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ECOFRIENDLY BIOPESTICIDES FOR THE MANAGEMENT OF RED FLOUR BEETLE, *Tribolium castaneum* (Herbst) IN STORED RICE

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ABSTRACT

In the present study essential oils like neem-azal, clove oil and acorus oil were tested at different concentrations for the management of red flour beetle, *Tribolium castaneum* (Herbst). Neem-azal at 2.0 per cent was found to be effective with LC_{50} value of 1.11 per cent and LT_{50} value of 2.4 days followed by acorus oil 15.0 per cent with LC_{50} value of 16.43 and LT_{50} value of 4.30 days against *Tribolium* larvae. Neem-azal at 2 per cent was found effective with LC_{50} of 0.99 per cent and LT_{50} value of 15.78 hours followed by clove oil 15.0 per cent with LC_{50} of 11.30 per cent and LT_{50} value of 45.07 hours against *Tribolium* adults.

KEYWORDS: Neem-azal, Clove oil, Acorus oil, LC_{50}, LT_{50}.

INTRODUCTION

The post harvest losses of food grains occur at different levels of storage from harvesting up to consumption. Abiotic and biotic factors are of major concern for losses of stored grains in the world. Among the biotic factors insect pests, rodents, mites and fungi contribute major portion of damage. Out of these post-harvest losses due to insects alone accounts for 2.0 to 4.2 per cent followed by rodents 2.50 per cent (IGMRI, 2020). Stored grains and their products are attacked by many insect pests as internal and external feeders.

The red flour beetle, *Tribolium castaneum* is an external feeder and feeds on broken rice, flour, cereals, meal, beans, spices and other stored products. Control of these insects relies heavily on the use of chemical insecticides and fumigants, which has led to problems such as negative impact on environment, residual toxicity, development of resistance to insecticides and lethal effects on non-target organisms (Abhijit *et al*., 2018). Indiscriminate and continuous use of synthetic insecticides and fumigants like phosphine in poorly sealed warehouses at sub lethal doses resulted in the development of resistance in many of the stored grain pests especially in bulk storage facilities and rice storage godowns. In view of resistance of stored grain pests, suitable alternative strategies like use of safer plant products like essential oils can be explored for the management of *T. castaneum* in stored rice.

The essential oils of spices, herbs, aromatic plants and their extracts possess insecticidal properties *viz.,* antifeedant, repellent and fumigant action. They are eco-friendly, relatively specific in mode of action, easy to use, less hazardous, less expensive, safer to non target organisms and readily available (Compolo *et al*., 2018)

Neem, *Azadirachta indica* contains many properties like insecticidal, ovicidal, antifeedant and growth inhibiting effects against many insect pests due to presence of triterpenoid, azadirachtin and other biochemical compounds such as nimbin, nimbidin and salanin (Choupanian *et al*., 2017). The essential oils isolated from the clove buds, *Syzygium aromaticum* is widely used and well known for its medicinal properties. It exhibits different insecticidal properties like inhibition of oviposition, insecticidal activity, prevention of adult emergence with isoeugenol being particularly active compound (Abo-El-Saad *et al*., 2011). Sweet flag, *Acorus calamus* is a herbaceous perennial and the rhizomes of the plants were found to have insecticidal, ovicidal, antifeedant and repellent activities with bioactive compounds like α-asarone and β-asarone (Abhijit *et al*., 2018).

In the present study, effect of essential oils like neem-azal, clove oil and acorus oil were studied against red flour beetle, *T. castaneum* in stored rice.

MATERIAL AND METHODS

Maintenance of insect culture

Tribolium mother culture was collected from rice storage godown of RARS, Tirupati, Chittoor district (13.6288°N, 79.4192°E), Andhra Pradesh and reared in plastic containers (11 cm × 8 cm) containing broken rice

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added with 5 per cent yeast in the insectary, Department of Entomology, S.V. Agricultural College, Tirupati under ambient storage conditions. The newly emerged F1 adults and larvae were separated and used for bioefficacy studies.

