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Research article

Evaluation of five medicinal plants for the management of *Sitophilus oryzae* in stored rice and identification of insecticidal compound

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ABSTRACT

Sitophilus oryzae is a kind of stored grain pest. This is controlled by using natural pesticides, which are more reliable, cost-effective, biodegradable, and eco-friendly than synthetic pesticides. Several plants show different insecticidal activities against various pests on their different parts (leaves, seeds, etc.). In this study, methanolic extracts of *Lantana camara* (leaves), *Carica papaya* (seeds), *Ricinus communis* (leaves), *Calotropis gigantea* (flowers), and *Gliciridia sepium* (leaves) were used to identify the best insecticidal activity against the rice weevil by doing mortality tests for one week with four replications under laboratory conditions. *Gliciridia sepium* leaves showed the highest insecticidal activity (100 ± 0) after seven days, and its extract was fractionated by using column chromatography and yielded 12 fractions. A contact bioassay of each fraction was performed, and fraction-11 showed the highest insecticidal activity against *Sitophilus oryzae* with a 100 % mortality after four days. Fraction-11 was analyzed by using GC-MS and FT-IR. Results revealed that the major constituent identified in fraction-11 was 4-C-methyl-myo-inositol. Therefore, 4-C-methyl-myo-inositol acts as a natural insecticide against rice weevils.

1. Introduction

Stored grains in silos and granaries are affected by insect infestation which in turn causes an extensive damage in wheat, rice and other grains. This can ultimately account in the loss of half of the harvest production, resulting in a huge financial loss [1]. Therefore, storage of grains under safety conditions is considered one of the most important stages of its distribution and export abroad. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is among the most widespread and destructive pests of stored cereals such as rice [2]. This is controlled by using natural pesticides since they are more suitable than synthetic ones due to their being biodegradable, eco-friendly, having no persistent properties, being cost-effective, and not being resistant.

The various parts of medicinal plants such as stems, flowers, leaves, and roots are environmentally friendly and an alternative for controlling pests [3–8]. These are capable of killing pests, inhibiting their growth, reproduction, and development [9]. Pyrethrum

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flowers [10], and neem leaves, bark, seeds and roots [11] are used to control mosquitoes, flies, and many other pests. Similarly, leaves of *Lantana camera*, *Ricinus communis*, *Gliciridia sepium*, seeds of *papaya carica*, and flowers of *Calotropis giganta* are shown to have insecticidal activity against the rice weevil at a different rate [12–14]. The highest insecticidal activity had been proven by contact mortality for one week at 4 triplications with control as methanol [15]. So, medicinal plants are alternatives to synthetic insecticides, and studies have focused more on natural organic pesticides made mainly from plant extracts that are cheap, eco-friendly, and non-toxic to humans [16].

In continuation to our previous study on insecticidal effect of Sri Lankan medicinal plants against *Sitophilus zeamais* Mostschulsky in stored maize [17], methanolic extracts of *Lantana camara* leaves, *Carica papaya* seeds, *Ricinus communis* leaves, *Calotropis gigantea* flowers, and *Gliciridia sepium* leaves were checked against *Sitophilus oryzae* in stored rice. However, *Gliciridia sepium* leaves showed the highest insecticidal activity (100 $\pm$ 0) after seven days. The extract of *Gliciridia sepium* leaves was subjected to column chromatography [18,19] and produced 12 fractions. Each fraction was analyzed by thin-layer chromatography (TLC), since TLC provides qualitative information that is useful for predicting the quantitative details [20]. Fractions 1–12 were tested for their insecticidal activities against *Sitophilus oryzae*. However, fraction-11 showed the highest insecticidal activity against *Sitophilus oryzae* with a 100 % mortality after four days. Fraction-11 was subjected to GC-MS and FT-IR analyses for identification purposes, and their data indicated its major compound which was identified as 4-C-methyl-myo-inositol. Therefore, 4-C-methyl-myo-inositol was suggested to act as a natural insecticide against rice weevils.

