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Extraction of bioactive compounds from *Streptomyces avermitilis* and *Azadirachta indica* and Evaluation against *Spodoptera litura*: A green approach

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Abstract

The tobacco caterpillar, *Spodoptera litura*, is a crucial polyphagous pest on plants, which is present throughout the South and Eastern world. Extract of *S. avermitilis* in combination with *A. indica* performed well than the extract alone from *Streptomyces avermitilis*. And combined extracts of bacteria and neem have maximum mortality (100 per cent at 250 ppm), LC₅₀ value (124.85 ppm), LC₉₀ value (305.4 ppm) and antifeedant rate (64.6 ± 15.69 per cent) than the extract from *S. avermitilis* alone (Mortality = 92 percent at 250 ppm; LC₅₀ = 93.61 ppm; LC₉₀ = 243.49 ppm; Antifeedant rate = 59.6 ± 15.13 percent). When *S. avermitilis* extracts at the concentration of 50ppm are treated to the *Spodoptera litura*, morphological changes are observed. The GCMS analysis observed five volatile compounds from Bacterial extract and seven bioactive compounds detected from neem extract. Hence, integrated pest control is the possible solution for many insects in the future. Our result gives the farmers a rational solution to the pest control problem due to their economic benefits and lowers social costs.

Keywords: *Spodoptera litura*, *Streptomyces avermitilis*, *Azadirachta indica*, integrated pest management, biopesticides, antifeedant activity

Introduction

Castor, *Ricinus communis* (Linnaeus), is a non-edible oilseed crop with a high industrial value. Castor is farmed on a total area of 1.5 million hectares, with an average yield of 1.5 million tons and productivity of 995 kg per hectare in 2007. Castor production losses due to insect infestations vary greatly depending on the season. From seeding through harvesting, castor is attacked by a variety of insects, including chewing pests such as the tobacco caterpillar, castor butterfly, and slug caterpillar. Tobacco caterpillars are regarded as a serious pests^[9]. The tobacco caterpillar, *Spodoptera litura*, is an important polyphagous pest on crops that are found throughout the South and Eastern world, infesting 112 plant species from 44 families, of which forty have been reported from India^[4]. It has a diverse host range of about 120 host plants, which includes crops, vegetables, weeds, and ornamental plants^[37]. Though it is a primary tobacco pest, it also affects cole crops, castor, cotton, chilies, sunflower, peanuts, legumes, amaranthus, and tomatoes. It was claimed that 15 egg masses and 400 to 500 larvae were found during outbreak conditions on tobacco, compared to four egg masses and 200 larvae per plant under normal conditions^[49].

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a polyphytophagous insect that inflicts destruction on many vegetables and field crops in China and other Asian countries^[43]. Although it was a sporadic tobacco pest in northern China for many years, it has progressively become a major issue in recent years^[13]. The leafworm, *Spodoptera litura*, produces agricultural economic losses ranging from 25.8 to 100 percent depending on crop stage and infection level in the field^[10].

Streptomyces avermitilis belongs to the Streptomycetes, a Gram-positive bacterial genus with a circular genome that yields airborne spores which generate macrocyclic lactones, such as oligomycin and secondary metabolites, including avermectin, which might be utilized as an anthelmintic and insecticide. The compounds derived from avermectin, which are used as anthelmintic agents are ivermectin, selamectin, doramectin, and abamectin. When given at a dosage rate of 300g/kg or less, they demonstrate excellent efficacy against nematodes and

arthropod parasites of domestic animals. However, Avermectins have the potential antibacterial and antifungal action as other macrolides and polyene antibiotics. They promote releasing and binding gamma-aminobutyric acid (GABA) at nerve terminals to inhibit the transmission of electrical activity in neurons and muscle cells. As a result, chloride ions enter into the cells, causing hyperpolarization and paralysis of the neuromuscular systems^[3].

Several species of *Streptomyces* produce Avermectin through the fermentation process. Nourishments are essential while synthesizing secondary metabolites^[52]. Carbon (C) and nitrogen (N) are the necessary components for the synthesis of avermectin from *S. avermitilis*. In several situations, glucose is thought to be a model for secondary metabolite synthesis. The C-source is quickly digested when adequate carbon sources are supplied to the microorganism^[24].

