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FT-IR Analysis of *Moringa oleifera* L. Leaf Extract and its Insecticidal activity against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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ABSTRACT: The objective of this study was to investigate the insecticidal properties of *Moringa oleifera* leaves with identification of chemical compounds within its extract using Fourier Transform Infrared Spectroscopy (FTIR). The research involved scanning the leaves' extract across a spectral range of 4000-400 cm^{-1} and identifying characteristic peaks in the FTIR spectrum. Spectral analysis confirmed the presence of several important chemical groups, including carboxylates, phenols, polyphenols, and hydroxyl groups within the extract. These compounds are known for their bioactive properties, which may contribute to the insecticidal effects observed in the study. In our experiment, notable results were obtained while assessing insecticidal efficacy of the *Moringa oleifera* leaves. The highest mortality rate of 100% was observed when dried leaves at concentrations of 10% and 20% were used against the targeted insect *Callosobruchus chinesis* infestation within a 48-hour timeframe. In contrast, lower mortality percentages of 38% and 72% were recorded when 5% and 10% dried leaves were used for the same duration of insect exposure. These results suggest that *Moringa oleifera* leaves contain bioactive compounds with insecticidal properties, as confirmed by FTIR analysis and insecticidal bioasssay. Further it suggests that botanicals serves as eco-friendly and sustainable insecticidal alternatives under storage conditions.

Keywords: *Moringa oleifera*, FTIR spectroscopy, Pulse beetle, insecticidal activity, Functional group.

INTRODUCTION

Moringa oleifera, commonly referred to as drumstick, containing diverse array of chemical constituents within its distinct plant parts. It belongs to Moringaceae family. It is extensively cultivated in India, Indonesia, Philippines, Africa, and various other parts of the world. Among the approximately 13 species in the *Moringa* genus found in tropical and subtropical regions, *M. oleifera* stands out as the most commonly cultivated species (Tayade *et al.*, 2022). This plant is well-known for its nutritional richness, the plant's leaves, seeds, flowers, and roots are utilized for various purposes (Padayachee and Baijnath 2012). It has been consumed as a food, fodder, medicines and several industrial applications (Fahey, 2005; Iqbal and Bhanger 2006). Moreover, owing to its economic significance, it also called "Miracle tree." Beyond its nutritional value, *M. oleifera* is recognized as a potent medicinal resource with efficacy in treating various ailments, including diabetes, cancer, and insomnia (Adebayo *et al.*, 2018). Additionally, it serves as a repository of bioactive chemical compounds, encompassing alkaloids, carotenoids, isothiocyanates, tannins, vitamins, polyphenols, phenolic acids, flavonoids, and saponins. The presence of these diverse compounds contributes to its extensive pharmacological properties (Leone *et al.*, 2015; Saini *et al.*, 2016; Mbikay, 2012; Saranya *et al.*, 2023).

Pulse beetles, *Callosobruchus sp.* belongs to family Bruchidae of order Coleoptera. It is cosmopolitan pests and widely distributed throughout the tropical and sub-tropical region (Giga and Smith, 1983). In India, 3 species of pulses beetle commonly found i.e. *C. chinensis*, *C. maculatus* and *C. analis* (Pruthi and Singh 1950). Amongst them, *C. chinensis* is considered to be the most economically damaging storage pest (Ahmed *et al.*, 2003). In developing countries, pulse production systems face about 20–25% post-harvest losses due to this pest (Maneepun, 2000). The grubs of pulse beetles pose great threat to legume seeds as they diminish both their weight and nutritional quality. These beetles infest grains from the field to storage through concealed infestations that are challenging to identify before storage. Since Pulses are rich source of protein, secondary invaders or intruders such as mites also start infesting fractured kernels and causes direct risk to human health by contaminating food and indirectly by mycotoxin (Maheshwari, 2023). For the management of these beetles, chemical fumigants and dusts are proven

to be effective in controlling (Kumari and Yadav 2021), but their application is impractical at the farm and household levels. When storing food grains for consumption, the use of pesticides may also carry the risk of harm to vertebrates (Bekele *et al.,* 1995). Injudicious pesticide usage harms the environment, disrupts the food chain through biomagnifications, and also leads to development of resistance in stored grain pests (Rajendran, 2003). Chemical insecticides, although frequently employed, come with adverse impacts on the environment and consumers, disrupting the delicate balance of ecosystems. Recognizing these concerns, botanical solutions emerge as a promising alternative ecofriendly approach for pest management (Morya and Kumar 2021).