2. Materials and methods

2.1. Collection and preparation of plant materials

Fresh, mature plant parts from leaves of *lantana camera*, *Ricinus communius*, *Gliciridia sepium*, flowers of *Calotropis giganta*, and seeds of *Carica papaya* were collected in Oluvil, Sri Lanka (latitude/longitude: 7.28°/81.87°). Then they were washed with tap water and air dried under shaded conditions in air-conditioned room at 25 °C (external temperature 30–31 °C and humidity 77–80 %) for 60 days. Then dried plant materials were pulverized well into powder by electric grinder and stored in airtight bottles till further use.

2.2. Extraction and concentration of plant extracts

Powder materials were soaked in methanol in a 1:10 (w:v) ratio for 24 h. Homogenization of mixing was done by using an ultrasound-assisted sonicator for 30 min. After that, we filtrated the solutions by using (what man's number one) filter paper. Extracts were concentrated by using a rotary evaporator at 45 °C and 70 rpm (Thermo Fisher Scientific, electrically heated with heating bath with facility for raising and lowering the height). The dried, concentrated crude extract was obtained for each plant material and stored at 4 °C until further use.

2.3. Stock solution (1000 ppm) preparation

The crude extract (50 mg) was dissolved in 50 mL of MeOH and used to prepare a 1000 ppm stock solution.

2.4. Rearing of Sitophilus oryzae

Parent stock of *Sitophilus oryzae* was collected from rice stores in the Sammanthurai area, in the eastern province of Sri Lanka (latitude/longitude: 7.36°/81.80°), identified the male and female by using their morphological characters, which are observed under a stereomicroscope.

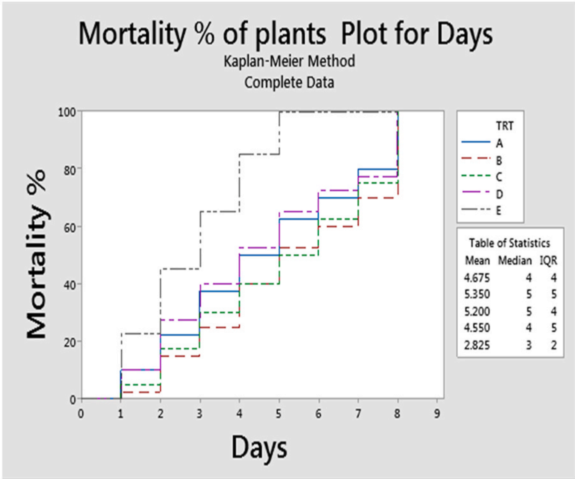
New insect generations were cultured by using 50 g of clean, disinfected rice samples in 4 jars with 20 unsexed adults in each jar. They were allowed to culture the new generations under laboratory conditions (25 $\pm$ 1 °C and 50–60 % RH), then it covered the mouths of the jars with aluminum foil by using the rubber band. Some holes were made in the aluminum foil to allow air. New generations were maintained throughout the experiment.

2.5. Contact bioassay

10 g of disinfected rice samples were poured into Petri dishes, then 10 mL of stock solutions were added to petri dishes, and the methanol was allowed to evaporate for 24 h. Rice samples were treated with methanol. After 24 h, 10 unsexed, one-week-old insects of Sitophilus oryzae were added to Petri dishes and covered with aluminum foil using the rubber band. There were 5 replications, including a control for each extract. The number of dead insects was counted every 24 h for one week [15].

A similar procedure was followed for the *Gliciridia sepium* leaf fractions. MeOH was used as a control.

$$\text{Mortality }\% = \frac{\text{Number of dead insects X }100\%}{\text{Number of total insects}}$$



TRT A: Leaves of *Lantana camara*
B: Seeds of *Carica papaya*
C: Leaves of *Ricinus communis*
D: Flowers of *Calotropis gigantea*
E: Leaves of *Gliricidia sepium*

Fig. 1. A plot of mean percentage of mortality of plants versus days.

