12 % whiteflies population reduction was recorded for imidachloprid 12.5 kg a.i. /ha. This was followed by *V. lecanii* (2.50 kg/ha) used as three foliar applications at 12 days interval (84.28 %), *V. lecanii* (5.00 kg/ha) (78.66 %), *V. lecanii* (3.75 kg/ha) (60.06 %) and *V. lecanii* (2.50 kg/ha) (45.70 %). Highest yield was record in imidachloprid (12.5 kg a.i. /ha) (12.05 q/ha) and *V. lecanii* (2.50 kg/ha) sprayed as three foliar applications at 12 days interval. The results of location II (Rajankuntte) indicated that imidachloprid (12.5 kg a.i. /ha) and *V. lecanii* (2.50 kg/ha –three foliar application at 12 days intervals) after 25 days after spray resulted in the highest reduction of whiteflies populations (90.19 and 87.85 %, respectively) compared to control, the yield obtained out of this two treatments were also more (11.35 and 11.02 %, respectively). The experiment conducted at location III (Arakere) also revealed that imidachloprid (12.5 kg a.i. /ha) and *V. lecanii* (2.50 kg/ha–three foliar application at 12 days intervals) after 25 days after spray were the best treatments. Reduction in whitefly populations resulted in increased yields i.e. 11.75 and 10.98%, respectively.

2.4.2. Mass-production of the entomopathogenic fungi (*Verticillium lecanii*) Zimm. Viegas

The use of fungi in the control of agriculturally harmful pests depends on different factors, including the ability to produce high concentrations of stable propagules at a reasonable cost **(Jaronski, 1986; Latgé *et al*., 1986).** Industrial production systems for some entomopathogenic fungi use a biphasic method in which the fungal inoculant – mycelia or hyphae – is produced in liquid culture, and then is transferred to solid substrates in order to increase conidial production **(Guillon, 1997).** There are several advantages of the biphasic fungal production system. For instance, the liquid culture can act as a

barrier against contaminants that might be present in the original culture stock. In addition, this system promotes increased competitiveness of the fungus, thus reducing the risk of the solid substrate being colonized by contaminating microorganisms. Additionally, this process ensures a uniform colonization of the solid substrate, resulting in homogeneous fungal growth. Finally, the colonization and production of conidia is faster, reducing the incubation time and economizing physical space **(Jenkins *et al*., 1998).** Although there are a large number of studies on the production of entomopathogenic fungi, the majority focus on the direct production on solid media, while few examine a biphasic culture system.

Romback (1989) studied that the development of simple and reliable production system follows the basic multiplication procedures of submerged liquid fermentation for the production of blastospores, which are short lived, and hydrophilic in addition the solid state fermentation **(Rousson *et al*., 1983)** for the production of aerial conidia. However, the most viable mass production technologies include making use of a diphasic strategy in which the fungal inoculum is pro-duced in liquid culture, which is further utilized for inoculating the solid substrate(s) for conidia production **(Burges and Hussey, 1981).**

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Vyas *et al.* (1991) reported that maize and germinated maize were found to be ideal substrates for culturing the fungus, *B. brongniartii* (Sacc.) Petch in the laboratory which yielded $4.8x10^8$ and $7.2x10^8$ conidia/g, respectively. Rice, wheat, greatmillet and germinated bajra were found to be on par with each other which yielded $2.2x10^8$ spores/g.

Vimaladevi (1994) cultured the mycopathogen on crushed sorghum enriched with 1% yeast extract. Sporulation was highest in crushed sorghum ($1.44x10^9$ conidia /g) after 8-9 days at 25º C.

Lopes *et al. (*1995) produced the fungus *N. rileyi* on rice, sorghum and soybean and stored it for three months at 40 ºC. Multiplication of the fungus on barley and a semi synthetic medium wherein maltose was replaced by cereal extract were found promising for cost effective production of mycopathogen.

Kulkarni (1999) studied the suitability of different cereal grains for mass multiplication of *N. rileyi*. Conidial growth increased with increase in duration after inoculation. Sorghum and rice grains were the most productive media with the yield of $13.45x10^8$ and $13.15x10^8$ conidia/g of substrate,

respectively followed by maize and bajra. Virulence of conidia harvested from these food grains varied significantly with respect to their infectivity to *Spodoptera litura* (Fab.). Conidia obtained from sorghum exhibited higher virulence by recording the lowest LC_{50} value followed by rice, maize and bajra.

Gopalkrishnan and Mohan (2000) tested 13 different synthetic media for sporulation of *N. rileyi*. Out of which Sabourauds Maltose Agar supplemented with 1 % yeast extract (SMAY), Carrot Agar yeast (CAY), Corn Meal Agar yeast (CMAY), Nutrient Agar yeast (NAY) and Czepecks dox Agar yeast (ZAY) showed sporulation. According to the authors, enrichment of synthetic media with yeast extract was must for mycelial growth and sporulation. SMAY and CAY were found to be suitable media for culturing and production of *N. rileyi.* Though, the spore yields in both the cases did not differ (0.56 g/100 ml of medium) but cost wise CAY was found cheaper. When compared to natural medium, the semi synthetic media were economically not feasible for large scale mass multiplication. Carrot was found to be the cheap and best suitable medium for the large-scale multiplication of *N. rileyi* ($6x10^{12}$ conidia/kg of substrate and Rs.1.0/g of conidia) followed by rice ($6x10^{12}$ conidia/kg and Rs.2.67/g) and pigeon pea ($1.12x10^{12}$ conidia/kg and Rs.14.39/g). On the contrary, the cost of production on SMAY, CAY, CMAY, NAY and ZAY was Rs. 284, 57.14, 170, 121.24 and 135.4 / g, respectively. Tapioca also supported the growth of the fungus but yields were lower ($0.10x10^{12}$ spores/kg and Rs. 50.00/g of spores). Media containing ragi, maize, paddy, bajra, sorghum, wheat, Bengal gram and jack seeds did not permit mycelial growth and sporulation.

Kulat *et al.* (2002) evaluated eight culture media for growth and sporulation of the fungus, *M. anisopliae*. Sporulation initiated in 5.66 and 7.66 days on Emerson's YPSS and Barners medium, respectively. While Sabourauds dextrose agar + yeast (SDA+Y) medium was found to be the best with highest radial growth (4.07 cm) followed by Emerson's YPSS medium (4.01 cm) at 10 days after inoculation. Highest spore count (9.43x106 spores/ml) of fungal suspension was observed in Barners medium followed by Emerson's YPSS medium (8.29x106) and SDA+Y medium (7.16x106) at 10 days after inoculation.

Leena *et al.* (2003) studied the mass production of the entomopathogenic fungi, *Paecilomyces farinosus* (Holmsk) and *P. lilacinus* (Thom.) Samson using by products of sugar industry and other agro-industrial by products and wastes. The suitability of the medium was assessed based on

specific parameters such as radial growth, biomass and spore production. *P. farinosus* recorded significantly greater diameter of growth circle (5.03 cm) on 4 % molasses medium followed by 6 and 5 % media. However, the mean biomass production (1.72 mg/100 ml) and spore production ($9.8x10^{10}$ spores/100 ml) were significantly higher on 6 % molasses compared to 4 % molasses broth and standard Potato Dextrose Broth (PDB). In case of *P. lilacinus*, 6 per cent molasses medium supported significantly less mycelial growth as compared to PDB medium, whereas, the biomass production (1.77 mg/100 ml) and spore production ($7.02x10^{10}$ spores/100 ml) were significantly higher on 6 % molasses. In spent wash broth at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 %, spore production of *P. lilacinus* was observed only at 10 and 20 % and it was evident that spent wash at higher concentrations (> 20%) was not suitable for multiplication. Among the oil cakes (coconut cake, cotton seed cake, sesamum cake, groundnut cake and neem cake), *P. lilacinus* produced significantly higher number of spores ($63.07x10^{10}$ spores/30g of substrate) on cotton seed cake followed by groundnut and coconut cakes whereas, *P. farinosus* produced a maximum of $18.9x10^{10}$ spores/30 g of substrate on groundnut cake followed by cotton seed and coconut cakes. Sugarcane press mud supported the growth as well as significantly greater spore production of both *P. farinosus* and *P. lilacinus* ($10.48x10^{10}$ and $8.44x10^{10}$ spores, respectively) compared to other agro-industrial by products and wastes tested (bagasse from sugar industry, tapioca rind from sago industry and coconut water from copra industry).

