Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2024; 12(4): 18-26

Received: 13-04-2024
Accepted: 20-05-2024

Nitu Sinha
Tea Chemistry and Pharmacology Laboratory, Department of Tea Science, University of North Bengal, Raja Rammohunpur, Bairatisal, West Bengal, India

Dr. Sonali Ray
Tea Chemistry and Pharmacology Laboratory, Department of Tea Science, University of North Bengal, Raja Rammohunpur, Bairatisal, West Bengal, India

Corresponding Author:
Dr. Sonali Ray
Tea Chemistry and Pharmacology Laboratory, Department of Tea Science, University of North Bengal, Raja Rammohunpur, Bairatisal, West Bengal, India

Different extraction processes of neem (*Azadirachta indica*) and its potential in controlling pest population

Nitu Sinha and Dr. Sonali Ray

DOI: https://doi.org/10.22271/j.ento.2024.v12.i4a.9345

Abstract
Neem, scientifically known as *Azadirachta indica*, is a flowering plant indigenous to the subcontinent of India and a common medicinal plant of the Meliaceae family. It has been recognized as a potential biocidal product with maximum efficiency in plant pest and disease management. The bioactive compound of neem is Azadirachtin. It influences insects' metabolic processes and acts as a larvicidal, insecticidal agent. Excessive and indiscriminate amounts of chemical pesticides are used to mitigate pest-related problems, leading to environmental pollution and the growth of resistant strains of pests. Biopesticides are regarded as a synthetic pesticide substitute to reverse the negative trend. The active components of neem extract possess increased regulatory activity and an exclusive mode of action through ecdysone disruption. Neem-derived biopesticides can control major pest populations (leaf miners, aphids, whiteflies, fungi, etc.). This review discusses various extraction processes of neem active components and their mode of action.

Keywords: Neem (*Azadirachta indica*), biocidal, extraction, mode of action, active components

Introduction

In modern agroecological systems, excessive use of chemical or synthetic pesticides results in an imbalance of ecological diversity and integrity, increasing the urge for plant-derived natural pesticides. Consequently, we observe over the past few decades, the application of plant-based insecticides has vividly increased. It is imperative to maintain the equilibrium of pest population, natural enemies, pest resurgence, and pest resistance against pesticides [1]. Chemical pesticides are organic pollutants that persist in the environment for extended periods and cause damage to no target organisms [2], such as natural predators and parasitoids.

Synthetic pesticides are most frequently used to replace labor and weeding. However, extremely hazardous pesticide usage and unsafe application practices adversely affect farmers' health. Pesticide residues are commonly detected in fish, snails, and frogs. Compounding the problematic use of insecticides, the pest population evolved insecticide tolerate genetic strains. Under the selective pressure of repeated application of chemical pesticides, resistance develops most rapidly. Eventually, resistant strains pass on to many populations from generation to generation. Finally, the efficiency of insecticides has vanished due to high levels of resistance [3]. As a plant-based pesticide, Neem gained much faith that insect resistance is improbable to produce.

Neem is native to the Indian subcontinent. From a morphological perspective, its height is around 50-100 m, and its expected growth requires around 130mm of rainfall per annum. It can be easily propagated using seeds or seedlings. The entire neem tree can be used, such as neem leaves, seeds, seed kernel, bark, neem oil, nee cake, etc. Neem tree yield can annually be around 37-50 kg of fresh fruits, from there approximately 24 kg of dry fruits. Dry fruits eventually turned into pulp, seedcoat, husk, and kernel. Lastly, it may give around two and a half kilograms of neem oil and three kilograms of neem cake [4].

In recent years, neem has acknowledged worldwide consideration for its pesticidal, insecticidal, repellent, and antifeedant effects against various pest populations. A long time ago, among Indian farmers, the insecticidal properties of neem got recognition. Over the wide variety of biocides, neem has the most promising and prominent effectiveness on pests, harmless to natural predators and parasitoids, and is eco-friendly [5].

Various phytoconstituents of neem plants, such as glycosides, flavonoids, alkaloids, and saponins, are antibiotics in nature. Due to the presence of these antibiotic substances, plants have their own defense mechanism against pathogens. So, the neem extract can be used as a growth inhibitor against harmful pathogens in agroecosystems [6]. Azadirachtin is the major ingredient of neem, exhibiting antifeedant and reproduction disorders in many species of insects [5].

In this review article, we discussed the several extraction processes of neem and their mode of action.

Habitat and distribution of Neem tree

The neem tree is most commonly found in South Asia and is native to these places. It is distributed throughout India, especially in tropical, subtropical, and semi-arid to wet tropical areas. It is also found in African countries, such as Somalia, Nigeria, Mauritania, Togo, etc., and South and Central America [5].

Chemical compounds in neem [7]

Neem trees and their plant parts are extensively used for insect control. Study for active constituents was commenced many years back, midway through the 20th century. Nimbin was the first bitter component that was extracted from neem oil. It contains lactone, acetoxy, an ester, methoxy, and aldehyde. About 135 components had been extracted and separated from various body parts of the neem tree. They were then divided into 2 significant catagories below:

Isoprenoids and others-Triterpenoids comprising protomeliacins, diterpenoids, limonoids, azadirone, and its byproducts, vilasinin kind of components, gedunin and its derivatives, and Csecomeliacins such as azadirachtin, salanin, and nimbin.

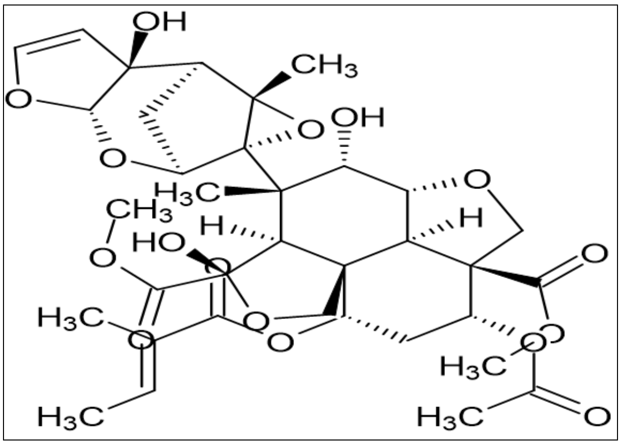

Fig 1: Chemical structure of Azadirachtin, major component of neem crude extract

Non-isoprenoids-These comprised of carbohydrates (Polysaccharides), proteins (Amino acids), sulphurous components, polyphenols such as flavonoids and their aliphatic compounds, coumarin, dihydrochalcone, glycosides, etc.

