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A Comprehensive Study on Extraction and Characterization of Bioactive Pesticidal and Insecticidal Compound from Azadirachta Indica (Neem)

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Abstract: *Research into the insecticidal effects of azadirachtin, a limonoid from the Neem (Azadirachta indica) has been ongoing for some 30 years. Its strong antifeedant, insect growth regulatory and reproductive effects are now well understood and documented. Antiffedancy varies markedly between species with mosquitoes being particularly sensitive to azadirachtin. The mode of action of azadirachtin lies in (i) effects on deterrent and other chemoreceptors resulting in antifeedancy and (ii) direct effects on most other tissues studied resulting in an overall loss of fitness of the insect. The complexity of the molecular structure of azadirachtin has precluded its synthesis for pesticide use although novel synthesis of the parent molecule is now almost complete and research into simpler mimetic substances is ongoing. Neem (Azadirachta indica is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has huge insecticidal property and is thus commercially exploitaed. During the last two decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and insecticidal applications of neem. It is now considered as a valuable source of unique natural products for botanical insecticides against various pests. This review gives a bird's eye view mainly on the biological activities of some of the neem compounds isolated, insecticidal actions of the neem extracts, applications of neem has an eco-friendly botanical insecticide in pest management along with their safety evaluation.*

Keywords: Azadirachtin indica; Neem leaves; Green Chemistry, Synthetic Insecticide, Limonoid; triterpense; Pest control; Antifeedancy; eproduction; Beta sitossterol Mode of action.

I. INTRODUCTION

Insecticide/Pesticides are chemical substances that are meant to kill pests.In general, a pesticide is a chemical or a biological agent such as a virus, bacterium, antimicrobial, or disinfectant that deters, incapacitates, kills, pests. This use of pesticides is so common that the term pesticide isoften treated as synonymous with plant protection product. It is commonly used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity The most commonly applied pesticides are insecticides to kill insects, herbicides to kill weeds, rodenticides to kill rodents, and fungicides to control fungi, mold, and mildew. A pesticide is any substance used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. Pesticides include herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds and mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice and rats. Because of the widespread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets. This make you and your skin look rejuvenated and youthful. Neem Pesticide / Insecticide formulation is a neem based botanical product that contains azadirachtin as an active ingredient. Azadirachtin is found to be very effective for over 600 species of insects. In developing countries, the losses of crops due to pest, plant disease and competition from weeds is great. In households, pest and insects such as mosquitoes, cockroaches, mice etc pose risks such as the destruction of furniture, clothing and to the causation of

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various diseases, most seriously; malaria.damages, also tend to have adverse effects on humans in various ways, most especially those produced from synthetic materials. These adverse effects of headache, dizziness, catarrh worth investigating. The insecticides range from agricultural to household pesticides. Every category has its own effect, both on the targeted pest/insect and the environment lives. This research targets insecticide produced from natural products and the need to choose these pesticides rather than those of synthetic origin.

