proceedings

Proceedings

Efficacy of Botanical Extracts Against Storage Insect Pests *Tribolium confusum* (confused flour beetle) and *Sitophilus oryzae* (Rice Weevil) †

B.G.B. Hemantha Piris [1], Kandiah Pakeerathan [2,*] and Sairam Sayanthan [1]

[1] Department of Biosystem Technology, Faculty of Technology, University of Jaffna, Ariviyal Nagar, 44000, Kilinochchi, Sri Lanka
[2] Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, 44000, Kilinochchi, Sri Lanka
* Correspondence: pakeerathank@univ.jfn.ac.lk; Tel.: +94-776969511
† Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021; Available online: https://iece.sciforum.net/.

Abstract: Storage insect pests are inflicting severe economic loss leading to food insecurity and hunger. Research evidence suggests that synthetic chemicals are toxic to humans and are creating deleterious impacts on biodiversity. Therefore, this study evaluated natural plant extracts as alternatives to synthetic insecticides in controlling storage insect pests in an eco-friendly manner. Fresh and dry leaves, and seed kernel extracts of *Azadirachta indica*, *Gliricidia sepium*, *Ocimum tenuiflorum*, *Cascabela thevetia* were tested against storage insect pests *Tribolium confusum* and *Sitophilus oryzae*. *Cascabela thevetia* fresh leaves, flowers, seed kernels, and fresh leaves of *A. indica*, *G. sepium*, and *O. tenuiflorum* were collected and aqueous extracts were derived. Two laboratory trials were conducted. The first one was to understand the toxicity of aqueous extracts on pests. Therefore, 100 adult insects of red flour beetles and rice weevils were topically applied with treatments at the rate of 10g/100 mL. The second trial was to test the repellent properties of the aqueous solutions. Therefore, extracts were applied over the sealed bait bags in a large closed container at the same rate. Immediately after treatment application, 100 adult insects of each group were released into the container containing baits, and the container closed. Both trials were arranged in a completed randomized design (CRD), replicated three times including the untreated control. From the first trial, the number of live insect per treatments was counted after 30 seconds, 1 minute, 5-minute, 10-minute, 1 h, 2 h, and 24 h, and mortality percentage were calculated. From the second trial, the number of insects inside the sealed bags was counted 24 h, 48 h, 5 d after treatment. The data were subjected to ANOVA using SAS 9.1. Tukey's HSD multiple comparison test was used to identify the best treatment combination. The result showed that *C. thevetia* dried seed kernels exhibited highly significant mortality of 50% against rice weevils and 100% against flour beetles, after an hour and one minute, respectively, at $P < 0.01$. *Ocimum tenuiflorum* and *A. indica* showed 100 % mortality against flour beetles after 24 h, but not in rice weevil. Repellency percentage was highly significant in *C. thevetia* dried leaf extract (91.30% and 75.00%) and fresh leaf extract (86.36% and 78.05 %) after 24 hours and five days, respectively at $P < 0.01$. In the repellency treatment, *C. thevetia* dried leaf and seed kernel extract showed highly significant repellency of 96.91% and 98.99%, respectively, against red flour beetles. The repellency percentage of *C. thevetia* dried leaf, seed kernel extract and *O. tenuiflorum* were 81.79%, 66.67%, and 59.15% after 24 hours, respectively, and significant at $P < 0.05$. The results suggest that leaf and seeds of *C. thevetia* have high insecticidal properties and can be used to repel storage insects in warehouses once potential toxicity to humans is determined.

Keywords: Cascabela thevetia; Ocimum tenuiflorum; Gliricidia sepium; Tribolium confusum; Sitophilus oryzae

Citation: Hemantha Piris, B.G.B.; Sayanthan, S.; Pakeerathan, K. Efficacy of Botanical Extracts Against Storage Insect Pests *Tri-bolium confusum* (confused flour beetle) and *Sitophilus oryzae* (Rice Weevil), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10529

