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Review Paper

Mode of action of Azadirachtin: A natural insecticide

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Abstract

Azadirachta indica is an important tree of meliaceae family and grow in tropical and subtropical areas in Southeast Asia. It is used for shade, reforestation and for production of natural insecticides and being used as medicinally (formulations or crude extracts) as well for various diseases since long back 3-4 decades in India specifically, its extract contains various biologically active components commonly known as tritepenoid or limonoids like, azadirachtin, salanin, nimbin, meliacin, valassin and gedunin etc., from different parts of the tree but, azadirachtin is very crucial in natural insecticidal/larvicidal activities (antifeedancy, growth regulator and reproduction effects) and many more like, antiallergenic, antifungal, anti-inflammatory and many more. Insect growth regulator (IGR), antifeedancy and reproductive effects are well understood and published in earlier literature. Also antifeedant effects are not consistent among various species of the insects; on the other hand, insect growth regulator (IGR), moulting, growth and reproductive effects are well consistent with different species. But still a lot remains to be known about the mode of action at cellular/molecular level and structure-property relationship of azadirachtin molecule and need some modern techniques like computational studies of the interaction. The mode of action of azadirachtin primarily consist of effects on the chemoreceptors which leads to antifeedancy and effects on juvenile and ecdysteroid hormones e.g., PTTH and allatotropins. Apart from these there are effects which are overall growth and development of the insects. In this article we are presenting the behavioural/physiological effects of the azadirachtin. These include other effects also like, neem resistance, growth reduction, and moulting deviations, enhanced mortality and categorically have direct effects on tissues and cell and indirect effects including endocrine system. It also gives some details regarding the studies conducted so far with interest of the mode of action of the azadirachtin at cellular level.

Keywords: *Azadirachta indica*, triterpenoids, antifeedency, IGR, mode of action, chemoreception, endocrine system, PTTH, physiological effects, neem resistance.

Introduction

Neem (*azadirachta indica)* is an evergreen and fast growing plant distributed particularly in India, Bangladesh, Pakistan, Thailand, Nepal, America, Africa and Southeast Asia. It has been used medicinally long back due to its medicinal properties and has a vital importance in India and becoming famous all over the world. It has maximum use as non-wood products like, bark, fruits, leaves, seeds, oil, gum and cake with numerous properties e.g., antifeedant, antiallergic, anti-inflamatory, antipyorrhoeic, antiscabic, antidermatic, antifungal, insecticidal, larvicidal, nematicidal, spermicidal and many other properties. Due to its medicinal importance, various formulations based on neem are being used for the treatment of various diseases. Neem has been considered as harmless for human, animals and other beneficial insects. This is approved by US Environmental Protection Agency and safe for the use on food crops[1].

The biologically active constituents of neem plant (from oil, leaf and bark etc.) and its various formulations are basically based on the tetracyclic triterpenoid tirucallol parent compound (Figure-1). These includes: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and others related compounds. Azadirachtin itself has around nine isomers (A-I), but A, B, and H-isomers are present in higher percentage. The various compounds present in neem are the results of many chemical processes like rearrangements, oxidation, reduction etc. from tirucallol molecule. Now after so many studies conducted on neem it generally accepted that azadirachtin a tetranotriterpenoid (called limonoid as well) molecule is the main bioactive metabolite responsible for numerous biological activities (particularly pesticidal). However more than 20-25 different active molecules have been isolated and characterized from the various sources of the neem so far and it is found after many experimental studies that these are responsible for some other activities associated with the neem. Various compounds extracted from neem plant are somewhat hydrophilic in nature but are highly soluble in organic solvents like, alcohols, ketones, esters[2] etc.

If we look the three dimensional structure of azadirachtin molecule (Figure-2-4), it is observed that it comprises the decalin moiety which is associated for the insect growth regulator (IGR) and many developmental effects[3]. On the other

hand, hydroxyl furan fragment is responsible for antifeedant effects and it is widely observed among the target species. Both these effects i.e., the antifeedant and regulatory effects are independent and no relation has been observed so far. However, they are related to the concentration of each other[4]. The mode of action of azadirachtin molecule has been established recently by Mordue and Blackwell[5]. The structure of azadirachtin was confirmed by NMR spectroscopy[6] and X-ray analysis of aderivative detigloyldihydoazadirachtin by Broughton[7]. Azadirachtin was isolated and identified by Butterworth[8] and Morgan[9]. A close analogue of azadirachtin, the marrangin (Aza-L) was isolated and structure was determined byKalinowski[10].

