
Non-target Effects of Botanicals on Beneficial Arthropods with Special Reference to *Azadirachta indica*

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Abstract

The 'green' agriculture through better ecological approaches in pest management is the stimulus for further research in alternate pesticides molecules barring synthetic ones, which led to increased search in plant sources. Among several options, neem, *Azadirachta indica*, has emerged as pinnacle of plant origin pesticides. Undoubtedly, the use of neem and its products in agriculture are increasing day by day, but its safety to the environment is always questioned. This review focuses on the biological activity of the plant origin insecticides on entomophages (parasitoids and predators), honeybees and the environment and provides ways and means to use them in integrated pest management. In general, the plant-derived pesticides, although effective against insect pests of agricultural importance, spare the beneficial fauna comparative to synthetic pesticide because of their capacities of biodegradable nature and innate low mammalian toxicity. Although some plant-derived products either in crude or in formulation showed slight to moderate ill effects to beneficial fauna including parasitoids, predators and honeybees during the application may greatly reduce the risk. No residual/persistent toxicity of neem or other botanical pesticides in the environment has so far been reported. In the context of organic agriculture, plant origin pesticides especially the neem-derived extracts/formulations and other bio-control agents are the befitting components to reduce the input costs and environmental risks posed by the synthetic chemical pesticides.

Keywords

Botanical insecticides • Non-target effects • Predator • Parasitoids • Honeybees • Environment

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1 Introduction

Entomophages, namely, parasitoids and predators, are natural enemies of insect pests in agroecosystems. Most insecticides used to protect the cultivated crops are relatively broad spectrum, killing the target insect pests and the non-target beneficial insects as well. But substances derived from plant resources have been generally considered safe compared to synthetic chemical insecticides. All the same, not all substances derived from plant resources are always safe. Sometimes adverse effects are also produced. Safety aspect of plant-derived substances is very important since there are many beneficial insects which contribute immensely for crop productivity and agricultural sustainability (Schmutterer 1992; Koul et al. 2004; Raguraman 2009; Isman 2013). Different kinds of parasitoids and predators play a very important role in natural control of insect pests. Many kinds of pollinators contribute to cross-fertilization of plants. Hence, it is important to protect all these from any harm. Conservation of beneficial insects is achieved by modification in insect control practices that allow beneficial insects to survive and promote their potential to suppress insect pests. This review focuses the impact and safety of plant origin insecticides to parasitoids and predators both in laboratory and field studies and envisages the strategies and application methods to achieve better pest management. For easy reading and documenting by the researchers, the plants are discussed alphabetically by scientific names under effects on parasitoids, predators, honeybees and environment.

2 Effect on Egg Parasitoids

Aqueous extract of *Acorus calamus* was reported to be safe to *Trichogramma japonicum* (Burman et al. 2003). With reference to neem, *Azadirachta indica*, as early as 1982, the side effects on egg parasitoids were studied in India by Joshi et al. (1982). They reported that 2 % of neem seed kernel extract (NSKE) applied on the egg masses

of *Spodoptera litura* did not repel the egg parasitoid, *Telenomus remus*. When the treatment was carried out of pre-oviposition of the parasitoid, the emergence of adult parasitoids was normal, but their duration of life was shorter than that of controls. On the other hand, spraying NSKE after oviposition, *T. remus* increased the fecundity of the wasps developed in treated eggs and prolonged their life as compared to that of untreated controls. Li et al. (1986) tested in the laboratory 29 insecticides including *Bacillus thuringiensis* and neem oil in order to study their side effects on *Trichogramma japonicum* and concluded that neem oil was the safest pesticide for the parasitoid.

Studies were made on the effect of host species on the sex ratio and parasitism rate of *Anastatus ramakrishnanae* in the laboratory and field in Tamil Nadu, India, with the pentatomid *Halys dentata* (on *Cassia marginata*, *Azadirachta indica* and *Casuarina equisetifolia*) and the coreid *Homoeocerus prominulus* (on *Cassia marginata*, *Prosopis spicigera* and *Acacia leucophloea*) as hosts. The order of parasitism was *C. marginata* > *A. indica* > *C. equisetifolia* with *H. dentata* and *C. marginata* > *P. spicigera* > *A. leucophloea* with *H. prominulus*. Higher rates of parasitism were recorded with *H. dentata* than *H. prominulus* throughout the year (Velayudhan et al. 1988).

Fernandez et al. (1992) conducted experiments with the eggs of yellow stem borer of rice, *Tryporyza incertulas*, by dipping for 30 s in Bordeaux, neem products and water. The eggs were exposed for 40 h of parasitization by the parasitoid, *Telenomus rowani*. The data revealed the highest mean number of parasitoid emergence of 65.8 % in water treatment, 59.90 % in 5 % aqueous NSKE, 31.4 % in 3 % neem oil and 38.2 % in Bordeaux treatment. Klemm and Schmutterer (1993) applied NSKE (2.5 and 3 %) against *Trichogramma* spp., egg parasitoids of *Plutella xylostella*. *T. principium* accepted neem-treated eggs in the laboratory and *T. pretiosum* in the field, but two treatments prevented the eclosion of adult parasitoids from treated *P. xylostella* eggs completely. Spraying of eggs with 0.2 % neem oil reduced the number of eggs parasitized

per female wasp by 13.3 %. Neem oil also reduced the emergence of *T. principium* from treated eggs by 45.1 %. However, neem seed kernel suspension (5 %) and neem oil 50 EC (3 %) were safe to the parasitoid *T. japonicum* in cotton ecosystem (Jayaraj et al. 1993).

Lyons et al. (1996) used the neem-treated eggs of *Ephesia kuehniella* in shell vials and offered to single females of *T. minutum* for parasitization by fixing the eggs with adhesive strips and held until all parasitoids had emerged from them; variable results were obtained. Azatin, neem EC (4.6 % AZA) and pure AZA were tested at concentrations of 50 g and 500 g/ha. At 50 g/ha, no significant effect was observed, but at 500 g/ha, Azatin and neem EC reduced the female survival by 64 and 40 %, respectively, whereas pure AZA showed neem oil effect. Likewise, at 500 g/ha the number of parasitized eggs was reduced by 89 % by Azatin, 29 % by neem EC and no reduction by AZA. The parasitoid development was reduced by all treatments. Cano and Gladstone (1994) studied the influence of the NSK-based extract NIM-20 on parasitization of eggs of *Helicoverpa zea* in a melon field in Nicaragua. Mass-reared *T. pretiosum* were released at six weekly intervals 1, 2, 6 and 24 h after application of NIM-20 at 2.5 g/l. No negative effect was observed as up to 84 % of the eggs of the pest were parasitized. Oswald (1989) treated the eggs of the coconut bug, *Pseudothrips wayi*, with aqueous NSKE (5 % w/v). The eggs were offered to the parasitoid *Oencyrtus albicrus* (Encyrtidae). There was a significant reduction in the number of wasps emerging from the treated eggs in comparison with controls. Srinivasa Babu et al. (1996) studied the effects of neem-based commercial insecticides such as Repelin and Neemguard on *T. australicum* in laboratory and field conditions. They reported that both the insecticides were relatively safe at lower concentrations, but higher concentrations adversely affected the parasitoids both in laboratory and in field. *Trichogramma chilonis* was affected by *A. indica* extracts (Stansly and Liu 1997). The egg parasitoid, *Trichogramma chilonis*, parasitized 45 % of eggs when host eggs were treated with neem formulation. However, emergence of *T. chilonis* from the parasitized eggs

treated anytime after parasitization was not affected (Markandeya and Divakar 1999).

Similar results were obtained against *T. japonicum* using Econeem and NeemAzal-T/S (0.1–1.0 %) (Lakshmi et al. 1998a), other neem-based pesticides in an IPM for rice pest management (Garg and Baranwal 1998) and egg parasitoid, *Tetrastichus pyrrillae* (Deepak and Choudhary 1998). On the whole, it has been assessed that neem products were fairly safe to *Trichogramma* spp. (Sreenivasa and Patil 1998; Sarode and Sonalkar 1999b). However, some neem formulations such as Nimbecidine (0.25–4.0 %), Neemgold (2.0–4.0 %) and Rakshak (1.0 %) are reported to possess adverse effects on parasitism (Lakshmi et al. 1998a).

Raguraman and Singh (1999) tested in detail the neem seed oil at concentrations of 5.0, 2.5, 1.2, 0.6 and 0.3 % for oviposition deterrence, feeding deterrence, toxicity, sterility and insect growth regulator effects against *Trichogramma chilonis*. Neem seed oil at 0.3 % deterred oviposition (parasitization) by the parasitoid, but the sensitivity varied considerably both under choice and no-choice conditions. Neem seed oil also deterred feeding at or above 1.2 % concentration both in choice and no-choice tests. In feeding toxicity tests, neem seed oil at 5 % concentration caused <50 % mortality to both males and females, but in contact toxicity tests, females were affected sparing males. No sterility effect was observed when the parasitoid was fed with neem seed oil-treated honey. Both pre- and post-treatment of host eggs revealed neem oil adverse effects on the development of the parasitoid.

Thakur and Pawar (2000) tested two neem-based insecticides (3 g Achook/litre and 2 ml Neemactin/litre), two biopesticides [1 g Halt (cypermethrin)/litre and 1 ml Dipel (*Btk*)/litre] and endosulfan (1.5 ml/l) in the laboratory for their relative toxicity to newly emerged adults of *T. chilonis*. Results revealed that neem-based pesticides and biopesticides were harmless, while endosulfan was slightly toxic to egg parasitoid. These observations also get support from the studies on different groups of chemicals, viz., insecticides, moult inhibitors and biopesticides against rice leaffolder *C. medinalis* and its

parasitoid *T. chilonis*. Sprays of monocrotophos 36 WSC applied at 2.0 ml/l caused 100 % larval mortality to *C. medinalis* 7 days after treatment followed by buprofezin 25 WP applied at 3.2 g/l with 66.66 % larval mortality and neem seed kernel extract (NSKE) 5 % (50 g/l) with 63.33 % larval mortality. Application of *Bacillus thuringiensis* subsp. *galleriae* (*Btg*) at 5.0 ml/l and NeemAzal-F 5 % at 1 g/l recorded 56.66 and 53.33 % larval mortality, respectively. More than 90 % emergence of *T. chilonis* was recorded from eggs treated with *Btg* and NeemAzal-F and NeemAzal-T/S followed by NSKE (89.80 %) and buprofezin (82.60 %). Only 73.80 % of adult *T. chilonis* emerged from monocrotophos treated host eggs after parasitization (Saikia and Parameswaran 2001). Similarly, in Thailand, Asian corn stem borer, *Ostrinia furnacalis*, was controlled by neem preparations, and it was observed that such treatments had no side effects on the parasitoid, *T. plasseyensis* wasps (Breithaupt 1995).

Rao et al. (2002) reported that Neemgold^R at 0.015 % had 50.33 % parasitism by *T. chilonis* on *C. cephalonica*. Jyothi and Sannaveerappanavar (2003) tested the aqueous NSKE and neem oil against *T. chilonis* in laboratory. NSKE at 2 and 4 % and neem seed oil at 4 % were moderately toxic to *T. chilonis* adults, causing 33.33, 31.24 and 29.58 % mortality, respectively. Neem oil at 2 and 5 % and NSKE at 4 % significantly reduced the parasitization of *C. cephalonica* to 49.22, 57.41 and 59.29 %, respectively, compared with 88.91 % in the control. NSKE at 2 % did not affect the rate of parasitization. NSKE at 2 and 4 % and neem seed oil at 2 % did not affect the emergence of the parasitoids from treated eggs. Solis et al. (2004) tested four concentrations of the neem seed oil (0.1, 0.5, 1.0 and 5.0 %) *Gryon gallardoi*, an egg parasitoid of *Leptoglossus zonatus* under laboratory conditions. The longevity of adults fed on sugar solution containing the neem oil concentrations was significantly reduced, the only exception being observed in females treated with 0.1 % of oil. Most likely, the vegetal extract caused antifeedant behaviour in adults, mainly when offered in high concentrations. However, the parasitoid emergence from

the host eggs treated with the neem product before parasitism did not suffer any influence, evidencing host acceptance and absence of repellence. The duration time of the immature stages and sex ratio of the parasitoid inside the host eggs topically treated with the product remained unchanged as well.

