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Effectiveness of Bottle Brush (*Callistemon lanceolatus* DC.) Leaf Extracts to Control the Nymph of Mealybug (*Phenacoccus manihoti* Matile-Ferrero)

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Abstract Mealybug (*Phenacoccus manihoti* Matile-Ferrero) poses a threat to cassava-growing regions of the world. A current global interest has focused on the discovery of non-chemical strategies and environmental friendly pest control approaches. The objective of this study was to investigate the efficacy in terms of toxicity and repellent properties of hexane, acetone and ethanol crude extracts from leaf of bottle brush (*Callistemon lanceolatus* DC.) against the nymph of mealybug (*P. manihoti*) by using leaf dipping method. Various concentrations of bottle brush extract, 0 (5% tween-20 water) 1, 2, 3, 4 and 5% (w/v) were applied. Then the insect mortality was observed at 24 and 48 hrs whereas, the repellent index (%RI) was observed at 24 hrs. The results showed that acetone extract from bottle brush was highly effective in killing the nymph of mealybug with the LC_{50} at 24 and 48 hrs as 3.54 and 2.28 %, respectively. Acetone and ethanol extracts of bottle brush at 2-5% concentrations showed very strong effect in repellence when the nymph of mealybug with >75% RI was found within 24 hrs. Therefore, the acetone extract of bottle brush leaf was highly containing insecticidal activity against nymph of mealybug in laboratory, then the next filed condition substance should be developed.

Keywords: plant extract, toxicity, repellent property, leaf dipping method

Introduction

The cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) is one of the most severe pests of cassava (*Manihot esculenta* (L.) Crantz) in the world (Bellotti *et al.,* 1999). Mealybug invasion can cause considerable economic damages on agricultural and horticultural plants (Miller *et al.,* 2005). Directs damages are usually a result of sap removal and toxins caused by the insect injection. Besides, indirect damages are associated with sooty mold growth caused by honeydew stains on plants (Mibey, 1997). In addition, the effects of plant viruses transmitted by mealybugs are also reported (Hoffmann, 2009). Chemical insecticides cause severe environmental pollution and bring about adverse effects on people and

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beneficial insects (Yi *et al*., 2011). Many insecticides are used for mealybug management but the increment of insecticide resistance is also a problem by using those insecticides (Wakgari and Giliomee, 2003). To solve those problem bases now there are several newer treatments available with more novel modes of action e.g. neonicotinoids, botanical insecticides, biosynthesis inhibitors and insect growth regulators (Daane *et al.,* 2006; Lo and Walker, 2010). Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little the environment or to human health (Isman, 2006). Danga *et al*. (2015) strongly reported that the extract of bottle brush (*Callistemon lanceolatus* DC.) had effectiveness to control bean eeetles (*Callosobruchus maculatus* (Fabricius)).

Callistemon is a genus of 34 species of shrubs in the family Myrtaceae, all of which are endemic to Australia (Goyal *et al*., 2012). Aqueous extracts of the leaves and flowers had antifungal and antibacterial activity (Singh and Shiva, 2014). The extract also showed cholinesterase activity. The essential oils from leaves possess antimicrobial, fungitoxic, antinociceptive and anti-inflammatory activities (Kumar *et al*., 2011), some chemical compounds commonly, could be found in leaf bottle brush as 1,8-cineole and β-pinene. (Singh and Shiva, 2014).

Objectives of this study was to investigate the efficacy in terms of toxicity and repellent properties of hexane, acetone and ethanol crude extracts from leaf of bottle brush (*C. lanceolatus*) against the nymph of mealybug (*P. manihoti*) by using leaf dipping method.

Materials and methods

Stock culture of mealybug

The tested insects were obtained from a culture of Phenacoccus manihoti Matile-Ferrero maintained on pumpkin in the laboratory of Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. Insect adult colony originally collected from the field in Kanchanaburi province, Thailand. and then, they were reared under laboratory condition prior to use nymph in various bioassays.

