

Research Article

Tenne P.C.R.K*, Karunaratne M.M.S.C

Phytochemical profile and bioactivity of essential oil from *Pimenta dioica* leaves on cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae): A farmer friendly solution for post-harvest pest management

<https://doi.org/10.1515/opag-2018-0033>

received November 15, 2017; accepted July 26, 2018

Abstract: *Pimenta dioica* (Allspice) leaf essential oil was isolated using hydro-distillation and insecticidal properties were evaluated for their ability to suppress post-harvest insect pest *Callosobruchus maculatus* in storage legumes and pulses. Four concentrations of leaf oil (0.5, 0.6, 0.8 and 1.0 v/v%) were tested to evaluate their contact and fumigation repellency as well as insecticidal activities. Volatile organic compounds of leaf oil were analyzed by headspace solid-phase micro extraction coupled with gas chromatography and mass spectrometry (HS-SPME/GCMS) using polar and medium polar fibers. All concentrations elicited significantly higher repellent effects on insects after 2 hour exposure when compared with those of the control with the highest concentration producing 98.0% and 92.0% contact and fumigation repellency effects. Contact and fumigation toxicity tests exhibited 100% mortality for the highest concentration after 6 and 12 hour exposure respectively giving low LC50 values of 0.3 (v/v%). Volatile oil analysis indicated that the leaf essential oil comprised of high amount of Eugenol (89.8%), Methyl eugenol (2.3%) and caryophyllene (4.1%) for the polar fiber. Eugenol (42.6%), Caryophyllene (27.9%), Humulene (6.8%) and 1,8 Cineol (8.1%) were obtained for the medium polar fiber. Results indicate that the leaf essential oil of *P.dioica* would be a safe, eco-

friendly alternative botanical insecticide in managing *C.maculatus* during storage.

Keywords: *Callosobruchus maculatus*, essential oil, *Pimenta dioica*, repellency, toxicity

1 Introduction

The primary food systems in the world have changed rapidly over the last 10 000 years. Cultivation of cereal and legume crops has become a promising approach to meet nutritional need for human survival (Hueston 2012). The practice of storing food grains was initiated following higher crop production, and long-term food storage appeared to be one of the major approaches to food hygiene and safety during storage of agricultural crop products (Aulakh and Regmi 2013). Reduction of post-harvest losses and quality deterioration during storage of grains has been considered as one of the most essential necessity to increase global food availability and accessibility, to ensure global food security (Ojebode et al. 2015).

Improper handling techniques, inclement weather and environmental circumstances, ravages by rats, insects and microbial agents are the most common forms of grain damage at storage facilities. This may account for a loss of 4-6% (Mezgebe et al. 2016). Of these, insects are the most severe damagers causing a storage loss of 80% (Palipane et al. 2009). Therefore, insect pest infestations in storage facilities are the most devastating and widespread problem throughout the world, causing detrimental impacts on the global economic food chain (Boxall et al. 2002).

Sri Lanka is still ranked as a developing country and economy primarily rely upon agriculture (Dharmasena

*Corresponding author: Tenne P.C.R.K, Department of Zoology, University of Sri Jayawardhenapura, Faculty of Applied Sciences, Gangodawila, Nugegoda, Sri Lanka,
E-mail: chathuriprathitha@gmail.com

Karunaratne M.M.S.C, Department of Zoology, University of Sri Jayawardhenapura, Faculty of Applied Sciences, Gangodawila, Nugegoda, Sri Lanka

and Abesiriwardena 2003). Lack of proper industrial and technical development in the production of protein rich-animal products, but limited economic access to these, may lead to the production of alternative protein sources. Sri Lanka's population of approximately 21.2 million obtains about 75% of its dietary proteins from plants (Senaratne et al. 1998).

Cowpea (*Vigna unguiculata*) is one of the most common alternative legume crop in Sri Lanka and is grown on 25,000 ha of the dry zone. The average annual yield is about 1600-1650 kg/ha. However, the plant is prone to injuries from 85 insect species at different stages of growth. Protein-rich edible cowpea seeds are stored in normal warehouse conditions at the farm level, providing storage facilities in order to use as human food and also as livestock feed (Kelida et al. 2015; Senaratne et al. 1998).

