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A COMPARATIVE EFFICACY OF METHANOLIC EXTRACTS OF AZADIRACHTA INDICA AND CASSIA FISTULA AGAINST TERMITES

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Abstract

Termites are insects consisting of 3000 species, of which 300 are considered pests. They feed on the decomposition of organic wastes like leaves, animal dung, and living or dead wood, causing significant economic damage. Fast-acting termiticides are frequently used to control termites. However, this practice has significant biological and environmental hazards. Medicinal plants have good insecticidal activity without any significant environmental risk. Therefore, the current study investigated the invitro toxic potential of Azadirachta indica and Cassia fistula *against termites. Fresh leaves of Azadirachta indica and* Cassia fistula *were collected from their habitat in the Kohat district. Leaves were identified, rinsed, shade dried, and ground, and the crude methanolic extract was prepared. Termites were also collected from their natural habitat in the Kohat district. Termite species were identified, and a 24-hour invitro assay was conducted in triplicates using Petri dishes and various concentrations of both extracts of selected medicinal plants.*

Both selected medicinal plants revealed a notable dose-dependent anti-termite activity. In comparison, Azadirachta indica showed a maximum activity (20.86±4.16) at a concentration of 8 mg/ml, while a great activity was observed by Cassia fistula (26.84±4.61) at the same concentration. Permethrin was used as a positive control, which revealed (25.23±4.7) mortality at the same concentration. The current study concluded that the Azadirachta indica exhibits anti-termite potential. Cassia fistula might contain some anti-termite insecticidal compounds which need to be isolated and explored further to replace the hazardous chemical substances against termites.

INTRODUCTION

Termites are small social insects consisting of 3000 species, of which approximately 300 are considered pest species. [1].exhibiting a pronounced colonial system with soldiers, workers, and reproductive cast (king and queen) in the natural environment [2]. Termites are integral members of the decomposer

community. They can degrade cellulose, which is the major component of wood [3]. Termites flourish in tropical and subtropical regions, contributing to the breakdown and recycling of one-third of the yearly deadwood. When they start destroying crops and wooden items, furniture construction materials, and forests, they cause economic loss [4]. Termites wreak havoc on plants by boring holes in their underground and above-ground parts and feeding on their seeds, leaves, stems, and lower branches of the plants [5]. Crops and local huts of impoverished subsistence farmers are devastated by these termites, which primarily impact developing countries in Asia. Termites attack trees, wooden structures, earth dams, underground power lines, telephone wires, wooden buildings, and wooden and paper furniture [4]. Termites play a significant role in preserving the world's unique ecological systems by recycling wood and plant matter (33% in the tropics), changing soil structure, and improving soil conditions and productivity by boosting soil organic matter[6]. They primarily attack agricultural products, decorative plants, cultivated fruits, forest nurseries, and man-made timber structures, including furniture for buildings, bridges, goods warehouses, and railway sleepers and poles.[7, 8].

Termites that construct their nests beneath and emerge to attack wooden structures in houses, woods, and crops are known as subterranean termites. Although many of these species have been identified, only a small number of them seriously destroy structures [9]. All the damage is done by worker termites, who are foraging for cellulose-rich items. Through trophallaxis and social grooming, these workers subsequently distribute food and nutrients to their other nest members, including nymphs, soldiers, and reproductive [10]. Subterranean termite control accounts for about 80% of total termite spending each year, with damage, repairs, and control worth about $11 billion annually in the United States alone. Termite control costs are expected to rise as living conditions improve and people's standards rise [11, 12]. The subterranean termite genus Heterotermes is indigenous to much of the world and is regarded as one of the most significant commercial termite pests [13]. In addition, it has been identified as a serious agricultural pest in the Indian deserts near the Pakistani border[14]. It can destroy adult trees without showing their presence by digging them out from the inside [15]. Pakistan is an agricultural production-based nation, and major and minor crops account for a sizeable part of its gross domestic product. However, due to high input costs, such as fertilizers and pesticides, Pakistan's crop revenue is significantly lower than that of other wealthy nations. The output of crops is currently declining, which eventually affects producers' gross revenue. One of the main pests in Pakistan, subterranean termites, cause significant harm to wheat, sugarcane, maize, tobacco, and fruit plantations [16].