Azadirachta indica, often known as Neem or Margosa, is a tree in the Meliaceae family and the Rutales order that has been utilized since the beginning of civilization and is one of the notable and adaptable plants on the earth. This tree originated in India and Southeast Asia, but it is now found worldwide, particularly in tropical and subtropical nations such as Brazil. Neem is a fast-growing perennial tree that grows to be small to medium-sized (10 to 15 m). Another noteworthy trait of this tree is its capacity to thrive in areas with high temperatures (up to 50 °C), little annual rainfall (400–800 mm per year), and poor and disturbed soils^[36]. Low temperatures, particularly below 14 °C, and frosts, on the other hand, facilitate the development of the Neem tree^[40]. Neem tree is a fundamental source of ninety-nine biologically active compounds, including azadirachtin, nimbin, nimbidin, and nimbolides and a maximum of those products have antifeedant, ovicidal, larvicidal, oviposition deterrent, growth-regulating, and repellent activities towards the insects^[42, 20, 11]. Neem designs have a substantial reproductive hang-up on rice hoppers^[31]. The neem oil having compounds are stated as strong antifeedants and growth inhibitors towards lepidopteran larvae^[30]. Deota and Upadhyay^[8] stated that azadirachtin, the lively element of *A. indica* against *S. litura* which, confirmed toxicity and antifeedancy.

Novel pesticides are those newly observed pesticides that affect unique insects, have an extraordinary mode of actions affecting unique biochemical sites, and provide greater ideal pest management. These novel pesticides have a very much less poisonous impact on useful insects, mammals, and the environment because of their green chemical origin and minimum doses for their application^[38].

So far there is no record of the additive or synergistic impact of *Streptomyces avermitilis* in combination with *Azadirachta indica* extract on feeding, survival, and development of *S. litura*. Hence, the existing work which was undertaken to assess the results of extracts combination, a newly advanced extract system consisting of *Streptomyces avermitilis* and *Azadirachta indica* extract (1:1 ratio) on feeding, biological activities against *S. litura*.

Materials and Methods

Collection of plant materials and Soil sample

The seeds of *Azadirachta indica* were collected from the surrounding areas of Sivakasi, Virudhunagar district, Tamilnadu, South India in July 2021. The seeds were authenticated by the Research Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, India. The alkaline soil was collected from a private lime factory located in

Srivilliputhur, Virudhunagar district, Tamilnadu, South India in July 2021 for bacterial isolation.

Extract from Bacterial culture

YMG Medium is the basic media for growing the mother culture of *Streptomyces avermitilis* from soil samples. This Medium contains 4.0 g yeast extract, 10.0 g malt extract, 4.0 g glucose, and 2.0 g Calcium carbonate (CaCO₃) (g/L), and the pH of the medium was adjusted to 6.5 before autoclave^[34]. The pH was corrected to 7.0 after autoclaving at 121 °C for 15 minutes. The media was then poured into plates and solidified to culture the sample. 1g of soil sample was suspended in 99ml of sterile distilled water. The sample was serially diluted in 10⁻¹ to 10⁻⁹ and diluted samples were spread on the plates by L- Rod. Then that inoculated plates are incubated at 37 °C for 24 hours. The spore-like culture was taken from the plate and inoculated into the seed medium. The medium consists of corn starch 30, soya flour 8, peanut meal 10, yeast extract 4, calcium chloride 0.03, and αamylase 0.04 (g/L). The pH was adjusted to 7.0 before autoclave. The medium was autoclaved at 121 °C for 15 minutes. The pH was adjusted to 6.8 after autoclave^[47]. After autoclave, a loopful of *Streptomyces avermitilis* was inoculated in the 40ml of seed medium. They were incubated at 28 °C in a shaker at 220 rpm for 24 hours. 5% of the culture was taken from the seed medium and inoculate into the fermentation broth. The fermentation broth consisted of corn starch 140, α amylase 0.1, soya flour 28, yeast extract 10, sodium molybdate dihydrate (Na₂MoO₄.2H₂O) 0.022, manganese sulfate monohydrate (MnSO₄.H₂O) 0.002, ammonium sulfate 0.25, cobalt chloride 0.02 (g/L). The pH of the medium was adjusted to 7.5 before autoclave. After autoclave, the pH was adjusted to 7.2^[47]. The 5% of the culture was inoculated into the broth and incubated at 28 °C in Shaker at 220 rpm for 10 days. After incubation, the culture was collected in tubes and centrifuged at 4 °C for 20 minutes at 8000 rpm. Because avermectin is an intracellular substance, cells had greater biomass and were collected as a pellet, whereas the supernatant was discarded. To thoroughly dissolve the cell biomass in the form of the pallet, it was diluted with 1ml of methanol. The mixture was centrifuged again for 20 minutes at 8000 rpm, and the supernatant was collected. The final test solution was prepared from this supernatant at a concentration of 0.0025 percent.