Moosa-Saber et al. (2004) evaluated the effects of NeemAzal on adult emergence and life table parameters of *Trichogramma cacoeciae*. The effect of NeemAzal on three developmental stages of the parasitoid was tested by dipping parasitized *Sitotroga cerealella* and *Cydia pomonella* eggs at the field recommended concentration 3, 6 and 9 days after parasitization corresponding to larval, prepupal and pupal stages. The emergence of adult parasitoids was adversely affected in both hosts, but the adverse effect was more in *S. cerealella* eggs compared with *C. pomonella*. The adult emergence was reduced by 73.30 and 33.76 % in *Sitotroga* and *Cydia* eggs compared with controls, respectively. Mitchell et al. (2004) evaluated extracts of neem seed kernel on scelionid egg parasitoid, *Gryon fulviventre* of a coreid *Clavigralla scutellaris*. Feeding by newly emerged wasps was dramatically reduced when honey was mixed with aqueous neem suspension, but 6-day survivorship of adults did not differ significantly from that of the control. Wasp oviposition behaviour was altered slightly when coreid eggs were treated with neem: the period of antennation was significantly extended, but time for drilling, oviposition and marking was unaffected. Neem-dipped eggs were accepted for oviposition and progeny emerged successfully from these treated eggs. Exposure of already parasitized eggs to neem did not interfere with progeny emergence, longevity or sex ratio. Thus, neem extract and egg parasitoids seem to be compatible and promising control strategies for *C. scutellaris*.

A. indica extracts were detrimental to parasitoid *Trichogramma pintoi* (Iannacone and Lamas 2003a). Boomathi et al. (2005) reported that the ethonolic extracts of neem + sweetflag and neem + sweetflag + pongamia at 0.12 and 0.18 % exhibited 34 % mortality of adult parasitoids and 79–81 % adult emergence of *T. chilonis* from

C. cephalonica. From a field trial, Thirumurugan and Koodalingam (2005) reported that cane crop sprayed with 5 % (NSKE) + *T. chilonis* at weekly interval had substantially reduced the internode borer and also registered higher yields of sugar. Masood and Mamoon-ur-Rashid (2006) reported that water extract and oil of neem seeds significantly affected the egg parasitization of *Trichogramma* spp. but did not show any negative effect on the adult emergence.

Hohmann et al. (2010) demonstrated that treating the hosts eggs of *Anagasta kuehniella* with aqueous neem seed extract (ANSE) at 15, 3 and 1.5 % and of an emulsifiable concentrate neem oil (ECNO) at 2.5, 0.5 and 0.25 % before parasitism was less deleterious to wasp emergence, especially for *Trichogramma annulata*. Pretreatments (24 h) of the host eggs with ECNO at concentrations varying from 0.5 to 0.25 % did not affect *T. pretiosum* longevity, but 2.5 % reduced *T. annulata* survival. Feeding wasps with honey mixed with 0.25 % ECNO negatively affected *T. annulata* survival.

Eyyüp and Başpınar (2012) sprayed NeemAzal-T/S for the control of *Liriomyza trifolii* larvae in tomato and showed that it had less impact on the parasitization by egg parasitoid in laboratory and greenhouse conditions. Usman et al. (2012) investigated the efficiency of *T. chilonis* alone, *T. chilonis* in combination with *Chrysoperla carnea* and neem extract against tomato fruitworm, *Helicoverpa armigera*, in field experiments. They found that treatment with Trichocard^R having 300 parasitized eggs in combination with *Chrysoperla* and neem extract was the most promising for effective management of *H. armigera* on tomato. Tunca et al. (2012) reported that azadirachtin, pyrethrum, capsaicin and d-Limonene were repellent to parasitoid *V. canescens* adults and therefore not compatible with parasitoid.

Mamoon-ur-Rashid et al. (2013) reported that neem oil at 1.5 and 2 % and neem seed water extract at 3 % significantly reduced the number of spotted bollworm larvae on treated leaves. Percentage parasitism of bollworm eggs by *T. chilonis* was significantly reduced when they were placed on leaves treated with 2 % neem oil,

and 3 % neem seed water extract, but adult emergence of *T. chilonis* was not affected by any of the neem treatments.

Rotenone from *Derris elliptica* was toxic to adults of *Edovum putleri*, an egg parasitoid of *Leptinotarsa decemlineata* (Obrycki et al. 1986; Hamilton et al. 1996). Rotenone caused mortality to *Trichogramma koehleri* (Iannacone and Lamas 2003a, b). Leaf extract of *Lantana camara* was safe to egg parasitoid *Trichogramma japonicum* (Burman et al. 2003). Nicotine sulphate from *N. tabacum* did not affect much the adults of *Encarsia formosa* (Heyler et al. 1992) but toxic to *Telenomus remus* (Chari et al. 1996). Water extract of *Pongamia pinnata* did not affect the emergence of egg parasitoid, *Trichogramma japonicum* (Burman et al. 2003). Ryanodine isolated from *Ryania speciosa* was safe to *Ooencyrtus kuwanai* and *Telenomus terebrans* (Tadic 1979). Water extract of *Vitex negundo* showed normal emergence of parasitoid, *Trichogramma japonicum* (Burman et al. 2003).

3 Effect on Larval Parasitoids

Aqueous extract of *Annona squamosa* was reported safe to parasitoid *Cotesia flavipes* (Reddy and Srikanth 1996). Neem, *Azadirachta indica*, oil was sprayed at 50 % as low volume application against the rice folder *C. medinalis*. The pest larvae were parasitized by ichneumonids, braconids and encyrtid groups of parasitoids in the field. Surprisingly the parasitization of the leaf folder larvae in neem oil-treated plots was double than control. This was due to the fact that most of the larvae could not spin the leaves together due to high toxicity of neem oil, thereby giving enough opportunity for parasitization. However, the neem oil had no side effects on parasitoids (Saxena et al. 1981). Such an increase in parasitoid population was also observed for the parasitoid *Diadegma semiclausum* than in control after the treatment of neem-based product 'Biosol' in cabbage plots (Chandra Mohan and Nanjan 1990). Similarly, various endoparasitic hymenoptera pupated and emerged normally from parasitized 4th and 5th instar *C. medinalis* larvae

that were reared on rice leaves treated with neem fractions or extracts (Schmutterer et al. 1983).

Schauer (1985) found that aphid mummies containing larvae or pupae of braconid parasitoids, *Diaeretiella rapae* and *Aphidius cerasicola*, were unaffected by 5 % neem seed kernel suspension. Neem seed oil was also quite safe for the natural enemies like *Lycosa pseudoannulata* and *Apanteles cypris* (Wu 1986); ichneumonid parasitoid, *Campoletis chlorideae* of *H. armigera* (Prasad et al. 1987); and external larval parasitoid, *Bracon hebetor* of pod borer, *Maruca testulalis* (Jhansi and Sundara Babu 1987). Other studies with *B. hebetor* also support the fact that neem is safer for this parasitoid as aqueous suspension, and an ethanolic extract of neem seed kernel (NSK) at 0.3, 0.6, 1.2, 2.5 and 5.0 % administered via food or by contact had no influence on the *B. hebetor* oviposition (parasitization) on *C. cephalonica*. Parasitoid eggs and pupae were also unaffected by the extracts tested. The parasitoid larvae, however, were killed by feeding on contaminated host larvae and also through contact with neem extracts. Thus, use of a minimum safety period is suggested for inundative release of *B. hebetor* in integrated pest management (Raguraman and Singh 1998). In order to determine the toxicity of oil extracts to *Chelonus blackburni* to explore the possibility of using parasitoid along with oils/extracts in integrated control programme of potato tuber moth, it was observed that all the vegetable oils including neem oil were safe to *C. blackburni*, an egg-larval parasitoid of *P. operculella* (Shilke et al. 1990).

Schneider and Madel (1992) reported that there was no adverse effect on adults of the braconid *Diadegma semiclausum* after exposure for 3 days or during their lifetime in cages to residues of an aqueous NSKE (0.1–5 %). The longevity of the wasps exposed to neem residues was even prolonged, but the difference between treated and untreated individuals was statistically not significant. Females of the braconid, derived from larvae developed in neem-treated larvae of *P. xylostella*, showed neem oil reduced fecundity or activity as compared to controls. Fresh extracts showed neem oil repellent effect. The influence of AZA on *Diadegma terebrans*, parasitoid of the

European corn borer, *Ostrinia nubilalis*, was investigated in the laboratory by McCloskey et al. (1993). These authors added sublethal doses (0.1 and 0.3 ppm) of AZA or ethanol (carrier solvent) to diets of second instar larvae of the pyralid. Both AZA concentrations showed neem oil significant difference of the parasitization percentage; host acceptance by the parasitoids was also not influenced. However, significantly higher mortality of parasitoids was observed in AZA-treated groups compared to untreated groups, especially after emergence from the hosts. The durations of the larval instars in the hosts were prolonged and the weight of pupae and adults from treated groups was reduced.

Lowery and Isman (1996) tested the effects of extracts from neem on aphids and their natural enemies. In field trials, populations of aphid natural enemies (predators and parasitoids) were not affected by application of neem insecticides, suggesting the compatibility of neem with biological control agents. Safety of natural enemies after neem application is also shown by the studies of Mani and Krishnamoorthy (1996) where encyrtid *Tetracnemoidea indica*, a dominant parasitoid of the pseudococcid *Planococcus lilacinus* on acid lime were exposed to acid lime leaves treated with 34 pesticides at field recommended doses. Fenvalerate (0.01 %) and NSKE (2 %) were non-toxic to the adult parasitoids. Similarly, there was no adverse effect of neem seed kernel water extract (NSKWE) (25 g/l) on the adult of *A. pluteae*, a parasitoid of leaf-eating caterpillar complex of cabbage (Bandara and Kudagamage 1996). It was also observed that after the spraying of the NSKWE and the two insecticides on the cocoons, there was no significant reduction in the adult emergence. Thus, NSKWE (25 g/l) had no adverse effect on *A. pluteae*. Similar results were obtained for *A. africanus* and *Telenomus remus* (Chari et al. 1997). Dobelin (1997) studied the side effects of NeemAzal-T/S (1.0 % azadirachtin) against two parasitoids of aphids, viz., *Aphidius colemani* and *Aphidoletes aphidimyza*. It was observed that neem products had no effects on these natural enemies.

Michelakis and Vacante (1997) advocated Neemark as a safe device for the control of

Phyllocnistis citrella that would not affect the parasitoid *Pnigalia* sp. They also implemented a biological control programme using *Ageniaspis citricola*, *Citrostichus phyllocnistoides* and *Semiela cher petiolatus*, along with Neemark. Stansly and Liu (1997) found that neem extract, insecticidal soap and sugar esters had little or no effect on *Encarsia pergandiella*, the most abundant parasitoid of *Bemisia argentifolii* in south Florida vegetable fields, and can contribute significantly to natural biological control of this and other whitefly species. Olivella and Vogt (1997) collected 10 species of leaf-mining Lepidoptera in apple orchards in southwestern Germany in 1996, the most abundant being *Phyllonorycter blancardella*, *Lyonetia clerkella* and *Stigmella malella* and a mining curculionid, *Rhamphus oxyacanthae*. Of these total parasitism by chalcidoidea and ichneumonoidea ranged from 10 to 29 %. Use of a neem preparation for pest control had no effect on the rate of parasitism.

