

Essential Oil and its Insecticidal Activity of Medicinal Aromatic Plant *Vetiveria zizanioides* (L.) Against the Red Flour Beetle *Tribolium castaneum* (Herbst)

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Abstract: Essential oil of Vetiver has versatile uses particularly as an inexpensive yet effective and eco-friendly tool to compact the essential role of biopesticides as well as insecticidal potentials. Insecticidal activity of the root extract of *Vetiveria zizanioides* (L.) in Petroleum ether, Ethyl acetate, Acetone and methanol against XSM, SMC, SRS and JTC strains of the red flour beetle, *Tribolium castaneum* (Herbst) was studied. The spongy root mass were dried, powdered and extracted by Steam distillation apparatus with the solvents below 60°C. Experimental extracts were applied on larvae and adult beetles in film residue methods and mortality was recorded after 24 h. In larval bioassay the highest toxicity was recorded for petroleum ether extract ($LD_{50} = 0.051 \mu\text{g}/\text{cm}^2$) in XSM strain and the lowest toxicity has been observed in Methanol extract ($LD_{50} = 11.351 \mu\text{g}/\text{cm}^2$) in SMC strain. In adults petroleum ether extract accessible highest toxicity ($LD_{50} = 58.69 \mu\text{g}/\text{cm}^2$) in JTC 12 strain and the lowest toxicity ($LD_{50} = 204.710 \mu\text{g}/\text{cm}^2$) also been observed for acetone extract in SRS strain. LD_{50} , 95% confidence limits and regression equations are presented.

Key words: Aromatic plant, essential oil, insecticidal activity, *Tribolium castaneum*, vetiver

INTRODUCTION

The insecticidal and acaricidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control (Bell *et al.*, 1990; Sahayaraj and Paulraj, 2000). Especially remarkable are tropical plants, from which hundreds of products of secondary metabolism with insecticidal properties have been extracted (Singh and Upadhyay, 1993; Shangwen, 1999, 2001). Chemical compounds from the roots of Vetiver grass possess repellent properties useful against ants, cockroach, bedbugs, headlice and moth (National Research Council, 1993; Handerson *et al.*, 2005a). Vetiver oil and many of its constituents are also repellent and toxic to termites (Zhu *et al.*, 2001a, b; Ibrahim *et al.*, 2004; Handerson *et al.*, 2005b). In addition, wood treated with nootkatone a compound and feeding deterrent against Formosan Subterranean termites. They are environmentally less harmful than synthetic pesticides and acting in many insects in different ways. Vetiver is a tall, tufted, perennial, scented grass, with a straight stem, long narrow leaves and a lacework root system that is abundant, complex, and extensive. It offers an inexpensive yet effective and eco-friendly tool to combat soil erosion. The roots have been used in Asia for centuries for their fragrance, and are woven into aromatic matting and screens. The roots of some cultivars and ecotypes possess essential oil that has been utilized as

fragrant material since ancient times. The plant also contains active ingredients used in traditional medicine and as botanical pesticide. Medicinal and Aromatic Plants (MAP) are two related plant groups that have in their chemical constituents that are either active in curing ailments (i.e., MP) or provide flavor and/or fragrances (i.e., AP) (Chomchalow, 2000). Again he strongly avowed that this plant seized aromatic compounds used mainly as flavors (e.g., in spices and herbs) and fragrances (e.g., in perfumery, cosmetics, soaps) (Chomchalow, 2000, 2001). However, from ancient times, these plants have also been used as raw materials for cosmetics, pharmaceuticals, botanical pesticides, disinfectants, insect repellents, herbal teas, herbal drinks, etc., Still, there is no works has been done in the similar kind of insecticidal activities on *Tribolium castaneum* (Herbst) stored pest. Hence, we have undertaken the following objectives of to study and evaluate the mortality rate (LD_{50}) of larval and adults of this experimental pest through the four different types of solvents (Pet. Ether, EtOH, Acetone and Methanol) based essential oil from *Vetiveria zizanioides* (L.)