Insect damage is the major constraint to cowpea grain production. Cowpea aphids (*Aphis craccivora*), leaf hoppers (*Empoasca* sp.), blister beetles (*Hyleu slugens*), green stink bugs (*Nezara viridula*) and cowpea beetles (*Callosobruchus maculatus*) cause the most severe grain damage in storage facilities (Tiroesele et al. 2014). Invasion and infestation by the insect pest *Callosobruchus maculatus* (Fabricius-1775) (Coleoptera: bruchidae), is the most serious by a beetle pest in stored cowpea, which may reduce both quality and quantity of stored cowpea during long term storage and their damage may reach 20-30% in tropical regions (Moreira et al. 2007). Significant loss of seed viability during storage of cowpea due to inhabitation and damage caused by *Callosobruchus maculatus* is the leading problem in the increment of pulse crop production in Sri Lanka (Ratnasekara and Rajapakse 2012).

The gravid adult female beetles lay eggs on the seed surface gluing them firmly (Nwanze et al. 1975). The eggs hatch within 3-4 days and larvae burrow inside the seed and feeds on the embryo. The mature larvae finally make a round window at the place where pupation occurs (Hagstrum et al. 2012). Feeding by larvae is responsible for a significant loss of physical and nutritional value of stored legumes (Mailafiya et al. 2014). *Callosobruchus maculatus* becomes most destructive due to its short life cycle. This damage also creates a condition that causes secondary infection. Thus, control of *C. maculatus* infestation during storage of legumes appeared as one of the promising event all over the world in order to achieve global food security.

Infestation control of stored legume pests has primarily relied upon the application of synthetic chemical insecticides. The direct application of synthetic chemical insecticides as repellents and toxicants is the most obvious approach to hygiene in storage structure, and is commonly used worldwide to control insect pests (Golob

1999). Methylbromide, ethylformate, sulfuryl fluoride are the most common fumigants responsible for rapidly killing of insect pest life stages within storage facilities (Isman 2006). Ease of handling, market availability, high residual activity and broad-spectrum activity on insects may have increased the extensive use of synthetic insecticides during storage (Rajashekar et al. 2012). However, regular and repeated use of these chemical compounds have been recognized to have several adverse effects on human health and survival, development of insect resistance and ecological imbalance depending on its chemical nature (Adesina and Ofuya 2015 ; Khani and Heydarian 2014).

By considering these facts, the entire scope for the development of safe, non-polluting pest control methods has been directed towards naturally occurring plant based insecticides. Since the 1980s, locally available plants or plant parts are being used to protect storage grains during 3 to 4 month storage (Talukder, 2009) as botanical insecticides. Botanical insecticides are naturally occurring chemicals extracted or derived from plants or plant parts that can be easily and cheaply produced by farmers in small scale storage facilities as crude extracts or powders (Rahman and Talukder 2006). Over 2000 plant species belonging to families such as Anacardiaceae, Apiaceae, Araceae, Brassicaceae, Myrtaceae, Pinaceae and Rutaceae have been screened to evaluate their potential repellent and toxic effects against insect pests (Rajendra and Sriranjini 2008). Pyrethrum, rotenone, neem and plant essential oils are the most common botanical insecticides that have been used to date. Various plant powders, extracts and essential oils have been reported to possess insecticidal activities in terms of repellents, toxicants, oviposition deterrents, and anti feedents against cowpea beetles and other storage insects (Isaman 2006).

Plant essential oils have shown many advantages over conventional insecticides owing to their low mammalian toxicity, rapid degradation and local availability (Pugazhvendan et al. 2012). Many of the plant volatile oils are composed of mixtures of monoterpenes, phenols and terpenes. Screening of phytochemicals including volatile organic compounds extracted from insecticidal plant parts has proven that these chemical compounds can be effectively used against storage insect pests to design new target molecules (Isman 2006).

In this regard, it is imperative to investigate, for the first time, the phytochemical profile and the repellent and insecticidal properties of the medicinally as well as culinary important plant, *Pimenta dioica* belonging to family Myrtaceae, a well renowned evergreen plant against *C. maculatus* to pave the way for the development of a highly effective natural post-harvest protectant to replace

hazardous synthetic insecticides in an ecologically sound and farmer friendly manner.

2 Methods

2.1 Rearing of *C. maculatus*

Cowpea (*Vigna unguiculata*) grains infested by *C. maculatus* were collected from the local market. Infested grains were then kept in transparent containers and covered with muslin cloth. Newly emerged healthy adult beetles were transferred into new containers and were provided with un-infested cowpea grains for oviposition and the laboratory cultures were continuously maintained at $28 \pm 2^\circ\text{C}$ and $84 \pm 2\%$ RH. Newly emerged one-day old adult cowpea beetles were used for all experiments.