Sharma et al. (1999) reported that the extracts from neem and custard apple kernels were effective against the spotted stem borer, *Chilo partellus*; Oriental armyworm, *Mythimna separata*; head bug, *Calocoris angustatus*; and the yellow sugarcane aphid, *Melanaphis sacchari* in sorghum, but neem extract was non-toxic to the parasitoids and predators of the sorghum midge with slight reduction in parasitism. But Sharma et al. (1984) reported that an active neem fraction of NSK had an adverse effect on larval parasitoid *Apanteles ruficrus* of Oriental armyworm *M. separata*. Injection of 2.5–10 µg of azadirachtin to newly ecdysed fourth and fifth instar larvae of host either partially inhibited or totally suppressed the first larval ecdysis of braconid *Cotesia congregata*, an internal larval parasitoid of tobacco hornworm *Manduca sexta* (Beckage et al. 1988). They also reported that the parasitoid growth was arrested, while the host larvae survived for 2 weeks or longer, following injection of azadirachtin, but their parasitoids never recovered and died encased within exuvial cuticle. Lamb and Saxena (1988) gave topical treatment to the females of ectoparasite *Goniozus triangulifer* at doses from 5 to 50 µg/l solution of neem seed bitters. The results indicated decreased

fecundity at 50 µg per female. When the rice plants were sprayed with 1,000 ppm neem seed bitters, very few larvae of leaf folder *Marasmia patnalis* sustained the development of *G. triangulifer* up to pupation stage. However, when 1,000 ppm neem seed bitters were sprayed three times, there was negative influence on parasitization by *G. triangulifer*. The studies further reported that when *Tetrastichus howardi* parasitized pupae of *M. patnalis* were dipped in 1,000 ppm of neem seed bitters, the adult emergence decreased significantly. However, topical application of 1,000–10,000 ppm of the neem seed bitters had no effect on *T. howardi*.

Loke et al. (1992) gave topical treatment of cocoons of *C. plutellae* with neem oil in the laboratory and found that neem inhibited adult eclosion significantly at 2.5 % concentration and no adult emergence was observed at 10 % level. Treated cocoons produced adults with reduced longevity but no morphological deformities. However, Osman and Bradley (1993) reported high mortality of larvae and morphogenetic defects of adult parasitoid *C. glomerata* developed from hosts treated with NSKE. Neem products did not affect adult parasitoids even after spraying with higher concentration, i.e. AZT-VR-K 2,000 ppm. Srivastava et al. (1997) reported that alcohol and hexane extracts of 17 neem ecotypes in India were found to be toxic to the egg, larval and pupal stages of the *B. brevicornis*. In general, the hexane extracts showed higher toxicity against the egg and pupal stages, whereas the alcohol extracts were more toxic against the larvae. Azadirachtin content of the neem ecotypes revealed no apparent correlation with the observed toxicity against different stages of the parasitoid.

Varied responses of parasitoids to various neem preparations have also been reported by several workers. For instance, Hoelmer et al. (1990) did experiments with parasitoids of *B. tabaci* and *Aphis gossypii* with the neem product Margosan-O. It was found that the aphid parasitoids, namely, *Lysiphlebus testaceipes* and *Aphelinus asychis*, were more sensitive to neem-treated surface, whereas the survival of the aphid parasitoid, *Eretmocerus californicus*, was the

same on treated and untreated *Hibiscus* foliage. The *E. californicus* pairs in sealed Petri dishes with treated and untreated foliage survived for 5 days. It was also observed that dipping of aphid mummies parasitized by *L. testaceipes* and also dipping the parasitized puparia of *B. tabaci* by *Encarsia formosa* and *E. transversa* did not affect the emergence of the parasitoids. However, when *E. californicus* parasitized white fly puparia was dipped, the emergence of parasitoids was reduced by more than 5 %. Similarly, Stark et al. (1990) on the other hand found that a highly purified and concentrated neem extract prevented adult of fruit flies emergence from puparia. However, the parasitoid *Opius* sp. emerged freely. Neem seed kernel extracts reduced the population of *Encarsia* sp. and *Aleurodiphilus* sp. (Price and Schuster 1991). *A. indica* leaf extract was safe to *Diaeretiella rapae* (Men et al. 2002). Loke et al. (1992) reported reduction in the rate of emergence of braconid, *Cotesia plutellae*, when cocoons were sprayed with *A. indica* oil.

Schmutterer (1992) found in laboratory experiments that concentrations of 10 and 20 ppm of azadirachtin or an azadirachtin-free fraction and of an enriched and formulated seed kernel extract of *A. indica* were only slightly harmful to *C. glomerata*, provided they were applied against the 5th instar of *Pieris brassicae*. Under these circumstances, numerous larvae of *C. glomerata* emerged from their hosts, pupated and hatched as normal adults. However, higher concentration (40 ppm) of azadirachtin and of the azadirachtin-free fraction as well as 50 and 100 ppm of the enriched product reduced the number of parasitoids considerably. The parasitoids were mainly killed by lack of food and died within their hosts. Larvae of *P. brassicae* under the influence of metamorphosis disturbance by neem products did not die immediately after uptake of active principles, but there was reduced food uptake, leading to increased intraspecific competition among the gregarious grubs of *C. glomerata*. Direct growth regulation effects of neem products against *C. glomerata* were not observed. Application of neem products against young (1st–3rd) larval instars of *P. brassicae* led to the death of the caterpillars together with the grubs of the parasitoid.

It is obvious from the studies available so far that neem and its various products/formulations do have some side effects especially against the larval parasitoids. We have some specific reports of such side effects available. Beitz and Hofmann (1992) studied the side effects of neem product AZT-VR-NR on the endoparasitic tachinid fly *Drino inconspicua* when the fly was exposed for 7 days with residues of neem product (45 g a.i./ha); it did not harm the adult flies, but fecundity was reduced by 18.5 % in comparison with control. Similarly, Serra (1992) observed no or only side effects of neem products on the parasitoids of the genera *Ganaspidium*, *Desorygma* and *Opius*, which emerged from the tomato leaf miner *Liriomyza sativae*. He also obtained similar results on the genera *Pseudapanteles* and *Glyptapanteles* that emerged from tomato pinworm, *Keiferia lycopersicella*. Moser (1994) observed no side effects of aqueous NSE (2.5 and 5.0 %) among natural enemies (coccinellids, syrphids, chrysopids and braconids) of *Aphis gossypii* on okra in Dominican Republic fields, but at the same time by slight harmful effects, viz., morphogenetic defects, delay of larval and pupal development was recorded in the laboratory experiments. Mineo et al. (2000) tested the side effects of azadirachtin mixed with mineral paraffin oil and a surfactant against the natural parasitoids of *P. citrella*. The observations revealed 16.67 % parasitoid larvae showing teratological symptoms.

Stark et al. (1992) studied the effect of azadirachtin on survival, longevity and reproduction of the three braconid parasitoids, namely, *Psystallia incisi* and *Diachasmimorpha longicaudata* from *Bactrocera dorsalis* and *Diachasmimorpha tryoni* from *Ceratitidis capitata*. The results revealed that all host larvae that were exposed to sand treated with azadirachtin pupated and adult eclosion was concentration dependent in both fly species, with little or no fly eclosion at 10 ppm. However, *P. incisi* and *D. longicaudata* successfully eclosed from pupae treated with 10 ppm azadirachtin. In all the cases after the exposure of azadirachtin, the adult eclosion was inhibited. Even life spans of parasitoids that emerged from treated flies were not significantly different from controls. The

azadirachtin had no effect on the longevity of parasitoid species tested in this study, indicating that the parasitoids were less sensitive to this chemical than were their hosts. The reproduction of *P. incisus* that developed in flies exposed to azadirachtin concentration of >20 ppm was reduced by 63.88 %. The reproduction of *D. longicaudatus* and *D. tryoni* was unaffected. This implies that neem-based products are safer at lower concentrations but induce adverse effects at higher levels of treatment, also obvious from the studies on neem products like Repelin and Neemguard that were tested on *Bracon hebetor* in laboratory and field conditions by Srinivasa Babu et al. (1996) to reveal their safety at lower concentrations against larval parasitoids. But at higher concentrations, both preparations adversely affected the development.

It is, however, also possible that parasitoids may be adversely affected due to lack of appropriate food. This is clear from the results of Jakob and Dickler (1996) where adults of the ectoparasitic, gregarious eulophid *Colpoclypeus florus*, an important parasitoid of the tortricid *Adoxophyes orana*, were not adversely affected by application of NeemAzal-S (25 and 100 ppm) in the laboratory and in the field, but 100 % of the larvae died, apparently due to lack of appropriate food on the neem-treated decaying larvae of the host. Schmutterer (1996) also described the varying sensitivity of bioagents to neem products, like eggs of predators such as coccinellids and chrysopids are not sensitive, but ectoparasitic gregarious larvae of *Bracon* sp. and *Colpoclypeus* sp. showed high mortality after contact with neem. Endoparasitic solitary or gregarious hymenopteran larvae were less endangered as their hosts protected them. Schmutterer suggested that often, lack of food in neem-treated hosts resulted in the death of parasitoids due to starvation.

Another aspect of interest is the IPM compatibility of neem with other products vis-a-vis the safety of natural enemies. The relative toxicity of pesticides to *Phyllocnistis citrella* and its parasitoid *Agéniaspis citricola* was compared by several bioassay methods. Azadirachtin (Neemix) + oil, diflubenzuron (Micromite) + oil, fenoxycarb (Eclipse) + oil and oil alone (FC 435–66) were

classified as IPM-compatible insecticides. Sprays of azadirachtin (Align) + oil, neem oil (Neemguard) and drenched imidacloprid (Admire) were ranked as semi-compatible insecticides (Villanueva and Hoy 1998).

Teggelli et al. (1998) studied the effects of Nimbecidine (5 ml/l) and Achook (5 ml/l), nuclear polyhedrosis virus (NPV) (1.5 ml/l) and some recommended insecticides on the emergence of *Campoletis chlorideae* from host larvae 3, 5, 8 and 11 days after parasitization (DAP). Among insecticides, Achook resulted in the highest adult emergence (42.33 %) at eight DAP, while fenvalerate, methomyl, malathion, chlorpyrifos and monocrotophos completely inhibited emergence. At 11 DAP, the biopesticides, namely, Nimbecidine, Achook and NPV, recorded the highest percentage of emergence (58.66, 56.33 and 53.33 %, respectively), while monocrotophos was most toxic (8.66 % adult emergence). The toxicity of all insecticides was lower on cocoons. Nimbecidine and NPV did not cause mortality 24 h after treatment. Similarly, *Hypomecis* sp. caused severe damage to *Azadirachta indica* in Akola, India, during October 1988. *Apanteles fabiae* and *Aleides* [*Aleiodes*] sp. were observed parasitizing *Hypomecis* sp. (Men 1999).

An IPM strategy for the control of *P. xylostella* was formulated by Facknath (1999) using neem with *C. plutellae*. Reddy and Guerro (2000) evaluated biorational and regular insecticide applications for the management of the diamond-back moth *P. xylostella* in cabbage. The IPM programme, based on the pheromone trap catch threshold of 8 moths per trap per night, included the utilization of *C. plutellae* (250,000 adults/ha), *Chrysoperla carnea* (2,500 eggs/ha), Nimbecidine (625 ml/ha), *Bt* (500 ml/ha) and phosalone (2.8 l/ha). The IPM programme induced a reduction of trap catches, egg and larval populations and, therefore, a low level of damage to the crop. All neem concentrations gave poor to very slight control of *Myzus persicae* when applied as contact action foliar sprays, with Pirimor^R providing the greatest contact kill. Neem at 180 ppm, when applied as a soil drench, gave total aphid control within 24 h, apparently

through systemic action. Aphid parasitoids and other beneficial insects were not affected by neem treatments, whereas Pirimor^R treatments reduced beneficial insect numbers. Although Pirimor^R would be the preferred choice for immediate aphid control through contact action in commercial crop production, neem still has a place in the control of aphids in situations such as organic crop production or in crops where resistance to other chemicals by aphids has resulted. Other uses may be in indoor and outdoor landscape situations where human health is of major concern and a long-lasting systemic method of aphid control desirable. In these cases, neem could be applied as a soil drench at concentrations of 180 ppm, possibly through existing irrigation systems (Holmes et al. 1999). Perera et al. (2000) studied the effect of three feeding deterrents: denatonium benzoate (5, 50 and 250 mg/l), azatin [azadirachtin] EC (0.01, 0.1 and 1 ml/l) and Pestistat^R (0.1, 1 and 2 ml/l) on the fourth instar larvae of important cabbage pests, *Chrysodeixis eriosoma* and *P. xylostella*, and on the parasitoid, *C. plutellae*. Results suggested that the three antifeedants were effective in managing cabbage pests, *C. eriosoma* and *P. xylostella*, and could be used in integrated pest management programmes.

