

## Fumigant and Contact Toxicity of *Tithonia Diversifolia* (Hemsl), *Tagetes erecta*, *Helianthus Annus* and *Bidens Sulphurea* Against *Tribolium Castaneum* (Herbst)

V.L. Premarathna, A.P. Henagamage, A.A.K. Karunathilake and H.A.C.S. Hapuarachchi  
*Uva Wellassa University, Badulla, Sri Lanka*

### Introduction

Since Sri Lanka is a developing and agriculture based country, it is crucial to minimize the postharvest losses in order to increase the national income. Post-harvest deterioration is principally caused by biological spoilage organisms including insects, fungi and small vertebrates (Golob *et al.*, 2002). The huge post-harvest losses and quality deterioration caused by insects and pests are major problems of assuring food security in developing countries. Therefore, effective and efficient controlling methods are required to eliminate this problem. Control of these insects and pests relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides, toxic residues, on stored grain, toxicity to consumer and increasing costs of application (Jbilou *et al.*, 2006). Therefore, the development of bio insecticides has been focused along with an urgent need for safe but effective biodegradable pesticides for a low cost.

This study was undertaken to screen the contact toxicity and the fumigant toxicity of *Tagetes erecta*, *Bidens sulphurea*, *Helianthus annuus* and *Tithonia diversifolia* which are highly abundant and underutilized herbaceous plants in Sri Lanka against *Tribolium castaneum* (Herbst). This method can be used as a cost effective, environmental friendly and efficient method to develop a bio insecticide as a value added product for grain storage pests.

### Methodology

Fresh leaves (L) and flowers (F) of *T. diversifolia* (TD), *H. annuus* (HA), *B. sulphurea* (BS) and *T. erecta* (TE) were collected from adjoining area of Badulla and were confirmed for the identification by the Herbarium, National Botanic Garden, Peradeniya.

Leaves of plants were dried under shady conditions for seven days. Fresh flowers and shade dried leaves of each plants were ground separately and were extracted using absolute methanol by shaking the plant materials with the solvent at room temperature for 72 hours. Plant material: solvent ratio was maintained at 1 g: 5 mL. Each extract was evaporated, by rotary evaporator at 40 °C to obtain the crude extracts.

Adult insects of *T. castaneum* were collected and confirmed by using their identical morphological characters. Insects were introduced in to a ventilated container containing wheat flour. After 72 hours, adult insects were removed and allowed to incubate at room temperature.

### Determination of the contact toxicity

Bioassay for mortality and repellency was conducted with two factor factorial design. 3.0, 5.0, 7.0, 9.0 and 11.0 mg of crude extracts were taken and dissolved in 1 ml of extracting methanol solvent. Solutions were introduced to 5 cm diameter Petri dish and air dried at room temperature for 15-20 minutes to remove solvents leaving extracts on the plates in

doses (weight of the extract/surface area) of 152.8  $\mu\text{g}/\text{cm}^2$ , 254.6  $\mu\text{g}/\text{cm}^2$ , 356.5  $\mu\text{g}/\text{cm}^2$ , 458.4  $\mu\text{g}/\text{cm}^2$ , 560.2  $\mu\text{g}/\text{cm}^2$  and 662.0  $\mu\text{g}/\text{cm}^2$ . Ten randomly selected adult insects of age 90 days were added to the Petri dishes and air tied using Para films. Dishes with absolute methanol but without extracts were maintained as the control and all the petri dishes were kept at 32 °C. Observations for mortality and repellency were taken at 24 and 48 hours for the treatments and the control. Each treatment and the control were replicated two times (Gunathilake *et al.*, 2011; Nikkon *et al.*, 2009).

Mortality percentages were reexamined at a randomly selected concentration series from 10 $\mu\text{g}/\text{cm}^2$  to 255 $\mu\text{g}/\text{cm}^2$ .

### Determination of the fumigant toxicity

According to the results of the contact toxicity, Whatman no. 1 filter paper discs (6.5 cm diameter) impregnated with each crude extract except *H. annuus* flower with the concentrations of 168.4 $\mu\text{g}/\text{cm}^2$ , 252.5 $\mu\text{g}/\text{cm}^2$  and 336.7 $\mu\text{g}/\text{cm}^2$ , were placed on the underside of the lid of baby food jars. Ten randomly selected adult insects of age 90 days were added in to the jar and all the bottles were incubated at 32 °C for 48 hours for the treatments and the control and each treatment and the control were replicated two times. Mortality percentage for each treatment and the control were recorded at the end of the relevant fumigant exposure periods (Paranagama *et al.*, 2004).

The best selected plants were reexamined under randomly selected concentration series to calculate the median lethal doses (LD<sub>50</sub> value).

