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Anti-phytopathogenic and SAR inducing properties of Neem: a review

Navodit Goel*, Kumari Anukrati, P K Paul
Amity Institute of Biotechnology, Amity University Uttar Pradesh, 48a, Knowledge park III,
Greater Noida, Uttar Pradesh, India - 201308
***Corresponding author: E-Mail: navoditgoel1985@gmail.com, Mobile: +91-9990033817**

ABSTRACT

Background: Plant protection has always been an interesting area of research for scientists for sustenance of quality agriculture. Several effective synthetic insecticides, fungicides and other microcidal chemical have been in practices for long now. However, this overzealous and indiscriminate use of these chemicals is harmful to the animals, field grown food, non-target flora and fauna and humans as well. Due to increasing awareness of the ill effects of these synthetic compounds on human and animal health as well as to the agro-ecosystem, efforts are being made to develop alternative and more environment-friendly methods of pathogen and pest management.

Scope: Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Neem (*Azadirachta indica*) is one such plant with almost every part of it *viz*. seeds, leaves, roots, bark, trunk and branches having multiple antimicrobial potency. Some constituents of the neem plant that effect disease control demonstrate systematic action in inducing resistance in certain plant species.

Conclusion: Several tests have indicated considerable promise for use of neem plant in diseases management. In this review, we have tried to discuss about the researchers conducted all over the world during past few decades in order to testify and validate the applicability of neem extracts and products as cost-efficient and eco-favoring biocide.

KEY WORDS: Neem, *Azadirachta indica*, systemic acquired resistance, biocide, plant protection.

1. INTRODUCTION

Plants possess a range of defenses that can be actively expressed in response to pathogens and parasites of various scales, ranging from bacteria, viruses to insect herbivores. The timing of defense response is crucial and can either make a plant susceptible or resistant to pathogens. In systemic acquired resistance (SAR) and induced systemic resistance (ISR), the plant defenses are preconditioned either by prior infection or treatment with elicitors that results in elevated resistance (or tolerance) against subsequent challenge by a pathogen or parasite (Vallad and Goodman, 2004). While SAR is characterized by broad-spectrum disease resistance and is mediated via a salicylic acid (SA) dependent process (Mauch-Mani and Metraux, 1998) induced systemic resistance (ISR) is mediated by a jasmonate/ethylene sensitive pathway (Pieterse and Van Loon, 1999). However, Metraux (2001) interprets that SAR and ISR are synonymous.

Benzothiadiazole (BTH) (Choi and Hwang, 2011) and β-aminobutyric acid (BABA) (Zimmerli, 2001; Ton, 2009; Slaughter, 2012) could induce SAR in plants leading to protection against pathogens (Percival, 2001). Harman (2004), also demonstrated that exogenous application of certain inducers elicits similar responses in plants. The activation of defense system is characterized by production of reactive oxygen species (ROS), synthesis of phytoalexins, accumulation of salicylic acid, enhanced expression of pathogenesis-related (PR) genes and activation of phenylpropanoid pathway, leading to the synthesis of higher phenolic compounds toxic to microbial pathogens (Durrant and Dong, 2004). Induction of SAR leads to priming of plants during which the plant defenses are activated by the further presence of the pathogen. Under conditions of disease pressure, primed plants exhibit a higher fitness than non-primed plants or defense-expressing plants. The enhancement of plant's defense capacity by priming is correlated with a potentiated expression of defense genes and *de novo* synthesis of antimicrobial compounds such as PR proteins, which are expressed in uninfected tissue in response to first infection (Ramos Solano, 2008).

Until recently, chemical means of disease control in plants was prevalent in common agricultural practices. However, environmental concerns and resistance of pests and pathogens to these chemicals has led to a steady increase in development of environment-friendly agrochemicals. Induction of SAR could be a suitable alternative to chemical crop protectants.

This review focuses on the implications of neem products/extracts in the induction of SAR in several plants as a method of plant protection and its future potency in meeting our expectations for the same.