Sufficient literature is available on neem and its uses in recent times. So this article is written for the purpose of brief introduction to the mode of action of azadirachtin present in the neem plant. We are also presenting some idea about the structure-property relationship responsible for various insecticidal and other activities of azadirachtin.

Antifeedant effects of azadirachtin

Azadirachtin is a chemical which shows antifeeding effects in insects by the mode of chemoreception (primary effect) and also food consumption by the insects is relatively reduced (secondary effect). These symptoms are shown because of the toxic nature of the azadirachtin.

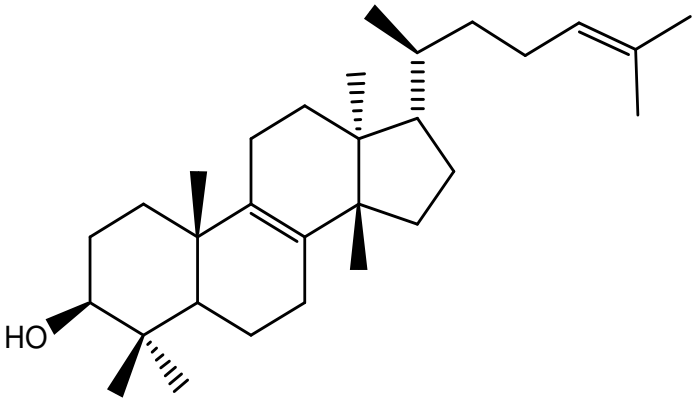

Figure-1: Structure of tirucallol.

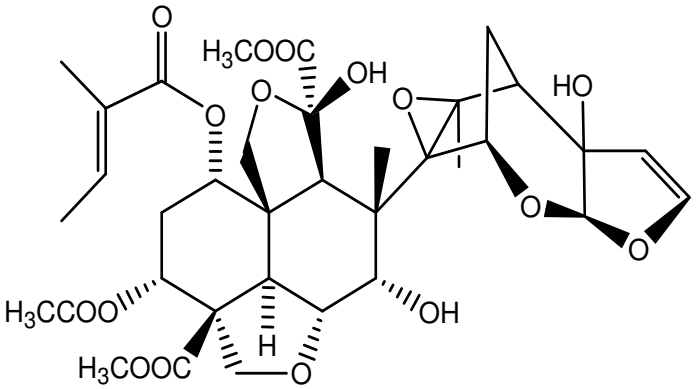

Figure-2: Structure of Azadirachtin-A.

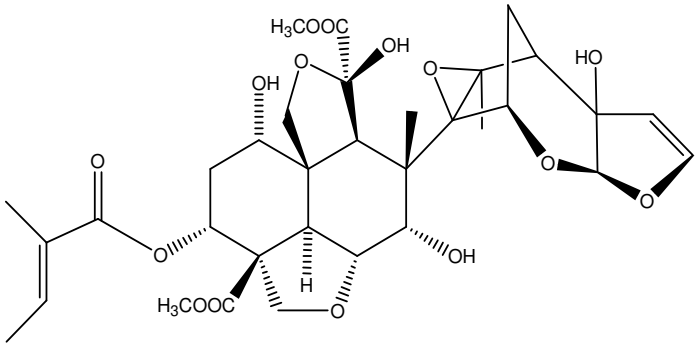

Figure-3: Structure of Azadirachtin-B.

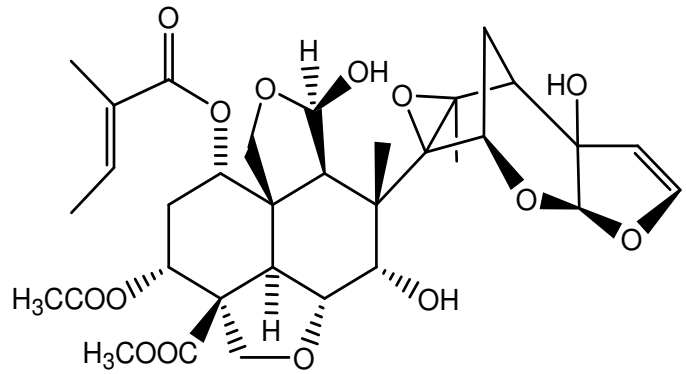

Figure-4: Structure of Azadirachtin-H.

