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Review

Neem and Environment: Integration of Neem with Bioagents in Insect Pest Management

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Of about five lakh plant species in the world today, the most useful one is the neem, *Azadirachta indica* A. Juss, which has been credited as a tree for solving global problems in view of its potential for use in agro-forestry and reforestation, improving soil fertility, excelling pest management and bettering health. Neem tree is supposed to originate in the forests of Karnataka (South India) or the dry island forests of Myanmar. Its natural distribution ranges from the Kanyakumari to Shivalik Hills in India. Now neem trees are found in 80 countries and its estimate is about 91 million trees. South Asian and Sub-Saharan regions constitute the main areas of distribution. Neem trees in India grow under different ecological regimes and very valuable forestry species. Of the several uses of neem, pesticidal value is the most important to ward-off pests and diseases on crops. Today more than 140 active principles have been identified, of which the most potent is azadirachtin. Neem derivatives affect about 500 species of insects. Neem's effects such as repellency, feeding and oviposition deterrence, growth inhibition, mating disruption and chemo-sterilization are more desirable than quick knock-down in integrated pest management programmes as they reduce the risk of exposing pests to natural enemies and leave no or untraceable residues in the environment. In fact, neem pesticides are commonly used to enhance the activity of native organisms in conservation biological control due to its bio-safety. NPV and Bt are highly compatible with neem products. In the case of parasitoids/predators, presampling and timing of application are necessary in order to avoid few ill effects of neem products on them. Neem is a perfect companion with other biopesticides in insect pest management.

Agasthiar, the great saint of ancient India, around 5000 years ago once made prophetic remarks to his disciples to sow the seeds of neem and let the benefits of its medicinal properties go to mankind for many more years to come. Neem, *Azadirachta indica* A. Juss belonging to Meliaceae is part and parcel of Indian medicine and culture. It is a botanical cousin of mahogany. According to a report by Board on Science and Technology for International Development, "this plant may usher in a new era in pest control, provide millions with inexpensive medicines, cut down the rate of human population growth and even reduce erosion, deforestation and the excessive temperature of an overheated globe" (National Research Council, 1992). Neem's other descriptions such as "nature's bitter boon", nature's gift to mankind", "the tree for many occasions", "the tree that purifies", "the wonder tree", "the tree for solving global problems" and "the tree of the 21st century" are recognition to its versatility.

In India, neem tree is called as *Margo, Limba*, *Limbo*, *Neem, Nim*, *Nimb*, *Nimba*, *Vembu, Vepa* or *Veppam* (and 100 more names); in Indonesia: *Imba*, *Intaran*, *Mimbo* and *Mindi*; in Iran: *Azad-drakht-I-hind* (free tree of India); in Sri Lanka: *Kohomba* and in Thailand: *Dao*, *Kwinin* and *Sadaoindia* (Schumutterer, 1995). Neem's products and derivatives have been found use in agriculture, forestry, medicine and household purposes.

On the basis of uses, the ancient Indian names for the neem tree include *probhadra* (very useful), *paribhadrak* (spreading its utility over large distances), *sarvobhadrak* (useful in every way) and *rajbhadrak* (best among all the useful trees), all of which point to its immense usefulness in Indian way of life (Dhaliwal *et al.,* 2004). The tree could provide shade to cattle and man, and the leaves are used as fodder for ruminants. The wood is used as fuel, timber for household furniture, and for agricultural implements. The seeds can provide oil for use in household lamps, lubricant and soaps. The seed cake, after washing, can be used in small amounts in poultry and cattle feed, as a source of organic manure, for conservation of nitrogenous fertilizers and for the elimination of nematodes (Koul, 1996; Koul, 2004). Thus, neem tree is the subject of concern for agricultural scientists, physicians and industrialists because its plant allelochemicals possess insecticidal properties (Saxena, 1989; Raguraman and Saxena, 1994; Jacobson, 1995;