2.6. Thin-layer chromatography (TLC) analysis

Silica gel precoated plates (Merck, PF_{254}; 20 × 20, 0.25 mm) were used for TLC. The crude extract was analyzed by TLC using 1:1 ethyl acetate: methanol as a mobile phase.

2.7. Separations by using chromatography techniques

A dried crude extract of *Gliciridia sepium* (30 g) was dissolved in a minimum amount of methanol and mixed with 50 g silica gel (70–230 mesh, Merck) by using a rotary evaporator. Dried crude was subjected to the column by using silica gel as the stationary phase and increasing the polarity of the solvent system as the mobile phase. The fractionation of crude was based on elute colour and TLC interpretation. 12 Fractions were obtained with increasing polarity of solvent, starting with hexane in combination with ethyl acetate. Afterward, increased polarity was obtained by combining methanol with ethyl acetate. The TLC system was used to separate the elutes, and it was performed by using the solvent systems hexane: ethyl acetate and ethyl acetate: methanol with their different ratios. Then TLC was observed under UV light [19].

2.8. Samples preparation from fractions 1-12

Each fraction (50 mg) was dissolved in 50 mL of methanol to get a 1000 ppm test solution.

2.9. Crystallization

The crystallization solvent must be immiscible, dissolve the compounds when heated, be volatile, non-toxic, and not flammable. After that, recrystallization characterization of the compound was performed using FT-IR and GC-MS analysis.

2.10. FT-IR and GC-MS analyses

Infrared (IR) spectrum was recorded in KBr disc on a FT-IR-8900 spectrophotometer, while GC-MS analysis was carried out in a combined 7890A gas chromatograph and mass spectrometer with EI ion source (single quadrupole, Agilent technologies).

2.11. Analysis of data

All the data were subjected to survival analysis using Minitab software at a 5 % level of significance.

1. Kaplan Meier curve - Used to estimate the % of survival/mortality at each time.
2. Log-rank test - Assessed if curves differ significantly (α −5%).

Table 1
Contact Insecticidal effect of different in methanol extracts on *Sitopholus oryzae* seven days.

Medicinal Plant	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Lantana camara	10.00 ± 4.73	20.00 ± 6.32	35.00 ± 7.54	45.00 ± 7.87	55.00 ± 7.86	62.50 ± 7.65	80.00 ± 6.32
Carica papaya	2.23.± 5.26	15.00 ± 5.65	25.00 ± 6.80	40.00 ± 7.75	52.50 ± 7.89	60.00 ± 7.75	70.00 ± 7.25
Ricinus communis	5.00 ± 3.45	17.5 ± 6.00	30.00 ± 7.25	40.00 ± 7.75	50.00 ± 7.91	62.5 ± 7.65	76.0 ± 6.85
Calotropis gigantea	10.0 ± 4.74	27.5 ± 7.06	40.0 ± 7.75	50.0 ± 7.91	65 ± 7.54	72.5 ± 7.06	76.0 ± 6.85
Gliricidia sepium	20.0 ± 6.32	40.0 ± 7.75	60.0 ± 7.75	77.5 ± 6.60	100 ± 0	100 ± 0	100 ± 0
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Mean percentage contact mortality ± SE for five replicates.
Concentration (1000 ppm)..

3. Results and discussion

3.1. Determination of mortality among those five medicinal plants

3.1.1. Kaplan meir curve

Mortality reached 100 % in the leaves of *Gliricidia sepium* after 5 days (Treatment E). The lowest mortality of 70.0 ± 7.25 % was observed in seeds of *Carica papaya* (Treatment B) after 7 days. Insecticidal activity order among those five medicinal plants was, *Gliciridia sepium* > *Lantana camera* > *Galotropis gigantea* > *Ricinus communis* > *Carica papaya* (Figure-1).

3.1.2. Log-rank test

3.1.2.1. With Gliricidia sepium

Test Statistics			
Method	Chi-Square	DF	P-Value
Log-Rank	38.2506	4	0.000

H0. :No differences between mortality curves.

H1. :There are differences between mortality curves.

$P < \alpha$ so Reject H_0

Therefore 5 % significantly confirmed that there were differences between the mortality curves of five medicinal plants.

