CHAPTER 2

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Rice (Oryza sativa L.) is one of the most economically important food crops. Biotic factors, particularly insect pests, are a major constraint in rice production. In Uttar Pradesh, India, gundhi bug (Leptocorisa spp.) is considered the major insect pest during the reproductive growth stages of the crop. This study on ‘Evaluation of bio-agents and botanical essential oils for the management of Leptocorisa oratorius (Fabricius) and L. acuta (Thunberg) (Hemiptera: Alydidae) in rice’ was based on the fact that the use of biological resources for the control of insect pests is becoming increasingly important. These resources can be found in almost any ecosystem and many have already been exploited, as well as, implemented as part of insect pest management strategies for various economic crops.

Biological resources refer to botanicals and bio-agents, which have been found to be effective in managing insect pests of various crops. In order to understand the use and effectiveness of certain plant products and bio-agents, a review on previous work was done. At the conclusion of the review, three botanicals (neem, Jatropha and castor) and two bio-agents (Beauveria bassiana and Bacillus thuringiensis) were selected to be evaluated against the gundhi bug. It was also important to grasp the knowledge on biology, behavior and ecology of Leptocorisa spp. Critical and salient findings of various scientists are presented below.

2.1 Taxonomy, biology, behavior and ecology of Leptocorisa spp.

2.1.1 Taxonomy

Panizzi et al (2000) and Siwi and Van Doesburg (1984) have illustrated the following taxonomic nomenclature for Leptocorisa spp. as phylum (Arthropoda), class (Insecta, order (Hemiptera), suborder (Heteroptera), infraorder (Pentatomorpha), superfamily (Coreoidea), family (Alydidae), genus (Leptocorisa) and species (acuta, oratorius, chinensis, among several others).
2.1.2 Biology and description

Hosamani et al (2009), Panizzi et al (2000) and Dale (1995) have reported exclusive details on the biology and description of *Leptocorisa* spp. The female bugs lay varying number of eggs during its lifetime. The number of eggs laid may range from 50 – 300. They are laid mostly in rows and in batches of as many as 19 eggs per batch. As observed by these workers, each egg was placed in contact with previous egg and cemented to the surface with a white gummy substance. They are oval in shaped, dorsally flat and elliptical with the surface being slightly granulate and shiny. The eggs appear blackish in color with an average length that ranged from 1.00 to 1.20 mm and a width of 0.80 to 0.86 mm. Incubation period may be from 6 – 8 days. Even though lack of operculum, a line of weakness was seen prior to hatching and its dorsal surface was neatly broken off from the micropylar, leaving a cup-shaped portion. Newly emerged nymphs are about 2mm long and pale green to yellowish in color. There are 5 nymphal instars and the average sizes in length (mm) from the second to the fifth instar are 6, 11, 14 and 16 respectively. The color may vary slightly from pale green to brownish green with each molt. The nymphal period takes about 15 to 30 days. The final molting takes place at night. Nymphs stop feeding about 5 hours prior to selecting a place to molt. The nymph then remains motionless in an extended position for about 2 hours before ecdysis. The actual process of molting takes only 2 minutes. Wing pads appear from the 3rd instar and increases in length as the nymphs molt into the 4th and 5th instars. A general description of the adult bugs is that they are slender and cylindrical with colors ranging from brown to green or brownish-green to brownish-orange. The size of the adults ranges from 15 – 19 mm. In the case of *L. oratorius*, the body is more robust with a variation in color ranging from green to brownish-orange. Its size ranges from 17.5 – 19 mm. A distinctive characteristic of this species is the presence of brownish to black ventro-lateral dots or spots on the abdomen. As with *L. acuta*, it can be easily separated from other species by the parameres (bifurcated at apex) and by the triangular projection of the 7th abdominal sternum in females. Its size ranges from 15 – 16 mm. The adults emerge after the 5th nymphal molt with fully developed wings after about 15 – 30 days from the time of hatching. Adult longevity may exceed 3 months.
2.1.3 Behavior and damage

Anonymous (2012b), Anonymous (1999a) and Dale (1995) presented distinct behavior patterns between the nymphs and adults of *Leptocorisa* spp. For instance, the first and second instars often raise their abdomen when on the panicle, but when on the leaves the whole body is straightened. The older nymphs and adults lower their abdomen flat against the panicle or leaf, draw the antennae and front legs together anteriorly against the substrate, and extend the middle and hind pairs of legs against the abdomen. Freshly hatched nymphs congregate around the empty egg cases for some time before they disperse for deeding. Nymphs feed voraciously and gregariously until the 4th instar. Rice bugs are diurnal but are most active during early morning and evening. During the day when it is too hot, they rest in shaded areas. When the plant is disturbed, nymphs drop to the lower parts of the plant while the adults fly a short distance. An infested field can be recognized by the rice bugs’ offensive odor. Rice bugs damage rice by sucking out the contents of developing grains, from pre-flowering spikelets to hard dough stage. The adults and nymphs have piercing-sucking mouthparts. Bugs feeding on developing seeds cause several different types of damage to rice. Early feeding from pre-fertilization through early milk stages causes the grains to become sterile or abort. Feeding during the milk and soft dough stages results in kernel shrinkage or slight discoloration. During the hard dough through the hard kernel stages, feeding seldom causes shrinkage but does create an entry site for opportunistic fungi and bacteria, which results in ‘pecky’ rice. Pecky rice is created by a combination of the bug’s injection of saliva and the fungi or bacteria, which enters the feeding site either on the mouthparts or post-feeding. Subsequently, discoloration and dark spots are formed typically associated with pecky rice.

Ishizaki *et al* (2007) have reported a detailed description on the feeding behavior of *L. chinensis*. When nymphs were released onto a rice plant, a series of typical behavioral phases were recorded. These phases started with antennation where the insect curved its antennae near the plant surface when it climbed up the stem. Upon reaching the panicle, the antennae were bent at right angles. The tips of the antennae lightly touched the plant surface. Next there was extension of rostrum as the insect lightly touched the plant
surface with the labium tip. Then the insect repeatedly dabbed the plant surface with the rostrum tip. As the insect walked slowly forward, it changed the angle and site at which it dabbed the plant surface with the rostrum tip. The antennae were bent during this behavior. Rostrum placing was next when the insect stopped dabbing, and the rostrum was kept motionless on the plant surface. The antennae were kept straightened for stylet penetration into the kernel. Finally, there was continuous sucking, while slightly and repeatedly moving the stylet in and out.

2.1.4 Ecology

Nugaliyadde et al (2000) studied the role of weed hosts on the survival of Leptocorisa oratorius. It was found that rice is the only host plant that could sustain the total community structure of L. oratorius and the peak populations synchronized with the crop from flowering through to harvesting. During the experiment, paddy bug eggs and young nymphs were not observed on weed hosts. It was also established that paddy bug could lay eggs and the emerging nymphs could develop up to adulthood on some weed species (Echinochloa spp., Panicum spp. and Cyprus spp.) under no-choice situation; however, the first generation adults thus developed could not reach maturity and lay eggs on the same weed host. Therefore, it is clear that availability of rice (especially at flowering stage) is essential for the sustenance of L. oratorius. During the flowering stage of rice, the insect multiples into large proportions and damages the developing grains. The highest proportion of mature females occurred at late booting through early heading stage, and the nymphal stages were confined to the heading through soft-dough stages of rice.

2.2 Management of gundhi bug, Leptocorisa spp.

Anonymous (2012b) and Jahn et al (2003) suggested the following techniques as a package of practice to prevent rice bug problems. Removal of weeds from fields and surrounding areas to prevent the multiplication of rice bugs during fallow periods. Level fields with even applications of fertilizer and water, which encourages rice to grow and develop at the same rate. Planting fields within a village at the same time also helps reduce rice bug problems. Many traditional photoperiod-sensitive rice varieties tend to
flower at the same stage, even when planted a week or more apart. Many ‘modern’ varieties are photoperiod insensitive and should therefore be planted (to the extent possible) at the same time. Some farmers set fires downwind of fields, so that the smoke blows over the field. However, even if the smoke disturbs rice bugs, they will return later. Capturing rice bugs, in the early morning or late afternoon, by net can be effective at low rice bug densities, although this method is labor intensive. Some wasps, grasshoppers and spiders attack rice bugs or rice bug eggs. Indiscriminate insecticide use disrupts biological control, resulting in pest resurgence. Begin scouting the field at pre-flowering and continue daily until the hard dough stage. Count the rice bugs in early morning or late afternoon from 20 hills while walking diagonally across a transplanted field. Adults often fly out of the way before you reach the rice plant, so counts may only reveal immature forms. Direct control may be required if there are more than 10 rice bugs for every 20 hills.

Anonymous (1999a) presented the following management options beginning with elimination of grassy weeds from rice, levees and surrounding areas by either cutting or burning reduces habitats for egg laying. There should be avoidance of staggered planting of fields in the area breaks continuous food source. Smoking the field by burning straw windward, and passing baskets or bags coated on the inside with sticky material are promising in repelling/capturing them. They suggested that netting and handpicking bugs will reduce their numbers. Also, putting attractants, such as arasan or anything having bad odor like dead snails or rats, will trap the bugs which can be burned or sprayed with chemicals to reduce their numbers. No modern varieties are resistant to this pest but varieties with panicles enclosed in the leaf sheath for longer time offer some mechanical resistance to feeding. Small wasps and long-horned grasshoppers are known to kill eggs, while fungal pathogens infect nymphs and adults. Spiders, crickets, lady beetles, and long-horned grasshoppers also feed on nymphs and adults. The use of recommended chemical insecticides can be applies as foliar sprays or dust formulations. Spraying or dusting at the flowering growth stage should be done in the early morning or evening on calm days. Malathion dust is most effective, followed by carbaryl dust. Acephate and
chlorpyriphos also give good results. They noted that granular insecticides are ineffective.

