American-Eurasian J. Agric. & Environ. Sci., 6 (3): 356-359, 2009
ISSN 1818-6769

Efficiency of Vetiver Grass Extracts Against Cowpea Weevil (*Callosobruchus maculatus* Fabr.)

Udomporn Pangnakorn

Department of Agricultural Science, Faculty of Agriculture Natural Resources and Environmental, Naresuan University, Phitsanulok, 65000, Thailand

Abstract: Roots of vetiver grass (*Vetiver zizanioides* Linn.) were collected, cleaned and dried at 25 C° for 3-4 days and powdered. Two methods were used in extraction, namely stream distillation and the other method extraction with three solvents (hexane, methanol and chloroform). Toxicity of the vetiver grass extraction was tested on cowpea weevil (*Callosobruchus maculatus* Fabr.) under laboratory conditions. A residue film test was tested the toxicity on the cowpea weevils. Mortality was observed daily for 24, 48 and 72 hours. The stream distillation of concentrations at 100 % and 90% showed highly efficient toxicity with 52.63% and 18.42% of cowpea weevils were killed, respectively. There was no significant difference of mortality when 50% to 80% of concentrations were applied. However the extraction with either of the three solvents: hexane, methanol and chloroform gave rather low mortality of cowpea weevils at 18.42, 15.78 and 7.89%, respectively.

Key words: Cowpea weevil · Vetiver grass · Mortality · Stream distillation · Residue film test

INTRODUCTION

Cowpea weevils *Callosobruchus maculatus* (Fabr.) damage cowpeas after harvest, attacks dried cowpeas and other related stored seeds. Damage appears as round holes in the peas. The weevils prefer dried cowpeas but will attack other beans and peas in storage. Pest control using some synthetic chemicals may cause health and environmental problems. Biopesticides provide alternative agent for plant protection to avoid those problem. Many essential oils from plants are insect repellents and have fungicidal activities [1]. The present work aimed to evaluate the insecticide activity of vegetable extracts against *C. maculatus* (Fabr.). Flowers, fruits and dry leaves of f ive vegetable species were used for extraction, in percolator, w ith solvent ethyl alcohol (30 and 50%). The mortality of the insects was evaluated after 48 hours after the extracts application. The results allowed the conclusion that the mortality of the insects is related to the increase of the time of exposition to the extracts and that the extracts of *Piper nigrum* and *Azadirachta indica* were the most effective in all exposition periods [2]. Also, Su [3] reported that extract of black pepper was found to be highly toxic to cowpea weevils *C. maculatus* (F).

Vetiver grass (*Vetiveria zizanioides*) is a tropical plant which grows naturally. In Thailand, vetiver grass can be found growing in a wide range of area from highlands to lowlands in various soil conditions. This species appears in a dense clump and grows fast through tillering. The clump diameter is about 30 cm. and the height is 50-150 cm. The leaves are erect and rather stiff with 75 cm. of length and 8 mm. of width. Vetiver roots are important and the most useful part. Most grass has fibrous roots which spread out from the underground part of the clump and hold the soil in an horizontal pattern. The roots that penetrate vertically into the soil are not deep. In contrast, the root system of vetiver grass does not expand horizontally but penetrates vertically deep into the soil, whether it be the main roots, secondary roots or fibrous roots. Root of vetiver grass has been used as a herbal plant, such as in aromatic form in wardrobe and insects repellents. The oil extract from the roots of *V.zizanioides* has been used in the perfume trade. Indigenous peoples have recognized vetiver for its medicinal uses, for thatching, mulch and feed and for soil and moisture conservation.

Since 2004 as a Food Safety Year, Thailand has had to highly completely with other countries in the world market. A wide variety of higher plants have been known

Corresponding Author: Dr. Udomporn Pangnakorn, Department of Agricultural Science, Faculty of Agriculture Natural Resources and Environmental, Naresuan University, Phitsanulok, 65000, Thailand

to provide new sources of natural pesticides [4]. This study was carried out to investigate the potential of using biopesticides from vetiver grass for controlling cowpea weevil.

MATERIALS AND METHODS

Plant Preparation: Fresh root vetiver grass was cleaned and air dried at 25°C for 3-4 days and powdered for sample extraction.

Plant Extraction

Steam Distillation Method: 500 g dried sample of root vetiver grass was placed in the plant chamber of the still and pressurized steam was generated in a separate chamber and circulated through the plant material. The heat of the steam (80-100°C) forces the tiny intercellular pockets that hold the essential oils to open and release them. As the steam cools, it condenses into water. The steam condenses on the root vetiver grass, both extracting and condensing the oil. The essential oil forms a film on the surface of the water. To separate the essential oil from the water, the film is then decanted or skimmed off the top.