Rachappa (2003) evaluated thirteen different grain media for mass production of *M. anisopliae*. Among them rice was superior grain media with conidial yield of 8.66 g/100g of substrate and spore count of $4.03x10^{9}$ conidia/g of substrate followed by sorghum, maize, wheat and barley. He also evaluated different containers for maximization of *M. anisopliae* spore production. Conidial yield per gram of substrate in flat bottle ($7.32x10^{9}$) and saline bottle ($7.26x10^{9}$), being at par with each other they were found to be the best containers. Polypropylene bags performed extremely poor by producing about 60.66 % less conidial yield than others. The contamination was low in saline bottle, flat bottle and conical flask (2-4%) as against 20 % in ordinary Polypropylene bags. Saline bottles were found to be the best for mass multiplication of the entomopathogenic fungus with highest spore yield ($7.26x10^{9}$/g of substrate) and least contamination (2%).

Mondal and Bhattacharya (2004) evaluated the growth and sporulation of Pantnagar isolate (PI) and Delhi isolate (DI) of *B. bassiana* on twenty one culture media. The media were prepared from broken grains of

pulses (bengalgram, blackgram, cowpea, frenchbean, greengram, lentil, pea and pigeonpea), cereals (maize, pearlmillet, rice, sorghum and wheat), oil seeds (groundnut and soybean) and agricultural by products (chopped sesbania and soybean stems, maize cob and sugarcane bagasse). To compare the performance of these media, two routinely used fungal growth media (potato dextrose broth or PDB and Sabouraud's dextrose broth or SDB) were prepared. Generally, sporulation by PI was the highest on pea. However, dry matter production was highest on groundnut and cowpea media. The dry mass production by DI was generally higher than PI in some media, the highest being on frenchbean followed by pea, soybean, cowpea, SDB and blackgram (0.816 to 1.000 g). However, for PI it was higher on lentil followed by frenchbean, greengram, soybean, pearlmillet, wheat and rice (0.237 to 0.684 g) compared to DI. The grain-based culture media have demonstrated great promise for mass production of the entomogenous fungi, particularly the PI.

Ajaykumar and Kanaujia (2005) studied the influence of different grain based media on sporulation, germination and virulence of *B. bassiana*. Among six grain based media, (*viz*. barley, fingermillet, maize, sorghum, soybean and wheat) highest spore production ($5.39x10^7$ conidia/ml) and spore viability (86.6%) was observed in fingermillet. The production and viability of conidia increased when the media was supplemented with sucrose (5.0 to 11.5% and 1.70 to 4.65%, respectively).

Bhide and Patil (2005) tested six different grain media for mass multiplication of *M.anisopliae*. Among the grain media, jowar was found most suitable with a production of 2.575x108 spores/g of substrate followed by bajra ($2.2x10^8$ spores /g). Other grain media tested such as wheat, rice, greengram were found to be on par with each other.

Nirmala *et al.* (2005) evaluated four isolates of each of *B. bassiana* (Bb), *M. anisopliae* (Ma) and *V. lecanii* (Vl) pathogenic agents to key pests. Biomass production in Potato Dextrose Broth (PDB) was comparatively higher in shake cultures than in stationary cultures for all isolates. In two-stage system of mass production, the maximum spore production on rice was observed in Bb5a, Ma4 and Vl3a isolates (96.0, 49.8 and $17.5x10^8$ spores/g) and maximum spores / g of rice were harvested in Bb5a, Ma2 and Vl2a isolates (28.00, 45.50 and 15.60 mg/g). Maximum viable spores / g of spore dust were recorded in Bb5a, Ma4 and Vl3a isolates ($4.7x10^{10}$, $2.5x10^{10}$ and $1.7x10^9$ spores /g). Taking into consideration the quantity of spore dust production and viable spores in the spore dust, Bb5a, Ma2 and Vl2a were

identified as potential isolates for large scale production of dry conidial powder.

Ramarethinam *et al.* (2005) reported that the fermentation method could be used as a standard method of the mass production of *V. lecanii* and there are different types of fermentation of which, the two most commonly used are submerged and semi solid fermentation methods. Under solid and liquid state cultivations, the entomopathogenic fungus, *V. lecanii* produced different types of spores. The aerial spores on cooked rice formed clusters on the tips of conidiophores whereas; the submerged spores were dispersed in the medium.

Sreeramkumar *et al.* (2005) cultured *Hirsutella thompsonii* Fisher on synthetic media like PDA, SDA, Potato carrot agar … etc. It was cultured on two different media viz. semi-solid and liquid media. Colonies on soybean agar produced the highest number of spores ($9.15x10^7$ spores /ml), whereas those on malt extract agar provided a significantly higher number of colony forming units ($4.78x10^9$ CFU/ml). Semi solid agar was not recommended for mass production of *H. thompsonii* due to its high cost. Ground corn coated with molasses was the best solid medium generating $3.07x10^7$ spores/ml with $2.64x10^7$ CFU/ml. Suitable liquid medium comprised 8 % polished rice, 2 % molasses and mineral salts.

Tamizharasi *et al.* (2005) evaluated suitability of molasses based media supplemented with different nitrogen sources for mass production of the entomopathogenic fungi, *B. brongniartii*, *B. bassiana* and *M.anisopliae.* Radial growth of all three fungi on molasses agar media differed significantly among different nitrogen supplements. The salts of $NaNO_3$ and KNO_3 supported the highest radial growth. Yeast extract, soyflour and defatted soymeal were the next best, while fertilizer grade urea inhibited the growth of *B. brongniartii*. Spore production of *B. brongniartii* and *M.anisopliae* did not differ significantly among molasses broth media fortified with different nitrogen supplements. In *B. bassiana* however, yeast extract supported significantly highest spore production followed by sorghum, maize, wheat and barley.

Ayyasamy and Baskaran (2006) studied the suitability of various synthetic and grain media for the cultural characteristics and mass production of the fungi, *P. farinosus* based on the biomass, radial growth, spore germination and mycosis on *Bemisia tabaci* (Gennadius). SMA yielded maximum biomass (730.2 mg), radial growth (80.2 mm), spore germination

(83.2 %) and infectivity (63 %). Among the grains tested for mass production, maize yielded maximum biomass of 448.20 mg, radial growth (74.00 mm), spore germination (81.20) and the highest infectivity of 63 %.

Sahayaraj and Karthick (2008) evaluated that the various agricultural products and by products such as grains, vegetable wastes, seeds, rice husk, straw dust and liquid media such as coconut water, rice and wheat washed water and rice cooked water for mass production of three entomopathogenic fungi; *Beauveria bassiana*, (Bals.) Vuil. *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii*. (Zimm) Viegas. Among the grains, wheat supported maximum spore production for *B. bassiana,* while sorghum recorded maximum spore production in *P. fumosoroseus* and *V. lecanii*. Similarly carrot, jack seeds and ladies finger also supported good growth and sporulation of all the three tested fungi. Coconut water supported maximum growth and sporulation.