Anonymous (1999b) highlighted the use of cane toad to control rice bug. An experiment was conducted to determine the potential of using dead cane toads (*Bufus marinus*) to control rice bug (*Leptocorisa* species). Adult cane toads were collected and killed one day before setting up the trial. A single dead toad was placed in half a coconut shell. The coconut shells were then suspended by a string to a wooden pole to the height of the rice panicles. Poles were spaced every 7 meters around the border of the rice plot just prior to the milky stage. Daily, for one week, the number of rice bugs settling on the dead cane toads was counted and it became clear from the first day that rice bugs were indeed attracted to the decomposing cane toads. Presumably the cane toad gave off an odor (yet to be identified), which the rice bugs were attracted to. On average, 11 rice bugs were attracted to a single cane toad each day over the trial period. However not all the rice bugs were attracted to the cane toads and an average of 18 bugs per square meter remained on the crop. This trial shows the potential for using this technique to reduce rice bug to a manageable level or monitor populations. Once the bugs settle on the toads they could be collected daily using a suitable container or net and destroyed. This is a unique form of pest control applicable to subsistence village based farming methods which requires no financial inputs, no chemical inputs and utilizes widely available natural materials to control an insect pest which can cause significant yield losses.

Ooi (1998) mentioned that farmers in Indonesia used animal based lures to test their ability to attract the rice ear bug. The most useful lures included putrescent crabs, putrescent toads, putrescent prawn, droppings of chicken and putrescent internal organs mostly of chicken. The farmers ultimately learned to appreciate the use of baits to monitor seasonal occurrence of the bug, hence avoiding the expensive prophylactic application of chemical insecticides. Although some work was done on the evaluation of *Beauveria bassiana*, *Metarhizium anisopliae* and neem, among others, for the management of *Leptocorisa* spp., none has been recommended to form part of any management strategy to date.
Research on the use of bio-pesticides for the control of *Leptocorisa* spp. is limited; however, the promising effects of neem (*Azadiracta indica*), jatropha (*Jatropha curcas*), castor (*Ricinus communis*), *Beauveria bassiana* and *Bacillus thuringiensis*, which have been studied recently for the management of different insect pests that attack various crops of economic importance, are presented hereafter.

### 2.3 Botanicals in managing major insect pests

The research of several workers on the use of neem (*Azadiracta indica*), jatropha (*Jatropha curcas*) and castor (*Ricinus communis*) plants for the management of various insect pests on different crops of economic importance has instigated the idea that managing *Leptocorisa* spp. in a similar manner can be achieved. A review on the different botanicals against various insect pests is presented below.

#### 2.3.1 Neem, *Azadiracta indica*

*Ikeura et al* (2013) studied the repellent effect of neem against the cabbage armyworm on leaf vegetables. In laboratory tests, the repellent effect of komatsuna and spinach treated with azadirachtin formulation or neem seed kernel oil cake for 7 days on the feeding cabbage armyworm were evaluated. The feeding repellent effect of azadirachtin formulation treatment was equivalent to that of commercial biological pesticide, while the effect of neem seed kernel oil cake treatment was higher. This result clarified that neem seed kernel oil cake has a high feeding repellent effect against cabbage armyworms. In field tests, although the feeding percentage for komatsuna and spinach controls was 70%, that for komatsuna and spinach treated with azadirachtin formulation and neem seed kernel oil cake was about 40% and 30%, respectively. These laboratory and field test findings demonstrated that despite having an affect less than that of azadirachtin, neem seed kernel oil cake is a high effective feeding repellent.

*Gnanamani and Dhanasekaran* (2013) conducted laboratory studies to determine the growth inhibitory effects of Azadirachtin against the 5th instar larvae of *Pericallia ricini* by oral and topical methods. Both methods revealed feeding deterrence in the *P. ricini* larvae and they decreased significantly the larval growth comparing with larvae in control
group. The highest anti-feedant activity and larval deformities were observed in 100ppm oral treatment and 300ppm topical treatment. In case of oral treatment, the 5th instar larvae were not successfully ecdysed and unable to develop into pupa. At topical application, larva-pupal intermediates were observed. No moult from larvae to pupa occurred and the body was still covered by the larval exuvium. This result indicated that Azadirachtin has anti-feedant properties and it disrupts the physiological events such as apolysis and ecdysis and finally it leads to death in the larval growth stage. The results showed that the Azadirachtin is promising as a bio-pesticide against *P. ricini*, and it could be an alternative for chemical pesticides.

**Achio et al (2012)** studied the insecticidal properties of extracts and powders prepared from different neem parts (seed, leaf, stem and root) against the *Macrotermes spp.*, *Phaseouslus spp.*, *Periplaneta spp.* and larvae of *Anopheles spp.* The lethal effect was more pronounced with the seed extract (40-55 %), followed by the leaf extract (30-45 %), the stem extract (30-40 %), and the root extract (10-30 %), as compared to the control (distil water) which registered 0 % mortality. The termites and the weevils were seen to be more susceptible to the various extracts, compared to the cockroaches and mosquito larvae. Oil extracted from the neem seed kernel showed even greater lethal properties on the insects, with a minimal lethal concentration, in all cases, being 0.50 % V/v. Again the termites and the weevils responded faster, recording total deaths within 2-5 minutes, compared to the cockroaches and the mosquito larvae where total deaths were experienced only after 30-90 minutes. It was also found out that there was a direct relation between the concentration and degree of lethal effectiveness of the oil. The neem, especially the seed oil, has great potential as natural biocide against termites and weevils.

**Mamoon-ur-Rashid et al (2012)** studied the impact of different neem oil concentrations on the development, longevity, fecundity and mortality of *Phenacoccus solenopsis* under ambient laboratory conditions using leaf and surface treatment bio-assay trials. Neem oil at 25000 and 30000 ppm on *Hibiscus* leaves killed 53% and 60% 2nd instar cotton mealybug nymphs, respectively after 24h treatment which was significantly greater
(P<0.05) than control. Neem oil at higher concentrations (20000, 25000 and 30000 ppm) adversely affected the duration for cocoon formation, pupal period, male longevity and shortened the male adult life duration and the longevity of mated and virgin females as compared with other treatments. However, neem oil concentrations did not have any significant effect on the oviposition and post-oviposition periods of females. Female fecundity and eggs per batch decreased by 73% and 50%, respectively when they were reared at 30000 ppm neem oil concentration as compared to those reared on untreated leaves. The detrimental effects of neem oil concentrations on cotton mealybug were sustained for three months under ambient laboratory conditions. They concluded that the neem oil solution can be stored up to three months under shaded conditions without deteriorating its effectiveness.

Roy and Gurusubramanian (2011) determined dose-mortality response of the three sucking pests of tea: Helopeltis theivora Waterhouse, Scirtothrips dorsalis Hood and Empoasca flavescens Fabricious, to neem formulation of varying azadirachtin content (300, 1500, 3000, 10000 and 50000 ppm) under field conditions. Of the neem formulations tested, 50000 ppm and 10000 ppm had the lowest LC50 values, highest slopes and relative potencies for the three target pests. Scirtothrips dorsalis was highly susceptible to neem formulation of varying azadirachtin content as revealed by the lower LC50 values (0.09 – 0.20 ppm), highest slopes (1.99-2.54) and mean percent reduction (46.8-75.8%) followed by E. flavescens (0.12-0.39 ppm; 1.92-2.38; 46.3-64.5%) and H. theivora (0.16-2.27 ppm; 1.48-2.25; 34.8-54.1%). The LC50 decreased as the azadirachtin content of neem formulation was increased, while pest damage lessened as the concentration of azadirachtin increased. They concluded that azadirachtin concentration is the determining factor in terms of its bioactivity; however, this may vary from insect to insect but in tea, 50000 ppm azadirachtin is ideal for managing the three major sucking pests.

Sharmah et al (2011) evaluated the efficacy of neem (Azadirachta indica A. Jass), soap nut (Sapindus trifoliatus L.) and their formulations and Malathion as standard insecticide against the rice bug (Leptocorisa oratorius Fabr.) during 2000-2001 on the basis of per...
cent reduction under field condition. The per cent reduction in number of rice adult bugs due to neem seed kernel powder, neem seed kernel powder + multani powder, multani powder, neem leaf powder, neem leaf powder + multani powder, neem leaf powder + ash, soap nut kernel powder, soap nut kernel powder + multani powder tested as dust were found to be 24.44, 14.78, 5.65, 17.06, 11.88, 6.54, 18.18 and 15.01 respectively for exposure period of ten days. The order of efficacy was malathion followed by neem seed kernel powder, soap nut kernel powder, neem leaf powder, neem seed kernel powder + multani powder, soap nut kernel powder + multani powder, neem leaf powder + multani powder, neem leaf powder + ash, multani powder for the exposure period of 3.

**Pinheiro and Quintela (2010)** evaluated the anti-feedant and insecticidal effects of two commercial neem (*Azadirachta indica*) oil formulations (Dalneem and Nim-I-Go) to *Oebalus poecilus* on irrigated rice. To evaluate the anti-feedant effect, both formulations were tested at 1% and 2% (v/v) concentration levels, while the insecticidal effect was evaluated at 0.5%, 1%, 2%, and 4% (v/v) concentrations of Dalneem, by ingestion. Both experiments were conducted with four replications by treatment, containing one panicle and adult insects segregated by gender (two insects per plot for the anti-feedant effect and five for the insecticidal effect). Both products were efficient, reducing the damage caused by insects. Insects fed less on the panicles treated with neem oil, causing lower number of feeding sheaths per panicle and lower percentage of damaged grains than the control. Spikelets weight was higher in panicles treated with neem oils. Females caused significantly higher damage than males in controls, for numbers of feeding sheaths and for the feeding deterrence index. Only at the 4% (v/v) concentration level, Dalneem caused adult mortality higher than in the control. Results showed that neem oil formulations, at \( \geq 1\% \) (v/v) concentration, can be used to reduce the quantitative and qualitative damages caused by *O. poecilus* in lowland rice.

