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N Abhijith
Department of Entomology, PhD Scholar, Department of Entomology, Sri Venkateswara Agricultural College, Tirupati, Chittoor, Andhra Pradesh, India

S Mohan
Professor, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Jeyarajan Nelson
Professor, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
N Abhijith
Department of Entomology, PhD Scholar, Department of Entomology, Sri Venkateswara Agricultural College, Tirupati, Chittoor, Andhra Pradesh, India

Bioassay to detect the toxicity of Sweet flag (*Acorus calamus*) 6 EC formulation against pests of stored spices *viz.*, rusty grain beetle, *C. ferrugineus* (Stephens) cigarette beetle, *L. serricorne* (F.) and red flour beetle, *T. castaneum* (Herbst)

N Abhijith, S Mohan and S Jeyarajan Nelson

Abstract
The toxicity of Sweet flag (*Acorus calamus*) 6 EC (Hexane extract) formulation was tested against three insect pests of stored products *viz*., *C. ferrugineus*, *L. serricorne* and *T. castaneum* in Post graduate research laboratory, Coimbatore during 2016. The SF 6 EC formulation was effective against *C. ferrugineus* and *L. serricorne* with 50 (45.19) and 66.67 (54.75) percent mortality at only 1 percent and 6 percent concentration after 36 and 60 Hours After Treatment (HAT) respectively. The cent percent mortality was observed at 3 percent and 8 percent concentration in *C. ferrugineus* and *L. serricorne* respectively. The concentration of 2 and 3 percent SF 6 EC was effective against *C. ferrugineus* whereas against *L. serricorne* it was 6 percent in Filter Paper Impregnation Method (FPIM). As chemical insecticides are causing many S the botanical extracts in stored pest management can be selected as surface treatment to avoid cross infestation of the stored products during storage.

Keywords: *Acorus calamus* 6 EC (Hexane extract), Malathion 50 EC, *Lasioderma serricorne*, *Cryptolestes ferrugineus*, *Tribolium castaneum*, HAT- Hours after treatment

1. Introduction
The postharvest food losses occur all from harvesting up to consumption. Abiotic and biotic factors are in major concern for losses of stored grains in the world, amid of biotic factors the insect pests contribute major portion by causing 30-40 percent damage. The traditional storage structures used in developing countries like India makes food grains more vulnerable to storage insects [1]. The high valued spice products are also attacked by several insect pests like *L. serricorne*, *S. paniceum* as internal feeders and *T. castaneum* as external feeder [2-3] and we also noticed *Cryptolestes ferrugineus* infestation as secondary feeder stored spices during the survey [4]. The chemical control is usually the choice of control to prevent contamination by insects but this may be feasible for the protection of grains used for seed purpose and the same when consumed insecticides may pose several problems like residues, bio magnification, health issues. As a substitute to this plant botanical extracts can be used as alternative method of control.
Sweet flag (*Acorus calamus*, F: Acoraceae) is the herbaceous perennial wetland plant distributed in the Himalayas, Manipur, Naga Hills and in South India. It is being used since 2000 years in Ayurveda to cure fever, asthma, bronchitis, cough and many digestive problems [5]. The rhizomes of the plants are also found to have insecticidal, ovicidal, antifeedant and repellent activities with bioactive compounds like α-asarone and β-asarone [6-9]. In this experiment, we tested the efficacy of Sweet flag 6 EC (Hexane extract) formulation (which was developed indigenously in TNAU, Coimbatore) against three stored-product insect pests reported during the survey of insect pests of spices.

2. Materials and Methods
2.1 Multiplication of insect culture: Adult insects collected during the survey were mass cultured species wise separately. The plastic bottles were labelled and holes were made over lid for aeration. The jar was half filled with wheat flour and dried ground brewer's yeast in the ratio of 95:5 respectively [10]. The plastic jars were cleaned and filled with fresh medium of wheat flour and dried ground brewer's yeast after completion of one life cycle.