Active components of various plant parts of *Azadirachta indica*

Leaves: It comprises constituents such as nimbanene, nimbiol, nimbin, nimbolide, nimbandiol, 6-desacetylnimbinene, amino acid, n-hexacosanol, ascorbic acid, 7-desacetyl-7-benzoylgedunin, 7-desacetyl-7-benzoylazadiradione, and 17-hy-droxyazadiradione [8].

Neem fresh leaves: It contains quercetin, polyphenolic flavonoids, and ß-sitosterol, [8].

Seeds: It comprises meliacins in the seed kernels, which include azadirachtin, gedunin, 7-desacetylgedunin, and desace-tylnimbin. However, its oil mainly encompasses nimbin, nimbidin, and nimbinin [9].

Flower: It contains flavonoids. Nimbicetin is similar to kaemferol [9].

Bark: Margosine is found in the bark; again, it is a bitter alkaloid. The volatile fatty acids occur in the bark. It comprises a combination of stearic and oleic acids and a minute quantity of lauric acid [9].

Morphology and systematic position of neem tree [10]

Neem *Azadirachta indica* A. Juss is a medium to the type of tree that generally ranges from 18-30m in height and with a large canopy of around 10-20m. The leaves are simply pinnate alternate and green. The flowers are tiny, pentamerous, mildly sweet, bisexual, and white or pale yellow in color. The fruits are ellipsoidal, very small, and greenish-yellow, purple, or yellow in color. Insecticidal potentials are found in neem leaves, seeds, bark, fruits, roots, and stems.

Systematic position [10]

- Kingdom-Plantae
- Phylum-Vascular plant
- Class-Magnoliopsida
- Order-Rutalae
- Suborder-Rutinae
- Tribe-Melieae
- Family-Meliaceae
- Subfamily-Melioideae
- Genus-*Azadirachta*
- Species-*indica*

Various extraction methods of active components of neem

The crude extract of neem tastes bitter and smells strongly due to triglycerides and large amounts of triterpenoid compounds, azadirachtin, salannin, meliantriol, nimbidin, and nimbin. Several methods have been developed to obtain the desired component from different neem plant parts, such as maceration, cold extraction, mechanical pressing, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), Soxhlet extraction, solvent extraction, etc. Both the traditional and modern extraction methods are listed below in Table 1.

Table 1: Extraction processes of neem

Sl. No.	Extraction procedure	Solvents	Extraction time	Yield	Pros	Ref
1	Sequential process of pressurized liquid extraction	Hexane, ethanol, and ethyl acetate	60 min		Extraction with different solvents provides extract with different antiproliferative potential	[11]
2	Response surface methodology	acetone, ethanol, methanol, distilled water, and boiling	59.25 min	TPC-4661.17 mg GAE/100 g dry weight	Comparatively better extraction of phenolic antioxidants contents from neem leaves	[12]

		water				
3	Soxhlet extraction	Hexane, ethanol, and methanol	1-3hr	Hexane-40.35% ethanol 42.65% methanol 42.89%	Maximum neem oil yield obtained	[13]
4	Pressurized Hot Solvent Extraction (PHSE)	Methanol	100min	210.93mg azadirachtin (per 100g of neem seed kernel)	improved extract yields in a shorter time and with less solvent usage.	[14]
5	Solvent extraction	n-hexane and ethanol	3hrs	Ethanol-41.11% and n-hexane-44.29%	Max neem oil yield with increasing temperature	[15]
6	Supercritical CO_2 extraction	CO_2, CO_2 + methanol	15 mins	85%	outstanding mass transport characteristics and simplicity in controlling with modifiers, pressure, or temperature	[16]
7	Microwave-assisted extraction (MAE), Room Temperature Extraction (RTE), and Reflux (RFX) Methods	Methanol, dichloromethane, and petroleum ether	20 mins	2.55%	When using MAE on leaves and leaf stems, the production of azadirachtin related to limonoids was more than twice as high as when using the RFX method.	[17]
8	Successive extraction	Ethanol	36hrs		Experiment at room temp.; no need for high temp.	[18]

Soxhlet extraction
Septiyani and Wibowo [19] reported Soxhlet extraction of neem leaves using different solvent systems. Ethanol, ethanol-water, water, ethyl acetate, and ethyl acetate-ethanol were used as solvents, and the extraction was conducted at 90 °C. Among all the used solvents, water-ethanol extract gave the highest yield at 1.39%. Siswomihaedjo *et al.* [20] described another report on soxhlet extraction. Neem leaves or stick powder was extracted for 10 hrs with 96% ethanol. Kumar *et al.* [21], Shewale and Rathod [22], Gupta *et al.* [23], Subramanian *et al.* [24], Benisheikh *et al.* [25], Mohapatra *et al.* [26], Awolu *et al.* [27], Babatunde *et al.* [28] showed in their study the neem plant parts extraction process using the same Soxhlet method.

Microwave-assisted extraction
Ezeonu *et al.*, m[29] reported aqueous and ethanolic extract using a microwave extraction. In this method, neem leaves, stems, bark, and seeds are grounded and placed in a microwave oven at 400-680 watts for 3 min. The cumulative extraction duration was 45 min. Extracts have fungitoxic activity. Nde *et al.*, [30] and Suttiarporn and Choommongko [31] reported a similar method of extraction process with hexane and methanol, ethyl acetate, ethanol, dichloromethane, and hexane, respectively. Compared with the conventional Soxhlet process for 10 hrs, it only takes 22-24 min. This method is a simple and potentially decent extraction process for bioactive components.

Supercritical CO_2 extraction
Ismadji *et al.* [32] described the mining of neem oil and numerous triterpenoid components (Azadirachtin, salannin, and nimbin) from its seed kernels by the supercritical CO_2 extraction process. Mongkholkhajornsilp *et al.* [33], Pater *et al.* [34], and Ajchariyapagorn *et al.* [35] reported the same method for extracting nimbin from neem seeds by means of CO_2 as a solvent system. The extraction parameters, such as optimum pressure, reaction temperature, and particle size of the solutes, were directly related to the yield rate.