In the past, termites could only be controlled by synthetic pesticides, particularly persistent organochlorine [17]. Their disadvantages include the development of pesticide resistance in the target pests and the maximal residual impact [18]. Botanical pesticides should come from nearby readily available plants, need little to no processing, and not become weeds or serve as hosts for pests that attack crops. They should also be minimally hazardous to non-target creatures, including people and beneficial insects [19]. The majority of plant oil plays a significant role in pest control agents.[20].

Termites are cosmopolitan, having the ability to adopt any habitat. They are found on every continent except Antarctica. Has the richest diversity in the tropical and subtropical areas [21]. The distribution of termites in the low area is influenced by environmental parameters such as temperature, moisture content, energy availability, soil type, nitrogen balance, methane, and carbon dioxide on a global scale [22].

Heterotermes indicola is one of the top three major pests in the United States [23]. It is reported to cause significant economic damage in Brazil and other regions of South America by damaging cash crops like cotton, rice, coffee, cassava, and mature forest trees like eucalyptus and pines. In Brazil, Heterotermes indicola specie is commonly found in sugarcane fields and is responsible for more than 10 tons of annual damage. [11, 24]. Heterotermes indicola (Wasmann) is one of the most destructive and economically significant subterranean termite pest species in Pakistan. Along with crops and orchards, it harms residential wooden structures. Due to its year-round activity ranks among the species with the most incredible tenacity [25].

Seven families of termites have been described viz; Hodotermitidae, Rhinotermitidae, Termopsidae, Kalotermitidae, Semitermitidae, Mastotermitidae and Termitidae [26].

Different synthetic chemical preservatives are sprayed on soft woods to prevent termite infestation and damage; however, these preservatives are harmful to people and animals and should only be used outdoors [27, 28]. Plant extracts were used against pests for agriculture in ancient China, Egypt, Greece, and India [17].

The extraction and production of several medicines and chemotherapeutics from medicinal plants, and traditionally used rural herbal treatments, has led to an increase in the dependence on their usage in industrialized nations [29]. Medical plants are now regarded as a critical resource in the treatment and prevention of many different diseases. Each plant contains several essential components that are useful in medicine and are utilized to create a variety of medications[30]. Compared to synthetic insecticides, bio-pesticides or plant-derived pesticides offer a more natural and ecologically friendly method of pest management[31].

Azadirachta indica also referred to as neem tree or Indian lilac, is a member of the Meliaceae family of mahogany trees. Tropical and semi-tropical climates are where it is commonly cultivated. [32]. Neem is a fast-growing tree, 15-20 m (49-66 ft) tall, rarely 35-40 m (115-131 ft). It is deciduous and sheds many leaves during dry winters. Branches spread widely. The dense crown is rounded and can reach a diameter of 20-25 m (66-82 ft). The neem tree is similar in appearance to its relative Chinaberry (Melia Azadirachta) [33]. Neem products can be suggested for many integrated pest control programs due to their relative specificity [34]. A variety of plant parts, including the an insecticide, larvicidal, antibacterial, antiviral, and spermicidal medications to treat both acute and chronic human ailments[35]. 200 insect species that attack grains, fruits, vegetables, crops, and ornamental plants may be influenced by neem extracts. Products made from neem are medium- to broad-spectrum insecticides for insects that consume plants. Neem has more than 100 chemical substances. Azadirachtin, salanine, and malential are among the potent chemical compounds[36].

The plant Cassia fistula, also known as (amalthas), is ubiquitous in gardens throughout most of Pakistan and may also be found in abundance in deciduous woods throughout the world [37].

