Acta fytotechn zootechn, 20, 2017(3): 54–59
http://www.acta.fapz.uniag.sk

Original Paper

Comparative sensitivity of maize weevil to essential oil of *Hoslundia opposita* Vahl leaves subjected to different drying regimes

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Article Details: Received: 2016-06-11 | Accepted: 2016-07-28 | Available online: 2017-09-30

http://dx.doi.org/10.15414/afz.2017.20.03.54–59

Botanical essential oils (EOs) are effective alternatives to over-dependence on synthetic insecticides in stored product protection. However, the burden of handling bulky fresh botanicals needed for EO extraction and the tendency of the freshly harvested botanical to rot in transit, if wrongly handled, can be bottlenecks to pilot scale application of EO. Therefore, this study evaluates the comparative efficacy of the EOs obtained from freshly harvested *Hoslundia opposita* Vahl (Lamiaceae) and plants exposed to different shade-drying regimes (1–5 days) against maize weevil, *Sitophilus zeamais* Motschulsky, under laboratory conditions. Evaluation included fumigant toxicity and repellence bioassays. At 2 hours after exposure (HAE), EO of freshly harvested *H. opposita* leaves caused significantly (P <0.001) lower mortality (53.30%) than the value observed in *H. opposita* leaves dried for 5 days (90.00%). The lethal time for 90.00% of the weevils (LT_{90}) obtained in EO from *H. opposita* leaves dried for 5 days (2.00 h) was significantly lower than 2.80 and 7.14 h obtained in EOs from the fresh and 1 day-dried leaves, respectively. At 1 HAE, EOs obtained from *H. opposita* leaves dried for 4 and 5 days caused 60.00% repellence which was significantly higher than 20.00% observed in fresh leaves. At 2 HAE, EOs obtained from *H. opposita* dried for 4 and 5 days caused significantly higher repellence (100.00 and 80.00%, respectively) than what was observed in fresh leaves (70.00%). The results imply that shade drying freshly harvested *H. opposita* leaves has no deleterious effects on the efficacy of its EO against maize weevil.

Keywords: bird gooseberry, *Hoslundia opposita* Vahl, botanical essential oils, drying time, insecticidal, maize weevil, *Sitophilus zeamais* Motschulsky

1 Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) is a cosmopolitan major insect pest of stored cereals in developing countries. Recent studies show that its hosts range has increased beyond cereals to other cereal products like pasta and processed tuberous crops. It can also feed on dried vegetables for survival in the absence of its preferred food (Babarinde et al., 2008b; 2013a,b). The use of botanicals for control of pests is receiving renewed attention because of several reasons. Such reasons include availability, low cost implications, reduced technical knowledge, target specificity, biodegradability and ecological safety and compatibility with other control strategies (Babarinde et al., 2008a, 2015; Maheswaran and Ignacimuthu, 2013). Essential oil (EO) is a preferred formulation for pest control because it is effective at comparatively low concentrations. More so, it is effective even without direct contact with the target organism (Moharramipour and Negahban, 2014; Babarinde et al., 2015).

There is contrasting view on the impact of drying methods on the chemical composition of botanical EO. While some authors reported variations only in the quantity, and claimed no disparities in the chemical composition (Omidbaigi et al., 2004; Khalid et al., 2008), other researchers gave contrary reports. For instance, Arabhosseini et al. (2006), Khangholi and Rezaeinodehi (2008), Sellami et al. (2011), Hanaa et al. (2012), Shahhoseini et al. (2013) and Usman et al. (2016) reported that drying methods or regimes have the tendency to affect both the yield and chemical composition of the EOs obtained from botanicals. It can therefore be concluded that the impact of drying methods and regime on the qualitative or quantitative composition of botanical EOs depend on the studied botanical species. Studies on the comparative bioactivity of the EOs obtained from botanicals exposed to varying drying regime against stored product insects are scarce in literatures. From literature search, studies on the bioactivity of EOs obtained from freshly harvested leaves against insect pests seem to be more numerous

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than those on the bioactivity of EOs obtained from dried leaves. There is paucity of information on comparative bioactivities of the EOs obtained from the freshly harvested botanical and the dried one against *S. zeamais* in the literatures. Recently, Usman et al. (2016) reported the comparative contact toxicity of the EOs obtained from the fresh and dried leaves of *Citrus meyeri* against *Callosobruchus maculatus* Fabricius.