Antimicrobial activity of Neem extracts: In India, traditionally neem has been used to control pests and pathogens of a wide range of crop plants and stored grains. Neem extracts of leaves and fruits are anti-feedent to insect pests of plants and stored food products (Schmutterer, 1995). Fostering advancement, studies in the last few decades have demonstrated the broad spectrum antimicrobial properties of neem against plant pathogens (Ogunwolu and Oddunlami, 1996; Govindachari, 2000; Gajalakshmi and Abbasi, 2004; Javed, 2007; Krishnamurthy, 2008; Wang, 2010; Bajwa and Ahmad, 2012).

Chopra (1952) and Rao (1986) could demonstrate the significant inhibitory effects of neem extracts on several bacterial strains. *Azadirachta indica* extracts could reduce *in vitro* growth of *Pestalotiopsis mangiferae* by

about 84.66 % (Rai, 1996). Coventry and Allan (2001) demonstrated a broad spectrum antibacterial and antifungal activity of neem seed extracts using microbial growth inhibition assays. The growth of potato tuber rot causing pathogen *Rhizopus oryzae* was effectively controlled by the aqueous leaf extracts of *Azadirachta indica* both *in vitro* and *in vivo* (Amadioha, 2001). Inhibition in *in vitro* spore germination of a number of pathogenic fungi such as *Pestalotiopsis theae, Colletotrichum camelliae, Curvularia eragrostridis, Botryodiplodia theobrome, Phyllactinia corylea, Peridiopsora mori, Pseudocercospora mori, Myrothecium roridum, Fusarium oxysporum* f.sp. *lycopersici, Fusarium solani, Rhizoctonia solani, Alternaria solani, Sclerotinia sclerotiorum, Aspergillus niger* and *Geotrichum candidium* (Saha, 2005; Maji, 2005; Agbenin and Marley, 2006; Haikal, 2007; Moslem and El-Kholie, 2009; Yeni, 2011, Chandrashekara, 2012; Kanherkar, 2013; Al-Hazmi, 2013) by different extracts from neem has been observed by various scientists and they have advocated neem to be used as a novel antifungal biocide for eco-friendly disease prevention in economically important crops like tea, tomato and mulberry. Similarly, Ashraf and Javaid (2007) reported that a 5-20% aqueous leaf extract of *A. indica* could reduce the *in vivo* growth of *Macrophomina phaseolina,* a soil-borne fungal pathogen known to cause root rot disease in more than 500 plant species, and also inhibit its growth by 34 to 85% in liquid culture. They hinted towards the possible application of neem extracts in management of charcoal rot of sunflower. Hoque (2007) suggested that neem extracts possess novel compounds containing antibacterial properties that can potentially be useful to control food borne pathogens and spoilage organisms.

Ethanol extracts of neem leaf showed considerable antibacterial activity against *Xanthomonas oxonopodis* and antifungal activity against *Fusarium oxysporum* (Perumal, 2008). Mondall (2009) signified that different concentrations of aqueous and alcoholic extracts of neem leaf inhibited the *in vitro* growth of seed borne fungi *Aspergillus* and *Rhizopus* at all ages. They intimated that the results are promising for protecting crop species against the fungal infestation and leading towards improvement of the crop in terms of yield and productivity. The presence of constituents like, azadirachtin and nimbin in the methanolic extracts of seed, leaf and bark of neem which exhibited antioxidant nature were prejudicial for microbes and could be possible nominee of being an effective bio-insecticide (Amal, 2009).

Significant levels of broad spectrum microcidal activity of neem extracts and neem based products have reflected their potency towards curbing down microbial multiplication which can be useful in controlling surface-dwelling plant pathogens. These neem products/extracts have shown direct inhibitory effects on the growth and sustenance of the phytopathogens and are able to kill them at low concentrations. In a few cases, where neem products are not efficient in controlling the microbial growth, it has been observed that the later's physiological functions are altered due to which the production of toxins in them is reduced to very low levels, hence disabling them in causing disease in plants (Ogbuewu, 2011). The researchers reported here formalize that neem extracts are able fungicides and bactericides and thus their implications in controlling crop pathogens can boost up the crop yield with minimum loss due to diseases.