Babu and Babu (2003) investigated the effects of neem-based formulations, viz., Neemguard and RD9 Repelin, on the developmental stages of *B. hebetor* and found that substantial reduction in hatching of treated eggs and growth disturbances but parasitoid pupae were spared. Dinesh et al. (2003) evaluated extracts of some locally available plant materials and propriety botanical formulations in comparison with a recommended insecticide (quinalphos: Ekalux^R) for their usefulness against the coffee mealy bug (*P. citri*) and their effect on natural enemies (parasitoid, *Leptomastix dactylopii*, and attendant ant, *Anoplolepis longipes* [*A. gracilipes*]). The treatments included extracts of Tulsi (*Ocimum sanctum* [*O. tenuiflorum*]), Bilva (*Aegle marmelos*), calotropis (*Calotropis gigantea*), marigold (*Tagetes erecta*), 'Universal biopesticide' formulation (containing *Aloe vera* [*A. barbadensis*], *Lantana camara*, *Calotropis gigantea*, neem

(*Azadirachta indica*) and *Vitex negundo*) and garlic. The proprietary products used were GB+ (100 % garlic preparation) and GB AG (77 % garlic and 22 % neem preparation). The treatments were effective against the mealy bug, and the parasitoid was relatively safe from its effect. Basappa and Lingappa (2004) tested various neem preparations, i.e. NSKE (5 %), neem leaf extract (NLE, 5 %), Margocide CK 20 EC (0.1 %), Achook (0.3 % water soluble neem powder) and Jawan (0.15 % neem extract) along with *Bougainvillea glabra* cold alcohol extract (CAE 30 %) against castor semilooper *Achaea janata* and its larval parasitoid (*Microplitis maculipennis* [*Snellenius maculipennis*]) under field conditions in Dharwad, Karnataka, India, during 1993 and 1994. NSKE, Margocide and Jawan were superior in reducing the larval population of castor semilooper at 3 days after spraying. Neem-based preparations were superior over *B. glabra* in reducing castor semilooper larval population at 3, 7 and 12 days after spraying. These botanicals also gave significantly higher seed yield than the untreated control. The highest cost/benefit ratio was recorded in NLE (1:2.80), followed by Margocide (1:2.20) and NSKE (1:1.94). A steady increase in *M. maculipennis* population was observed in all treatments after 3 days of spraying. Ahmad et al. (2003) did not recommend the use of parasitoids in combination with *A. indica* preparations since the application to the soil had a long-lasting effect on the rate of parasitization and on survival (as demonstrated with the parasitoid, *Diaeretiella rapae*).

Haseeb et al. (2004) evaluated the field doses of neem insecticides against *C. plutellae* under laboratory conditions. Agroneem (4.8 mg a.i./l), Neemix (20 mg a.i./l) and Ecozin (20 mg a.i./l) caused only 11.1, 16.7 and 5.6 % adult mortality, respectively. Vijay Bhardwaj et al. (2005) studied the adults of *D. fenestralis* [*D. fenestrale*] and *C. plutellae* by transferring into vials containing filter paper soaked in solutions of malathion (0.05 %), fenvalerate (0.01 %), fipronil (0.007 %), cypermethrin (0.015 %), Achook [*Azadirachta indica* extract] (0.3 %) and cypermethrin (0.0075 %) + *Bt* [*Bacillus thuringiensis*] (0.15 %).

Parasitoid mortality was evaluated after a 6-h exposure period and after 24 and 48 h posttreatment periods. *C. plutellae* mortality was highest with malathion (74.44 %), followed by fipronil (57.77 %), cypermethrin (27.77 %), fenvalerate (15.55 %), cypermethrin + *Bt* (14.44 %) and Achook (4.44 %). Malathion was also the most toxic to *D. fenestralis*, followed by fipronil, cypermethrin, cypermethrin + *Bt*, fenvalerate and Achook (94.44, 68.88, 67.77, 25.55, 24.44 and 3.33 % mortality, respectively), proving the safety of neem-based insecticide (Achook). Charleston et al. (2005) tested the effect of two botanical pesticides, viz., aqueous leaf extracts from the syringa tree *Melia azedarach* and commercial formulation from the neem tree *Azadirachta indica*, Neemix 4.5, in the laboratory and in a glasshouse on two species of parasitoids, *C. plutellae* and *Diadromus collaris*. No direct negative effect was recorded on the longevity of the parasitoid species. However, hind tibia length was found to be significantly shorter in male *C. plutellae* that emerged from *P. xylostella* that had been exposed to syringa extracts. In the glasshouse, a significantly higher proportion of *P. xylostella* was parasitized by *C. plutellae* on plants treated with neem than on the control plants. Rowell et al. (2005) reared six parasitoid species on diamondback moth larvae and pupae collected in northern Thailand. These included the larval parasitoid *C. plutellae*, a larval-pupal parasitoid *Macromalon orientale* Kerrich and pupal parasitoids *D. collaris* and *Brachymeria excarinata*. These parasitoids were effectively integrated in the pest management protocol of *P. xylostella* using simple presence-absence sampling for lepidopterous larvae, and the exclusive use of *Bacillus thuringiensis* or neem resulted in the highest yields of undamaged cabbage compared with a control or weekly sprays of cypermethrin (local farmer practice).

Adarkwah et al. (2011) reported that the commercial preparation of neem, Calneem[®], was safe to the larval parasitoids *Habrobracon hebetor* and *Venturia canescens*, while it controlled their hosts, rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and tropical warehouse moth *Cadra cautella* Walker

(Lepidoptera: Pyralidae) in stored rice and wheat, respectively, in the laboratory. Kumar et al. (2010) tested two commercial neem products, viz., NeemAzal-T/S (1 % azadirachtin) as foliar application and NeemAzal-U (17 % azadirachtin) as soil application, in laboratory bioassays against about-to-emerge adults and adults of an aphelinid, *Eretmocerus warrae*, an efficient parasitoid of the whitefly *Bemisia tabaci*. They found that the longevity of the adult parasitoids was only affected after 36 h contact with high-dose residues of NeemAzal-T/S (10–15 ml l⁻¹) in a dry-residue bioassay test. But the experiments indicate that ecto-/endoparasitoids are principally highly vulnerable to neem but show in addition that soil application could reduce negative side effects compared to plant spraying and hence improve selectivity.

Alvarenga et al. (2012) treated the neem seed cake (NSC) extract to control the Mediterranean fruit fly along with its larval parasitoid *Diachasmimorpha longicaudata*. They found that NSC affected parasitoid emergence negatively. The effect of parasitism coupled to NSC did not provide greater reduction in the medfly emergence than when parasitism was used alone. However, each of these 2 methods affect a different life stage of medfly larvae and pupae, respectively, and their joint use may increase the probability of controlling medfly populations in field.

Leaf extract of *Datura metel* was safe to *Cotesia flavipes* (Reddy and Srikanth 1996). Aqueous leaf extract of *M. azedarach* did not show direct negative effect on the longevity of the parasitoid species of *Cotesia plutellae* and *Diadromus collaris* (Charleston et al. 2005). Nicotine sulphate from *N. tabacum* was toxic to *Apanteles congregata* (Barbosa et al. 1991).

4 Effect on Predatory Insects

Acetone extracts of *Aframomum melegueta* exhibited adverse effect on eggs, reduced the efficiency of predation in larvae and showed poor rate of pupation of Coccinellids, *Cheilomenes lunata* and *Cheilomenes vicina*

(Ofuya and Okuku 1994). Leaf extract of *Ailanthus excelsa* did not affect the predators such as coccinellids, chrysopids and syrphids (Patel et al. 2003). Leaf extract of *Aloe vera* was safe to grubs, pupae and adults of predators such as coccinellids and syrphid fly (Balikai and Lingappa 2004). Water extract of *Annona squamosa* was safe to *Chrysoperla carnea* and *Onus insidiosus* (Isman and Leatemala 2004). Methanolic extracts of leaves of *Atlantia monophylla* were found to be safe to aquatic mosquito predator, *Diplonychus indicus* (Sivagnaname and Kalyanasundaram 2004).

Bioactivity of neem, *A. indica*, against predatory insects and spiders is discussed under subheadings: Earwigs, Crickets, True Bugs, Ants, Beetles, Syrphids, Cecidomyiids, Lacewings, and Predatory Spiders and Mites.

4.1 Earwigs

NeemAzal-F was tested against the European earwig *Forficula auricularia* by Sauphanor et al. (1995). This earwig is a polyphagous crop pest and a predator at the same time. In peach and apricot orchards, for instance, it causes serious damage by feeding on ripening fruit, whereas in apple orchards it can be used against harmful populations of various aphid species. Adults of *F. auricularia*, exposed to 50 ppm AZA on glass plates (standardized method of IOBC/WPRS) in the laboratory, did not show increased mortality or reduced ingestion of food; fecundity was also not adversely influenced. On the other hand, second instar nymphs treated with 25, 50 or 250 ppm of NeemAzal-F could not complete their metamorphosis and died. They also exhibited reduced food intake and extended stadia. Neither repellent nor phagodeterrent effects were observed. Under field conditions in a peach orchard, the nymphal population of the earwig was reduced by 70 % when sprayed with NeemAzal-F at a concentration of 50 ppm. Hence, NeemAzal-F could be applied in peach or apricot orchards when a reduction of the nymphal population of the earwig is required but avoided if high numbers of the predator are desirable, for instance, in apple orchards to obtain a

significant reduction of aphids. Earlier, Schauer (1985) and Eisenlohr et al. (1992) observed that *F. auricularia* had no side effects by neem products.

4.2 Crickets

The cricket *Metioche vittaticollis*, preying upon eggs of rice leaffolders in Asia, was not affected by spraying neem seed bitters (containing AZA and other active ingredients) at 10,000 ppm (8 l/ha: ultra low volume spray) in field trials in the Philippines (Lamb and Saxena 1988).

4.3 True Bugs

Chelliah and Rajendran (1984) tested the toxicity of seven insecticides against *Cyrtorhinus lividipennis*. The least toxic of the sprays was 0.07 % endosulfan, which was effective against rice hoppers, followed by 5 % neem oil; the corrected mortality percentages were 34.72–36.90 on 1 day after spraying, 7.40–38.11 on the second day and 20.01–20.03 on the third day. The other sprays tested (0.075 % quinalphos, 0.04 % chlorpyrifos, 0.07 % phosalone, 0.08 % monocrotophos and 1.0 % carbaryl) were highly toxic to the bugs exhibiting 36.07–53.96 % mortality on the third day. Sharma et al. (1984) observed that *Orius* sp., a predator of sorghum midge, *Contarinia sorghicola*, was unaffected by an active neem fraction.

A slight harmful effect of neem oil was reported to the mirid bug, *C. lividipennis* by Saxena et al. (1984). But Fernandez et al. (1992) conducted a trial in greenhouse against *C. lividipennis* using four treatments, viz., neem oil 3 %, aqueous NSKE 5 %, endosulfan and water, and observed no mortality in the case of neem oil and aqueous NSKE, while endosulfan induced cent per cent mortality. Serra (1992) reported malformed nymphs of the predator, *Nesidiocoris* sp., after spraying of neem seed water extract 4 % and neem oil 2 % in the laboratory. It was observed that these neem products had no significant effect on the field population of this bug. The toxicity of all the sprays diminished 5 days after spraying. Similarly, a delayed moulting and morphogenetic

defects after spraying of Margosan-O on third instar nymphs of the pentatomid predator *Perillus bioculatus* of the Colorado potato beetle in the USA have been recorded (Hough and Keil 1991). Krishniah and Kalode (1992) reported that the LC50 for neem oil was 50 % for the black mirid bug, *Tytthus parviceps* was 2.88 %, whereas for its prey, the green rice leafhopper, *Nephotettix virescens*, was only 1.39 %.

Drescher and Madel (1995) reported increased mortality in test populations of the anthorcid *Orius majusculus* after NeemAzal-T/S treatment (concentrations 1:50 to 1:200), and after oral intake, the rate of emergence of first instar nymphs was reduced by 3 %. Neem oil's repellent or phagodeterrent effect was observed when treated eggs of *Sitotroga cerealella* served as food for the bugs. According to the guidelines of IOBC/WPRS for standardized tests on the side effects of pesticides, NeemAzal-T/S at 1:50 was 'slightly harmful' under laboratory conditions. They also reported neem oil's negative effect on fecundity, sex ratio, rate of emergence or behaviour.