**Lynn et al (2010)** tested for toxicity of the plant-derived natural pesticide azadirachtin and two types of commercial neem (*Azadirchta indica* A. Juss) based formulations, Neema (liquid type) and Neema-plus (pellet type) on the mortality rate and developmental inhibition of the sweet potato whitefly (*Bemisia tabaci*) and root-knot
nematode (*Meloidogyne incognita*). When *B. tabaci* adults were fed leaves containing 5 or 10 ppm of azadirachtin solutions, the rates of female oviposition, subsequent egg hatch, and adult eclosion were significantly reduced to 23.1, 53.2, and 26.6% of the control, respectively. Further studies revealed that the rates of adult colonization, oviposition and egg hatch were reduced to 78.2, 47.0, and 71.2% by Neema foliar spray and 31.3, 34.1, and 66.8% by soil treatment with Neema-plus relative to the control. When isolated soil nematodes were exposed to various concentrations of azadirachtin, Neema, and Neema-plus, the immobility of juvenile nematodes showed no change at 2 h after treatment, whereas a reduction of 36.3% was observed at day 1 with 10 ppm of azadirachtin. Soil treatments with neem formulations significantly reduced the numbers of soil nematodes and plant root-knots; the reduction with Neema was 12.1 and 9.0%, and with Neema-plus 26.4 and 24.6% of the control, respectively. Furthermore, soil treatment with Neemaplus greatly improved the growth of cucumber plants in nematode-infested pots. These results showed that azadirachtin and neem-based formulations were highly effective on the developmental inhibition of both whiteflies and root-knot nematodes. Thus, soil application of the neem-based formulations would be applicable for the control of both leaf-sucking and soil pests.

Irigaray *et al* (2010) evaluated the commercial formulation of azadirachtin, Align® against different developmental stages of the European grape berry moth, *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae). When administered orally, Align reduced the fecundity and fertility of adults treated with 1, 5, and 10 mg litre-1. At the highest doses, fecundity and fertility were zero, but longevity was not affected. An LC$_{50}$ of 231.5 mg litre-1 was obtained when Align was sprayed on eggs less than 1 day old. Hatching of all egg classes was significantly reduced, and this reduction was more pronounced for eggs less than 24 h old. LC$_{50}$ values of 2.1 mg litre-1 for first instar and 18.7 mg litre-1 for third instar were obtained when Align was present in the diet. Larvae reared on a diet containing different concentrations of Align did not molt into adults at the highest concentrations (0.3, 0.6, 1.2), and 50% molted at the lowest concentration (0.15). Phenotypic effects included inability to molt properly and deformities. The combination of acute toxicity and low, effective concentrations of Align observed in this
study could lead to the inclusion of insecticides containing azadirachtin in integrated management programs against this pest.

_Senthil-Nathan et al (2009)_ investigated the effects of two different neem products (Parker Oil™ and Neemas) on mortality, food consumption and survival of the brownplanthopper, *Nilaparvata lugens* Stal (BPH) (Homoptera: Delphacidae). The LC$_{50}$ (3.45 ml/L for nymph and 4.42 ml/L for adult in Parker Oil™ treatment; 4.18ml/L for nymph and 5.63 ml/L for adult in Neemas treatment) and LC$_{90}$ (8.72 ml/L for nymph and 11.1 ml/L for adult in Parker Oil™ treatment; 9.84 ml/L for nymph and 13.07 ml/L for adult in Neemas treatment) were identified by probit analysis. The LC$_{90}$ (equal to recommended dose) was applied in the rice field. The effective concentrations of both Parker Oil™ and Neemas took more than 48h to kill 80% of the *N. lugens*. Fourth instar nymph and adult female were caged on rice plants and exposed to a series (both LC$_{50}$ and LC$_{90}$) of neem concentrations. Nymph and adult female *N. lugens* that were chronically exposed to neem pesticides showed immediate mortality after application in laboratory experiment. The quantity of food ingested and assimilated by *N. lugens* on neem-treated rice plants was significantly less than on control rice plants. The results clearly indicate the neem-based pesticide (Parker Oil™ and Neemas), containing low lethal concentration; can be used effectively to inhibit the growth and survival of *N. lugens*.

_Kraiss and Cullen (2008)_ evaluated direct spray treatments of two neem formulations, azadirachtin and neem seed oil, under controlled conditions for effects on survivorship, development time and fecundity in *A. glycines* and *H. axyridis*. Both azadirachtin and neem seed oil significantly increased aphid nymphal mortality (80 and 77%, respectively) while significantly increasing development time of those surviving to adulthood. First-instar *H. axyridis* survival to adulthood was also significantly reduced by both neem formulations, while only azadirachtin reduced third-instar survivorship. Azadirachtin increased *H. axyridis* development time to adult when applied to both instars, while neem oil only increased time to adult when applied to first instar. Neither neem formulation affected the fecundity of either insect.
**Das et al (2006)** evaluated the efficacy of different concentrations of the commercial neem-based insecticide, Nimbicidine® against the eggs of the red flour beetle *Tribolium castaneum* (Herbst). The insecticide significantly inhibited the hatching, pupation and adult emergence of the beetle. The latent effects of Nimbicidine® on the next generation progenies were expressed by significant reductions in the growth of larvae, pupation and adult emergence coupled with lengthened developmental period, but the sex ratio was unaffected.

**Ma et al (2005a)** tested two extracts from neem (*Azadirachta indica* A. Juss. (Meliaceae)) seeds, azadirachtin and oil, and a mixture of neem oil and abamectin on second-instar nymphs of the rice bug *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae) using choice and no-choice conditions in field cages. In a choice test, treatment with the mixture of neem oil and abamectin was most effective in reducing the survival of *L. chinensis*, followed by azadirachtin at 60 ppm, 30 ppm and 3% neem oil, whereas all treatments except neem oil caused 100% mortality within 3 weeks in a no-choice test. When second-instar nymphs had choices of treated and untreated plants within a treatment, no differences in yield and sum of dead and stained grains were found between those two choices, indicating that nymphs neither caused significant reduction in yield nor reduced the quality of untreated plants. Regardless of treatment, the difference in overall yield between treated and untreated plants under choice conditions was not statistically significant (P>0.05). Our results indicate that neem-based formulations, used alone or in combination with abamectin, have the potential to be integrated into the existing programs to control the rice bug.

**Sutherland et al (2002)** used bioassays and field tests to examine the efficacy of crude neem kernel extracts and several commercially available neem products against *Oebalus poecilus* in Guyana. Bioassays revealed that the extracts exhibited a low contact kill as against the commercial products. Anti-feedant and ovipositional deterrent tests demonstrated good activity in reducing stinkbug feeding damage but not oviposition. Field-testing of all compounds highlighted that the commercial product showed promise in reducing stinkbug damage when applied at 2.5 l ha⁻¹. It was concluded that
Azadirachtin is an ideal candidate substance for inclusion into an IPM program for small rice stink bug predominantly because of its low toxicity to natural enemies.

### 2.3.2 Jatropha

Jide-Ojo et al (2013) examined the phytochemical composition of leaf extracts as well as biological effects of juice; leaf extracts and seed oil of *Jatropha curcas* against *Sitophilus zeamis*. The study also investigated the inhibition of oviposition, progeny production and grain damage, insecticidal effects and mammalian toxicity of the extracts. Compared to other phytochemicals, the concentration of saponin and cardiac glycoside were higher in the leaf extract. All extracts of *J. curcas* (0 – 100 ppm) investigated showed a dose-dependent inhibition of oviposition, progeny production and promote significant (P < 0.001) insect mortality. Grains pre-treated with seed oil produced the highest result for all the parameters. The seed oil (100 ppm) produced 93% (P < 0.001) protection against grain damage by *Sitophilus zeamis*.

Bashir and El Shafie (2013) tested *Jatropha* seed oil concentrations of 5%, 10%, 15% and 20% for biological activity against the third nymphal instar of the desert locust, *Schistocerca gregaria*. All tested concentrations caused significant (p< 0.05) mortality in the experimental insects ranging from 22.4% to 59.2% after 7 days of application. The LD₅₀ values for treated nymphs at 48, 72, and 96 hrs were 3.12%, 6.57% and 9.85%, respectively. Oil concentration of 10% resulted in a delay of the development time from the 5th to 6th nymphal instar by 5 days, where treated nymphs had completed development in 16.50 days compared with only 11.33 days in the untreated control group and 12.00 days in the group treated with hexane. The same concentration (10%) also significantly reduced the per cent of egg hatch. The concentration of 5% caused a significant anti-feedant effect on the treated nymphs as compared to the untreated control group.

Bhagat and Kulkarni (2012) conducted laboratory experiments to determine the effect of fifteen botanical extracts of seed oils, root and leaves of three Jatropha species *viz.* *J. nana*, *J. gossypifolia* and *J. glandulifera* against third instar larvae of tobacco cutworm *S. litura*. The highest larval mortality was observed to seed oils of all three species and
methanol extract of *J. glandulifera* leaves, while remaining extracts showed moderate effects. In anti-feedent studies five extracts viz. seed oil, methanol and petroleum ether extract of leaves and root of *J. gossypifolia*, seed oil and methanol extract of leaves from *J. glandulifera* showed pronounced activities. Adulticidal activity performed for natural enemies like lady bird beetle and *Cryptolaemus montrouzeri* showed no toxicity for extracts and less toxicity to seed oils.