Published: 1 July 2021

1. Introduction

A pest can be any living organism (animal or plant) that causes economic damage to crops, livestock, stored foods, humans, and their wealthy livings [2]. Among the pests, insects that are causing tremendous damage to stored products play a major role in food shortage and food insecurity [1]. The Red flour beetle *Tribolium confusum* (confused flour beetle) and *Sitophilus oryzae* (Rice Weevil) are more dangerous stored product pests and can destroy large quantities of stored rice and flours in both short term and long term storage by feeding, and deteriorate the quality by excreting fecal, shedding off exuvia, excretion of gelatinous substances while laying eggs and pupating [11]. Control or management of stored product insects is important, unless, otherwise, contaminated food products create financial, legal, aesthetic, ethical problems [1]. Traditionally, inert materials (eg: wood ash) and fumigation were practiced for household level and commercial level management of this menace, respectively. Fumigation at the commercial level using toxic chemicals gives quick and prominent results [8], but overuse of noxious synthetic insecticide caused dramatic impacts to the living and non-living things on the earth. Even though the completely advanced hi-tech preventive and curative methods such as electronic nose, near-infrared spectroscopy, nanoparticle-based inert dust, acoustic technology are being practiced in commercial level storage pest control [3], botanicals or plant-based extracts proved to be eco-friendly and affordable mean to manage stored product pests, even, by poor rural farmers [7;10;12;14&15]. Lower middle-income country Sri Lanka has recently banned importing synthetic agrochemicals and fertilizers, therefore, agricultural scientist's responsibility is to invent cheap natural organic pesticieds to overcome the pest problem for 70% of farming communities living in the rural areas. Based on the above-mentioned criticisms, an investigation was planned with an objective to test and formulate plant-based insecticide against storage insect pest using abundantly available underutilized herbs *C. thevetia* (yellow oleander), A. indica (Neem), *O. tenuiflorum* (Thulsi or basil), *G. sepium* (Gliricidia) in Sri Lanka.

2. Materials and Methods

The investigation was carried out at the Entomology Laboratory of the Department of Agricultural Biology, Faculty of Agriculture, the University of Jaffna from November 2020 to March 2021. The detailed information of materials used, and methods followed are described below.

2.1. Sample Collection and preparation of botanical extracts

Flowers, fruits, and leaves of *C. thevetia*, and leaves of *A. indica*, *O. tenuiflorum*, *G. sepium* were obtained directly from the herbal garden at the department of Agricultural Biology, Faculty of Agriculture, University of Jaffna. Samples were surface sterilized using 2% NaOCl and double-distilled water, respectively. Water-based extracts were derived from finely ground collected fresh and 14 days' air-dried samples using protocol presented by Mihaylova and Lante (2019) [5]. Then a series of centrifuges and filtration was followed until getting solid free aqueous extract and the concentration was 10g/100mL. The aqueous extract was stored at 4 °C for further testing.

2.1.1. In-vitro assay on storage insects

Experiment-01: Rice weevils and red flour beetles were derived from the rearing unit of the same laboratory. The nearly equal size and weight of 100 weevils/per container and beetles/per container were released into separate testing containers. Then the 100% (10g/100mL) concentration of prepared plant extracts was topically applied using a hand sprayer at the rate of 500 l/Ha. The experimental setup was arranged in randomized block design and replicated four times along with non-treated control. The number of dead insects in each container after 30 seconds, 1 minute, 5-minute, 10-minute, 1 hour, 2 hours, and 24 hours after treatment administration were recorded.

Experiment-02: About 90 gunny bags of 500g capacity were prepared and filled with 250 grams of fresh rice and flour separately in each bag. The small holes were made physically on each bag to facilitate the insect to enter. Then every five bags were distantly placed in large plastic containers, and four bags in every container was treated by single-plant extracts and one left as control. The same procedure was followed for all the treatments. Once treated, nearly equal size and weight of 100 weevils/per container and beetles/per container were released into the treatment containers where baits were kept and the container was closed. The experimental setup was arranged in randomized block design and replicated four times. After 24 hrs, 48 hrs, 72 hrs, and five days of insect release, the number of insects present in every bag in every container was counted and the repellent percentage was calculated.