Extract preparation

Fresh leaves of bottle brush (*C. lanceolatus*) was air-dried and ground into finely powder in blender. Powder samples (0.5 kg) were extracted with 4 L of hexane using soaking method allowed to stand at room temperature about 3 days, then filtered through filter paper. After filtering, the hexane was removed at 50°C using a rotary evaporator, to obtain the crude hexane extract. The remaining materials were then extracted with 4 L of acetone and ethanol respectively. By the same method, acetone and ethanol crude extracts were also obtained. The final yield of crude extracts (1 g) were re-suspended in 20% Tween-20 in water to a volume of 10 mL, to make a 10% (w/v) stock solution and was kept at 10°C for subsequent use. This stock solution was further diluted with water to obtain the 1, 2, 3, 4 and 5% extract solutions

Bioassay

The insecticidal toxicity of the crude extracts against C. lanceolatus nymph was investigated by using leaf dipping method when the okra fruit was used as host plant in the experiment. Okra fruits were dipped with the extract solution 0 (5% tween-20 in water), 1, 2, 3, 4 and 5%, and they were left at room temperature to air-dry. When after the fruit was placed in plastic Petri dish (13 cm diameter) and covered with fine cloth. Twenty C. lanceolatus nymphs were then introduced in each plastic Petri dish containing a treated okra fruit, and covered with fine cloth. The experiment was performed as no-choice test and the percent mortality of the nymph was observed at 24 and 48 hrs.

The repellent tested was investigated through choice tests. The same concentrations of extract solutions as referred in the no-choice test were performed, if only each treatment and control were applied on each side of okera fruit. After 24 hrs, the number of insects was counted in the treated (T) and control (C). The percentages of repellent index (%RI) was calculated by the following formula: %RI = [(C-T) / C+T)]×100 (Pascual-Villalobos and Robledo, 1998).

Statistical analysis

Data for the bioassay was corrected for mortality in the control using Abbott's formula (Abbott, 1987). The experiment was designed in three completely randomized replicates. The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). Value of LD50 (median lethal dose) was calculated by the probit method.

Results and Discussion

The efficacy in terms of toxicity and repellent properties of hexane, acetone and ethanol crude extracts from leaf of bottle brush (*Callistemon lanceolatus* DC.) against the nymph of mealybug (*P. manihoti*) by using leaf dipping method showed that acetone extract from bottle brush was highly effective in killing the nymph of mealybug with the LC_{50} at 24 and 48 hrs for 3.543 and 2.275% mortality that more than caused by ethanol (4.468 and 3.554%) and hexane (5.070 and 3.811%) extracts, respectively, with significantly different (Table 1). Many documents regarding various plant extracts presented their effectiveness against different target insects. Soliman *et al*. (2005) reported that ethanol extracts from various wild plant proved as the most efficiency against Aphis gossypii followed by acetone, hexane, ethyl acetate and finally diethyl ether plant extracts. Ethanol extract of bottle brush at 5% concentration showed extremely repellent, when 2-4% concentrations showed 78.9-92.2%RI to mealybug nymph was found within 24 hrs. Whereas, acetone extract of bottle brush at 2-5% concentrations gave also high repellence with 76.1-94.4%RI. Besides, hexane extracts of this plant presented moderate repellence when less than 64.4%RI was found (Figure 1). Viswan *et al*. (2014) reported that the essential oil of bottle brush was higly repellent and adulticidal activity was against coleopteran beetle, *Lasioderma serricorne*. Plants belonging to the family Myrtaceae were identified as containing botanical insecticides (Papachristo and Stamopoulos, 2002). Therefore, the acetone extract of bottle brush leaf had highly insecticidal activity against nymph of mealybug in laboratory, then its effectiveness in filed condition should be developed.