Information about the pesticidal properties of *Hoslundia opposita* Vahl (Lamiaceae) leaves is scarce in the literatures except for Babarinde et al. (2014; 2017ab) who recently reported the bioactivity of its EO against rust red flour beetle, *Tribolium castaneum* Herbst and cowpea seed bruchid, *Callosobruchs maculatus* Fabricius. To the best of our knowledge, this is the first report of the bioactivity of *H. opposita* EO against *S. zeamais*. This study was designed in order to compare the sensitivity of *S. zeamais* to EO from freshly harvested *H. opposita* Vahl (Lamiaceae) with the EOs obtained from *H. opposita* subjected to different drying periods. The thrust of this work was the need to examine the comparative efficacy of EO obtained from fresh leaves with from the dried leaves. This is because conveyance of fresh leaves from the place of harvest to the place of extraction can be tedious and the harvested plants may rot in transit if the botanicals are not well handled and long duration is required. Therefore, the objectives of this study is to evaluate the effect of drying periods of *H. opposita* leaves on fumigant toxicity and repellence of the EO obtained from the leaves against maize weevil, *Sitophilus zeamais*.

2 Material and methods

2.1 Insect culture

Sitophilus zeamais was obtained from the Stored Product Entomology Unit of the Department of Crop and Environmental Protection Laboratory, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Nigeria. The insect was raised on uninfested Tsolo (a local yellow maize variety) bought at Sabo Market, Ogbomoso. The maize was not pre-treated with any insecticide. Approximately 300 g maize was weighed into each of six 1 L capacity plastic jars and 30 mixed sexes *S. zeamais* adults were introduced into each jar. The jars were covered with muslin cloth and tied with robber band to allow aeration and prevent either escape of the introduced weevils or intrusion of unwanted species. The insect culturing jars were put inside a wooden cupboard in the laboratory (28 ±2 ºC temperature and 70 ±3% relative humidity). A period of 14 days was allowed for mating and oviposition of the insects, after which the introduced parental generation was removed from the cultures. The insect cultures were maintained throughout the experimental period from which adults of known age were obtained when needed for bioassay.

2.2 Procurement of Hoslundia opposita essential oil

Freshly harvested *Hoslundia opposita* leaves were obtained from the premises of the University of Ilorin, Ilorin, Nigeria in September, 2015 at 7 am and subjected to different shade-drying periods (1, 2, 3, 4 and 5 days) under shade at 28±2 ºC temperature and 70 ±3% relative humidity on a wooden laboratory bench. Extraction of the EOs was done at the Department of Chemistry Laboratory, University of Ilorin, Ilorin, Nigeria using hydro distillation method with the use of Clevenger type apparatus (Babarinde et al., 2014), using 200 g each of either the freshly harvested or the dried leaves. The EOs obtained from the freshly harvested leaves and the leaves exposed to the different drying periods were separately stored in labeled glass sample bottle at 4 °C until use.