Repercolation
Lee *et al.*, [36] showed in their study the neem leaf extraction process using the repercolation method at room temperature. In this process, 52 g of dried leaves powder was added to methanol. The extract was filtered, and an access amount of solvent was evaporated to get the dry neem extract.

Solvent extraction
It is one of the most prevalent extraction procedures. Subapriya *et al.* [18], Sitasiwi *et al.* [37], and Kim *et al.*[38] described the neem leaf extraction process using ethanol and methanol as a solvent system at room temperature, with no need for a higher temperature during extraction.

Pesticidal effect of neem
Neem extract is highly effective against insects. The chief active component is azadirachtin, it exhibits agro-medicinal properties. It disrupts the feeding ability of insects, acts as a deterrent, is repugnant, is responsible for insect sterility, acts as an oviposition deterrent, and reduces sperm production in male insects. Overall, it has larvicidal [39], insecticidal [40-55], and acaricidal effects. Bioactive components of neem, nimbidin, and nimbin are present in neem leaf and are the main reason for the bitter taste of neem. In Table 2 below, we discussed the neem plant part, its active components, and its mode of action.

Table 2: Mode of action and doses of neem-derived biopesticide:

Sl. No.	Mode of action	Major components	Target pests	Dosage LC50	Exposer time	Ref.
1	Antibacterial	Phenolic and flavonoids	Foodborne and Contaminated Bacteria	50mg/ml.	18-24 hrs	[56]
2	Growth inhibitory		Klebsiella, Salmonella, and *Staphylococcus aureus*	Klebsiella (12.5 mg/ml) and Salmonella (6.2 mg/ml) respectively	24hrs	[57]
3	Molluscicides		Snail intermediate hosts of schistosomiasis and selected non-target aquatic animals	Neem leaf B. Pfeifferi (0.0319%-adults, 0.0196%-neonates, and 0.0064%-egg-masses B. Truncatus. -(0.0203%-adults, 0.0103%-neonates, and 0.0025%-egg-masses Neem seed B. Pfeifferi (0.0285%-adults, 0.0133%-neonates, and 0.0043%-egg-masses Stored seed extract 0.0321%-adults, 0.0185%-neonates, and 0.0044%-egg-masses	24hrs	[58]
4	Insecticidal		*Ceratitis capitata*	888ppm		[59]
5	Aphicide		Grain Aphid (*Sitobion avenae*)	0.34to 1.10%	3 days	[60]

6	Insecticide		Brassica pod midge (*Dasineura brassicae Winn.*)	20g/ha		[61]
7	Insecticide		*Oxya chinensis*	14.93 to 55.66mg/mL.	72hrs	[62]
8	Insecticide		*Mahanarva fimbriolata*	NeemAzal (0.014%), Nimkol (0.225%), and aqueous extract were (0.611%) respectively.	10days	[63]
9.	Insecticide	Azadiractin	Sawfly, *Athalia lugens proxima* Klug.	Azadiron (0.1359) > Bioneem (0.1531)> NLE (0.1833)> Neemarin (0.3207) > Neemgold (0.3208) > Econeem (0.6397) > DNSKP (0.6868) > Azadirachtin (0.7124)> Neemazal (0.7421) > Nimbicidine (0.9025) > NSKE (0.9554)	4hrs	[64]
10	Insecticide		*Trichogramma chilonis*	2 ml/liter	24hrs	[65]
11	Insecticide		*Spodoptera litura*	5 ml/l	3-7days	[66]
12	Insecticide		*Syllepte derogata* (Fabricius), a cotton phyllophagous pest.	4.03 10^4 ml/l-for leaf dipping method and 51.13 ml/l-for larval immersion.	24hrs	[67]
13	Insecticide		*Corcyra cephalonica* (Staint.)	0.16% (a.i) v/w	15days	[68]
14	Insecticide		The leafminer (*Liriomyza sativae*)	10 g/100 mL	24hrs	[69]
15	Aphicide		Grain Aphid (*Sitobion avenae)*	0.34 to 1.10%.	3days	[70]
16	Insecticide		cotton mealybug, *Phenacoccus solenopsis*	1.5 and 2.0%	72 hrs	[71]
17	Insecticide		Cotton-sucking pests aphid, whitefly, jassid, and thrips	Neem oil and Neem powder -1% and 2% respectively	3,7,14 days	[72]
18	Insecticide		*Myzus persicae*	1, 5 and 10%	2days	[73]
19	Insecticide		*Nilaparvata lugens*, brown planthopper	40%	72hrs	[74]
20	Insecticide	Azadiractin	*Plutella xylostella* (Linn.)	15 and 20 ppm	24hrs	[75]