Effects as insect growth regulator (IGR)

On larval insects the growth regulatory effect of azadirachtin is well known and causes inhibition in various growth processes, malfunctions and mortality of the insect larvae depending on the concentration of the azadirachtin. Hence, by increasing the concentration of azadirachtinat the larval stage, reduced longevity, fecundity, wingless adults, incomplete moulting process, pupae results with various deformities and overage larvae etc., these effects are described with a wide variety of insect species like Lepidoptera[11,12]. Diptera[13,14] Orthoptera[15,16] Hemiptera[17,18] Coleoptera[12,19]and Hymenoptera[20,21].

Effects on the reproduction system

Azadirachtin treatment given to male and female insects clearly shows the antifeedant and insect growth reglator (IGR) effects followed by the adverse effects on the reproduction system like, effects on ovarian development, oogenesis, fecundity, egg viability[22], fat body protein and ovarian protein levels (S. exempta) and reduced oviposition. Also, synthesis of vitellogenin and its uptake by oocytes[23] affected as well.

Below 100ppm level of the active azadirachtin content *aphids* are not sensitive to primary antifeedant effects but, secondary effects are clearly observed. When the low concentration (5ppm) of azadirachtin was administered to female insects, it was observed that their fecundity reduced rapidly within 48 hours of azadirachtin containing diets administration. On the

other hand, male potency reduced to around to 80% with the treatment of 0.125mg to each insect (*O. fasciatus*). Size of the testes of the desert locusts are also reduced with the low concentration of azadirachtin during the time of their development and reduction in the male sperm cells.

Chemoreception effects

Chemoreceptor is a specialized class of sensory cells, which responds to a particular chemical substance and play a very important role in insects. Chemicals which inhibit the phytophagous insects are responsible for the plant defence. The action of these chemicals includes behavioural responses, inhibition and internal physiological changes in the insects. While behaviour related to feeding are the results from neural system and chemical senses of the insects.

On the other hand, contact chemoreceptors present on the tarsi, mouthparts and oral cavity are mainly affected. The detailed neurophysiological effects of azadirachtin have been conducted by Blaney and Simmonds. They have conducted these tests on the larvae of *Locust* and *Lepidopterous*[24]. The results related to the feeding behaviour are due to the blockage input from the receptors. These receptors in general respond to phagostimulants or other interactions. The main effect of azadirachtin is on the sugar and deterrant cells[24]. The other mode of action of azadirachtin on other types of cells is still not known.

The rate of firing is reduced in sugar cells, but it has no any harmful effect. The detailed mechanism of azadirachtin required some further studies. On the other hand, secondary antifeedant effects, also important and provide some valuable details regarding the central regulating mechanism of the chemoreceptors. It is already established by many studies that internal milieu can affect chemosensory responsiveness after feeding and which involves hemolymph-azadirachtin interaction.

After many studies, it was found that, there was not a single response by chemoreceptors rather show a number of other associated responses against azadirachtin and was observed that there was a complete or partial inhibition of feeding in the insects. Inhibitory action induced by azadirachtin is also important with regard to the oviposition sites by the phytophagous insects. Hence neem oil and its seed extract were studied to see deterrent effects on oviposition sites[25].

Endocrine effects by azadirachtin

Azadirachtin have detrimental effects on the endocrine system of the insects and causes reduced or delay in some of the important developmental hormones[26], which are very vital to the development, reproduction and moulting processes of the insects. These hormones are mainly ecdysteroids like, ecdysone (Figure-5), ecdysterone, turkesterone and 20-hydroxyecdysone etc.

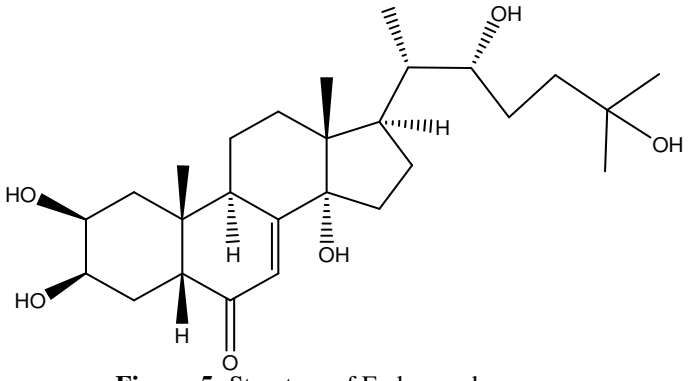

Figure-5: Structure of Ecdysone hormone.