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), comm only called fall armyworm (FAW) is a moth whose larvae feed on more than 100 different plant species [1]. It is known to be a serious pest of maize, rice, sorghum, tuff, sugarcane, and a wide range of other crops including peanuts, soybean, and some non-food crops such as cotton [2]. In maize, larvae of FAW destroy the fresh leaves which impair the growth and ultimately reduce the yield of the crop. Until 2016, there was no recorded incidence of FAW invasion in West Africa [3-5]. Outbreaks of FAW were reported in January 2016, in southwest Nigeria, Ghana, Benin, Sao Tomé, and Togo, [6]. However, FAW is now repted to be present in over 40 countries in tropical and southern Africa including Madagascar [2]. About 98% of local farmers in Ghana reported in a household survey that maize is affected by FAW and the reported damage to maize amounted to 26.6% [7]. Economically, Ghana potentially losses US$177 m annually due to damage by FAW. To mitigate the loss due to FAW infestation, many farmers employ the use of synthetic chemical insecticides. However, the use of synthetic chemical insecticides has unintended detrimental consequences including residues in food and water [8,9]. It is also known that synthetic pesticides (insecticides and herbicides) kill beneficial insects [10,11]. Aside from this, pesticides including organophosphates and carbamates can cause serious health effects [9,12]. This necessitates the development of alternative effective eco-friendly insecticides. Environmentally acceptable pesticides are known for their relatively low toxicity to non-target organisms and biodegradability due to their botanical origin. This makes it possible to incorporate such insecticides into integrated pest management programs [13,14]. Due to the rise in the advocacy for the use of safer insecticides, several research works have been conducted in search of plant sources of safe insecticides. For instance, asteroid-like tetranortriterpenoid (azadirachtin) obtained from the Indian neem tree (Azadirachta indica), has been identified as a key insect repellent in vegetables [13]. Moreover, the importance of neem in agriculture [9], toiletry, pharmaceutical and medicine [15], has grown considerably because of its numerous bioactive ingredients. It has been found that neem has antiallergenic, antidermatic, antifeedant, antiviral, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, insecticidal, larvicidal, antiimplantation and nematicidal properties [16]. Studies have shown that neem biopesticides were effective against pests in stored grains like rice, wheat, corn, and legumes as well as in foodstuffs like potato and tomato in India [9,17]. Neem has an insecticidal effect on insects that suck plant sap and those which chew plant parts. The active ingredient in neem, azadirachtin, serves as a growth regulator as well as a feeding and oviposition deterrent. It acts as a growth regulator by reducing the level of ecdysone, a hormone that disrupts the moulting process of the insects and thus prevents larvae from developing into adults [18]. Azadirachtin is a mixture of seven isometric compounds being Azadirachtin- A to -G with Azadirachtin-A as the dominant and Azadirachtin-E as the most effective growth regulator [19].

II. METHODOLOGY

Extraction of Neem Oil (Active Ingredient)

The primary active compounds for pesticidal action are found in neem oil, which is extracted from neem seed kernels. The two most common methods of extraction are cold pressing and solvent extraction.

Cold Press Extraction-This is the most common and organic method for extracting neem oil.

Procedure:-(1) Feed the ground neem seed powder into a cold-pressing machine. (2) Apply mechanical pressure to the powder to extract the oil. (3) The cold-pressed oil is collected in containers. (4) The oil is then filtered to remove solid particles or impurities.

Advantages: This method preserves the bioactive compounds, such as azadirachtin, which are essential for pesticidal action. It is also more environmentally friendly, as no chemicals are used.

Solvent Extraction-This method is typically used when large quantities of neem oil are required. Procedure:- Mix the neem seed powder with a solvent (e.g., hexane, ethanol, or petroleum ether).Let the mixture sit for 48–72 hours with occasional stirring to facilitate the extraction of the oil.After extraction, filter out the solid particles.Remove the solvent

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using a distillation process, leaving behind concentrated neem oil. Formulation of Neem-based Insecticide-To create a usable neem-based insecticide or pesticide, the neem oil must be formulated into a solution that can be easily applied to plants. The formulation process typically involves diluting the neem oil and adding emulsifiers. Basic Neem Oil Pesticide Preparation- Neem Oil Solution: Dilute neem oil with water to achieve a solution with 1%–2% concentration of active ingredients. A higher concentration can be used for more severe pest problems.

Emulsification: Since neem oil is hydrophobic (doesn't mix well with water), an emulsifier is needed to make the mixture stable. Common emulsifiers include:Liquid soap (preferably castile soap),Soapnut extract,Lecithin (a natural emulsifier).Mix the emulsifier with the water before adding neem oil. Stir or shake well to ensure a uniform emulsion.

Sprayable Neem Pesticide Solution-Materials: 1–2 teaspoons of neem oil,1 liter of water,1 teaspoon of liquid soap or an emulsifier (optional)

Procedure: (1) Mix the water and soap/emulsifier. (2)Slowly add neem oil to the mixture while stirring.(3)Stir the solution well to ensure even distribution.(4)Filter the solution using a fine cloth or strainer.The resulting neem insecticide can be stored in spray bottles or larger containers for application.