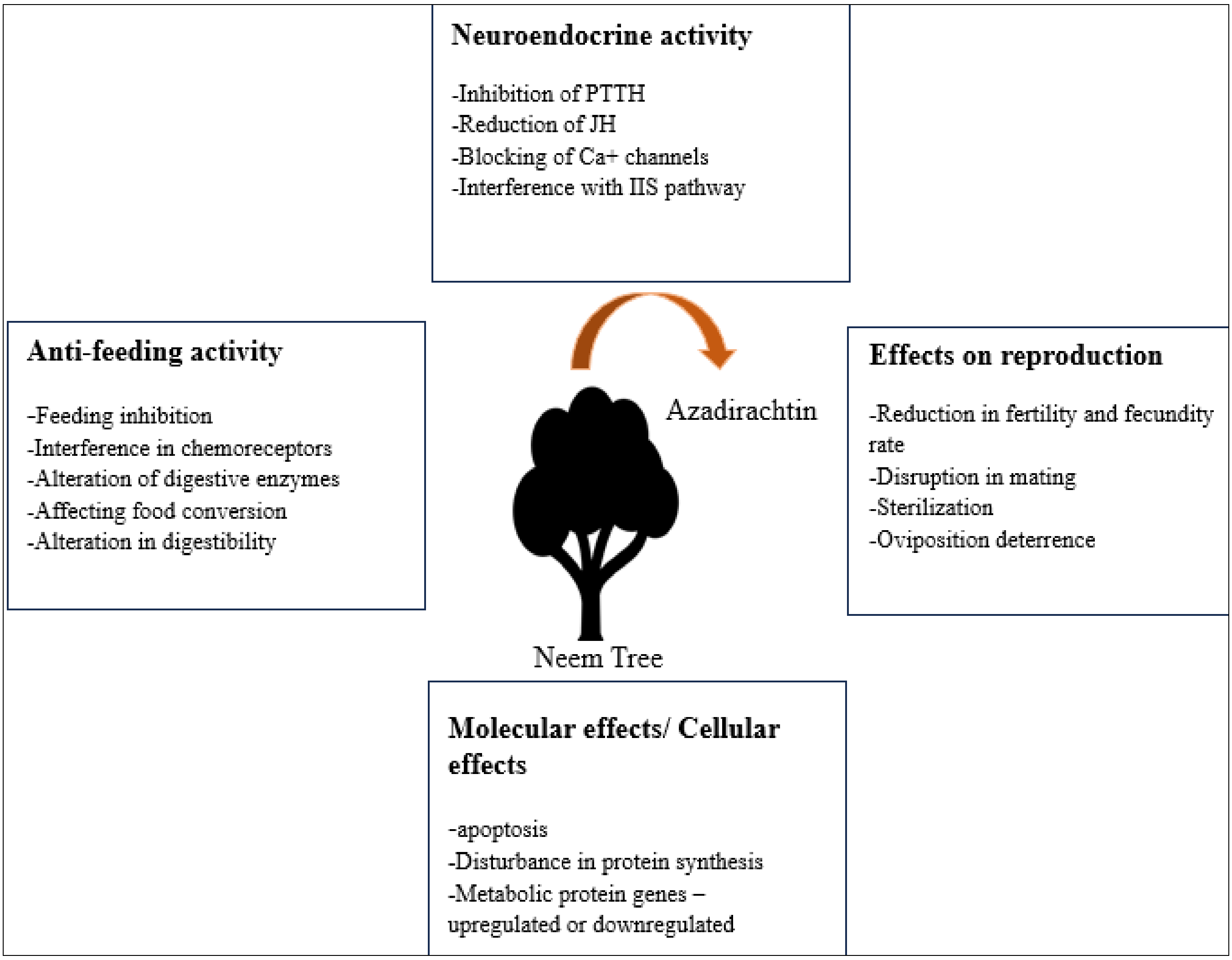

Fig 2: Mode of action of Azadirachtin (The major component of neem) on pests

Table 3: The complete mechanism of Azadirachtin's role against insects:

Sl. No	Effects	Target	Mechanism of its role	Example	Ref.
1	The primary or gustatory antifeedant	Mouthpart and other chemoreceptors	It activates specific 'deterrent' cells in chemoreceptors and prevents the firing of ' sugar' receptor cells, which normally trigger feeding. Feeding deterrents inhibit the consumption ability and results malnutrition and ultimately death of these animals.	*Schistocerca gregaria, Spodoptera littoralis, S. frugiperda, Heliothis virescens,* and *Helicoverpa armigera*	[87,88]
2	Secondary antifeedant	Gut	Inhibition of peristalsis Reduced production of an enzyme Midgut not replaced	Aphids of tobacco seedlings treated with azadirachtin - feeding activity suppressed and delayed.	[87,88]
3	Growth and moulting (IGR)	Cuticle structure	Direct effect-interference with juvenile hormone and moulting hormone levels Indirect effect-modification of haemolymph ecdysteroid levels	Insects	[87] [79]
4	Sterility	Reproductive organ	Enlarged amount of yolk resorption and degenerate ovaries. It prevents the fat body from synthesising vitellogenin and the eggs from absorbing it. decreased fecundity and sterility in the end.	Effect of azadiractin on locust	[87]
5	Neuroendocrine system	Neurosecretory system	interferes with peptide hormone transport and release mechanisms (PTTH, allotoinhibins, and allatostatins)		[87]
6	Cell growth	Cell line	Cell toxicity and the suppression of *in vitro* cell growth.	Sf9 cells, *Aedes albopictus* cells	[87]
7	Cytotoxicity	Cell division in differentiated tissues	Both direct cell death and mitotic blockage	After azadirachtin therapy, the Mexican bean beetle, Epilachna varivestis, showed severe tissue degradation in the areas where active cell division was taking place.	[87]
8	Inhibition of Protein synthesis	Transcription	Impacts the transcriptional level, where the machinery involved in protein synthesis is activated for a critical purpose.	suppression of the production of trypsin by the midgut and fat body cells, the production of detoxifying enzymes by the midgut and fat body cells, and the production of 20-mono-oxygenases for the catabolism of ecdysone in insecticide-resistant insects.	[87]
9	Antimitotic	Chromosomal aberration	a rapid rise in the mitotic index accompanied by the emergence of several abnormal mitotic figures	cultured insect cells (Sf9)	[89]

Mode of action

Acaricide
Venzon *et al.* [76] reported neem-based pesticides as an acaricide. The research assessed the potential of these insecticides to affect the two-spotted spider mite pest population (*T. urticae* Koch) in leafy minor crops over time and the onset of phytotoxicity symptoms.

Larvicide
Hernandez-Trejo *et al.* [51] reported larvicidal activity of phenolic constituents present in neem extract on the second-stage larvae of *Spdoptera frugiperda*. It showed a 60% mortality rate. Solvent extractions are less pollutant for the environment as they are not residual. Nicoletti *et al.* [77] described another study about the larvicidal effect of neem cake extract against *Aedes albopictus* larvae. The larvicidal efficiency of the phytocomplex together was significantly higher than the isolated compounds of extract.

Adverse effects on insect growth regulation (IGR) and development
Azadirachtin has a growth-regulating effect on larval insects [78]. It causes malfunction at different stages of larval growth, development [79], and eventually death of the insects depending on the concentration of it. The successive concentration of azadirachtin may cause reduced fecundity [80, 81], nutritional indices [82], deformed wings and body pigmentation [83], incomplete moulting, other deformities, overage larvae, etc.

Antifeedant effect of azadirachtin
Ramesh Babu P.B and Lalan Gupta [84] and Mir S. Mulla and Tianyun Su [85] reported in their study the insecticidal effect of azadirachtin derived from neem extract. It directly affects the chemoreceptor (Primary consequence) organs of the insects and deterrent from food consumption (secondary consequence), thus resulting in antifeedant among them and also ultimately reducing the fitness of the insects [8, 6].