Van Hanh and Keun Kim (2008) investigated the production of aerial conidia of *Lecanicillium lecanii* 41185, a highly virulent fungus, by solid-state fermentation to be used as a biocontrol agent against aphids. Among several agro-industrial solid media, steamed polished rice was found to produce the highest amount of aerial conidia. The optimal conditions for aerial conidia production were determined to be 28.5% moisture content in the rice, 25^{o}C culture temperature, rice pH of 6.0, 75% ambient relative humidity, 4-dold seeding culture, 0.6% KNO_3, and 12 d of culture time. The conidia yield increased from 5.7×10^9 conidia/g polished rice to 18.2×10^9 conidia/g polished rice following the application of these optimized conditions.

Karthikeyan and Selvanarayanan **(2011)** evaluated the suitability of certain culture media for certain fungi. Three synthetic media *viz.*, Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Rose Bengal Agar (RBA) and three natural substrates *viz.*, water hyacinth, rice bran and spent mushroom paddy straw were chosen. The suitability of the medium was assessed based on the specific parameters namely colony growth in diameter, spore density and biomass production. The maximum colony growth attained by *B. bassiana* (42.00 mm) and the highest spore density (4.52×10^7 spores/ml) and biomass production (466.33 mg) were obtained in the synthetic medium, PDA. Among natural substrates, rice bran amended medium achieved the highest colony growth (46.33 mm) and yielded more spore density of 4.86 $\times10^7$ spores/ml and the biomass of 485.00 mg. and *V. lecanii* achieved the maximum colony growth (47.67 mm) and spore density of 4.73 $\times10^7$

spores/ml and biomass of 484.67 mg on RBA and on rice bran, it is recorded as 48.67 mm, 5.06 $\times 10^7$ spores/ml and 492.52 mg, respectively.

Mehta *et al*. (2012) studied that the entomopathogenic fungi like *Verticillium lecanii* and *Metarhizium anisopliae* were cultured in different media to produce highest biomass of fungus. The agricultural products and organic products were used for the total biomass production in Kota district. The different media used for the production of biomass were vegetables, cereals, pulses, rice washed water, boiled rice water, straw dust, fruits etc. For the production of biomass of fungi grains media, organic media and non-synthetic media have been used. The maximum biomass production for both *Metarhizium anisopliae* and *Verticillium lecanii* were observed in yeast extract media (36.96 g in 250 ml and 30.82 g. in 250 ml, respectively).

Bhanu, *et al.* (2012) stated that the success of microbial control of insect pests depends not only on the isolation, characterisation and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the biocontrol program. *Beauveria bassiana* is an entomopathogenic fungus which is used against a number of insect pests. To develop an efficient method for the utilization of this fungus as a bio control agent, various grains and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth were screened. Pea amended media produced maximum biomass of the tested fungus, while SDB produced significantly higher spore production of the fungus. Highest conidial count (9.06×10^7 conidia/ ml) was observed on cowpea media followed by soybean.

2.4.3. Virulence of entomopathogenic fungi passage and subculturing through artificial media and an insect host

Virulence of entomopathogenic fungi can be affected by repeated subculturing in artificial media or passage through insect hosts. Constancy of fungal virulence following successive subculturing on artificial media is a desirable trait for production of biocontrol agents **(Brownbridge *et al*., 2001; Vandenberg and Cantone, 2004).** Attenuation or enhancement of virulence of entomopathogenic fungi following repeated subculturing in artificial media or passage through insect hosts have been previously reported **(Kawakami, 1960; Schaerffenberg, 1964; Fargues and Robert, 1983).** Some studies have reported that successive cultures in artificial media cause attenuation in fungal virulence. In contrast, some studies reported that no decline was observed in virulence of fungi subcultured in artificial media. Single passage

Table1: *In vitro* and *in vivo* studies assessing subculturing on the virulence of certain entomopathogenic fungi.

Type of Assay	Fungus	Repeats (subcultures)	Result	References
In vitro	*B. bassiana*	16	decreased virulence against *Leptinotarsa decemlineata*	**Schaerffenberg (1964)**
	B. bassiana	----------	no decreas in virulence against *Bemisia argentifolii*	**Brownbridge *et al.*(2001)**
	B. bassiana	2	significant reduction in virulence	**Quesada-Moraga and Vey (2003)**
	Verticillium lecanii	2 or 3	Significant reduction in virulence	**Nagaich (1973)**
	Aschersonia aleyrodis	19	lost virulence against *Trialeurodes vaporariorum*	**Fransen *et al*. (1987)**
	fumosoroseus	30	no change in virulence against *Diuraphis noxia* or *Plutella xylostella*	**Vandenberg and Cantone (2004)**
	M. anisopliae	12	no decrease in virulence against *Tenebrio molitor*	**Ansari and Butt (2011)**
In vivo	*B. bassiana*	----------	became more virulent againest *Alphitobius diaperinus*	**Steinkraus *et al.* (1991)**
	B. bassiana	2	increase in virulence	**Quesada-Moraga and Vey (2003)**
	M. anisopliae	----------	virulence was restored	**Shah *et al*. (2005)**
	M. anisopliae	1	increased virulence against *Rhipicephalus microplus*	**Guedes–Frazzon *et al*. (2000)**
	Culicinomyces clavisporus	----------	increased virulence against *Aedes aegypti*	**Cooper and Sweeney (1986)**
	P. fumosoroseus	15	deacreased virulence against *Diuraphis noxia* and no change in virulence against *P. xylostella*	**Vandenberg and Cantone (2004)**
	Nomuraea rilleyi	12	no significant decrease or increase in virulence	**Ignoffo *et al*. (1982)**
	Aspergillus flavus	---------	no changes invirulence, increased the number of conidia, decrease in the day for fungal growth	**Scully and Bidochka (2005)**

of entomopathogenic fungi through a suitable host can restore or increase the virulence, however some workers reported that virulence can only be increased after two or more successive passages **(Butt *et al*., 2006).** The effect of repeated *in vitro* subculturing and *in vivo* passaging on viability, morphological characteristics and virulence, varies within entomopathogenic

fungi strains. There are many studies assessing the effect of repeated *in vitro* subculturing and *in vivo* passaging on the virulence of entomopathogenic fungi, and several different observations have been reported. Some positive and negative results obtained from different studies have been shown in Table (1).

Nagaich (1973) reported that the loss of virulence of *Verticillium lecanii* (Zimm.) after two or three subcultures as cause for decay in the quality of stocks, thereby rendering the product commercially unviable **(Butt *et al*. 2006).**Very few studies have been carried out to determine the capabilities of the host in restoring or increasing fungal virulence of attenuated fungal cultures in artificial media (**Wasti and Hartman 1975; Butt and Goettel 2000). Guedes–Frazzon *et al.* (2000)** found that after single-step re-isolation of *M. anisopliae* strains (M5 and E6S1) previously employed on engorged *R. microplus* females strongly elevates their virulence. However, this work was not conclusive in determining the effects of different passages through a suitable host in increasing fungal virulence because the overall effect was only measured in the zero and first passages through a suitable host.

Hajek *et al*. (1990) suggested that the rate of subculturing has an impact on virulence, while the absolute length of time in axenic culture does not influence virulence. For some unknown reason, virulence may be temporarily restored in some subcultures but the overall trend is a decline.