Cassia fistulas have some of therapeutic applications, especially in traditional remedy. It is an extraordinary plant with beautiful yellow flowers which are regularly used as decorations. The seeds of this species are used to treat gastritis and diarrhea. They're also used to repel insects. Chia seeds can also be used to treat bile and stimulate appetite skin diseases, syphilis, leprosy, tuberculosis are all treated with root. [38].

Based on these properties the present study was designed to identify the prevalent termite specie in district Kohat and asses the selected medicinal plant anti-termite potential of two selected medicinal plant methanolic extracts like Azadirachta indica (Neem) and Cassia Fistula (Amalthas) to identify the most effective plant and compare the activity with the available chemical insecticide. Therefore, natural plant products can be useful substitutes for synthetic pesticides to prevent various health risks.

Method Aand Materials

3.1 Study Area

This study was conducted in District Kohat Khyber Pakhtunkhwa Pakistan. Kohat district is the southernmost of the three districts of Peshawar Division, located between 32 74 and 33 53 north latitude and 70 34 and 72 17 east longitude. The district has a total area of 2,545 square kilometers (983 square miles).

Collection of Termites

The collection of termites was made from the natural habitat of District Kohat, Khyber Pakhtunkhwa Pakistan. The worker termites were collected from dead wooden logs and mud mounds respectively by collection trap units with some modification. And identified with the help of taxonomic keys to the genera. Live specimens were kept in Petri dishes with soaked cotton to plug in the laboratory at a temperature of 27 ± 1C and relative humidity of 80±5%. They were fed on Whatman filter paper (NO 1) for one week before the experiment to remove debris and other particles. All termites were evaluated in a week of collection and only active and healthy termites were used for the experiment.

Figure 2 Collection of termites

Plant Sample Collection:

Fresh leaves of Azadirachta indica leaves were collected from Kohat, Khyber Pakhtunkhwa, Pakistan. Fresh leaves of Cassia Fistula were collected from Dera Ismail Khan District Punjab, Pakistan. These plants were identified by Mr. Zafar Iqbal Assistant Professor Department of Botany at Kohat University of Science and technology. He followed the flora of Pakistan and related floras to identify both species. The plant material was rinsed with distilled water and air dried on the laboratory table at room temperature of 27-37 ◦C for one Month.

Figure 3.3.1 Azadirachta indica

Figure 3.3.2 Cassia Fistula

Crude Extract Preparation

For the preparation of plant extracts, a considerable amount of freshly washed leaves of Azadirachta indica (Neem) and Cassia Fistula (Amalthas) were kept for shaded drying for several days. Dried leaves of selected plants were crushed in an electric blunder. Then about 250 gm powder of each plant's leaves was mixed with 250 ml methanol in two bottle flasks and allow for 21 days. The mouth of each flask was closed with cotton plug aluminum coils. Each plant leaves extract was filtered with filter paper to obtain 100% filtrate. The dried remains were collected by evaporating the solvent with the aid of a rotary vacuum evaporator and stored in the refrigerator. The extracts were concentrated on a rotary evaporator by removing the excess solvent under a vacuum to prepare the crude extract of selected medicinal plants in the laboratory of the Pharmacy Department KUST. The stock

solution was prepared by dissolving 1g crude extract in 100ml distilled water and different concentrations (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml) were prepared by applying the formula C1V1=C2V2
Were,
C1: Concentration of Stock Solution: C2: required concentration: V2: required volume and V1: Volume to be removed .5 Invitro Assay
The in-vitro assay was used to evaluate the toxic potential of plant extract. Filter paper Whatman No 1, 2.5×2.5 Inches) were pre-weighed and treated with 1.5 ml of each plant extract (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml) air dried and kept in Petri dishes (3×3 Inches). This experiment was conducted in triplicates and positive control was treated with chemically available insecticide permethrin and Negative control was treated with only water. Ten worker termites were placed in each Petri dish. The Petri dishes were kept in a dark environment at 27±1 C and 80±5 RH. The Percentage of mortality was calculated in each plant extract and positive group at every dose treatment after every three hours for 24 hours.