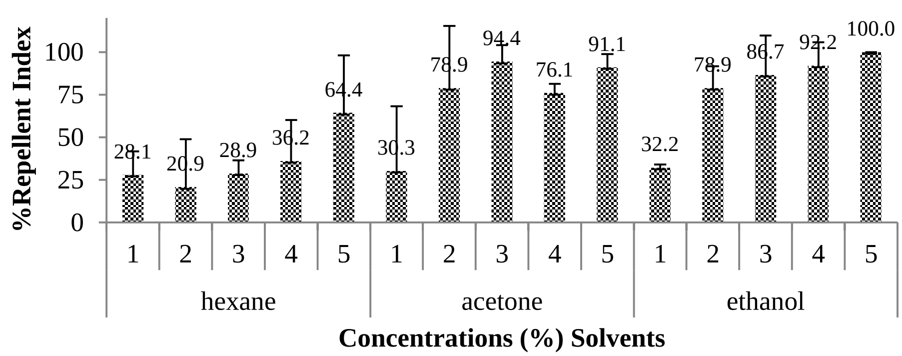

Figure 1. Percentage of repellent index (RI) of bottle brush (*Callistemon lanceolatus* DC.) leaf extracts at various concentrations against mealybug (*Phenacoccus manihoti* Matile-Ferrero) by dipping method at 24 hrs.

Table 1. Percentage of mortality of mealybug (*Phenacoccus manihoti* Matile-Ferrero) after feeding with host plants treated with bottle brush (*Callistemon lanceolatus* DC.) leaf extracts at various concentrations by dipping method at 24 and 48 hrs.

Time	Essential oils	%Mortality[1/]						CV	LC_{50}	Slope	SE
		Concentration (%)									
		0	1	2	3	4	5				
24 hr.	Hexane	0.0±0.0^{C}	12.2±10.4^{Ca}	15.8±1.3^{Cb}	15.2±9.1^{Cc}	34.4±11.2^{Bb}	51.0±10.1^{Ab}	39.01	5.070	0.367	0.041
	Ethanol	0.0±0.0^{C}	0.0±0.0^{Ca}	8.0±2.7^{Cc}	28.8±1.1^{Bb}	33.9±11.8^{Bb}	49.2±1.4^{Ab}	25.10	4.768	0.510	0.051
	Acetone	0.0±0.0^{F}	10.6±3.5^{Ea}	27.9±5.0^{Da}	49.1±2.8^{Ca}	55.0±2.4^{Ba}	71.4±0.0^{Aa}	8.17	3.543	0.483	0.051
	%C.V.	-	83.3	19.5	17.8	23.1	10.2				
48 hr.	Hexane	6.2±5.5^{D}	21.4±3.6^{Ca}	25.9±8.5^{Cb}	33.7±8.3^{Cb}	52.7±6.1^{Bb}	68.1±12.6^{Ab}	22.90	3.811	0.357	0.035
	Ethanol	6.2±5.5^{D}	1.7±2.9^{Db}	17.9±8.6^{Cb}	51.1±4.1^{Bb}	62.2±6.8^{Ab}	71.0±4.2^{Ab}	15.95	3.554	0.515	0.040
	Acetone	6.2±5.5^{D}	13.3±6.7^{Da}	43.9±3.7^{Ca}	78.7±11.9^{Ba}	86.8±9.7^{ABa}	94.4±9.6^{Aa}	15.45	2.275	0.690	0.046
	%C.V.	-	38.5	24.9	15.9	11.3	12.1				

1/ Means in row with the same time followed by the same capital letter and column followed by the same common letter are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

Conclusion

From this study, the efficacy in terms of toxicity and repellent properties of hexane, acetone and ethanol crude extracts from leaf of bottle brush (*Callistemon lanceolatus* DC.) against the nymph of mealybug (*P. manihoti*) by using leaf dipping method. It showed that acetone extract was highly effective in killing the nymph of mealybug with the LC_{50} at 24 and 48 hrs as 3.543, and 2.275 %, respectively. Acetone and ethanol extracts at 2-5% concentrations also showed very strong effect in repellence when the nymph of mealybug with >75% RI was found within 24 hrs.