2.3 Bioassays of insect

Two bioassays (fumigant toxicity and repellence) were used to evaluate the comparative sensitivity of maize weevil, *S. zeamais*, to the EOs. The concentrations used for the bioassays were determined by preliminary (dummy) experiments

2.3.1 Fumigant toxicity bioassay

Fumigant toxicity bioassay was done as described by Babarinde et al. (2014), using Whatman filter paper (approximately 4 cm^2 area) folded and glued to the inner surface of the lid of 0.75 L capacity fumigation chamber. EOs (25 µL) obtained from the freshly harvested *H. opposita* leaves and the leaves exposed to different drying period were separately applied to the filter paper glued to the lid of the chamber and covered for 20 min prior to the introduction of the insects. The control was 0.75 L capacity fumigation chamber without EO application. Thereafter, ten 1- to 5-day old *S. zeamais* adults were separately introduced into the both EO-treated and control fumigation chambers and then covered again. The experiment was replicated three times. Data were taken half-hourly for 5 hours on the mortality of *S. zeamais*. Insects were adjudged dead when they were unable to move their legs and antennae.

2.3.2 Repellence bioassay

Area preference test previously described by Babarinde et al. (2014; 2017a) was used for the repellence bioassay using 5 µL EO obtained from the *H. opposita* leaves exposed to different drying regimes. The test arena was a 9 cm diameter Petri dish with Whatman No 1 filter paper (9 cm diameter) cut into equal halves and joined together with an adhesive tape. The EO concentration (5 µL) was applied onto one half paper disc using a pre-set micro applicator, while the other half was left untreated. Thereafter, ten 1- to 5-day old *S. zeamais* adults were introduced to the centre of the test arena.

The experiment was replicated three times. Repellence data (numbers of insects present in control and treated halves) were taken at 1 and 2 hours after treatment. Percentage repellence (PR) value was calculated thus:

$$PR = (Nu - N_t/Nu + N_t) \times 100,$$

where:

N_u – number of insects on untreated disc
N_t – number of insects on treated disc

2.4 Experimental design and statistical analyses

The experiment was laid out in Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) and significant means were separated using Studentized Neuman Keuls (SNK) at 5% probability level. Probit analysis was used to determine the lethal time for 50% and 90% (LT_{50} and LT_{90}) of the assayed weevils. All statistical analyses were done using SPSS Software (SPSS, 2006).

3 Results

3.1 Fumigant toxicity of the essential oils against *S. zeamais*

The results of the toxic effect of drying regimes on the EO obtained from the leaves of *H. opposita* against *S. zeamais* is presented in Table 1. Throughout the experimental period, it was observed that mortality observed in untreated control was significantly (P <0.05) lower than mortality observed in experimental set up with EO treatments. When *S. zeamais* adults were exposed to EO for 0.5 h, the percentage mortality (13.33–36.67%) was significantly (Df = 6, 20; F = 7.515; P <0.001) higher than 0% mortality observed in the untreated control. Also, at 1 hour exposure period, percentage mortality observed in weevils treated with the EO of leaves dried for 4 and 5 days (56.67% and 50.00%, respectively) was significantly (Df = 6, 20; F = 9.492; P <0.001) higher than mortality observed in weevils treated with the EO of 3-day drying period (23.33%) and the untreated control (3.33%). At 1.5 hour exposure period, all EO caused significantly (Df = 6, 20; F = 15.97; P <0.001) higher fumigant toxicity (46.67-73.33%) than what was observed in the untreated control (3.33%). At 2 hours after exposure (HAE), mortality observed from the EO of freshly harvested *H. opposita* leaves (53.3%) was significantly (Df = 6, 20; F = 14.828; P <0.001) lower than 90% mortality observed from *H. opposita* leaves dried for 5 days. At 2.5–5 HAE, mortality due to exposure of weevils to EO-fumigated chambers was not significantly affected by drying regime of the botanicals, but values (70.00–100.00%) were significantly higher than mortality observed in the untreated control (3.33%).

Means were compared along the drying regimes. Values with different alphabets for the same bioassay duration (1 or 2 h) are significantly (P <0.05) different using SNK. Repellence classes inserted into the bars: Class I = 0.1– 0% Class II = 20.1 – 40%; Class III = 40.1–60.1% Class IV = 60.1–80%; Class V = 80.1–100%. ANOVA Results {(1 h: Df = 5,17; F = 8.057, p = 0.002); (2 h: Df = 5,17; F = 27.00, P <0.001)}.

