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Phytochemical Studies of Neem Seed Kernel Extract and Its Efficacy on Leaf Miner (*Liriomyza trifolii*)

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ABSTRACT

Plant extracts are a great source of molecules with insecticidal activity, which are used for pest control in several crop production systems. The work aims to evaluate the toxicity of an aqueous extract of neem seed kernel extract (NSKE) against larvae of Liriomyza trifolii (Burgess). The effect of neem seed kernel extract on the food utilization, development and survival of leaf miner larvae was evaluated by feeding larvae on an artificial diet supplemented with different concentrations of the extract (2%, 4%, 6%, 8%, and 10%). The effects observed were dose-dependent and the highest concentration evaluated (10%) was the one that affected food utilization patterns by altering the nutritional indices, larval weight gain, development time, and survivor. In-vitro assays were performed to detect saponins, lectins, and trypsin inhibitors in NSKE and trypsin inhibitors were detected. The data obtained indicated the existence of a potential molecule in the tested extract of neem seed kernel to be used as an alternative insecticide to be integrated into the management of L. trifolii.

Keywords: *Liriomyza trifolii, neem seed kernel extract, saponins, trypsin*

INTRODUCTION

The over-reliance on synthetic pesticides as the sole measure for pest control has created several problems (e.g., resistance, residues, pollution, health hazards, etc.). Plant extracts proved to be effective as natural pesticides [1]. They are cheap, safe and Integrated Pest Management (IPM) compatible. [13] In this respect, more than 2000 species of higher plants possess insecticidal properties against various insect pests and vectors of plant diseases. Among such plants, the neem tree (*A. Indica A. Juss*) is becoming a potential source of natural insecticides [35].

The longterm effects of indiscriminate and harmful use of synthetic pesticidshave led to the development of alternative, cost effective, nontoxic, biodegradable and environmentally friendly biological control approaches [10]. These non-chemical and biological pest control methods are relevant not only for organic farming but also in attaining basic principles of sustainable integrated pest manageme [4]. Neem tree extracts were researched for their versatile properties and were widely used against a wide range of pest species [8], such as meliatriol, nimbin, azadirachtin, nimbinin, azadirachtol, nimolicinoic acid, azadirone, salannin, etc. [16], the most commonly discussed molecule of which is azadirachtin. NSKE is the least dangerous to humans among the currently advertised botanical insecticides and shows very low toxicity to beneficial plants. Therefore, it is very promising for the control of many pests. Azadirachtin may also overlap with mitosis in the same way as colchicine, and has similar histopathological effects on insect intestinal epithelial cells, muscles and fatty tissues, culminating in reduced mobility and reduced flight activity [32]; [1] [27]. Several studies have established the effect of neem oil on various insect groups. Neem

oil has exhibited action against Lepidoptera among the major insect groups: antifeeding effect and increased larva mortality [20] [22] [29]. In addition to its medical applications, neem has aroused interest in many other areas. In the cosmetics and hygiene sector, neem is used in the composition of face masks, lotions, sunscreens, soaps, and toothpastes [21]. Products derived from neem can contribute to sustainable development and pest control problems in agriculture [18]. These products benefit from the natural properties of neem as a powerful insect growth regulator (IGR) that also affects many other organisms (such as nematodes and fungi) and can act as a plant fertilizer [6]. The current research work was carried out for a qualitative screening of neem (*A. indica A. Juss*) seed kernel (NSKE) extracts as potential natural insecticides against leaf miner.

MATERIALS AND METHODS

Preparation of neem extract

The whole neem tree contains bitterness to varied extents, but a higher concentration of it is found in the neem kernel. Neem kernel is a valuable source of major limnoid. It is therefore essential to understand the scientific method of fruit collection and depulping to get kernels.

Collection of Neem Fruits

The neem yields fruits from May to August 2013 year. Being rich in carbohydrates neem fruits gets attacked by fungi when coming in contact with soil. Such fruits may damage the quality of the final products prepared from these fruits. Hence it is strongly recommended to avoid contact of neem fruits with soil. As the fruit rips it must be depulped as early as possible.

Depulping of Neem Fruits

Depulping is a process to remove seed coat and pulp from the neem seed. It is done by hand and using a mechanical depulper. Rub the ripe neem fruits between palms in the bucket of water and wash the seed. Use clean water for depulping. After depulping and cleaning, dry the neem seeds in the shade in a thin layer. Keep the neem seeds in a cool and dry place. If processed properly these neem seeds can be stored for about 6-12 months.

Separation of the kernels

The dried neem seeds are ground slightly by hand and the outer shell of the seed is removed. Kernels are present inside the shell which are separated and then made into powder using a grinder. It should be pounded such that no oil comes out of it. This coarse powder is used for further studies on the extraction of Azadirachtin.

Qualitative estimation of phytochemicals

Qualitative phytochemical analysis of aqueous extracts of neem seed kernel extract was carried out on the extract using the standard procedure to identify the constituents as described by Sofowora [26], [7].