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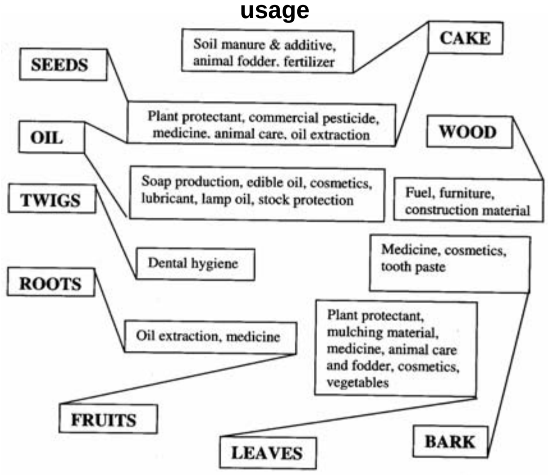
Kraus, 2002; Jayaraj and Ignacinuthu, 2005; Chandrasekaran, 2007; Raguraman, 2007; Raguraman and Ganapathy, 2009; Singh *et al.*, 2009) source of nitrogenous fertilizer, plant disease control, elimination of nematodes, its use in Ayurvedic, siddha, dental care and allopathic medicines and in manufacture of soaps (Fig. 1) (Koul and Wahab, 2004).

Fig 1. Parts of neem tree and their potential usage



Source: Koul (2004)

Diversity

Neem, the genus Azadirachta has two species, i.e. *A. indica* A. Juss. (syn. *Melia indica* Brandis, *Melia azadirachta* L.) and *A. excelsa* (Jack) Jacob (Syn. A. *integrifolia* Merr., *Melia excelsa* Jack) (Dhaliwal *et al.*, 2004). A. *indica siamensis* Vales is indigenous to Thailand (Oo, 1987). Loose panicle, open and long flowers and flowering in during April in *A. indica* and dense panicle, stout and bigger flowers and flowering during November/December in *A. indica siamensis* are the main distinguishing features. Other than the genus *Azadirachta, Melia azaderach* L., which is known as China Berry is more prevalent in China, India and North Eastern Asia.

Neem in India grows in marginal, degraded and wastelands from even plains to high hills up to 1000 meters. Neem is found naturally in the forest areas such as southern tropical dry deciduous forest, northern and southern tropical thorn forests, and tropical dry evergreen forest proving its suitability for agro-forestry and social forestry (Swaminathan and Raguraman, 2009). As the neem grows under different ecological regimes, the fruiting takes place during different months of the year with consequent variation not only in morphological features but also in extract yields and chemical constituents. In Tamil Nadu, neem trees set for flowering from April to June and mature fruits from May to August.

Chemistry

Neem is bitter in taste. The bitterness is due to the presence of an array of complex compounds called "triterpenes" or more specifically, "limonoids". About 140 bioactive compounds have been isolated from various parts of neem tree; still more are being isolated. This formidable array of highly bioactive compounds makes neem a unique plant with potential application in agriculture, medicine and conservation of environment. The limonoids in neem belong to nine basic structure groups: azadirone (from oil), amoorastaitin (from fresh leaves), vepinin (from seed oil), vilasinin (from green leaves), gedunin (from seed oil and bark), nimbin (from leaves and seed), nimbolin (from kernel), and salanin (from fresh leaves and seed), and the aza group (from whole seed) (Kraus, 2002). Azadirachtin and its analogues have fascinated researchers for the past three decades because of their phagorepellency, growth inhibition, and chemosterilising effects on insect pests (Saxena, 1989; Schmutterer, 1995, Koul and Wahab, 2004; Raguraman *et al.*, 2004).

Azadirachtin content in neem could vary considerably due to ecotypic or genotypic differences. There are also vast differences in extract yields and bioefficacy of neem ecotypes, i.e. those growing in dry areas have higher bioactivity than those growing near the sea (Singh, 1987) and major bioactivity occurs due to the presence of a limonoid, azadirachtin. Trees growing in Northern India are bigger in size than those growing in Southern India. Biologically active materials are concentrated in the seed of the tree and hence seed quality and yield are very important. Singh *et al*., (1998) reported 0.35 per cent azadirachtin A in *A. indica* and *A. indica siamensis*, and 0.38-0.56 per cent in A. *excelsa.* There have been vast differences in azadirachtin content of seeds obtained from different countries (Ermel, 1995). However, the highest amount of azadirachtin was detected in samples from South and Southeast Asia *i.e.* India, Myanmar and Thailand (Table 1).