2.2 Extraction of essential oil

Fresh leaf powder (350 g) of *P. dioica* was subjected to hydro-distillation using a modified Clevenger type apparatus for a 3 hour period to yield leaf essential oil. The oil was stored in glass vials in a refrigerator at 4°C . Four solutions at concentrations of 0.5, 0.6, 0.8 and 1.0 v/v% were prepared using acetone as the solvent.

2.3 Contact repellency effect

Contact repellency of the *P. dioica* leaf essential oil was tested by using a single choice cup bioassay suggested by Mohan and Fields (2002) with some modifications

(Figure 1). Four concentrations at 0.5, 0.6, 0.8 and 1.0 v/v% were admixed with 25 g of fresh cowpea seeds in small perforated plastic cups separately (height 9cm, diameter 7cm). One day old, 20 adult beetles were then introduced into each plastic cup. The top 2/3 height of the cup was perforated with holes large enough to allow the beetles to pass through if they were repelled by the treatment. These holes were covered with masking tape to let the introduced beetles settle down inside the cup. Before the onset of each experiment, the masking tape was removed. The bioassay apparatus was placed inside a larger transparent container (height 25 cm, diameter 10 cm) covered with muslin cloth to trap the insects that escaped through the perforations. The same setup was made as the control by admixing the seeds, only with acetone. The number of repelled insects trapped inside the large container was counted after 2 hours. This experiment was replicated five times.

2.4 Fumigation repellency effect

A similar bioassay apparatus mentioned in the contact repellency test was used to test fumigation repellency of leaf essential oil with some modifications (Figure 2). The bottom of the small plastic cup was removed and replaced by Whatman® filter papers (diameter 3 cm). Four concentrations (0.5, 0.6, 0.8 and 1.0 v/v %) were applied to the filter papers separately and covered with mesh to prevent insects contacting them. One day old twenty adult beetles were then introduced into the small plastic cup and all repelled insects were counted after 2 hours of exposure. The control was treated only with acetone. This experiment was replicated 5 times.