Ibrahim *et al.* (2002) showed that the nutrition can influence the sugar composition at the surface of *Metarhizium anisopliae* conidia but no clear pattern was established between carbohydrate groups and adhesiveness. Besides the number of conidia adhering, attenuation may also influence the speed of germination. Faster germination has been correlated with higher virulence in *M. anisopliae* and *Paecilomyces fumosoroseus* **(Altre *et al*., 1999; Inglis *et al*., 2001).**

Farooq *et al*. (2005) investigated the nutrition influenced growth, sporulation and virulence of the insect pathogenic fungus, *Metarhizium anisopliae*. Virulent conidia were produced on susceptible insect hosts, 1% yeast extract, 2% peptone, osmotic stress medium (OSM) and C: N (10:1) medium. Several strain independent markers were identified and could be used to predict the virulence of *M. anisopliae* conidia. Virulent conidia typically had high levels of spore bound Pr1, an important cuticle degrading protease, and high germination rates. The authors also showed for the first time that virulent conidia have an endogenous C: N ratio below 5.2:1. Real

time PCR revealed that virulent conidia from insects contained significantly higher levels of transcripts of pr1 A and other pathogenicity-related genes than inoculum from artificial media. Of the artificial media studied, 1% yeast extract medium yielded the most virulent conidia, these had higher levels of transcripts of these pathogenicity-related genes than the least virulent conidia from the high conidia yielding C: N (35:1) medium (=SDA), however, the levels were significantly lower than those in insect-derived conidia. The study showed for the first time that the passaged inoculum is virulent irrespective of the original culture medium or insect host. Virulent conidia were consistently produced on OSM even though growth and sporulation were poor. The starvation conditions, whether *in vivo* or *in vitro*, results in de-repression of Pr1 and that elevated levels of this enzyme enhance fungal virulence.

Abid *et al*. (2010) evaluated the radial growth, sporulation, germination, spore-bound Pr1 and virulence of the strains of entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*, on different nutritional conditions, with the objective to produce virulent spores, which may kill the host quickly. Variations in nutritional requirements existed among the fungal species as well as fungal strains. Except for *M. anisopliae* (EBCL 02049), all the selected fungi cultured on osmotic stress media attained lower colony growth (<32 mm), with low sporulation in *M. anisopliae* (strain 406) and *B. bassiana* (EBCL 03005). High sporulation was observed on potato dextrose agar of all the strains of entomopathogenic fungi, but high germination was observed only in the strains of *M. anisopliae* (93-98%). All studied strains except *I. fumosorosea* (EBCL 03011) harvested from *O. varians* cadavers resulted in highly virulent spores with high spore-bound Pr1 activity. Exposure of larvae to suspensions of fungal spores (*in vivo*) of the strains of *M. anisopliae* and *B. bassiana* by immersion resulted in high level of fetal infection (≥ 98%). The results indicated that the influence of nutritional conditions on fungal growth and sporulation is strain dependent and serial subculturing of entomopathogenic fungi on PDA significantly affects the level of infection. Comparatively, *in vivo* spores harvested from *Ocinara varians* cadavers resulted in virulent spores with elevated levels of spore-bound Pr1.

Adames *et al*. (2011) assessed the virulence of strain M379 of the fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) after different passages through a suitable host and at different concentrations for the control of both acaricide-susceptible and resistant strains of the tick, *Rhipicephalus* (formerly *Boophilus*) *microplus* Canestrini (Ixodida: Ixodidae) *in vitro*. The highest value of LC_{50} for the

susceptible strain corresponded to zero passage with 7.68×10^7 conidia/ml followed by the fourth passage with 2.68×10^7, which reduced by 2.87-fold of the lethal concentration. When comparing LC_{50} values of the fourth vs. the seventh passage (2.59×10^5 conidia/ml**)**, the lethal concentration was reduced (103.47-fold) by the seventh passage. In addition, in the resistant strain, the LC_{50} corresponded to zero passage was 4.95×10^7 conidia/ml followed by the fourth passage with a value of 7.86×10^6, which reduced by 6.30-fold of the lethal concentration. When comparing LC_{50} values of the fourth vs. the seventh passage (1.04×10^5 conidia/ml) in the resistant strain, the lethal concentration was reduced (75.58-fold) by the seventh passage. These results suggest that the number of passages on *M. anisopliae* through a suitable host increased its virulence on both *R. microplus* strains. When comparing LC_{50} of the zero passage through a suitable host of both acaricide-susceptible and resistant strains, the highest LC_{50} values corresponded to the susceptible strain (7.68×10^7 conidia/ml) followed by the resistant one with a value of 4.95×10^7, showing that on the resistant strain the lethal concentration is reduced by 1.55-fold. When comparing the fourth passage, the highest values of LC_{50} corresponded to the susceptible strain with 2.68×10^7 conidia/ml followed by the resistant one with 7.86×10^6 conidia/ml, showing for the resistant strain a 3.41-fold reduced lethal concentration. Moreover, when comparing the seventh passages, the highest values of LC_{50} corresponded to the susceptible strain with 2.59×10^5 followed by the resistant with 1.04×10^5 conidia/ml, revealing for the resistant strain a 2.49-fold reduced lethal concentration. These results suggest that the resistant strain needs a lower concentration of conidia than the susceptible strain. In this case, the acaricide-resistant strain is more susceptible to *M. anisopliae* of zero- and seven-passage strains.

Asghar (2013) investigated the effects of repeated subculturing of *Beauveria bassiana* and *Metarhizium anisopliae in vitro* and passages through insects on their virulence against *Uvarovistia zebra*. The virulence of both fungi was reduced after four subcultures in Potato Dextrose Agar, but this reduction was not quite significant for *B. bassiana.* Attenuated fungi obtained from the fourth subculturing were passaged through third instar nymphs of *Uvarovistia zebra.* The insect passage was repeated 2 times and virulence of the fungi was evaluated by mortality in a bioassay. Following passage, there was a small but non-significant increase in the virulence of the fungi.

2.4.4. Formulation of *Verticillium lecanii* (Zimm.) Viegas spores as a mycoinsecticide

As a general rule, pesticides are formulated products that containing active ingredient(s) or active ingredients and co-formulants or additives with specialized characteristics that are needed to make and keep the pesticide efficient in many aspects. Biopesticides are no exception to this rule and formulation may even be more consequential as they contain living organisms. There are many different types of formulations depending on the active ingredient(s), the production process, the target(s) and the application method. This is true for biopesticides as well. In the chemical industry, formulation technology has a long history and many different types of formulations have been developed. Most information is, however proprietary and confidential. The knowledge of formulating chemical pesticides is not available to biocontrol workers, but neither is it very appropriate. Of course, there is some similarity in requirements for both types of products, and in biocontrol we could learn from the chemists, but mostly, formulation is a new research area for biopesticides and there are no "off the shelf" solutions available. New technologies have to be developed for each of the different pathogens. First and foremost we need to ask the question "why do we need formulation?" The four main objectives in formulating a microorganism are:

- To stabilize the propagules that are collected from the production process by means of the downstream process so that they ultimately can be packaged, stored and shipped to the end-user,
- To make a user-friendly product that can be applied economically by the enduser and can be effectively delivered to the target,
- To protect the propagule, once applied, against harmful environmental influences, thereby maintaining and even improving its persistence at the target site and
- To minimize the risks of exposure to the applicator during loading, mixing and applying the product as well as to the worker in the crop, and to the consumer in the case of food crops. **(Ravensberg, 2010)**

Forschler and Nordin (1989) reported that a commercial wettable powder formulation of the entomogenous fungus *B. bassiana* (AGB 6178) applied at 2 × 109, 2 × 1010 and 1 × 1011 conidia / ml against *Plectodera scalator* (F) (Coleoptera : Cerambycidae) in cotton caused more than 60 % mortality of the adults.

Zhang *et al.* (1992) tested the wettable powder formulation of *B. bassiana* at (50×10^9) spores / g against *Ostrinia furnacalii* Gn. under laboratory conditions and the results showed 95 % mortality, of the insect-pest.

Injection of powder formulation of *B. bassiana* can be advantageous because better fungus host contact occurs and lower doses are required **(Stimac *et al.*, 1993).** A maximum of 70 % reduction of the population of *Solenopsis inviota* Buren occurred when colonies were injected with 0.3 g of a powder formulation containing 50×10^9 spores of *B. bassiana*. In colonies with more than 10000 ants, application of 25 g of formulation (1 part conidia + 4 parts diatomaceous earth) by injection into nest or surface treatment caused ant mortality of between 70 and 92 %.