Table 1. Azadirachtin and oil content of neem seed kernels from Asian countries

Country	Azadirachtin (mg/g)	Oil (%)
Iran	2.75	45.4
India	5.14	47.6
Myanmar (Burma)	6.10	48.8
Sri Lanka	3.40	50.1
Thailand	5.20	45.0
Yemen	4.44	49.7

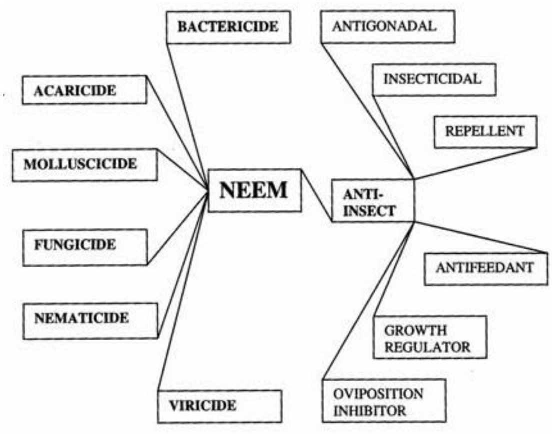
Source: Ermel (1995)

Rengasamy *et al.* (1996) reported that azadirachtin content in neem seed kernels from eight agroecological regions of India varied from 0.14 to 1.66 per cent. The ecotypes growing in regions with moderate climate, red laterite and shallow medium black soils and altitudes less than 500 m above mean sea level were rich in azadirachtin content, whereas ecotypes growing in high altitude alluvial soils with extreme hot and cold climates had very low azadirachtin content. The azadirachtin content of neem fruits collected from an agroclimatic zone in Rajasthan varied from 0.19 to 0.67 per cent (Gupta and Prabhu, 1997). Similarly, azadirachtin content in neem seeds collected from 12 different locations in Tamil Nadu varied from 3.47 to 6.70 g/kg of kernel and oil content varied from 261 to 436 g/kg of kernel (Sridharan and Venugopal, 1998). These studies indicate a negative influence of total rainy days during fruiting season (April to August on the azadirachtin content and significant positive influence of sunshine hours during off-season (September to March) on the oil content of the seeds.

Neem as Biopesticide

Neem and its allelochemicals have variety of effects on insect pests, pathogenic fungi and bacteria and nematodes (Fig. 2). It has a long history of use primarily against household and storage pests in Asian continent. However, a breakthrough was made by Pradhan *et al.* (1962) who successfully protected the crops by spraying them with 0.1 per cent neem seed kernel suspension during a locust invasion in Northern India. Till date neem products have been evaluated against 450-500 species of insects in different countries around the world and 413 of these are reportedly susceptible at different concentrations (Schmutterer, 1995). In India alone, neem has been evaluated against 103 species of insects, 12 nematodes and many pathogenic fungi (Arora and Dhaliwal, 1994; Schmutterer and Singh, 1995; Suresh *et al.,* 2004).