Extract from Neem Seed

The dried (15 – 20 days in the shade at the room temperature, 27 to 37 °C day time) seed (150g) were powdered in the size of 355µm using Mortar and pestle and extracted with a 60:40 combination of Ethanol (450mL): Hexane(300mL) in a soxhlet apparatus (boiling point range 70 °C) for 6 hours^[46]. The extract was filtered through a Millipore filter (0.45µm) and centrifuged at 8000 rpm for 20 minutes. The supernatant was collected and stored at 4 °C. The final test solution was prepared from this extract at a concentration of 0.0025 percent.

Preparation of *Streptomyces avermitilis* extract in combination with *Azadirachta indica* extract

The final mixed solution was prepared at a concentration of 0.005 percent by combining *Streptomyces avermitilis* and *Azadirachta indica* (1:1). By the same way five different concentrations (0.005, 0.01, 0.015, 0.02, 0.025) of mixture solution was prepared. And also the alone extract of

Streptomyces avermitilis was prepared at the concentration of 0.005, 0.01, 0.015, 0.02, and 0.025 for separate treatment.

Gas Chromatography (GC) and Mass Spectroscopy (GC-MS)

The extracts from *Streptomyces avermitilis* and *Azadirachta indica* were analyzed via GC, using a DB-S capillary column. The oven was preheated at 50 °C. The GC-MS conditions were identical to those of the GC. The electron ionization was tuned to 70 eV, the particle source temperature to 275 °C, and the output rate was set to 29–400 atomic mass units sec⁻¹.

Larvae collection

Tobacco Cutworm (*Spodoptera litura*) second instar larvae were collected from the plant of *Ricinus communis* at private agricultural land located on Sengamala Nachiar Puram, Virudhunagar District, Tamilnadu and confirmed by the Research Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi, Virudhunagar district, Tamilnadu. Larvae were maintained in plastic and enamel trays. They were maintained at 28 ± 2 °C and 75% - 85% relative humidity.

Larvicidal Bioassay

Bioassay was performed with fifth instars of *S. litura* were using at 50, 100, 150, 200, and 250 ppm concentrations of *Streptomyces avermitilis* extract alone and the *Streptomyces avermitilis* extract on with the combination of *Azadirachta indica* extract. Sterile castor leaves (75 – 125 cm²) were sprayed with five different extract concentrations and air-dried for 10 minutes to eliminate excess moisture. The control leaves were treated with sterile distilled water that contained 0.025 percent ethyl alcohol at a ratio of (1:1). The treated leaves were put in a (9 x 5 x 4 cm³) bioassay chamber. The

bioassay chamber was incubated at 28 ± 2 °C, with 95 percent humidity, and a photoperiod of 15:9 (L: D). All treatments used a minimum of 25 larvae per concentration, and these treatments were performed ten times (n = 250). Every 24 hours, the dried leaves were removed and replaced with new untreated castor leaves. From the fourth post-treatment day to the tenth, mortality was detected. The formula (1) was used to determine the percentage of mortality and Probit analysis ^[12] was used to calculate treatment doses for biological studies.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

Antifeedant bioassay

Leaf disc no choice bioassay method was used to investigate antifeedant effects of plant extracts. Fresh castor leaf discs (1350sq.mm) were dipped in 50, 100, 150, 200, and 250 ppm concentrations of bacterial extract alone and bacterial extract in combination with plant extract. The leaf discs that had been soaked in the water acted as a negative control and Abamectin (1.9% EC obtained from Tropical agro system private limited, Chennai, Tamil Nadu) was used as a positive control. Leaf discs were placed in individual Petri plates (9 cm dia). The wet filter paper was placed in each petri dish to prevent the leaf discs from drying out too quickly. A single pre-starved third instar larva of *Spodoptera litura* was placed in each Petri plate. For 24 hours, the larva was allowed to feed on the treated discs. For each treatment, five replicates were maintained. At the end of the experiment, the aid of a leaf area meter was used to measure the unconsumed area of the leaf disc and the percent antifeedant activity was assessed using Singh and Pant's ^[44] formula.

$$\text{Percent antifeedant activity} = \frac{\text{Leaf disc consumed by the larvae in control} - \text{Leaf disc consumed by the larvae in treated}}{\text{Leaf disc consumed by the larvae in control} + \text{Leaf disc consumed by the larvae in treated}} \times 100$$

Statistical analysis

The mean and Standard deviations were calculated using SPSS (16.0 for windows) software from the replication data. The probit analysis calculated the LC₅₀ and LC₉₀ data ^[12].