Statistical analyses were performed using Minitab 14.0 versions and SAS 9.1 versions statistical packages.

### Results and Discussion

Mean mortality percentages (M%) with different types of plant crude extracts at 24 hours exposure time indicate that all most all the plant extracts show a certain bioactivity even at the 152.8 $\mu\text{g}/\text{cm}^2$  concentration. There was no any mortality observed in the control dishes even without food and air supplement. The results of ANOVA show that there is no significant interaction between concentration and the crude type to the mortality rate ( $p > 0.05$ ).

According to the results of the contact toxicity, mean mortality (M%) percentages with different type of plant crude extracts, there is a very low activity of fresh flowers of *H. annuus* on the target insects even at the 560.225  $\mu\text{g}/\text{cm}^2$  concentration at 24 hours exposure time. Therefore, the crude from fresh flowers of *H. annuus* was not considered for further analysis ( $p < 0.05$ ).

The highest fumigant toxicity values were shown by crude extract from fresh flowers of *T. diversifolia* and dry leaves of *T. erecta* with the resulted mean mortality percentages. Also considerable fumigant toxicity was observed from the fresh flowers of *T. erecta*.

Table 1: LD<sub>50</sub> values in contact toxicity and fumigant toxicity

Crude extract Plant	Plant part	Extract code	Contact LD <sub>50</sub> (µg/cm <sup>2</sup> )	Fumigant LD <sub>50</sub> (µg/cm <sup>2</sup> )
<i>T. diversifolia</i>	leaves	TD-L	115.429	319.511
	flowers	TD-F	121.463	196.368
<i>H. annuus</i>	leaves	HA-L	38.925	361.699
	flowers	HA-F	346.834	-
<i>B. sulphurea</i>	leaves	BS-L	224.334	363.440
	flowers	BS-F	51.716	342.425
<i>T. erecta</i>	leaves	TE-L	13.129	109.044
	flowers	TE-F	293.873	349.028

Resulted LD<sub>50</sub> values from the Probit analysis based on the mortality (Table 1) indicates the leaves of *T. erecta* (TE-L) has the least LD<sub>50</sub> values in both contact and fumigant assays: 13.129 µg/cm<sup>2</sup> and 109.044 µg/cm<sup>2</sup> respectively. Therefore, it has the highest activity against the target insect. Further, leaves of *H. annuus* (HA-L) and flowers of *B. sulphurea* (BS-F) also show considerable activities for contact toxicity. Flower of *T. diversifolia* (TD-F) also shows considerably high fumigant toxicity. These results indicate high fumigant and contact toxicity of all four underutilized plant species, *T. diversifolia* (Hemsl), *T. erecta*, *H. annuus* and *B. sulphurea* against grain storage pest *T. castaneum* (Herbst) and the potential agricultural and industrial applications via the development of value added products.

### Conclusion

All the crude types show high contact toxicity and *T. diversifolia* flowers and *T. erecta* leaves show high fumigant toxicity against *T. castaneum*. The selected plants can be used to develop a fumigant against the target insect. However more studies and field trials should be undergone to get effective applications.

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Plant part	Extract code	LD <sub>50</sub> (µg/cm <sup>2</sup> )	LD <sub>90</sub> (µg/cm <sup>2</sup> )
Leaves	HA-L	38.022	361.049
Flowers	HA-F	316.874	316.874
Leaves	BS-L	224.734	303.540
Flowers	BS-F	31.716	313.422
Leaves	TE-L	13.129	109.041
Flowers	TE-F	201.872	319.078

Resulted LD<sub>50</sub> values from the Probit analysis based on the mortality (Table 1) indicates the leaves of *T. erecta* (TE-L) has the least LD<sub>50</sub> values in both contact and fumigant assays (13.129 µg/cm<sup>2</sup> and 109.041 µg/cm<sup>2</sup>, respectively). Therefore, it has the highest activity against the target insect. Further, leaves of *T. erecta* (HA-L) and flowers of *B. subspina* (BS-F) also show considerable activities for contact toxicity. Flower of *T. erecta* (TE-F) also shows considerably high fumigant activity. These results indicate that rootstock and contact toxicity of all four identified plant species *T. erecta* (Herbst) and the potential uses and *B. subspina* against grain storage pest *T. castaneum* (Herbst) and the potential agricultural and industrial applications via the development of value added products.

#### Conclusion

All the crude types show high contact toxicity and *T. erecta* flowers and *T. erecta* leaves show high fumigant toxicity against *T. castaneum*. The selected plants can be used to develop a fumigant against the target insect. However, more studies and field trials should be undergone to get effective applications.

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