Discussion
The traditional Soxhlet technique has numerous benefits over other extraction processes. Most notably, it can extract components at a consistent pace and does not require many solvents.

Both polar and semi-polar components are present in neem leaves. The choice of solvent system is a significant breakthrough. The use of solvents with various polarity levels helps to obtain all active constituents during extraction processes. Various organic compounds of different parts of plants have diverse attractions to the polarity of the solvents. The yield rate of extractions is directly associated with the appropriateness of the polarity array of the obtained components from extraction and their solvents.

Neem leaves active component is azadirachtin, and the stick is tannin. Both the components have antibacterial effects using Soxhlet extraction.

It had been reported that the binary solvent system had a sophisticated attraction for azadirachtin from neem leaves and neem oil at a specific solvent ratio and extraction time.

Among modern extraction methods, the supercritical fluid extraction process is emphasized as a fast, effective, and competent method for extracting bioactive components. It is extensively used in large industrial production houses. CO_2 is the most widely utilized solvent system since it is inexpensive, safe, and has low to medium critical qualities.

Neem-derived beneficial phytocomponents are basically secondary metabolites. These play a pivotal role in plant defense mechanisms. The same strategy was applied to extract these metabolites using various extraction processes to formulate biopesticide for agroecosystems.

The neem-derived extract contains numerous bioactive components of the limonoids group. Among this group, azadirachtin is the utmost effective and also regulates the insect growth mechanism. It also has an insecticidal effect on pest juvenile stages.

The efficacy of various neem-derived insecticides varies according to the pest's developmental stage. Early instar larvae are more susceptible than adult forms.

Neem-treated crops become unattractive or unpalatable to insects because of their bitter taste and smell, which affects pests' growth, feeding, survival, and, most importantly, reproduction.

Azadiractin, the main bioactive component of neem biopesticides, is photo and thermo-labile. Under high solar radiation and temperature, it easily deteriorates. Subsequently, there is a need for the stabilization of formulations.

Azadirachtin affects the insect's growth and development in many ways. Primarily, it interrupts cell division by preventing or altering the development of new accumulations of organelles or cytoskeletons. Furthermore, it stops spermatozoa from developing and hinders the transport and release of neurosecretory peptides. Secondarily, metabolically active and large amounts of protein-producing cells are inhibited, such as insect cell lines with maximum proliferation, midgut cells for digestion, and midgut and fat body cells for detoxication.

The future aspect of neem as biopesticides

Neem is the greatest option for a natural, non-synthetic substitute for plant pesticides. Over the years, extensive research has demonstrated its pesticidal effectiveness. It is a more affordable and environmentally responsive insecticide than commercially produced by chemical synthesis.

Conclusion

Collaborative and advanced pharmacological research should be done to use the phytoconstituents of crude neem extract as a source of possible bioactive components for enhanced human and environmental health in the future. The advanced study must investigate the neem extracts derived major repellence-causing biochemical constituents and their specific biochemical pathways of action on test pest organisms. At a particular cell cycle stage, azadirachtin may inhibit protein synthesis by preventing or altering transcription or translation at the molecular level. Azadirachtin has a novel mode of action with the most minor toxicity to humans and the environment. Supplementary studies should be done on classifying and documenting binding sites using microarray, proteomics, and other techniques.

Acknowledgments

This work was supported by the Swami Vivekananda Merit Cum Means scholarship to the first author and the University of North Bengal.

Author Contributions

Nitu Sinha drafted the manuscript and Dr. Sonali Ray conceived the article along with final drafting. The authors read and approved on the final version of the manuscript.

Disclosure statement

There is no conflict of interest among the authors regarding the publication of the manuscript.

References

1. Shannag HK, Capinera JL, Freihat NM. Effects of neem-based insecticides on consumption and utilization of food in larvae of *Spodoptera eridania* (Lepidoptera: Noctuidae). Journal of Insect Science. 2015;15(1):152.
2. Radha R. Comparative studies on the Effectiveness of Pesticides for Aphid control in Cowpea. Research Journal of Agriculture and Forestry Sciences. ISSN. 2013;2320:6063.
3. Lim GSoon, Bottrell DG. Neem pesticides in rice: potential and limitations. International Rice Research Institute; c1994.
4. Lokanadhan S, Muthukrishnan P, Jeyaraman S. Neem products and their agricultural applications. Journal of Biopesticides 2012;5:72.
5. Archana B, Tabassum P, Pranita D. Review on Neem (*Azadirecta indica* A. Juss) as a potential source of organic pesticide. International Journal of Ayurveda and Pharma Research. 2017;5(4):12-5.
6. Shrivastava DK, Swarnkar K, Raghavendra Rao GE. Antifungal Activity of leaf extract of Neem (*Azadirachta indica* Linn). IntJCurrMicrobiolAppSci 2014;3:305-8.
7. Naniwadekar MY, Jadhav AS. Process Development of Pesticide Production from *Azadirachta indica* A. Juss. vol. 1. 2012.
8. Alzohairy MA. Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complementary and Alternative Medicine 2016;2016.
9. Reddy IVS, Neelima P. Neem (*Azadirachta indica*): A Review on Medicinal Kalpavriksha. International Journal of Economic Plants 2022;9(1):059-63.
10. Eid A, Jaradat N, Elmarzugi N. A Review of chemical constituents and traditional usage of Neem plant (*Azadirachta indica*). vol. 2. 2017.
11. Santos KS, Barbosa AM, Freitas V, Muniz AV, Mendonça MC, Calhelha RC, *et al*. Antiproliferative activity of neem leaf extracts obtained by a sequential pressurized liquid extraction. Pharmaceuticals. 2018;11(3):76.
12. Hismath I, Wan Aida WM, Ho CW. Optimization of extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves. Int Food Res J 2011;18(3):931-939.
13. Tesfaye B, Tefera T. Extraction of Essential Oil from Neem Seed by Using Soxhlet Extraction Methods. Advanced Engineering, Management and Science 2017;3(6):239870.
14. Jadeja GC, Maheshwari RC, Naik SN. Extraction of natural insecticide azadirachtin from neem (*Azadirachta indica* A. Juss) seed kernels using pressurized hot solvent. The Journal of Supercritical Fluids. 2011;56(3):253-8.