Preparation of different concentrations

The essential oils *viz.,* Neem-azal, Clove oil and Acorus oil used in this study were procured from Indian Scientifics, Tirupati. Different test concentrations of essential oils were prepared using acetone as a solvent by serial dilution method. Neem-azal of 1.5 and 2.0%, Clove oil of 10.0 and 15%, Acorus oil of 10 and 15% were prepared. Three replications of each treatment were maintained along with an untreated control.

Bioassay studies using diet incorporation method

The efficacy of essential oils was evaluated against larvae of *T. castaneum* using diet incorporation method (Ganesh *et al.,* 2020). The Rice grains were mixed with formulations at different test concentrations. Two hundred (200) µl of formulation was added to 20 grams of broken rice and the plastic containers of measurement (11 cm × 8 cm) were shaken manually for 5 minutes for uniform distribution. Each treatment was replicated thrice and 20 larvae (6-7 days old) were released into each plastic container. The containers were closed with muslin cloth for sufficient ventilation and kept in ambient laboratory conditions of 30°C temperature and 70 per cent relative humidity. The mortality counts were taken at 1, 3, 5, 7 and 10 days after exposure. Insects were considered dead which were without any leg or antenna movements after prodding with a fine brush.

Bioassay test using filter paper impregnation method

The Filter Paper Impregnation Method (FPIM) was used for testing contact toxicity of formulations (Manal *et al.,* 2018). The filter papers (Whatman) were trimmed into appropriate sizes and laid in a labelled Petri dish. Three replications of each treatment were maintained along with an untreated control.

Essential oils of different concentrations were evaluated using filter paper impregnation method against the adults of *T. castaneum*. All oils were diluted to different concentrations using water as solvent for neem-azal and acetone as solvent for other formulations. One ml of each concentration was applied to filter paper which was placed in Petri dishes and allowed for evaporation of solvent. After evaporation of solvent, 20 adults were released into each Petri dish. Each treatment was replicated three times along with untreated control. Adult mortality was recorded at 24, 48 and 72 hours after treatment.

Statistical Analysis

Based on the per cent mortality count, LC_{50}, LC_{90} and LT_{50} values were calculated through probit analysis using the SPSS statistical package for determining their effectiveness against larvae and adults of *T. castaneum.* The larval mortality and adult mortality were subjected to angular transformation. The data was statistically analyzed using SPSS software.

RESULTS AND DISCUSSION

Among the different essential oils against *T. castaneum* larvae, neem-azal at 1.5% and 2.0% was found to be highly effective with 100 per cent mortality at 10 DAT followed by acorus oil 15% and clove oil 15% with 86.66 and 76.66 per cent respectively (Table 1). Whereas larvae pupated after 10 days in untreated control.

Probit analysis revealed LC_{50} at 1.11, 19.54 and 16.43 per cent for neem-azal, clove oil and acorus oil respectively and LC_{90} at 6.42, 244.49 and 110.74 per cent for neem-azal, clove oil and acorus oil respectively (Table 3). LT_{50} of 2.40, 4.31 and 4.30 days were obtained for neem-azal @ 1%, clove oil @ 10% and acorus oil @ 10% respectively (Table 4).

Bioassay test against *Tribolium* adults revealed complete 100 per cent mortality at both concentrations of neem-azal followed by acorus oil @ 15% (90%) and clove oil @ 15% (83.33%) compared to zero per cent mortality in untreated control at 72 HAT (Table 2).

Probit analysis revealed LC_{50} at 0.99, 11.30 and 11.68 per cent for neem-azal. clove oil and acorus oil respectively and LC_{90} at 2.08, 25.25 and 26.32 per cent for neem-azal, clove oil and acorus oil respectively (Table 5). LT_{50} of 15.78, 45.07 and 45.76 hours were obtained for neem-azal, clove oil and acorus oil respectively (Table 6).