%Morality= ODP÷TP×100

Where,
ODP: Observed dead population; TP: Total Population

Statistical Analysis:
By using one-way ANOVA, the mortality ratio percentage of termites was calculated. The result of mortalities such as mean and standard deviation was analyzed statistically by using statistical software, after recording the maximum mortality rate LD50 was calculated by LD50 Calculator |AAT Bioquest.

RESULTS
In the present study, the pest termite identified was Heterotermes was a major pest to crops and buildings. For the identification of soldiers, termites were collected from different localities of the Kohat district. And identified with the help of the identification key. The external morphology of different body parts (1) Body (2) head (3) thorax (4) abdomen (5) antenna and legs were matched with identification keys. After identification, the prevalence of Heterotermes indicola specie was recorded in six localities of the Kohat district in different associated fruit trees. Plant leaves extract of two plant species Azadirachta indica and Cassia fistula Figure were used as insecticides against Heterotermes indicola.

Prevalence of Termites in various localities of Kohat district:
A total of 300 samples of termite species were collected from the different localities. Samples from each infected plant were collected. Three plants were selected for sample collection. The termite samples were identified into three genera, namely Odontotermes, Heterotermes, and Microtermes.
Microtermes were small compared to the other genera. It is distinguished by its mandibles, which were strongly curved inward at the tip, and its antenna which had 12 to 14 segments. In odontotermes, the left mandible was equipped with one robust and forwardly directed tooth, whereas the right mandible was with a relatively small tooth. Heterotermes have a large head of yellowish-brown color with well-developed mandibles. Those characteristics are utilized to identify the genera and species.
Relative percentage occurrence of these genera indicated that the Heterotermes were the most abundant, among the samples collected from target trees in different localities of the Kohat district.

Table 1 Percentage of termite genera in various localities of Kohat district:

Termites Genera	Percentage (%) of Occurrence
Odontotermes	25%
Heterotermes	58%
Microtermes	17%

Heterotermes were encountered frequently in the roots and stems of trees, and it was observed at the base of the sample collection. They require a source of moisture in their environment. They satisfy this need

by nesting in or near the soil and tend to reach their food sources from underlying soil through tunnels in wood. Heterotermes were more abundant in three plants from different localities. Odontotermes was found less frequently in three selected plants and Microtermes was the least abundant encounter termite.

Table 2 Termites genera on Psidium guajava, Prunus domestica, Prunus armeniaca:

Associated Tree	Termites genera	Percentage (%) of Occurrence
Psidium guajava	Odontotermes	20%
	Heterotermes	57%
	Microtermes	23%
Prunus domestica	Odontotermes	12%
	Heterotermes	73%
Prunus armeniaca	Microtermes	15%
	Odontotermes	43%
	Heterotermes	44%
	Microtermes	13%

Figure 4 Heterotermes indicola

Effect of Azadirachta indica leaves extract on termites:

Leaves extract of Azadirachta indica methanolic solvent showed 9.58±1.86, 10.35±1.22, 17.55±4.58, 21.25±4.55, and 21.67±4.47 percent mortality by feeding on 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control respectfully in trail one of a 24-hour experiment where is in -ve control there is no significant mortality was observed i.e. 00±00. After trail II percent mortality rate on same concentration (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control) was 10.35±1.22, 16.52±2.07, 25.68±3.82, 26.25±4.76 and 23.39±4.67 while in -ve control observed mortality was 00±00. After trail III of the experiment, mortality rate was noted for all the concentration, which was 7.71±, 1.36, 12.68±1.86, 29.5o±4.28, 33.02±4.51, and 30.63±4.96, while is in -ve control there is no significant mortality observed i.e., 00±00. The p values for the concentration 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control each is 0.0072, 0.0181, 0.0006, 0.0158, 0.0116.