**Nuchsuk et al (2012)** studied the larvicidal effects of the crude protein extract and purified toxin (Jc-SCRIP) from the seed coat of *Jatropha curcas* Linn. against the third instar larvae of mosquitoes, *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say and compared with seed kernels of *J. curcas* and *Ricinus communis*. At various concentrations of purified toxin and crude protein extract, the larval mortality of both *Ae. aegypti* and *Cx. quinquefasciatus* were positively correlated with increased exposure time. The larvae of *Cx. quinquefasciatus* were more susceptible to the toxin and both extracts than the larvae of *Ae. aegypti*. After 24 hours of exposure, the extract showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with LC$_{50}$ values of 3.89 mg/ml and 0.0575 mg/ml, respectively. The toxin, Jc-SCRIP, showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with LC$_{50}$ values of 1.44 mg/ml and 0.0303 mg/ml, respectively. These results indicated that the crude protein extract and Jc-SCRIP were more toxic to the third instar larvae of *Cx. quinquefasciatus* than that of *Ae. aegypti*. The potent larvicidal activities of the seed coat extract and the Jc-SCRIP toxin from *J. curcas* suggest that they may be used as bioactive agents to control the mosquito population.

**Nisar et al (2012)** determined the effect of leaf and seed extracts of *Jatropha curcas* Linn. in various polar and non polar solvents against workers and soldiers of subterranean termites, *Odontotermes obesus* (Ramb.) on mortality and tunneling behaviour. The extracted materials were added to soil in 1, 5 and 10% concentrations. A control was also maintained with solvents alone. The studies showed that all extracts had significantly low LT$_{50}$ in hours than their respective control treatments. LT$_{50}$s among various polar and non polar solvents were statistically different (*P$<0.05$). The reduction in tunnel length
(mm) was observed in all extracts and was significantly different from their respective controls.

Khani et al (2012) concluded that petroleum ether extract of black pepper (Piper nigrum) and physic nut (Jatropha curcas) have insecticidal efficacies against rice moth (Corcyra cephalonica). The C. cephalonica larvae at 16 days old were shown to have similarities in susceptibility to petroleum ether extract of P. nigrum and J. curcas with LC$_{50}$ values of 12.52 and 13.22 μL/mL, respectively. In a bioassay using no-choice tests, the parameters used to evaluate anti-feedant activity were Relative Growth Rate [RGR]; Relative Consumption Rate [RCR], efficiency on conversion of ingested food [ECI] and Feeding Deterrence Indices [FDI]. Both extracts showed high bioactivity at all doses against C. cephalonica larvae and anti-feedant action was increased with increasing plant extract concentrations. The petroleum ether extract of P. nigrum and J. curcas showed strong inhibition on egg hatchability and adult emergence of C. cephalonica at the lowest concentration.

Habou et al (2011) evaluated the insecticidal activity of Jatropha curcas on various crop pests with oil concentrations of 0.5, 1, 2.5, 5, 10 and 15% on the black bean aphid (Aphis fabae Scop.). Further field tests were done with 5 and 7.5% oil concentrations on the main pests infesting cowpea crops (Vigna unguiculata L.). The results obtained in the two tests demonstrated the biocidal effect of the treatments applied, which increased with the concentration. On the black bean aphid, the biocidal effect increased during the hours following the application of oil before reaching a peak after 4 days. In the field, there was reduction in the level of attack by aphids (Aphis craccivora) by 10 and 50% respectively compared to the control. Also, 50% and 75% fall in the number of thrips (Megalurothrips sjöstedti) and bugs (Anoplocnemis curvipes), respectively were observed under the same conditions. This treatment made it possible to greatly increase yields compared to the untreated control.

Katoune et al (2011) assessed the insecticidal properties of J. curcas oil against cowpea insect pests at the ICRISAT research station, Sadoré, Niger. Four concentrations (2.5%,
5%, 7.5% and 10%) of physic nut oil extract were evaluated as field sprays along with an untreated control (water spraying) and a conventional insecticide (Deltamethrin Decis® EC) treatment. Deltamethrin and physic nut oil at 7.5% gave the highest seed yields, with more than 1000 kg ha\(^{-1}\). Both treatments, alongside the one with 10% oil, sustained significantly lower thrips (\textit{Megalurothrips sjostedti}) infestation than the water-sprayed control. All oil extract treatments and the Deltamethrin treatment sustained significantly lower infestation by \textit{Clavigralla tomentosicollis} bugs than the untreated control, with the lowest infestation occurring with 7.5% oil. Furthermore, correlations between oil concentration and thrips and bug infestation were negative and significant, while correlation between oil concentration and seed yield was not significant, due to a phytotoxic effect of oil at high concentrations.

\textbf{Kshirsagar (2010)} investigated the insecticidal activity of \textit{Jatropha} seed oil against \textit{Callosobruchus maculatus} infesting cowpea (\textit{Phaseolus aconitifolius} Jacq). It was found that the eggs of \textit{C. maculatus} were more susceptible to \textit{Jatropha} oil and showed mortality to all selected dosage. \textit{Jatropha} seed oil was highly toxic to the eggs of \textit{C. maculatus} (\(F = 76.17; p< 0.001\)) at all dosage levels compared to other pre-adult stages (larvae: \(F = 65.13; p< 0.001\), Pupae: \(F = 17.43; p< 0.001\)).

\textbf{Singh and Sushilkumar (2008)} evaluated \textit{Jatropha curcas} oil and its toxic fraction at different dilutions i.e., 1%, 5%, 10% and 20% against termite, \textit{Microcerotermes beesoni}. The maximum wood protection against termites of both the treatments was obtained at their highest concentration i.e. 20%. The weight loss ranged from 18.77% to 48.80% at concentrations of 20% to 1% of \textit{Jatropha curcas} oil formulation. The protection afforded by toxic fraction was enhanced, with the % weight loss ranging from 10.48 to 35.19. However, all the treatments proved to be effective over the control (50.84%).

\textbf{Arnubio et al (2006)} studied leaf extracts of \textit{Jatropha gossypiifolia} L. (Euphorbiaceae) against larvae of three lepidopteran species, \textit{Busseola fusca} (Fuller) (Lepidoptera: Noctuidae), \textit{Ostrinia nubilalis} Hubner (Lepidoptera: Pyralidae) and \textit{Sesamia nonagrioides} Lef. (Lepidoptera: Noctuidae), which are important pests of maize in
Africa, Europe and Mediterranean countries, respectively. Leaf extracts were shown to be highly toxic to neonate larvae of *B. fusca* and *O. nubilalis* quickly after they were ingested. In contrast, no effect was found on fourth instar *O. nubilalis* and a low level of toxicity was observed on neonates of *S. nonagrioides*. Given the toxicity of *J. gossypiiifolia* to larval neonates of *B. fusca* and *O. nubilalis*, this extract can be used for the control of these species when they are colonizing the plant.

**Adebowale and Adedire (2006)** evaluated jatropha seed oil at various serial dilution ranging from 0% to 2% (v/w) at 0.5% intervals for anti-ovipositional activity and long-term protective ability of treated cowpeas against the seed beetle *Callosobruchus maculatus*. The oil significantly (*P* < 0.05) reduced oviposition by *C. maculatus* in no-choice test in all the concentrations tested. The number of eggs laid by the seed beetle reduced from an average of 54.33 ± 3.53 in the control to only 4.00 ± 1.53 in 2% oil-treated seeds. There was no adult emergency in all the oil. However in choice-tested seed, 6.67 ± 1.33 eggs were laid in cultures treated with 2% oil, while 21.67 ± 1.45 were laid in control cultures. In dual-choice tests, oviposition was significantly reduced at all the oil concentration evaluated. *Jatropha curcas* oil also offers a 12-week protection for treated seeds since there was neither seed damage nor adult emergency in treated cowpea seeds.

**2.3.3 Castor**

**Fouad (2013)** evaluated the toxicity and repellent activity of essential oils of camphor (*Eucalyptus globules*), castor (*Ricinus communis*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and mustard (*Brassica rapa*) against *B. incarnatus* adults at concentrations of 0.5, 1, 2 and 4% and acetone (control) were applied. All essential oils with 4% concentration repelled the *B. incarnatus* adult except castor oil. The percentage of repellence was higher for cinnamon with 2 and 4% concentration compared with other essential oils and concentrations. In residual film experiment, the cinnamon oil had the highest toxicity rate on *B. incarnatus* adult followed by clove, camphor, mustard and the lowest effect was by castor oil.
Ede and Demissie (2013) reported the characterization of biocide from Jatropha (Jatropha curcas) and castor (Ricinus communis L) seeds oil. The biocide potential of the oil seeds was evaluated against termite (Odontotermes obesus) and cockroach (Blattella germanica). The bioassay study showed that Jatropha 10% oil caused 100% mortality in 48 and 72 hrs against termite and cockroach, respectively. Castor 10% oil caused 100% mortality in 60 and 72 hrs against termite and cockroach, respectively. The LD$_{50}$ was determined to be 0.64% and 1.24% for termite and cockroach, respectively for jatropha oil after 72 hrs exposure. It was determined to be 1.43% and 1.08% for termite and cockroach respectively for castor oil. The biocidal potential of the oil is statistically significant (p<0.05) when compared with blank and solvent controls at all concentration tested.