Morphological effects

For adult emergence, treated and control pupae were maintained separately in a glass trough. The deformity was noticed between the treatment and control. Adults from the treated and control had their morphological abnormalities were compared.

Table 1: Larvicidal activity of Bacterial Extracts alone and Bacterial Extracts in combination with Neem extracts against fifth instar larvae of *S. litura* at different concentrations.

Scientific name/ Family	Percent Mortality (ppm)					LC ₅₀ (ppm)	95% (LCL-UCL)	LC ₉₀ (ppm)	95% (LCL-UCL)	Slope±SE	x ² (df=3)	Reg. equation
	50	100	150	200	250							
<i>Streptomyces avermitilis</i>	16	28	52	76	92	93.61	2.016-4.157	243.49	18.4-4.92	3.087±0.546	5.698	y=0.416x-6.09
<i>Streptomyces avermitilis</i> + <i>Azadirachta indica</i>	28	44	64	84	100	124.85	2.191-4.411	305.2	21.29-6.71	3.301±0.567	4.136	y=0.3607x-6.92

Control = no mortality; LC₅₀ lethal concentration that kills 50% of the exposed larvae; LC₉₀ lethal concentration that kills 90% of the exposed larvae; UCL upper confidence limit. LCL lower confidence limit; x²= chi-square; d.f. = ° of freedom.

Antifeedant activity

The antifeedant activity of Bacterial extract and plant extracts was determined using the antifeedant index. Normally, a higher antifeedant index indicates a slower rate of feeding.

The antifeedant activity varied greatly depending on the concentration of extracts utilized in this study. Based on the leaf area eaten by *Spodoptera litura*, the antifeedant effects of Bacterial extract and plant extract were assessed. The

antifeedant effect of several concentrations of extracts was analyzed and listed in Table 3. The number of positive signs showed the effective antifeedant for the samples. Among five concentrations tested the 200 and 250 ppm concentrations from the extracts of *Streptomyces avermitilis* in combination with *Azadirachta indica* and 250 ppm concentration from the extract of *Streptomyces avermitilis* alone was performed well

against the third instar larvae of *Spodoptera litura*. The maximum antifeedant activity was recorded in the extract of *Streptomyces avermitilis* in combination with *Azadirachta indica* (64.6), the extract from *Streptomyces avermitilis* alone has 59.6 and the positive control insecticides abamectin have 72.6 of antifeedant activity against *S. litura*.

Table 2: Screening of *Streptomyces avermitilis* extract alone and *Streptomyces avermitilis* extract combined with *Azadirachta indica* extracts at five different concentrations for antifeedant activity against *Spodoptera litura*.

Scientific name/ Family	Concentration (ppm)				
	50	100	150	200	250
<i>Streptomyces avermitilis</i>	++	++	+++	+++	++++
<i>Streptomyces avermitilis</i> + <i>Azadirachta indica</i>	++	+++	+++	++++	++++

- No antifeedant activity; + Antifeedant activity below 25%; ++ Antifeedant activity between 25-50%; +++ Antifeedant activity between 50-75%; ++++ Antifeedant activity above 75%.

Table 3: Antifeedant activity of *Streptomyces avermitilis* extract alone and *Streptomyces avermitilis* extract combined with *Azadirachta indica* extracts against *Spodoptera litura*.

Scientific name/ Family	Percent Antifeedant (ppm)					Antifeedant activity (%)
	50	100	150	200	250	
D ₂ H ₂ O (Controls)	0	0	0	0	0	0.00 ± 0.00
TiCl ₄	1	1	2	2	3	1.8 ± 0.72
Abamectin (Positive control)	45	59	76	88	95	72.6 ± 18.05
<i>Streptomyces avermitilis</i>	41	45	59	71	82	59.6 ± 15.13
<i>Streptomyces avermitilis</i> + <i>Azadirachta indica</i>	45	52	61	76	89	64.6 ± 15.69

Morphological effects

Visible metamorphic abnormalities were observed on the larvae when treated with 50 ppm concentrations of the

Streptomyces avermitilis extracts alone. Deformities were noted in the pupa and wings of the adults (Fig. 1).



Fig 1: Morphological effects on Pupa and Adult (a), Healthy pupa of *Spodoptera litura* (b), Treated pupae of *Spodoptera litura* (c), Healthy adult of *Spodoptera litura* (d), and Treated adult of *Spodoptera litura*.