Results of present study are in agreement with findings of Abhijith *et al.,* (2018) who studied efficacy of acorus oil using filter paper impreganation method and recorded 100 per cent mortality in *Lasioderma serricorne* and *Cryptolestes ferrugineus* adults when treated with acorus oil 10 per cent after 60 hours after treatment. Rashmi *et al.* (2019) recorded repellent activity of neem oil against *T. castaneum* which showed 88.31, 95.08 and

Table 1. Effect of essential oils on larval mortality of red flour beetle, *T. castaneum*

Treatment	Per cent larval mortality*				
	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT
Neem-azal 1.5%	26.66 (31.00)[b]	53.33 (46.92)[b]	83.33 (66.14)[a]	96.66 (83.86)[a]	100.00 (90.00)[a]
Neem-azal 2.0%	36.66 (37.22)[a]	70.00 (57.00)[a]	93.33 (77.71)[a]	100.00 (90.00)[a]	100.00 (90.00)[a]
Clove oil 10.0%	16.66 (23.86)[c]	36.66 (37.22)[d]	43.33 (41.15)[d]	53.33 (46.92)[d]	70.00 (56.79)[c]
Clove oil 15.0%	23.33 (28.78)[b]	36.66 (37.22)[d]	53.33 (46.92)[c]	73.33 (59.00)[c]	76.66 (61.22)[c]
Acorus oil 10.0%	23.33 (28.78)[b]	33.33 (35.22)[d]	50.00 (45.00)[c]	56.66 (48.85)[d]	63.33 (52.78)[d]
Acorus oil 15.0%	26.66 (31.00)[b]	40.00 (39.23)[c]	63.33 (52.78)[b]	73.33 (59.00)[c]	86.66 (68.86)[b]
Untreated control	0.00 (0.91)[d]	0.00 (0.91)[e]	0.00 (0.91)[e]	0.00 (0.91)[e]	0.00 (0.91)[e]
SEd	11.04	13.79	18.66	20.67	23.80
F value	2.79	4.01	4.07	4.88	3.84

DAT-Days after treatment; *Mean of three replications; Figures in parentheses are angular transformed values; Means followed by same letters are not significantly different by DMRT

Table 2. Efficacy of essential oils on adult mortality of red flour beetle, *T. castaneum* in stored rice

Treatment	Per cent adult mortality*		
	24 HAT	48 HAT	72 HAT
Neem-azal 1.5%	73.33 (59.00)[b]	90.00 (71.57)[b]	100.00 (90.00)[a]
Neem-azal 2.0%	90.00 (71.57)[a]	100.00 (90.00)[a]	100.00 (90.00)[a]
Clove oil 10.0%	36.67 (37.22)[c]	50.00 (45.00)[c]	60.00 (50.85)[c]
Clove oil 15.0%	76.67 (61.22)[b]	80.00 (63.43)[c]	83.33 (66.14)[b]
Acorus oil 10.0%	33.33 (35.22)[c]	53.33 (46.92)[c]	60.00 (50.85)$_c$
Acorus oil 15.0%	73.33 (59.00)[b]	80.00 (63.43)[c]	90.00 (75.00)[ab]
Untreated control	0.00 (1.07)[d]	0.00 (1.07)[d]	0.00 (1.07)[d]
SEd	21.18	22.69	27.36
F value	2.86	3.62	2.92

HAT-Hours after treatment; *Mean of three replications; Figures in parentheses are angular transformed values; Means followed by same letters are not significantly different by DMRT

Table 3. LC_{50} and LC_{90} of three essential oils against *T. castaneum* larvae at 2 DAT