Reduction of disease incidence in crops by neem extracts: Numerous studies have lately emphasized on the ability of extracts from different parts of neem plant in inhibiting the *in vitro* as well as *in vivo* growth of crop pests and pathogens, hence reducing the disease incidence in plants grown under controlled and field conditions and during storage. A 2% aqueous emulsion of clarified neem seed oil was found to be fungicidal against apple pathogens *Botrytis cinerea* and *Glomerella cingulata* (Moline and Locke, 1993). Treatment of banana fruit infested with *Fusarium oxysporum* with aqueous leaf extract of *A. indica* could effectively control the disease with minimum percentage loss in fruit weight (Singh, 1993).

Efficacy of neem extracts against several rice pathogens has been demonstrated during various researches. Ganguly (1994) reported that aqueous neem leaf extract inhibited mycelial growth and spore germination of *Helminthosporium oryzae* and *pyricularia oryzae* responsible for blast and brown spot of rice plant respectively. Furthering these results, Amadioha (2000; 2002) were able to control rice blast *in vitro* and *in vivo* with water and ethanolic leaf extracts, and oil extract of seeds neem which were able to significantly reduce the *in vitro* radial growth of *Pyricularia oryzae* and *Cochliobolus miyabeanus* and significantly reduce the development and spread of blast disease in rice plants in the greenhouse and brown spot disease in fields respectively.

The fungicidal properties of aqueous leaf extracts of neem against *A. alternate* from pear fruits with 85% control of fruit rot *in vivo* were demonstrated by Srivastava (1997). Hot aqueous and oil extracts of neem seeds significantly reduced spore germination, growth of *Colletotrichum indemuthianum in vitro* and the size of pathogen induced lesions in cowpea (*Vigna unguiculata*) when applied either before or after the occurrence of infection in plants (Amadioha and Obi, 1998). Wet rot disease of *Amaranthus* sp. caused by *Choanephora cucurbitarum* was controlled using root, bark and fruit extracts of *A. indica* and it was considered that it could be a possible source of pathotoxicity as they are systematic and easily biodegradable (Olufolaji, 1999). Neem seed kernel extract was found effective not only in controlling the powdery mildew disease but also in increasing the grain yield of blackgram under pot culture experiment (Rettinassababady, 2000).

Management of several fungal (*Alternaria solani*, *Fusarium oxysporum*) (Patil, 2001; Agbenin, 2004; Hassanein, 2008; Sallam, 2011), bacterial (*Xanthomonas campestris*) (Reddy, 2012) and nematodal (*Meloidogyne javanica*, *Meloidogyne incognita*) (Javed, 2008; Hadian, 2011) diseases in greenhouse and field grown tomato plants by the application of extracts from various parts of neem have been achieved during diverse investigations during the last decade and implications of these products in the form of an effective biocide has been highly recommended. Pepper plants grown in controlled as well as natural environment when sprayed with aqueous suspensions of neem oil and then inoculated with *Xanthomonas campestris* pv. *vesicatoria* depicted lesser disease symptoms than the water-treated controls. Weekly foliar sprays of neem oil significantly reduced disease severity on the foliage of inoculated field-grown pepper plants in both years of a two-year study with no cytotoxic effects in the host plants (Abbasi, 2003). Madhusudhan (2005) observed that spraying a 5% neem oil solution in tomato and Bell pepper could increase their resistance against *Tobamovirus* infection. The number of virus-induced local lesions were reduced significantly in the neem treated plants as compared to the controls. The incidence of root-rot disease in cucumber (*Cucumis sativus*) seedlings was noted to decrease when they were soaked in 15% extracts concentration and sown in soil artificially infested by *F. solani* alone or with *F. solani* and *Trichoderma harzianum* (Haikal, 2007).