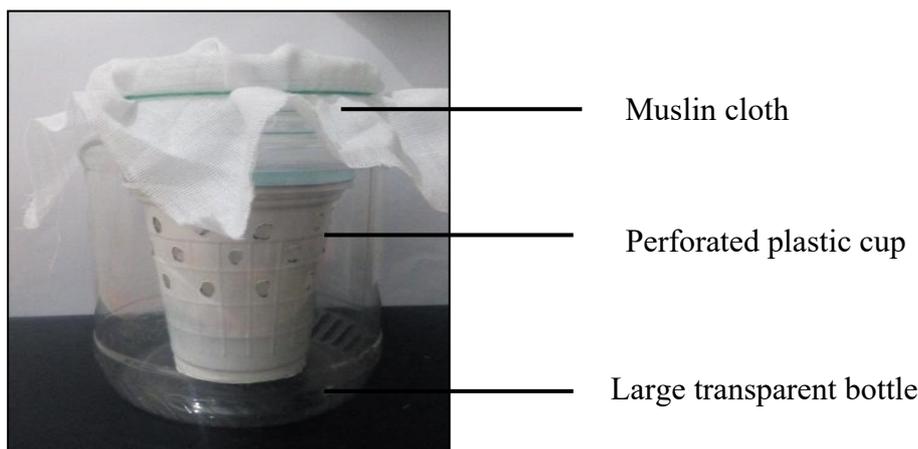


Figure 1: Contact repellency bio assay

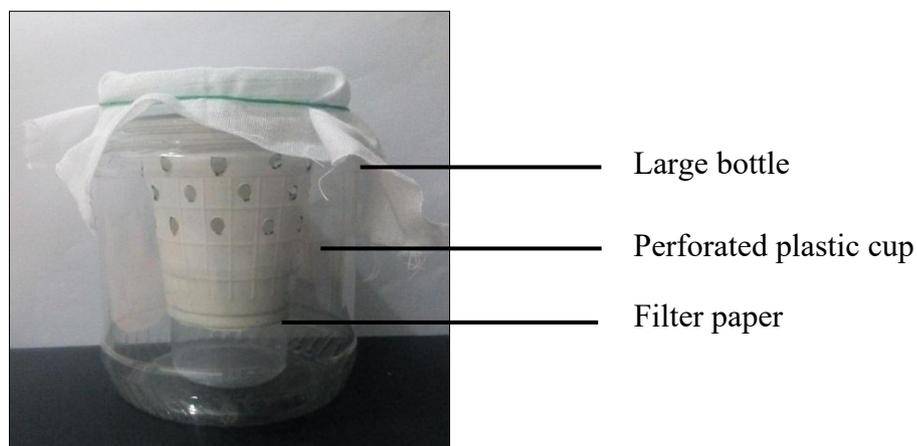


Figure 2: Fumigation repellency bioassay

2.5 Contact toxicity effect

Contact repellency was assayed by using the method of Huang et al. (1999) with slight alterations. Four concentrations at 0.2, 0.3, 0.6 and 0.8 v/v% were applied evenly on the inner surface of cleaned glass vials (10 ml) and on the screw caps and the solvent (acetone) was allowed to evaporate completely. Then 20 newly emerged (0-24 hours) adult insects were introduced and the screw cap was tightened to prevent insect escape. All dead insects were counted after 30 minutes, 6 and 12 hours after exposure and 5 replications were made for each treatment. Only acetone was used in the control.

2.6 Fumigation toxicity effect

Whatman No 1 filter paper strips (diameter 6 mm) were impregnated with 0.2, 0.3, 0.6 and 0.8 v/v% concentrations and kept for 1 minute for the solvent (acetone) to evaporate. Subsequently, each filter paper was placed on the underside of the screw cap of the glass vials (volume 10 ml) and covered with nylon mesh to prevent the insects contacting the strips. Twenty newly emerged (0-24 hours) beetles were then introduced into each glass vial which were screwed tightly. A similar control setup only with acetone was made. Insect mortality was recorded after 1/2, 1 and 2 hours after exposure and replicated 5 times.

2.7 Screening of volatile organic compounds in leaf essential oil

Freshly prepared *P. dioica* leaf essential oil (40 μ l) was placed inside the headspace vial (diameter 2 cm, height

6.7 cm, volume 12 ml) and conditioned. Head space solid phase microextraction (HS-SPME) fiber was injected towards the oil and the setup was kept at room temperature for 30 minutes to adsorb emitted chemical compounds from the oil. Both polar and medium polar HS-SPME fibers were used for the extraction. Identification of components was based on retention times and MS spectral library data at the Chemistry Department of University of Sri Jayewardenepura, Sri Lanka.

2.8 Statistical data analysis

Statistical data analysis was carried out using statistical package of “Minitab 17” version. Data obtained for the repellency bioassays were subjected to one-way analysis of variance (ANOVA). Multiple comparison test (Tukey) was used to separate mean values of the experiments, where significant differences existed ($p < 0.05$). Probit analysis was used to estimate LC_{50} values to determine the lethal concentrations needed to kill 50% of adult *C. maculatus*.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Contact and fumigation repellency effect on adult beetles

Contact and fumigation repellent effects of *P. dioica* leaf essential oils on adult *C. maculatus* after 2 hour of exposure are shown in Table 1. According to the results, all concentrations elicited significantly higher repellent effects compared to the control. Both contact and

fumigation repellency increased progressively with the increase in concentration. The highest concentration manifested very strong contact and fumigation repellent actions by producing 98.0 and 92.0 rates respectively. The lowest concentration produced more than 50% repellent activity in both tests.

Data in Table 2 demonstrated the contact and fumigation toxic effect of *P. dioica* leaf essential oil against *C. maculatus* after 6 and 2 hours after exposure respectively. Results suggested that, the highest percentage mortality of 100% observed at the highest concentration after 12 hour exposure in contact toxicity test where as in fumigation toxicity 100% mortality observed after 2 hours exposure. There was no statistical difference between the control and the lowest concentration in both tests. In addition, mean percentage mortality was at 0.6%v/v in contact toxicity test which is

higher than 75%, thus indicating the strong insecticidal action of oil extracted from *P. dioica* leaves.

Figure 3 depicts the mean percentage mortality of adult *C. maculatus* 1/2, 6 and 12 hours after treatment (HAT) at different concentrations in the contact toxicity test. More than 80% of mortality was observed at higher concentration (0.8% v/v) while the highest percentage mortality of 100% was recorded 12 HAT.

