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Research Article

# Assessment of *Adiantum incisum, Alternanthera pungens* and *Trichodesma indicum* as Bio-insecticides against Stored Grain Pests

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**Abstract:** This study investigated the insecticidal potential of *Adiantum incisum* Forssk (Pteridaceae), *Alternanthera pungens* Kunth (Amaranthaceae) and *Trichodesma indicum* L. (Boraginaceae). Aqueous, methanolic and n-hexane extracts of whole plants (roots, stem and leaves) were prepared using maceration technique. Insecticidal activities of aqueous, methanolic and n-hexane extracts of three plants (10, 20 and 30 mg/mL) were evaluated by impregnated filter paper insecticidal assay. Methanol and hexane extracts of *Adiantum incisum* gave effective $LD_{50}$ (15.3 mg/mL) against *Callosobruchus chinensis* and *Sitophilus oryzae* ($LD_{50}$22 mg/mL) after 24 hours respectively. Present research elucidates the important phytochemicals (alkaloids, saponins and tannins) in three plants by FTIR and good insecticidal activity ($LD_{50}$ < 25 mg/mL in 24 hours) against *Callosobruchus chinensis* and *Sitophilus oryzae*. Current study promotes further investigations for using these plant extracts as anti-feedants, repellents, fumigants and formulation of non-toxic bio-insecticides.

**Keywords:** *Adiantum incisum*, *Alternanthera pungens*, FTIR, insecticidal activity, phytochemistry

## 1. INTRODUCTION

Annual food grain losses due to insect attack accounts for about 5% of world food production [1]. Stored grain pests are the major cause of decrease in commercial value, quality, seed viability and weight of grains. About 70% of these devastating pests of food grains belong to the order Coleoptera [2]. *Tribolium castaneum* (red flour beetle), *Sitophilus oryzae* (rice weevil) and *Callosobruchus chinensis* (bean weevil) belong to order Coleoptera. *Tribolium castaneum* infest almost every stored cereal or cereal product including maize and wheat flour. It secretes harmful quinones which damage flour and flour products [3]. *Sitophilus oryzae* is the most damaging and widely spread cereal pest particularly in areas with warm climate. It infects many cereals; e.g., wheat, maize, barley, rice and sorghum [2]. *Callosobruchus chinensis* damages important leguminous grains and infect different beans like chickpea, green black and red gram, soybean, pea and peanut [4].

Stored grain insects are usually controlled by synthetic insecticides. However, there are many disadvantages of using these synthetic insecticides, including insect resistance, environmental problems, effects to non-target organisms and damaging human health and quality of stored grains. Plants based insecticide can be a good alternative to chemical insecticides because of a number of advantages including target specific activity, bio-degradability, environmentally safe and non-toxic [5]. Plants, due to the presence of different phytochemicals like phenolics, terpenoids and alkaloids, have been effectively used as insect anti-feedants, insect repellents and insecticides. Phyto-constituents also provide protection to plants against plant attacking insects. Plants have developed sophisticated mechanisms of defense. Mostly effective chemical production is involved in defense pathways [6, 7]. Terpenoids present in plants act as neurotoxic for insects. Plant based insecticides find applications in domestic and agricultural practices [8].

FTIR (fourier transform infrared spectroscopy) is widely employed to analyze pesticide products and identifying their functional groups and

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chemical components [9]. Because of the fingerprinting ability, FTIR is useful for analyzing the phytochemicals present in medicinal plants. Different phytochemicals such as alkaloids, flavonoids, saponins, tannins, phenols and terpenes are responsible for conferring the biological activities to plants. FTIR analysis of medicinal plants can give an idea about different phytochemicals present in plants in short time through reliable and fast method [10]. Different functional groups have characteristic bio-constituents which are responsible for treatment of various ailments. Thus FTIR, through revealing the functional groups of medicinal plants, also gives an idea about the different biological activities of respective medicinal plants. For example, plants having carboxylic acid are usually responsible for the treatment of fever, stomatitis, jaundice and joint pains. Medicinal plants having sulphur groups are usually responsible for disinfectant properties [9].

