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Research Application Summary

Insecticidal properties of neem seed extracts: A review

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Abstract

The use of botanical pesticides such as azadirachtin found in the neem seed extract to control insect pests has increased globally due to its ability to maintain ecological safety. The objective of this review was to explore on different ways in which neem oil, the main extract from the neem seed, controls insect pests. Azadirachtin was found to be both a systemic and contact pesticide, hence repelling goeze on canola plants, while preventing settling and oviposition of the pea aphids. Antifeedant effect was achieved in lepidoptera family using an azadirachtin concentration of 1-50 and 100-600 ppm in cleoptera, homoptera and hemiptera families. Females of blanch showed less feeding than the males when treated with neem oil. The sugar receptors of desert locusts were blocked by an azadirachtin concentration of 0.01ppm and above, while the ability of the peach-potato aphid to transmit potato leafrol virus was reduced by the active substance. The fecundity, oviposition and the number of eggs laid by *Drosophila melanogaster* were reduced using a single spray of neem oil. Fecundity of aphid nymphs and the oviposition of cabbage aphids were reduced by azadirachtin. The pupation and eclosion of *Drosophila melanogaster* dropped significantly when the concentration of azadirachtin was increased. Early exposure of Drosophila melanogaster to azadirachtin slowed down the development of the adults by lowering the number of organisms, while delaying larval and pupal development. Resistance of pests to purified azadirachtin was found to be minimal, but zero to refined neem seed extracts. Neem seed extracts were reported to be biodegradable and non-toxic to humans, animals and natural enemies of pests. The efficacy of neem seed extracts in the control of insect pests, such as aphids, was found to be lower than that of the synthetic pesticides; hence a combination of neem oil with one or more other botanical pesticides is recommended in the attempt to increase the efficacy of botanical pesticides.

Key words: Azadirachtin, fecundity, oviposition, sugar receptors

Résumé

L'utilisation de pesticides botaniques tels que l'azadirachtin découverte dans l'extrait de graines de Neem pour contrôler les insectes nuisibles a augmenté globalement en raison de sa capacité à maintenir la sécurité écologique. L'objectif de cette étude était d'explorer les différentes manières dont l'huile de neem, l'extrait principal de la graine de Neem, contrôle les insectes nuisibles. L'azadirachtin s'est avéré être à la fois un pesticide systémique et de contact, qui repousse la goeze des plantes de canola, tout en empêchant l'installation et l'oviposition des pucerons de pois. L'effet anti-nutritif a été réalisé contre la famille Lépidoptère utilisant une concentration d'azadirachtin de 1 à 50 et 100 à 600 ppm dans les familles de Cleoptera, d'homoptère et d'hémiptera. Les femelles de Blanch ont montré moins de capacite d'alimentation que les mâles lorsqu'ils sont traités avec de l'huile de neem. Les récepteurs de sucre des sauterelles du désert ont été bloqués par une concentration en azadirachtin de 0,01 ppm et plus, tandis que la capacité du puceron à transmettre le virus de l'enroulement des feuilles de pommes de terre a été réduite par la substance active. La fécondité, l'oviposition et le nombre de Drosophila Melanogaster ont été réduits à l'aide d'un seul spray d'huile de neem. La fécondité des nymphes et l'oviposition de pucerons de chou ont été réduites par Azadirachtin. La nymphose et l'éclosion de Drosophila Melanogaster ont considérablement diminué lorsque la concentration d'azadirachtin a été augmentée. Une exposition précoce de Drosophila Melanogaster à Azadirachtin a ralenti le développement des adultes en abaissant le nombre d'organismes, tout en retardant le développement des larves et des pupes. La résistance des parasites à l'azadirachtin purifiée a été jugée minimale, atteignant zéro avec des extraits de graines de neem raffinés. Les extraits de graines de Neem seraient biodégradables et non toxiques pour les humains, les animaux et les ennemis naturels des parasites. L'efficacité des extraits de graines de Neem dans le contrôle des insectes nuisibles, tels que les pucerons, a été jugée inférieure à celle des pesticides synthétiques ; Par conséquent, une combinaison d'huile de neem avec un ou plusieurs autres pesticides botaniques est recommandée dans la tentative d'accroître l'efficacité des pesticides botaniques.