Fig 2. Neem as biopesticide



Source: Koul (2004)

Insect pest management

Neem based insecticides exhibited a wide range of biological effects on the target insects. The repellent and antifeedant effects of neem have been reported against a wide range of insect pests including desert locust, *Schistocerca gregaria;* migratory locust, *Locusta migratoria*; rice planthoppers, *Nilaparvata lugens* and *Sogetella furcifera*; the leaf folder. *Cnaphalocrocis medinalis;* and the ear-cutting caterpillar, *Mythimna* separate (Saxena, 1989). *N. lugens* fed on neem sprayed rice seedlings had poor growth and low symbiont load; and even starved nymphs avoided feeding on plants sprayed with neem oil (Raguraman and Saxena, 1994; Raguraman and Rajasekaran, 1996). Concentrations ranging from 0.001 to 0.4 per cent of various neem seed kernel (NSK) extracts have generally been found to deter the feeding of most of the insects evaluated so far (Singh, 2000). The growth inhibitory effects of neem derivatives result in various developmental defects and even mortality. The larvae of various lepidopterous pests like *P. xylostella*, *Spodoptera frugiperda*, *Helicoverpa zea*, *Pectinophora gossypiella* and *Ephestia kuehniella* studied in various developing countries in Asia showed impaired development on neem treated diet (Saxena, 1993).

Neem products also affect insect vigour, longevity and fecundity. Neem compounds sterilized the females of *Epilachna varivestis* and *Leptinotarsa decemlineata*, while reproductive maturation was inhibited in *N. lugens* males. At higher concentrations, most females did not emit normal male eliciting signals (Saxena, 1998). Neem products have also been found to act a ovipositional deterrents for *Dacus cucurbitae*, *Helicoverpa armigera*, *Spodoptera litura*, *Callosobruchus* spp., *etc*. (Parmar and Singh, 1993). Ovicidal activity of neem products has also been reported in other insect species including *Corcyra cephalonica*, *Earias vittella* and *S. litura* (Arora and Dhaliwal, 1994).

A number of studies have been carried out in several countries of Asia to evaluate neem alone or in combination/alternation with conventional insecticides and other approaches against insect pests of agricultural crops. In the Philippines, plots treated with a 2:10 neem cake and urea mixture applied at 120 kg/ha had lower incidence of ragged stunt, grassy stunt and tungro viruses and yielded significantly more than control plots in both dry and wet seasons. Also, weekly spraying of 50 per cent neem oil-custard apple mixture in 4: 1 proportion (v/v) using ultra low volume sprayer at 8 litres/ha from seedlings to the maximum tillering stages significantly reduced tungro incidence and increased grain yield (Abdul Kareem *et al*., 1987). In field trials conducted in India, neem treatments were found effective against brown planthopper, green leafhopper, yellow stem borer, rice gall midge, rice leaf folder and grasshopper (Dhaliwal *et al*., 1996; Raguraman and Rajasekaran, 1996). Sprays of NeemAzal 5% @ 1.0 and 0.5 ml/l were as effective as monocrotophos 5-6 ml/l against rice leaf folder, Cnaphalocrocis medinalis and yellow stem borer, Scirpophaga incertulas, respectively (Dhaliwal *et al.*, 2002).

Neem products applied in combination with synthetic pyrethroids effectively controlled cotton pests (Gupta *et al.*, 1999). An azadirachtin-rich insecticide, RD-9 Repelin, controlled the bollworm complex on cotton in Punjab and in Andhra Pradesh on par with quinalphos (Dhawan and Simwat, 1996). Alternate application of neem products and conventional insecticides made at the economic threshold level of 6-8 proved effective against *Bemisia tabaci* adults (Mann *et al.*, 2001). NeemAzal and Rakshakgold @ 2 l/ha when alternated with endosulfan and chloropyriphos effectively checked *B. tabaci* below economic threshold. In field trials conducted in Karnataka, neem seed kernel powder extract (4%) was found to be effective against *P. xylostella* and *Crocidolomia binotalis* (Moorthy and Kumar, 2000). Thus, neem has bright prospects in managing insect pests of major agricultural crops as integral component of biointensive pest management.

Integration of Neem Pesticides with Bioagents

Although neem pesticides are environment-friendly, they are not as effective as insecticides because of their low toxicity, slow action and quick degradation. Hence, integrating them with other components of IPM, *viz.*,entomophages (parasitoids and predators) and entomopathogens (viruses, bacteria, fungi and protozoans) will be more useful for sustainable crop protection. A recent review indicated that neem products are safer to egg and larval parasitoids and predators including spiders and can be used along with entomopathogens in various agro-ecosystems (Raguraman *et al.*, 2004; Jayaraj and Ignacimuthu, 2005).