This study investigated the FTIR analysis of three important folklore medicinal plants for assessment of phytochemicals. Insecticidal activity of aqueous, methanolic and n-hexane extracts of three plants has also been evaluated for potential applications in bio-pesticides.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plants

Three plants *Adiantum incisum* Forssk*, Alternanthera pungens* Kunth and *Trichodesma indicum* L. were collected from Kund village in July 2013. Kund is a small village located near Union Council i.e. Nara. Nara is the Union Council of Tehsil Kahuta. Tehsil Kahuta is one of the seven tehsils of Rawalpindi District. Plants were identified from Department of plant sciences, Quaid-i-Azam University Islamabad and specimens of the collected plants were saved in herbarium (Fig. 1). The Voucher Numbers of Plants are as follows; *Adiantum incisum* 2978-ZB, *Alternanthera pungens* 1487-BA and *Trichodesma indicum* 2564-MA.

### 2.2 Extracts Preparation

Shade dried whole plants were powdered and extracted with three different solvents of distinct polarity index, water (9.0), methanol (5.1) and *n*-hexane (0.0). Aqueous, methanol and *n*-hexane extracts of *Adiantum incisum, Alternanthera pungens* and *Trichodesma indicum* were prepared by soaking plant powder in methanol and *n*-hexane respectively for 15 days. Extracts were placed in shaking incubator at 120 rpm and at temperature 25°C. Aqueous extracts were placed for seven days only to avoid fungal contamination. Solutions were filtered with single layer of gauze cloth followed by Whatman filter paper no. 41. (8 µm) Clear extracts obtained were evaporated to dryness in a rotatory evaporator (SIGMA-ALDRICH) at 30°C ± 2. Dried extracts were kept in refrigerator at 4°C for further experimental procedures.


**Fig. 1.** Specimens of three plants saved in herbarium.

### 2.3 Insecticidal Assay

Rice, wheat porridge and mung beans insects were collected from Rawalpindi city, Pakistan during the month of March, 2014. All these stored grains were domestically infected with different insects listed as Rice insects: *Tribolium castaneum* (Tenebrionidae), Wheat porridge insects: *Sitophilus oryzae* (Dryophthoridae) and Mung bean insects: *Callosobruchus chinensis* (Bruchidae). Insects were identified and reared in lab with the respective food medium (*T. castaneum* is reared on a medium consisting of wheat flour: corn meal: yeast (50: 50: 2); *S. oryzae* on whole wheat with high moisture contents i.e. more than 12%; *C. chinensis* on chickpea) at room temperature (29-31°C) in plastic bottles. Necks of the bottles were covered with gauze cloth. Insecticidal activity of aqueous, methanol and *n*-hexane extracts of *Adiantum incisum, Alternanthera pungens* and *Trichodesma indicum* was determined using impregnated filter paper assay against different mentioned insects according to the method reported [1]. Filter paper was cut according to the size of 60mm petri plates and placed inside it. Different doses of plants extracts (10, 20 and 30 mg/mL) were poured (1 mL) over the filter paper. Samples were allowed to dry and then mature adult (about 7-8 weeks old) insects were released on these filter papers. Negative controls were prepared by applying only solvents over the filter paper and set under similar conditions. Standard pesticide Permethrin (SIGMA-ALDRICH) (10 mg/mL) was used as positive control. All the petri plates (samples and controls) with insects were placed in laboratory at room temperature (25°C ± 2). Experiment was done in triplicates and the mortality reading was taken after every 24 h. It was carefully examined if recovery of insects occurs or not. Mortality was confirmed by touching a hot needle with the insect's body. Abbott's formula was used to correct the mortality percentage:

$$Pr = (P_0 – Pc / 100 - Pc) \times 100$$

Where Pr = Corrected Mortality (%)

$P_0$ = Observed Mortality (%)

Pc = Control Mortality (%)