III. LITERATURE REVIEW

NEEM:-

Neem (Azadirachta indica A. Juss) (Meliaceae) is native to the Indian sub-continent and well known as the 'Botanical Marvel', 'Village Pharmacy', 'Wonder Tree', 'all-can-treat-tree' and 'Gift of Nature'. Neem has great potential in the fields of pest management, environmental protection and medicine. All parts of neem like seed, flower, bark, and leaf possess insecticidal activity but seed kernel is the most effective. The products derived from neem tree act as powerful Insect Growth Regulators (IGR) and also help in controlling several nematodes and fungi (Subbalakhmi et al., 2012). Neem (Azadirachta indica) is a medicinal tree native to the Indian subcontinent, known for its antibacterial, antifungal, and insecticidal properties. It is widely used in traditional medicine, agriculture, and skincare for its health and ecological benefits.

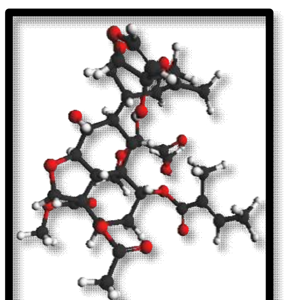

Figure:- Chemical structure of Azdirachtin Indica

History of Azadirachtin (NEEM):

Warden (1888) was the first to study the chemistry of neem oil that contains sulphur. Later, margosonic acid margosopicrin were isolated from neem oil. Chemical investigations carried out after 1940, yielded the isolation of the major bitter principle of neem, nimbin in a crystalline form (Siddiqui, 1942). Later on, other compounds like nimbinin from bark, nimbosterol from flowers, nimbocinone from leaves, nimbidin and nimbinin from oil and aldobiuronic acid from neem gum were isolated. Azadirachtin is the most potent locust antifeedant isolated during the year 1968. Neem possess insecticidal, nematicidal, fungicidal, bactericidal (Schmutterer, 1995), and molluscicidal properties (Mehlhorn et al., 2011a).

Bio-Pesticidal Activity Of Neem:

Neem Oil

Neem oil extracted by cold-pressing the seed kernels of neem is highly effective against soft-bodied insects and mites. The presence of disulphide in neem oil is a major contributor to its bioactivity. The most significant insecticidal and therapeutic properties of this agro-medicinal neem component are illustrated in Figure 2. Neem oil contains more than a dozen azadirachtin analogs, but the major contributor to the insecticidal activity is azadirachtin. The remaining triterpenoids including nimbin, salannin, and their derivatives contribute little to the efficacy (Isman, 2006). Interestingly, neem oil is non-toxic to mammals, birds and fishes and exhibits fewer chances of resistance, due to its multiple mode of action on insects. Many formulations of neem seed oil exhibit antifeedant, ovicidal, larvicidal, insect growth regulatory, and repellent activity against insect pests. The larvicidal property of neem oil against mosquitoes has long been investigated. a multitude of components that minimize the chance of resistance to synthetic insecticides in mosquitoes. One such study investigated the potential of neem oil as an eco- friendly alternative for the control of malaria.

Neem Active Pesticidal Components

Neem parts constituting leaf, seed, bark, flower, and oil possess a multiplicity of components that are responsible for its multiple pesticidal activities.

Azadirachtin

The main component of neem oil, leaves, flowers, and fruits with insecticidal properties is Azadirachtin. It constitutes 0.1-0.3% of neem seeds and was first isolated from A. indica by Morgan et al. at Keele University, England (Sclimutterer, 1985). It is a complex tetranortriterpenoid limonoid with repellent and pesticidal properties. Biosynthesis of triterpenoids from A. indica initiates with azadirone and a C-ring opening, which culminates in Azadirachtin formation. Azadirachtin, along with other related triterpenoids such as Azadirachtin B, salannin and nimbin, are the active ingredients in neem plant based bioinsecticides and they act by disrupting the growth and development of insects and by deterring their feeding. It is considered as a botanical pesticide with exceptional growth regulating and biocidal efficacy along with deterrent effects on the ovipositing and feeding of insects (Morgan, 2009). An attempt to evaluate the exact molecular mechanism of insecticidal activity of azadirachtin on Monochamus alternatus, a pine sawyer beetle, has indicated enrichment of differentially expressed genes (DEGs) in 50 pathways. 920 and 9984 unique genes were found to be up ling and down regulated significantly. Such detailed gene profiling to assess the azadirachtin internalization with M. alternatus, can promote the development of efficient azadirachtin derived herbal pesticides (Lin et al., 2016).