3.1.2.2. Without Gliricidia sepium

Test Statistics			
Method	Chi-Square	DF	P-Value
Log-Rank	2.41368	3	0.491

H0:. No differences between mortality curves.

H1:. There arze differences between mortality curves.

$P > \alpha$ Can't reject H_0.

Therefore 5 % significantly confirmed that there were no differences between the mortality curves of five medicinal plants.

When comparing the insecticidal activity of those five medicinal plants (Table 1), *Gliricidia sepium* showed the highest insecticidal activity against *Sitophilus oryzae*. Already, this plant material has been identified as an insecticide plant [21]. By using statistical analysis, this *Gliricidia sepium* showed significantly different insecticidal properties when compared with other plant materials. However, when considered without *Gliricidia sepium*, they didn't show any significant differences among themselves.

In this study, we had been looking for some medicinal plants around the garden area of our village. By looking at those plant materials, including literature reviews and collected plants, In the *Lantana camera*, dodecanol, pyrrolizin, 1-docosene, and nonadecene showed insecticidal activity [22]. Methanolic leaf extracts of *L. camara* are highly polar, and due to this, they exhibits better efficiency in extracting various polar phytol compounds. Leaf extracts of *L. camara* show antibacterial, antifungal, antioxidant, antiviral, allelopathic, and other properties and activities [23]. In the seed extracts of *Carica papaya*, the polyhydroxy amide is an active component. Researchers have already demonstrated that papaya seed aqueous extract has larvicidal activity against Aedes

Table 2
Contact Insecticidal effect of fractions 1–12 (concentration 1000 ppm) from *Gliricidia sepium* on *Sitopholus oryzae* in seven days.

Fractions	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
F1	7.5 ± 4.60	17.5 ± 6.00	27.5 ± 7.06	42.5 ± 7.82	57.5 ± 7.82	72.5 ± 7.06	85 ± 5.65
F2	10 ± 4.74	20 ± 6.32	32.5 ± 7.41	42.5 ± 7.82	52.5 ± 7.90	65.0 ± 7.54	80.0 ± 6.34
F3	2.50 ± 2.47	10.0 ± 4.74	17.5 ± 6.00	27.5 ± 7.06	37.5 ± 7.65	50.0 ± 7.91	62.5 ± 7.65
F4	5.0 ± 3.45	10.0 ± 4.74	17.5 ± 6.00	25.0 ± 6.85	35.0 ± 7.54	50.0 ± 7.91	65.0 ± 7.54
F5	7.50 ± 4.16	15.0 ± 5.65	22.5 ± 6.60	32.5 ± 7.41	45.0 ± 7.87	60.0 ± 7.75	75.0 ± 6.85
F6	7.5 ± 4.16	12.5 ± 5.23	17.5 ± 6.0	25.0 ± 6.85	32.5 ± 7.41	42.5 ± 7.82	52.5 ± 7.89
F7	12.5 ± 5.23	25.0 ± 6.85	40.0 ± 7.75	55.5 ± 7.87	67.5 ± 7.41	82.5 ± 6.00	97.5 ± 2.47
F8	15.0 ± 5.65	30.0 ± 7.25	47.5 ± 7.90	62.5 ± 7.66	82.5 ± 6.01	100 ± 0	100 ± 0
F9	12.5 ± 5.23	25.0 ± 6.85	37.5 ± 7.65	55.0 ± 7.87	70.0 ± 7.25	87.5 ± 5.23	100 ± 0
F10	17.5 ± 6.0	35 ± 7.54	50.0 ± 7.91	67.5 ± 7.41	82.5 ± 6.0	82.5 ± 6.00	100 ± 0
F11	22.5 ± 6.61	45 ± 7.87	70.0 ± 7.25	100 ± 0	100 ± 0	100 ± 0	100 ± 0
F12	17.5 ± 6.00	37.5 ± 7.65	55.0 ± 7.87	70.0 ± 7.25	85.0 ± 5.65	100 ± 0	100 ± 0
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