Spectroscopic techniques play a crucial role in analyzing the structures of chemically related systems by examining the interactions between light and matter. An array of spectroscopic methods, such as vibration and fluorescence spectroscopy, operating across diverse spectral ranges, serve as sensitive tools for extracting chemical information and determining structural and physicochemical properties. These techniques primarily involve assessing the energy of radiation absorbed or emitted by molecules (Movasaghi *et al.,* 2008; Jain *et al.,* 2016). Fourier Transform Infrared (FT-IR) spectroscopy, a branch of vibration spectroscopy, is a non-destructive method that measures fundamental molecular vibrations. This versatile technique finds application in the analysis of gases, liquids, and solids (Rébufa *et al.,* 2015; Joshi *et al.,* 2019). Past studies utilized to study moringa powder to assess diverse parameters, including mineral content, protein levels, moisture content (Rébufa *et al.,* 2015) and sorption potential (Araújo *et al.,* 2010). The present study combines FTIR analysis with insecticidal bioassays, shedding light on the chemical compounds present in the *M. oleifera* leaf extract and their role in combating insect infestations.

METHODOLOGY

A. Preparation of M. Oleifera leaves Extract and FTIR spectral measurements

M. oleifera leaves were collected. The collected leaves were washed thoroughly under running water and then washed twice with double distilled water to remove dust particles and then air dried at room temperature to remove residual moisture. 20 g dried leaves were boiled in 100 ml distilled water in an Erlenmeyer flask, and then cooled at room temperature. The extract was centrifuged at 3000 rpm for 10 min and filtered via Whatman filter paper no.1. The obtained extract was characterized by FTIR.

FTIR works on the principle of absorption and emission of infrared radiation to obtain infrared spectrum of any solid, liquid or gas. It was used to identify the characteristic functional groups present in the extract. A small quantity of *M. oleifera* leaves extract was used for mid-IR spectrum with Bruker, Germany infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1}. The peak values of the FTIR were recorded.

B. Assessment of insecticidal efficacy of M. oleifera leaves against C. chinesis

The research on insecticidal activity was carried out with laboratory culture of *C. chinesis*, obtained from already infested cowpea seeds. The culture was maintained in jar under an ambient temperature of 28 ± 3°C and 70 – 75 % relative humidity. The insect culture was maintained as described by Adenekan and Shosanya (2006). The untreated seeds were cleaned and sorted according to sizes as checked by their weights. These were kept in the refrigerator at a temperature of 10°C in order to prevent insect infestation until they were needed for the experiment. The *moringa* leaves were collected and air-dried. The untreated cowpea seeds kept in the refrigerator were taken out and allowed to acclimatize for about twenty-four hours under ambient laboratory condition before being used. 100 g of seeds were placed in each jar. There were six treatments replicated four times. Each treatment consists of 1, 2, 5, 10 and 20 percent dried leaves of *moringa* and untreated cowpea seeds served as the control. 20 adult of *C. chinesis* less than 12 hours old were introduced in each jar. Data were collected on the percent mortality after infestation at 8, 12, 24, 36 and 48 hours.

RESULT AND DISCUSSION

The mid-IR spectrum (4000-400 cm^{-1}) based on transmission versus Wavenumber of *M. oleifera* leaves extract indicated in Fig. 1. The Mid-IR spectra is divided into 4 regions which includes (A) The single Bond region (2500-4000 cm^{-1}) (B) The double Bond region (1500-2000 cm^{-1}) (C) The triple Bond region (1500-600 cm^{-1}) (D) the finger print region (1500-600 cm^{-1}). The functional groups and its quantified frequencies were studied based from references (Nandiyanto *et al.,* 2019; Coates, 2000). The results can be concluded as follow:

(a) The spectrum consists of more than five absorption bands which indicate analysed chemical is not a simple material.

(b) The sharp absorption peak at 3248.14 cm^{-1} is due to the stretching of hydroxyl groups that are present in the extract.