Kareem et al. (1988) reported the effect of neem seed kernel extract on population of predatory mirid and spiders in rice and compared with those of monocrotophos (0.75 kg a.i./ha). It was revealed that the populations of mirids and spiders were also lower in plots treated with monocrotophos than in plots treated with neem, 48 days after treatment. Safety of neem formulations and insecticides to *Microvelia douglasi atrolin-eata*, studied for a predator of planthopper in rice ecosystem, revealed that Neemix (2 and 4 %) and Rakshak (0.2 and 0.5 %) were the safest neem formulations, whereas phorate and carbofuran (1 kg a.i./ha) granular application and quinalphos spray at 0.5 % were the least toxic to the predator (Lakshmi et al. 1998b). Also neem formulations vis-à-vis insecticides were safe to *C. lividipennis* after the application of Neemgold at 0.5 % and Neemix at 2.0 % even after 72 h exposure (Lakshmi et al. 1998c), though chlorpyrifos and monocrotophos recommended for rice caused 100 % mortality within 24 h of exposure. Ghelani et al. (2000) tested various synthetic and botanical pesticides for their contact toxicity to the eggs

and nymphs of *R. fuscipes*. The data on mortality of eggs and nymphs revealed that all the synthetic insecticides were more toxic than botanical insecticides. Among synthetic insecticides, quinalphos was highly toxic, while endosulfan was least toxic. However, among botanical insecticides, nicotine sulphate was least toxic to the eggs and nymphs of *R. fuscipes*.

Sahayaraj and Paulraj (1999a, b) observed toxic effects of leaf extracts of *Azadirachta indica*, *Vitex negundo*, *Pongamia glabra* and *Calotropis gigantea* on different life stages of reduviid predator *Rhynocoris marginatus* by contact and stomach toxicity studies. Tedeschi et al. (2001) studied the side effects of three neem formulations (Neem-Amin EC, Stardoor and B.P. 20/S) on the mirid predator, *M. caliginosus*, in the laboratory. Direct toxicity tests on first instar nymphs exposed to fresh dry residues on glass plates at different doses demonstrated that all the products were harmful to the insects with LD50 values much lower than the maximum recommended rate (1.217, 0.264 and 1.083 mg a.i./l instead of 15, 31.5 and 80 mg a.i./l for Neem-Amin EC, Stardoor and B.P. 20/S, respectively). Moreover, a reduction of fecundity of the surviving females was assessed with Neem-Amin EC and B.P. 20/S. High mortality was recorded when the insects were introduced onto the plants just after the treatment, but no significant differences compared with the controls were observed 5 days after the treatment. The experiments showed that azadirachtin being biodegradable, thus having short persistence, makes this active ingredient a promising component in integrated pest management programmes, if time gap is guaranteed between the treatment and the introduction of the predator.

Sahayaraj and Karthikraja (2003) studied the effect of azadirachtin on egg hatchability, nymphal mortality and biological control potential of *Rhynocoris marginatus* on the cotton pest *Aphis gossypii*. The biopesticide at specific concentration did not affect the biological control potential of *R. marginatus*. Jaastad et al. (2009) stated that azadirachtin did not significantly affect the most important predatory hemipterans, *Anthocoris nemorum* and *Pachydiplax longipennis*, while the

omnivorous *Psallus ambiguus* and *Aphelinus mali*, mainly regarded as beneficials, were negatively affected by azadirachtin treatment.

4.4 Ants

Hellpap (1985) tested the neem product (AZT-VR-K) fed larvae of *Spodoptera frugiperda* to the colonies of the ant, *Ectatomma viridum*. The ants accepted the neem-treated larvae. The predatory earwigs, *Doru taeniatum*, were also exposed to the armyworm larvae. It was observed that after 7 days of exposure, there was significant difference in mortality among earwigs fed with treated larvae of armyworm. Schmidt and Pesel (1987) reported that worker ants were resistant when sprayed with neem products. On the other hand, feeding of AZT-VR-K and MTB/H20-K-NR to the red forest ant *Formica polyctena* led to a stimulation of egg production when low concentrations were used. In contrast, higher concentrations reduced the number of eggs drastically, sometimes down to zero after a few weeks. This effect could be reversed if feeding of neem products was stopped and untreated food supplied instead. Use of neem-based products with predatory ants, *Oecophylla smaragdina*, gave excellent control of fruit flies, *Bactrocera cucurbitae*, in organic agriculture system, but it was not sufficiently active to manage *Aulacophora* spp. (Rohan 2000).

4.5 Beetles

In laboratory experiments, adult *C. septempunctata*, kept on neem oil-treated glass plates according to IOBC/WPRS guidelines, did not show increased mortality or reduction of fecundity compared to untreated control, but the metamorphosis of the larvae was interrupted (Schmutterer 1981). In the same insect species when treated in laboratory and semi-field trials with AZT-VR-K (1,000 ppm) and a combination of it with neem oil (250–30,000 ppm), there was no effect on the emergence of first instar larvae from treated eggs

(Kaethner 1990). Spraying on adults had no adverse effects on fecundity and activity (fitness), whereas the same treatment on fourth instar larvae under laboratory conditions induced mortality, especially of pupae that developed from treated larvae. Numerous adults that emerged from surviving pupae exhibited morphogenetic defects of their wings. In contrast, spraying on two coccinellid species including *C. septempunctata* in field cages did not result in any side effects. In laboratory studies of Lowery and Isman (1996), topical treatment of early second instar larvae of *C. undecimpunctata*, using 1 % neem oil, did not result in reduced pupation or emergence of adults as compared to controls.

Margosan-O had no harmful effect on *Delphastus pusillus* preying on *Bemisia tabaci* and *Scymnus* sp. preying on *Aphis gossypii* and *Myzus persicae* (Hoelmer et al. 1990). Margosan-O also did not show any adverse effect against predatory carabid beetle *Platynus dorsalis* when their soil habitat was treated with the neem product (Forster 1991). Saleem and Matter (1991) observed that the neem oil acted as temporary repellent against the predatory staphylinid beetle *Paederus alfieri*, the coccinellid *C. undecimpunctata*, and the lacewing *Chrysoperla carnea* in cotton, but otherwise neem oil had no adverse effect on the predators of *Spodoptera littoralis*. That neem oil had no adverse effect on predators is also obvious from the studies of Kaethner (1991), as it was found harmless to the eggs, larvae or adults of *Chrysoperla carnea* and *C. septempunctata*.

Mohapatra et al. (1991) observed even 24 % concentration of neem oil had no significant adverse effect on the coleopteran predators in rice, and Matter et al. (1993) demonstrated that although neem oil had residual activity for up to 6 days, yet it had no effect on survival or behaviour of larvae of *C. undecimpunctata* except for a prolongation of the fourth instar larva. Consumption of the aphids by this predator was unaffected. Eisenlohr et al. (1992) reported that NeemAzal-F had neem oil effect on oviposition of coccinellids in peach orchards, though residual toxicity of some insecticides and neem seed kernel extracts against the predatory beetle *Brumoides suturalis* has been

recorded (Chandrababu et al. 1997). It was found that NSKE extract and endosulfan exhibited low toxicity to *B. suturalis* larvae and adults.

An interesting study of Patel and Yadav (1993) on the toxicity of some botanical and chemical insecticides to *Cheilomenes sexmaculata* and its hyperparasite *Tetrastichus coccinellae* shows that among the botanicals, nicotine sulphate (0.05, 0.04 and 0.03 %), Repelin (0.5, 0.75 and 1.0 %) and Neemark (0.05, 0.2 and 0.4 %) were highly toxic to adults of *T. coccinellae*, whereas they were absolutely safe to *C. sexmaculata*. In a detailed study of mortality and predation efficiency of *Coleomegilla maculata* following applications of neem extracts, it was observed that the toxicity of the neem extracts to *C. sexmaculata* was almost 100 % when both neem formulations were used at 10 % concentrations. The azadirachtin contents in neem oil (v/v) and neem seed kernels (w/v) were 13.7 and 91.0 ppm, respectively. Malathion was also tested at the field rate of 2.85 g a.i./ha (Roger et al. 1995). Adult mortality rate of the coccinellids after 72 h was 100 % following malathion treatments. Neem oil toxicity was observed after the treatments with the aqueous suspension of ground neem seeds. The predation efficiency of *C. sexmaculata* was also evaluated after topical application of these three insecticides at sublethal doses. Fifteen minutes after treatment, adult coccinellids were provided with 30 aphids for 24 h. The aqueous suspension of ground neem seeds caused 50 % reduction in the number of aphids consumed.

Stark and Wennergren (1995) opined that toxicity of pesticides to bioagents might not be straightforward, but the susceptibility of various life stages should be estimated for noteworthy concussions. Banken and Stark (1997) studied the stage and age influence on the susceptibility of *C. septempunctata* after direct exposure to neem product 'Neemix', where first instars were treated by direct application of 0, 40, 100, 200, 400, 600 and 1,000 ppm and fourth instars were treated with 400, 600, 800 and 1,000 ppm azadirachtin, the active ingredient in Neemix. The LC50 for first and fourth instars were estimated as 1,120 ppm and 520 ppm azadirachtin, respectively. These values were much higher than the

recommended rates for control of aphids (3 weekly applications of 20 ppm), suggesting that Neemix might be used in IPM programmes because application rates that control aphids should result in appreciable mortality of predators. Fourth instar larvae of *C. septempunctata* were innately more sensitive to the growth-disrupting effects of acute exposure to Neemix than 151 instars. It is possible for early instars to sustain the effects of Neemix as long as the pesticide is detoxified before the onset of pupation. These results suggest that it is extremely important to examine more than one life stage of a species to estimate the total effect of pesticides. Banken and Stark (1998) also studied the exposure and the risk of neem products against *C. septempunctata* using direct sprays, residues on leaves and pesticide-contaminated prey. The pesticide alone and the predator caused significant decrease in aphid population. However, no significant ($P < 0.05$) interaction between the predator and the pesticide was detected, indicating that the chemical and biological control agents were not working synergistically. Furthermore, exposure to the pesticide in microcosms significantly reduced or completely eliminated oviposition in adult *C. septempunctata*, and all of the larvae exposed to 100 or 600 ppm died within 10 days of treatment. Although survivorship of adult ladybird beetles was unaffected, exposure to Neemix resulted in a severe reduction in fecundity or complete sterility depending on the concentration.

Mani et al. (1997) studied the effect of 5 % neem seed kernel extracts on the predator *Cryptolaemus montrouzieri* and observed no detrimental effect on the progeny production. Dhaliwal et al. (1998) tested Achook and Nimbecidine for the control of insect pests on cabbage. The neem formulations were evaluated at 1, 2 and 4 kg/ha and compared to 0.5 kg a.i./ha of endosulfan used as treated control. Among these, endosulfan was the most effective against all the insect pests, followed by Achook and Nimbecidine. The feeding efficiency of the *C. septempunctata* on *L. erysimi* treated with neem-based insecticides was higher than for aphids treated with endosulfan. Studies on

L. erysimi control by Neemol and nicotine sulphate applied alone or in combination with chemical insecticides dimethoate and methyl-O-demeton in mustard (Vekaria and Patel 2000) have also revealed that both plant products were less toxic to the predators, *Diaeretiella rapae* and *C. septempunctata*, than the chemical insecticides. Chakraborti and Chatterjee (1999) also found that all formulations of neem were safe to the ladybird predators even at the highest concentrations (9 ml a.i./l).

Prasad and Logiswaran (1998) compared the toxicity of different insecticides to the adult of *C. sexmaculata* and reported that the neem oil is the safest insecticide based on LT 50 values. It was concluded that the less toxic phosalone, monocrotophos or neem oil could be integrated with the release of *C. sexmaculata* in the field. Azadirachtin and dichlorvos also induced lowest toxicity to the predator *C. montrouzieri* (Sundari 1998), and Neemix and Multineem had least effect against predatory coccinellids (Mishra and Mishra 1998).