Treatment	LC_{50} value (%)	Fiducial limits (95 %)		LC_{90} value (%)	Fiducial limits		Slope ± SE	Intercept ± SE	Chi square value
		Upper	Lower		Upper	Lower			
Neem-azal	1.11	1.33	0.95	6.42	15.17	4.06	1.68 ± 0.26	-0.07 ± 0.05	2.78
Clove oil	19.54	31.29	14.79	244.49	133.10	104.45	1.16 ± 0.20	-1.50 ± 0.20	4.39
Acorus oil	16.43	45.16	10.97	110.74	468.08	41.95	1.55 ± 0.20	-1.88 ± 0.21	5.80

LC: Lethal concentration

Table 4. LT_{50} of three essential oils against *T. castaneum* larvae

Treatment	LT_{50} value (Days)	Fiducial limits (95 %)		Slope ± SE	Intercept ± SE	Chi square value
		Upper	Lower			
Neem-azal @ 1.0%	2.40	3.52	1.25	2.31± 0.18	-0.88± 0.12	19.08
Clove oil @ 10.0%	4.31	5.96	2.79	2.06± 0.14	-1.31± 0.12	30.28
Acorus oil @ 10.0 %	4.30	6.73	2.32	1.78± 0.13	-1.14± 0.11	42.96

LT: Lethal time

Table 5. LC_{50} and LC_{90} of three essential oils against *T. castaneum* adults in contact toxicity test

Treatment	LC_{50} value (%)	Fiducial limits (95 %)		LC_{90} value (%)	Fiducial limits		Slope ± SE	Intercept ± SE	Chi square value
		Upper	Lower		Upper	Lower			
Neem-azal	0.99	1.07	0.93	2.08	2.44	1.85	4.00± 0.32	0.04± 0.64	1.30
Clove oil	11.30	15.17	8.54	25.25	57.01	17.95	3.67± 0.29	-3.86± 0.31	10.29
Acorus oil	11.68	15.60	8.95	26.32	58.79	18.70	3.63± 0.29	-3.88± 0.32	9.50

LC: Lethal concentration

Table 6. LT_{50} of three essential oils against *T. castaneum* adults in contact toxicity test

Treatment	LT_{50} value (HAT)	Fiducial limits (95 %)		Slope ± SE	Intercept ±SE	Chi square value
		Upper	Lower			
Neem-azal @ 1.5 %	15.78	11.34	24.67	3.15 ± 0.58	-3.77 ± 0.89	4.21
Clove oil @ 10.0 %	45.07	66.22	33.83	1.23 ± 0.37	-2.05 ± 0.62	0.48
Acorus oil @ 10.0 %	45.76	60.41	35.63	1.46 ± 0.38	-2.42 ± 0.62	0.28

LT: Lethal time

97.81 per cent repellent activity at 1, 2 and 3 per cent neem oil respectively. Mantzoukas *et al.* (2020) reported insecticidal activity of neem oil @ 3 per cent against *Tribolium confusum*, *Oryzaephilus surinamensis* and *Plodia interpunctella* larvae and pupae with 100 per cent mortality.

Shukla *et al.* (2009) reported that acorus rhizome powder (5 mg/g seed) was found more efficacious with 100 per cent ovicidal activity and completely inhibited F_1 adult emergence at a lower dose than that of leaf powders on chick pea seeds against *Callosobruchus chinensis.* Ali *et al.* (1983) reported that neem oil @ 1.00 per cent had brought 100.00 per cent grub mortality accounting to zero per cent adult emergence in green gram.

Rajasri *et al*., (2014) studied efficacy of neem formulations on pulse beetle and reported that all the

neem formulations *viz.*, NSK powder, neem cake, neem leaf powder, neem oil and neem-azal against *C. chinensis* in stored black gram up to 15 months of storage and among all neem formulations neem-azal found effective as compared to other formulations.

The essential oils *viz*., neem-azal @ 2%, acorus oil @ 15% and clove oil @ 15% were found to be highly effective against red flour beetle larvae and adults in stored rice and hence these safer biopesticides can be used for ecofriendly management of *T. castaneum* in stored rice.

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