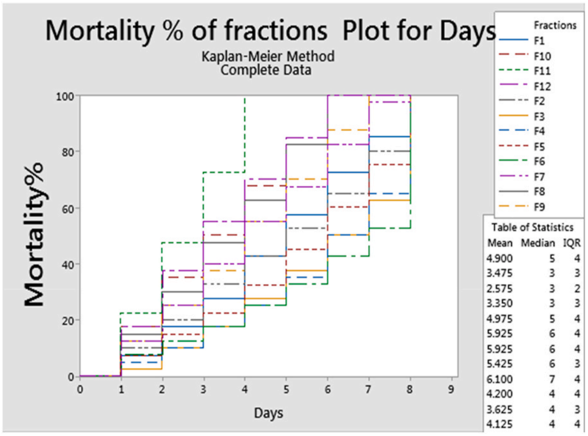


Fig. 2. A plot of mean percentage of mortality of fractions versus days.

aegypti. Phytochemical constituents such as flavonoids, alkaloids, and tannins are the reason for larvicidal activity in the seeds and peels of *C. papaya* [24]. An aqueous leaf extract of *R. communis* has excellent insecticidal activity with flavonoids content. Quercetin is the major insecticide compound within flavonoids [25]. Methanolic extracts of the seeds and leaves of *R. communis* have shown insecticidal activity. The compound responsible for the insecticidal property in this case is ricin [26]. *C. gigantea* root bark showed the highest insecticidal activity in a methanolic extract. The probable existence of flavonoids, terpenoids, phytosterols, and tannins in the leaf extract of *C. gigantea* showed the toxic effect on Herbst [27]. Due to the presence of phytochemicals in *C. gigantea* flowers they are effective against the mealy bug. Syringic acid, syringaldehyde, and hydroxybenzoic acid are the responsible and effective biopesticides against the mealy bug [28]. Leaf extracts of *G. sepium* show larvicidal, ovicidal, and pupicidal activity against the malarial vector [18, 21]. Leaf extract of *G. sepium* has the potential to be used as a bioinsecticide for papaya mealy bugs [29].

3.2. Determination of mortality among those 12 fractions

3.2.1. Kaplan- meir curve

Mortality became 100 % in F11 of *Gliricidia sepium* after 4 days.

The Lowest mortality of 52.5 ± 7.89 % was observed in fraction-6 (F6) of *Gliricidia sepium* after 7 days, while the highest mortality of 100 ± 0 was observed in fraction-11 (F11) after 7 days (Table 2). The insecticidal Activity order of fractions was, F11> F12 > F10 > F8 > F9 > F7 > F1 > F2 > F5 > F4 > F3 > F6 (Figure-2).

3.2.2. Log-rank test

Test Statistics			
Method	Chi-Square	DF	P-Value
Log-Rank	150.386	11	0.000

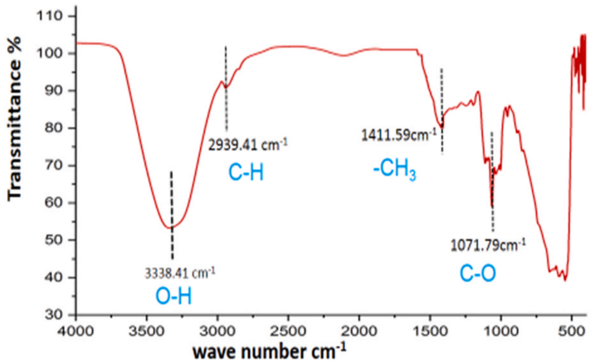


Fig. 3. FT-IR spectrum for fraction-11.