Nahunnaro (2008) enumerated the antifungal effect of *Azadirachta indica* seed oil against yam rot pathogen (*Rhizopus stolonifer*) and proposed that neem oil probably reduced the penetration of the fungus into host cells and hence reduced damage and consequent weight loss. Joseph (2008), Siva (2008) and Nwogbaga and Utobo (2012) remarked significant control of wilt and leaf spot diseases in Brinjal by application of aqueous, ethanolic and acetonic extracts of neem leaves and seeds and mentioned that being non-pollutive, cost effective, non-hazardous and easily available, neem extracts could prove biologically based environmentally safe alternatives for formulating integrating disease management schedule for brinjal. Reduced incidence of *A. flavus* after the application of neem cake in soybean during storage conditions and maintenance of high germination percentage of the seeds over a storage period of 6 months was noted by Krishnamurthy (2008). Vimala and Suriachandraselavan (2008) reported that application of 5% extract of need seed kernel was potent in reducing the incidence of powdery mildew disease of bhendi and increasing its net yield by preoccupying the infection site and deprive the same to the pathogen.

Pretreatment of the fruits of plum (*Prunus salicina*) or Yali pear (*Pyrus bertschneideri*) with Neem seed kernel extract during storage and then inoculation with the post-harvest spoilage pathogens effectively protected them from disease (Wang, 2010). Their findings point towards possible prevention of disease either initiating a defense response in the host fruits or by prevention of pathogen spore germination on them. Spraying of the cabbage plants with the neem seed extracts significantly reduced the numbers of pests as compared to the control plants. Significantly, more of the pests infested the control plants than the treated plants ($p < 0.01$). The mean weight of cabbage heads on the sprayed plots was significantly heavier than that of the control unsprayed plots. The use of *A. indica* seeds extract increased yield by 37.05% (Baidoo and Adam, 2012).

The diverse findings clearly suggest that reduction of disease in the infected plants and its control in spread to the uninfected ones by neem extract formulations has a vast future. The incidence of a number of bacterial, fungal and viral diseases could be controlled or reduced successfully by tactful entailment of neem extracts. Inspite of being lethal to the phytopathogens, no visible significant target-plant-health-deteriorating observations have been registered during investigations related to control of various plant diseases. These observations provide new dimensions to the applicability of crude or solvent based neem extracts/products in the form of effective biopesticides.

Neem extract induced SAR: There have been several reports of direct or indirect effects of extracts from different parts of neem on target pests and pathogens (Schmutterer, 1995; Amadioha, 2000). They may directly inhibit the growth of pests and pathogens at the site of application or may induce defense responses in the host plant both locally as well as systemically which would result in the synthesis of broad spectrum antimicrobial compounds possessing the ability to control infection by the invading pathogens.

Neemazal, a product of neem, has been found to induce resistance in pea (*Pisum sativum* Linn) against *Erysiphe pisi*. Neemazal could induce hypersensitive reaction (HR), as evidenced by browning of host cells associated with appressoria and increased phenylalanine ammonia lyase (PAL) activity in pea leaves (Singh and Prithviraj, 1997). A commercial product from neem seeds “Margosan-O” was able to induce a temporal and differential expression of defense proteins chitinase, β-1,3-glucanase and peroxidase in oranges. When these plants were challenge inoculated with green mold pathogen, *Penicillium digitatum*, the enhanced immune reactions resulted in to lower disease incidence (Fajardo, 1998). Aqueous leaf extract of *Azadirachta indica* induced resistance in barley against the stripe pathogen *Drechslera graminea* through biochemical changes in the host plant. Vigorous inhibition of soil borne pathogenic fungal growth was achieved and the treated leaves exhibited significantly high activity of enzymes phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) along with rapid and distinct accumulation of fungitoxic phenolic compounds (Paul and Sharma, 2002).

