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RESEARCH ARTICLE

Formulation and Evaluation of *Achyranthes bidentata* Root Extract Based Herbal-insecticide

Nuri Farzina Rahman[1], Kangkana Sharma[2], Sahaniyaz Laskar[1], Sahjahan Ali[1], Md. Asif Ahmed[1], Wearank Terang[1], Ningthoujam Dabung Meitei[1], Haider Khan[1], Md. Iliyash Ahamad[1] and Rituraj Bharadwaj[1*]

[1]Rahman Institute of Pharmaceutical Sciences and Research, Tepesia, Sonapur – 782402, Assam, India; bharadwajrituraj@gmail.com

[2]Department of Zoology, Gauhati University, Jalukbari, Guwahati – 781014, Assam, India

Abstract

Pesticides are the chemicals most commonly used by humans in the production of many foods worldwide. Most of the pesticides are chemical derived which not only causes different diseases in the human population rather affects the environment too. The natural pesticide obtained from plant-based sources can be considered as an alternative. *Achyranthes bidentata* is chosen for this current study to formulate an effective pesticide based on plant origin. The root extract of *A. bidentata* has been prepared and it has been subjected to its phytochemical screening. The functional group present in the extract has been determined by using FTIR and the volatile constituents have been detected by using GCMS. The insect-repellent property of the extract has been investigated using weevil and found to be efficacious. FTIR analysis showed hydroxyl, amine, amide, carbonyl, nitrile, alkanes, alkenes and nitro groups, while GCMS analysis showed benzene, 1-phenyl ethyl alcohol, limonene, toluene. In the insect repellent study, it has been found that A 3% to 15% concentration of *A. bidentata* extract and duration time of 24, 48 and 72 hours caused 88.33% grain weevils after 72 hours at 10% extract concentration. It can be concluded from the performed studies that, *A. bidentata* root extract can be effectively used as an insecticide for agricultural purposes.

Keywords: *Achyranthes bidentata,* Pesticide, Root Extract, Weevil

1. Introduction

The rapidly developing world continuously demands huge agricultural productivity by adopting an organic mode. Researchers are highly encouraged to investigate cost-effective herbal pesticide groups due to the high expense of chemical pesticides and the environmental risk they pose. The oldest type of pest management is plants, which rely on their built-in defences against herbivores that have evolved over millions of years. Bio-pesticides, insect pheromones produced in nature, plant extracts and oils, plant growth regulators and insect growth regulators, etc. Among them, low toxicity, rapid biodegradation, targeting insects and maintaining ecological balance are the best properties of bio-pesticides[1,2]. Botanical insecticides made from plants are potential alternatives to traditional pest control methods for the present and the future. They are safe for both animals and the environment, have broad-spectrum activity, and have a rather precise mechanism of action[3]. The co-evolution of plant species with microbes and herbivores led to the production of plant-based poisons[4]. Plants have a wide range of chemical defences against insects as a result of their co-evolution with insects. A variety of plants have been examined for their insecticidal properties that are mostly utilized for meals, cosmetics, spices, and crop protectants and some of them are shown to be effective because botanicals are consumer-friendly and less likely to cause ecological damage[5].

*Author for correspondence

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The first pure plant pesticide was employed in the 17th century when tobacco leaf nicotine proved to be fatal to insects. There are several botanical pesticides on the market right now that are sold all over the world. In contrast, synthetic pesticides (organochlorines, organophosphates, carbamates and pyrethroids) replace natural pesticides, causing soil contamination, long-term contamination and damage to wildlife (fish, birds), the impact of biological control and pollination, and disease[6]. As a result, more botanical insecticide products are on the market, which is changing the situation and leading to an increase in bio-pesticide products. To fully capitalize on each bio-pesticide's desirable qualities, future work must thus focus on strategies to optimize its use[7].

The newly active chemical compounds found in plants offer fundamental building blocks for the creation of new pesticides, which calls for the isolation and screening of these naturally occurring bioactive chemicals to determine their activity. Plant-derived pesticides include various chemicals that reduce the likelihood that pests would develop resistance, as opposed to synthetic insecticides, which are based on a single component. Crop protection is part of large crop production and is required in specific areas such as horticulture, greenhouses, organic farms, homes, parks and gardens. This is the case even with deliberate efforts to breed or modify plants to make them resistant to insects and diseases. The development of secondary metabolites for host resistance, disease, and plant competition has given plants the ability to protect crops. As described in the article[6], they are also compatible with other less effective solutions for insect control such as pheromones, oils, detergents, entomopathogenic fungi, predators and parasites.

The medicinal plant *Achyranthes bidentata* is used in the treatment of many diseases such as miscarriage, dysmenorrhea, abdominal and knee pain, muscle and bone weakness, gonorrhoea, oedema, headache, dizziness, toothache, sun sores, hematemesis and bleeding. Recent clinical studies have shown that the joint of *A. bidentata* can protect cartilage by reducing inflammation and apoptosis and promoting cytokine production. *A. bidentata* have also been shown to have anti-cancer, anti-inflammatory, anti-arthritic, antioxidant and wound-healing properties. According to recent pharmacological studies on this herb, PIM1, CYP1B1 and HSPA2 are the main targets of *A. bidentata* joints in the treatment of osteoarthritis. Herbs have proven effects on the immune system, nervous system, bone metabolism and growth. According to toxicological studies, it is seen that *A. bidentata* joints are not toxic in terms of their medicinal properties.