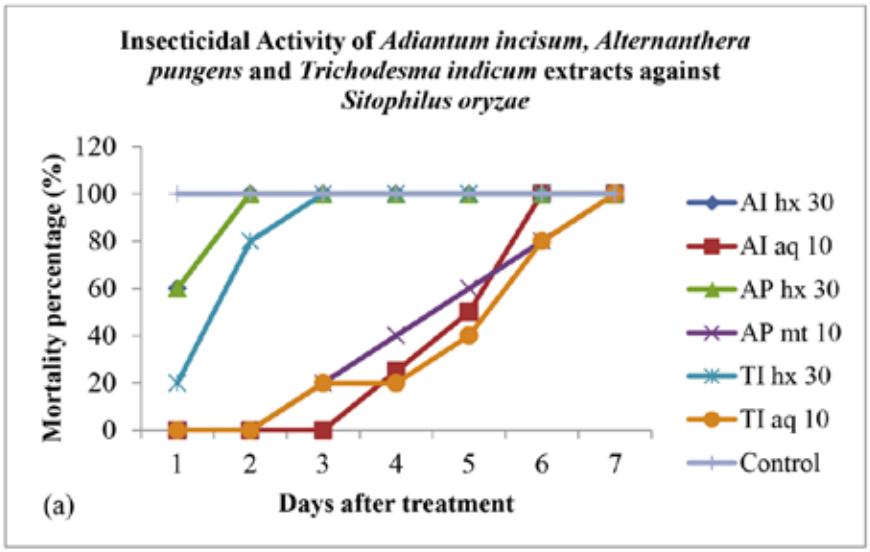
### 2.4 Fourier Transform Infrared Spectroscopy

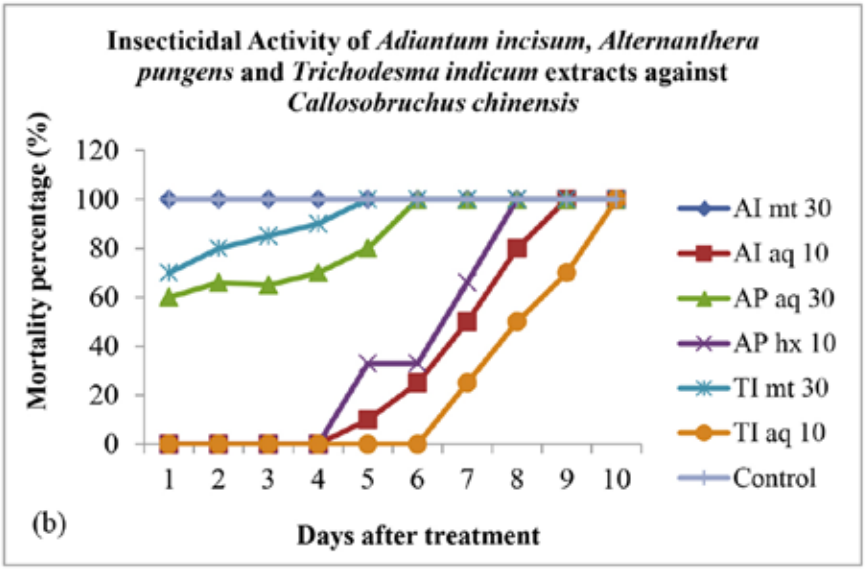
FTIR analysis was carried out by Potassium bromide pellet method as reported [11] with some modifications. FTIR analysis was done with crude plant powder of *Adiantum incisum, Alternanthera pungens* and *Trichodesma indicum* Potassium bromide and respective plant powder was mixed in a ratio of 1:100. Mixture was then placed in hydraulic press to obtain the potassium bromide pellet. Spectra of FTIR was recorded with FTIR 8400 (Shimadzu Japan) at room temperature (20 ± 3°C) at spectral range of 4000-400 $cm^{-1}$.