15. Liauw MY, Natan FA, Widiyanti P, Ikasari D, Indraswati N, Soetaredjo FE. Extraction of neem oil (*Azadirachta indica* A. Juss) using n-hexane and ethanol: Studies of oil quality, kinetic and thermodynamic. ARPN Journal of Engineering and Applied Sciences. 2008;3(3):49-54.
16. Tonthubthimthong P, Douglas PL, Douglas S, Luewisutthichat W, Teppaitoon W, Pengsopa LE. Extraction of Nimbin from neem seeds using supercritical CO2 and a supercritical CO2-methanol mixture. The Journal of supercritical fluids. 2004;30(3):287-301.
17. Dai J, Yaylayan VA, Raghavan GSV, Pare JRJ, Liu Z, Be JMR. Influence of Operating Parameters on the Use of the Microwave-Assisted Process (MAP) for the Extraction of Azadirachtin-Related Limonoids from Neem (*Azadirachta indica*) under Atmospheric Pressure Conditions. Journal of Agricultural Food Chemistry 2001;49(10):4584-4588.
18. Subapriya R, Bhuvaneswari V, Nagini S. Ethanolic neem (*Azadirachta indica*) leaf extract induces apoptosis in the hamster buccal pouch carcinogenesis model by modulation of bcl-2, BIM, caspase 8 and caspase 3. Asian Pacific Journal of Cancer Prevention 2005;6(4):515-520.
19. Septiyani R, Wibowo C. Identification of active compounds and testing the antioxidant properties of neem leaf extract. InAIP Conference Proceedings 2019 Apr 17 (Vol. 2094, No. 1). AIP Publishing.
20. Siswomihardjo W, Badawi SS, Nishimura M, Hamada T. The difference of antibacterial effect of neem leaves and stick extracts. Int Chin J Dent 2007;7:27-9.
21. Kumar R, Sharma S, Devi L. Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extracts of *Azadirachta indica* of Bundelkhand Region. International Journal of Life-Sciences Scientific Research 2018;4(4):1925-1933.
22. Shewale S, Rathod VK. Extraction of total phenolic content from *Azadirachta indica* or (neem) leaves: Kinetics study. Preparative Biochemistry and Biotechnology. 2018;48(4):312-320.
23. Gupta SP, Singh M, Ahmed I, Sharma DN, Kumar P. Effect of Temperature and Time Intervals on the Solvent Extraction of Essential Oil from *Azadirachta indica* (Neem) Leaf Powder by Using Soxhlet Extraction Method. World J Pharm Res 2018;8(3):939-946.
24. Subramanian S, Salleh AS, Bachmann RT, Hossain MS. Simultaneous extraction and separation of oil and azadirachtin from seeds and leaves of *Azadirachta indica* using binary solvent extraction. Natural Product Sciences 2019;25(2):150-156.
25. Benisheikh AAG, Muhammad FM, Kelluri H, Aliyu ZM, Mallam UB, Jibrin MW. Phytochemical extraction and antimicrobial studies on crude leaf extract of *Azadirachta indica* (Neem) in Semi-Arid Region of Borno State, Nigeria. International Journal of Research & Review 2019;6(12):516-522.
26. Mohapatra A, Ranjan M, Horta SS. Antimicrobial activity of neem (*Azadirachta indica*) leaf extracts. International Journal of Research in Agricultural Sciences 2014;1(2):143-148.
27. Awolu OO, Obafaye RO, Ayodele BS. Optimization of solvent extraction of oil from neem (*Azadirachta indica*) and Its Characterizations. J Sci Res Rep 2013;2(1):304-314.
28. Babatunde DE, Otusemade GO, Efeovbokhan VE, Ojewumi ME. Chemical composition of steam and solvent crude oil extracts from *Azadirachta indica* leaves. Chemical Data Collections 2019;20:100208.
29. Ezeonu CS, Imo C, Agwaranze DI, Iruka A, Joseph A. Antifungal effect of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on fungal rot diseases of yam and cocoyam. Chemical and Biological Technologies in Agriculture 2018;5:1-9.
30. Nde DB, Boldor D, Astete C. Optimization of microwave assisted extraction parameters of neem (*Azadirachta indica* A. Juss) oil using the Doehlert' s experimental design. Ind Crops Prod 2015;65:233-240.
31. Panawan Suttiarporn and Vachira Choommongkol. Microwave-Assisted Improved Extraction and Purification of Anticancer Nimbolide from *Azadirachta indica* (Neem) Leaves. Molecules 2020;25(12):1-15.
32. Ismadji S, Kurniawan A, Ju YH, Soetaredjo FE, Ayucitra A, Ong LK. Solubility of azadirachtin and several triterpenoid compounds extracted from neem seed kernel in supercritical CO 2. Fluid Phase Equilib 2012;336:9-15.
33. Mongkholkhajornsilp D, Douglas S, Douglas PL, Elkamel A, Teppaitoon W, Pongamphai S. Supercritical CO 2 extraction of nimbin from neem seeds: A modelling study. J Food Eng 2005;71(4):331-40.
34. Patel MJ, Tripathy S, Mukhopadhyay KD, Wangjam T, Cabang AB, Morris J, *et al*. A supercritical CO2 extract of neem leaf (*A. indica*) and its bioactive liminoid, nimbolide, suppresses colon cancer in preclinical models by modulating pro-inflammatory pathways. Molecular carcinogenesis. 2018;57(9):1156-1165.
35. Ajchariyapagorn A, Kumhom T, Pongamphai S, Douglas S, Douglas PL, Teppaitoon W. Predicting the extraction yield of nimbin from neem seeds in supercritical CO2 using group contribution methods, equations of state and a shrinking core extraction model. The Journal of Supercritical Fluids. 2009;51(1):36-42.
36. Lee JW, Ryu HW, Park SY, Park HA, Kwon OK, Yuk HJ, *et al*. Protective effects of neem (*Azadirachta indica* A. Juss.) leaf extract against cigarette smoke-and lipopolysaccharide-induced pulmonary inflammation. International Journal of Molecular Medicine. 2017;40(6):1932-40.
37. Sitasiwi JA, Isdadiyanto S, Muflichatun Mardiati S. Effect of ethanolic Neem (*Azadirachta indica*) leaf extract as an herb contraceptive on Hepato-somatic Index of the male mice (Mus musculus). InJournal of Physics: Conference Series 2018 (Vol. 1025, p. 012043). IOP Publishing.
38. Kim WH, Song HO, Jin CM, Hur JM, Lee HS, Jin HY, *et al*. The methanol extract of *Azadirachta indica* A. juss leaf protects mice against lethal endotoxemia and sepsis. Biomolecules & Therapeutics. 2012;20(1):96.
39. Mantzoukas S, Ntoukas A, Lagogiannis I, Kalyvas N, Eliopoulos P, Poulas K. Larvicidal action of cannabidiol oil and neem oil against three stored product insect pests: effect on survival time and in progeny. Biology. 2020;9(10):321.
40. Scott IM, Kaushik NK. The toxicity of a neem insecticide to populations of Culicidae and other aquatic invertebrates as assessed in in situ microcosms. Archives of environmental contamination and toxicology. 2000;39:329-36.
41. Kwasi I, Boadu O, Samuel DRI, Tulashie K, Anang MA, Kpan J. Production of natural insecticide from Neem