Mots clés: Azadirachtin, fécondité, oviposition, récepteurs de sucre

Introduction

The repercussions of use of synthetic pesticides, including their effects on insect pests resistance, chemical residue in agricultural produce and effects on non-target insects, have led to the preference of ecologically safe pest control alternatives in crop production worldwide (Stanley *et al.,* 2014). Neem oil is a major example of a currently best performing and most promising botanical pesticide (Kova íková and Pavela, 2019). It is the main extract obtained from the seeds of neem tree (Campos e*t al.,* 2016). The neem tree (*Azadirachtaindica* A. Juss) belongs to the Meliaceae family and is native of the Indian subcontinent (Campos *et al.,* 2016).

The neem seed extracts contain at least 100 biologically active compounds (Campos *et al.,* 2016), although azadirachtin is the main active ingredient (Campos *et al.,* 2016). Azadirachtin is a steroid-like tetra-nortriterpenoid that exhibits a wide range of bioactivity to hundreds of insect pests from various orders (Shannag *et al.,* 2015). It has been found to cause 90% of the effect on most pests (Campos *et al.,* 2016). Depending on the insect order, azadirachtin affects pests by causing antifeedant effect, suppressing reproduction, blocking sugar receptors and negatively affecting metamorphosis (Shannag *et al.,* 2015).

The effectiveness of neem oil is influenced by method and time of application, method of extraction, concentration applied, insect species, origin of the neem tree, temperature and humidity (Shannag *et al.,* 2015). Neem oil also has to be formulated so as to boost its efficacy and decrease its potential phytotoxicity, while improving its storability (Stanley *et al.,* 2014). Unfortunately, information related to use and effectiveness of neem products in insect pest control is scattered world over, making it difficult to consolidate the achievements and highlight the remaining knowledge gaps. The objective of this review was to explore on different ways in which neem oil, the main extract from the neem seed, controls insect pests.

Active substances in neem seed oil. Neem extracts are hydrophilic, but have easily soluble inorganic solvents like ketones and alcohol (Dhra *et al.,* 2018). The active ingredients in neem are found exclusively in the different parts of the neem plant: leaves, seeds and kernels. They include azadirachtin, nimbin, nimbidin, nimboids, nimbidol, sodium nimbinate, gedunin, salannin and quercetin.

Azadiractin is the best and most potent insect growth regulator and antifeedant. It is a limonoidallelo chemical present in fruits and the tissues of the neem tree (Ogbuewu *et al.,* 2010). Azadirachtin was first isolated from tropical neem seeds by Butterworth and Morgan (1968), while its full structure was determined later by Ley *et al*. (1985). The efficacy of salannin, nimbin and 3-tigloyl-azadirachtin (azadirachtin B) is directly related to azadirachtin content, although the other compounds also possess biological activities that contribute to the efficacy of the azadirachtin (Mordue and Nisbet, 2000). Although azadirachtin has nine isomers, isomers A, B and H are the most abundant (Dhra *et al.,* 2018). The seed kernel is the most important part that has the highest concentration of azadirachtin (40%) (Chaudhary *et al.,* 2017).

Insecticidal value and control mechanism. The neem plant is an important source of botanical pesticides; about 540 different species of insect pests are susceptible to neem products (Schmutterer, 2009). These insects mainly belong to the order Lepidoptera, Diptera, Homoptera, Hemiptera (field crop pests) and Coleoptera (storage crop pests). Below are the mechanisms by which Azadirachtin acts in the control of insect pests:

Systemic and contact effects: Azadirachtin reportedly moves systemically to all growing plant parts; thus inhibiting the settling behavior of the insects (Pavela *et al.,* 2004). However, azadirachtin is found to be less systemic due to the water insoluble nature of the neem oil. Therefore, the neem oil must be formulated to make it systemic (Stanley *et al.,* 2014). Azadirachtin is also a contact insecticide that exhibits translaminar activity (Campos *et al*., 2016). Goeze (*Phyllotreta crucuferae*) posed more attacks on canola plants that were not treated with neem extracts, compared to the treated plants. This was because azadirachtin in neem oil repelled the goeze that tried to invade the treated canola plants. Pea aphids (*Acyrthosiphon pisum*) were deterred to land, prob or oviposit on plants treated with neem seed oil (Mikami and Ventura, 2008).