2. Materials and Methods

2.1 Materials

Methanol, Chloroform, Hydrochloric acid, Quercetin, Gallic acid, Folin-ciocalteau reagent, sodium carbonate, Sodium nitrite, aluminium chloride, Ferric chloride, Sulphuric acid, sodium hydroxide, Potassium iodide, iodine, Sodium hydroxide have been procured from Himedia, India. All chemicals used in the study were analytical grades.

2.1.1 Plant Material Collection

Achyranthes bidentata plants were collected from the nearby Golaghat district around the Dergaon area, Assam, India in April 2023. After fresh samples were collected, the spines around the eaves were removed. Then wash the roots 2-3 times with fresh water and then with 0.05w/v hydrogen peroxide. After pre-treatment, the root samples were cut into pieces and air-dried. The dried pieces of *A. bidentata* roots were then collected and stored in an airtight container. The dried pieces of *A. bidentata* roots were ground to a fine powder using a cutter mill and the powder was sieved to a different size mesh to obtain coarser and fine powders with a particle size range of 0.51, 1-1.18, 1.18-1.4 mm. Finally, each size of powdered *A. bidentata* roots was kept in a small labelled plastic container till their use in extract preparation.

2.1.2 Preparation of Crude Extracts

Extraction was done by the method described by Debnath *et al.*,[8] briefly, 50g of *Achyranthes A. bidentata* roots powder sample was soaked in 500 ml absolute methanol using an Erlenmeyer flask as a cold maceration at a ratio of 1:10 w/v. The mixture was placed in an orbital shaker with continuous shaking at room temperature (220°C) with a stirring speed of 150 rpm to ensure a good extraction of the active ingredients in the sample, thus increasing the efficiency of the method and shortening the yield time. A week later, remove the

residue with muslin and again Whatman filter paper No. -1 using a separating funnel. The residue was pressed again continuously to extract as much as possible. After extraction of the plant sample, the filtrates were taken into the round bottom flask and then concentrated by evaporating the solvent with a rotary evaporator at 45°C. The crude *A. bidentata* extract was then collected through a glass beaker and made dry by drying in a hot air oven at 38°C for 24 hours to remove any residual solvent. Afterwards, partial results of *A. bidentata* root extract in a station and a refrigerator at 40°C until used for insect bioassays and other tests.

2.1.3 Yield Determination of Achyranthes bidentata Extract

The per cent yield of crude methanolic extract of *Achyranthes bidentata* roots was determined using the method described by Gahlot *et al.,* and Felhi *et al*[9,10]. The crude extract sample from the oven was weighed using electronic balance and yield was calculated using the following equation:

Yield (%) = [weight (g) of extract/weight (g) of powdered sample] X 100

2.1.4 Phytochemical Screening of Achyranthes bidentata Extract

Phytochemical analysis is the process of identifying available phytochemicals. The methanolic extract of *Achyranthes bidentata* roots was subjected to qualitative tests for the presence or absence of various phytochemical components present in the extract namely alkaloids, flavonoids, polyphenols, tannins, saponins, terpenoids and steroidal compounds. The phytochemical screening was carried out by chemical test according to the standard procedures[11,12].

2.1.5 Total Flavonoid Content Determination

After the qualitative analysis of the extract, the analysis of flavonoids was performed. The total amount of flavonoids present in *Achyranthes bidentata* extracts was determined using the aluminium chloride calorimetric method described in article[13]. Briefly, standard solutions of 0, 50, 100, 150, 200 and 250 mg/L quercetin were prepared with distilled water as standard solution. Then, 0.1 g of sample extract was taken and dissolved in 100 ml of distilled water and turned into 1 mg/ml extract solution. Then add 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution to each test tube. After 5 minutes, 0.3 ml of 10% aluminium chloride was added. Add 2 ml of 1M sodium hydroxide for 6 mins.

Finally, it is diluted to 10 ml with distilled water and mixed well, like orange-yellow. After 20 minutes, absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Shimadzu 1800). From the recorded absorbance readings, a calibration curve is constructed by plotting the quercetin concentration versus the quercetin absorbance as a standard, and the amount of flavonoids present in the extract is determined by linear regression analysis. Experiments were performed in triplicate to minimise external variability. Data on total flavonoids are expressed in mg quercetin equivalents per gram dry mass of *A. bidentata* extract.