Although the EOs from *H. opposita* leaves dried for 1–5 days had lower LT_{50} values (1.28–1.77 h) compared withthe value observed in the EO from freshly harvested leaves (2.22 h), the difference was not significant, taking into consideration the overlapping of their Fiducial limits. However, the LT_{90} (2.00 (1.84–2.19) h) obtained from the EO of the *H. opposita* leaves shade-dried for 5 days was significantly lower than 2.80 (2.52–4.79) h and 7.14 (5.13–9.27) h obtained for the EOs from the freshly harvested and 1 day-dried *H. opposita* leaves, respectively (Table 2).

3.2 Repellent properties of the essential oils against *S. zeamais*

The result of repellence test is represented in (Fig. 1). At 1 HAT, EOs obtained from *H. opposita* leaves shade-dried for 4 and 5 days caused Class III repellence (60.00%) which was significantly (Df = 6, 20; F = 8.057; P <0.001) higher than Class I (20.00%) observed in freshly harvested *H. opposita* leaves. At 2 HAE EOs obtained from *H. opposita* dried for 4 to 5 days caused significantly

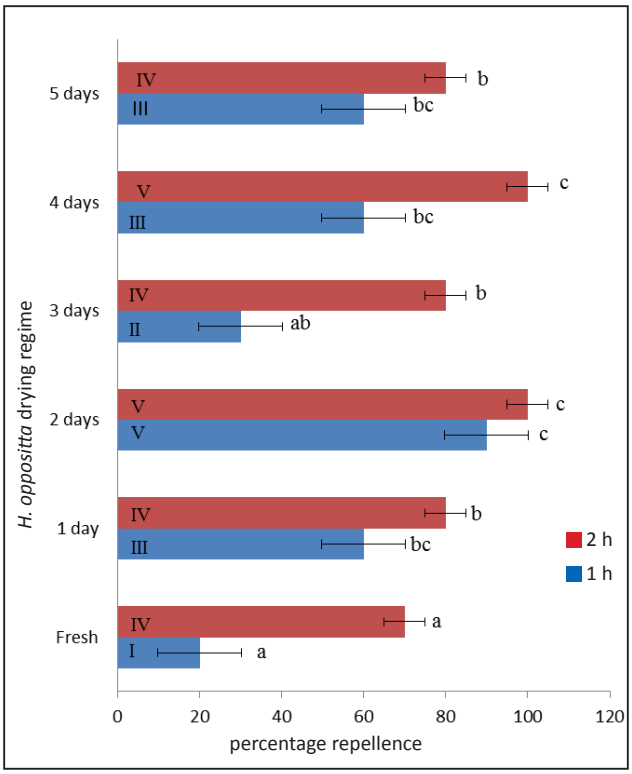

Figure 1 Repellence of essential oils obtained from *Hoslundia opposita* leaves exposed to different drying regimes against *Sitophilus zeamais*

Table 1 Fumigant toxicity of essential oils obtained from *Hoslundia opposita* leaves exposed to different drying regimes against *Sitophilus zeamais*