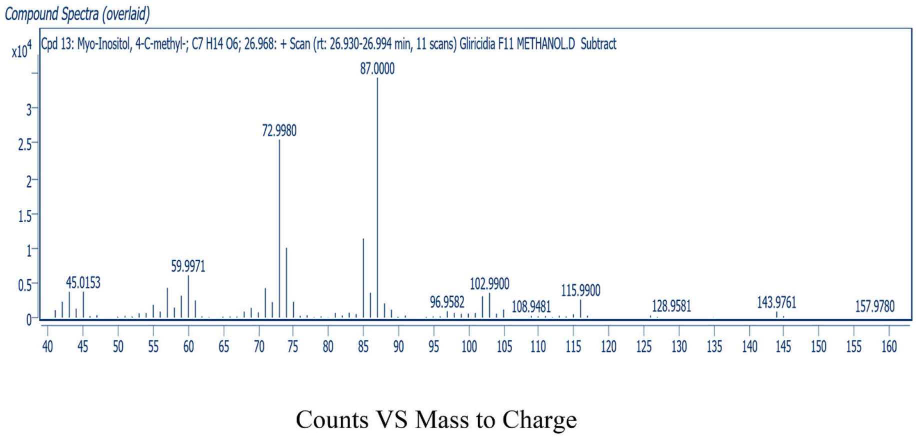


Fig. 4. Compound spectra of 4-C-methyl-myo-inositol.

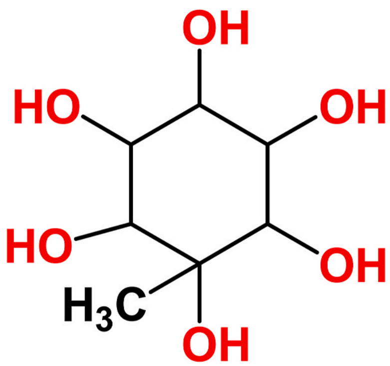


Fig. 5. Chemical structure of 4-C-methyl-myo-inositol.

H0:. No differences between mortality curves.

H1:. There is differences between mortality curves.

$P < \alpha$ Reject H_0 .

Therefore 5 % significantly confirmed that there were differences between mortality curves of 12 fractions.

In column chromatography, a gradient solvent system was used and started with a hexane/ethyl acetate composition. Because it's standard, good for ordinary compounds, and best for difficult separations. Using cotton wool to avoid disturbing the sample. Silica gel is the stationary base, and when using it, wear glasses because it causes silicosis. Fractionation of that particular plant extract based on

Table 3
Compound summary datasheet.

	Name	Cpd	Hits	Mass
472–95–7	4-C-methyl-myo-inositol	13	5	194.0790

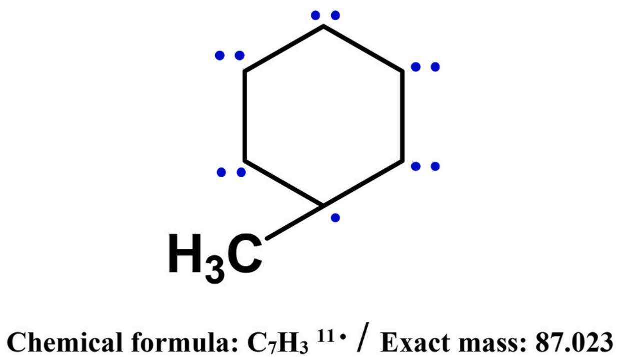


Fig. 6. Chemical structure of Base peak compound.

that polarity. Within that, several compounds are presented. By using that gradient solvent system, highly polar fraction F12 obtained 80%–100 % MeOH with EtOAc. However, the highest insecticidal activity fraction, F11, was collected at 60%–80 % MeOH with EtOAc. Details of fractions concerning solvent combination are available in supplementary material.

3.3. FT-IR analysis

The FT-IR spectrum of fraction-11 showed four peaks (Figure-3) at 3338.14 cm^{-1}, 2939.41 cm^{-1}, 1411.59 cm^{-1}, 1071.79 cm^{-1} for O–H bond, C–H bond stretching, -CH3 bending, and C–O stretching, respectively. The very broad and strong peak at 3338.41 cm^{-1} is for the alcoholic O–H group. Next, a very small peak at 2939.41 cm^{-1} for the C–H bond in alkane groups. At 1411.59 cm^{-1}, this peak was responsible for –CH3 groups and was a small peak. Finally, a very strong narrow peak was at 1071.79 cm^{-1} which indicated a C–O group. Below that 1000 cm^{-1} was the fingerprint region and it was shown a complicated spectrum.