MATERIALS AND METHODS

Test insect: Four strains of *T. castaneum* viz. XSM, SMC, SRS and JTC were collected from the Crop Protection and Agricultural research station, where the stocks were maintained since 1990. XSM, strain was

originally collected from flourmills at and other three strains were collected from Crop Protection Lab., Department of Agriculture and environmental Science. Mass cultures were maintained in glass jars (1000 mL) and subcultures were in beakers (500 mL) with food medium and kept in an incubator at $30 \pm 0.5^\circ\text{C}$. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 15:5 was used as food medium throughout the experimental period.

Production of vetiver oil: Spongy root mass of certain cultivars of *Vetiveria zizanioides* contains trace amount of essential or volatile oil, known as vetiver oil or 'khus oil', which can be extracted by steam distillation. The dried roots, after washing and drying, can be distilled immediately, or are stored for 12-24 months so enzymatic process can increase oil yield. The process of distillation consists of soaking the root mass prior to packing in the distillation unit, and steam is allowed to pass through for a period of six hours or more. The steam distillation produces around 0.3-1.0% of oil although higher percentages can also be obtained. A resinoid is also produced by solvent extraction for perfumery work. The principal constituents include vetiverol, vetivone, khusimone, khusitone, terpenes (e.g., vetivenes), and sesquiterpenoids. Its chemical compounds were reviewed by Fuehrer (1970). Distillation is long and involves high pressure using water and steam distillation method.

Oil extraction: Freshly prepared Vetiver oil were mixed with following procedure Extractions were done in a Soxhlet's apparatus with four solvents using the process described by Mehmet and Hakki (2004) and Harborne (1998) below 60°C . The solvents were petroleum ether, ethyl acetate, and methanol used serially on the same stock of spongy root in vetiver. After completing extraction, the mixed solvent was removed from the extract with a vacuum rotary evaporator.

Mortality assay: Film residue method (Busvine, 1971) was used to test the mortality of the larvae and adults of *T. castaneum*. The extracted materials were weighed and dissolved in acetone for dosing. For larval bioassay the experiments were done in glass vials (10 mL, 2.7 mm dia.) in which 0.2 mL of each dose was dropped and air-dried. Then 2.0 g of food was added and ten first instar larvae were released in each vial in four replicates for each dose. A control batch was maintained with the same number of larvae. The doses for all strains were similar and they were 48.530, 4.853, 0.485 and 0.048 $\mu\text{g}/\text{cm}^2$ for petroleum ether extract, 61.080, 6.108, 0.610 and 0.061 $\mu\text{g}/\text{cm}^2$ for ethyl acetate extract, 41.63, 4.163, 0.416 and 0.041 $\mu\text{g}/\text{cm}^2$ for acetic extract, and 26.04, 2.604, 0.260 and 0.026 $\mu\text{g}/\text{cm}^2$ for methanolic extracts.

Adult bioassays were done in petridishes (90 mm). One ml of each dose was dropped separately, covering

uniformly the whole area of the petridish. After air dried four plastic rings (30 mm diam) were placed inside the petridish and 10 adult beetles were released within each ring. The rings within the petridish were served as replications. The doses were chosen after an ad-hoc bioassay done before the actual experiments. The doses for XSM-1, SMC-2 and SRS-3 strains were similar and they were 4367.99, 436.79, 43.68 and 4.37 $\mu\text{g}/\text{cm}^2$ for petroleum ether extract; 5497.95, 549.80, 54.98 and 5.50 $\mu\text{g}/\text{cm}^2$ for Ethyl Acetate extract, 3746.64, 374.66, 37.47 and 3.75 $\mu\text{g}/\text{cm}^2$ for Acetone extract; and 2343.98, 234.40, 23.44 and 2.34 $\mu\text{g}/\text{cm}^2$ for Methanol extract. In case of JTC-4 strain the doses were 10060.30, 1006.03, 100.60 and 10.06 $\mu\text{g}/\text{cm}^2$; 14147.52, 1414.75, 141.48 and 14.15 $\mu\text{g}/\text{cm}^2$; 12268.60, 1226.86, 122.69 and 12.27 $\mu\text{g}/\text{cm}^2$; and it was 3109.08, 310.91, 31.09 and 3.11 $\mu\text{g}/\text{cm}^2$ in the same orders of extractions respectively. Larval and adult mortality was recorded after 24 h of treatment. The mortality percentage was corrected using Abbott's formula (Abbott, 1925). The observed data was subjected to Probit analysis.