2.2. Data analysis

The insect mortality percentage and repellency percentage were calculated using the following formulas.

$$Mortality\ \% = \frac{Number\ of\ death\ insects}{\text{Number of live insects released}} * 100\ \%$$

$$Repellency\ \% = \frac{Number\ of\ insects\ in\ un-treated\ samples - Number\ of\ insect\ in\ treated\ samples}{Number\ of\ insects\ in\ un-treated\ samples} * 100\ \%$$

The data were subjected to ANOVA using SAS 9.1 student version, and Tukey's HSD multiple comparison test was performed to identify the best treatment at $P < 0.05$ using the same software.

3. Results and Discussion

These experiments were conducted to test the efficacy of botanical extracts on the major storage pests rice weevil and red flour weevil. The results of the study reveal that the plant extracts show significant results among the different treatment combinations and control at $P < 0.05$.

The results of the investigations on Rice weevil show that at least 50 % mortality was not observed in any of the treatment tested until an hour, whereas, after an hour, the significantly highest mortality of 50 % was observed in *C. thevetia* dried seed kernel extract treatment (Figure 1). Even though the 6% mortality was observed after one minute of treatment in *C. thevetia* Dried Seed kernel extract, it was observed that weevils were actively moved immediately after application, and tried to escape from the treatment container. Among the treatments, *C. thevetia* dried Seed kernel and leaf extracts can have potentially high quantity active ingredients (phytochemicals) to repel or kill the weevils. In the repellency experiment (Table1), the results show that repellency percentage was highly significant ($P < 0.01$) in *C. thevetia* dried leaf extract and fresh leaf extract with the values of 97.96% and 88.89 %, respectively, after 24 hours of treatments administration. Whereas in other treatments the repellency percentage was significant and above 50 % at $P < 0.05$.

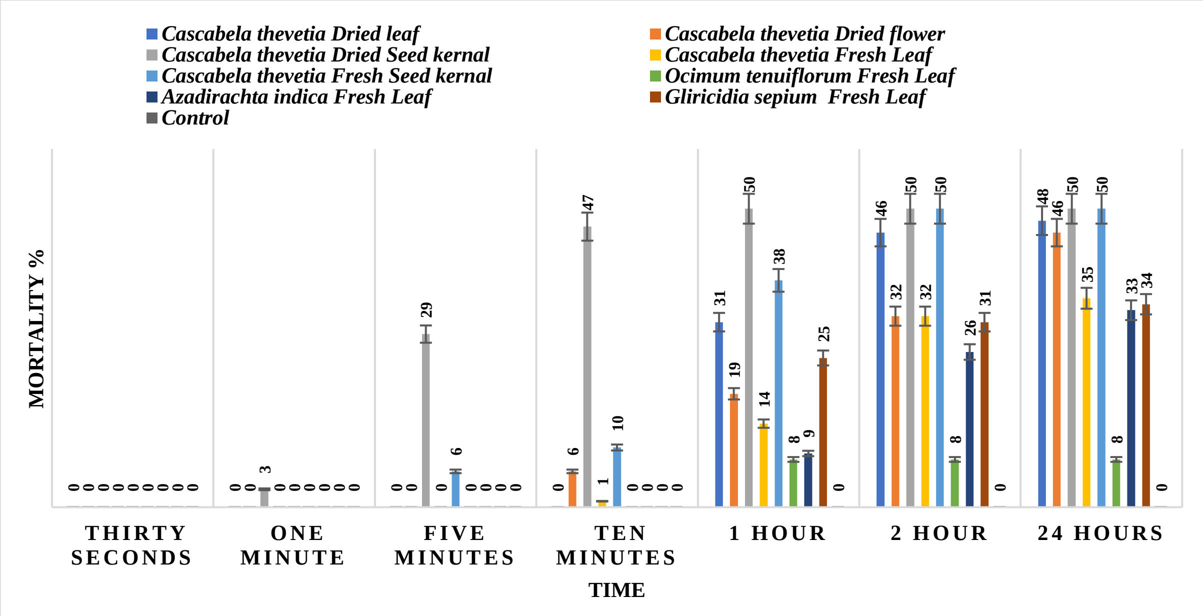

Figure 1. Mortality % of rice weevil to different plant extracts.