Mean percentage mortality of adult *C. maculatus* at different concentrations is shown in Figure 4. Here also, more than 80% of mortality was observed at the highest concentration 1/2, 1 and 2 HAT.

Probit analysis carried out for both contact and fumigation toxicity tests showed the lowest LC50 value at the highest concentration after 12 hour exposure and 2 hour exposure respectively by giving 0.34±0.01 and 0.39±0.01 (v/v%).

Table 1: Contact and fumigation repellency effect of leaf essential oil of *P. dioica* on *C. maculatus* after 2 hour exposure in the contact repellency test

Concentration (v/v%)	*Mean % Repellency ±SD	
	Contact repellency	Fumigation repellency
Control	0.0±0.0 ^a	0.0±0.0 ^a
0.5	64.0±2.7 ^b	72.0±2.7 ^b
0.6	76.0±2.2 ^c	75.0±3.5 ^b
0.8	87.0±2.7 ^d	83.0±2.7 ^c
1.0	98.0±4.2 ^e	92.0±2.7 ^e
Probability	P<0.05	P<0.05

*Means followed by the same letters are not significantly different according to the Tukey's test at P<0.05

* Means percentage Repellency ± SD for five replicates (n= 100)

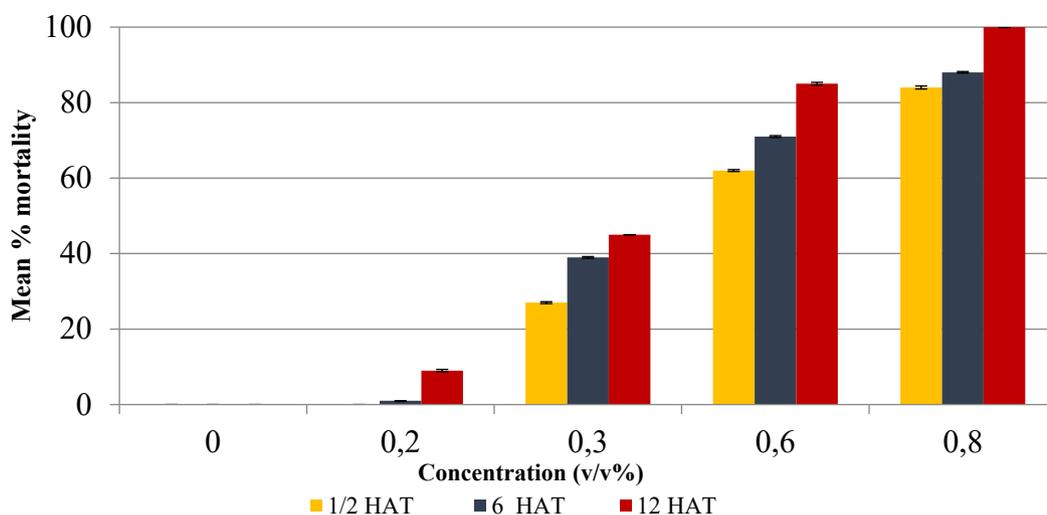


Figure 3: Mean percentage mortality of oil from *P. dioica* on *C. maculatus* after 1/2, 6 and 12 hours in the contact toxicity test HAT- Hours After Treatment

Table 2: Contact and fumigation toxic effect of *P. dioica* leaf essential oil on *C. maculatus* after 12 and 2 hours after exposure

Concentration (v/v%)	*Mean % Mortality \pm SD	
	Contact toxicity	Fumigation toxicity
Control	0.0 \pm 0.00 ^a	0.0 \pm 0.0 ^a
0.2	7.0 \pm 3.5 ^a	7.0 \pm 2.7 ^a
0.3	46.0 \pm 23.5 ^b	41.0 \pm 5.4 ^b
0.6	85.0 \pm 3.5 ^c	66.0 \pm 6.5 ^c
0.8	100.0 \pm 0.0 ^d	100.0 \pm 0.0 ^d
Probability	P<0.05	P<0.05

*Means followed by the same letters are not significantly different according to the Tukey's test at P<0.05

* Means percentage Repellency \pm SD for five replicates (n= 100)