leaves (*Azadirachta indica*). Pelagia Research Library 2011;1:33-8.
42. Shah FM, Razaq M, Ali Q, Shad SA, Aslam M, Hardy IC. Field evaluation of synthetic and neem-derived alternative insecticides in developing action thresholds against cauliflower pests. Scientific reports. 2019;9(1):7684.
43. Kumar R, Kranthi S, Nagrare VS, Monga D, Kranthi KR, Rao N, *et al*. Insecticidal activity of botanical oils and other neem-based derivatives against whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on cotton. Int J Trop Insect Sci 2019;39:203-210.
44. Liang GM, Chen W, Liu TX. Effects of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae). Crop protection. 2003;22(2):333-40.
45. Santhosh K, Kumar A, Author C, Scholar MS. Comparative efficacy of selected insecticides and neem products against chickpea pod borer [*Helicoverpa armigera* (Hubner)]. The Pharma Innovation Journal 2022;11:1558-1562.
46. Akol AM, Sithanantham S, Njagi PG, Varela A, Mueke JM. Relative safety of sprays of two neem insecticides to *Diadegma mollipla* (Holmgren), a parasitoid of the diamondback moth: effects on adult longevity and foraging behaviour. Crop protection. 2002;21(9):853-9.
47. Stark JD, Rangus TM. Lethal and sublethal effects of the neem insecticide formulation, 'Margosan-O', on the pea aphid. Pesticide Science. 1994;41(2):155-60.
48. Stark JD. Population-level effects of the neem insecticide, Neemix, on *Daphnia pulex*. Journal of Environmental Science and Health, Part B. 2001;36(4):457-65.
49. Ahmad S, Ansari MS, Muslim M. Toxic effects of neem based insecticides on the fitness of *Helicoverpa armigera* (Hübner). Crop Protection. 2015;68:72-8.
50. Sutherland JP, Baharally V, Permaul D. Use of the botanical insecticide, neem to control the small rice stinkbug *Oebalus poecilus* (Dallas, 1851) (Hemiptera: Pentatomidae) in Guyana. Entomotropica 2002;17(1):97-101.
51. Trejo HA, Herrera RR, Galindo SA, Badillo LCM, Gallegos FAC, Valdez AJA, *et al*. Insecticidal capacity of polyphenolic seed compounds from neem (*Azadirachta indica*) on *Spodoptera frugiperda* (JE Smith) larvae. Journal of Environmental Science and Health, Part B. 2021;56(12):1023-30.
52. Baidoo PK, Ansah BD, Agbonu I. Effects of Neem (*Azadirachta indica* A. Juss) Products on Aphis craccivora and its Predator Harmonia axyridis on Cowpea. vol. 2. 2012.
53. Hossain MB, Poehling HM. Effects of a neem-based insecticide on different immature life stages of the leafminer *Liriomyza sativae* on tomato. Phytoparasitica. 2006;34:360-9.
54. Satti A, Ellaithy M, Mohamed A. Insecticidal activities of neem (*Azadirachta indica* A. Juss) seeds under laboratory and field conditions as affected by different storage durations. Agriculture and Biology Journal of North America 2010;1:1001-1008.
55. Aníbal F. Cóndor_Golec. Effect of neem (*Azadirachta indica* A. Juss) insecticides on parasitoids. Rev Peru Biol 2007;14:69-74.
56. Yehia HM. Methanolic extract of neem leaf (*Azadirachta indica*) and its antibacterial activity against foodborne and contaminated bacteria on sodium dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Am Eurasian J Agric Environ Sci. 2016;16:598-604.
57. Banna QR, Parveen F, Jalaluddin Iqbal M. Growth inhibitory effect of ethanolic neem leaves extract on Klebsiella, Salmonella and Staphylococcus aureus. Bangladesh J Pharmacol 2014;9(3):347-50.
58. Ahmed AAM, Ahmed SM. Extracts of Leaves and Seeds of the Neem Tree, *Azadirachta indica*, As Environment-Oriented Molluscicides for Combating Schistosomiasis. Proceedings of Workshop on African Freshwater Malacology, 2003, p. 235-49.
59. Vendramim JD, Mastrangelo T. Sublethal effect of neem extract on Mediterranean fruit fly adults. Rev Bras Frutic 2013;35:93-101.
60. Aziz MA, Ahmad M, Nasir MF, Naeem M. Efficacy of different neem (*Azadirachta indica*) products in comparison with imidacloprid against english grain aphid (*Sitobion avenae*) on wheat. Int J Agric Biol 2013;15:279-84.
61. Pavela R, Kazda J, Herda G. Effectiveness of Neem (*Azadirachta indica*) insecticides against Brassica pod midge (*Dasineura brassicae* Winn.). J Pest Sci (2004) 2009;82:235-40.
62. Li L, Song X, Yin Z, Jia R, Zou Y. Insecticidal activities and mechanism of extracts from neem leaves against *Oxya chinensis*. Arq Bras Med Vet Zootec 2019;71:1-10.
63. Garcia JF, Grisoto E, Vendramim JD, Botelho PSéM. Bioactivity of Neem, *Azadirachta indica*, Against Spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae) on Sugarcane. J Econ Entomol 2009;99(6):2010-4.
64. Chandel BS, Sengar HS. Insectifuge effect of neem, *Azadirachta indica* A. Juss and their derivatives against sawfly, *Athalia lugens* proxima Klug. (Hymenoptera: Tenthredinidae). International Journal of Zoology Studies. 2018;3(1):53-9.
65. Asrar M, Ali S, Mushtaq N, Zia K, Bibi R, Akhtar I, *et al*. Toxicity of neem seed extract and different insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Journal of King Saud University-Science. 2022;34(6):102135.
66. Singh B, Singh N, Singh S. Efficacy of Neem based biopesticides and chemical insecticide against *Spodoptera litura* on cauliflower under field condition in Gurugram District of Haryana. Plant Arch 2019;19:3039-42.
67. Ganda H, Togbé EC, Houndété TA, Boukari ET, Gogan M, Dagbénonbakin GD, *et al*. Effectiveness of neem seed oil (*Azadirachta indica* A. Juss: Meliaceae) on *Syllepte derogata* Fabricius, Lepidoptera: Pyralidae. Journal of Applied Biosciences. 2018;129:13029-38.
68. Pathak CS, Tiwari SK. Insecticidal Action of Neem Seed (*Azadirachta indica* A. Juss) Acetone Extract Against the Life-Cycle Stages of Rice-Moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae). World Journal of Agricultural Sciences 2012;8(5):529-536.
69. Costa EM, Silva FEL, Araujo EL. Effect of aqueous neem seed extract via irrigation on larvae of *Liriomyza sativae* in melon crop. Hortic Bras 2018;36:353-6.
70. Aziz MA, Ahmad M, Nasir MF, Naeem M. Efficacy of different neem (*Azadirachta indica*) products in comparison with imidacloprid against english grain aphid (*Sitobion avenae*) on wheat. Int J Agric Biol