GC-MS characterization of chemicals

Bacterial extract

Five bioactive compounds were identified in the Bacterial extract. The molecular formula, molecular weight (g/mol),

retention time (RT), and chemical structures of the active compounds are shown in Table 4 along with their peak area. The peak size was proportional to concentration and was higher for Trimethyl [4-methyl-4-oxo-2-pentyl] phenoxy]

Silane (42.71%). The following compounds were identified include, 1, 4- Bis(trimethylsilyl)benzene (32.45%), Benzo[h]quinolone, 2,4-dimethyl (19.17%), (E)-2-

Bromobutyloxychalcone (2.61%), N-Methyl-1-adamantaneacetamide (3.06%).

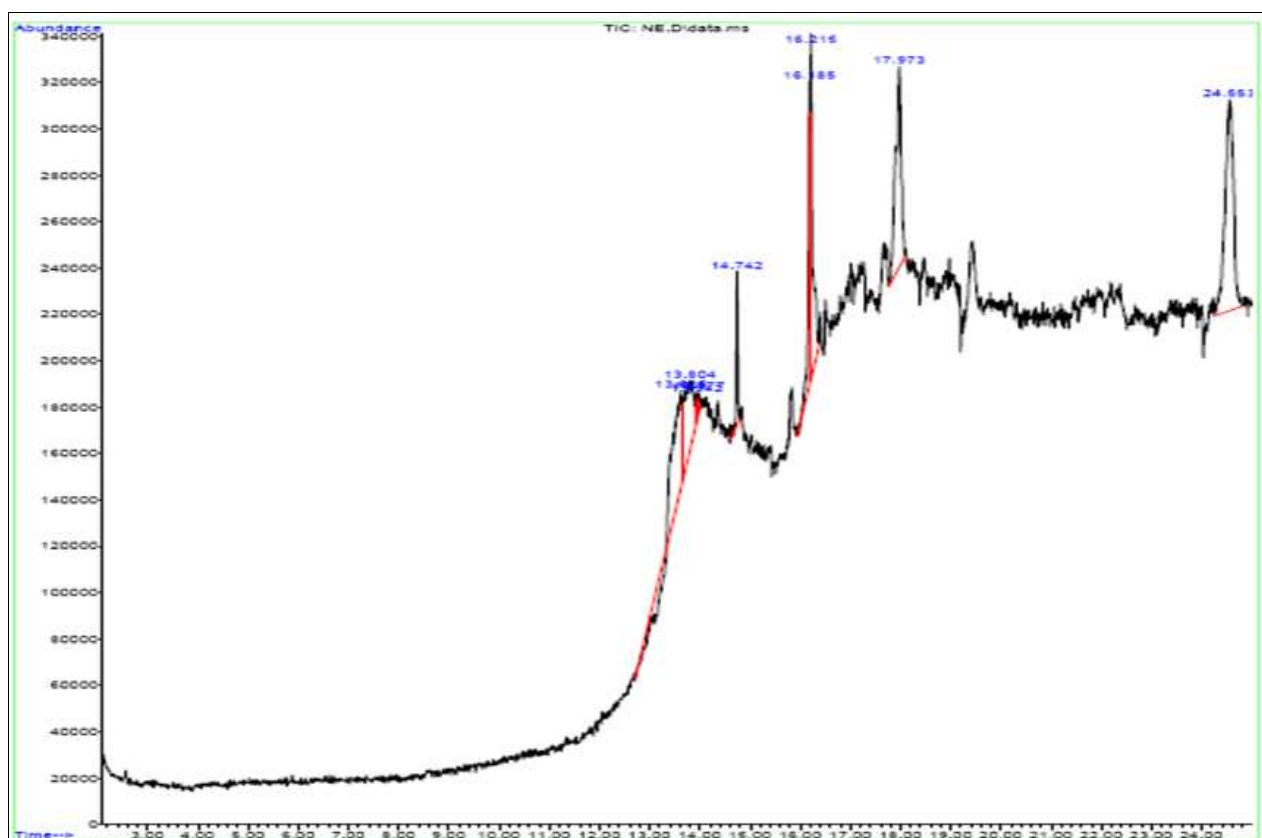

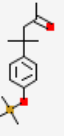

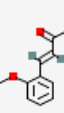
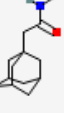


Fig 2: Identification of Bacterial compounds through GC-MS analysis

Table 4: Chemical compositions of Bacterial extract

S. No	RT	Compound name	Mol. For. / Mol. Wit. /Structure	Peak area total%
1	16.799	1,4 Bis(trimethylsilyl)benzene	 C ₁₂ H ₂₂ Si ₂ (222.47)	32.45
2	18.100	Trimethyl[4-methyl-4-oxo-2-pentyl]phenoxy]Silane	 C ₁₅ H ₂₄ O ₂ Si (264.43)	42.71
3	20.136	Benzo[h]quinolone, 2,4-dimethyl	 C ₁₅ H ₁₃ N (207.27)	19.17
4	20.839	(E)-2-Bromobutyloxychalcone	 C ₁₉ H ₁₉ BrO ₂ (359.3)	2.61
5	20.929	N-Methyl-1-adamantaneacetamide	 C ₁₃ H ₂₁ NO (207.31)	3.06