Table 3 Morality rate of termites in different concentrations of Azadirachta indica methanolic extract:

Concentrations	Activity			95% Confident Interval		
	Trial I (%±SD)	Trial II (%±SD)	Trial III (%±SD)	P values	Lower	Upper
2mg/ml	9.58±1.86	10.35±1.22	7.71±1.36	0.0072	5.8407	12.586
4mg/ml	10.35±1.22	16.52±2.07	12.68±1.86	0.0181	5.4436	20.923
6mg/ml	19.20±4.58	21.90±3.82	23.60±4.28	0.0006	30.407	37.513
8mg/ml	21.25±4.55	26.25±4.76	29.50±4.51	0.0158	12.166	41.514
-ve Control	00±00	00±00	00±00	-	-	-

+ve Control	21.67±4.47	23.39±4.67	30.63±4.96	0.0116	13.418	37.042

Effect of Cassia Fistula leaves extract on termites:
Leaves extract of Cassia Fistula methanolic solvent showed 16.07±2.39, 12.1±2.16, 11.88±4.63, 12.o1±3.24and 21.67±4.47 percent mortality by feeding on 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control respectfully in trail one of 24-hour experiment where is in -ve control there is no significant mortality was observed i.e. 00±00.Atfer trail II percent mortality rate on same concentration (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control) was 8.07±0.87, 22.63±2.44, 24.57±4.73, 26.25±4.76 and 23.39±4.67 while in -ve control observed mortality was 00±00. After trial III of the experiment, mortality rates were noted for all concentrations, which was 7.77±1.26, 13.7±0.93, 19.52±4.51, 24.31±4.47, and 28.75±5.24 while is in -ve control there is no significant mortality observed i.e., 00±00. The p values for the concentration 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control each is 0.0595, 0.0388, 0.0094, 0.0428 and 0.0074.

Table 4 Rate of mortality of termites in different concentration of Cassia Fistula methanolic extract:

Concentrations	Activity			95% Confident Interval		
	Trial I (%±SD)	Trial II (%±SD)	Trial III (%±SD)	P values	Lower	Upper
2mg/ml	11.07±2.39	8.07±0.87	7.77±1.26	0.0595	-1.0581	22.331
4mg/ml	12.1±2.16	14.63±2.44	13.7±0.93	0.0388	2.0476	30.239
6mg/ml	11.88±4.63	24.57±4.73	19.52±4.51	0.0094	17.08	41.74
8mg/ml	12.01±3.24	26.25±4.76	24.31±4.47	0.0428	1.6727	40.041
-ve Control	0±0	0±0	0±0	-	-	-
+ve Control	21.67±4.47	23.39±4.67	30.63±4.96	0. 0074	15.43	33.776

4.4 Comparative rate of mortality of termites at Azadirachta indica, Cassia fistula, and Permethrin.
As a comparison between the methanolic leaves extract of two medicinal plant and the positive control using permethrin as insecticidal activity in triplet and compared. All the plant and positive control showed the differential anti-termite result in doses and time dependent manner. Leaves extract of Azadirachta indica methanolic solvent showed, 9.21±1.51, 13.18±1.84, 21.90±4.62, 25.18±4.16 and 25.23±4.79 percent mortality by feeding on 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control respectfully in triplet trails of 24-hour experiment where is in -ve control there is no significant mortality was observed i.e. 00±00. Leaves extract of Cassia Fistula methanolic solvent showed percent mortality rate on same concentration (2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control) was 8.97±1.48, 10.43±1.72, 18.65±4.23, 20.85±4.61 and 25.23±4.79. The p values for the concentration 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and +ve control each is 0.0084. 0.0738, 0.0509, 0.0597 and 0.0587.