Singh and Singh (1996) tested different neem-based formulations and synthetic insecticides on aphidophagous coccinellids on *Brassica juncea*. Achook (WSP), RD-9 Repelin, NeemAzal-T/S, Neemgold, Neemta 2100 and Nimbecidine at 0.03 % were quite safe to coccinellids than the synthetic insecticides such as endosulfan 35EC, fenvalerate 20EC, dimethoate 30EC and Chess 25EC. The order of safety was maximum in Achook followed by RD-9 Repelin, NeemAzal-T/S, Neemgold, Neemta 2100, Nimbecidine, endosulfan, Chess, fenvalerate and dimethoate during the first experimental trial (1994–1995) and Neemgold followed by Achook, Annona 20 EC, Neemta 2100, Achook EC, NeemAzal-T/S, endosulfan, Nimbecidine, Chess 25, fenvalerate and dimethoate during second experimental year (1995–1996). However, Imtiaz et al. (1998) found two neem extracts (RB-a and RB-b at 6,7,8,9 and 10 %) and an extract of bakayan (*Melia* sp.) berries (1, 2, 3, 4 and 5 %) toxic to the coccinellid *Coccinella* sp. and reported that 10 % RB-a and RB-b induced the highest mortality (85.7 and 82.5, respectively). Neem oil was, however, quite safe for natural enemies *Aphytis melinus* and *Chilocorus nigrita* predating

Aonidiella aurantii (Krishnamoorthy and Rajagopal 1998).

Simmonds et al. (2000) investigated the effect of crude neem seed extract, a formulation of azadirachtin (Azatin), a pyrethrum extract and one of the two naphthoquinones isolated from *Calceolaria andina* Benth on the foraging behaviour of the *C. montrouzieri* larvae and adults. All the botanicals influenced the foraging behaviour of *C. montrouzieri*, at one or more concentrations. Larval and adult foraging behaviour was influenced most by neem that also affected larval behaviour; the predators contacted fewer treated leaves and spent less time on treated than on untreated leaves. Larvae also consumed fewer mealy bugs treated with naphthoquinones.

Ma et al. (2000) assessed the toxicity of several biorational pesticides and chemicals to *H. armigera* and *H. punctigera* and also on the major predators in cotton ecosystem. Moderate dose-dependent control was obtained in plots treated with neem seed extract, azadirachtin (AZA) at rates of 30, 60 and 90 g/ha. Plots treated with Talstar EC (bifenthrin) applications achieved the best results, followed by treatment with alternation of chemicals (methomyl, bifenthrin, thiodicarb and endosulfan) and biorational insecticides (neem oil, azadirachtin and *Btk*). Predators, including coccinellids, chrysopids, Araneae and hemipterans, were insensitive to AZA, toosendanin (Tsdn) and *Bt* applications. In contrast, chemicals were very toxic to predators. The toxicity of azadirachtin to predaceous insects attacking bollworm, *H. armigera*, by exposing *Menochilus signatus* and lacewings, *Harmonia conformis*, to neem oil (50 and 200 ppm) and endosulfan (50 and 200 ppm) through prey, which had consumed one or the other of these compounds, showed that endosulfan decreased predation rates by *H. conformis* at 50 ppm. However, azadirachtin, when ingested with prey, did not affect predation rates between 50 and 200 ppm concentrations (Oi et al. 2001). Neither of these pesticides caused direct mortality to adult beetles or lacewing larvae at the tested concentrations. Azadirachtin at both concentrations delayed pupation of *M. signatus* and extended duration of the larval stage, which

increased the number of prey consumed by the predator causing serious mortality of the pupae. However, pupal lacewings were all killed by 200 ppm azadirachtin treatment and 50 % at 50 ppm azadirachtin treatment, distinctly reducing the population of the next generation.

Two ladybird beetles, *Cycloneda sanguinea* and *Harmonia axyridis*, were tested in the laboratory to eight fungicide formulations commonly used in citrus production in Florida, USA. Both benomyl and the combination of copper and petroleum oil proved toxic to larvae of *C. sanguinea* that were exposed to concentrations corresponding to recommended field rates, either as leaf residues or in topical spray applications. Larvae of *C. sanguinea* also suffered lethal effects when exposed to neem oil as a leaf residue, but not after topical application. No compound appeared repellent to adult beetles of either species (Michaud 2001). Jalali and Singh (2001) reported that at field recommended rates, endosulfan and neem-based product (Replin) were safe to adults of *C. sexmaculata* immediately after spraying, giving 20 and 0 % mortality in a semi-field test up to 25 days after spraying on cotton plants. Residues of Repelin^R were also safe to grubs.

Lok Nath and Singh (2003) evaluated the safety of four plant extracts, one neem formulation and a synthetic insecticide (dimethoate) to the ladybird beetle (*C. septempunctata*) and syrphid flies (Syrphidae) preying on *H. coriandri* infesting coriander (*Coriandrum sativum*) in Kumarganj, Faizabad, Uttar Pradesh, India, during 2001/2002. One day after spraying, Pride of India (*Lagerstroemia indica*) seed kernel extract (PSKE 1 %) was the safest to both predators, followed by karanj (*Pongamia glabra* seed kernel extract (1 %), neem (*A. indica*) seed kernel extract (1 %), buken (*Melia azedarach*) seed kernel extract (1 %) and Neemarin (1 %). Dimethoate (0.03 %) was highly toxic to the ladybird beetle and syrphid larvae. A similar trend was evident at 3, 5 and 7 days after spraying. Thus, the botanical extracts, especially PSKE, were safer and more environment-friendly insecticides when used on coriander crop for aphid control. Chakraborti (2004) reported that detopping of affected shoots with pests at 16 days after transplanting (DAT)

followed by application of neem cake at 3 and 1 kg/m² at 20-day intervals or foliar application of neem oil at 10 ml/l + azadirachtin at 4 ml/l at 7-day intervals beginning at 17 DAT were found to give better control of *A. gossypii*, *Scirtothrips dorsalis* and *Polyphagotarsonemus latus* on chilli crop, and at the same time, they spared coccinellids, syrphids and spiders compared to chemical control.

Balikai and Lingappa (2004) tested the aqueous extracts of some selected plant products during post rainy seasons in Bijapur, Karnataka, India, against the potential predators of aphids (*C. sexmaculata*, *C. septempunctata*, *C. carnea*, *Syrphus* sp. and *Ischiodon scutellaris*). The plant products were 2–3 times less toxic than malathion. Endosulfan was as toxic as 5 % *Datura metel* whole plant extract, 0.05 % Neem soap and 2 % *Pongamia pinnata* kernels. The botanicals such as 5 % *Ricinus communis* leaves, 5 % *Argemone mexicana* whole plant and 2 % *Prosopis juliflora* leaves were less effective to the predators. The plant products 5 % *Catharanthus roseus* leaves, 5 % *Pongamia pinnata* leaves, 5 % *Azadirachta indica* kernels, 5 % *Vitex negundo* leaves and 5 % *Adhatoda vasica* leaves were safe to natural enemies, and hence they can be effectively utilized in sorghum ecosystem for aphid management. Abudulai et al. (2004) studied the effects of Neemix 4.5 EC (at 210.4 g azadirachtin/ha) on the predators of *N. viridula* eggs on cowpea plants in the fields. Egg predation was not significantly different between the neem-treated and water-treated eggs in 2000 and 2001. Similarly, the percentage of predation on eggs in treated and untreated plots was not significantly different in both years. During the study, red imported fire ants (*Solenopsis invicta*) were regularly seen preying on egg masses of *N. viridula* in the field. Also, *Coccinella septempunctata*, *Coleomegilla maculata lengi* and other coccinellid larvae (Coccinellidae) were observed preying on eggs. Other predators of eggs were *Geocoris punctipes*, *Conoderus falli*, *Oecanthus celerinictus* and *Gryllus* sp. Silva and Martinez (2004) studied the effects of the neem seed oil aqueous solution on survival and performance of egg, larva and adult stage of the coccinellid predator

C. sanguinea under laboratory conditions. In a first trial, eggs and 2nd instar larvae were sprayed with the neem solutions at 0, 0.5 and 2.25 ml/l. Spraying the eggs did not affect egg hatch or larvae survival and development. When the larvae were sprayed, significant mortality was observed only at the higher concentration, and larval development and predatory capacity were not affected. Also, adults that emerged from treated larvae showed no alterations on sex rate, fecundity, fertility and longevity, thus indicating that at the tested concentrations, the neem oil does not reduce the reproductive potential of the species.

Hamd et al. (2005) conducted greenhouse experiments at Khartoum (Sudan) with the aphid predator *Hippodamia variegata* and NSKE (25 g/l), NeemAzal-T/S (1 % azadirachtin A) and fenvalerate (Sumicidin[®] 20EC). An equivalent of 400 l/ha was applied with a plastic hand sprayer, containing 0.2 l Sumicidin (40 g/ha fenvalerate), 1.6 l NeemAzal (16 g/ha azadirachtin A) or 10 kg of neem seed powder (ca. 30 g/ha azadirachtin A was applied as neem seed water extract). The beetles were fed on *Aphis gossypii* reared on cucumber leaves. Four different stages of the predator were sprayed topically with the test preparations. The preparations varied in their effects on the different stages of the predator. NSKE was less harmful to the predator than NeemAzal-T/S. In topical treatments with NeemAzal-T/S, the corrected mortality (%) was eggs 37.7, larvae 40.0, pupae 38.2 and adults 16.7; with neem seed water extract, eggs 15.1, larvae 26.7, pupae 29.4 and adults 10.0; and with fenvalerate, eggs 86.8, larvae 100, pupae 73.5 and adults 100. Feeding L2 larvae and adults on contaminated aphids resulted in the following corrected mortalities (%): Sumicidin 100, NeemAzal ca. 40 and neem seed water extract 20/17 (larvae/adults). Feeding on aphids treated via the soil with neem seed water extract resulted at maximum in 26.4/16.7 % corrected mortality (larvae/adults), while no effects on the longevity of adults could be observed. So, in contrast to Sumicidin, neem preparations (being effective against pests) proved to be harmless for the beneficial beetle.

Larvae of the seven spotted ladybird beetle *Coccinella septempunctata* were treated with

azadirachtin, and the impact on haemogram was investigated for 1, 30 and 60 min after treatment. Total haemocyte count increased 1 min after treatment with azadirachtin. Overall, azadirachtin was relatively safe for *C. septempunctata* larvae (Anjum et al. 2007).

Swaminathan and Hussain (2010) tested the side effects of botanicals, viz., neem seed kernel extract, eucalyptus oil and neem oil, against aphidophagous coccinellids, *Adonia variegata*, and found that neem seed kernel botanicals NSKE 10 % caused the highest mortality followed by neem oil (5.0 %), and the posttreatment effect (1 day after) evinced maximum reduction in feeding for NSKE (10 %) followed by neem oil (5 %).

4.6 Syrphids

Field trial conducted using neem emulsifiable concentrate for the control of sorghum aphid *Melanaphis sacchari* did not show any adverse effect on syrphid larvae and adults of coccinellids (Srivastava and Parmar 1985). Third instar larvae of the hover fly *Episyrphus balteatus* were mostly killed when treated with 100 ppm of an enriched seed kernel extract MTB/H20-VR-K synergized with sesame oil combined in a ratio of 1:4 (Schauer 1985). The larvae/pupae of syrphid flies seem to be more sensitive to neem products than those of other predators. Eisenlohr et al. (1992) reported that the number of syrphid larvae was not reduced in the field after spraying of NeemAzal-F on peach trees infested by *Myzus persicae*, but the survival of adults derived from larvae collected in the field on treated trees and held afterwards in the laboratory was quite low. Lowery and Isman (1996) observed that adult emergence of *Eupeodes fumipennis* was reduced by neem oil (0.5, 1, 2 %) to 35, 24 and 0 %, respectively, in comparison with controls.

4.7 Cecidomyiids

Lowery and Isman (1996) reported that the number of larvae of predaceous cecidomyiids was reduced in the field after application of

neem seed extract and neem oil (1 %) as compared to controls.