RESULTS

The calculated LD_{50} , 95% confidence limits and regression equations are presented in Table 1. In larval bioassay the LD_{50} of petroleum ether extract of roots were 0.051, 0.024, 0.045 and 0.071 $\mu\text{g}/\text{cm}^2$ for XSM, SMC, SRC and JTC 12 strains respectively, which are lowest in comparison to the other extracts. Thus, it proved to be the most toxic to *T. castaneum* larvae. The methanolic extract was least toxic for XSM ($\text{LD}_{50} = 4.187 \mu\text{g}/\text{cm}^2$) and SRC ($\text{LD}_{50} = 11.351 \mu\text{g}/\text{cm}^2$) strains, whereas for SMC the lowest toxicity was recorded in acetic ($\text{LD}_{50} = 0.339 \mu\text{g}/\text{cm}^2$) and for JTC strain in ethyl acetate ($\text{LD}_{50} = 10.580 \mu\text{g}/\text{cm}^2$) extracts.

In adult bioassay again petroleum spirit extract was the most toxic (LD_{50} is 198.57, 316.85, 85.46 and 58.77 $\mu\text{g}/\text{cm}^2$ for XSM, CR1, FSS II and CTC 12 strains respectively). The acetic extract was least toxic for XSM ($\text{LD}_{50} = 1000.40 \mu\text{g}/\text{cm}^2$), JTC ($\text{LD}_{50} = 306.57 \mu\text{g}/\text{cm}^2$) and JTC ($\text{LD}_{50} = 279.13 \mu\text{g}/\text{cm}^2$) strains. In case of SRC ethyl acetate extract was the least toxic ($\text{LD}_{50} = 383.01 \mu\text{g}/\text{cm}^2$). Four solvents extracted compounds and their toxicity also acted differently on strains of *T. castaneum*. For larvae the LD_{50} is more or less uniform in petroleum ether extract of the spongy roots of *Vetiveria zizanioides* (L.). Ethyl acetate and acetic extracts showed a slight and methanolic extract in some cases showed major variation in toxicity with the strains. In adults toxicity varied widely with the strains of beetle used. This could be due to the resistance capability of the strains to plant extracts. The insignificant χ^2 values for the regression coefficients suggest no heterogeneity of the data.

Table 1: LD₅₀, 95% confidence limits, regression equations and experimental values of the Vetiver essential oil extract in different solvents treated on larvae and adults of *T. castaneum*