## 3. RESULTS AND DISCUSSION

Insecticidal assay was carried out with different domestic insects. Permethrin was used as positive control and it showed 100% mortality after 24 hours in case of all three insects. Methanol extract of *Adiantum incisum* was found to be the most effective (100% mortality after one day and $LD_{50}$ 15.3 mg/mL after 24 hours) against *Callosobruchus chinensis* while aqueous extract of *Trichodesma indicum* was least effective (25% mortality after 7 days) against the said species as shown in (Fig. 2a). Hexane extracts of all plants were effective against *Sitophilus oryzae* (Fig. 2b) as compared to their methanol and aqueous counter parts. Hexane extract of *Adiantum incisum* exhibited highest insecticidal activity (100% mortality after 2 days and $LD_{50}$ 22 mg/mL after 24 hours) among all the plant extracts against *Sitophilus oryzae*. Lowest activity was exhibited by aqueous extract of *Trichodesma indicum* (40% mortality after 4 days). Aqueous extract of *Alternanthera pungens* showed highest insecticidal potential against *Tribolium castaneum* (100% mortality after 10 days $LD_{50}$<10 mg/mL after 24 hours) with the lowest insecticidal activity shown by aqueous extract of *Trichodesma indicum* (0% mortality after 10 days). Results are shown in Fig. 2c.

FTIR was carried out with plant powder of *Adiantum incisum*, *Alternanthera pungens* and *Trichodesma indicum*. Through the analysis of FTIR data, presence of many different phyto-constituents was indicated in selected plants. Broad peaks in spectra of three plants in the range of 3146-1640 ($cm^{-1}$) indicate the presence of $NH_3^+$ in amino acids. Sharp peaks in the range of 1400-1310 ($cm^{-1}$) reveals that $COO^-$ functional group is present in all



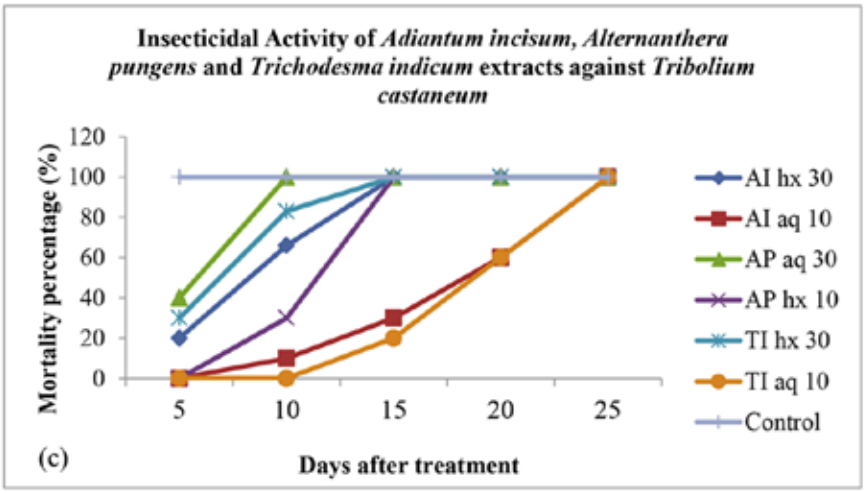




**Fig. 2.** Insecticidal activities shown by different extracts of selected plants against: (a) *Callosobruchus chinensis*; (b) *Sitophilus oryzae*; and (c) *Tribolium castaneum*. AI, AP & TI are *Adiantum incisum, Alternanthera pungens* and *Trichodesma indicum,* respectively. aq, mt and hx are aqueous, methanol and n-hexane extracts, respectively, while 10, 20 and 30 indicate doses used, i.e., 10 mg/mL – 30 mg/mL.