Table 5 Comparison between Azadirachta indica, Cassia Fistula and Positive Control group mortality percentage (%)

Concentrations	Activity			95% Confident Interval		
	A. Indica (%±SD)	C.Fistula (%±SD)	Difference (%±SD)	P values	Lower	Upper
2mg/ml	9.21±1.51	8.97±1.48	0.24±0.03	0.0084	-0.785	8.885
4mg/ml	13.18±1.84	10.43±1.72	3.37±0.12	0.0738	-1.183	13.17
6mg/ml	21.90±4.62	18.65±4.23	3.25±0.39	0.0509	-0.3472	19.587
8mg/ml	25.18±4.16	20.85±4.61	4.75±0.45	0.0597	-0.7279	21.695
-ve Control	0±0	0±0	0±0	-	-	-
+ve Control	25.23±4.79	25.23±4.79	0.0±0.09	0.0587	-2.2888	22.302

Discussion

Researchers around the world have used plants to control various insects. Earlier studies have shown that various plants have been used to control insects and are both effective and environmentally friendly. We studied compounds in plants that show various properties, including antimicrobial properties against viruses and pathogens. The current study showed that two medicinal plants, Neem (Azadirachta indica) and Cassia Fistula, have toxic and potent growth-retardant effects that cause mortality in target species. Termites are important in recycling and decomposition of decaying materials, but they also pose a serious threat to crops and buildings. Plant roots, orchards, and wooden buildings under construction (Ahmed & Farhan., 2006). Termites wreak havoc on agriculture, wildlife sanctuaries and historic sites, and negatively affect the economy (Rashid et al., 2012). There is considerable termite loss in annual and perennial crops and structures (Verma, Sharma & Parsad., 2009, Guan et al., 2011). Termites are known to feed on mammalian feces and play a significant role as reuses, and their foraging activity increases soil nutrients and aeration. Termite tunneling and nesting activities increase soil chemical and physical properties as well as microbial activity, depending on soil type, organic matter, and water content (Freymann et al., 2008). Cassia Fistula is locally known as Amalthas. The green parts of plants were evaluated for its toxic effect on the termite development. The methanolic extract of Cassia Fistula leaves has very toxicology, adult emergence inhibit, and anti-termite activity. Azadirachta indica is locally known as Neem. The leaves extract of neem has shown insecticidal effect on termites. The insecticidal activity of Azadirachta indica attributes to toxic substances such as alkaloids. Extracts of Azadirachta indica possess inhibitory properties of metabolism of Heterotermes indicola. But the mortality rate due to the plant extract correlates with the dose and exposure time to the extract. The toxicity of these crude plant extracts against the termites could depend on the several factors such as chemical composition of the crude leaves extract of plants and the vector susceptibility. The mortality also depends on the time of exposure and treatment with concentrations. The experiment was conducted to figure out the taxological effect of plant leaves extract against termites. In all cases, considerable differences in the insect mortality differs from plant to plant. As observed during this study, the result reached its maximum value in the case of Azadirachta indica. It shows that plants crude extracts have broad spectrum because of the several phytochemicals which cause mortality. The effect of plant extracts on termites indicated that these materials are good alternatives for synthetic chemical insecticides. Studies have determined that some botanical compound such as alkaloids, nicotine, calotropin and harmaline in the extracts produced high mortality against some termites.

Time interval plays a significant role in the effect of phytochemicals in the plant crude extracts. In the first three hours, their effect is minimum that may be due to the fewer intakes of phytochemicals by the termites but as the time proceeds, the phytochemicals effect getting strong on them, which slowly and gradually cause mortalities.

Conclusion

From the current study. It's concluded the effect of Azadirachta indica methanolic extract was shown high anti-termites potential and the Cassia fistula have some anti-termites insecticidal effect. The plant leaves extract is effective against termites. The phytotoxicity was high at 8mg/ml and was lowest at the 2mg/ml for both plant Azadirachta indica and Cassia Fistula in Compare with the positive control Permethrin shown mortality as compared to negative control.

It is concluded that the Azadirachta indica show high potential anti-termite activity while Cassia fistula might contain some insecticidal some anti-termite insecticidal activity which need to isolated and explored further to replace the hazardous chemical substance against termites.

The current study reveal that the prevalence of subterranean termites is very high in Kohat district. These finding can help us to constitute an effective alternative to harmful chemical insecticide that persist in the environment and cause environmental toxicity for other non-targeted organisms.

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