When increasing the time, the repellency percentage was declined (Table 1). But the repellency percentage was highly significant in *C. thevetia* dried leaf extract (91.30% and 75.00%) and fresh leaf extract (86.36% and 78.05%) after 24 hours and five days, respectively. Among the treatments, the *O. tenuiflorum* aqueous extract has the longer effect to keep the weevils away, rather than killing the weevils (in the preliminary experiment time 84% of insects had escaped from the experiment area within ten minutes in open conditions). This is an indication that the botanicals alter the behavior of the weevils towards the repellency than kill. The death of weevils may be due to the high concentration of Cardiac glycosides (thevetin A, thevetin B, Oleandrin, Neriifolin) [4]. Rajapakse (2009) reported that the presence of highly poisonous cardiac glycosides in *C. thevetia* causes cardiovascular system and also gastrointestinal tract failure which will lead to the death of organisms [9].

Saljoqi et al. (2006) reported that botanical extracts of *Melia azadarach*, *Perthenium hysterophorus*, *Phlogocanthus thyrsiflorus*, *Vitex trifolia*, *Zanthoxylum acanthopodium*, and *A. indica* were on rice weevils and tested result showed that plant powder *M. azadarach* recorded the highest mean mortality of 80.54% at 35 days after treatment [13]. But current study used aqueous extract and repelled more than 90% of the weevils within 24 hours.

The results summarized in Figure 2 show that mortality was observed after Thirty seconds, One-minute, Five-minute, Ten-minute, One-hour, Two-hours, Twenty-four hours of treatments on red flour beetles. Immediately after treatment administration, in thirty seconds, highly significant 96% mortality was recorded in *C. thevetia* dried seed kernel extract at $P < 0.01$. After 24 hours of treatment administration, 100% mortality was recorded in all the treatments except control. Therefore, it indicates, red flour beetles are highly sensitive and susceptible to phytochemicals. In the repellency treatment (Table 2), *C. thevetia* dried leave and seed kernel extract showed highly significant repellancy of 96.91% and 98.99%, respectively (Table 2). Whereas *O. tenuiflorum* fresh leaves extract and *A. indica* fresh leaf extracts showed significant and more than 80% mortality at $P < 0.05$. When increasing the time, the repellent percentage declined and only *C. thevetia* dried seed extracts showed more than 80% of repellency even after five days of treatment. Mortality percentage observed was high in red flour beetle than rice weevil because it may due to red flour beetles are delicate insects than Rice weevils [8].

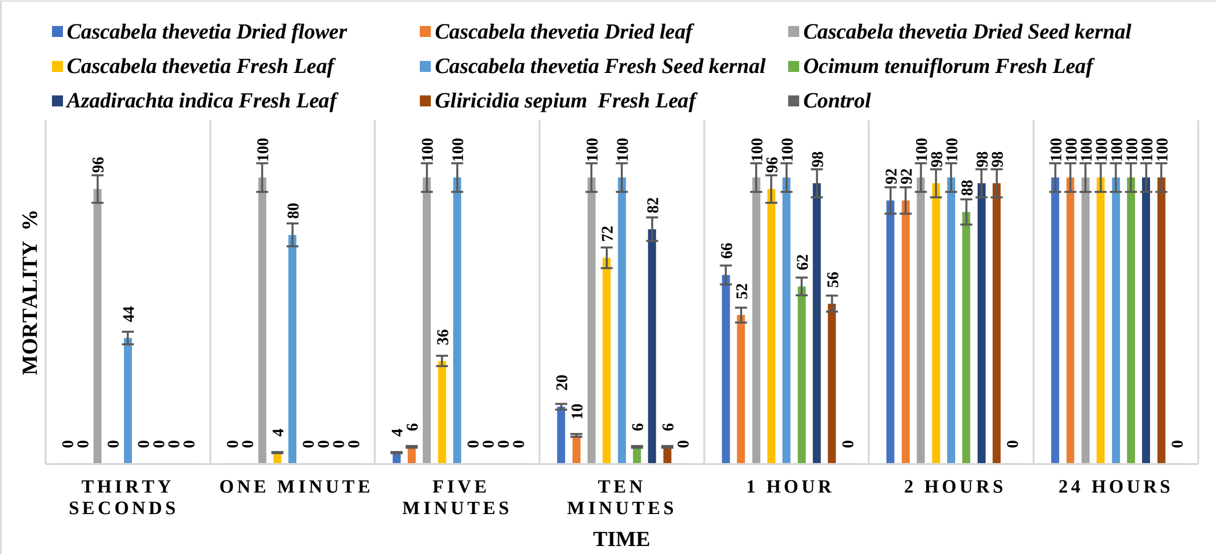

Figure 2. Mortality % of red flour beetle to different plant extracts.