Various strains	Solvent(s)	LD ₅₀ μg/cm ²	95% CL Upper limit	Regression equations	X ² df
Larval bioassay					
XSM	Pet. Ether	0.051	0.006	Y = 4.7354+0.54558X	0.769 (2)
	EtOH	0.632	0.150	Y = 3.6248+0.76372X	1.342 (2)
	Acetone	0.591	0.315	Y = 3.7375+0.71240X	0.462 (2)
	Methanol	4.187	0.285	Y = 3.3092+0.64873X	0.625 (2)
SMC	Pet. Ether	0.024	0.004	Y = 4.7878+0.54786X	1.108 (2)
	EtOH	0.041	0.130	Y = 4.6719+0.5334X	1.095 (2)
	Acetone	0.339	0.202	Y = 3.9432+0.7443 X	0.214 (2)
	Methanol	0.071	0.008	Y = 4.4077+0.6942X	0.262 (2)
SRC	Pet. Ether.	0.045	0.012	Y = 4.6040+0.6072X	0.082 (2)
	EtOH	0.398	0.167	Y = 4.0214+0.6113X	0.696 (2)
	Acetone	0.115	0.163	Y = 4.3621+0.6019X	1.392 (2)
	Methanol	11.351	0.040	Y = 3.6589+0.41961X	0.503 (2)
JTC	Pet. Ether.	0.071	0.213	Y = 4.45160+0.6506X	0.130 (2)
	EtOH	0.760	2.683	Y = 4.5592+0.2761X	0.338 (2)
	Acetone	1.200	0.285	Y = 4.4127+0.5995X	0.072 (2)
	Methanol	10.580	0.110	Y = 4.8933+0.4519X	0.437 (2)
Adult Bioassay					
XSM	Pet. Ether.	85.46	339.735	Y = 2.0045+1.3035X	1.235 (1)
	EtOH	1947.52	960.681	Y = 0.9501+1.4524 X	2.857 (1)
	Acetone	389.09	799.358	Y = 1.7822+1.0725X	3.371 (1)
	Methanol	326.33	213.741	Y = 1.9832+1.41558X	3.008 (1)
SMC	Pet. Ether.	175.067	573.474	Y = 2.3069+1.07685X	1.719 (1)
	EtOH	1892.606	4252.741	Y = -1.1179+1.7718X	3.062 (1)
	Acetone	7148.368	6776.66	Y = 0.8865+0.94725X	1.205 (1)
	Methanol	31.859	1351.006	Y = 3.0606+0.83701X	3.517 (1)
SRC	Pet. Ether.	56.6137	129.008	Y = 1.6968+1.70991X	1.810 (1)
	EtOH	383.01	990.901	Y = 2.3492+0.80584X	0.522 (1)
	Acetone	97.788	1547.731	Y = 1.51261+1.3464X	3.634 (1)
	Methanol	177.626	599.527	Y = 2.61212+0.9499X	2.671 (1)
JTC	Pet. Ether.	37.788	98.060	Y = 2.86467+1.2069X	3.793 (1)
	EtOH	306.57	279.13	Y = 2.9661+0.8893X	0.481 (1)
	Acetone	118.55	13506.85	Y = 2.4657+0.7036 X	0.020 (1)
	Methanol	42.374	230.329	Y = 3.59937+0.7021X	2.133(1)

DISCUSSION

4 Pesticidal Role of Vetiver Oil: Vetiver oil is known to repel insects; people in India and elsewhere have placed vetiver root among their clothes to keep insects away (Hiremath *et al.*, 1997; Ravi *et al.*, 2007). It also repels flies and 13 cockroaches and may make a useful ingredient in insect repellents (National Research Council, 1993). The two tricyclics sesquiterpenoids - zizanal and epizizanal - isolated from vetiver oil show insect repelling activity (Jain *et al.*, 1982). Babprasert and Karintayakit (1996) reported that at least six compounds (α , β -vetivone, khusimone, zizanal, epizizanal and (C)-(1S, 10R)-1, 10-dimethylbicyclo[4,4,0]-dec-6-en-3-one) were repellent to insects. Recently, studies of Zhu *et al.* (2001a, b) found three other vetiver oil compounds that were repellent to the Formosan subterranean termite including: nootkatone, zizanol and bicyclovetivenol. Zhu *et al.* (2001b) discovered that compound volatility was inversely proportional to repellent effectiveness. Vetivones (α and β) were the least volatile and the most effective repellents. The volatility of nootkatone is similar to α and β vetivone.

Vetiver oil is known to repel insects; people in India and elsewhere have placed vetiver root among their clothes to keep insects away ([http://tnnt.es.sortment.com/what is vetiver_rtco.htm](http://tnnt.es.sortment.com/what_is_vetiver_rtco.htm)). It also repels flies and cockroaches and may make a useful ingredient in insect repellents (National Research Council, 1993). It has been used to repel moths (Chomchalow, 2001). The two tricyclic ses-quiterpenoids - zizanal and epizizanal - isolated from vetiver oil show insect repelling activity (Jain *et al.*, 1982). Maistrello and Henderson (2001) were of opinion that some of the components of vetiver oil, such as nootkatone - a sesquiterpene, which has been found to repel and even kill termites, may have important industrial applications, as insecticide or insect repellent, or eventually, other products may be developed. It also has some anti-fungal properties (Thubthimthed *et al.*, 2000) and perfected activity against *Formosan subterranean* termites opined by (Koren and Gregg, 2006.).