Solvent Extraction: Extraction of chemicals from the plant materials was performed using the Soxhlet apparatus. 20 g dried sample of root vetiver grass was placed in cloth bags and put in the extraction machine. The solvent was added to plant chamber and pressurized steam with heat of the steam at 80-100°C. As the steam cools, it condenses into water. The steam condenses on the root vetiver grass, both extracting and solvent. Once the extraction of 1,500 ml of hexane was complete, the solvent was changed to chloroform and methanol. The following solvents in polarity level were hexane, chloroform and methanol, each of the solvents taking 7-10 days for extraction. The vetiver grass extracts were concentrated for testing on *Callosobruchus maculatus* (F).

Insect Preparation: Cowpea weevil (*C. maculatus* F.) culture was reared on mungbean seeds in glass jar at temperature of 30±2 °C, relative humidity of 70±10 % and light at 12:12 (L: D) of day light. The mungbean seeds were frozen at 1-2°C for 3 days for killing the other insects' eggs or parasites contaminated on seed before feeding. The cowpea weevil eggs were collected everyday until hatched to adult within 20 days.

Efficacy Test of Root Vetiver Grass Against Cowpea Weevil: The extractions both steam distillation method and solvent extraction, were diluted with acetone in various concentrations and were tested on cowpea weevils under residue film test

Residue Film Test: The residue film test was used for efficacy test against the cowpea weevil 1 ml of the oil film was dropped on to the vial tube with micropipette and was shaken to spread the oil film inside the tube. A group of ten 2-3 day-old adult female cowpea weevils was placed in the tube and then covered with nylon for air flow. The experimental were done under 4 replications / treatment and were kept at the room temperature. Mortality was observed daily for 24, 48 and 72 hours within 3 days.

RESULTS AND DISCUSSION

The efficacy test of extracted volatile oils from the root of vetiver grass with steam distillation method is shown on Table 1. The results show that the residual contact toxicities to cowpea weevil (*C. maculatus* F.) at 100 and 90% of concentration were highly efficient with mortality rate of 52.63 and 18.42% respectively. The lower concentration from 50 until 80% showed only 2.65 to 7.89% of mortality against cowpea weevil. Particularly, less than 50% had no effect on mortality of cowpea weevil. The highest efficiency of vetiver grass extraction revealed on 100% of concentration which gave significantly high mortality (LSD=1.0950) among the treatments. According to the extracted volatile oils from the root of vetiver grass produced a thin layer of film on the top of the extraction. Although allowing them to stand for a long time it did not cause clear separation with no color like water. These events are different from extracted volatile oils from lemon grass where the yellow layer will be clearly separated from water [5]. Therefore, the extraction of volatile oils from the root of vetiver grass with steam distillation method could not be evaluated on concentration. However, in this study 100% of concentration is presumed. When it was diluted in various concentrations, the active ingredient could not show efficacy as good as the concentration at 100%. However, Reddy and Singh [6] reported neem oil volatiles at a concentration of 200 µl against cowpea weevil (*C. maculatus* F.) caused 100 percent mortality within an hour.

Table 1: Residual contact toxicities of extracted volatile oils from the root of vetiver grass to cowpea weevil (*C. maculatus* F.)

	Mortality[1]			
Treatment	24 hour	48 hour	72 hour	df
Control	0.00	0.00	2.50 cd	ns
50%	0.00	0.00	0.00 d	ns
60%	0.00	2.65	2.65 cd	ns
70%	5.26	5.26	7.89 bc	ns
80%	0.00	2.65	7.89 bc	ns
90%	2.65	10.52	18.42 b	ns
100%	10.52	18.42	52.63 a	*

[1] = Corrected mortality [8]

LSD = 1.0950 ns = non significant, * = highly significant

Table 2: Residual contact toxicities of solvent extracts from the root of vetiver grass to cowpea weevil (*C. maculatus* F.).

	% Mortality after 72 hours[1]		
Treatment	24 hour	48 hour	72 hour
Control	0.00	2.65	2.65 ns
Hexane	2.65	10.52	18.46 ns
Chloroform	2.65	5.26	7.69 ns
Methanol	0.00	7.89	15.78 ns

[1]= Corrected mortality (Abbot 1925)

LSD = 2.0250 ns = non significant

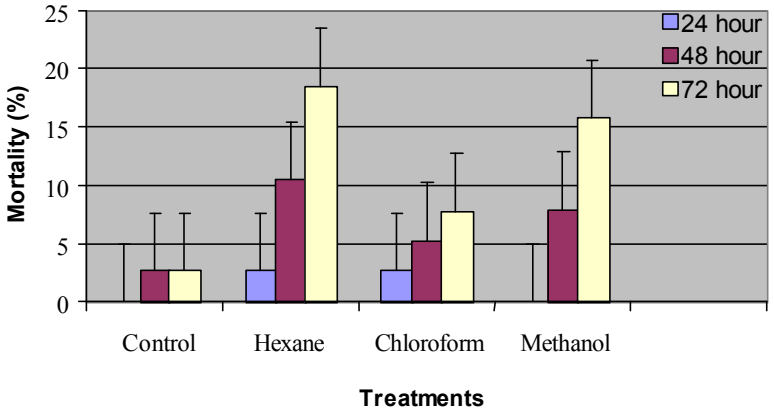

Fig. 1: Toxicities of different solvent extracted from the root of vetiver grass against to cowpea weevil (*C. maculatus* F.) after treatment at 24, 48 and 72 hour