Mortality at hours after exposure										
Drying Period	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
0 day (Fresh)	26.67 ±3.33bc	40.00 ±5.77bc	53.33 ±3.33b	53.33 ±3.33b	76.67±14.53b	100.00 ±0.0b	100.00 ±0.0c	100.00 ±0.0b	100.00 ±0.0b	100.00 ±0.0c
1 day	23.33±3.33bc	40.00±5.77bc	53.33±8.82b	63.33±14.53bc	70.0 ±17.32b	76.6±12.02b	76.67±12.02b	76.67±12.02b	80.00±11.55b	86.67±6.67b
2 days	20.00 ±0.00bc	40.00 ±5.77bc	56.67 ±6.67b	76.67 ±6.66bc	96.67 ±3.33b	100.00 ±0.0b	100.00 ±0.0c	100.00 ±0.0b	100.00 ±0.0b	100.00 ±0.0c
3 days	3.33 ±3.33b	23.33 ±3.33b	46.67 ±3.33b	66.67 ± 3.33bc	86.67 ±3.33b	86.67 ± 3.33b	86.67 ± 3.33b	100.00 ±0.0b	100.00 ±0.0b	100.00 ±0.0c
4 days	36.67 ± 8.82c	56.67 ±8.82c	73.33 ±6 .68b	73.33 ±6.68bc	80.00 ±0.0b	83.33 ± 3.33b	100.00 ±0.0c	100.00 ±0.0a	100.00 ±0.0b	100.00 ±0.0c
5 days	23.33 ±3.33bc	50.00 ±5.77c	70.00 ± 5.75b	90.00 ± 5.77c	100.00 ±0.0b	100.00 ±0.0b	100.00 ±0.0c	100.00 ±0.0b	100.00 ±0.0b	100.00 ±0.0c
Untreated control	0.00 ±0.00a	3.33 ±3.33a	3.33 ± 3.33a	3.33 ± 3.33a	3.33 ±3.33a	10.00 ±5.77a	3.33 ±3.33a	3.33 ±3.33a	3.33 ± 3.33a	3.33 ±3.33a
ANOVA Results	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20	*Df* = 6, 20
	F = 7.515	F = 9.492	F = 15.97	F = 14.828	F = 13.714	F = 27.303	F = 52.689	F = 87.667	F = 63.000	F = 2163.667
	P = 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Data are means ± standard errors. Values with different alphabets along the column are significantly (P <0.05) different using SNK.

Table 2 Lethal time (LT_{50} and LT_{90} (h)) of essential oils obtained from *Hoslundia opposita* leaves exposed to different drying regimes against *Sitophilus zeamais*

Drying regime	LT50 (95% FL)	LT90 (95% FL)	Regression equation	Slope (± SE)	Chi square	*Df*
0 day (Fresh)	2.22 (0.97–2.47)	2.80 (2.52–4.79)	Y = -4.43 - 4.20X	-4.20 (±1.06)	22.30	7
1 day	1.32 (0.22–2.48)	7.14 (5.13–9.27)	Y = -0.21 - 0.52X	-0.52 (±0.41)	2.32	7
2 days	1.63 (1.31–1.80)	2.33 (2.19–2.55)	Y = -1.72 - 2.99X	-2.99 (±0.58)	11.89	7
3 days	1.57 (1.25–1.83)	3.02 (2.64–3.64)	Y = -0.89 - 6.31X	-6.31 (±0.14)	20.45	7
4 days	1.77 (0.00–2.56)	3.26 (0.64–13.07)	Y = -1.12 - 2.48X	-2.48 (±0.49)	33.54	7
5 days	1.28 (1.10–1.42)	2.00 (1.84–2.19)	Y = -0.70 - 2.84X	-2.84 (±0.25)	7.85	7

SE – Standard error; FL – Fiducial Limit

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(*Df* = 6, 20; *F* = 27.00; *P* <0.001) higher repellence (100.00 and 80.00%, respectively) than what was observed in freshly harvested leaves (70.00%).

4 Discussion and conclusions

It was observed that regardless of the drying regime, EO obtained from *H. opposita* leaves had significant fumigant toxicity against *S. zeamais* compared with the untreated control. Toxicity of *H. opposita* was exposure period-dependent. This was because the weevils had no escape route from the experimental unit, and so, the longer the period of exposure, the higher the toxic effect of the EO that penetrated into their body systems. This agrees with Lira et al. (2015) who reported the fumigant toxicity of EO from *Alpinia purpurata* inflorescences against *S. zeamais*. The observation was also in line with Babarinde et al. (2014) who reported the fumigant toxicity of the EO obtained from the freshly harvested *H. opposita* leaves against *T. castaneum*. Santos et al. (2015) recently reported the toxicity of plant oils from southwestern Amazon against *S. zeamais*. The fumigant toxicity of the EOs implies that they could be used in controlling *Sitophilus* species infesting cereals at small concentrations without direct contact with the target organisms.