4.8 Lacewings

Neem seed kernel suspension 2 % sprayed on tobacco plants conserved *Chrysopa scelestes*, an egg and larval predator of *S. litura* (Joshi et al. 1982). The adults of the lacewing *Brinckochrysa scelestes* (*Chrysopa scelestes*) were repelled from egg laying on cotton plants after they were sprayed with various commercial neem products of Indian origin and aqueous NSKE (Yadav and Patel 1992). First instar larvae of the predator emerged normally from treated eggs. Polyphagous predator, *Chrysoperla carnea*, treated in laboratory and semi-field trials with AZT-VR-K (1,000 ppm) and with a mixture of this product with neem oil (250–30,000 ppm) induced neem oil toxicity on eggs or adults; the fecundity of the latter was also not significantly affected (Kaethner 1990, 1991). The number of eggs (fecundity) laid by adult females developed from treated larvae was normal. The mortality of larvae fed with neem-treated aphids did not differ from that of controls. On the other hand, 79 % mortality of larvae occurred after topical treatment in the laboratory. In contrast, spraying of potato plants together with larvae of *C. carnea* in screenhouses did not result in any toxic or morphological effects.

Vogt (1993) did not find any significant influence of NeemAzal-F on the larvae of the lacewing in field trials. In laboratory experiments of Hermann et al. (1997), high mortality of larvae and pupae of *C. carnea* occurred if larvae were kept on NeemAzal-T/S (0.3 and 0.6 %) contaminated glass plates (IOBC/WPRS standardized tests), but practically no mortality was found in semi-field trials. Vogt et al. (1997) also studied the effectiveness of NeemAzal-T/S at 0.3 % against *Dysaphis plantaginea* on apple and on its side effects on *C. carnea*. A single application of NeemAzal-T/S in April gave very good control of *D. plantaginea* for about 5–6 weeks. After this period, *D. plantaginea* built up new colonies and *Aphis pomi*, too, increased in abundance. Yield

losses caused by *D. plantaginea* were significantly lower in the neem-treated plot than in the untreated control plot. The side-effect test revealed that in the field NeemAzal-T/S was harmless to larvae of *C. carnea*. Neem seed extract was also found safe to *C. carnea* in comparison to nine insecticidal products (Sarode and Sonalkar 1999a) where chlorpyrifos, deltamethrin and cypermethrin were found highly toxic to *Chrysoperla*. There was no mortality of *C. carnea* due to neem-based pesticides like NSKE at 5 %; Neemark, Achook and Nimbecidine each at 0.003 % and neem oil at 1 % (Deole et al. 2000). On the contrary, Srinivasan and Babu (2000) evaluated NSKE and commercial neem products, viz., NeemAzal-T/S, NeemAzal-F, Nimecicine, Neemgold, TNAU neem product 0.03 % EC, TNAU neem product neem oil 60 EC and Indeem against eggs, grubs and adults of *C. carnea*. The products caused 14.66–25.33 % egg mortality compared to 8.00 % in untreated controls and 6.66–16.66 % grub mortality compared to 3.33 % in controls. The longevity of treated adults ranged from 18.66 to 20.66 days in treatments, while it was 23.66 days in control. Fecundity was also affected slightly by all neem products (599.66 to 741.66) as against 874.66 eggs in controls.

Ingawale et al. (2005) determined the effective and safer insecticides and plant products for the control of lucerne aphids (including *Acyrtosiphon pisum* and *A. kondoi*) and their effect on the pest predators (ladybirds and *Chrysoperla carnea*) in an experiment which was conducted during November 2002 in Rahuri, Maharashtra, India. The treatments comprised sprays of malathion 0.05 %, DDVP [dichlorvos] 0.05 %, deltamethrin 0.0075 %, dimethoate 0.03 %, neem [*Azadirachta indica*] seed extract 5 %, neem leaf extract 10 %, nirgudi leaf extract 10 %, Econeem 2 %, Nirma 2 % and untreated control. Deltamethrin, dimethoate, dichlorvos, malathion and Econeem gave consistently superior results over other treatments for the control of aphids at 2 and 7 days after spraying (DAS). At 15 DAS, only deltamethrin and dimethoate gave the best results. The untreated control recorded the maximum number of ladybirds followed by the botanical insecticides. As

regards *C. carnea* larvae, Econeem at 2 DAS and nirgudi leaf extract at 7 and 15 DAS showed the highest population.

Khan et al. (2013) tested neem oil and *Chrysoperla carnea* in different combinations against aphids in canola. Among the treatments, they found that module consisting of neem oil 2% + *C. carnea* proved very effective in reducing the aphid population and neem oil concentrations relatively safe to predators and suitable for use in integrated pest management of aphids in canola.

5 Effect on Predatory Spiders and Mites

Saxena et al. (1984) reported that the wolf spider *Lycosa (Pardosa) pseudoannulata*, an important predator of leafhoppers in rice fields in Asia, was not harmed by neem oil and alcoholic or aqueous NSKE. In fact, neem oil (3%) and aqueous NSKE (5%) were quite safe for the spiders, though endosulfan induced 100% mortality of the predators (Fernandez et al. 1992). NSKE, neem oil or neem cake extract (10%) treated rice plots had better recolonization of spider *L. pseudoannulata* than in monocrotophos (0.07%) treated plots after 7 days of treatment (Raguraman 1987; Raguraman and Rajasekaran 1996). The same neem products also spared the predatory mirid bug, *C. lividipennis* (Mohan 1989). The population of *L. pseudoannulata* and *C. lividipennis* were reported to be unaffected by different neem seed kernel extracts in paddy crop (Saxena 1987, 1989; Shukla et al. 1988; Jayaraj et al. 1993; Mariappan et al. 1993). Similar observation on rice crop was made by Nirmala and Balasubramaniam (1999) who studied the effects of insecticides and neem-based formulations on the predatory spiders of rice ecosystem. It was observed that feeding efficiency of *L. pseudoannulata* was higher than *T. javana* in all the treatments except in NSKE against green leafhopper *Nephotettix virescens* as prey, whereas rise in body weight was obtained in both predator species when they were treated with neem products, indicating the safety of neem to spiders. Babu et al. (1998) also reported that a combination

of seedling root dip in 1% neem oil emulsion for 12 h + soil application of neem cake at 500 kg/ha + 1% neem oil spray emulsion at weekly intervals gave an effective level of control of green leafhopper (*Nephotettix virescens*) infesting rice (var. Swarna). A combination of neem oil + urea at a ratio of 1:10 when applied three times at the basal, tillering and panicle initiation stages gave a superior level of control of brown planthopper (*Nilaparvata lugens*). The treatments, urea + nimin [neem seed extract] and a seedling root dip with 1% neem oil emulsion + neem cake at 500 kg/ha + 1% neem oil spray emulsion at weekly intervals was equally effective against *N. lugens*. All neem products had little effect on predators, *C. lividipennis* and *L. pseudoannulata* (Sontakke 1993; Babu et al. 1998). NSKE sprays at 5, 10 and 20% were also substantially safe for spiders and ants in cowpea ecosystems (Sithanantham et al. 1997).

Mansour et al. (1986) studied the toxicity of NSKE from different solvents on the spider *Cheiracanthium mildei* and found that NSKE 2% did not affect the spiders. But at 4% concentration, the sequence of toxicity of the extracts was pentane > acetone > ethanol > methanol and water; the latter two solvent extracts were non-toxic. Mansour et al. (1993) reported that the commercial products, namely, Margosan-O, Azatin and RD9 Repelin, showed no toxicity to the spider. Wu (1986) and Serra (1992) observed that the neem products were not at all toxic to predatory spiders. Nanda et al. (1996) observed the activity of natural enemies in cucurbit fields, where neem-based pesticides were applied for the control of *Henosepilachna vigintioctopunctata*. Natural enemies observed in considerable numbers were *Tetrastichus* sp., *Chrysocoris johnsoni*, *Tetragnatha* sp., *Oxyopes* sp. and orb-web spiders, and neem product did not inflict any harm to them. Lynx spider, *Oxyopes javanus*, was less sensitive to neem oil (50% EC) than *L. pseudoannulata* (LC50 values = 9.73 and 1.18%, respectively) (Karim et al. 1992), thereby confirming that neem oil was the safest pesticide for spiders (Wu 1986). In corn (Breithaupt 1995) and cabbage fields (Saucke 1995) in Papua New Guinea, no significant effect was observed

against *Oxyopes papuanus* from aqueous NSKE (2 %) or NeemAzal-S treatments. Serra (1992) did observe adverse effects from NSKE 4 % applied on unidentified spiders in tomato fields in the Caribbean.

The bioefficacy tests of neem derivatives against the predatory wolf spiders (*L. pseudoannulata*), jumping spider (*Phidippus* sp.), lynx spider (*Oxyopes* sp.), dwarf spider (*Callitrichia formosana*), orb spider (*Argiope* sp.), damselflies (*Agriocnemis* sp.) and mirid bug (*C. lividipennis*) showed that neem seed kernel extract and neem oil were relatively safer than the insecticides to *L. pseudoannulata*, *Phidippus* sp. and *C. lividipennis* in field conditions (Nanda et al. 1996). Markandeya and Divakar (1999) evaluated the effect of a commercial neem formulation (Margosan 1500 ppm) in the laboratory against two parasitoids and two predators. The formulation was tested at the field recommended dose of 10 ml/l. The neem formulation Margosan 1,500 ppm was safe to all the four bioagents studied, viz., *T. chilonis*, *B. brevicornis*, *L. pseudoannulata* and *C. sexmaculata*. Spider population in rice ecosystem was the lowest in carbofuran treatment and highest in neem cake treatments. The mean predator population of *Ophionea indica*, *Paederus fuscipes*, *Lycosa* sp. and coccinellid beetles was significantly higher in plots with *Azolla* at 5 t/ha, with or without neem cake at 1.51/ha, in field trials conducted in southern Tamil Nadu, India, under lowland rice irrigated conditions (Baitha et al. 2000).

Predatory mites and spiders also showed appreciable tolerance to neem-based insecticide sprays both in laboratory and field. The effect of Neemguard obtained from *A. indica* seed kernels on the predacious mite *Phytoseiulus persimilis* and the predatory spider *Cheiracanthium mildei* was investigated in laboratory experiments. Neemguard had no toxic effect on *C. mildei* or *P. persimilis* (Mansour et al. 1997). Azadirachtin (Nimbokill 60 EC)-based products were relatively safe to the predatory mite *Amblyseius fallacies* (Kain and Agnello 2002). Rao et al. (2003) reported that neem oil, Biobit^R + flufenoxuron, profenofos + Biobit and lambda-cyhalothrin + Biobit showed the best control of

the rice leaffolder and also conserved the spiders in the field. Biobit treatment alone resulted in a leaf folder damage of 17.55 % folded leaves with predator population 4.33–5.33 predator/ five hills.

Punzo (2005) conducted an experiment to evaluate the effects of azadirachtin on the mortality, growth and immunological function of the whipscorpion, *Mastigoproctus giganteus*, a large arachnid predator commonly found in arid areas and agroecosystems in southwestern USA and Florida. Ingesting prey injected with 1.0 and 10.0 mg/l of neem seed extract containing azadirachtin resulted in significant mortality over the 30-day test period. Ingestion of prey injected with 10 ppm azadirachtin gave significant mortality in both protonymphs and adult females. The ingestion of prey treated with 1.0 and 10 mg azadirachtin significantly decreased the size of *M. giganteus* nymphs. The pupae of azadirachtin-treated insects often exhibit deformities to the head and thoracic appendages.

Water extract of *Calotropis gigantea* was evaluated to *Chrysoperla carnea* and reported to exhibit normal egg hatchability and less larval and adult mortality (Patil et al. 1997). Aqueous suspension of *C. gigantea* did not affect hatchability and incubation period of *Rhynocoris marginatus* (Sahayaraj and Paulraj 1999a). Leaf extract of *Catharanthus roseus* was safe to grubs, pupae and adults of predators such as coccinellids, chrysopids and syrphid fly (Balikai and Lingappa 2004). Patil et al. (1997) also reported the safety of *C. roseus* water extract to *C. carnea*. Methanol and ethanol crude extracts of *Chenopodium ficifolium* were harmful to beneficial insects (Quang et al. 2010). Matter et al. (1993) tested the oils from *Citrus aurantium*, *Melia azedarach* and *Melia volkensis* on *Coccinella septempunctata* in the laboratory; none of them affected the adult survival and behaviour and also the consumption of aphids. *Chrysanthemum coronarium* whole plant extract was safe to beneficial natural enemies (Mourad et al. 2008). Acetone extracts of *Cymbopogon citrates* exhibited adverse effects on eggs, reduced efficiency of predation in larvae and induced poor rate of pupation on the Coccinellids,

Cheilomenes lunata and *Cheilomenes vicina* (Ofuya and Okuku 1994).