Neem extract

The identified compounds were showed in Table 5. The peak size was proportional to concentration and was higher for 1H-Indole, 5-methyl-2-phenyl (29.96%). Compounds also identified included: Anthracene, 9,10-dihydro -9,9,10-

trimethyl (18.93%), 2,3-Dihydroxypropyl elaidate (18.89%), 2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl), (4a.alpha, 7beta, 8a.beta) (8.57%), Cis-11-Hexadecenal (4.09%), 9,17 octadecadienal,(z) (0.49), 9,12 octadecadionic acid(z,z) (0.78%).

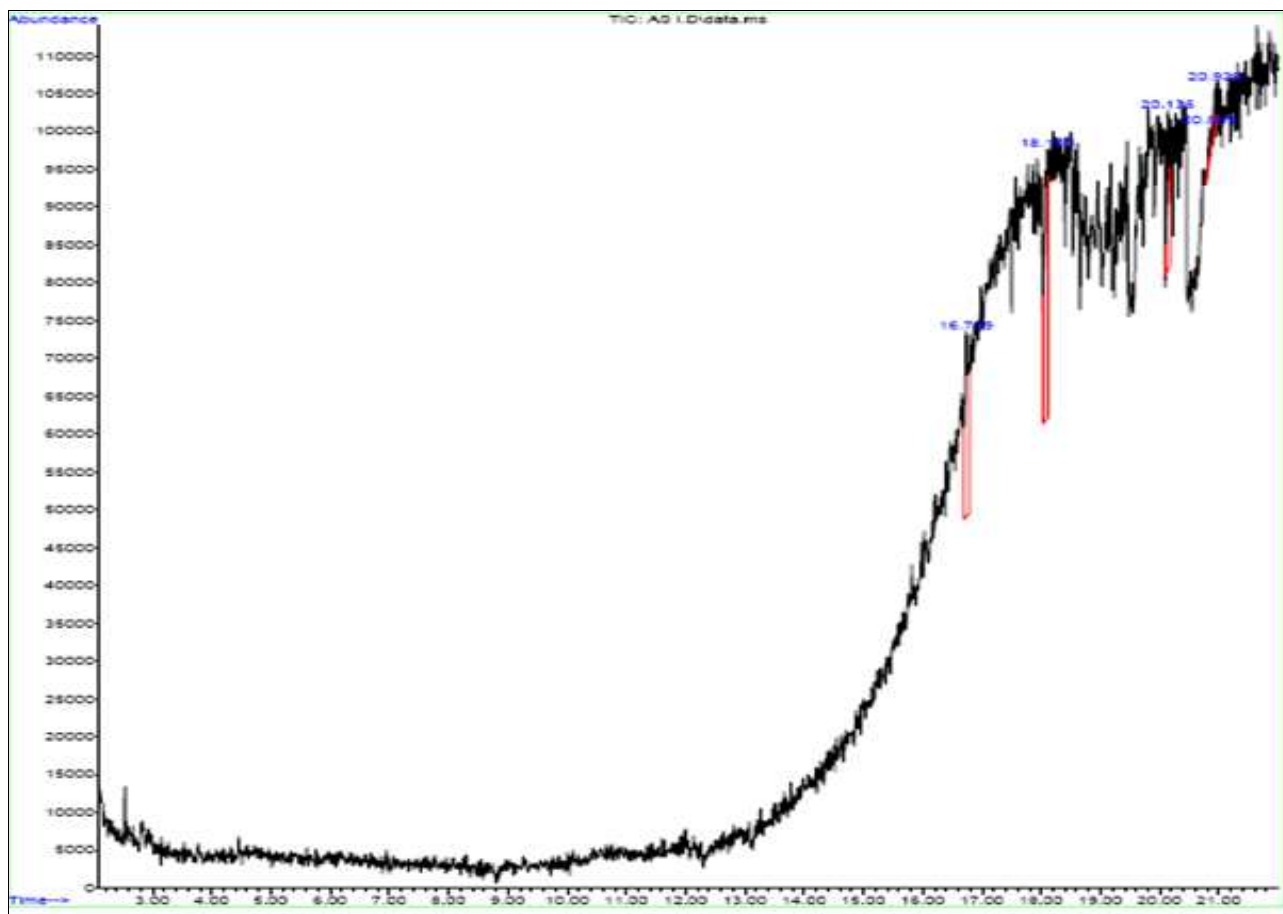
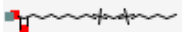
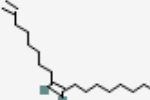
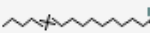
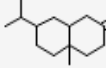
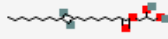
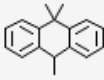
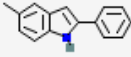


Fig 3: Identification of Neem compounds through GC - MS analysis

Table 5: Chemical compositions of Neem extract.