2.1.6 Total Phenol Content Determination

Total phenolic content in *Achyranthes bidentata* extracts was determined spectrophotometrically using the described Folin-Ciocalteau reagent method using gallic acid as standard[14,15]. The method is based on the reduction of phenolic hydroxyl groups formed under simple conditions using the Folin-Ciocalteu phenol reagent to produce a blue chromophore that can be detected spectrophotometrically. Briefly, a 1000 mg/L or 1 mg/ml solution was prepared by placing 0.1 g of dry burdock root extract in 100 ml of distilled water. Mix 1 ml of extract with 5 ml of diluted Folin-Ciocalteau reagent. The solution is mixed well and incubated for 5 minutes at room temperature. After incubation, add 4 ml of sodium carbonate solution to increase its alkalinity and incubate again in a 50°C water bath for 5 minutes. Folin-Ciocalteau reagents are sensitive to reducing compounds to reduce the oxidation state, causing a blue colour to form during the reaction. Measure the absorbance of the reaction mixture at 760 nm using a UV-Vis spectrophotometer (Shimadzu-1800, λmax 35). Extractions were performed in triplicate. The calibration curve was prepared using gallic acid as a standard and the amount of phenolic compound in the extract was determined from the standard curve. All phenolic content data for *A. bidentata* extracts are expressed as milligrams of Gallic Acid Equivalent weight (GAE) per gram of dry mass.

2.2 FTIR Analysis of *Achyranthes bidentata* Roots Extract

For FTIR analysis, 10 mg of dried *Achyranthes bidentata* roots extract was mixed with 100 mg of potassium bromide and the resulting pellet was placed in an FTIR spectroscope. The functional groups were analysed for the scanning extract sample with a scan range from 400 to 4000 cm-1 and the peak values of FTIR were recorded (Perkin Elmer)[16].

2.2.1 GCMS Analysis of the Volatile Content

A GCMS analysis was carried out to find any volatile phytoconstituents in the methanolic root extract. The root extract was analysed by GC-MS using the described GC SHIMADZU QP2010 system and gas chromatograph interface to a mass spectrometer (GC-MS) as reported[16].

2.3 Bioassay

2.3.1 Method of Rearing Insects

Grain weevils were collected from grain storage and reared in the laboratory at room temperature of 25±2°C using wheat grain as a food media. The wheat grain used for the experiment was cleaned and disinfested by keeping it in a refrigerator below 0°C to eliminate possible internal infestation or to kill any storage insect pests. Briefly, 5 kg of weevil-infested wheat grains were purchased from the local market and then grain weevils were collected. The weevil was then transferred into six (n=6) clean and transparent glass jars with torn tiny holes to allow air passage. The separation of weevils was performed by ventilating the grain to the air and picking it up. The collected grain weevils were then allowed to breed for 5-6 weeks to supply similar-aged adult weevils. Each experimental glass jar contained 50 g of experimental cereal grains and was infested with 20 adult weevils. Then they were kept at room temperature. After a specified day when the treatments were exposed, all parent weevils were counted and removed. Data were recorded based on the number of adult insects alive and adult insects dead (parental insect mortality)[17,18].

2.3.2 Treatment and Design

The studies were designed in a factorial format with two factors: Concentration and botanicals. Factor A featured locally accessible botanicals, including *Achyranthes bidentata*, and Factor B included varied concentrations of the botanicals, including untreated control (0%) and 3.0%, 9.0%, and 15.0%. So, the formula for the number oftreatment combinations was 1 botanical X 4 concentrations[17,18].

2.3.3 Toxicity Testing

Experimental bioassay with toxicity test using the spray method. The parameter is the mortality of the weevil at 24, 48 and 72 hours. The liquid extract of *Achyranthes bidentata* root is sprayed on the grain and let to dry and grains without liquid extract are only sprayed with water and also air dried. Five pairs of grain weevils were placed in a Petri dish holding 10 grams of grain that had been treated with various concentrations of botanical extracts. In the untreated medium, the same number of insects was retained as a control. Adult mortality was noted from each replication, and using Abbott's technique, the original statistics were then adjusted and converted to a percentage[16,17].

$$\text{Corrected Mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 10$$

2.3.4 Repellence Test

Petri dishes are divided into two parts treated and untreated. Afterwards, we spread the plant seed extract over half of the rice using a straw. After the treatment halves are air-dried, ten lines are placed in the middle of each Petri dish. The number of insects in each section was counted from one hour to three hours[17,18]. The data were expressed to Percentage Repulsion (PR) by the following formula:

$$\mathbf{PR = (NC\text{-}50) \times 2}$$

Where, NC = the percentage of insects present in the control half.

Positive (+) values expressed repellency and negative (-) values attractancy.

2.3.5 Application of Achyranthes bidentata Extract on Insects

For observing the mortality effects of methanolic *Achyranthes bidentata* roots extract against granary

weevils, initially different concentration of test solutions of the extract was prepared by serial dilution. The number of insects that died was assessed at concentrations of (0% (control), 3%, 9%, and 15%), and at different time intervals of 24, 48 and 72 hrs. Each concentration of *A. bidentata* extract was prepared and calculated by measuring the weight of crude *A. bidentata* extract and mixed with 40 ml of distilled water.

$$\text{Weight of crude extract (g)} = \frac{\text{volume of water (40ml)} + \text{percent dosage}}{100}$$

For the application of methanolic *Achyranthes bidentata* roots crude extract on grain weevils, 50 g of disinfected wheat seeds were put into each glass jar and five different rates of *A. bidentata* extract test solution of (0% (control), 3%, 9%, and 15%), which were prepared before, were added onto this clean wheat grain in each jar separately and shaken well to ensure even distribution and coating. 20 randomly selected laboratory-reared adult grain weevils were introduced into each treatment including the untreated control and maintained under laboratory conditions. The experiments were conducted in triplicates for each concentration of botanical extracts and control treatments. The infested experimental wheat samples were sieved every day for weevil count and mortality data were assessed after 24, 48 and 72 hours of exposure time. Adult weevils that did not respond to pointed metal probes or needles were considered dead. Adults who died during each measurement period were counted and discarded, while survivors were returned to their treatment. On the 3rd day, the remaining weevils (dead and alive) were counted and discarded. Per cent Cumulative insect mortality rate was calculated as follows:

Cumulative mortality (%) = [Cumulative number of dead insectsa/total number of insects] x 100

$$\text{Corrected mortality (\%)} = \frac{\text{Cumulative number of dead insects}}{\text{total number of insects}} \times 100$$

But this cumulative percentage of insect mortality observations was corrected using the equation given below by control mortality using Abbott's formula to take account of natural mortality in untreated controls[19,20] as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

3. Results and Discussion

3.1 Yield of *Achyranthes bidentata* Extract

From a 50 g dried *A. bidentata* powder sample macerated with 500 ml methanol (1:10 w/v), 2.71 g of crude *A. bidentata* extract was obtained. Thus, 5.42% is the obtained yield. The optimum percentage yield of *A. bidentata* extract at different maceration periods according to the method described earlier was 5.42% or 2.71 g. Previous studies reported that (1:10 w/v) of dry weight to solvent volume ratio is used as an ideal[17,21]. According to the study done by Jajere, 100 g powdered *A. bidentata* roots macerated with 800 ml methanol (1:8 w/v) for two days yielded 3.60 g of crude extract or 3.6% in percentage[22]. In this case, as shown above, the maceration time taken from 3-9 days yields better as compared with the maceration time of 2 days used in the previous study. Therefore, the soaking time needed for extraction determines the yield of *A. bidentata* extract. The difference in yield of the extract is also affected by other factors such as sample-to-solvent ratio used, milling condition, age of the plant and geographical location in which the plant material was harvested. It has been reported previously, that the effect of the size of the particle and powdered sample to solvent ratio on the yield of one of the well-known insecticide active components extracted from Derris elliptica roots using soaking extraction and found that a fine particle size of 0.5-2 mm yielded high which creates a large surface area for an efficient mass transfer when compared with a coarse particle size of 2-5 mm[23]. A sample-to-solvent ratio of 1:10 (w/v) yielded 1.22% of rotenone as compared to a solvent-to-sample ratio of 1:3.3 (w/v) yielded 0.19% at 0.5-2 mm particle size extracted for 50 hrs. Azwanida also reported that methanol was a solvent for flavonoids at a weight ratio of 1:10 w/v for 1 hour. The 1:10 (w/v) methanol extract showed greater

antioxidant power compared to the water extract (1:10 w/v). Crude methanolic extract of *A. bidentata* roots obtained through maceration extraction has a dark greenish colour and, a sticky solid nature with a pH of 5.79.

3.2 Qualitative Analysis of Phytochemicals

The results of the qualitative phytochemical analysis of *Achyranthes bidentata* roots extract are presented in Table 1. Results of chemical analysis due to colour change shows that the roots of the *A. bidentata* plant contain saponins, alkaloids, flavonoids, steroids, glycosides, Phenols, terpenoids and tannins in different concentration. The persistent and high concentration of foam in *A. bidentata* extract indicates that *A. bidentata* roots are rich in saponins. The presence of saponins in *A. bidentata* roots enables the use of the plant to defend against different herbivores and improves its use as an anti-feedant and insecticide activity for different life stages of pests. Plants rich in saponins may inhibit herbivores from eating plants and may lose their motility, resulting in pest death due to the high toxicity of saponins. Saponins also indirectly affect pests by forming different forms with various digestive enzymes and damaging the mucous fibres of various cells in the intestinal tract. Likewise, saponin molecules bind to cholesterol and cause cytotoxicity. Therefore, failure of moulting in insects is due to this saponin-enzyme complex[24]. The bright yellow colour found in *A. bidentata* root extract indicates that *A. bidentata* root extract contains a large number of flavonoids. The phenolics and flavonoids present in the root of *A. bidentata* provide this plant with good antimicrobial activity and also a defence mechanism against bacteria, viruses, parasites or herbivores.

Table 1 shows that steroids, glycosides and terpenoids were moderately present in the methanolic *A. bidentata* root extract. Tannins, alkaloids and phenols were weakly present in the *A. bidentata* root extract. Generally, Phytochemical screening results have indicated that the methanolic extract of *A. bidentata* root is rich in saponins and flavonoids followed by terpenoids, steroids and glycosides. That's why *Achyranthes bidentata* root has active substances, especially saponins, which expand its use to reduce damage to stored crops that can affect stored crops, pests, insects and other diseases, these diseases cause problems by affecting their nutrition, growth and development birth of an offspring.