Azadirachtin kills soft-skinned insects upon contact. Neem oil forms a coating over the bodies of insects including the hard-skinned, hence blocking breathing openings which eventually leads to suffocation and death of the insects (Concklin, 2011).

Antifeedant effects: Azadirachtin is best known for its antifeedant effect on insect pests (Dhra *et al.,* 2018). The concentration of azadirachtin to achieve antifeedant effect is different across various orders and species of insects. Members of the lepidoptera family (moths and butterfly) are most affected since their antifeedant effect is achieved by a very small concentration of the neem oil (1-50 ppm) (Dhra *et al.,* 2018). The effectiveness is even higher on lepidopterous insects during their immature stages of development (Concklin, 2011).

Members of Cleoptera, Hemiptera and Homoptera families are less sensitive to neem oil since the antifeedant effect is achieved by 100-600 ppm concentration of the neem oil (Mordue and Nisbet, 2000). In the three dimensional structure of azadirachtin, the hydroxyl fragment is the one responsible for the antifeedant effects on insect pests (Dhra *et al.,* 2018).

Recently, it has been reported that female insects respond to azadirachtin differently from the males (Mikami and Ventura, 2008). For instance, females of Blanch (*Chrotogonus homalodemus*) exhibited less feeding when treated with neem extracts than the males (Mikami and Ventura, 2008). Antifeedant and regulatory effects are independent, though concentration of one affects the concentration of the other (Dhra *et al.,* 2018). The crude neem extracts reduces feeding and probing of the treated plants by the aphids for around 4 days after spraying (Pavela *et al.,* 2004).

Azadirachtin also causes physiological effects, like reduced food consumption, which are more consistent than the antifeedant effects (Schmutterer, 2009). This results into secondary antifeedant effects, which in turn result to hormonal imbalance and complication of the physiological systems like inhibition in the production of digestive enzymes (Schmutterer, 2009). Reduction in feeding and food uptake by insects translates to lower plant damage (Kraiss and Cullen, 2008). For example, locusts treated with azadirachtin produced less fecal pellets due to reduced feeding (Mordue and Nisbet, 2000).

The mean dry weight of food intake by southern armyworm moth (*Spodoptera eridania*) larvae treated with higher concentrations of pure neem oil (7.8 ml per litre) and Azatrol (31.2 ml per litre) declined by about 13.9 and 8.8% as compared to the control experiment, respectively (Shannag *et al.,* 2015). Physiological effects can be either indirect (via the endocrine system to block the release of morphogenetic peptide hormones, that control corpora allata and prothoraic glands), or direct (when azadirachtin enters into the cells of insects and inhibit cell division and protein synthesis) (Mordue and Nisbet, 2000).

Blockage of sugar receptors: Azadirachtin reportedly stimulates 'deterrent' cells of chemoreceptors and block the firing of 'sugar' receptors that stimulate feeding, hence making the insects to starve to death by only deterring feeding (Mordue and Nisbet, 2000). Dessert locusts are very sensitive to azadirachtin concentrations of 0.01 ppm and above, hence failing to feed on discs impregnated with sugar. Interrupting the feeding activity can reduce the ability of insects to transmit infections to plants, although azadirachtin does not prevent the spread of such infections. For instance, there was reduced ability of peach-potato aphid to transmit potato leafroll virus (PLRV) to tobacco plants treated with neem oil because the azadirachtin reduced the time required by the pest to transmit the disease (Mordue and Nisbet, 2000). Low doses of azadirachtin were found to reduce the chances of green rice leafhopper to transmit tungro virus in rice fields (US National Research Council Panel, 1992).