The seeds of *A. squamosa* were reported to have insecticidal and abortifacient properties. The crude oils from *Vetiveria zizanioides* at 2.5% concentrations significantly reduced the leaf damage caused by larvae

(Chomchalow *et al.*, 1970) and petroleum ether extract of oil reduced the weight and length of subterranean termites (Maistrello and Henderson, 2001). Acetone extracts from fresh and stored leaves were toxic to adult *C. maculatus*, whereas the ethanol extracts were not active (Ibrahim *et al.*, 2001). But in the present study Vetiver oil extracts in acetone was less active against adults of *T. castaneum*. Ravi *et al.* (2007) recorded essential extract and activity of sesquiterpenes phytochemical activity showed at 1.5 % concentration the highest mortality in *H. armigera* (43.33%) and 36.66% mortality at 1% concentration in *S. litura*. Larval development of SRS and JTC strains of *T. castaneum* significantly affected by the essential oil of *V. zizanioides* (Chomchalow, 2001,2003) and the weight and development of pupae and adults in both strains was significantly affected by some plant products treatments (Sahayaraj and Paulraj, 2000). Adults *T. castaneum* were repelled by contact with food medium treated with 2 and 5 g leaf dust/10 g flour, for essential oil from Vetiver. Extracts of *V. zizanioides* had repellent and anti-oviposition properties when applied to *Ceratitis capitata* (Misra, 2000). The depletion in free amino acids had an adverse effect on protein turnover, resulting in delayed metamorphosis (Duk, 1990; Thubthimthed *et al.*, 2000). Isolated two compounds from the seed, which were found to be toxic to fruit fly. The petroleum ether extract of the vetiver oil of this species yielded 13 known adjacent, and 4 non-adjacent bistetrahydrofuranic acetogenins, and the compounds squamocin and squamostatin A (Miller, 2003). Four non-adjacent bistetrahydrofuranic acetogenins, named squamostatins-B to -E, were isolated from the petroleum ether extract of Vetiver essential oil by (Mishra *et al.*, 1992; Rao and Suseela, 1998; Babprasert and Karintayakit, 1996). Flavonoids isolated from aqueous extracts showed antimicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against *C. chinensis* at a concentration of 0.07 mg/mL (Ndemah *et al.*, 2002). Adults of *T. castaneum* were repelled by contact with food medium treated with 2 and 5-vetiver grass oil dust/10 g flour (Vanden *et al.*, 2007).

The present experiments showed that *T. castaneum* larval mortality was the most in petroleum spirit extract of seed, whereas ethyl acetate and acetone extracts were moderately toxic and methanol extract was the least toxic for XSM and SMC strains, but it remained toxic for CR and JTC strains. In adults acetone extracts showed less toxic to all strains used. Petroleum spirit extract remained most toxic for SMC-II and JTC strains, whereas methanol extract was most toxic for other two strains i.e., XSM and JTC strains.

Vetiver plant oil is a complex mixture of compounds, some of which are potent biopesticides. Commercial insecticides and repellents with less mammalian toxicity are desirable for pestiferous insects. Isolation or chemical synthesis is currently the only viable approach to obtain these natural products, but this is not economically

practical (Hyun *et al.*, 2005). Our approach is to modify vetiver oil, enriching the desired products, thereby alleviating the need for their isolation. Simple chemical modification (such as oxidation or reduction) is used to target different compounds present in vetiver oil so that the end product is directly useful for pest control suggested by Nix *et al.* (2005) and Chauhan and Raina, (2006).

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