A previous study showed confirmation that *A. bidentata* root extract contains saponins, glycosides, flavonoids, and tannins[25,26], but no steroids. The difference is that the test method and the use of solvent extraction are not the same. This change is also due to changes in the environment in which the plant is collected, seasonal changes that may change the plant content, changes during extraction or storage, and the drying process. The investigation also reports use of methanol can extract more active biological compounds since methanol extract inhibits the growth of microbes more than aqueous extract. Studies done by Hammuel, also show that of methanolic extract of *A. bidentata* roots is rich in alkaloids followed by flavonoids, steroids, terpenoids, glycosides and saponins with the absence of tannins[27]. Studies also done by Chigodi, (2013) indicate the methanolic extract of the roots of the *A. bidentata* plant indicates the presence of tannins, alkaloids, glycosides, saponins, flavonoids, reducing sugars, terpenoids, phlobatannins, steroids and Coumarins[28].

3.3 Quantitative Analysis of Phytochemicals

All flavonoid content is expressed in terms of Quercetin Equivalents (QE), determined by the well-known aluminium chloride calorimetry method. The absorbance reading of different quercetin concentrations was used to generate a calibration curve, and the total flavonoid content of the extract was calculated using a linear regression equation.

Table 1. Results of qualitative analysis of *A. bidentata* roots extract

Phytoconstitutent	Colour Observed	Result
Saponin	----	+ + + +
Terpenoid	Reddish Brown	+ +
Steroid	Red	+ +
Tannin	Blue-Black	+
Glycoside	Yellow	+ +
Flavanoid	Yellow-Orange	+ + +
Phenols	Blue-black	+
Alkaloid	Reddish/Brown precipitate	+

The absorbance readings for *A. bidentata* extract from UV Spectrophotometer in triplicate were 0.104, 0.098, and 0.103. Therefore, the mean absorbance for *A. bidentata* extract was 0.102 and the concentration of quercetin, mg/L from the regression line was: C = 108.71 mg/l. This implies that 1.087 ± 0.002 mg of quercetin acid equivalent/the g of the dry mass of total flavonoid was obtained from 0.1 g of methanolic extract of *A. bidentata* roots. Flavonoids exhibit a wide range of biological properties and therapeutic activities including insecticidal, antifungal, antiviral, antibacterial, antitumor, anti-inflammatory, hepato-protective, anti-hypertensive, anti-rheumatism, antidiuretic, oestrogenic, antiallergic and anti-carcinogenic activities which are associated with their antioxidant and free-radical scavenging properties. Important phenolics which have insecticidal, repellent and feeding deterrent functions are flavonoids[29].

3.4 Total Phenolic Content Determination

The Total phenol composition of *Achyranthes bidentata* roots extract was determined using the Folin-Ciocalteu's reagent as described in the method section using Gallic acid as the standard. The absorbance readings for *A. bidentata* crude extract from UV Spectrophotometer in triplicate were 0.527, 0.623, and 0.514. Therefore, the mean absorbance for *A. bidentata* extract was 0.5547. The concentration of Gallic acid, mg/l was: C = 0.563 mg /g. Thus the total phenolic content of the methanolic extract of *A. bidentata* roots was found to be 0.563 ± 0.068 mg GAE/gram of dry mass *A. bidentata* extract. This implies that 0.563 mg of Gallic acid equivalent/g of dry mass of total phenolic compounds was obtained from 0.1 g of methanolic extract of *A. bidentata* roots. Phenolics are used as a defence against pathogens, antioxidant, prophylactic and therapeutic agents to cure many diseases[30].

3.5 FTIR Analysis of *Achyranthes bidentata* Extract

The functional group present in the methanolic root extract of *Achyranthes bidentata* has been summarized in Table 2 and Figure 1. FTIR analysis shows the extract contains hydroxyl groups (O-H), amines (-NH, C-N), amides (NH-C=O), carbonyls (C-O), nitriles (C≡N), alkanes (C-H, C-C), alkenes (C=C) and nitro groups (N=O). The Spectral values have a strong broad sharp peak centred at 3325.56 cm^{-1} which indicates the hydroxyl group is a dominant functional group in *A. bidentata* root extract. These functional groups signify the presence of alcohols, phenolics, amines, amides, nitriles, alkanes, alkenes and nitro compounds in *A. bidentata* root extract. Therefore, the presence of these functional groups confirms that *A. bidentata* root extract contains basic phytochemicals that amplify its pesticide usage.

3.6. GCMS Analysis

The GCMS analysis of the root extract has revealed the presence of Benzene, 1-Phenylethanol, Lemonene and Toluene. The details of the GCMS analysis are summarized in Table 3 and Figure 2.

3.7 Bioassay Test

3.7.1 Toxicity Test

This experiment evaluated the toxic effects of the natural biopesticide *A. bidentata*, and its various concentrations (0% (control), 3%, 9%, and 15%), every 24 hours after treatment on grain weevil until death. The results are shown in Table 4 as well as in Figure 3. It was quite evident that all of the concentrations possessed the lowest mortality at 24 hours and the highest at 78 hours. At 24 hours, the lowest mortality (0%) was measured in control (0%) i.e., untreated grain, and the highest mortality (45.81%) was produced in 15 % concentration. At 72 hours, 15% concentration caused the highest mortality (79.48%) followed by 9% concentration (66.51%) due to its high concentration and toxicity, and the lowest mortality (12.22%) was measured in control (0%), i.e., untreated grain. In addition, the highest daily mortality (17.83%) was observed at 15% concentration, and the lowest daily mortality rate (3.33%) was observed in the control (0%). However, the lowest and highest mortality rates recorded in gene therapy were 3% and 15%, respectively. It is clear from the results that the mortality of insects increases as the concentration level of plants increases. So, the order of toxicity of three concentrations and control on rice weevil, *S. sitophilus* L. could be ranked as 15%>9%>3%>control (0%).