(c) No specific peak for aldehyde has been found at between 2700and 2800 cm^{-1}.

(d) In triple Bond region (2500- 2000 cm^{-1}) peak at 2117.38 indicated C≡C bond in the Extract.

(e) In double Bond region (1500-2000 cm^{-1}), there is a number of bands at 1866.95, 1749.32, 1643.35, 1560.04 and 1523.49 cm^{-1}.

(e) Peak at 1643.35 cm^{-1} is due to C=C stretching associated with alkene or presence of Amide group (1680-1630 cm^{-1}) or secondary amine >N-H band (1650-1550 cm^{-1}).

(f) Peak at 1749.32 indicated presence of carbonyl compound which may be due to Ester (1750-1725 cm^{-1}) or Alkyl Carbonate (1760-1749 cm^{-1}).

(g) Peak at 1560.04 indicates presence of Carboxylate (1610-1550 cm^{-1}) in the leaves Extract.

(h) Peaks at 1523.49 and 1866.95 indicates presence of Aliphatic nitro compounds (1560-1540 cm-1) and five membered ring anhydride (187—1820 cm^{-1}) respectively.
(i) In the fingerprint region (1500-600 cm^{-1}) peaks are present at 1396.67, 1367.69, 1158.08 and 1088.87 cm^{-1}.
(j) Peak at 1158.08 and 1088.87 indicates aromatic C-H in planar bend (1225-950 cm^{-1}). Peak at 1088.87 might be peak due primary amine, CN stretch.
(k) 1396.67 and 1367.69cm^{-1} indicates presence of Phenol or tertiary alcohol, OH bend (1410-1310 cm^{-1}).

The results of the insecticidal activity of *M. oleifera* leaves were recorded (Table 1). The insecticidal activity was examined over varying time intervals at different concentrations of dried *M. oleifera* leaves. The following results were observed: When a 1% concentration of air-dried *M. oleifera* powder was used, no mortality among the target insects was recorded after 8 hours of exposure. However, at subsequent time intervals (12, 24, 36, and 48 hours), mortality rates of 2%, 8%, 14%, and 28% were observed, respectively. For a 2% concentration, no immediate mortality occurred within the initial 8 hours. Nevertheless, mortality rates increased progressively, with 6% at 12 hours, 14% at 24 hours, 26% at 36 hours, and 38% at 48 hours. At 5% concentration, 4% mortality was noted after 8 hours. Subsequently, mortality rates increased substantially to 14% at 12 hours, 32% at 24 hours, 56% at 36 hours, and 72% at 48 hours. At 10% concentration of *M. oleifera* leaves significant results were noticed. Mortality rates were 12% at 8 hours, 30% at 12 hours, 44% at 24 hours, and 68% at 36 hours were observed, with a remarkable 100% mortality achieved at the 48-hour mark. The highest concentration tested, which was 20% air-dried *M. oleifera* leaves, produced mortality rates of 18% at 8 hours, 40% at 12 hours, 60% at 24 hours, and 74% at 36 hours, reaching 100% mortality after 48 hours of exposure. In the control group, which did not receive any *M. oleifera* treatment, no mortality was observed at the 8 and 12-hour checkpoints. However, as time progressed, mortality rates gradually increased, with 4% at 24 hours, 8% at 36 hours, and 14% at 48 hours, indicating the natural progression of insect infestation without treatment. These findings provide valuable insights into the time-dependent insecticidal effects of different concentrations of air-dried *M. oleifera* leaves and underscore its potential as a natural and effective means of insect pest control.

The present finding agrees with Madukwe *et al.* (2012), who pointed out that *M. oleifera* can be used as a biopesticide due to the leaves being completely safe for consumption and having no known negative side effects or toxic constituents. Similar results were obtained by Adenekan *et al.* (2013), when *M. oleifera* leaves extract were used against *C. maculatus* showed 85 percent mortality within 24 hours of infestation. Owolabi *et al.* (2014) reported that Insecticidal activity of essential oil extracted from *Morinda lucida against C. maculatus.* Mohammed and Iddriss (2022) reported that cowpea seeds treated with neem leaf powder tasted bitter, due to the presence of an active ingredient known as azadirachtin, causing the seeds to taste bitter. Hence, moringa leaf powder was preferred, since it does not cause negative affect the taste of the seeds after storage. Loebel (2002) demonstrated antioxidant activity of leaves of *Moringa oleifera* was due to their high amounts of polyphenols. It prevents oxidative damage to major biomolecules and gives significant protein protection against oxidative damage. Similarly, Anita *et al.* (2012) claimed that the seeds of the moringa contain coagulant lectin and the crushed leaves of the plant can kill insects by disrupting digestion and causing moulting.