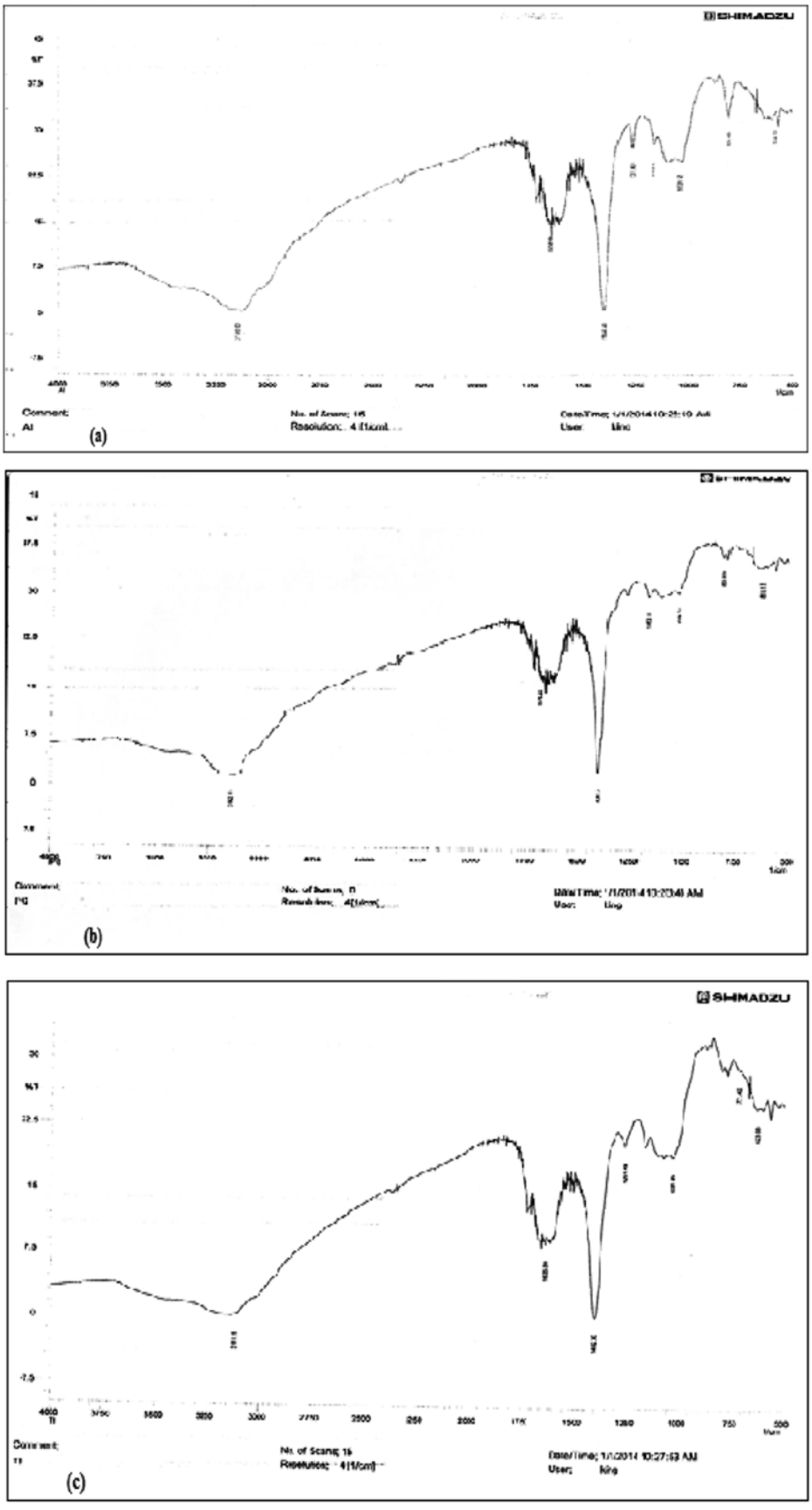



**Fig. 3.** FTIR spectra of (a) *Adiantum incisum,* (b) *Alternanthera pungens,* and (c) *Trichodesma indicum.*

**Table 1.** FTIR Interpretation for *Adiantum incisum*, *Alternanthera pungens* and *Trichodesma indicum.*

| Peak values ($cm^{-1}$) | | | Range ($cm^{-1}$) | | | Assignment of functional groups | | | Possible compounds | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A.I.*** | **A.P.** | **T.I.** | **A.I.** | **A.P.** | **T.I** | **A.I.** | **A.P** | **T.I.** | **A.I.** | **A.P.** | **T.I.** |
| 3146 | 3142 | 3111 | 3146-1640 | | | $NH^{3+}$ | | | Saponins, Alkaloids | | |
| 1700 | 1676 | 1635 | 1700-1655 | | | C=O | | | Flavonoids, Anthraquinones, Coumarins, Cardiac Glycosides | | |
| 1398 | 1400 | 1402 | 1620-1398 | | | COO- | | | Tannins | | |
| 1262 | --- | 1261 | 1300-1262 | ---- | | C-O-C | ---- | C-O-C | Saponins, Glycosides, Coumarins, Tannins | ---- | Saponins, Glycosides, Coumarins, Tannins |
| 800 | 800 | --- | 1515-420 | --- | | Benzene ring | | --- | Flavonoids, Tannins, Glycosides, Coumarins, Anthraquinones | | --- |
| --- | 550 | 550 | --- | 565-465 | | --- | C-C=O | | --- | Tannins | |

*A.I., A.P. and T.I. are *Adiantum incisum*, *Alternanthera pungens* and *Trichodesma indicum,* respectively.

plant samples. Results are shown in Table 1. FTIR spectra of three plants are depicted in Fig. 3. By comparing the basic chemical structure of different phytochemicals with the functional group revealed by FTIR spectra indicated that alkaloids, saponins, tannins, glycosides and flavonoids were present in all of the plant samples.

Plants protect themselves against the insects without side effects on environment due to active components. This forms the basis for utilizing plants as insecticidal agents. Methanol extract of *Adiantum incisum* was most effective against *Callosobruchus chinensis*. Rajashekar et al. [12] reported significant insecticidal activity of *Decalepis hamiltonii* (Apocynaceae) methanol extract against a number of insects including *Callosobruchus chinensis, Rhyzopertha domonica* (Bostrichidae), *Sitophilus oryzae, Stigobium pancieum* (Anobiidae) and *Tribolium castaneum*. Hexane extracts of all plants showed good insecticidal ability against *Sitophilus oryzae* and *n*-hexane extract of *Adiantum incisum* was most effective among the plant samples tested. Previous study suggested low activity of *Trichodesma indicum* ethanol extract against *Tribolium castaneum* [13] which is in agreement with our present study. Amongst all the three plants, *Adiantum incisum* has the highest insecticidal activity, followed by *Alternanthera pungens* and *Trichodesma indicum* has shown lowest insecticidal potential.