3.7.2 Repellence Test

The repellent effect of natural bio and its different concentrations (3%, 9%, and 15%) at different hours after treatment on grain weevil were evaluated in this

Table 2. FTIR analysis of *Achyranthes bidentata* extract

SL No	ABSORPTION	APPEARANCE	GROUP	COMPOUND CLASS	COMMENTS
1	3325.56	Strong, Broad	O-H stretching	alcohol	Intermolecular bonded
2	1631.64	strong	C=C stretching	α,β-unsaturatedketone	
3	1550.87	medium	C=C stretching	cyclic alkene	
4	1517.05	strong	N-O stretching	nitro compound	
5	1418.12	medium	O-H bending	carboxylic acid	
6	1357.82	medium	O-H bending	phenol	
7	1251.06	medium	C-N stretching	amine	
8	1029.64	strong	S=O stretching	sulfoxide	
9	1016.19	strong	C-F stretching	fluoro compound	
10	845.42	strong	C-Cl stretching	halo compound	
11	826.54	medium	C=C bending	alkene	trisubstituted
12	693.85	strong	C=C bending	alkene	disubstituted (cis)
13	675.71	strong	C-Br stretching	halo compound	

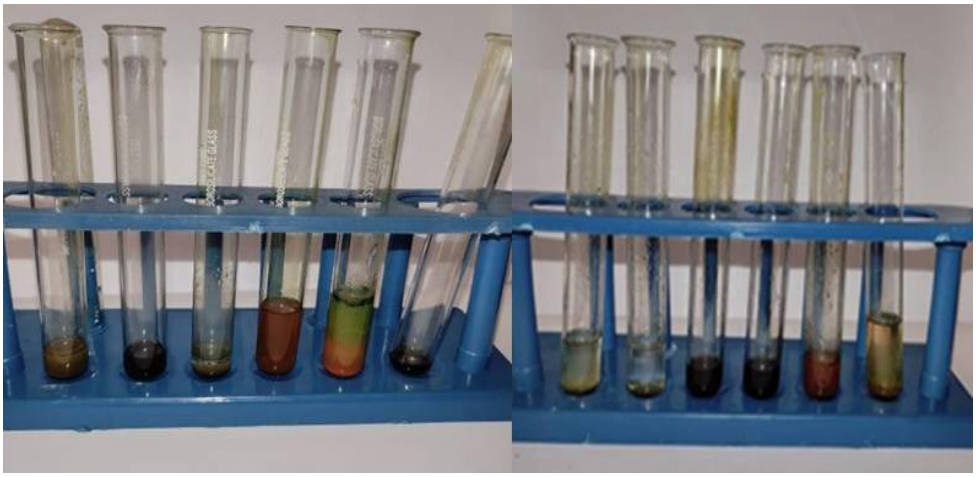

Figure 1. Qualitative phytochemical screening of *Achyranthes bidentata* root extract.

Table 3. GCMS analysis *Achyranthes bidentate* extract

Sl.no.	RT	Scan	Height	Area	Area%	Norm%	Name
1	29.552	4509	1,023,888	114,054.1	0.059	0.18	Benzene
2	38.456	6289	1,014,609	60,783.5	0.032	0.09	1-Phenylethanol
3	44.858	7569	3,014,078	105,036.7	0.055	0.16	Lemonene
4	49.545	8506	1,086,128,896	64,494,716.0	33.515	100.00	Toluene

experiment and presented in Tables 5 and Figure 3. The different concentrations (3%, 9%, and 15%) of the botanicals at different hours after treatment caused statistically significant repellent effects on grain weevil. Among 3 concentrations and 3 times, i.e., 1, 2, and 3 Hour After Treatment (HAT), the highest number of insects (71.11%) was repelled by 15% concentration at both 1 and 2 HAT, and the lowest (26.67%) was repelled by 3% concentration at 3 HAT. Again, the highest average repellent intensity per hour (66.67%) was caused by high concentration (15%) followed by medium concentration (9%) repelling (58.52%), and