The insects mass cultured were used for further used for experiments.

2.2 Preparation of filter papers for bioassay: The Filter Paper Impregnation Method (FPIM) was used for testing contact toxicity of formulations. The filter papers (Whatman) were trimmed into appropriate sizes (9cm) and dipped in different percent solution of respective formulations, shade dried and laid in a labelled Petri dish of 9cm in diameter [11-13]. Malathion 50 EC formulation as standard check and untreated water dipped filter paper maintained as control.

2.3 Bioassay on cigarette beetle, *L. serricorne* and rusty grain beetle, *C. ferrugineus*: The bioassays was performed to asses contact toxicity of sweet flag at concentrations *viz*., 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 percent and *viz*., 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0 percent against cigarette beetle, *L. serricorne* and rusty grain beetle, *C. ferrugineus* respectively. Ten freshly emerged adults of *L. serricorne* and *C. ferrugineus* were released onto the filter paper and covered with the lid.

2.4 Record of observation

Mortality was recorded at 12 hrs interval for three days. The moribund insects were considered as dead. The experiment was replicated three times.

2.5 Data analysis

The data recorded in different treatments were subjected to analysis of variance (ANOVA) using AGRESS software.

3. Results

3.1 Assessment of contact toxicity of botanical pesticide, Sweet Flag (*Acorus calamus*) 6 EC on cigarette beetle, *L. serricorne*: The insecticidal activity of Sweet Flag (SF) 6 EC (Hexane extract) on adults of *L. serricorne* are shown in Table 1. The highest mortality of 26.67 percent was recorded in 10 percent concentration of Sweet Flag (SF) 6 EC on 12 Hours After Treatment (HAT) followed by 9 (16.67%), 8 (16.67%) and 7 (10.00%) percent concentration while the standard check Malathion 50 EC @ 1 percent and untreated control recorded cent percent and zero percent mortality respectively (Table 1). However, on 24 HAT the cumulative adult mortality increased to 40.00 in 10 percent, 33.33 in 9 percent, 30.00 in 8 percent concentration (Table 1). On 72 HAT the concentrations of 10, 9 and 8 recorded cent percent mortality, which were on par with the standard check Malathion 50EC, 1 percent, while only 16.67 percent mortality was recorded in untreated control.

3.2 Assessment of contact toxicity of botanical pesticide Sweet Flag (*Acorus calamus*) 6 EC on rusty grain beetle, *C. ferrugineus*: The insecticidal activity of SF 6 EC on adults of *C. ferrugineus* are shown in Table 2. The highest mortality of 70.00 percent was recorded in 3 percent concentration of Sweet Flag (SF) 6 EC on 12 HAT followed by 2 (46.67%), 1 (16.67%) and 0.8 (6.67%) percent concentration while the standard check Malathion 50 EC @ 1 percent and untreated control recorded 90.00 percent and zero percent mortality respectively (Table 2). However, on 24 HAT the cumulative adult mortality increased as 96.67 in 3 percent, 76.67 in 2 percent, 16.67 in 1 percent concentrations respectively. On 72 HAT the concentrations of 3, 2 and 1 recorded cent percent, 96.67 percent and 70.00 percent mortality respectively, The 3 percent concentration which were on par with the standard check Malathion 50 EC, 1 percent, while only 16.67 percent mortality was recorded in untreated control.

3.3 Assessment of contact toxicity of botanical pesticide Sweet Flag (*Acorus calamus*) 6 EC on red flour beetle, *T. castaneum*: There was no mortality observed at any concentration in *T. castaneum* adults and it was found to be resistant to SF 6 EC

Table 1: Insecticidal action of sweet flag 6 EC on *Lasioderma serricorne* adults