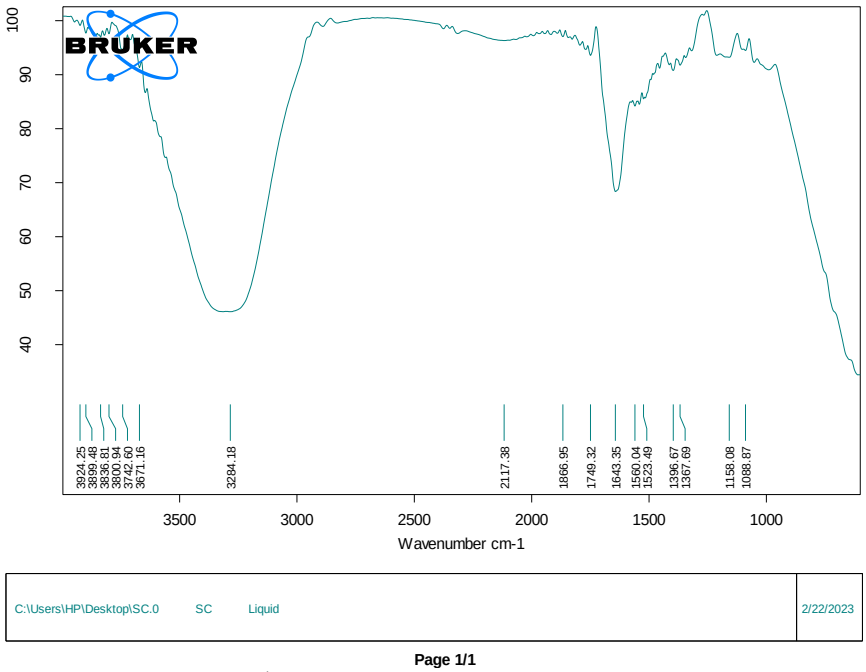

Fig. 1. Mid-IR spectrum (4000-400 cm^{-1}) based on transmission versus Wave number of *M. oleifera* leaves extract.

Table 1: Mortality after infestation of Pulse beetles treated with *M. oleifera* dried leaves.

% Dried Leaves	% Mortality after infestation				
	8 hrs	12 hrs	24 hrs	36 hrs	48 hrs
1%	0	2	8	14	28
2%	0	6	14	26	38
5%	4	14	32	56	72
10%	12	30	44	68	100
20%	18	40	60	74	100
Control	0	0	4	8	14

CONCLUSIONS

In conclusion, the present study provides compelling evidence for the insecticidal potential of *M. oleifera* leaves, reaffirming their value as a biopesticide. The research combined FTIR analysis with insecticidal bioassays, shedding light on the chemical compounds present in the *M. oleifera* leaf extract and their role in combating insect infestations. The FTIR spectra confirmed the presence of bioactive chemical groups such as carboxylates, phenols, polyphenols, and hydroxyl groups, which are recognized for their insecticidal properties. The bioassay results revealed that the effectiveness of moringa leaves is both concentration and time-dependent. The highest mortality rate of 100% was achieved with 10% and 20% dried leaf concentrations within 48 hours, signifying the potent insecticidal effects of these extracts. Furthermore, the moringa leaves do not compromise the taste of stored seeds, making them a practical choice for pest management in food storage as an eco-friendly and sustainable alternative for insect pest control. Moreover, the study emphasizes the antioxidant properties of *M. oleifera* leaves, attributed to their high polyphenol content, which helps protect vital biomolecules and proteins from oxidative damage. In light of these findings, Moringa holds promise as a sustainable and eco-friendly solution in the ongoing quest for effective insect pest control under storage conditions.

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Conflict of Interest. None.

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