Table 1. Repellency effect of botanicals on rice weevils.

Aqueous extraction	After 24 hours	After 48 hours	After 5 days
Cascabela thevetia Dried flower	61.11^{cd}	66.67^{b}	59.15^{b}
Cascabela thevetia Dried Leaf	97.96^{a}	91.30^{a}	75.00^{a}
Cascabela thevetia Dried seed	71.79^{bc}	43.75^{d}	38.71^{c}
Cascabela thevetia fresh leaf	88.89^{a}	86.36^{a}	78.05^{a}
Cascabela thevetia Fresh seed	63.01^{c}	66.67^{b}	57.14^{b}
Ocimum tenuiflorum Fresh Leaf	70.13^{c}	63.01^{b}	70.13^{ab}
Azadirachta indica Fresh Leaf	78.05^{b}	64.86^{b}	64.86^{ab}
Gliricidia sepium Fresh Leaf	61.11^{cd}	55.07^{c}	41.27^{c}
Non treated (Control)	36.16^{e}	10.37^{e}	0.00^{d}

Values with the same alphabets are not significantly different according to the Tukey's HSD at 95% confidence interval.

Table 2. Repellency effect of botanicals on red flour beetle.

Aqueous extraction	After 24 hours	After 48 hours	After 5 days
Cascabela thevetia Dried flower	75.00^{bc}	71.79^{bc}	50.75^{bc}
Cascabela thevetia Dried Leaf	96.91^{a}	91.30^{a}	66.67^{b}
Cascabela thevetia Dried seed	98.99^{a}	95.83^{a}	81.79^{a}
Cascabela thevetia fresh leaf	75.00^{bc}	71.79^{bc}	50.75^{bc}
Cascabela thevetia Fresh seed	79.52^{bc}	78.05^{bc}	43.75^{cd}
Ocimum tenuiflorum Fresh Leaf	85.06^{b}	85.06^{b}	59.15^{bc}
Azadirachta indica Fresh Leaf	82.35^{b}	79.52^{bc}	46.15^{cd}
Gliricidia sepium Fresh Leaf	61.11^{d}	52.94^{d}	46.15^{cd}
Non treated (Control)	15.00^{e}	0.00^{e}	0.00^{e}

Values with the same alphabets are not significantly different according to the Tukey's HSD at 95% confidence interval

Znuncio et al. (2016) reported that secondary metabolites such as alkaloids, amides, chalcones, flavones, phenols, etc. present in neem and gliricidia cause insect death. Flavonoids are the most lethal compounds present in the gliricidia fresh leaves, and among flavonoids, biochanin and pinocembrin are more toxic to stored insects [6]. As mentioned above, the various concentrations of cardiac glycosides (thevetin A, thevetin B, Oleandrin, Neriifolin) present in the *C. thevetia* plant could be the reason for the high mortality in red flour beetles when it contacts directly.

This study found that different storage insects behave differently to the plant extracts. Therefore, further study with more category of storage insect is needed to determine the LD 50%.

5. Conclusion

This study found that dried *C. thevetia* seeds and leaf extracts cause highly significant mortality and repellent effect in both rice weevils and red flour beetles. *Cascabela thevetia* seeds and leafs are more potential sources to prepare insect protectants. *Cascabela thevetia* seeds are more toxic to human, therefore, further study related to side effects assessment is recommended.