Ramos-Lopez et al (2012) determined the composition of hexane leaf extract of Ricinus communis by gas chromatography–mass spectrometry (GC-MS) and obtained four fatty acids: linolenic acid (47.76%), linoleic acid (15.28%), palmitic acid (13.01%), and stearic acid (1.73%). The insectistatic and insecticidal activities of the two major components, linolenic acid and linoleic acid, on the larval development of Spodoptera frugiperda were assessed. The larval viability fifty (LV$_{50}$) values of linolenic acid and linoleic acid were $0.849 \times 10^3$ ppm and $0.857 \times 10^3$ ppm, respectively. Thus, both fatty acids evaluated were found to have the insectistatic and insecticidal activities against S. frugiperda.

Kodjo et al (2011) tested the effect of different treatments of R. Communis plant extract (20%) and oil emulsion (5% and 10%) and their persistence (0, 3 and 5 days after application; DAA) on mortality and oviposition behaviour of P. xylostella. R. communis products had strong larvicidal effect on P. xylostella, with 100% mortality recorded on 3rd instar larvae treated with 10% oil emulsion in both ingestion and contact toxicity tests. Aqueous extracts were significantly less toxic with the highest mortality rates ($67.49 \pm 1.98\%$ and $70.86 \pm 0.85\%$) recorded with seed kernel extract and the lowest with the root extract ($53.98 \pm 1.21\%$ and $54.87 \pm 1.88\%$), in topical toxicity and ingestion toxicity experiments, respectively. The adult emergence was significantly affected with the lowest emergence rate recorded in 5% oil emulsion, $57.72 \pm 72\%$ and $49.98 \pm 0.98\%$. 

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in topical toxicity and ingestion toxicity tests, respectively. No significant different was noted between Dursban and aqueous extract treatments. Among emerged adults from larvae treated with oil and aqueous extracts, a 44–79% abnormal development as wings and legs deformation were observed.

**Ramos-Lopez et al (2010)** determined the insecticidal and insectistatic activities of methanol, hexane and ethyl acetate extracts of the seeds and leaves of *R. communis*, castor oil and ricinine at different concentrations against *S. frugiperda*. This study demonstrated for the first time, that the castor oil and ricinine are active ingredients of *R. communis* that acts against *S. frugiperda* and that each of the seed extracts exhibited better insecticidal and insectistatic activity than the leaf extracts. The half maximum larvae viability concentration (LVC<sub>50</sub>) were 0.38 × 10<sup>3</sup> ppm for the ricinine, 0.75 × 10<sup>3</sup> ppm for a methanol extract of seeds, 1.97 × 10<sup>3</sup> ppm for an ethyl acetate seed extract, 2.69×10<sup>3</sup> ppm for the castor oil, 4.83 × 10<sup>3</sup> ppm for a methanol extract of leaves, 5.07 × 10<sup>3</sup> ppm for an ethyl acetate extract of leaves, 9.95 × 10<sup>3</sup> ppm for a hexane extract of seeds and 10.01 × 10<sup>3</sup> ppm for a hexane extract of leaves.

**Nasr et al (2010)** examined the biocidal activity of castor oil plant (*Ricinus communis*) in controlling the whitefly *Bemisia tabaci* (Genn) (Homoptera: Aleyrpdidae). Castor oil was extracted from seeds using different solvents, hexane, acetonitril and methanol. Assays were performed with adults and larvae. Mortality of the pest increased with increasing concentrations of castor oil. The lethal concentrations of the castor oil extracts were higher for adult fly than the immature stage. The insecticidal values of castor oils were compared with other formulations based on Azadirachtin (neemix, 4.5% EC) and Pyrethrum 5% SC. Emulsions of castor seed oil gave effective control against the whitefly *Bemisia tabaci* (Genn).

**Mushobozy et al (2009)** evaluated five plant oils from sesame (*Sesamum indicum*), oil-palm (*Elaeis guineensis*), cotton (*Gossypium hirsutum*), castor (*Ricinus communis*) and maize (*Zea mays*) at a dosage of 5 ml/kg of common bean seeds and a control of malathion dust 2% active substance (a.s.) at a dose 0.5 g/kg of seeds for their ability to
suppress the populations of *Zabrotes subfasciatus*. Castor and palm oils resulted in effective protection comparable to that of malathion. There were a significant low percentage seed damage and weight loss in seeds treated with malathion, castor and palm oils. Total number of weevils in these treatments was least, compared to other plant oils studied. All treatments did not show adverse effect on germination capability of the seeds. This study showed that it is possible to use castor or palm oils to protect common bean seeds against *Z. subfasciatus* infestations.

Tinzaara *et al* (2006) tested crude extracts of chinaberry tree (*Melia azedarach* L.), mexican marigold (*Tagetes* spp.), water hyacinth (*Eichornia crassipes*, Martius) and Castor oil (*Ricinus communis* L.) for their effect on weevil mortality, settling responses and oviposition in the laboratory. All extracts of the botanicals did not show significant effects on weevil mortality compared to controls. Weevil settling responses on corms treated with extracts of botanicals compared to controls were statistically similar after 1 h and 72 h of observation. Oviposition was significantly low on corms treated with *M. azedarach*, *Tagetes* spp and *R. communis* compared to controls. Oviposition on corms treated with water hyacinth extracts was not statistically different from oviposition on controls. The data indicates that botanicals possess limited insecticidal properties but the potential of *M. azedarach*, *Tagetes* spp and *R. communis* to control the weevil through preventing oviposition needs further investigation.

2.4 Bio-agents in managing major insect pests.

The research of several workers on the use of *Bacillus thuringiensis* and *Beauveria bassiana* for the management of various insect pests on different crops of economic importance has instigated the idea that managing *Leptocorisa* spp. in a similar manner can be achieved. A review on the different bio-agents against various insect pests is presented below.

2.4.1 *Bacillus thuringiensis*

Anitha *et al* (2011) characterized *Bacillus thuringiensis* isolates rhizospheric cotton soils by using acetate selection process from eight different locations in South India. The fact
that *B. thuringiensis* indices were higher in proportion in soil samples taken from Tamil Nadu and Karnataka than from Andhra Pradesh, indicates the abundance of *B. thuringiensis* populations in the cotton rhizosphere. Biochemical typing of the isolates designated eight local isolates (BtNg13, BtCo1, BtHyb7, BtAm2, BtRm5, BtWr3, BtPl4, BtN 9), which belong to subspecies *kurstaki*, the most prevalent subspecies. Toxicity assays on American boll worm larval (*F*1) populations collected from the Andhra Pradesh, Bangalore and Coimbatore regions, with a susceptible insect strain against different isolates of *B. thuringiensis kurstaki* spore-crystal mix, revealed distinct susceptibility patterns and specificity. The highest susceptibility was observed in the *F*1 populations of Coimbatore, followed by Bangalore and Hyderabad populations, in comparison with the susceptible insect strain. Significant differences were observed (*p* < 0.0005 and CD = 5.3975) among Btk local isolates, *H. armigera* biotypes, Btk spore-crystal mix concentration and their interactions, through the Multifactorial ANOVA analysis. The toxicity of local *B. thuringiensis* isolates was higher than that of HD-1 (reference *B.t.k* strain). Indigenous *Btk* isolates have an enormous potential for the management of *H. armigera* in terms of development of resistance to HD-1. The present study would serve as a baseline data for future resistance monitoring of *B. thuringiensis* strains in *H. armigera* in Southern India.

**Zibae et al (2010)** examined potential effects of medicinal plants, *Artemisia annua* and *Lavandula stoechas* and the insect pathogenic bacterium *Bacillus thuringiensis* var. *kurstaki* on activities of digestive enzymes (α-amylase, α- and β-glucosidase, lipase and proteases) and lactate dehydrogenase (LDH) in *H. cunea* by using two hosts, mulberry and sycamore. Results showed that *B. thuringiensis* var. *kurstaki* and plant extracts when administered orally, affected the digestive enzyme profiles of *H. cunea*. Combined effect of *B. thuringiensis*, *A. annua* and *L. stoechas* extracts on mulberry decreased the activities of digestive enzymes in a dose-related manner, except for β-glucosidase and lipase. When larvae were treated by different concentrations of the mentioned insecticides, LDH activity increased i.e. the higher activity was obtained by *B. thurengiensis* alone and *B. thurengiensis* and *L. stoechas* extracts together. The least activity was observed in the case of *L. stoechas* extracts alone on both hosts.
Physiological analysis would be particularly informative when using combination of biopesticides to enhance the efficiency of a safe management process.

**Xie et al (2010)** isolated 218 *Bacillus* isolates from the dissected guts of 100 diapausing larvae of the silkworm, *Bombyx mori*, collected from silkworm farmers in the Hangjiahu area of Zhejiang Province. Six isolates were identified as *Bacillus thuringiensis* strains. The strain named as W015-1 is highly toxic to the lepidopteran *Plutella xylostella*, and is deposited in the HITAR *Bacillus* Collections with Accession No. 20050509W015. Larvicidal assays of the trypsin-activated form of Cry1Ac22 were carried out and demonstrated high insecticidal activity against larvae of *Plutella xylostella* (LC$_{50}$: $4.135 \times 10^8$ cfu/mL; 95% FL: 3.368~5.122×108 cfu/mL), which was much higher than that of model strain HD 73. W015-1 and the reference strains are dissimilar with differing in plasmid profiles, cry genotypes and crystal proteins. Thus, it is believed that *Bt* W015-1 could be a potential biopesticide alternative to *Btk*.