When powder formulation of *B. bassiana* containing diatomaceous earth and $10^4 - 10^{11}$ conidia / g. were applied directly onto ants in colonies containing 1 g of ant workers (about 800 ants), doses of $>10^4$ conidia / ml caused mortality ranging from 36 to 100 %. More than 99 % of adults were killed by application of a dose of 10^6 conidia / ml **(Stimac *et al.*, 1993).**

Murphy *et al.* (1998) reported that Botanigard® a commercial wettable powder formulation of *B. bassiana* applied at 0.5 and 1.0 lb / acre against the sweet potato whitefly on poinsettra showed that treatment at 0.5 lb / acre was sufficient to maintain aleyrodid populations below damaging levels.

The greater reduction (5.1 larvae/plant and 5.9 larvae/plant) of *Plutella xylostella* (L.) of 4^{th} instar larval populations were obtained from wettable powder treatments of *B. bassiana* at higher rate (5.0×10^{13} spores/ha) and from single application of emulsifiable suspension, respectively. Two applications of wettable powder at higher rate (5.0×10^{13} spores/ha) resulted in lower larval counts of 4.3 larvae / plant **(Vandenberg *et al.*, 1998).**

Ota *et al.* (1999) evaluated two formulations of *B. bassiana* as an emulsifiable suspension formulation (ES) and wettable powder against the whitefly on tomato. The results showed that both formulations of *B. bassiana* are found useful for the management of whiteflies attacking tomatoes.

The talc based formulations of *M. anisopliae*, *B. bassiana* and *B. brongniartii* (Sacc.) Petch obtained by mixing in proportion 1:2 to 1:5 with carrier material (talc) to achieve 4.6×10^8 conidia / g in formulation showed high virulence against *Holotrichia consanguinea* (Blanch.) and *Maladera*

insanabilis (Brenske.). *M. anisopliae* and *B. brongniastii* recorded LT_{50} of 7.95 – 16.20 and 9.93 – 13.98 days, respectively when third instar larvae were exposed to their highest dose of inoculum (5.0×10^7 conidia/g). *B. bassiana* was found to be weak pathogen against both insects **(Sharma *et al.*, 1999).**

Orozco *et al.* (2000) reported that commercial formulation *i.e.*, wettable powder of *B. bassiana*, (Mycotrol) ® increased the mortality of whitefly nymphs and adults when compared to control.

Ramarethinam *et al.* (2000) evaluated a commercial wettable powder formulation (Priority) ® of *Paecilomyces fumosoresus* (Wize) Brown and Smith against the red spider mite, *Oligonychus coffea* in tea under laboratory conditions. The mortality of *O. coffeae* ranged from 58.2 to 64.83 % on 10^{th} day after spraying and 75.68 to 95.68 on the 15^{th} day after spraying.

Nankinga and Moore (2000) reported that the application of *B. bassiana* formulated as wettable powder at the rate of 2×10^6 conidia / ha proved to be most effective in reducing the banana weevil population by 65.72 % within 8 weeks after a single application.

Two formulations of *B. bassiana* isolate IMI 330194 (oil palm kernel cake based formulations of conidia (OPKC) and conidial powder (CP)) were tested against adult banana weevils and showed the same level of weevil mortality of 75.5 % compared with only 1 % mortality in the control **(Godonou *et al.*, 2000).**

Sood *et al.* (2001) evaluated a commercial wettable powder formulation of *B. bassiana* (Daman®) against third instar larvae of *P. xylostella* at 2×10^7 cfu / ml and showed it was more effective in bringing mortality.

Easwaramoorthy *et al.* (2003) reported that formulation of the *B. bassiana* cultured on molasses yeast broth was prepared using pressmud and lignite as carrier material. In a laboratory bioassay, pressmud formulation caused higher mortality of third instar grub *Holotrichia serrata* (Fab.) than lignite formulation.

(Biopower)®, a talc based commercial formulation having *B. bassiana* as active ingredient was evaluated against the diamondback moth, *P. xylostella* on cauliflower in greenhouse condition. All the dosages had shown a significantly higher efficacy when compared to the untreated control. A

mortality rate ranging between 47 to 92 % was recorded **(Ramarethinam *et al.*, 2002b).**

Field efficacy of talc based conidia formulation of *M. anisopliae* was evaluated against white grubs in potato. The *M. anisopliae* formulation applied at 5×10^{13} conidia / ha along with chlorpyriphos 20 EC at 200 g a.i. / ha was found effective with exhibiting maximum reduction in plant mortality (75-80%) and tuber damage (63.7%) by way of controlling the grub population (56.5%), which resulted in highest tuber yield of 155 q / ha **(Bhagat *et al.*, 2003).**

The laboratory evaluation of *N. rileyi* formulations *viz.*, oil formulation (sunflower oil + Tween®-80 (0.02%), wettable powder (talc) and crude formulation at 2×10^8 conidia / ml concentration against third instar larvae of *S. litura*. At the end of 10^{th} day, the cumulative mortality was 95.00 % in oil formulation followed by wettable powder and crude formulation by recording 83.10 and 77.00 %, respectively **(Nagaraja, 2005).**

Oil based formulations recorded lower LC_{50} and LT_{50} values compared to WP formulations **(Ramegowda, 2005).** Of eight WP formulations crude WP had registered lowest LC_{50} value of 80.09×10^3 conidia / ml followed by talc based WP and rice flour. Among oil formulations, safflower oil (1.42×10^4 conidia/ml) recorded lowest LC_{50} values followed by groundnut and sunflower oils. The cumulative % mortality percentage after nine days was relatively higher with oil formulations compared to wettable powder formulations. Among the WP formulations crude formulation recorded 82 % mortality followed by talc and rice flour. Among oil formulations groundnut oil registered highest mortality of 96 % followed by sunflower and safflower oils.

2.4.5. Storability, viability and germination of mycopathogen formulations

The most important challenge in the development of mycopathogen formulation is the storage stability of conidia. A stability of 12-18 months at ambient temperature is a minimum requirement to increase the market competitiveness **(Ramegowda, 2005).**

Laboratory produced sclerotia (mummified cadavers of *Heliothis virescens (F.)*) and conidia of *N. rileyi* were held in field conditions for 281 days. Conidia were infectious after being held on the surface of the soil and in

glass vial for 138 and 209 days, respectively **(Spernkel and Brooks, 1977).** Cadavers held on the surface of soil began sporulating after 47days. Infectious conidia were found on these cadavers for the entire sampling period (281days). Infectious conidia were also present on cadavers buried 10 cm in soil upto 194 days.

Daust *et al.* (1983) studied the effect of formulation on the viability of *Metarhizium* conidia. Among fourteen oils (12 are botanicals and two non-botanicals) evaluated, conidial viability declined in all oils over a two months period at 19°C and 26°C. However, viabilities were considerably more at 4°C, viability of conidia stored in all oils were considerably lower than those of dry conidia stored at 4°C. The liquid vehicles like petroleum based oils, organic acids and water were even more detrimental to conidial survival than botanical oils. In contrast to liquid formulations, conidia stored at 10 % granules or 20 % dusts retained high viabilities over a 12 months period at 4°C.

Prior *et al.* (1988) investigated the effect of storage conditions on survival of *B. bassiana* incoconut oil at room temperature (25°C) and in refrigerated condition (2°C). The conidia lost their viability in six days under room temperature, where conidia found to survived up to forty days under refrigerator conditions. Author reveals that conidial suspension in oil was effective for field application because of its non-drying properties. The oil formulation of *B. bassiana* exhibited the additional advantage of prolonged conidial survival.