Bharathi (2004) recorded a multifold increase in chitinase, *β*-1, 3 glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and phenol accumulation in plants treated with a formulation of neem extract, chitin, *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* for the management of fruit rot infection in chillies. Aqueous leaf extract of neem provided the control of *Alternaria* leaf spot pathogen *(Alternaria sesami)* of sesame (*Sesamum indicum* L: *Syn. S. orientale* L). Foliar application of the extract led to the changes in plant metabolism as leaves of the treated plants exhibited notably high level of enzymes phenylalanine ammonialyase (PAL), peroxidase (PO) and content of phenolic compounds (Guleria and kumar, 2006).

Trilogy, a product of neem induced resistance in Cucumber against *Podosphaera xanthii*. The elicited leaves exhibited significantly high activities of PAL and TAL along with rapid accumulation of antifungal phytoalexins (Aboellil, 2007). They added that the plant extract which was easily available for controlling plant diseases was non-pollutive, cost effective, non hazardous and didn't disturb ecological balance. During their field trials on black gram (*Vigna mungo*), Venkatesan (2010) recorded the induction of resistance in the plants sprayed with neem extracts as evident from the increased accumulation of pathogenesis-related (PR) proteins, phenols and other defense related compounds in the treated plants as compared to the controls. 43.5 % reduction in disease incidence in chickpea against *Ascochyta rabiei* by prior application of aqueous leaf extract of *Azadirachta indica* (Ghanzafar, 2010) and increase in POX activity by methanol as well as water extracts of neem leaves leading to SAR (Sarwar, 2011) in them have been reported. War (2011) observed critical changes in PO, PPO, total phenol content and H_2O_2 in groundnut leaves treated with neem oil and concluded that neem oil influenced the metabolic system of the host plant and induced the oxidative response that could defend the plant against a variety of stresses.

Several recent studies on efficacy of neem extracts in induction of SAR in tomato have provided influential results. Aqueous leaf extracts of neem have been implicated in successful induction of resistance in tomato and its rescue from wilt caused by *Fusarium oxysporum* as evident from elevated activities of peroxidase, catalase and superoxide dismutase and induced expression of newer peroxidase isoforms (Farag Hanaa, 2011) and by the elevated levels of total soluble proteins and total phenols and qualitative as well as quantitative differences in the protein profiles of treated and untreated leaves (Arzoo, 2012). The results obtained by Bhuvaneshwari (2012) had shown that neem fruit extract induced systemic resistance in barley seedlings against *D. graminea*. The concentration of salicylic acid (SA) and activities of PAL and Peroxidase (PO) were observed to be significantly higher in untreated leaves of seedlings given a single leaf treatment with neem fruit extract. Fruit extract of neem induced defense response through enhanced activities of phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), polyphenol oxidase (PPO), peroxidase (PO) along with isoenzymes of PPO and PO in tomato (Bhuvaneshwari and Paul, 2012). Surendra (2012) reported elevated levels of PO, PPO and PAL in mustard after foliar application of extract from neem seed kernels, thereby inducing resistance against *Alternaria brassicae*. The aqueous leaf extracts of neem induced the defense related enzymes such as PPO, PO and β-1,3-glucanase in both pre- and post- inoculation of *Xanthomonas oryzae* indicating that the induction of defense enzymes are responsible for the control the bacterial blight disease rather than antagonism by neem extract in rice.

Goel et al. (2013, 2014) and Goel and Paul (2015) reported the induction of SAR mediated by elevated levels of peroxidase and polyphenol oxidase both locally and systemically in tomato plants treated with aqueous neem fruit extract. Neem treatment could induce additional PO and PPO isoforms in the treated plants which were absent in control plants. Moharam (2013) elaborated the role of neem seed oil in inducing resistance in Okra through the activation of biochemical defense system involving changes in activities of peroxidase, polyphenyl oxidase and chitinase and increase in total proteins and total phenols content both at the site of application as well as away from it.