Treatments	Cumulative adult mortality (%)					
	12 HAT	24 HAT	36 HAT	48 HAT	60 HAT	72 HAT
T1 Sweet flag 6EC, 3%	0.00 (0.52)^{f}	0.00 (0.52)^{h}	0.00 (0.52)^{i}	10.00 (18.44)^{h}	13.33 (21.42)^{f}	13.33 (21.41)^{g}
T2 Sweet flag 6EC, 4%	6.67 (14.96)^{e}	13.33 (21.42)^{g}	13.33 (21.41)^{h}	23.33 (28.88)^{g}	26.67 (31.09)^{e}	26.67 (31.09)^{f}
T3 Sweet flag 6EC, 5%	3.33 (10.52)^{e}	13.33 (21.42)^{g}	26.67 (31.09)^{g}	33.33 (35.26)^{f}	36.67 (37.27)^{d}	36.67 (37.27)^{e}
T4 Sweet flag 6EC, 6%	3.33 (10.52)^{e}	16.67 (24.09)^{f}	36.67 (37.27)^{f}	46.67 (43.09)^{e}	66.67 (54.75)^{d}	73.33 (58.91)^{c}
T5 Sweet flag 6EC, 7%	10.00 (18.43)^{d}	20.00 (26.57)^{e}	50.00 (45.00)^{e}	63.33 (52.74)^{d}	83.33 (65.92^{b}	93.33 (74.66)^{c}
T6 Sweet flag 6EC, 8%	16.67 (24.09)^{c}	30.00 (33.21)^{d}	56.67 (48.83)^{d}	70.00 (56.80)^{c}	86.67 (68.63)^{b}	100.00 (89.48)^{b}
T7 Sweet flag 6EC, 9%	16.67 (24.09)^{c}	33.33 (35.26)^{c}	63.33 (52.73)^{c}	73.33 (58.91)^{c}	100.00 (89.48)^{a}	100.00 (89.48)^{ab}
T8 Sweet flag 6EC, 10%	26.67 (31.09)^{b}	40.00 (39.23)^{b}	80.00 (63.46)^{b}	93.33 (75.41)^{b}	100.0 (8.48)^{a}	100.00 (89.48)^{ab}
T9 Malathion 50EC, 1%	100.00 (89.48)^{a}	100.00 (89.48)^{a}	100.00 (89.48)^{a}	100.00 (89.48)^{a}	100.00 (89.48)^{a}	100.00 (89.48)^{a}
T10 Control	0.00 (0.52)^{f}	0.00 (0.52)^{h}	0.00 (0.52)^{i}	0.00 (0.52)^{i}	6.67 (14.96)^{g}	6.67 (14.96)^{h}
SEd	0.17	0.30	0.56	1.18	1.55	1.760
CD(0.05)	0.36	0.63	1.16	2.45	4.42	3.67

Values are mean of three replications

HAT =Hour(s) after treatment

Figures in parentheses are transformed arcsine values

In a column means followed by same letter(s) are not significantly different (p=0.05) by LSD

Table 2: Insecticidal action of sweet flag 6 EC on *Cryptolestes ferrugineus* adults