Mechanism of Action

Azadirachtin is structurally similar to the insect hormones known as "ecdysones" which are responsible for metamorphosis in insects. The feeding behavior in insects is dependent on the neural inputs received from the chemical sensors of the insects, for example, the taste receptors in the mouthparts, tarsi and oral cavity. These sensors integrate a "sensory code" that is delivered to the central nervous system. Manifestation of antifeedancy by azadirachtin occurs through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in insects by firing the "sugar" receptor cells (Jennifer Mordue Luntz et al, 1998).

Nimbolide

Two main active ingredients; Nimbolide B and Nimbic acid B also demonstrate herbicidal activity of neem. Their allelopathic and phytotoxic activity was observed in a study where they inhibited the growth of lettuce, crabgrass, alfalfa, jungle rice, and barnyard grass. The allelopathic activity increased with an increase in the concentration of active compounds, but the intensity varied with different species of weed (Kato- Noguchi et al., 2014).

Salannin

Salannin is an active component of neem with insect-growth regulating and antifeedancy activity. Salannin deters feeding. increases the larval stage duration and causes delayed molt, leading to decreased pupal weight that results in larval and pupal mortality. This has been demonstrated in an early study on Oxya fuscovittata where salannin caused delayed molting and nymphal mortality (Govindachari et al, 1996). The bioactivity observed was more prominent in azadirachtin as compared to salannin, however, a combination of azadirachtin with salannin and nimbin can provide insect growth-regulating activity with increased efficacy.

Neem Based Nano-Biopesticides

In agricultural practices, herb-based insecticides have the disadvantage of getting degraded when exposed to sunlight, due to low shelf life. Moreover, the active ingredients of neem cause non-specific toxicity. Aqueous extracts of neem leaves have shown toxicity to Oreochromis niloticus, by causing telangectiasis, bend in secondary lamellae, pyknosis, secondary lamellae shortening, and necrosis. Therefore, it is imperative to consider eco-toxicological properties of active ingredients of bio-pesticides (Alim and Matter, 2015). To overcome this, nano- biotechnology offers great potential, as it involves the production of unique nanoformulations that have the ability to improve the physiochemical stability, degradability, and effectiveness of natural products (Perlatti et al., 2013).

Adnan, S., Uddin, M., Alam, M., Islam, M., Kashem, M., Rafii, M., et al. (2014). Management of mango hopper, idioscopus clypealis, using chemical insecticides and neem oil. Sci. World J. 2014:709614. doi: 10.1155/2014/ 709614

An experiment was conducted in Field Laboratory, Department of Entomology at Bangladesh Agricultural University, Mymensingh, during 2013 to manage the mango hopper, Idioscopus clypealis L, using three chemical insecticides, Imidacloprid (0.3%), Endosulfan (0.5%), and Cypermethrin (0.4%), and natural Neem oil (3%) with three replications of each. All the treatments were significantly effective in managing mango hopper in comparison to the control. Imidacloprid showed the highest efficacy in percentage of reduction of hopper population (92.50

± 9.02) at 72 hours after treatment in case of 2nd spray. It also showed the highest overall percentage of reduction (88.59 ± 8.64) of hopper population and less toxicity to natural enemies including green ant, spider, and lacewing of mango hopper. In case of biopesticide, azadirachtin based Neem oil was found effective against mango hopper as 48.35, 60.15, and 56.54% reduction after 24, 72, and 168 hours of spraying, respectively, which was comparable with Cypermethrin as there was no statistically significant difference after 168 hours of spray. Natural enemies were also higher after 1st and 2nd spray in case of Neem oil.

Ahmad, K., Adnan, M., Khan, M. A., Hussain, Z., Junaid, K., Saleem, N., et al. (2015). Bioactive neem leaf powder enhances the shelf life of stored mungbean grains and extends protection from pulse beetle. Pak. J. Weed Sci. Res 21, 71–81.