Author Contributions: K.P. contributed more. K.P. conceived the research idea and K.P. and S.S. guided the research; B.G.B.H.P. conducted experiments; B.G.B.H.P. and K.P. wrote the manuscript; and S.S. and K.P. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: This research was carried out in JICA research and laboratory complex, Faculty of Agriculture, University of Jaffna. The authors thanks to Japanese government for providing equipments facilities through JICA grant.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abd El-Aziz,S.E. Control strategies of stored product pests. *J. Entomol.* **2011**, *8*, 101–122, doi:https://doi.org/10.3923/je.2011.101.122.
2. Department of Health, 2010 [https://www1.health.gov.au/internet/publications/publishing.nsf/Content/ohp-enhealth-manual-atsi-cnt-l~ohp-enhealth-manual-atsi-cnt-l-ch5~ohp-enhealth-manual-atsi-cnt-l-ch5.1]. (Accessed on 17 March 2021).
3. Hagstrum,D.W.;Athanassiou,C.G. Improving Stored Product Insect Pest Management: From Theory to Practice. *Insects* **2019**, *10*, 332. https://doi.org/10.3390/insects10100332.
4. Karthik,G.; Iyadurai,R.; Ralph,R.; Prakash,V.; Abhilash,K.; Sathyendra,S.; Abraham, O.C.; Truman,C.; Reginald, A. Acute oleander poisoning: A study of clinical profile from a tertiary care center in South India. *J. Fam. Med. Prim. Care* **2020**, *9*, 136–140, doi:https://doi.org/10.4103/jfmpc.jfmpc_632_19.
5. Mihaylova,D.;Lante,A. Water an Eco-Friendly Crossroad in Green Extraction: An Overview. *Open Biotechnol. J.* **2019**, *13*, 155–162, doi:https://doi.org/10.2174/1874070701913010155.
6. Nukmal,N.; Pratami,G.;Rosa,E.;Sari,A.;Kanedi, M. Insecticidal Effect of Leaf Extract of Gamal (*Gliricidia sepium*) from Different Cultivars on Papaya Mealybugs (*Paracoccus marginatus*, Hemiptera: Pseudococcidae). *IOSR-JAVS* **2019**, *12*, 4–8, doi:https://doi.org/10.9790/2380-1201030408.
7. Obeng-Ofori, D. The use of botanicals by resource poor farmers in Africa and Asia for the protection of stored agricultural products. *Stewart Postharvest Rev.* **2007**, *3*, 1–8, doi:. https://doi.org/10.2212/spr.2007.6.10.
8. Dixon,R.K.;Morrison,F.O. Small chamber tests of insecticidal fumigants. *Can. J. Zool.* **1970**, *48*, 1411–1418, doi:https://doi.org/10.1139/z70-239.
9. Rajapakse,S. Management of yellow oleander poisoning. *Clin. Toxicol. (Philadelphia, Pa.* **2009**, *47*, 206–212, doi:https://doi.org/10.1080/15563650902824001.
10. Rajashekar,Y.; Bakthavatsalam,N.;Shivanandappa,T. Botanicals as grain protectants. *Psyche J. Entom.* **2012**, 13, doi:https://doi.org/10.1155/2012/646740.
11. Rees. D. Insects of stored products, CSIRO publishing, Australia, 2004, 1-181.
12. Said, P.P.;Pashte,V.V. Botanicals: The protectants of stored grains pests. *Trends in Biosci.* **2015**, *8*, 3750–3755.
13. Saljoqi, A.U.R.; Afridi, M.K.;Khan, S.A;Rehman S. Effects of six plant extracts on rice weevil *Sitophilus oryzae* L. in the stored wheat grains. *J. Agric. and Biol Sci* **2006**, *1*, 61–65.
14. Sharaby,A. Evaluation of some Myrtaceae plant leaves as protectants against the infestation by *Sitophilus oryzae* L. and *Sitophilus granarius* L. *Insect Sci. Appl.* **1988**, *9*, 465–468.
15. Trivedi,A.; Nayak,N.;Kumar,J. Recent advances and review on use of botanicals from medicinal and aromatic plants in stored grain pest management. *J. Entomol. Zool. Stud.* **2018**, *6*, 295–300.

16. Znuncio,J.C.;Mourão,S.A.;Martínez,L.C.;Wilcken,C.F.;Ramalho,F.S.;Plata-Rueda,A.;Soares,M.A.;Serrão,J.E. Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predator, *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Sci. Rep.* **2016**, *6*, 1–8, doi:https://doi.org/10.1038/srep3026.