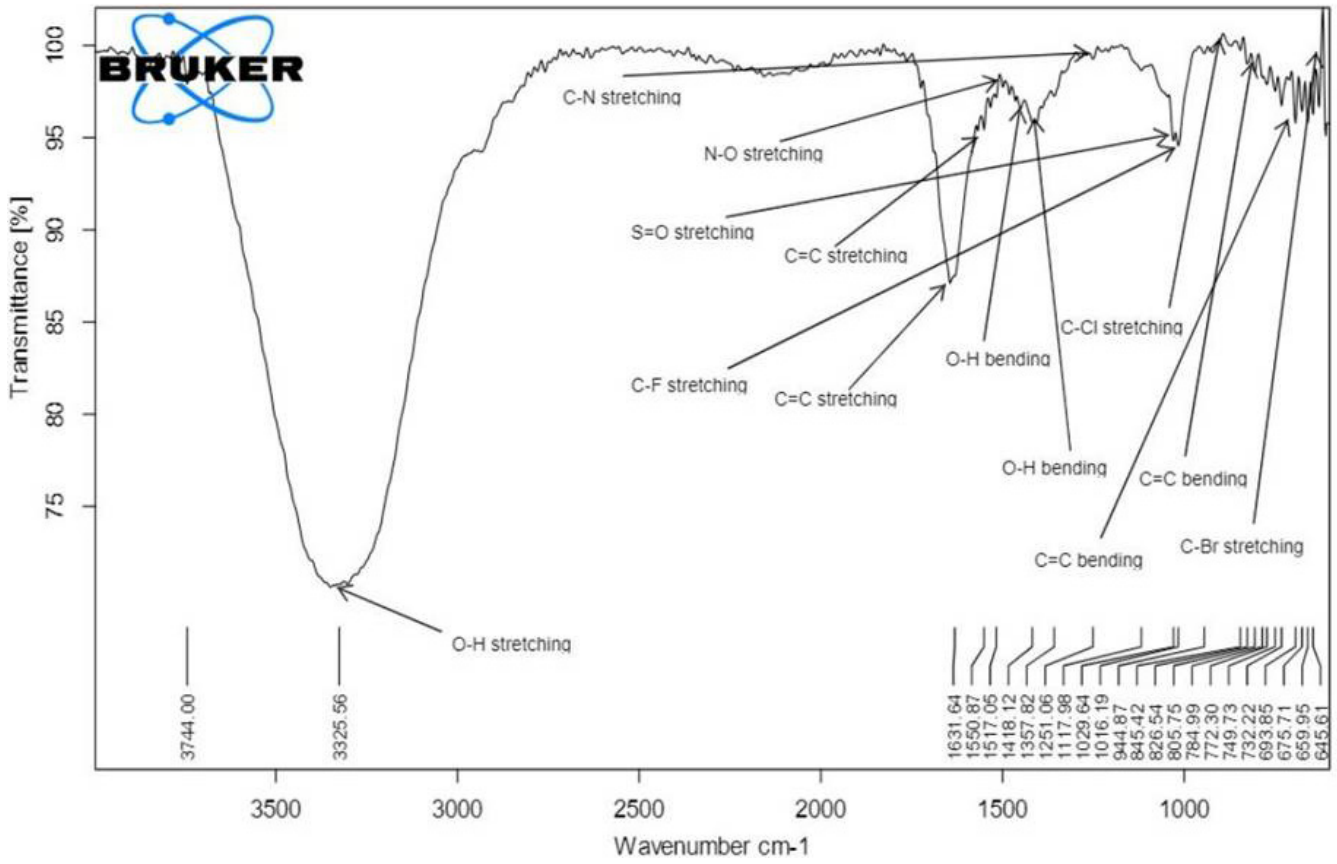

Figure 2. FTIR analysis of *Achyranthes bidentata* root extract.

Table 4. Effect of different concentration of botanical on the mortality of the test insect

Concentration(%)	Mortality of the Test Insect (%)			
	24 HAT	48 HAT	72 HAT	Mortality perday
3%	30.86	48.64	57.72	12.97
9%	35.31	54.57	66.51	15.38
15%	45.81	67.65	79.48	17.83
0	3.33	6.67	12.22	3.33
Mean	28.83	44.48	53.98	12.37

the lowest (36.30%) was caused by low concentration (3%). The repellent effect of different concentrations of all botanicals increased with the increase of concentrations. According to repellent intensity, the order and repellency class of various concentrations of botanicals could be expressed as 15%>9%>3% and IV>III>II respectively. In all cases there were changes in the mortality of grain weevils in all doses of *A. bidentata* extract and therefore mortality rates were increased by increasing both the concentration of *A. bidentata* extract and exposure duration. However, the variation of mortalities of grain weevils due to the length of days was slight compared to the variation of mortalities based on the dose of *A. bidentata* extract.

4. Conclusion

The findings of this study hence show *Achyranthes bidentata* roots have good insecticide activity in killing grain weevils. The significant difference in mortality results obtained in the treated samples indicated the possible potential of *A. bidentata*