Plant products are receiving greater attention as prophylactics against several species of plant-parasitic nematodes. Numerous experiments have shown the potential nematicidal value of plant parts and their by-products when incorporated into soil or when the plants themselves are interplanted as seedlings among crop plants. Various products (oils, cakes, extracts, etc.) prepared from the leaves and seeds of the neem plant (Azadirachta indica A. Juss) (Family Meliaceae) have been reported as effective protectants against nematode pests when used as root-dips and seed treatments. Nemato-toxic compounds of the neem plant, especially the azadirachtins, are released through volatilization, exudation, leaching and decomposition. The modes of action of these compounds are complex, and a number of mechanisms in relation to nematode management are yet to be fully explored. This review critically assesses the potential of these products in the management of nematodes in tropical agriculture.

Akhtar, M. (2000). Nematicidal potential of the neem tree Azadirachta indica (A. Juss). Integr. Pest Manag. Rev. 5, 57–66. doi: 10.1023/A:1009609931223

Pesticides are widely used in agricultural and other settings, resulting in continuing human exposure. Epidemiologic studies indicate that, despite premarket animal testing, current exposures are associated with risks to human health. In this review, we describe the routes of pesticide exposures occurring today, and summarize and evaluate the epidemiologic studies of pesticide-related carcinogenicity and neurotoxicity in adults. Better understanding of the patterns of exposure, the underlying variability within the human population, and the links between the animal toxicology data and human health effects will improve the evaluation of the risks to human health posed by pesticides. Improving epidemiology studies and integrating this information with toxicology data will allow the human health risks of pesticide exposure to be more accurately judged by public health policy makers.

Alavanja, M. C., Hoppin, J. A., and Kamel, F. (2004). Health effects of chronic pesticide exposure: cancer and neurotoxicity∗ 3. Annu. Rev. Public Health 25, 155–197. doi: 10.1146/annurev.publhealth.25.101802.123020

Plant-based medicines are useful in the treatment of cancer. Many breast cancer patients use complementary and alternative medicine in parallel with conventional treatments. Neem is historically well known in Asia and Africa as a versatile medicinal plant with a wide spectrum of biological activities. The experiments reported herein determined whether the administration of an ethanolic fraction of Neem leaf (EFNL) inhibits progression of chemical carcinogen-induced mammary tumorigenesis in rat models. Seven-week-old female Sprague Dawley rats were given a single intraperitoneal injection of N-methyl-N-nitrosourea (MNU). Upon the appearance of palpable mammary tumors, the

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rats were divided into vehicle-treated control groups and EFNL-treated groups. Further, we found that EFNL treatment effectively upregulated proapoptotic genes and proteins such as p53, B cell lymphoma-2 protein (Bcl-2)-associated X protein (Bax), Bcl-2-associated death promoter protein (Bad) caspases, phosphatase and tensin homolog gene (PTEN), and c-Jun N-terminal kinase (JNK). In contrast, EFNL treatment caused downregulation of anti-apoptotic (Bcl-2), angiogenic proteins (angiopoietin and vascular endothelial growth factor A [VEGF-A]), cell cycle regulatory proteins (cyclin D1, cyclin-dependent kinase 2 [Cdk2], and Cdk4), and pro-survival signals such as NFκB, mitogen-activated protein kinase 1 (MAPK1). The data obtained in this study demonstrate that EFNL exert a potent anticancer effect against mammary tumorigenesis by altering key signaling pathways.

IV. RESULTS AND DISCUSSION

Discussion

The extraction of bioactive pesticidal and insecticidal compounds from Azadirachta indica (neem) involves methods like solvent extraction, cold pressing, and supercritical fluid extraction. These methods isolate key compounds such as azadirachtin, nimbin, and salannin, which are responsible for neem's insecticidal properties. Azadirachtin acts as an insect growth regulator, disrupting feeding and reproduction in pests. The characterization of these compounds is done using techniques like HPLC, GC-MS, and NMR, which help identify and quantify the bioactive substances. Neem-based insecticides are eco-friendly and effective alternatives to chemical pesticides, offering sustainable pest management solutions. Neem (Azadirachta indica), widely known for its pesticidal and insecticidal properties, is a tropical evergreen tree that has been used for centuries in agriculture, medicine, and pest control. The plant is rich in bioactive compounds, primarily found in its leaves, seeds, bark, and fruit. These compounds, particularly azadirachtin, are known to be highly effective in pest management by disrupting the growth and reproduction of insects.