**Borgio (2010)** studied RNAi mediated gene knockdown in sucking and chewing insect pests as no effective *Bt* toxins are known against sap-sucking homopteran pests such as aphids, leafhoppers etc. The RNAi was applied to block different proteins biosynthesis by sucking insect pests. To achieve the objectives, clones were selected and transcribed to dsRNA. The transcribed dsRNAs were digested with RNase III to prepare siRNAs. Fifty micro liter volumes of test samples containing either control reagent or siRNA in varying quantities were mixed with the insect diet (1ml and 1g for sucking and chewing pests). Five different concentrations, 40, 20, 10, 5, 1 μg/ml of siRNA from a gene were applied to find out the concentration required to kill 50% of insects. The siRNA treatment resulted in specific gene silencing (not significant) and consequently brought very less mortality percentage. The present results suggest that feeding of siRNAs through an artificial diet can be exploited for the screening of siRNAs for insect pests control and functional genomic studies in both sucking and chewing insect pests.

**Eswarapriya et al (2010)** evaluated the genotype of indigenous *Bt* isolate through phase contrast microscopy, biochemical characterization, plasmid profile, SDS and insecticidal
activities. The plasmid and protein profile differed from the reference strain *Bacillus thuringiensis entomocoids*. However, biochemical characterization revealed the isolate as *Bt*. The insecticidal toxicity of the isolate for *Plutella xylostella* (Diamond black moth) was similar to that of the reference of strain causing high toxicity. Further characterization is necessary to develop this strain as biopesticides.

Valicente et al (2010) tested different media to grow *B. thuringiensis*. The seed culture (strain 344, *B. thuringiensis tolworthi*, belonging to Embrapa Maize and Sorghum Microorganism Bank) was produced using shake flasks and grown in LB medium plus salts during 18 hours, incubated on a rotary shaker at 200 revolutions per minute (rpm) at 30°C for 96 hours. Medium 1 was composed of: Luria Bertani (LB) plus salts (FeSO₄, ZnSO₄, MnSO₄, MgSO₄), and 0.2% glucose; medium 2 was composed of 1.5% glucose, 0.5% soybean flour plus salts; and medium 3 was composed of liquid swine manure at 4% and 0.2% glucose. All three media were sterilized and inoculated with *B. thuringiensis tolwothi* (seed culture) at a stirrer speed of 200rpm, for 96 hours at 30°C. The pH was measured at regular intervals. Media 1 and 2 showed a tendency to shift toward a basic pH and medium 3 to an acidic pH. Media 1 and 2 showed the highest number of viable spores, 2.0 x 10⁸ c.f.u/mL, within the 96 hours of incubation, however medium 2 showed a biomass dry weight of 1.18g/L. During the fermentation period, medium 1 showed the highest spore concentration, 1.4 x 10⁹ spores/mL after 96h of fermentation. Efficiency against *S. frugiperda* first instar larvae showed that all *Bt* produced in all three media killed above 60% in the highest concentrations.

Zakeel et al (2009) collected and isolated *B. thuringiensis* strains present in surface and sub-surface soil samples from Nochchiyagama by 0.25M sodium acetate selection method. Isolated *B. thuringiensis* was grown on Luria Bertani agar medium and stained by Gram staining procedures. Sixty isolates of *B. thuringiensis* were identified by Coomassie Blue staining procedure and characterized based on colony morphology, crystal shape, plasmid profile and bioassay. Results revealed that sub-surface samples had more *B. thuringiensis* counts than surface soils. This study also indicated that *B. thuringiensis* was abundant in soils contaminated with animal wastes. All the isolates
formed ‘pan cake’ shape circular colonies with smooth or serrate margins with varying diameter. Fifty five isolates were found to have rod shape crystals, 4 were spherical shape and only one isolate had rhomboidal shape crystal. Out of 60 isolates, 36 isolates were toxic to the third instar larvae of *Aedes aegypti*.

**Ozturk et al (2009)** isolated five *B. thuringiensis* strains from soil samples and evaluated them in terms of their novel activities according to the following criteria: parasporal inclusion morphology, SDS-PAGE, plasmid DNA patterns, toxicity against *Ephestia kuehniella*, and detection of *cry1, cry2, cry3, cry4, cry5, cry7, cry8, cry12, cry21* and cyt type genes with PCR. One strain, named as F21, gave positive results with both *cry1* and *cry2* general primers in PCR. However, two strains, named as F16 and F19, gave positive results only with *cry2* general primers. The PCR amplified region of *cry1* gene for F21 showed 97% similarity to *cry1Ac* and *cry2* gene for F21, F16 and F19 showed 96% similarity to *cry2Ab*. The F21 and F19 isolates exhibited the highest toxic activity against *Ephestia kuehniella*, resulting in 83% and 80% mortality respectively. Three *B. thuringiensis* isolates produced typical inclusions, which were spherical, bipyramidal and cuboidal in shape, associated protein bands being approximately 130 and 65 kDa. The most larvaecidal isolates for *E. kuehniella* were F21, F19 and F16, with LC50 values of 1.08, 1.48, and 2.17, respectively.

**Shahid et al (2003)** used biopesticides based on fungus *Metarhizium anisopliae* and bacteria *Bacillus thuringiensis* on stem borer and leaf folder of rice, which have reduced the population of these pests effectively both in laboratory and in the field. In the laboratory bioassays CAMB biopesticides were very useful on insect larvae of *Helicoverpa armigera*. By using the combination of fungal and bacterial formulation the increase in mortality was studied and it was 100% after 96h. In the field trials a significant effect on stem borer and leaf folder was observed.

**Pinto and Fiuza (2003)** used PCR and bioassays screening of *Bacillus thuringiensis* isolates from rice fields of Rio Grande do Sul, specific to lepidopterans and coleopterans. The screening isolates showed that 56.51% were potentially lepidopterans specific and
21.73% were coleopteran specific, with a homogeneous distribution in the rice field. Bioassays against *Spodoptera frugiperda* larvae showed the highest corrected mortality (25%) with *Bt* 2027-1 isolate selected by the presence of *cry9* genes. The toxicity bioassays carried out with *S. frugiperda* using purified proteins of *Bt* aizawai HD68 indicated an LD$_{50}$ of 0.95 µg/larva. Two *Bt* isolates carrying the *cry3* genes caused a 100% mortality to *Oryzophagus oryzae* larvae.

*Singh et al (2000)* evaluated *Bt* based biopesticides, *viz.* Dipel 8L, Biolep and Halt in bioassay studies against third instar larvae of *Plutella xylostella* (Linnaeus). The LC$_{50}$ values for the three products were 0.013, 0.039 and 0.22 percent, respectively. The slope values for the probit-regression equation were 1.28, 1.49 and 1.43 for Dipel 8L, Biolep and Halt, respectively. Dipel 8L was found to be most promising in the bioassay studies and was used in further studies for food consumption and indices of growth of *P. xylostella* larvae as affected by feeding on *Bt* treated food. There was a concentration dependent decrease in the amount of food consumed by the surviving larvae. The indices of growth like consumption index, relative growth rate, approximate digestibility and efficiency of conversion of ingested food to body substance of such larvae were also adversely affected.

*Travers et al (1987)* were able to isolate *Bacillus thuringiensis* from environmental samples with a background of 109 bacteria per g of soil. Our selection process differed significantly from classical selection methods which permit only the desired organism to grow. In our process, germination of *B. thuringiensis* spores was selectively inhibited by sodium acetate, while most of the undesired spore formers germinated. Next, all of the non-sporulated microbes were eliminated by heat treatment at 80°C for 3 min. The surviving spores were then plated on a rich agar medium and allowed to grow until they sporulated. Of random colonies picked from agar, 20 to 96% were crystal-forming *Bacillus* species. Further, *B. thuringiensis* and *B. sphaericus* were routinely selected by this method.
2.4.2 Beauveria bassiana

Broglio-Micheletti et al (2012) evaluated different isolates and concentrations of conidia of *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883 and *Beauveria bassiana* (Balsamo) Vuillemin, 1912 for control of the cattle tick. The conidial suspensions were prepared from fungi grown in rice medium in polypropylene bags. The tests were performed by immersion of engorged females collected from animals not treated with acaricides. The mortality rate caused by the fungus *M. anisopliae* ranged from 92 to 100%, while for *B. bassiana* it was 44 to 100%; mortality was greater at concentrations of 10⁸ and 10⁹ conidia mL⁻¹ suspension. The isolates *B. bassiana* Fitossan 1 and *M. anisopliae* PL 43 (both at 10⁹ conidia mL⁻¹) and Fitossan 4 (10⁸ conidia mL⁻¹) within 14 days of treatment killed 100%, 92% and 88% of the engorged females, respectively. In general there was no difference in the weight of eggs from females treated with the same isolate at concentrations of 10⁷, 10⁸ and 10⁹ conidia mL⁻¹, although isolates of *M. anisopliae* had lower weight. The efficiency of control ranged from 0% (control with water) to 31.30% (*M. anisopliae* Fitossan 4, 10⁸ conidia mL⁻¹).

Kaur et al (2011) tested the virulence of *Beauveria bassiana* against second, third and 4th instar larvae of *Spodoptera litura* using three concentrations i.e. 2.03 × 10⁸, 4.03 × 10⁶ and 1.47 × 10⁵ spores/ml. All the treatments resulted in significantly higher mortality than control. Besides mortality, sub-lethal effects were also evaluated on larvae that survived fungal infection. Significant decrease in larval period was observed due to infection as compared to control. The life span of females emerging from treated larvae was half that of the control females. In addition to this, inhibitory effects were also manifested as reduced reproductive potential. The eggs descended from treated larvae showed significant decrease in hatchability. *B. bassiana* also induced pupal and adult deformities. A significantly higher number of deformed adults were observed at lower concentrations as compared to the highest concentration.