Dried pellets (with or without added bran) formulations of *B. bassiana* stored at room temperature for 5 months. After 5 months of storage all pellets sporulated profusely. Mean recovery of conidia from pellets without bran was 1.77×10^8 conidia /pellet, while that of pellets with bran was 2.54×10^8 conidia / pellet. Conidial production was significantly greater in bran added formulation **(Knudsen *et al.*, 1990).**

Several different harvesting procedures *viz.,* filtering of mycelium produced in air lift containers from the culture medium, washing with deionized water, spraying with sugar solutions and incubating for 18 h at 4°C before drying were used to obtain dry mycelium preparations of entomopathogenic fungi, *M. anisopliae* and *B. bassiana*. The conidial production of treated mycelia stored for 1.5 and 4.5 months at 4°C was not significantly different for any procedure. For dry mycelium of *M. anisopliae*, maltose and sucrose treated preparations produced more conidia than preparations sprayed with dextrose solution, with water spray or not sprayed

B. bassiana preparations dried soon. Dextrose treated mycelia were superior to other treatments when stored at room temperature. Conidial production was greater after a storage period of 15 weeks at 4°C than for freshly prepared material in pure mycelium and alginate preparations of *M. anisopliae*. For pure mycelium preparation, there was an about 100 fold reduction in conidial production after room temperature storage compared with 4°C storage. For alginate formulation (5 g of sodium alginate with 10 ml of 100% ethanol and 4% $CaCl_2$, fungi culture which was mixed dried on trays) the reduction was about 10 folds **(Periera and Roberts, 1991).**

In case of *B. bassiana*, higher conidial production occurred after storage at 22°C. There was no significant difference between storage at room temperature and at 4°C in all formulations. The results confirm the observation that *B. bassiana* mycelium can be stored for longer periods of time than *M. anisopliae*. But, in both fungi, corn starch formulation (500 ml mycelia paste + 25 g at gelatinized corn starch, later hardened by drying for 3-4 h) provided some protection for the material stored at room temperature which may be related to the presence of sugars in the formulations. The conidia of *B. bassiana* formulated as fat pellets, dispersible powder (with talc) and oil suspension (in Shellsol®) are evaluated for viability in two temperature condition *i.e.*, at 4°C and 25°C stored for 45 days. Initial germination was 90.7 %. There was no significant difference in germination between treatments after 15 days of storage, but conidia formulated in shells at 25°C showed a drop in germination after 15 days from 90.7 to 69 % by day 30 and subsequently to 55.3 % at 45 days. The same formulation kept at 4°C dropped from 94 % on the 15^{th} day of storage to 80.3 % on 30^{th} day and 77 % at day 45. The conidia formulated as DP at fat pellets give more than 89 % germination at both temperatures after 30 days of storage. The highest conidial viability after 45 days was recorded with the conidia in fat formulation (84.7% at 25°C and 91.3% at 4°C) as reported by **(Hidalgo *et al.*, 1998).**

(Biopower®) a talc based formulation of *B. bassiana* was evaluated for its shelf life in three locations having different agro-climatic conditions *viz.*, Ooty, Coimbatore and Chennai. Greater conidial viability and better survivability upto 11 months were observed in Ooty and upto 9 months in Coimbatore, where the average minimum and maximum temperature ranges between 5 – 25°C and 19.23 – 34.7°C, respectively, when compared to Chennai (upto 8 months) having an average minimum of 25°C and maximum of 36°C **(Ramarethinam *et al.*, 2001).**

The conidial germination of *N. rileyi* N812 in different oil formulations was studied for 36 hours storage at room temperature. The fungus *N. rileyi* N812 conidial germination was more than 80 % in presence of sunflower oil, diesel: sunflower oil mixture (7:3), diesel: groundnut oil mixture (7:3) and Tween®-80 (0.1%). The less % germination percentage was seen in safflower oil and its combinations with diesel, groundnut oil which can be attributed to the high viscosity of oil which aggregated spores and reduced the germinations **(Nahar *et al.*, 2004).**

Among nine vegetable oils and seven WP formulations of *N. rileyi* studied, conidia of *N. rileyi* lost 99 % of their viability within a day of storage in vegetable oils **(Ramegowda, 2005).** The viability of conidia after one year of storage was 22.21 % in refrigerated condition, while it was only 15.64 % at ambient room temperature. Rice flour, talc and sorghum flower emerged as the best among carrier materials evaluated, while skimmed milk powder and gram flour appeared to be non-suitable.

Wiwat (2004) reported that the sugars like maltose (control), D-glucose, lactose, Sucrose was evaluated for conidial germination and conidial production of *N. rileyi*. Among these all the four sugar sources influenced the conidial germination uniformly upto 95 %. In case of conidial production, lactose is found superior with 1.80×10^9 conidia per ml over other sugars which given conidial production ranging from 1.1 to 1.3×10^9 conidia / ml. Also, Different additives *viz.*, glycerol, KCl, sucrose and their combinations were evaluated for survival of *N. rileyi* in liquid formulations after storage for 56 weeks at 4°C. The treatment involving 20 % w/v KCl + 10 % w/v sucrose recorded highest survivability upto 56.43 %, whereas other two treatments *viz.*, 30 % v/v glycerol and 20 % w/v KCl + 10 % v/v glycerol recorded zero % survivability**.** Also, twelve different oil based formulations of *N. rileyi* were evaluated . For conidial germination on the day of formulation and two weeks after formulation at two environmental conditions of 4°C and 30°C. Most of the oil formulations are resulted in more than 62 % germination after two weeks of storage at 4°C, where in case of 30°C only peanut oil and castor oil were found to have germination upto 30 % and the remaining oils recorded germination ranging from 0 to 18 %. After storage period of 17 weeks, at 4°C, the oils *viz.*, shellsol T®, Paraffin oil, castor oil were found to be superior by recording germination of conidia up to 72.20, 76.27 and 79.64 %, respectively. Whereas remaining oils were found ineffective to store as they recorded less than 50 % germination. Bentonite clay and its combination with sugars at the ratio of 7:1 parts were found effective by recording conidial germination of *N. rileyi* after 23 weeks of storage at 4°C as they recorded

more than 80 % of conidial germination, where the vice versa combination *i.e.,* 1 parts of clay and 7 parts of sugars recorded less than 60 % conidial germination. Clays like (aluminium silicate), bentonite soil and bentonite recorded high germination percentages of about 75.91, 72.61 and 69.11, respectively after storage for 34 weeks at 4°C. After storage period of 40 weeks at 4°C, the clay aluminium silicate and bentonite recorded 78.54 and 68.08 % germination, respectively. Meanwhile, different water based, oil based and WP formulations were evaluated for their shelf life at two environments *viz.*, 4°C and 30°C. Among water based formulations, 30 % glycerol, 20 % KCl + 10 % glycerol solution, 20 % KCl + 10 % sucrose solution were found superior as they recorded more than 8 weeks of shelf life with 80 % viability of conidia at 4°C, whereas, at 30°C they are able to be viable for less than one week period. In case of oil based formulations, shellsol T, paraffin oil, mineral oil and castor oil were found effective by providing shelf life upto 2 weeks with 80 % viability and more than 6 weeks with 40 % viability under 4°C where remaining oils were shown ineffective in maintaining viability. All the oils were ineffective in maintaining shelf life at 30°C more than 1week. In case of WP formulations, it is found that aluminium silicate was able to maintain 80 % viability upto 43 weeks at 4°C, wherein it is less than 1 week in 30°C. It is followed by bentonite and its combination with lactose, maltose, glucose and sucrose as proportion of 7:1 which were able to maintain more than 80 % viability up to 23 weeks at 4°C wherein at 30°C, they were found effective upto 1-3 weeks **.**