The induction of SAR in plants mediated by enhanced expression of PR genes and the defense related proteins by neem extracts has been a breakthrough finding as far as stabilizing neem extracts in the form of efficient biocides is concerned. Their ability to elicit certain biochemical reactions within the host cell leading to activation of pathways which have greater role to play in plant's rescue is a matter of exploitation and application. Induction of natural mechanism like SAR in plants by natural means becomes all the more important because it allows us to reduce the application of synthetic chemical pesticides which have severe detrimental effects on ecological balance as well as the consumers. The efficacy of the probable neem based biocide in inducing SAR in the target plants provides a sustainable and reliable substitute to the effective but harmful chemical pesticides and could prove to be a boon to the agricultural arena, particularly to the farmers who are directly exposed to toxic chemicals.

2. CONCLUSIONS

Neem extracts and products have shown multidisciplinary action against phytopathogens, starting from direct growth inhibition, preventing establishment and subsequent growth of pathogen on the host plant or a successful induction of SAR in them. A large number of researches in the past few decades have been conducted to verify the relevance of neem extracts for inhibiting pathogen growth and/or induction of SAR and thus protecting the plants in a natural manner. Enough scientific evidence has been gathered from several hundred references on the

subject to warrant the launching of a well coordinated multidisciplinary research and development programme leading to agricultural, industrial and commercial exploitation of this plant. Neem based pesticides are gaining more attention because of their non-toxic and environmental friendly nature. The need of biocides (as an alternative to the prevalent chemical inducers) for natural elicitation of plant defense has furtherised this approach amongst the scientists around the globe. The implications of plant products/extracts for plant disease management have emerged as a logical, effective and long lasting approach. Extracts from neem are now known to reduce disease spread in crops/plants grown either in greenhouses or in fields, in seeds and fruits during storage. They may directly inhibit the pathogen growth, preoccupy the probable infection sites on host surface or initiate a potent defense mechanism in host plants so as to prevent infection from a wide range of disease-causing bacteria, fungi, viruses and other microbes by the generation of anti-pathogenic environment inside the cell. Moreover, they have long shelf-life making them easy to be stored. The popularity of botanical pesticides is increasing and some plant products are being used globally as green pesticides. Formulations from neem extracts could provide easily available, cost-effective, eco-friendly and harmless means of disease management in plants. Most of the studies have provided promising results. A number of commercial formulations from neem products like Nim80, Neemas, Parker oilTM, Margosan-O, dk-bioneem, multineem, neem oil, azacel, alpaste and larvocel have also egressed into the markets.

Despite so many researches being carried out for the exploitation of neem and its extracts in the form of a suitable broad spectrum biocide, several aspects of its successful plunging in plant protection remain untouched. For instance, persistent levels of neem products in food and effective concentration of neem products is not defined yet. Also, biochemical mode of action of these products is still not clear. In addition to the target pathogen, the application of neem extracts may kill various beneficial phylloplane dwelling organisms necessary for ecological balance. Furthermore, before implications of neem products for large scale plant protection, it is mandatory to key out the target host genes of these products. It is necessary to figure out the genes which are exclusively essential for natural plant processes but are amenable to be down regulated by neem products which may otherwise lead to inadvertent loss of indispensable physiological functions of the host plant and decelerate their food producing ability.

Although neem based products are easy to prepare, cheap and highly effective, commercialization of neem products for serving as a nontoxic plant protector is concerned, it is crucial to make sure that the starting material can be obtained in sufficient quantities to meet market demand, and on a consistent basis. Unless it is extremely abundant in nature, it will be necessary to cultivate it to obtain sufficient biomass for extraction. At present, neem products are harvested in India from among the estimated 25 million existing trees. However, many of those trees have been intentionally planted in towns and villages. The aftereffect of prolonged usage of a product such as development of resistance in the microbes is also a questionable possibility.

In the context of agricultural pest management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post harvest protection of food products in developing countries. As far as neem products are concerned, they have proved successful guardians of plants time and again, yet a lot more advanced and rigorous research is required to validate their long lasting potency for the purpose.

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