These hormones are synthesized inside the insects by the cholesterol metabolism. Phytoecdysteroid hormones are also act as protecting agents against herbivore insects. These effects (ecdysis and apolysis) are linearly related to time and dose of the azadirachtin content. Also, these include death of the insects without moulting or during the process. Here the azadirachtin mainly targets the epidermal cells where ecdysteroids are produced.

These features lead to the curiosity for the ecdysteroids levels and further mode of action of azadirachtin on the prothoracic gland of the larvae of the insect. It is concluded from the in-vitro studies that Azadirachtin doesn't act on the prothoracic gland and the prothoracicotropic hormone (PTTH) level was found unequal. From these findings, it may be concluded that, azadirachtin interacts primarily on the PTTH releasing sites in the corpora cardiaca. Also acetylcholine as well has inhibiting effects on the release of the ecdysone[27]. It is worth to note that, the activity (ecdysone release stimulation) of antagonist of acetylcholine (atropine) is blocked by the azadirachtin at a particular concentration[28].

Juvenile hormones on the other hand, are also affected in the presence of azadirachtin, but this is quite complex to give a plausible mechanism, as it is simultaneously related to other processes like, neurosecretion and ecdysone release during the moulting. Some groups have conducted studies related to the effects of azadirachtin on juvenile hormones and concluded that, supernumerary occurs during moulting[29]. This supernumerary was due to the increase in the juvenile hormones levels[30]. The azadirachtin primarily hinders the release of the allatotropins and thereby blocking the synthetic and release processes of the juvenile hormones. This leads to the overall deficiency of juvenile hormones in the body of the insects. This deficiency disturbs the oogenesis process[31] and crochets destruction in the larvae of *M. sexta*[32].

Therefore from the above discussions, it can be concluded that azadirachtin's effects can't be determined from the endocrine system only, rather it targets various other organs and specific types of tissues with different mode of actions. Although some of the azadirachtin effects are very clear like, effects on moulting, metamorphosis and juvenile hormones effects.

Physiological effects

There are several physiological changes in insects have been reported other than the endocrine and chemoreception effects. These include after azadirachtin treatment like, lack of crochets[32], differentiation of tissues[33], imaginal discs, very early variation of wing discs leads touneverted tanned pupalwing discs[34], cuticle black patches etc., are the results of the disturbances in the juvenile hormones and ecdysone levels in hemolymph at the metamorphosis stage. Therefore, we can systematically categorise the effects of azadirachtin in two types, direct and indirect effects.

Indirect effects of azadirachtin include endocrine system. Neurosecretion of the brain has been inhibited by the presence of azadirachtin and this is completed by the process of blocking of the release of PTTH and allatostatins. These hormones mainly regulate the functioning of the prothoracic glands and corpora allata respectively. Ecdysone hormone (help in moulting) from this gland is responsible for new cuticle formation and ecdysis. On the other hand, juvenile hormones regulate the juvenile stages at every moulting stage. Also deposition of yolk in the eggs are regulated by the these hormones and any deviations in the levels of these hormones can lead to various observable effects in insects like, disruption of moult and some negative effects on the sterility of the insects.

While direct effects include the inhibition of cell division and particular protein synthesis for example, paralysis of flaccid muscles, necrosis of cells of midgut and decreasing effects on the regenerative cells of the gut and deficiency in some enzymes production. Also it is worth to note that as far as physiological effects are concerned, they are consistent if we compare different species particularly with regard to the antifeedant effects[35].