The oils shellsol and ondina oil, in the mixture or individually gave highest conidial germination values of *M. anisopliae* var. *acridum* after 24 hrs of incubation. Peanut oil, Tween®-80 and Agral® also gave high germination values after 24 h and 48 h (above 99%). Only soybean oil was significantly different from the other oils after 24 hrs, but not after 48 h. Viability of conidia in medium term storage (40 weeks) was better at 10°C than 27°C for all tested oil based formulations and there were more significant differences between formulations stored at 27°C than formulation stored at 10°C; they remained viable above 97 %. Pure dry conidia and peanut oil were the only formulations that maintained conidial viability higher than 90 % in both temperatures after 40 weeks of storage **(Alves *et al*., 2002).**

Storing the condia of *M. anisopliae* under refrigeration provided longer life compared to ambient temperature during entire storage period. Reduction in Cfu was from 250 to 176.5 × 10^6 / g after 180 days of storage under refrigeration which amounts to loss in viability of 29.5 %. The corresponding loss in ambient temperature was 50 %. Among the different carrier materials

evaluated, Cfu count was least affected by attapalgite and kaolinite followed by sorghum flour. On the contrary, fly ash adversely affected the Cfu count **(Rachappa, 2003).**

Ezzati-Tabrizi *et al*. (2009) evaluated the wettable powder as prepared on the basis of aerial conidia of two isolates of the entomopathognic fungus, *Beauveria bassiana*. Viability and pathogenicity of conidia products against the second-instar larvae of *Thrips tabaci* in four cases; Conidial-product Maintained in Refrigerator (CMR), Conidial-product Maintained in Laboratory (CML), New Formulated Conidia (NFC) and New Conidia without formulation (NC). Analysis of corrected seven-day total mortality data revealed that there were significant differences among these product-cases in their pathogenicity to thrips larvae. Recorded mortality rates for CMR, CML, NFC and NC showed that the pathogenicity of CML was lower compared to three other cases for both isolates. In the next step, inorganic salts ($MgCl_2$, NH4PO_4, KH2PO_4, $MgSO_4$ and NaCl) were added at a rate of 0.1 M into the both CMR and CML products. Bioassay results indicated that caused total mortality of thrips larvae increased with the addition of salts. the results showed that applied carriers and salts have positive effect on preserving conidia viability and pathogenicity to the second-instar larvae of the onion thrips.

Jana (2009) investigated the impact of different carriers and storage temperatures on conidia of the fungus *Beauveria bassiana* strain I 101. The aim of the research was to reach the information on the best storage conditions for the spores shelf-life. The spores were formulated in three types of carriers (one nutritive and two inert carriers that differed in grain size) and the experiment proceeded at temperatures of 22, 4 and -20°C. The evaluation was based on vitality bioassays including germination and growth index assessment and the bioassay of virulence based on the target organism *Tenebrio molitor*. The germination differed markedly between temperatures as well as between carriers. Nutritive carrier was found to be the most suitable for storage of *B. bassiana* conidia in all aspects, especially when kept at both low temperatures. The germination rate was 97.67% after storage at 4°C for 90 days; the initial germination rate was 97.33%. On the contrary, worse results were mostly achieved in unformulated conidia stored at 22°C (germination rate 12.33% after 90 days).

Hsia *et al.* (2014) evaluated the conidial viability of entomopathogenic fungi as influenced by temperature and additives. Initially, five fungal isolates *i.e. Metarhizium anisopliae* (isolates- MPs, MaBg and MaCc1a), *Beauveria*

bassiana (isolate- BbGc) and *Paecilomyces fumosoroseus* (isolate- PfPx) were screened by exposing conidia of each isolate to wet heat and oven heat stress through a series of temperature. Isolate MPs showed the best tolerance to the heat stress. The conidial germination of this isolate was 100%, when conidia were exposed at 30 to 35ºC temperature for all exposure intervals. Thereafter, the effect of additive was investigated on conidial viability of the isolate MPs. A total of four commonly used components and their recommended percentage used for water-dispersible granules (WG) have passed the test. Tersperse®2700 (a dispersant), 1-naphthalene sulfonic acid, sodium salt (a wetter), lignosulfonic acid, sodium salt (a dispersant-cum-binder), sodium acetate (a disintegrant), sodium alginate and sodium glutamate (as nutritive sources as well as protectant) were selected as basic components for WG-conidia formulation as they were not harmful to MPs with germination beyond 80%, when conidia were exposed to these additives. Terwet®1004 and alginic acid failed to obtain more than 80% conidial germination; hence they were excluded as ingredients of WG for causing adverse effects on conidial viability. The results indicate that the conidia of this isolate might be useful as active ingredient to produce commercial WG-conidia formulation.

2.4.6. Efficacy of *Verticillium lecanii* (Zimm.) against hemipterous insects

The suspension of *Verticillium lecanii* with concentration of 16 x 10^6 spores/ml, when used against the coffee green bug, *Coccus viridis* (Green) in Tamil Nadu, gave 16.6 % morality. The sprays containing surface active agents like Teepol®, Triton x® 100 at 0.03 % or glycerol at 0.1-0.3 % gave 48.9, 79.9 and 22.4 to 31.0 % mortality, respectively **(Easwaramoorthy and Jayaraj, 1978).**

Verticillium lecanii (Zimm.) (Monilales: Moniliaceae), an entomopathogenic fungus found world wide, has been used successfully as biological control agent against various species of aphids for number of years **(Hall, 1982).**

(Khalil *et al*., 1983) in Czechoslovakia, found that when the fungus *V. lecanii* was applied at concentration of 10^8 spores/ml in sprays to plants in glasshouse was highly effective against the aphid species, *Aphis fabae* on sugar beet and *Myzus persicae* on cucumber.

Application of the fungus, *Paecilomyces persimilis* (Wize) followed by three introductions of two *Encarsia formosa* / plant and treatment of

Ashersonia species gave good control as did the application of *V. lecanii* against *B. tabaci* **(Landa, 1984).**

Quinden (1984) tested the mycotal formulation of the fungus *Verticillium lecanii* applied to tomato crop against greenhouse whitefly, *Trialeurodes Vaporariorum* at the rate of 5 x 10^{11} spores/ha which gave significant control of the aleyrodid only 16 days after application.

The effect of a range of humidities on the transmission and sporulation of a commercial preparation (Vertalec®) of *V. lecanii* was investigated in greenhouse at 20°C on capsicum against aphid *M. persicae* by **(Milner and Lutton, 1986).** They found that at least 36 hours is required for infection at 100 % relative humidity. After 96 hours of spraying, 94.5 % of *M. persicae* were infected.

Field trials were carried out in Tamil Nadu by **(Jayaraj, 1989)** to determine the effectiveness of *V. lecanii* in controlling the coffee scale *Coccus viridis*. The fungus caused 73.1 % mortality of the pest when applied at 1.6 x 10^6 spores / ml twice at an interval of two weeks. The maximum mortality of 97.6 % was obtained when the surfactant, Tween 20® was added to the spore suspension. High volume spray was more effective than low volume. The addition of 0.1 % glycerol enhanced the effectiveness of the fungus.

Suklova (1989) reported that *V. lecanii* + Boverin® (*Beauveria bassiana*) gave 98 % control of whitefly. Optimum condition for these fungi was 80-90 % relative humidity and a temperature of 26-28°C.

Evaluation of the entomopathogen *V. lecanii* in the control of the aphid, *M. persicae* on chrysanthemum was conducted by **(Hincapie *et al*., 1990).** Three strains of the fungus *viz.,* VL-A, isolate from *M. persicae*, VL-GC isolated from *Erinnyis ello* and VL-MR from *Trialeurodes vaporariorum* Weshw were used. VL-A caused 100 % mortality compared to 37.5 % for VL-GC and 30 % for Vl-MR. Three concentrations of VL-A was evaluated ($1x10^4$, $1x10^6$ and $1x10^8$) and the mortality increased from 39.5 to 100 %.