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References

Abbott, W. S. (1987). A method of computing the effectiveness of an insecticide. Journal of the American Mosquito Control Association 3:302-303.

Bellotti. A. C., Smith, L. and Lapointe, S. L. (1999). Recent advances in cassava pest management. Annual Review of Entomology 44:343-370.

Daane, K. M., Bentley, W. J., Walton, V. M., Malakar-Kuenen, R., Millar, J. G. and Ingels, C. A. (2006). New controls investigated for vine mealybug. California Agriculture 60:31-38.

Danga, S. P. Y., Nukenine, E. T., Younoussa, L., Adler, C. and Esimome, C. O. (2015). Efficacy of *Plectranthus glandulosus* (Lamiaceae) and *Callistemon rigidus* (Mytaceae) leaf extract fractions to *Callosobruchus maculatus* (Coleoptera: Bruchidae). Journal of Insect Science 15:139.

Goyal, P K., Jain, R., Jain, S. and Sharma, A. (2012). A review on biological and phytochemical investigation of plant genus Callistimon. Asian Pacific Journal of Tropical Biomedicine 2:1906-1909.

Hoffmann, H. (2011). Aphids, mealybugs and scales; common sapsuckers in the home garden. Department of Agriculture and Food of Western Australia. 499 pp.

Isman, M. B. (2000). Plant essential oils for pest and disease management. Crop Protection 19: 603-608.

Kumar, S., Kumar, V. and Prakash, O. M. (2011). Antihyperglycemic, antihyperlipidemic potential and histopathological analysis of ethyl acetate fraction of *Callistemon lanceolatus* leaves extract on alloxan induced diabetic rats. Journal of Experimental and Integrative Medicine 1:185-190.

Lo, P. L. and Walker, J. T. S. (2010). Good results from a soil-applied insecticide against mealybugs. New Zealand Winegrower 14:125-127.

Mibey, R. K. (1997). Sooty moulds. In: Ben-Dov, Y. and C.J. Hodgson, eds. Soft Scale Insects: Their Biology, Natural Enemies and Control, Amsterdam and New York: Elsevier. pp. 275–290.

Miller, D. R., Miller, G. L., Hodges, G. S. and Davidson, J. A (2005). Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. Proceedings of the Entomological Society of Washington 107:123-158.

Papachristos, D. P. and Stamopoulos, D. C. (2002). Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthosecelides obtectus* (Say). Journal of Stored Product Research 38:117-128.

Park, B. S., Choi, W. S., Kim, J. H., Kim, K. H. and Lee, S. E. (2005). Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents. Journal of the American Mosquito Control Association 21:80-83.

Pascual-Villalobos, M. J. and Robledo, A. (1998). Screening for anti-insect activity in Mediterranean plants. Industrial Crops and Products 8:183-194

Singh, S. and Shiva, S. (2014). Genus Callistemon: an update review. World Journal of Pharmacy and Pharmacetical Sciences 3:291-307.

Soliman, M. M. M., Hassanein, A. A and Abou-Yousef, H. (2005). Efficiency of various wild plant extracts against the cotton aphid *Aphis gossypii* Glov. Aphdidae: Homoptera). Acta Phytopathologica et Entomologica Hungarica 40:185-196.

Wakgari, W. M. and Giliomee, J. H. (2003). Natural enemies of three mealybug species (Hemiptera: Pseudococcidae) found on citrus and effects of some insecticides on the mealybug parasitoid *Coccidoxenoides peregrines* (Hymenoptera: Encytridae) in South Africa. Bulletin of Entomological Research 93:243-254.

Yi, D., Cui, L., Liv, Y., Zhauang, M., Zhauang. Y., Fang, Z. and Yang, L. (2011). Transformation of cabbage (*Brassica oleracea* L. var. *capitata*) with Bt cry1Ba3 gene for control of diamondback moth. Agricultural Sciences in China 10:1693-1700.

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