Insect reproduction suppression: Azadirachtin affects the reproductivity of insects by affecting the development of the ovaries, oogenesis, egg availability, vitellogenin and its uptake by oocytes, fecundity, and also reduces oviposition (Dhra *et al.,* 2018).Adults are killed by disrupting mating and sexual communication, hence reducing fecundity (Concklin, 2011). The fecundity and oviposition of Drosophila melanogaster, treated with a single spray of neem oil reduced together with the number of eggs laid as the disinclination of this compound by the flies increased over two successive generations (AGRA, 2019). The fecundity is affected in insects exposed to the neem treatments as well as sterilizing effects on Homopterans. The vigor of insects is also affected, resulting to overall loss of fitness of insects.

Treated male adults cannot copulate and females cannot recognise the male pheromone and this reduces their mating ability (Kraiss and Cullen, 2008). Azadirachtin significantly increases aphid nymphal mortality (80%) while significantly increasing development time of those surviving to adulthood (Kraiss and Cullen, 2008). An azadirachtin concentration of 5 ppm reduced the fecundity of female aphids within 48 hours of administration. In male aphids, potency was reduced to about 80% when treated with 0.125 mg of each insect. There was reduced sizes of testes and amount of sperms in desert locusts when treated with low concentrations of azadirachtin (Dhra *et al.,* 2018). Fecundity of carmine spider mite, productivity of storage pests, egg number and availability in Corcyra cephalonica (rice moth) and oviposition of vegetable leaf miner were significantly reduced by neem extracts (Karnavar, 1987). The oviposition of cabbage aphids was affected in treated plants by denying them favourable site for egg laying. Fecundity of the aphids nymphs was also reduced (Pavela *et al.,* 2004).

Insect metamorphosis effects: Azadirachtin affects the metamorphosis of insects resulting to various morphogenetic defects and mortality, depending on the concentration; with the larval and nymphal stages of the insects being the most susceptible (Kraiss and Cullen, 2008). There is a delayed growth and development of the insects as deaths of the insects in these stages occur (Kraiss and Cullen, 2008).

Azadirachtin is known to act against the insect juvenile hormone and 20 hydroxyecdysone (AGRA, 2019). High concentrations of azadirachtin lead to incomplete molting process in the larval stage, various deformities in the pupal stage and wingless adults. This, therefore, leads to reduction in longevity and fecundity of insect pests (Dhra *et al.,* 2018). For instance, as the concentration of azadirachtin was increased, pupation and eclosion rates in Drosophila melanogaster dropped significantly.

Moreover, the azadirachtin caused some morphological abnormalities like heavily pigmented larvae, pupa-adult intermediate and malformed adults (Morakchi and Bendjazia, 2016). The body weight of all the developmental stages of Drosophila melanogaster was reduced by 10-20% when treated with azadirachtin. Early exposure of Drosophila melanogaster to azadirachtin slowed down the development of adults by decreasing the number of organisms and delaying larval and pupal development (AGRA, 2019).

Insect pest resistance against Neem seed extract. Although pests have developed resistance against many active ingredients of pesticides, azadirachtin in neem seed oil has successfully prevented development of such insect resistance (Kova íková and Pavela, 2019). Mordue and Nisbet (2000) argued that azadirachtin reduces production of the detoxification enzyme as a protein synthesis inhibitor, therefore, managing insect resistance. Also, azadirachtin was found to increase the efficacy of other biopesticides, hence reducing the chances of insect pests developing resistance mechanisms against such biopesticides (Dara, 2017).

It is also believed that neem oil contains a group of active ingredients possessing different chemical characteristics. However, due to the low residue power of neem oil, multiple applications are required to control pests. This eventually increase the selection pressure on the pest population, resulting to development of resistance by the pests (Campos *et al.,* 2016). Green peach aphids were reported to develop a low to medium resistance against purified azadirachtin under repeated application of the bio-pesticide. However, the aphids developed no resistance to the refined neem seed extracts (Dara, 2017).

Conclusion and Recommendations

This review had shown that neem seed extract (neem oil) controls different insect pests using various mechanims. Apart from its effectiveness in controlling these pests, neem oil has also exhibited zero or very minimal effects to the environment and as chemical residue in agricultural produce. Unlike other pesticides, neem extracts are little known to induce resistance in insect pests. Therefore, using neem oil as a botanical pesticide will help save the environment and the human health.

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