Treatments	Cumulative adult mortality (%)					
	12HAT	24 HAT	36 HAT	48 HAT	60 HAT	72 HAT
T1 Sweet flag 6EC, 0.2%	0.00 (0.55)^{f}	0.00 (0.55)^{g}	6.67 (15.34)^{f}	10.00 (18.44)^{f}	13.33 (21.13)^{f}	20.00 (26.56)^{fg}
T2 Sweet flag 6EC, 0.4%	0.00 (0.55)^{f}	6.67 (15.34)^{f}	13.33 (21.41)^{e}	16.67 (24.09)^{e}	16.67 (24.09)^{f}	23.33 (28.66)^{f}
T3 Sweet flag 6EC, 0.6%	0.00 (0.55)^{f}	10.00 (18.44)^{e}	23.33 (28.88)^{d}	23.33 (28.88)^{d}	23.33 (28.88)^{e}	30.00 (33.21)^{e}
T4 Sweet flag 6EC, 0.8%	6.67 (15.34)^{e}	16.67 (24.09)^{d}	23.33 (28.88)^{d}	26.67 (31.09)^{d}	33.33 (35.06)^{d}	40.00 (39.04)^{d}
T5 Sweet flag 6EC, 1%	16.67 (24.09)^{d}	16.67 (24.35)^{d}	43.33 (41.17)^{c}	50.00 (45.19)^{c}	50.00 (45.19)^{c}	70.00 (56.58)^{c}
T6 Sweet flag 6EC, 2%	46.67 (43.09)^{c}	76.67 (61.12)^{c}	86.67 (68.67)^{b}	86.67 (68.87)^{b}	90.00 (71.58)^{b}	96.67 (79.51)^{b}
T7 Sweet flag 6EC, 3%	70.00 (57.01)^{b}	96.67 (80.04)^{b}	100.00 (89.45)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}
T8 Malathion 50EC, 1%	90.00 (71.57)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}	100.00 (89.45)^{a}
T9 Control	0.00 (0.55)^{f}	0.00 (0.55)^{g}	0.00 (0.55)^{g}	0.00 (0.55)^{g}	6.67 (15.34)^{g}	16.67 (24.35)^{g}
SEd	0.42	1.17	1.39	1.51	1.49	1.75
CD(0.05)	0.87	2.47	2.91	3.17	3.14	3.67

Values are mean of three replications
HAT =Hour(s) after treatment
Figures in parentheses are transformed arcsine values
In a column means followed by same letter(s) are not significantly different (p=0.05) by LSD

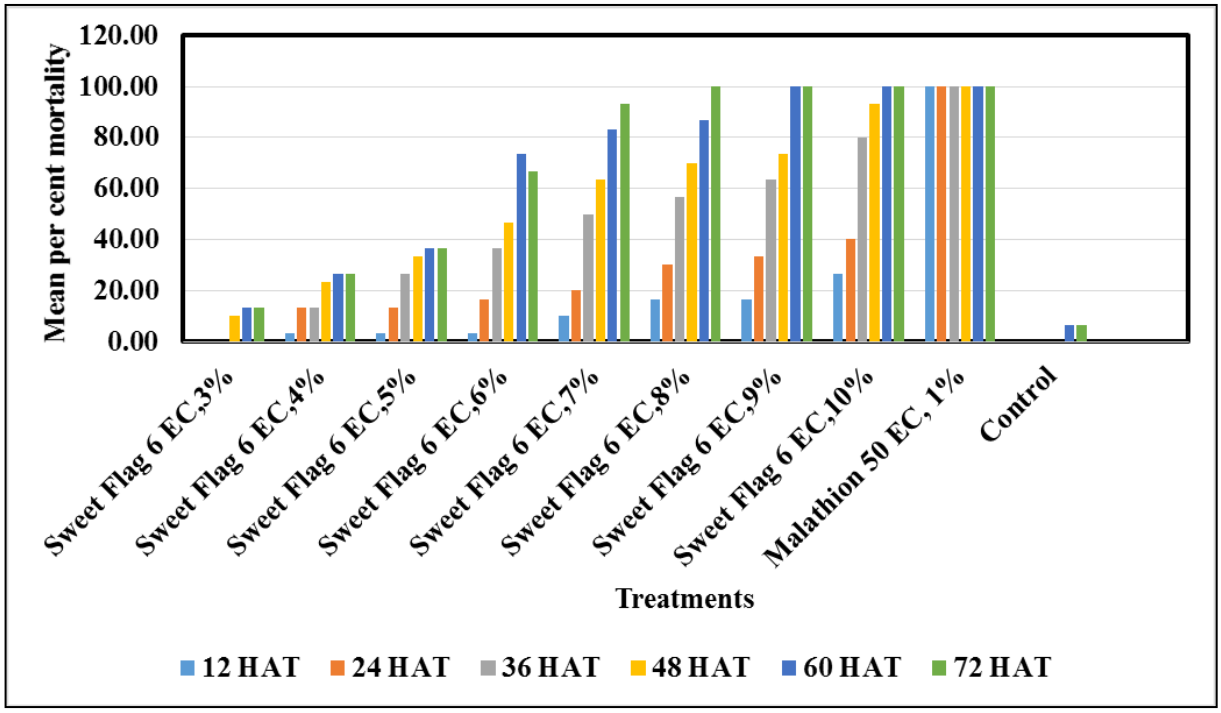

Fig 1: Insecticidal action of sweet Flag 6 EC of *L. serricorne* adults by Filter Paper Impregnation Method (FPIM)