S. No	RT	Compound name	Mol. For. / Mol. Wit. /Structure	Peak area total%
1	13.625	9,12 octadecadionic acid(z,z)	 C ₁₈ H ₃₂ O ₂ (280.4)	0.78
2	13.977	9,17 octadecadienal,(z)	 C ₁₈ H ₃₂ O (264.4)	0.49
3	14.742	Cis-11-Hexadecenal	 C ₁₆ H ₃₀ O (238.41)	4.09
4	16.185	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl), (4a.alpha, 7beta, 8a.beta)	 C ₁₄ H ₂₄ O (208.34)	8.57

5	16.215	2,3-Dihydroxypropyl elaidate	 C ₂₁ H ₄₀ O ₄ (356.5)	18.89
6	17.973	Anthracene, 9,10-dihydro 9,9,10 trimethyl	 C ₁₇ H ₁₈ (222.32)	18.93
7	24.553	1H-Indole, 5-methyl-2-phenyl	 C ₁₅ H ₁₃ N (207. 27)	29.96

Discussions

Chemical insecticides cause elevated toxicity to treasured organisms in an ecosystem and also affect parasitoids, predators, soil organisms, which include earthworms, and soil-borne microbes. The quality of the soils is strongly related to the presence of the soil creatures [27]. Usage of chemical insecticides to control the pests or weeds leads to the soils' infertility [5]. At the same time, resistance to numerous pesticides is a global problem [23, 50] especially in the case for *H. armigera* and *S. litura*, which maximum pesticides have didn't effectively control [53].

S. avermitilis produces eight associated components of avermectins, the most effective components, Bla and B1b, were utilized in the medical, veterinary, and agricultural fields [18]. Avermectins represent a unique category of macrocyclic lactones that have incontestable nematicidal, acaricidal, and insecticidal activity [1]. Avermectins (abamectin and ivermectin) are poisonous to nearly all insects, although tolerance varies and dying cases may be uncommonly slow (it takes 24 h to 30 days) [45].

The *S. avermitilis* extract alone mortality of 92, 76, 52, 28, and 16 percent was recorded at concentrations of 250, 200, 150, 100, and 50 ppm respectively. These results support the current study compared to previous reports. Whereas, Abamectin has less mortality alone (77.33, 67.22, 43.00, 35.20, and 30.66 percent were recorded at concentrations of 250, 200, 150, 100, and 50 ppm respectively) against third instar larvae of *S. litura* [41]. And the LC₅₀ and LC₉₀ are 93.63 and 243.49 obtained from the extract of *Streptomyces avermitilis*. This is much higher than the LC₅₀ and LC₉₀ value of the abamectin compound alone (LC₅₀ = 33 and LC₉₀ = 123) done by Ahmad and Gull [1].

The antifeedant activity of plant extracts towards insects has been studied in lots of countries. Quantification of the antifeedant impact of botanicals is of terrific significance inside the area of insect pest management. From the ecological factor of view, antifeedants are very crucial since there by no means kill the goal insects at once and permit them to be had to their natural enemies and assist with inside the protection of natural balance. A higher antifeedant index generally suggests a reduced rate of feeding. Antifeedant is a chemical that inhibits the feeding without killing the insect pests directly, at the same time as it stays close to the dealt with foliage and dies through starvation [51, 32]. Antifeedants provide the first line of crop safety in opposition to notorious insects. Any substance that reduces food intake via way of means of an insect may be taken into consideration as an

antifeedant or feeding deterrent [19]. In general, antifeedants have profound negative results on insect feeding behavior [17]. Wankhede *et al.* [48], found the maximum antifeedant activity observed by karanji oil (61.51), followed by neem oil (49.51%) and sesame oil (18.38%). The antifeedant activity of *S. avermitilis* extract alone against *S. litura* is 59.6, *S. avermitilis* extract in combination with neem extract against *S. litura* is 64.6 and the positive control has higher activity (72.6) than extracts.