3.4. GC-MS spectroscopic analysis

Fraction-11 from *Gliricidia sepium* was subjected to GC-MS analysis (Figure-4), and showed molecular ion peaks at *m/z* 87.000, 143.071, and 108.508 for $[C_7H_3]^{+\cdot}$, $[C_7H_{11}O_3]^{+\cdot}$, and $[C_7H_8O]^{+\cdot}$, respectively. This was compared with NIST library data, which predicted that the insecticidal activity compound was 4-C-methyl-myo-inositol (Figure-5) (*m/z* 194.079, $C_7H_{14}O_6$). Here does not indicate that the peak for mass value is 194.079; it may be due to that mass-to-charge ratio range alignment (Table 3). Anyhow determined that molecular weight from that data, which was provided along with an additional compound summary data sheet along with GC-MS results. From that compound spectra, the highest peak (*m/z* 87.00) was the base peak of that particular compound (Figure-6). By using these characterization details, we can determine that the fraction contains mainly a 4-c-methyl-myo-inositol compound. Therefore, this compound acts as a natural insecticide against rice weevils.

In this work, plant material was washed using 450 °C tap water to remove sand particles and debris. The purpose of usiultrasonic-sound-assisted sonicator is to increase the permeability of the cell wall and agitate the particles in the solution. Conditions for the priority evaporator are 45 °C and 70 rpm. The temperature should be lower than the methanol boiling point. Commonly referred to as methanol, it is used as a solvent for plant extraction because of its high polarity, which produces high extraction yields. Nowadays, the Sri Lankan government has banned some agrochemicals due to their side effects, so we have focused more on natural insecticides. In this research, we found that insecticidal compound in the methanolic leaf extract of *Gliricidia sepium*. That fraction F11 contains a major constituent, 4-C-methyl-myo-inoitol, which is responsible for insecticidal activity. It belongs to the inositol family. Research reviews of that particular compound show several activities. A water-ethanol leaf extract of *A. indica* contains 4-c-methyl-myo-inositol. It has been shown several medicinal roles, such as myo-neuro-simulant, methyl donor, methylguanidine inhibitor, myo-protective, myocardiocontractant, and myocardiodepressant [30]. Methanolic leaf extract of *Dolichandrone yields the* highest percentage of 4-C-methyl-myo-inositol [31]. By looking at GC-MS analysis data of methanolic extract of fruits of *Tetrapleura tetraptera*, the highest percentage of laminitol was found. It's a D-4-C-methyl-myo-inositol compound. Its isomer is 4-C-methyl-myo-inositol [32]. Fractionation of an ethanolic extract of *Strychnos ligustrina* Blume wood is an alternative to a natural antimalarial drug [33]. Methanolic leaf extract of *Chamaecrista nigricans (Leguminosae)* contains the major constituent 4-c-methyl-myo-inositol. It has been used to prevent ovarian hyperstimulation syndrome in experimental rats.

4. Conclusion

By considering our research work within these five medicinal plants, *Gliricidia sepium* was shown to have 100 % mortality (100 ± 0) after 4 days. Moreover, fraction-11 from a methanolic leaf extract of *Gliricidia sepium* had the highest insecticidal activity (100 ± 0) against *Sitophilus oryzae* after 3 days. Purification and characterization of that particular fraction by using FT-IR and GC-MS analysis predicted that the majority of the compound responsible for that insecticidal activity was 4-c-methyl-myo-inositol. To further the process of this research, we suggested not only checking out the insecticidal property of these compounds against other pests but also by using various solvents as well.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Mohammad Yasin Mohammad: Writing – review & editing, Investigation. **Haroon M. Haniffa:** Methodology. **Ashok K. Shakya:** Writing – original draft. **Rajashri R. Naik:** Writing – original draft. **Tharsika Sivaranjan:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30793.

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