Parasitoids: The safety of neem products (NSKE 2%) on the egg-laying behaviour of the parasitoid *Telenomus remus* on *Spodoptera litura* egg-masses was shown by Joshi *et al.,* (1993). Neem oil 50 EC (3%) was reported safe to *Trichogramma japonicum* (Jayaraj *et al.,* 1993). Aqueous, ethanolic and hexane extracts of neem seed kernel were found to deter oviposition and feeding of *Trichogramma chilonis* Ishii but no physiological and IGR effects were noticed (Raguraman and Singh, 1995, 1999). Azatin, Neem EC and Azadirachtin at 50 g/ha, had no significant effect on *T. minutum* Riley (Lyons *et al.,* 1996). However, Saminathan (2000) observed that direct exposure of *T. chilonis* reduced the adult emergence (15.5-18%) and parasitization (44-64%). The unfortified oils reduced the population of *Bracon brevicornis* up to 34% 7 days after treatment in field trials. Raguraman and Singh (1998) suggested combined use of the larval parasitoid, *Bracon hebetor* by presampling for stage of the parasitoid in the field and release of the adult parasitoid after 4-5 days of neem sprays.

Predators: Among various predators, spiders are more tolerant to neem products. There was no toxicity of certain neem products to the spider *Chiracanthium* mildei under lab conditions. The wolf spider, *Pardosa pseudoannulata*, a potential predator on rice leafhoppers is not harmed by spraying neem oil and NSKE showing good compatibility with botanical pesticides. Ineffectiveness of neem extracts against mirid bug *Cyrtorhinus lividipennis* (Reuter) was reported by several workers. Dhaliwal et al., (1999) reported that neem formulations were comparatively safer to the predator *Coccinella septempunctata* L.

Nucleo Polyhedro Virus (NPV): Although (NPV) has been reported effective against many insects, its prolonged incubation period, slower action and sensitivity to ultra-violet rays are few bottlenecks in using this pathogen against economic pests. Neem bitter 0.1% in combination with SlNPV and crude sugar (1%) inflicted significantly higher mortality on S. litura and this combination recorded the shortest LT50 (Jayaraj and Ignacinuthu, 2005).

Bacteria: An additive effect of a neem product viz., AZT-VR-K was observed with various B. *thuringiensis var. kurstaki*, which resulted in enhanced mortality of *Spodoptera frugiperda* larvae (Hellpap and Zebitz, 1986). Combined use of NeemixR and *B. thuringiensis* var. kurstaki significantly increased the mortalityof *H. virescens* larvae much more than the individual treatment. Increased efficacy of mixture of AlignR (neem formulation) and XentariR (*B. thuringiensis* formulation) against *P. xylostella* was reported by Mau *et al.* (1995).

Fungi: The growth of the fungi *Metarhizium anisopliae* var. anisopliae and Beauveria brongniartii was not affected by a neem product, NeemarkR (Vyas *et al*., 1999). Combinations of sub-lethal concentrations of NSKE with sub-lethal concentration of entomo pathogenic fungi showed efficacy on the mortality of *S. litura* and *H. armigera* (Saminathan, 2000).

Conclusion

Neem and bioagents are nature's twin gifts to mankind for their utility in the IPM of agricultural pests, without endangering agro-ecosystem. In fact, conservation biological control has been most commonly used to enhance the activity of native organisms. NPV and Bt are highly compatible with neem products. In the case of parasitoids/predators, presampling and timing of application are necessary in order to avoid the ill effects of neem products, if any, on them. It is obvious that new millennium will look forward to "integrated biological control" that will include natural enemies *viz-a-vis* other biopesticides synchronizing with ecological and behavioural aspects of pests. In the context of organic farming neem based pesticides would play a vital role or even the befitting component as an alternative to synthetic pesticides in pest management along with biocontrol agents.

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