Table 2 showed the efficacy of residual contact toxicities of three solvents: hexane, methanol and chloroform from the root of vetiver grass on cowpea weevil. The hexane and methanol vetiver grass extracts indicated mortality rate after 72 hours of application at 18.42, 15.78% respectively. Although there were no significance differences among the three solvents when compared to the untreated, these showed that the extraction with solvents gave high % mortality the same as at 90% concentration with steam distillation method. Similarly, Chungsamarnyart and Jiwajinda, [5] reported that the extracted root of vetiver grass showed high efficiency on larva tick nearly to 100 % but gave lower insecticidal activity on adult tick. Additionally the extraction with chloroform had a toxic effect of only 7.89% mortality on cowpea weevil. It may be due to the toxic compound extraction with hexane and methanol having a much higher insecticidal activity compared to the extraction with chloroform. Ho *et al.* [7] reported that the *n*-hexane extract of dried fruit of star anise has higher contact toxicity to the stored products beetles *Tribolium castaneum* (Herbst) than *Sitophilus zeamais* (Motsch).

Figure1 shows the toxicity of extracted volatile oils from the root of vetiver grass with solvents by using residue film test. The result showed that hexane gave highest adult mortality followed by methanol extract after 72 hours of application. Although, the extraction with methanol showed low mortality after 24 hours, but the result gave high mortality after 72 hours. This activity is in parallel with the report by Marngar *et al.* [9] that methanol extracts gave much higher insecticidal activity compared to the aqueous extracts. Meantime the extraction with chloroform resulted in the lowest insecticidal activity.

CONCLUSION

Extracted volatile oils from the root of vetiver grass with stream distillation were found to have insecticidal effect against cowpea weevils (*Callosobruchus maculatus* F.). The 100% of concentration extraction was shown to have the highest mortality. However, the effectiveness is decreased when the extraction was diluted. Meantime the activity of extraction with hexane and methanol showed efficiency mortality trends on adult cowpea weevil in the experimental. Therefore root of vetiver grass show the potential to be developed into biopesticide for controlling stored product pest, particularly larva of insects. The study should be useful in development of plant extracts to reduce the use of synthetic chemicals in preventing crop pests.

ACKNOWLEDGEMENT

The author thankfully acknowledges the funding of Higher Education Commission and also wishes to thank to Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand for supporting sample of insects in this study.

REFERENCES

1. Isman, M., 1999. Pesticides based on plant essential oils. Pesticide Outlook, 10(2): 68-72.
2. Almeida, S.A. de, F. de A.C. Almeida, N.R. dos Santos, M.E. R Araújo and J.P. Rodrigues, 2004. Activity insecticide of vegetable extracts on *Callosobruchus maculatus* (fabr.,1775) (Coleoptera: Bruchidae) R. bras. Agrociência, jan-mar, v. 10, n. 1, pp: 67-70.
3. Su, H.C.F., 1977. Insecticidal properties of black pepper to rice weevils and cowpea weevils. J. Econ. Entomol., 70(1): 18-20.
4. Grainge, M. and S. Ahmed, 1988. Handbook of Plants with Pest-control Properties. Wiley-Interscience, New York.
5. Chungsamarnyart, N. and S. Jiwajinda, 1992. Acaricidal activity of volatile oil from lemon and citronella grasses on tropical cattle ticks. Kasetsart J. (Nat.Sci.) 26(5): 52-56.
6. Reddy, A.V. and R.P. Singh, 1998. Fumigant toxicity of neem (*Azadirachta indica* A. Juss.) seed oil volatiles against pulse beetle, *Callosobruchus maculatus* Fab. J. Appl. Ent., 112: 607-611 12: 1-5.
7. Ho, S.H., Y. Ma, P.M. Goh and K.Y. Sim, 1995. Star anise, *Illicium verum* Hook f. as a potential grain protectant against *Tribolium casta*neum (Herbst) and *Sitophilus zeamais* Motsch. Postharvest Biol. Tech., 6: 341-347.
8. Abbott, W.S., 1925. Method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
9. Marngar, D., B. Kharbuli, D. Syiem and H. Kayang, 2002. Insecticidal Activity of Some Extracts Against the Rice Grasshopper, *Oxya hala*. *In* Proceedings International Conference on Biopesticides 3 April 21-26, 2002, Kuala Lumpur, Malaysia.