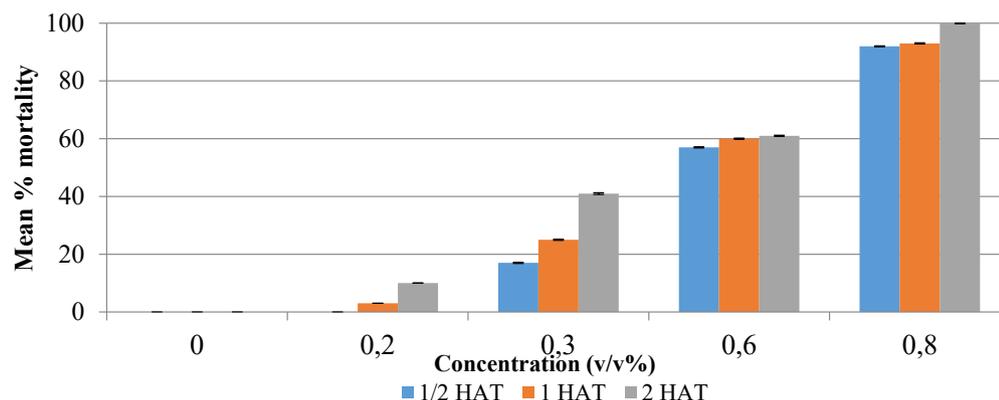


Figure 4: Mean percentage mortality of oil from *P. dioica* on *C. maculatus* after 1/2, 6 and 12 hours in the contact toxicity test HAT – Hours After Treatment

3.2 Screening of volatile organic compounds in leaf essential oil

Phytochemical constituents extracted from *P. dioica* leaf essential oil using HS-SPME medium polar fiber are presented in Table 3. Eugenol (42.6%) and caryophyllene (27.9%) were detected in higher amounts.

Time

Results presented in Table 4 revealed that the polar HS-SPME fiber extract higher amount of eugenol and caryophyllene.

4 Discussion

Pimenta dioica leaf essential oil elicits very high repellent and insecticidal activities on *C. maculatus* populations in a dose dependent manner. It is of great interest that the highest concentration demonstrated very high responses in insects in both contact (98%) and fumigation (92%) repellency tests even after 2 hour exposure. Application of fumigants is common in storage systems and a

convenient tool for monitoring storage insects with the ability to kill or remove a wide spectrum of insects by penetrating in to storage commodities. Results of the present study indicated highest fumigation repellent effect and therefore essential oil can be used as a potential botanical fumigant. Moreover, results obtained for contact and fumigation toxicity revealed 100% mortality of the beetle after 12 hour and 2 hour exposure. It was also quite remarkable to observe that over 80% of adult mortality for both toxicity tests happened only half an hour after treatment. The probit analysis revealed quite low LC50 values of 0.3 (v/v%) for contact toxicity after 12 hour and 0.3 v/v% for fumigation toxicity after 2 hour exposure, thus indicating that the essential oil is extremely effective both as a contact and a fumigation toxicant. These findings therefore, substantiate the highly effective role of *P. dioica* in the management of *C. maculatus* in storage. More importantly, no previous studies have been reported to date on the toxic and repellent properties of *P. dioica* leaf essential oil against storage insect pests including *C. maculatus*. In some previous studies (Kim et al. 2003) reported the potential insecticidal activity of extracts from

Table 3: Solid- Phase Microextraction of *P. dioica* leaf oil with Medium Polar SPME fiber

Medium polar HS-SPME fiber		
Volatile compound	Percentage (%)*	^a RT
Pinene	0.454	3.830
1,8 cineole	8.184	5.302
Linalool	0.532	5.477
Terpinene	0.183	6.139
Eugenol	42.612	10.478
Caryophyllene	27.908	11.261
Beta- Guaiene	0.331	11.396
α -Humulene	6.867	11.631
Naphthalene	1.769	12.438
Total		77.97

*Data expressed as percentage of the total peak area

^aRT = Retention Time

Table 4: Solid- Phase Microextraction of *P. dioica* leaf oil with Polar SPME fiber

Polar HS-SPME fiber		
Volatile compound	Percentage (%)*	^a RT
1,8 cineole	2.322	5.250
Eugenol	89.803	10.218
Methyl eugenol	2.399	10.710
Caryophyllene	4.139	11.015
α -Caryophyllene	0.919	11.448
Naphthalene	0.419	12.277
Total	99.582	

*Data expressed as percentage of the total peak area

^aRT = Retention Time

cinnamon (*Cinnamomum cassia*) bark oil, horseradish (*Cochleria armoracia*) oil and mustard (*Brassica juncea*) oil against *Callosobruchus* sp. Within one day after treatment. Essential oils extracted from *Dennettia tripetela* and *Piper guineense* achieved 100% mortality of adult *C. maculatus* and *Sitophilus zeamais* in 24 hours. According to these studies, 100% mortality of adult *C. maculatus* was observed after 3 days on neem (*Azadirachta indica*) oil (Ahamed et al. 2003).