Whole plant extract of *Datura metel* was safe to grubs, pupae and adults of coccinellids, chrysopids and syrphid fly (Balikai and Lingappa 2004). Rotenone from *Derris elliptica* caused mortality of larvae and adults of *Coleomegilla maculata* and *Chrysoperla carnea* (Hamilton and Lashomb 1997), *Perillus bioculatus* (Goldstein and Keil 1991), *Dolichogenoidea* and *Chrysoperla externa* (Iannacone and Lamas 2003a, b). But rotenone was safe to all the three instars of generalist predator, *Metacanthus tenellus* (Oliver and Bringas 2000).

Leaf extract of *Ipomoea carnea* was safe to predators such as coccinellids and chrysopids (Patel et al. 2003). Patil et al. (1997) evaluated the effect of *Lantana camara* to *C. carnea* and reported normal egg hatchability and low mortality of larvae and adults. Leaf extracts of *L. camara* and *Jatropha curcas* were safe to predators such as coccinellids, chrysopids and syrphids (Patel et al. 2003). Leaf extract of *Justicia adhatoda* (syn. *Adhatoda vasica*) was safe to grubs, pupae and adult stages of predators such as coccinellids, chrysopids and syrphid fly (Balikai and Lingappa 2004).

Leaf extract of *Melia azedarach* showed low mortality of mirid predator (Jazzar et al. 1999). Leaf extract of *Mentha spicata* and *Thevetia nerifolia* was safe to coccinellids, chrysopids and syrphids fly (Patel et al. 2003). Acetone extract of *Momordica charantia* exhibited adverse effects on eggs, reduced efficiency of predation by larvae and induced poor rate of pupation on coccinellids, *Cheilomenes lunata* and *C. vicina* (Ofuya and Okuku 1994).

Nicotine sulphate from *Nicotiana tabacum* did not affect adults of *Leptomastidea abnormis*, *Scymnus* sp. (Viggiani 1973) and *C. septempunctata* (Singh et al. 1985). But nicotine sulphate was found to be toxic to *Phytoseiulus persimilis* (Stenseth 1990), to *Amblyseius eharai* (Kashio 1983), to *Hyposoter annulipes* (Heneidy et al. 1988), to *Campoletis chloridaeae* (Gunaseena et al. 1990), to *Dacnusa sibirica* and *Diglyphus isaea* (Heyler et al. 1992) and to *Apanteles africanus*

(Chari et al. 1996). On the contrary the nicotine from *N. tabacum* was safe to *Curinus coeruleus* (Diraviam and Viraktamath 1993).

Patil et al. (1997) evaluated the effect of leaf and whole plant extracts of *Parthenium hysterophorus* and found that the extracts did not affect eggs, larvae and adults *C. carnea*. Water extract of *Pongamia pinnata* did not influence hatching and incubation periods of eggs of *Rhynocoris marginatus* (Sahayaraj and Paulraj 1999). Derivatives of *P. pinnata* were least harmful to beneficial insects (Jayaraj 1991; Saminathan and Jayaraj 2002). Leaf or seed extracts of *Parthenium hysterophorus*, *Pongamia pinnata*, *Prosopis juliflora* and *Ricinus communis* and *Vitex negundo* were safe to grubs, pupae and adults of predators such as coccinellids, chrysopids and syrphid fly (Balikai and Lingappa 2004).

Ryanodine isolated from *Ryania speciosa* was safe to predators such as *Tetranychus urticae* and *Amblyseius longispinosus* (Schicha 1975), *Stethorus loxtoni*, *Stethorus nigripes* and *Stethorus vagans* (Walters 1976) and *Typhlodromus helenae* (Schicha 1977). Koodalingam et al. (2009) tested the sublethal concentrations of *Sapindus emarginatus* and observed that the kernel extract was found to be safe for two non-target aquatic larvae of *Chironomus costatus* and the nymphs of *Diplonychus rusticus*.

Alpha-terthienyl from roots of *Tagetes* species possessed all the desirable properties of a good insecticide/pesticide. It is fast acting, non-toxic, economic and property of degradation makes it more user friendly and safe (Manish et al. 2001). *Trichilia havanensis*-derived limonoids namely azadirone and 1,3+1,7-di-*O*-acetyl-havanensin did not show any toxic effect on two natural enemies *Chrysoperla carnea* and *Psytalia concolor* (Pilar et al. 2006). Patil et al. (1997) reported that extracts of *Thuja occidentalis* and *Vitex negundo* did not significantly reduce egg hatching and spared the adults *C. carnea*. Water extract of *V. negundo* did not affect the hatchability and incubation period of *Rhynocoris marginatus* (Sahayaraj and Paulraj 1999a, b). Acetone extract of *Zingiber officinale* exhibited adverse effects on eggs, reduced predatory efficiency of larvae

and induced poor rate of pupation to *Cheilomenes lunata* and *C. vicina* (Ofuya and Okuku 1994).

6 Effect on Honey Bees, Other Social Wasps and the Environment

Allophylus edulis extracts did not show any toxic effect against beneficial insect honey bee *Apis mellifera* (Lucia et al. 2009). Oil from *Allium sativum* was toxic to beneficial insects (Olkowski et al. 1995). Bees were moderately harmed by spraying *A. indica* extracts before flowering (Schmutterer and Hoist 1987). Neem products were found to be harmless to spiders, butterflies, ladybird beetles, wasps and bees (Saxena 1987; Schmutterer and Hoist 1987). Neem extracts had minimal toxicity on non-target organisms such as predators and pollinators (Naumann and Isman 1996) and degraded rapidly in the environment (Barrek et al. 2004). *A. indica* oil spray treatments were found to have no effect on adult honeybee populations (Melathopoulos et al. 2000). Neem seed oil (1 %) showed reduction in pollinator population 1 day after application (Harjindra Singh et al. 2010).

Azadirachtin, one of the more potent bioactive compounds from *A. indica*, was, in general, harmless to butterflies, bees, ladybirds and wasps (NRC 1992) and has very low mammalian toxicity and is relatively safe to beneficial insects (Gandhi et al. 1988). Azadirachtin was toxic to bee larvae, though less toxic to adults (Peng et al. 2000). Azadirachtin seems to be selective, non-mutagenic and readily degradable, with low toxicity to non-target and beneficial organisms, and causes minimal disruption to the ecosystem (Sundaram 1996). Azadirachtin can cause direct mortality, as it was determined for larvae of *Apis mellifera* and *Phormia terraenovae* (Rembold et al. 1981; Wilps 1987).

Commercial products from *A. indica* are reported to be harmless to natural enemies, pollinators and other non-target organisms (Ranga Rao et al. 2008; Singh and Singh 1996). *A. indica* formulations were found to be quite safe to spiders (Samiayyan and Chandrasekharan 1998).

A. indica-based insecticides had negligible effects on beneficial insects and low environmental impacts (Schmutterer 1995; Haseeb et al. 2004; Greenberg et al. 2005; Isman 2006). *A. indica* preparations are said to be safe for bees; they do affect the foraging behaviour and flight distances of bumble bees. The sublethal doses of azadirachtin affected the foraging distance of bumble bees (Karise et al. 2007). NeemAzal-T/S, a formulated product of *A. indica*, was proved to be harmless for the beneficial beetle (Hamd et al. 2005). When bees were caged in cotton field after spray, NeemAzal did not cause any mortality of honey bees 1h after spray (Mann and Dhaliwal 2001).

An exclusive review by Boeke et al. (2004) expressed that *A. indica* provides many useful compounds that are used as pesticides and could be applied to protect stored seeds against insects. However, in addition to possible beneficial health effects, such as blood sugar-lowering properties and antiparasitic, anti-inflammatory, antiulcer and hepato-protective effects, also toxic effects are described. Also, they presented toxicological data from human and animal studies with oral administration of different neem-based preparations. The nonaqueous extracts appear to be the most toxic neem-based products, with an estimated safe dose (ESD) of 0.002 and 12.5 µg/kg bw/day. Less toxic are the unprocessed materials seed oil and the aqueous extracts (ESD 0.26 and 0.3 mg/kg bw/day and 2 µl/kg bw/day respectively). Most of the pure compounds show a relatively low toxicity (ESD azadirachtin 15 mg/kg bw/day). For all preparations, reversible effect on reproduction of both male and female mammals seems to be the most important toxic effect upon subacute or chronic exposure. From the available data, safety assessments for the various neem-derived preparations were made, and the outcomes are compared to ingestion of residues on food treated with neem preparations as insecticides. This leads to the conclusion that, if applied with care, use of neem-derived pesticides as an insecticide may be encouraged.

Clytostoma callistegioides, *Dolichandra cynanchoides*, *Dodonaea viscosa*, *Macfadyena unguis-cati*, *Phytolacca dioica*, *Prosopis juliflora*,

Salvia procurrens and *Salvia guaranitica* extracts did not show any toxic effects on the beneficial insect honey bee *Apis mellifera* (Lucia et al. 2009). *Chrysanthemum coronarium* whole plant extract was safe to beneficial natural enemies, humans and the environment (Mourad et al. 2008). Rotenone from *Derris elliptica* was toxic to bumble bees (Marletto et al. 2003). *Pongamia pinnata* (seed oil 1 %) showed reduction in pollinator population 1 day after application (Harjindra Singh et al. 2010).

7 Conclusions

From the foregoing, it is summarized that plant-derived insecticides show slight to moderate ill effects on parasitoids, predators and honeybees as obviously they also belong to the same class Insecta. But its innate biodegradability and low mammalian toxicity put them at height of 'safe/green' insecticides. In the case of parasitoids, certain guiding principles are suggested in accordance with multi-array activities of neem products in insects. Parasitoids are also susceptible, when they come in direct contact with plant origin insecticides including neem products. In such circumstances, blanket application of neem/any botanical product without understanding the behaviour of the parasitoid may adversely affect the beneficial capacity of the parasitoid. For example, the inundative release of the egg parasitoid *T. chilonis* should be resorted 3–4 days before/after neem product application. The external larval parasitoids are no exception to the ill effects, if they are in direct contact with neem products. To avoid this, for inundative releases, application of neem products may be followed by the release of the parasitoids and spraying may be avoided if the parasitoids are in larval stages in the field. Hence, pre-sampling is suggested to know the stage of the parasitoid, be it internal or external, for timing the application of botanical-based insecticides.

In the case of predatory insects and spiders, certain degree of selectivity is nevertheless apparent, as adult insects show no or relatively low sensitivity as in the case of earwigs, crickets, true

bugs, beetles, lacewings and wasps. This can be explained by the fact that growth-disrupting compounds affect the first line juvenile instars of insects. The fecundity of neem-treated adult and predaceous parasitic insects and the fertility of their eggs are also not or only slightly affected by neem, in contrast to some phytophagous species. In some cases, the predation efficiency may be reduced. Nymphal/larval instars of beneficial insects are sensitive to neem products when topically treated, and reduction in food ingestion, delayed growth, difficulties in moulting, teretological and morphogenetic defects, reduced activity and increased mortality are normally observed in the laboratory. But far less drastic or even no effects are observed under semi-field or field conditions. This is partly due to the fast breakdown of the active principles under field conditions.

Neem and entomophages are twin gifts in IPM without endangering the agroecosystem. In fact, conservation biological control most commonly used the activity of native organisms. Hence, pre-sampling the parasitoids/predators is necessary in timing the application of botanical insecticides including neem products in order to avoid ill effects, if any. The 'integrated biological control' will include natural enemies vis-à-vis other botanical insecticides for organic production of agricultural products and to avoid the ill effects of synthetic chemical pesticides.

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