Steroids (Liebermann-Burchard's test)

1 ml of the extract was dissolved in 10 ml of chloroform and equivalent concentrated sulphuric acid was contained on the sides of the test tube. The top layer turns red, and the sulfuric acid layer reveals white fluorescence in color. It has suggested the presence of steroids. [9]

Terpenoid (Thionyl chloride test)

2 ml of extract and concentrated H2SO4 were applied to 2 ml of acetic anhydride. Black, green ring structures indicate the presence of terpenoids. [3]

Fatty Acids

0.5 ml of extract is mixed with 5 ml of ether. This extract was put to evaporate on filter paper, and the filter paper was dried. The advent of transparency means that fatty acids are present on filter paper. [3]

Tannins (Ferric chloride)

A few drops of 1 percent lead acetate were applied on 2 ml of extraction. A yellowish solubilize had the presence of tannin. [30]

Saponins (Foam test): Stirred 5 ml of extract for15 minutes in a graduated flask, combined with 20 ml of distilled water. Foam formation is indicative of saponin activity. [17]

Anthocyanins

Add 2 ml of aqueous extract and 2 ml of 2N HCl and NH3. The emergence of blue-violet pink-red changes indicates the presence of anthocyanins. [23]

Leucoanthocyanins: 5 ml of aqueous extracts added

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with 5 ml of alcohol isoamyl. The topmost layer of anthocyanins from leuco is red. [23]

Coumarins

Adding 3 ml of 10% NaOH to 2 ml of aqueous yellow extract indicates the presence of coumarins. [24]

Emodins

2 ml NH4OH and 3ml Benzene were added to the extract.The red color appearance suggests emodin involvement. [24]

Alkaloids (Mayer's test)

1gram powder sample of NSKE was taken in a conical flask and ammonia solution (3ml) was applied. Free alkaloids were predicted to live for a few minutes. Chloroform (10ml) was applied to the hand-shaken conical flask and then filtered. The chloroform was evaporated by eater bath from crude extract, and Mayer's reagent [36] (3ml) was applied. Immediately a cream color precipitation was obtained which showed the existence of alkaloids. [7]

Flavonoids (Shibita's reaction test)

The stock solution (1ml) was put into a test tube and a few drops of dilute NaOH solution was applied. The test tube showed an intense yellow colour. It became colorless when a few drops of dilute acid were added which suggested the presence of flavonoids.

Statistical analysis

The statistical analysis of the generated data was carried out using [26] with the program SAS 9.2 software.

RESULTS AND DISCUSSION

The findings of this analysis showed that neem seed kernel extract (NSKE) contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and sugar reduction. Anthraquinones have not been detected from the extract (Table 1). This is consistent with [5] study in which the aqueous extract of Azadirachta indica NSKE contains pentosis and carbohydrates in addition to the chemical constituents found. Anthraquinones, ketones and monosaccharides have also not been detected. Quantitatively, the plant NSKE extract also showed a higher proportion of saponins, and moderate concentrations of tannins and glycosides, while flavonoids, alkaloids, reduced sugars and terpenes were present at low concentrations (Table 1).

Table -1: Qualitative analysis of neem seed kernel extract

Phytochemical analysis	Qualitative test	Water extract Neem seed kernel extract(NSKE)
Steroids	Liebermann Burchard test	+
Terpenoids	Thionyl chloride test	+
Tannins	with Ferric chloride	+
Saponin	Foam test	+
Anthraquinones	Bontrager's test	-
Alkaloid	with Mayer's test and Dragendoff's test	+
Flavonoids	with NaOH, with Mg/HCl	+
Phenolic compounds	with lead acetate	+
Reducing sugars	Benedict's test	+
Cardiac glycosides	Legal test	+
Fatty acids	With ether and filter paper	-
Leucoanthocyanins	With isoamyl alcohol	-
Coumarins	With NaOH	+
Emodins	With NH_4OH and Benzene	+

+ = present, - = absent.

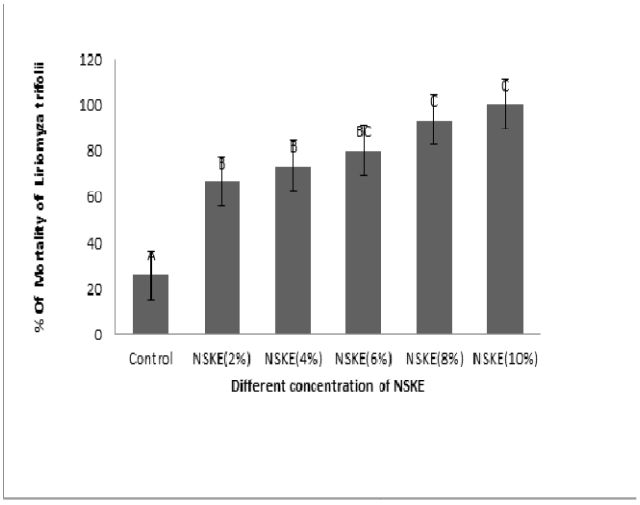

Fig.1. Efficacy of botanicals against Liriomyza trifolii in tomato different superscripts differs significantly at 5% level.