With reference to the findings of the present study on contact and fumigation toxicity, 100% mortality of insects was obtained within 12 hour and 2 hour exposure respectively. Therefore, it can be stated that the essential oil of *P. dioica* elicited a higher toxic effect within a very

short time period.

Pimenta dioica essential oil consists of eugenol, pinene, caryophyllene, cineole, linalool and methyl eugenol, which may have considerable insecticidal effects. According to a somewhat similar study carried out on phytochemical constituents has revealed that eugenol, α -pinene, caryophyllene, 1,8 cineole, linalool and humulene are present in *P.dioica* essential oil (Dharmadasa et al. 2015). However, in the present study, compounds such as methyl eugenol, α phellandrene and β phellandrene were also detected along with the other chemical constituents.

It has been revealed by (Koul et al. 2008) that eugenol is highly toxic to *Sitophilus granaries* and *Musca domestica*. Eugenol, in fact is a substituted phenolic

compound and many phenolic compounds are reported to have a considerable insecticidal effect. Methyl eugenol, although found in low quantities (2.4%) in the present study is reported to be a better toxicant than monoterpenes such as limonene and 1,8 cineole. Monoterpenes being more volatile are known to be much more effective as insect fumigants. Cited literature has also depicted the potential of linalool and 1,8 cineole against rice weevil, *Sitophilus oryzae*. Linalool being a constituent of several plant essential oils has been shown to act on the nervous system of the insect, affecting ion transport and the release of acetyl cholinesterase (Kostyukovsky et al. 2002). The results of the present study suggest that the chemical constituents found in the plant leaf oil may be exhibiting very strong insecticidal activities as repellents and toxicants either singly or in combination.

The overall study reveals that *P. dioica* leaf essential oil can be highly recommended as a safe, and eco-friendly alternative botanical agent in managing *C. maculatus* during storage of legumes and pulses as a potential repellent as well as toxicant. To widen the knowledge of the biological activity of this plant on the pest, it is worthwhile extending the present research further to investigate the effectiveness of the identified volatile constituents against this pest as well as against other storage insect pests. More research should be carried out in order to gain substantial knowledge on the long-term effects of this plant on *C. maculatus* as well as other storage insect pests with the view to commercializing this natural plant product or its derivatives in future.

Conflict of interest: Authors state no conflict of interest.