Cellular mode of action of azadirachtin

Although, many azadirachtin's effects has been understood in more details with various studies conducted. These studies have been conducted by many research groups worldwide like, antifeedant, IGR, moulting and some other effects. But, still the exact mechanism of action of azadirachtin is quite difficult to establish or have some unavoidable reasons for the researchers at the cellular level. So, yet biochemical mode of action of azadirachtin need further studies to have sufficient data to ascertain exact mechanisms. Studies showed that around 75% of the azadirachtin content is expelled rapidly out of the body (hemolymph) of the insects after labelled 22, 23-dihydroazadirachtin treatment with a period of 24 hours. Rest of the residue remains bound to the various specific tissues[36]. In a study of dry mount autoradiography of tissues of *L. migratoria* was treated with [3H]-dihydroazadirachtin, showed specific and dense labelling of corpora cardiac, neurilemma of brain[37] and tubules of malpighian[36]. But most of the residue content was found attached in malpighian tubules tissues as compared to corpora cardiac, gut, ovary and other body parts. While the autoradiography studies showed that neurosecretory axon of corpora cardiaca tubules were densely labelled. Later, the labelled material was extracted from the malpighian tubules and was found intact, which suggests that the high affinity for binding at basal infoldings of the tubules of malpighian excretory cells[36]. These malpighian and corpora cells are important in excretion and cleaning of allelochemicals etc., from the haemolymph. Some of the Tritiated dihydroazadirachtin content from the *L. migratoria* was come out as intact and eventually converted to more polar metabolite particularly in the gut, while malpighian tubules extract was the original molecules[31,36]. In some other species however, dihydroazadirachtin conversion to polar metabolites is more rapid and excreted very soon after the treatment[38]. Structure-activity studies of azadirachtin were also conducted by various research groups like, Ramasaki and Klocke[39], Rambold[37], Blaney et al.[40], Simmond et al.[41], with regard to the antifeedancy, IGR and other effects.

At the conclusion point, it is important to give some details regarding the structure-properties relationship that, decalin and dihydrofuran ring of azadirachtin are important to contribute to the activity of the azadirachtin molecule as a pesticide, while hydroxyfuranacetal is responsible for antifeedant effects. Also C_7 and C_{11} hydroxy groups (-OH) play a role for potency and for the transport phenomena hydrophobic moiety is required. At present more mechanistic studies are being conducted by many authors worldwide to have more detailed perspective at molecular level. With the help of better understanding of the mode of action of the azadirachtin at cellular level would lead to the synthesis of azadirachtin analogues with better properties thanazadirachtin like, better photochemical and hydrolytically stabilities.

Resistance against neem formulations

So far there is no any report of the resistance of the neem formulations in the insects. And the main reason for this advantage is that, the neem formulations are being used is almost crude products having many more related components in the neem kernel or the oil and these related compounds to azadirachtin maybe having different mode of actions giving them multi bioactivities like, antifeedancy, repellancy, growth and development, sterilizing, inhibition in ovi position and reduced reproductively. Additionally some neem formulations produce some different physical effects in the insects like, autotomy by the application of neem oil.

Conclusion

At this stage, we have still some better understanding about the bio-efficacies of the azadirachtin like, antifeedant effects but results are not much consistent among various insect species. IGR on the other hand, are more consistent with the species. IGR effects haveled to decrease in the level of ecdysteroid hormones of the haemolymph and which because of the blockage of the PTTH release. These deficiencies of hormones

bring about the decrease of the other important hormones which are vital for the moulting, growth and development for the insect as a whole like, allatotropin and juvenile hormones. The exact mode of action at molecular level still unknown and need some modern studies including techniques like, quantum mechanical treatment (computational study) can help in better understanding of the mode of action of azadirachtin, which inturn bring the neem formulations to the next level for the commercialization purpose. Also other chemical components than azadirachtin need separate studies for more clear action mechanisms.

Table-1: Azadirachtin's effects on insects[42].

Name of the effects	Targeted organs/tissues	Mechanism of action
Antifeedancy	Mouthparts and other chemo-receptors, gut	Sugar cells inhibition and stimulation of deterrent cells, inhibition of peristalsis, reduced production of the enzymes.
IGR effects	Cuticle	Reduced ecdysteroid and juvenile hormones with the blockage of morphogenic peptide hormones.
Cellular processes	Cell division	Inhibition of mitosis, meiosis metaphase and blockage of cell division
	Muscles	Tone of the muscles reduced
	Synthetic cell system	Effects on digestive system in the gut and synthesis of vital protein.
Sterility	Reproduction system	Reduction of ecdysteroid and juvenile hormones and it leads to reduced eggs.

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