No previous study has been reported for FTIR analysis of any of these plants. Many reports are available where FTIR has been employed for detecting phytoconstituents in different plant samples, e.g., Saxena and Saxena [12] reported the presence of flavonoids and phenols in plant *Acorus calamus* (Acoraceae) on the basis of FTIR analysis. FTIR and UV-Vis was performed on *Bougainvillea Glabra* Choisy (Nyctaginaceae) which indicated phenols and flavonoids in this plant species [14]. FTIR was also carried out for medicinal herb *Warburgia ugandensis* (Canellaceae) which showed the presence of various functional groups and these functional groups were attributed to the presence of various phytochemicals including alkaloids, saponins, tannins, flavonoids, steroids and terpenes, polyphenols and cardiac glycosides in this plant. These studies reflect FTIR as a potent tool for phytoconstituents detection. FTIR analysis of three plants reveals that *Adiantum incisum* is richest in terms of phytoconstituents with different peaks conferring presence of important phytochemicals including saponins, coumarins and glycosides which might account for the good insecticidal activity shown by this plant. Besides the possible compounds revealed by FTIR, some unknown compounds can also be attributed to the significant insecticidal activity

## 4. CONCLUSIONS

FTIR spectra of plants indicated presence of important phytoconstituents saponins, alkaloids,

tannins and flavonoids in all three plants. While insecticidal assay revealed that methanol extract of *Adiantum incisum* was found to be most effective against *Callosobruchus chinensis*. Hexane extracts of all plants showed good insecticidal ability against *Sitophilus oryzae.* Extracts of *Alternanthera pungens* were found to be most active against *Tribolium castaneum*. For long-term effectiveness of these extracts especially against internal feeders, further experiments within the presence of food-medium are required.

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