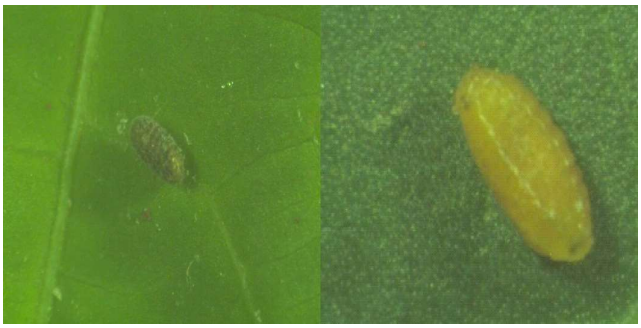

Fig.2. Effect of NSKE at 10% concentration against Liriomyza trifolii in both treated and untreated samples

The findings of this analysis showed that neem seed kernel extract (NSKE) contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and sugar reduction. Anthraquinones have not been detected from the extract (Table 1). This is consistent with [5] study in which the aqueous extract of Azadirachta indica NSKE contains pentosis and carbohydrates in addition to the chemical constituents found. Anthraquinones, ketones and monosaccharides have also not been detected. Quantitatively, the plant NSKE extract also showed a higher proportion of saponins, and moderate concentrations of tannins and glycosides, while flavonoids, alkaloids, reduced sugars and terpenes were present at low concentrations (Table 1). The presence of these phytochemical components may be responsible for the antimicrobial activity of the plant leaf extract. These findings are consistent with the [2] which found similar constituents exhibiting antiprotozoal and antibacterial activity. Flavonoid has also been reported to have a higher potential benefit to human health [14]. Another phytochemical study of the leaf extract Azadirachta indica was reported by Imran [12] in which petroleum ether, chloroform and methanol extracts were found to contain only relatively higher amounts of glycosides, triterpenes and fatty acids. This may explain why it is important to select a solvent for the extraction of a specific constituent. Phytochemicals in Neem seed kernel extract (NSKE) were found to be steroids, terpenoids, tannins, saponin, alkaloid flavonoids, phenolic compounds, reducing sugars, cardiac glycosides, coumarins and emodins were present. Whereas anthraquinones, Fatty acids, and Leucoanthocyanins were absent in the tested samples as shown in Table-1 and the presence of these phytochemicals suggested that the activity of chlorophyll is enhanced using this extract. Through this the pesticide action of neem seed kernel extract is enhanced. Similar effects are exhibited in both the control and treatment groups in Fig-1. 2% NSKE was very minimal efficacy; however, 10% NSKE was very effective in controlling the growth of Liriomyza trifolii larva in tomato plants (Fig-2). The efficacy of NSKE was enhanced by increasing the concentrations from 2-10%, however, all concentrations were significantly ($p< 0.05$) effective in controlling the larval growth as compared to the control (Fig-1).

Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, saponins and related active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceuticalindustry. Thesesecondarymetabolites are reported to have many biological and therapeutic properties. Recently several studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems etc [15]. Alkaloids, flavonoids, and glycosides have been reported to exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity, etc. The phytoconstituents are well known for their curative activity against several human problems such as ulcers, swollen liver, malaria, dysentery, diarrhea, etc. A variety of herbs and herbal extracts contain different phytochemicals with biological activities that can be of valuable therapeutic index. Much of the protective effect of herbal plants have been attributed to phytochemicals, which are non-nutrient compounds. [25]

Phytochemical test results showed high scores for saponins, and moderate scores for tannins and glycosides, whereas alkaloids, terpenes, and flavonoids had low scores. According to Anyanwu and Dawet [2], these constituents present in plants are known to have anti-protozoal and anti-bacterial activity. Flavonoids, in particular, are of possible benefit to human health [14]. Azadirachtin extracts from Neem seeds, leaves and bark have been reported to have high biological activity against insect pests, but have very low toxicity to mammals and the environment in general [33] [19] [33]. Licensed Neem insecticide formulations Neemros ® and Neemroc EC ® have also been shown to be effective against insect pests. The widespread use of the neem plant is thus due to the existence of these bioactive compounds, which can explain many of its common uses against various diseases.

Very few integrative reports of all phytochemical constituents of neem seed kernel extract are available

to date, and researchers have reported selected phytochemicals in their studies. Thus, an overall phytochemical constituent approach of analysis was studied in these experiments.

CONCLUSION

Neem seed kernel extract was able to control the growth of Liriomyza trifolii leaf miner. The present study provides evidence that solvent extract of neem seed kernel extract contains agriculturally important bioactive compounds and this justifies the use of plant species as traditional insecticides for the treatment of insects. Further, purification, identification, and characterization of the bio-active chemical constituent'scompounds would be our priority in future studies.

Consent And Ethical Approval

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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