Ugine (2011) investigated how temperature and *Beauveria bassiana* (Balsamo) Vuillemin infection impacted *L. lineolaris* fecundity and longevity, with the goal of determining what temperatures optimized *B. bassiana*’s control potential. *B. bassiana*-
treated adult females and controls were maintained at constant 18 °C, 21 °C, 25 °C, 30 °C or 32 °C. The number of eggs laid was determined daily until death. \emph{B. bassiana} caused large reductions in the total number of eggs laid (45–76%) at all temperatures; however, there was no effect of infection on the daily rate of egg production. There was not a sub-lethal effect of infection on egg production on the last full day of life. Intrinsic rates of natural increase and population doubling times were marginally affected by treatment with \emph{B. bassiana}; doubling times increased with treatment a mean of 3.5 days (range 0.5–7.7 days). There was a temperature dependent trend in the length of the pre-oviposition period and the days to death after treatment with \emph{B. bassiana}. As temperature increased from 18 °C to 32 °C, the number of days to death converged on the pre-oviposition period. The maximum difference was observed at 18 °C (5.3 days different) compared to 30 °C and 32 °C (0.3 days). These results strongly suggested that treatment of tarnished plant bugs with \emph{B. bassiana} should be performed when plant bugs are in diapause or when temperatures are cool to minimize reproduction during the disease incubation period.

\textbf{Kodjo et al (2011)} evaluated the efficacy of 5 fungal isolates from the genera of \emph{Beauveria, Verticillium, Paecilomyces} and \emph{Metarhizium} to the green leafhopper, \emph{Empoasca decipiens} nymphs under laboratory conditions. A single exposure concentration (1 x 10^7 conidia/ml) assays for each isolate were first conducted by contact with treated disc leaf for 5 min. These were followed by multiple-concentration assays on three of the most pathogenic isolates using four test concentrations ranging from 1 x 10^3 to 1 x 10^6 conidia/ml. All isolates proved to be highly virulent to late fifth-instar nymphs of \emph{E. decipiens}, with mortality ranging from 60 to 98% and the mean survival time (MST), from 4.30 to 6.09 days. Among the tested isolates, three isolates, \emph{M. anisopliae} (Ma43), \emph{B. bassiana} (Bba113) and \emph{P. fumosoroseus} (Pfr12) were the most pathogenic to the nymphs, resulting in significantly higher mortality and lower MST values. In the multiple-concentration assays, nymphal mortality was dose-dependent. The LC_{50} values ranged from 0.8 to 5.0 x 10^5 conidia/ml with the third- and fifth-instar nymphs being more susceptible than the first-instar. All tested strains were able to complete their life
cycle by forming conidiospores on the dead insects; in general one to two days after the host had died.

Filho et al (2011) assessed the efficiency of formulated Beauveria bassiana-based myco-insecticides to control Myzus persicae (Sulzer) in cabbage under field conditions. Aqueous conidial suspensions (0.01% Tween 80 + 0.01% v/v Agral) of three fungal isolates were sprayed twice at different dates, each with 2.0 x 10^9 viable conidia per potted plant using screened cages. The number of nymphs and adults of M. persicae per leaf was significantly reduced in plots treated with isolates CG 864 and PL 63, with control efficiency ranging from 57% to 60%. Further field trials using screened cages with isolate CG 864 formulated as oil dispersion reduced the aphid population by 85-87% as compared to the control, whereas a 71% reduction was seen in plants treated with the aqueous conidial suspension 20 days following the first spray. The last experiment was conducted in a commercial cabbage field (without cages), in which the fungus was applied at three different dates, each with an equivalent of 1.0 x 10^13 viable conidia/ha. The reduction in the number of aphids per leaf was more evident between four and five weeks following the first spray, resulting in 76-83% and 57-65% control efficiency for oil dispersions and unformulated conidia, respectively. However, with the exception of imidacloprid-treated plants, rapid aphid re-infestation was observed in all treatments. In this study, the stand-alone use of myco-insecticides for aphid control was not a satisfactory strategy, although utilization of B. bassiana in IPM strategies remains a field to be explored.

Karthikeyan and Jacob (2010) assessed the biological efficiency of white muscardine fungus, Beauveria bassiana against Rice Blue Beetle, Leptispa pygamea Baly (Coleoptera: Chrysomelidae) under both laboratory and field conditions. Results of the experiment revealed that the fungus had a cumulative adult mortality of 56.67 to 80.00 per cent at 105 to 109 spores/ml and the LC_{50} value was 2.26 x 104 spores / ml under laboratory conditions. Under field conditions, the fungus brought about 61 to 72 per cent reduction of damage over untreated control and was superior to all tested biopesticides, paraffin oil @ 2%, neem oil @ 2% and azadirachtin 1% @ 0.004% and were on par with
efficacy of tested synthetic chemical insecticides, chlorpyriphos @ 0.05 % and carbaryl @ 0.2 % against the beetle. The persistent toxicity of B. bassiana was on par with other eco-friendly insecticides viz., azadirachtin 1% (Econeem) and neem oil.

Prasad and Syed (2010) investigated one microbial biopesticide Beauveria bassiana (Balsamo), vUILIemin, of fungal origin against one of the most notorious pest Helicoverpa armigera (Hubner) which alone shares a major portion of damage to various agricultural crops. When four different concentrations (0.1, 0.125, 0.2 and 0.25 x 10^8 conidia per ml) of fungus Beauveria bassiana (Balsamo), vUILIemin were used against 3rd larval instar of Helicoverpa armigera (Hubner), 86.7 percent mortality was observed at highest dose level against 23.20 percent in control. ANOVA results further supported the mortality data; F value was significant at 1 percent level for different concentrations. Besides causing such heavy mortality, treated instar revealed various morphological abnormalities such as extensive hair growth, swollen body and lethargic larvae covered with fungal mycelium. These abnormal larvae were unable to moult in fourth instar. Thus the results shows that inclusion of this fungal biopesticide in the pest control strategy against Helicoverpa armigera (Hubner) can be a successful step in removing chemical pesticides from the environment.

Shefik (2010) evaluated the effect of the entomopathogenic fungi Beauveria bassiana on the various developmental stages of Phthorimaea operculella. Both first and second instars larvae were more susceptible than the third and fourth instars. The infected prepupae and pupae resulted in marked decreases in the emergence and longevity of moths, deposited eggs and egg hatchability. An obvious increase in the pupal duration was observed and malformed adults were also recorded. The latent was markedly obvious, especially in high doses of B. bassiana.

Loc and Chi (2007) studied Metarhizium anisopliae (M.a) and Beauveria bassiana (B.b) to exploit their potential for controlling the diamondback moth (DBM). The results in laboratory and greenhouse showed that all of four selected isolates of M. anisopliae and B. bassiana which have been isolated from different naturally infected insects were found
to be pathogenic to the tested DBM. The M.a (OM3-STO) isolate which was isolated from naturally infected DBM exhibited the highest infectivity to DBM. In the field experiments, all of four selected isolates of *M. anisopliae* and *B. bassiana* were found to be effective for controlling the DBM. The efficacy could be seen from 5 DAT. Among them, M.a (OM3-STO) isolate, which was isolated from naturally infected DBM exhibited highest efficacy for controlling the DBM, next is B.b (OM2-SDO) and then M.a (OM1-R). The cauliflower yield of the three above fungal treatments was not significantly different as compared to that of the specific bio-insecticide (Crymax_35WP) treatment. There was 73.2, 68.2 and 66.7 percent increased in cauliflower yield in M.a (OM3-STO), B.b (OM2-SDO) and M.a (OM1-R) treatment, respectively, as compared to untreated control. These results indicated that M.a (OM3-STO), M.a (OM1-R) and B.b (OM2-SDO) have good potential as microbial control agents for diamondback moth of cruciferous crops.

**Loc and Chi (2005)** conducted experiments in the greenhouse and field to evaluate the efficacy of some new isolates of *Metarhizium anisopliae* (M.a) and *Beauveria bassiana* (B.b) against rice ear bug (REB), *Leptocorisa acuta*. The results in greenhouse showed that all of 12 selected isolates of *M. anisopliae* and *B. bassiana* which have been isolated from naturally infected insects during 2003-2005 were found to be pathogenic to the tested REB. The mortality percentage of *L. acuta* caused by *B. bassiana* and *M. anisopliae* isolates ranged from 57.5 to 77.7% and from 74.7 to 87% at 10 DAT, respectively. Among 12 new selected isolates, M.a (OM3-BD), M.a (HG3-B) and M.a (HG5-BD) exhibited higher pathogenicity to REB as compared to the rest. In field experiments, all of 12 selected isolates of *M. anisopliae* and *B. bassiana* were found to be effective for controlling REB, the efficacy was seen from 7 DAT and reached to its highest peak at 14 DAT. The field mortality of *L. acuta* caused by *B. bassiana* and *M. anisopliae* isolates ranged from 45.3 to 74.9% and from 63.6 to 86.6% at 10 DAT, respectively. *M. anisopliae* showed better efficacy against REB as compared to *B. bassiana*. Among 12 new selected isolates of M.a and B.b which have been tested, M.a (OM3-BD), M.a (HG3-B) and M.a (HG5-BD) exhibited higher efficacy against the REB as compared to the rest.
Wraight and Ramos (2005) applied commercial biopesticides based on the fungal pathogen *Beauveria bassiana* strain GHA and the bacterial pathogen *Bacillus thuringiensis tenebrionis* alone and in combination (tank mixed) against larval populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, in small plots of potatoes. Interactions between the two products were evaluated in terms of pest-control efficacy. *Beauveria bassiana* (formulated as Mycotrol) was applied at low and medium label rates of 1.25 and 2.5x10^{13} conidia/ha, and *B. thuringiensis* (formulated as Novodor) was applied at low and high label rates of 40.3 and 120.8x10^{6} Leptinotarsa units/ha. Two weekly applications of the bacterial pesticide alone provided 50–85% control of beetle larvae within 14 days after the initial application, while applications of the mycopesticide alone produced no greater than 25% control. Maximum control, in nearly all tests, was produced by the combination of the two products. The combined treatments produced a statistically significant 6–35% greater reduction in larval populations than would have been predicted had the two biopesticides acted independently. These results indicate that *B. thuringiensis* and *B. bassiana* have strong potential for integrated biologically based management of Colorado potato beetle.