Khalil *et al*. (1990) studied the effectiveness of *V. lecanii* against the aphid *M. persicae* in laboratory, on one year old potted peach plants. The fungus was effective at concentrations of 10, 10^5 and 10^7 spores/ml with number of aphids alive/cm leaf area after 25 days being 0.68, 0.61 and 0.57, respectively, compared with the control value of 1.91.

Meade and Bruce (1991) recorded the mortality of various instars of *B. tabaci* and *T. vaporariorum* resulting from exposure to *V. lecanii*. The mortality of nymphs of all three instars of both species due to fungal infection was significantly higher than that in other treatments.

Masuda and Kikuchi (1992) investigated the pathogenicity of *V. lecanii* isolates on aphids, *A. gossypii*, *M. persicae* and whitefly *T. vaporariorum*. Both isolates MGV118 and MGV145 were pathogenic to larvae and adults of *T. vaporariorum* than MGV145. MGV145 was stronger than that of MGV118 on the apterous adults of *A. gossypi* and adults of *M. persicae*. The mortalities caused by the two isolates were almost the same (96-100%) at higher concentration (10^7 and 10^8 conidia/ml).

According to **(Nier *et al.*, 1993),** the pathogencity of *V. lecanii* against nymphs of *T. vaporariorum* and *B. tabaci* at the concentration of 3.2 x 10^6 conidia / ml gave 91 and 100 % mortality, respectively, but a suspension containing 1 x 10^7 conidia / ml resulted in infection rate of 78 per cent. *B. tabaci* was found more susceptible than *T. vaporariorum*.

Two years of laboratory and field assessments using the entomopathogenic fungus, *V. lecanii* against Saskatoon berry leaf aphid, *Acryrthosiphon macrosiphum* (Wilson) showed 70 % aphid kill, three days after treatment. After five days of treatments, it showed 90 to 100 % aphid kill, three days after treatments. After five days of treatment, it showed 90 to 100 % aphid mortality as compared to 35 % in control groups. Field tests using two application of *V. lecanii* showed a significant decline in aphid population as compared to that on the water treated plants **(Miranpuri and Khachatourians, 1995).**

Ekesi *et al.*, (1998) investigated the effect of *B. bassiana, M. anisopliae, V. lecanii* and *P. fumosoroseus* against *Megalurothrips* sp. The observed mortality ranged from 29 and 100 % in *B. bassiana* and 54 and 100 % in *M. anisopliae*, 29 to 68 % in *V. lecanii* and 13 % in *P. fumosoroseus.*

Effect of *B. bassiana, P. farinosus, and M. anisopliae, V. lecanii, V. fusisporum* and *Hirsutella* sp. against *Taeniothrips inconsequens* Pear was also studed **(Brownbridge *et al.*, 1999).** (Bio power®) a commercial formulation of *V. lecanii* cause 43.56 % mortality on *Scirtothrips dorsalis* (Hood) on chilli. All the commercial formulations irrespective of dosage tested have shown a significantly higher efficacy when compared to the untreated control, a significantly higher mortality percentage which was 42.8

– 86.4 in the Biocatch® (*V. lecanii*) treatment on thrips and 61.2 to 87.2 % in Priority® (*P. fumosoroseus)* treatment on the two spotted spider mite were recorded **(Ramarethinam *et al.*, 2002a).**

Mote *et al.* (2003) reported that *V. lecanii* at 0.3 % recorded appreciable kill of thrips, *T. tabaci* infesting gerbera in polyhouse of 18.33, 63.33 and 91.67 % at 3, 7 and 14 daya after treatment. Survey on potential microbial agent against thrips showed natural epizootics or fungal infection on *T. palmi* infesting cucumbers and the fungi was identified as a *V. lecanii* and this caused approximately 20 % of thrips mortality **(Krishnamurthy and Eswara- Reddy, 2004).**

Five different spore concentrations of *V. lecanii* viz., 1 x 10^9, 1 x 10^8, 1 x 10^7, 1 x 10^6 and 1 x 10^5 spores / ml were evaluated against different stages (1^{st}, 2^{nd} larvae and adult) of *S. dorsalis* in laboratory. Among the various concentrations tested, spraying of fungus at 1 x 10^9 spores / ml recorded maximum mortality at all stages (33%, 49% and 59% and least at 1 x 10^5 spores / ml (8%, 14% and 21%) **(Ganga-Visalakshy *et al.*, 2004).**

Evaluation of *V. lecanii* against mealy bugs *Maconellicoccus hirsutus* (Green) and *Planococcus citri* (Rissso) on grape and citrus was reported by an **(Anonymous, 2005).** *V. lecanii* at 1, 2 and 4 g/l, *V. lecanii* at 1-4 g /l, *V. lecanii* at 4 g / l, *V. lecanii* at 1x10^9 spores/ml (Pure culture) did not cause any mortality of mealybugs.

Nirmala *et al.* (2006) in Bangalore studied the pathogenicity of twelve fungal isolates belonging to *B. bassiana*, *M. anisopliae* and *V. lecanii* against *A. craccivora*; *A. gossypii* and *R. maidis* using the detached leaf bioassay technique. All the twelve isolates of the three fungi were found to be pathogenic to *A. craccivora* and *A. gossypii* at a concentration of 1 × 10^7 spores / ml. All isolates except Bb3 and Bb4 of B. *bassiana* were pathogenic to *R. maidis*. The mortality ranged from 2 to 74 % in *A. craccivora*, 14 to 80.8 % in *A.gossypii* and 6 to 50% in *R. maidis*. Bb5a isolate of *B. bassiana* caused highest mortality percentage in *A. gossypii* (80.8%) and *R.maidis* (50%) indicating the broad spectrum in action. VII isolate of *V. lecanii* recorded the maximum mortality of (80.8%) of *A. craccivora. R. maidis* was relatively less susceptible to the three fungi than *A. craccivora* and *A.gossypii*.

Anitha (2007) reported that the botanicals neem oil and mycopathogens *Verticillium lecanii* recorded 2.56 and 3.33 mean number of aphids/3 leaves and these treatment are next best after oxydemeton methyl (a check) which

recorded mean number of 1.15 aphids / 3 leaves, during the first spray. During 2^{nd} spray, these treatments are found significant in controlling the aphids on okra. Regarding the overall mean, the treatment of neem oil show the number of 5.03 of aphids/ 3 leaves.

Banu and Gopalakrishanan (2012) evaluated the activity of formulations of a native entomopathogenic fungus, *L.lecanii* stored at room temperature against the cotton mealy bug, *Paracoccus marginatus* infesting cotton in India. Time mortality responses (LT_{50}) for different formulations were calculated using probit analysis. The results revealed that the LT_{50} was found to be increased with increase in duration of storage. At the end of six months of storage, oil and talc based formulations recorded LT_{50} values of 10.21 (9.21 – 11.33) and 10.31 (9.43 – 11.29), respectively. No significant difference in the virulence of spores formulated in talc and oil was noticed as there was overlapping in fiducial limit was observed. But when tested under pot culture condition, oil based formulation recorded significantly higher mortality of *P. marginatus*. Spraying of oil based formulation of *L. lecanii* twice at 15 days interval recorded a maximum of 75.00 % mortality. The results indicate that a comprehensive evaluation of entomopathogenic fungus in agriculture using oil based formulation is advisable particularly in organic cultivation.

Mangoud *et al*. (2012) investigated the relative toxicity of different compounds against the seychellarum mealybug, *Icerya seychellarum* (Westwood) (Hemiptera: Monophlebidae) and the vedalia beetle, *Rodalia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) on mango leaves under laboratory conditions. The obtained data indicated that different compounds (Biofly®, NeemAzal® and Super Mesrona oil®) gave medium effects on nymphs, and adult females of *I. seychellarum* and immature and mature stages of their predator *R.cardinalis*, compared with Malathion which gave highly effects on the mealybug and its predator using direct exposure technique**.**