References

- Adesina J.M., Ofuya T.I., Oviposition deterrent and egg hatchability suppression of *Secamone afzelii* (Schult) K. Schum leaf extract on *Callosobruchus maculatus* (Fabricius) (Coleoptera:Chrysomelidae), *Jordan Journal of Biological Science*, 2015, 8, 95-100
- Ahmed K.S., Itino T., Ichikawa T., Duration of developmental stages of *Callosobruchus chinensis* L.(Coleoptera: Bruchidae) on azuki bean and the effects of neem and sesame oils at different stages of their development. *Pakistan Journal of Biological Sciences*, 2003, 6, 932-335
- Aulakh J., Regmi A., Post-harvest food losses estimation-development of consistent methodology, *Food and Agriculture Organization*, 2013
- Boxall R.A., Brice J.R., Taylor S.J., Bancroft R.D., Technology and management of storage. *Crop Post-Harvest: Science and Technology, Principles and Practices*, 2002, 1, 141-232
- Dharmadasa R.M., Abeysinghe D.C., Dissanayake D.M.N., Fernando N.S., Leaf Essential Oil Composition, Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content of *Pimenta dioica* (L.) Merr (Myrtaceae): A Superior Quality Spice Grown in Sri Lanka, *Universal Journal of Agricultural Research*, 2015, 3, 49-52
- Dharmasena C.M.D., Abeysiriwardena D.D.Z., Effect of variety, grain moisture and packing material on weight and storage pests of rough rice during storage, *Annals of the Sri Lanka Department of Agriculture*, 2003, 57-67
- Golob P., The use of spices and medicinals as bioactive protectants for grains, *Food & Agriculture Organization of the United Nations*, 1999
- Hagstrum D.W., Phillips T.W., Cuperus G., *Stored Product Protection*. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, 2012
- Hueston W., Overview of the global food system: Changes over time/ space and lessons for future food safety, in *improving food safety through a one health approach: workshop summery*, National Academies Press, Washington, 2012
- Isman M.B., Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world, *Annual Review of Entomology*, 2006, 51, 45-66
- Kedia A., Prakash B., Mishra P.K., Singh P., Dubey N.K., Botanicals as eco-friendly biorational alternatives of synthetic pesticides against *Callosobruchus* spp.(Coleoptera: Bruchidae)—a review, *Journal of Food Science and Technology*, 2015, 52, 1239-1257
- Kim S.I., Roh J.Y., Kim D.H., Lee H.S., Ahn Y.J., Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*, *Journal of Stored Products Research*, 2003, 39, 293-303
- Kostyukovsky M., Rafaeli A., Gileadi C., Demchenko N., Shaaya E., Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests, *Pest Management Science*, 2002, 58, 1101-1106
- Koul O., Walia S., Dhaliwal G.S., Essential oils as green pesticides: potential and constraints, *Journal of Environmental Science and Technology*, 2008, 4, 63-84
- Mailafiya D.M., Maina Y.T., Degri M.M., Gadzama U.N., Traders' perception of food grain storage and pest management in Dalwa market, Borno State, Nigeria, *Journal of Agricultural and Crop Research*, 2014, 2, 62-70
- Mezgebe A.G., Terefe Z.K., Boshia T., Muchie T.D., Teklegiorgis, Y., Post-harvest losses and handling practices of durable and perishable crops produced in relation with food security of households in Ethiopia: Secondary data analysis, *Journal of Stored Products and Postharvest Research*, 2016, 5, 45-52
- Mohan S., Fields P.G., A simple technique to assess compounds that are repellent or attractive to stored-product insects, *Journal of Stored Products Research*, 2002, 38, 23-31
- Moreira M.D., Picanço M.C., Barbosa L.C.D.A., Guedes R.N.C., Campos M.R.D., Silva G.A., Martins J.C., Plant compounds insecticide activity against Coleoptera pests of stored products, *Pesquisa Agropecuária Brasileira*, 2007, 42, 909-915
- Nwanze K.F., Horber E., Pitts C.W., Evidence for ovipositional preference of *Callosobruchus maculatus* for cowpea varieties, *Environmental Entomology*, 1975, 4, 409-412
- Ojebode M.E., Olaiya C.O., Adegbite A.E., Karigidi K.O., Ale T.O., Efficacy of some plant extracts as storage protectants against *Callosobruchus maculatus*, *Journal of Biotechnology and Biomaterials*, 2016, 127, 2

- Palipane K.B., Wijayaratne L.K.W., Fernando, M.D., Control of insect pests under ware-house conditions using smoke generated from partial combustion of rice (paddy) husk, *Journal of the National Science Foundation of Sri Lanka*, 2009, 37(2), 125-134
- Pugazhvendan S.R., Ross P.R., Elumalai K., Insecticidal and repellent activities of plants oil against stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Asian Pacific Journal of Tropical Disease*, 2012, 2, 412-415
- Rahman A., Talukder F.A., Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*, *Journal of Insect Science*, 2006, 6,1-10
- Rajashekar Y., Bakthavatsalam N., Shivanandappa T., Botanicals as grain protectants, *Psyche: Journal of Entomology*, 2012
- Rajendran S., Sriranjini V., Plant products as fumigants for stored-product insect control, *Journal of Stored Products Research*, 2008, 44, 126-135
- Ratnasekera D., Rajapakse R.H.S., The potential use of indigenous plant materials against *Callosobruchus chinensis* L. and *Callosobruchus maculatus* L.(Coleoptera, Bruchidae) in stored legumes in Sri Lanka, *Journal of Biopesticide*, 2012, 5, 88-94
- Senaratne R., Dayatilake G.A., Subasinghe S., Studies in Sri Lanka on cowpea: N₂ fixation, growth, yield, and effects on cereals, nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia, *International Atomic Agency* 1998, 117
- Talukder F., Pesticide resistance in stored-product insects and alternative biorational management: a brief review, *Sultan Qaboos University Research Journal-Agricultural and Marine Sciences*, 2009, 14, 9-15
- Tiroesele B., Thomas K., Seketeme, S., Control of cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), using natural plant products, *Insects*, 2014, 6, 77-84