Result: The results of extraction and characterization of bioactive pesticidal and insecticidal compounds from Azadirachta indica (neem) have demonstrated significant potential in developing natural pest control solutions. Some key findings are:

1. Extraction Yield: Solvent extraction methods (using solvents like methanol, ethanol, and acetone) have successfully yielded a range of bioactive compounds from neem, with methanol being one of the most effective solvents for extracting azadirachtin and other limonoids. Cold pressing has also been effective in extracting neem oil, which contains a high concentration of these bioactive compounds.
2. Bioactive Compounds Identified: Through various chromatographic and spectroscopic techniques, compounds like azadirachtin, nimbin, nimbolide, salannin, and azadiradione have been isolated. Azadirachtin is the most studied and potent compound, exhibiting insecticidal properties by acting as an insect growth regulator, while other limonoids contribute to the overall efficacy of neem-based insecticides.
3. Characterization: Characterization of neem extracts using techniques like HPLC, GC MS, and NMR has confirmed the presence and quantified the amounts of these 29 | Page bioactive compounds. HPLC has been particularly effective in quantifying azadirachtin, while GC- MS and NMR have helped elucidate the structure of various triterpenoids and limonoids.
4. Insecticidal Activity: Neem extracts have demonstrated strong insecticidal activity against a wide range of pests, including aphids, caterpillars, and termites. Azadirachtin has been shown to inhibit feeding, disrupt molting, and prevent reproduction in insects, making it a highly effective biopesticide.
5. Eco-friendliness: The extracted neem compounds have been found to be environmentally safe, biodegradable, and non-toxic to humans and animals, offering a sustainable alternative to synthetic chemical pesticides. These results underline the potential of neem as a valuable source of bioactive insecticidal compounds, supporting its use in integrated pest management (IPM) systems for sustainable agriculture.

V. CONCLUSION

The production of natural insecticides and pesticides derived from neem seeds represents a promising and sustainable solution to modern agricultural challenges. As awareness of the environmental and health risks associated with synthetic chemical pesticides grows, neem-based products offer a viable alternative that aligns with the global shift toward organic farming and eco-friendly practices.Neem seeds, rich in bioactive compounds like azadirachtin, nimbin, and salannin, provide a natural, effective method of pest control. The extraction of these compounds through methods like cold pressing or solvent extraction leads to the creation of neem oil, which can then be formulated into a variety of pesticide products—ranging from foliar sprays to soil treatments and even eco-friendly repellents.The process of extracting neem oil involves several stages, including seed collection, drying, crushing, grinding, and the actual extraction of the oil. These are increasingly being enhanced by emerging technologies like supercritical CO2 extraction, microwave-assisted extraction, and enzyme-assisted methods, which promise higher yields, better quality, and improved sustainability. Such innovations not only improve the efficiency of production but also ensure the preservation of neem's potent pesticidal compounds.Moreover, neem-based products are gaining recognition for their versatility. They are not only effective against a wide range of pests but also pose minimal risk to beneficial insects, wildlife, and human health, making them an integral part of Integrated Pest Management (IPM) strategies. Neem products also contribute to soil health, making them a valuable tool in regenerative agriculture practices.Looking ahead, the future of neem-based pesticides is bright. Increasing demand for organic products, stricter regulations on synthetic chemicals, and innovations in formulation and application techniques will all drive growth in this sector. Research in areas such as nanoformulations, precision agriculture, and biological pest resistance management will continue to enhance the efficacy and environmental compatibility of neem-based insecticides.steps. In conclusion, the extraction of neem seeds for the production of natural insecticides/pesticides represents a sustainable, economically viable, and environmentally responsible approach to pest management. As the world embraces organic farming and seeks greener alternatives, neem-based solutions are poised to play a critical role in shaping the future of global agriculture.

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