2013;15:279-84.
71. Rashid MURM, Abdullah MK, Hussain S. Toxic and residual activities of selected insecticides and neem oil against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) under laboratory and field conditions. Pakistan Entomologist 2011;33:151-155.
72. Jarwar AR, Abro GH, Khuhro RD, Dhiloo KH, Malik MS. Efficacy of neem oil and neem kernal powder against major sucking pests on brinjal under field conditions. European Academic Research. 2014;2(6):7641-58.
73. Déla MA, Koffivi KG, Komina A, Arnaud A, Philippe G, Adolé GI. Evaluation of neem leaves-based preparations as insecticidal agents against the green peach aphid, *Myzus persicae* (Sternorrhyncha: Aphididae). African Journal of Agricultural Research. 2014;9:1344-52.
74. Salleh NH, Mohamed AR, Abdullah S, Sohaimi KS, Munirah AN, Yusoff NA, *et al*. Optimization of Insecticides Extracted from Neem Leaves on *Nilaparvata lugens*, Brown Planthopper. Journal of Advanced Research in Engineering Knowledge. 2018;4(1):1-7.
75. Ahmad N, Ansari MS, Hasan F. Effects of neem based insecticides on *Plutella xylostella* (Linn.). Crop Protection 2012;34:18-24.
76. Venzon M, Togni PHB, Perez AL, Oliveira JM. Control of two-spotted spider mites with neem-based products on a leafy vegetable. Crop Protection 2020;128:105006.
77. Nicoletti M, Serafini M, Aliboni A, D'Andrea A, Mariani S. Toxic effects of neem cake extracts on Aedes albopictus (Skuse) larvae. Parasitol Res 2010;107:89-94.
78. Kraiss H, Cullen EM. Insect growth regulator effects of azadirachtin and neem oil on survivorship, development and fecundity of *Aphis glycines* (Homoptera: Aphididae) and its predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). Pest Manag Sci 2008;64:660-8.
79. Schlüter U, Bidmon HJ, Grewe S. Azadirachtin affects growth and endocrine events in larvae of the tobacco hornworm, *Manduca sexta*. Journal of insect physiology. 1985;31(10):773-7.
80. Pandey JP, TRK. Neem based insecticides interaction with development and fecundity of red cotton bug, *Dysdercus cingulatus* Fab. International Journal of Agricultural Researcg 2011;6:335-46.
81. Boulahbel B, Aribi N, Kilani-Morakchi S, Soltani N. Activity of neem oil in Drosophila melanogaster: toxicity and delayed effect on the progeny. J. Entomol. Zool. Stud. 2015;3:306-10.
82. Shannag HK, Capinera JL, Freihat NM. Effects of neem-based insecticides on consumption and utilization of food in larvae of *Spodoptera eridania* (Lepidoptera: Noctuidae). Journal of insect science. 2015;15(1):152.
83. Suyog Pandey SP, Pandey JP, Tiwari RK. Effect of some neem based insecticides on wing shape and pigmentation in lemon-butterfly, *Papilio demoleus* L. World Applied Science Journal 2011;13:1356-60.
84. PB Ramesh babu, Gupta L. Studies on the anti-feedant effect of neem leaf using synthetic insecticide. International Journal of Pure and Applied Mathematics 2018;119(12):611-20.
85. Su M, Mulla MS. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J. Am. Mosq. Control Assoc. 1999;15:133-52.
86. Abdullah Fauziah SP. The feeding response of *Epilachna indica* (Coleoptera: Coccinellidae: Epilachninae) towards Extract of *Azadirachta indica*. Journal of Entomology 2008;5:77-90.
87. Mordue AJ. Present concepts of the mode of action of azadirachtin from neem. InNeem: Today and in the new millennium 2004 (pp. 229-242). Dordrecht: Springer Netherlands.
88. Jennifer Mordue A, Nisbet AJ. Azadirachtin from the Neem Tree *Azadirachta indica*: its Action Against Insects. An Soc Entomol Brasil 2000;29:615-32.
89. Salehzadeh A, Akhkha A, Cushley W, Adams RL, Kusel JR, Strang RH. The antimitotic effect of the neem terpenoid azadirachtin on cultured insect cells. Insect biochemistry and molecular biology. 2003;33(7):681-9.