Percentage repellence also progressed with exposure period and was significant at 1 and 2 HAT. Higher repellence observed at a later exposure period than early exposure period implies that the components of *H. opposita* EO did not volatilize because the repellency chamber was closed. Also, there was hyper excitability due to the olfaction of the EO at the early exposure period, but the insects attained stability in their responses to the EO with an increase in exposure period (Babarinde et al., 2014). Repellent property of the EO shows that they can be used to control non-resident *S. zeamais* populations. The repellent property of the EOs obtained from *Pistacia lentiscus* (Anacadiaceae) leaves, and some tropical and Mediterranean botanical species against *S. zeamais* has been reported by some authors (Conti et al., 2010; Bougherra et al., 2015) From the results of the two bioassays, EO obtained from *H. opposita* leaves dried for 4 or 5 days appear to be significantly better than the EO obtained from freshly harvested *H. opposita*. This result contradicts the result of Usman et al. (2016) who reported similarity between the contact toxicity of the EO obtained from both fresh and dried leaves of *Citrus meyeri* against *C. maculatus*. The disparity in the results of the two experiments could be attributed to the differences in the studied organisms, the botanicals and the bioassays evaluated. While Usman et al. (2016) evaluated contact toxicity against *C. maculatus* using *C. meyeri*, the present study reports the evaluation of fumigant toxicity and repellence of *H. opposita* against *S. zeamais*.

Bioactivities of EOs against stored product insects have been associated with the chemical constituents present in the EOs. For instance, Pimienta-Ramírez et al. (2016) reported that *Eupatorium glabratum* EO and two of the main components of the oil, α-pinene and α-phellandrene, were toxic against *S. zeamis*. In another study, fumigant toxicity of EO obtained from *Aphyllocladus decussatus* Hieron, *Aloysia polystachya* Griseb, *Minthostachys verticillata* Griseb Epling and *Tagetes minuta* L, which are rich in ketones and their major components: a-thujone, R-carvone, S-carvone, (-) menthone, R (+) pulegone and E-Z- ocimenone were evaluated against *S. zeamais*. M. verticillata oil was the most toxic and all ketones showed insecticidal activity against the weevil (Herrera et al., 2014). Although, the scope of this study does not include the elucidation of the bioactive components present in each EO, the comparative disparity in the bioactivity of the EOs could be due to the variations in the components present in each EO. According to Shahhoseini et al. (2013) in a study on the effect of different drying methods on the EO of Lemon verbena (*Lippia citriodora*), the highest content of EO and total monoterpenes were obtained at 30 °C oven drying, neral and geranial being maximized at that temperature. However, increasing temperature of oven had a negative effect on oil content and monoterpenes like neral and geranial. Also, Teles et al. (2013) evaluated the EO content and composition of fresh and dry leaves of spearmint (*Mentha villosa* Huds.) and reported that the drying methodologies affected the composition of the EOs. Rahimmalek and Goli (2013) also reported that the yield and composition of the EOs obtained from *Thymys daenensis* subsp. *daenensis*. Celak leaves were affected by the drying treatments.

In conclusion, the results indicate that the EOs obtained from either fresh or dried *H. opposita* leaves have promising potentials as an effective insecticide against *S. zeamais* adults. However, it is recommended that when there is the need for drying of the freshly harvested botanical in order to reduce the botanical bulkiness and ease of conveyance, shade-drying should be preferred to high temperature oven-drying or sun-drying. This is because shade-drying can preserve the thermo-labile components of the botanical EOs, which might be negatively affected by high temperature drying (Agah and Najafian, 2012).

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