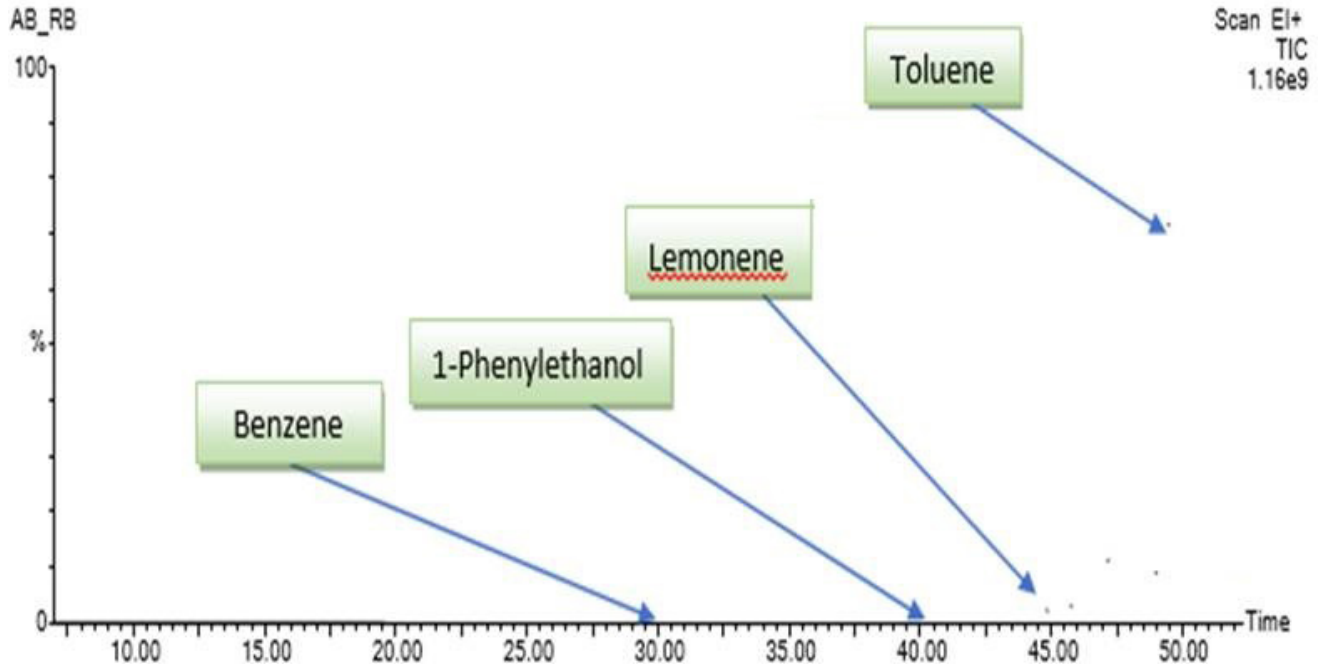

Figure 3. GCMS analysis of *Achyranthes bidentata* root extract.

Table 5. Repellent effect of different concentration of botanical on the test insect

Concentration(%)	Repellency Rate			RepellencyPer Hour	RepellencyClass
	1 HAT	2 HAT	3 HAT		
3%	33.33	48.89	26.67	36.30	II
9%	66.67	60.00	48.89	58.52	III
15%	71.11	71.11	57.78	66.67	IV
Mean	57.04	60	44.44	53.83	

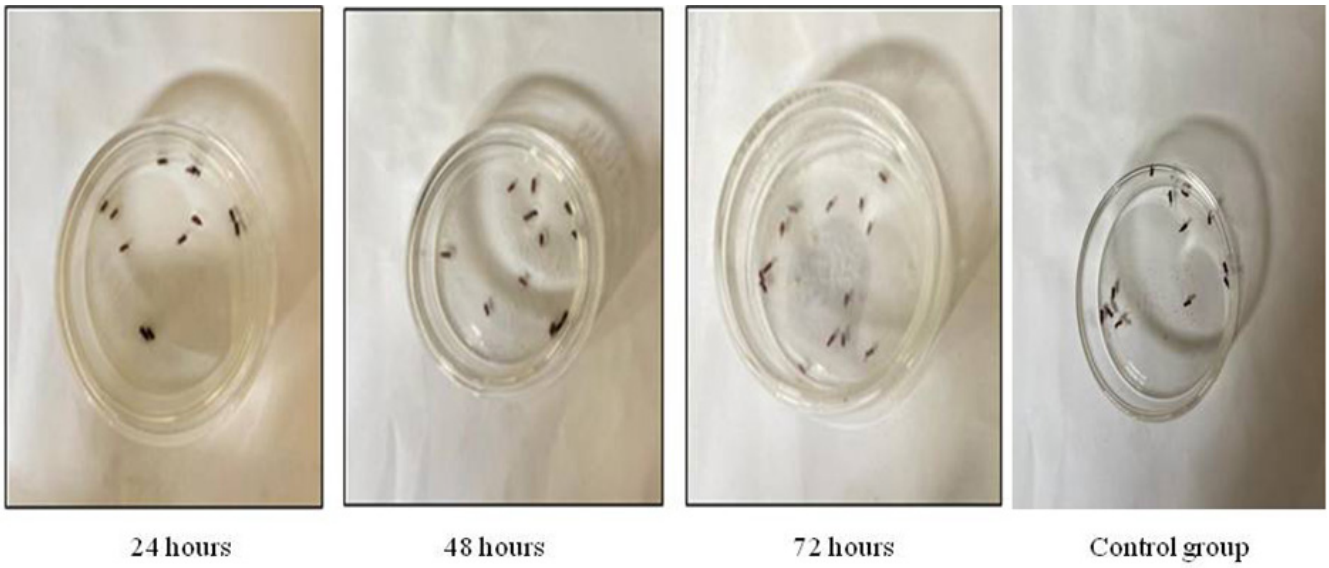

Figure 4. Treated groups (Grain Weevils) observed after 24 hours, 48 hours and 72 hours respectively.

extract as an alternative agent for the control of granary weevil. Therefore, the plant can be used with integrated pest management methods. This shows *A. bidentata* root extract has a good potential for higher concentrations in killing weevils and can be used as a grain protectant which in turn replaces synthetics such as malathion.

5. References

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