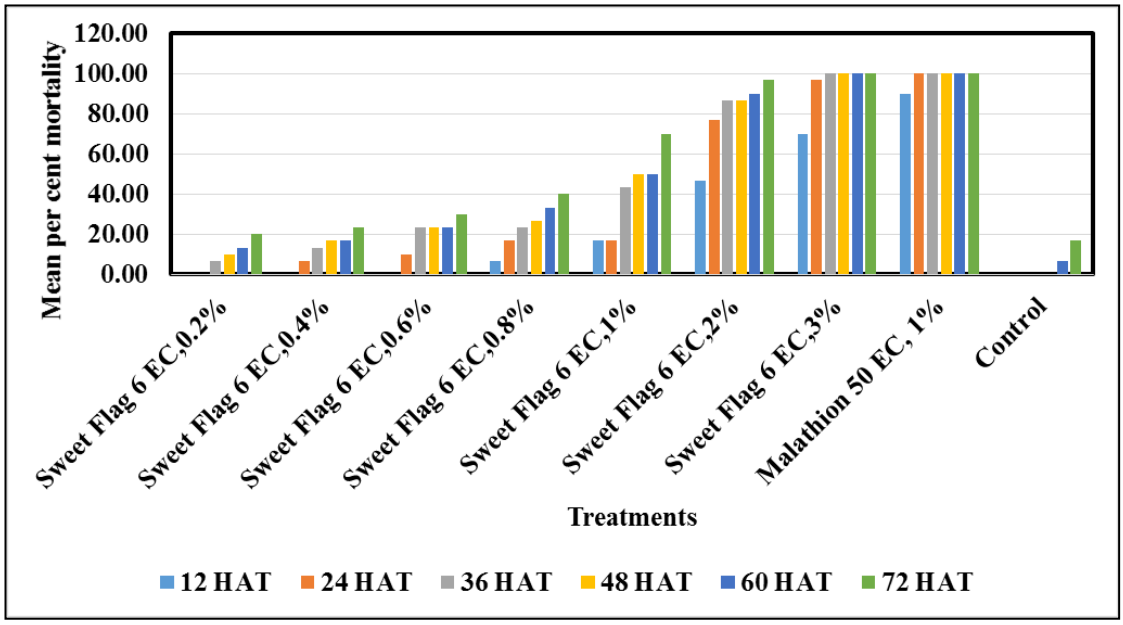

Fig 2: Insecticidal action of sweet Flag 6 EC of *C. ferrgineus* adults by FPIM

4. Discussion

The cent percent mortality was observed in *L. serricorne* and *C. ferrugineus* adults at 8, 9, 10 percent and 3 percent concentration of SF 6 EC respectively (Table 1 and 2), the mortality was on par with standard check Malathion 50EC (Fig. 2) and studies by Park *et al.*, 2003 [14] also shown that the presence of (Z)-asarone as the principal mortality factor, this study proves that the mortality caused by SF 6 EC due to presence of the alkaloid 'asarone' in the formulation.

5. Conclusion

Current study on testing efficacy of the Sweet flag (*Acorus calamus*) 6 EC on three storage insect pests revealed that the *C. ferrugineus* adults were more susceptible at lower concentrations than *L. serricorne* and *T. castaneum* adults found to be resistant. The study may be useful in controlling the stored product insect by surface treatment in godowns as an alternative to synthetic insecticides, further the efficacy of the botanical can be tested on other stored product insects also.

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