The plant kingdom is a wealthy supply of biologically lively natural chemicals. More than 10,000 secondary metabolites had been chemically recognized from the plant kingdom [26]. Neem products which include neem seed kernel extract, neem leaf extract, neem oil, and neem cake, are broadly used as insect repellents and insecticides towards a wide variety of pests [22, 29]. Azadirachtin, a ring C- Seco tetranortriterpenoid, is the essential active source and is the maximum remarkable natural insect antifeedant found till to-date [21]. Besides azadirachtin, neem oil additionally includes extraordinary fatty acids, particularly oleic acid, linoleic acid, linolenic acid, palmitic acid, and stearic acid [33].

In combination with pungam oils (Ponneem), the neem oil is performed well than individual neem and pungam oil treatments against *S. litura* [35]. But there's no record on the additive or synergistic impact of *Streptomyces avermitilis* combined with *Azadirachta indica* extract on *S. litura*. In the present study, the effect of mortality, LC₅₀, LC₉₀, and antifeedant activity was found in the combination of *S. avermitilis* and *A. indica* extracts treatment which was significantly high compared to the *S. avermitilis* extract alone. Insect growth regulation properties of plant extracts are very specific in nature, due to the fact insect growth regulator works on the juvenile hormone. The enzyme ecdysone performs a primary function in the shedding of old pores and skin and the phenomenon is referred to as ecdysis or molting. When the plant compounds input are into the body of the larvae, the function of ecdysone is suppressed and the larva fails to molt, last at the larval level and ultimately dying [28]. Arivoli and Tennyson [2] reported that several deformities in body length, Head size, darkened coloration on wings of the *S. litura* adults which emerged from the pupae treated with five different plant extracts. In the present study, the adult from 50ppm concentrations of the *Streptomyces avermitilis* extracts alone treated pupae that had no fully developed wings.

Řezanka *et al.* [39], isolated many odorous compounds from *S. avermitilis* culture are 2, 3-dimethyl tetrahydrofuran

and methyl 2-furan carboxylate and, also the Hexa-membered, heterocyclic compound 5, 6-dimethyl-tetrahydro-2-pyrone. 5-(2-methylenecyclohexyl)-2-pentanone and 3, 4, 4a, 5, 6,7,8,9 octahedron-4a-methyl-2H-benzocycloheptene-2-one were also found in the mixture. By the GCMS analysis Kamaraj *et al.* [25] identified ten chemical compounds in the NGE included: 3, 7,11, 15- tetramethyl-2 hexadecane-1-ol, 3, 7-dimethyl-1, 7-octadien-3-amine, Dimethyl 4-aminobenzene-1, 2-dioate, Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester, n-hexadecanoic acid, Linoleic acid, 1,4 Dioxaspiro [4.5]decane and Ricinoleic acid methyl ester. Hossain *et al.* [15] found different chemical compounds from Omani neem in hexane crude extract are gamma-elemene, (2E)-3,7,11,15-tetramethyl-2-hexadecan-1-ol, methyl petroselinate, phytol, methyl isoheptadecanoate, hexadecamethylcyclooctasiloxane, butyl palmitate, 2,6,10,14-tetramethylheptadecane, nonadecane, isobutyl stearate, oxalic acid, 2-ethylhexyl tetradecyl est, heptacosane, eicosane, 7-hexyl (10.01%), heptacosane, 7-hexyl and octacosane. Literature search exhibits that the maximum of predominate compounds was diagnosed in different crude extracts are biologically energetic molecules [16]. These excessive percentages of chemical substances have been diagnosed and characterized in different organic crude extracts of neem which have been formerly started from some of the different medicinal plant species [14]. These recognized active compounds are taken into consideration to plant protection systems [6]. Some of the chemical substances had been removed and recognized in different crude extracts from neem samples those compounds are presently used as herbal antioxidants, antimicrobial agents, and in the method of various medicines [7].

This study showed the combination of extracts performed well and the isolated compounds could be used as a vital source of pesticides and for the agrochemical industry.

Conclusion

In conclusion, widespread usage of insecticide is inefficient and unsustainable for future perspectives. Many insecticides do the job they are supposed to do they suppress pest populations. However, health and environmental consequences render them ineffective. Furthermore, due to insect resistance, most synthetic and natural pesticides are vulnerable to ineffectiveness. As a result, integrated pest control is the only feasible option for the future. Hence, our proposed pesticide provides a rational solution to the pest control problem to the farmers due to their economic benefits and lower social costs. Further in silicon studies are needed to find the binding ability of discovered compounds from Bacteria and neem to insects.

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