Samuels and Coracini (2004) screened isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against 4th instar and adult *Blissus antillus* with the aim to develop a biological control program for this important pasture pest. Ten fungal isolates were initially screened and three isolates were chosen for further investigation. To determine virulence, insects were inoculated by immersion in concentrations of 5x10^{8} conidia mL^{-1}. Mortality was evaluated for 10 days. *B. bassiana* ARSEF 792 was the most virulent isolate to both nymphs and adults, causing 53 and 78% infection, respectively, and values for LT_{50} of 7.8 and 5.0 days, respectively. Germination studies were carried out to confirm viability and determine speed of germination as a pathogenicity factor. The production of conidia on the cadavers of insects infected with the three selected isolates was determined. The production of conidia on rice media was also evaluated. *B. bassiana* CG 24 produced the highest number of conidia on insects cadavers (14.9x10^{7} conidia per insect) and also on rice media (10.6x10^{9} conidia per g).
Legaspi et al (2000) performed laboratory and field evaluations on the use of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (strain GHA) against the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae). We also performed bioassays against the sugarcane borer, *Diatraea saccharalis* (F.) (Pyralidae), a key pest in other sugarcane growing areas. In the bioassays, *E. loftini* was substantially more susceptible to *B. bassiana* than *D. saccharalis*, based on both 5-d LD$_{50}$ values and survival times. A commercial oil-based formulation of *B. bassiana* was evaluated in the field using the following treatments: oil alone (control), *B. bassiana* + oil, and *B. bassiana* + Silwet L-77 carrier at an application rate of $5 \times 10^{13}$ spores per hectare. Neither numbers of *E. loftini* per stalk, nor stalk damage ($\approx 20\%$ bored internodes) were significantly affected by treatment. The application of *B. bassiana* + Silwet significantly affected the numbers of internodes showing high damage, but not those with low or medium damage. Analysis of yield data and juice quality showed no significant treatment effects. We conclude that the application of *Beauveria* + Silwet offers the best chances for reducing damage caused by *E. loftini* of those treatments tested. However, reductions in insect incidence or damage did not result in measurable increases in yield or sugar quality, probably because of insufficient coverage. Effective control of stalk boring pyralids in sugarcane using *B. bassiana* will likely require improvements in delivery technology.

Poprawski et al (2000) tested conidial suspensions of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith for pathogenicity to third-instar nymphs of *Trialeurodes vaporariorum* (Westwood) reared on cucumber and tomato plants. Nymphs were highly susceptible to infection by both fungi after a one-time application of conidia onto cucumber plants. In contrast, insects reared on tomato plants were significantly less susceptible to infection. We hypothesized that the glycoalkaloid tomatine might have been involved in anti-microbiosis on tomato leaves. Tomatine mixed with Noble agar at 5 concentrations was tested for its effects on germination of conidia of both fungi. Germination of conidia of *B. bassiana* was only slightly affected at the two highest concentrations of tomatine. In contrast, germination of conidia of *P. fumosoroseus* was completely inhibited at 500 and 1,000 ppm of
tomatine. The in vitro tolerance of tomatine by *B. bassiana* contradicted our in vivo data. Sequestered tomatine by *T. vaporariorum* nymphs would explain, at least partially, the insect’s defense against the pathogens. That little in vitro inhibition of *B. bassiana* was found supported the hypothesis that *B. bassiana* was inhibited only in vivo, after the penetration process. Inhibition of *P. fumosoroseus* might have occurred on the insect’s cuticle before penetration, as evidenced by the complete inhibition of spore germination in vitro in the presence of tomatine at 500 and 1,000 ppm. An explanation for the differential in vitro sensitivity of *B. bassiana* and *P. fumosoroseus* to tomatine is being sought.

**Tuan and Gabriel (1996)** evaluated the virulence of three isolates of *Beauveria bassiana* (Bals.) Vuill. to the rice bug, *Leptocorisa oratorius* (F.). All isolates tested were able to infect *L. oratorius* in the laboratory. GLH 9 isolate was most potent with LC$_{50}$ of 4.65 x 10$^9$, 2.22 x 10$^9$ and 1.3 x 10$^9$ conidia/ml for third instar, fourth instar, fifth instar and adults, respectively. LT$_{50}$ was 7.95 days (third instar); 5.52 days (fourth instar); 3.37 days (fifth instar) and 2.89 days (adults). The test also showed that immature stages (3rd - 4th instars) were more resistant to fungal infection than late instar (5th) and adults. The results of the study revealed that *B. bassiana* is highly pathogenic to *L. oratorius* under laboratory conditions.

**2.5 Reports on combinational studies with eco-friendly agents**

At the National Symposium on ‘Microbials in Insect Pest Management’ held in February 2000 in Chennai, India; it was concluded that combinational studies, such as botanicals with microbials, should be focused on when searching for alternatives to chemical insecticide for the control of insect pests. It was highlighted that *Bt* and Neem Azal were found to be more effective on *Spodoptera litura*, as well as, additive and synergistic interactions of nematodes, *Steinernema carpocapsae* and *Bt* showed considerable effect on the root grub, *Holotrichia serrata* (*Ignacimuthu, 2000*).

The research of several workers on the use of botanicals in combination with bio-agents for the management of various insect pests on different crops of economic importance is presented below.
Rondelli et al (2013) evaluated the insecticidal activity of Beauveria bassiana (Bals.) Vuill. and castor bean oil and mixtures of both components against the diamondback moth. To do so, we separately used castor bean oil (at 2% concentration), the isolate ESALQ-447 and a commercial formulation (Boveril® WP), and a mixture of castor bean oil with the isolate and the B. bassiana product formulation, totaling six treatments with a control. Assays were carried out under greenhouse with the respective treatments sprayed on cabbage plants infested with four second instar larvae of P. xylostella. The evaluated parameters were larval mortality and pupal and larval viability. All treatments reduced larval viability in relation to the control, however, only the ESALQ-447 isolate or a mixture of the isolate with castor bean oil reduced pupal viability, significantly reducing the pest population levels in the next generation. Castor bean oil mixed with B. bassiana, however, did not augment pest mortality.

Srinivasan (2012) reported that bio-pesticides have high compatibility with other pest management techniques and even synergistic effects are evident. He revealed that there is evidence available for the synergistic action of neem with microbial pesticides such as NPVs of tomato fruit worm and common army worm, and entomopathogenic fungi (B. bassiana) against common army worm.

Bajwa and Ahmad (2012) studied the effect of azadirachtin in combination with Nuleopolyhedrovirus on the enzyme activity of the midgut of Spodoptera litura. It was concluded that enzyme activities of the gut were decreased both when azadirachtin and NPV were used individually and in combination. Gut enzymes, acid phosphatases, alkaline phosphatases, adenosine triphosphatases and lactate dehydrogenases activities were reduced when S. litura larvae were fed with a diet of castor leaves which were pretreated with azadirachtin and NPV. A maximum weight loss was also noted.

Amutha et al (2010) revealed that compatibility of isolates of Beauveria bassiana with azadirachtin formulations were investigated; however contradictory results were reported. For instance, neem oil was found compatible with B. bassiana by several authors but was reported to be inhibitory by other authors. They suggested that the observed differences could be due to inherent variability of chemicals to biological creatures.
Depieri et al (2005) studied the compatibility of a commercial formula of emulsible neem oil (Azadirachta indica A. Juss.) and of aqueous extracts of neem seeds and leaves with Beauveria bassiana (Bals.) Vuill. *in vitro*. Three experiments were conducted to evaluate the effect of each product on the fungus vegetative growth and on conidia production and viability. The products were incorporated to a culture medium (BDA+E) and distributed into petri dishes, in the following concentrations: 0.15%; 1.5% and 15% (leaf aqueous extract), 1%; 2% and 4% (seed aqueous extract) and 0.5%; 1% and 1.5% (emulsible oil). Vegetative growth and conidia production were the basis for characterization of the aqueous extracts of seeds and leaves and of the emulsible oil, using the T classification model for compatibility of products. Seed and leaf extracts were less harmful to *B. bassiana* than the emulsible oil. Under the tested concentrations, the oil was not compatible with *B. bassiana*, inhibiting conidia vegetative growth significantly and decreasing production and viability of conidia, particularly at higher concentrations.

At the conclusion of this review, it was quite apparent that the botanicals and bio-agents studied against the different insect pests were employed as single components per treatment, either using different dose rates or in comparison with others. Very few compatibility studies have been done; as such, diversifying the cause of insect mortality is relatively unemployed. Using a single botanical or bio-agent is exposing them to the evolving problem of insect resistance, such as the case with *Bacillus thuringiensis* and many chemical insecticides.

Insect pest management is moving towards bio-intensive Integrated Pest Management (IPM) where the use of chemical insecticides should only be at the time of pest outbreaks when population density will exceed economic threshold levels. Bio-intensive IPM will prevent the over exposure of a single botanical or bio-agent to any particular insect pest. As such, it is mandatory to find out suitable and compatible botanicals with bio-agents for specific insect pests. Therefore, investigations during this study aimed at finding effective botanicals and bio-agents, either alone or in combination, for the management of gundhi bug, *Leptocorisa* spp.