

Use of Botanical Extracts for the Management of *Sitophilus oryzae* L. in Stored Paddy Grains

B. Vinothkumar₁*, K. Elanchezhyan₂ and V. Muralitharan₃ ¹Hybrid Rice Evaluation Centre, Gudalur, The Nilgiris ²Rice Research Station, Ambasamudram ³Department of Agricultural Entomology Tamil Nadu Agricultural University, Coimbatore - 641 003

Laboratory studies were conducted to study the effect of plant powders and acetonic extracts of four plants species Ruta chalepensis L., Eupatorium adenophorum (Spreng.)King & H.Rob., Ocimum sanctum L. and Zingiber officinale Rosc. against rice weevil, Sitophilus oryzae L (Coleoptera: Curculionidae) infesting paddy grains. Among them, leaf powders of E. adenophorum, O. sanctum and R. chalepensis @ 5 g per 25 g of seeds, were significantly superior with cent percent adult mortality on 14 days after treatment . Insecticidal activity of acetonic extracts of the plants was assessed by seed treatment method, filter paper diffusion method (direct contact application) and fumigation methods. The insect responses towards the treatment depended on the type of plant, the time of exposure and the mode of application. Among them acetonic extracts of E. adenophorum, and R. chalepensis @ 3 % v/w were effective with cent percent adult mortality 3 days after treatment. E. adenophorum, and R. chalepensis @ 2 % also recorded cent percent adult mortality on 5 and 7 days after treatment, respectively, whereas O. sanctum @ 3 % recorded 100 percent mortality on 7 days after treatment. In a test with the filter paper diffusion method, E. adenophorum leaf extract showed 100% mortality within three days after treatment at 8.5 mg /cm, whereas, only 88.67% mortality was observed in R. chalepensis, 65.89% in Z officinale and 61.67 % in O. sanctum at this dosage. E. adenophorum leaf extract having volatile toxic chemicals as indicated by 43.00 per cent adult mortality in fumigation bioassays.

Key words: Ruta chalepensis, Eupatorium adenophorum, Ocimum sanctum, Zingiber officinale, Sitophilus oryzae, botanical insecticides

Cereals are vital components of both human and livestock diets. After harvesting, unprocessed cereal product, will be stored for various lengths of time. Subsequent to the initial storage period it may also be stored at processing premises. Whilst in storage, cereals are at the risk to infestation by a wide range of stored product insects and mites (Wakefield, 2006). Stored-product insects can cause post harvest losses, estimated from 9% in developed countries to 20% or more in developing countries (Phillips and Thorne, 2010). The rice weevil, Sitophilus oryzae L. (Coleoptera: Curculionidae) is one of the most pests which causes heavy losses of stored grain both quantitatively and qualitatively throughout the world (Park et al., 2003). Earlier the control of insect pests in storage was largely based on synthetic insecticides and fumigants (pirimiphos methyl and phosphine) which led to the development of insecticide resistant strains, increasing cost of application, lethal effects on non-target organisms in addition to direct toxicity to users (Best and Ruthven, 1995). Their adverse environmental effects and the need to maintain sustainable environment created the need for environmental-safe, degradable and target specific insecticides. Because of this, much effort has been focused on plants or their constituents as potential

sources of commercial insect control agents (Han et al., 2006). The use of plant materials in pest control is an important alternative to the use of synthetic insecticides (Aranson et al., 1989). Plants are rich source of chemical compounds with various medicinal and insecticidal properties (Arnason et al., 1989). The insecticidal activity of many plant derivatives against several stored product pests has been demonstrated (Usha Rani and Udaya Lakshmi, 2007). Many plant chemicals have larvicidal, pupicidal and adulticidal activities, most being repellants, ovipositional deterrents and antifeedants against both agricultural pests and medically important insect species. Present study was undertaken to assess the potential of Ruta chalepensis L., Eupatorium adenophorum (Spreng.)King & H.Rob. , Ocimum sanctum L. and Zingiber officinale Rosc. against S. oryzae. The aim in selecting these plants was to identify the plants effective as natural pest control agents that can be grown easily in farmstead so that the pesticides of natural origin that are economically viable and environmentally safe are easily available for users.

Materials and Methods

Insect culture

The culture of rice weevil *S. oryzae*, was maintained in the laboratory without exposure to

*1Corresponding author email: drbvinothkumar@gmail.com

any insecticides on rice grains (variety: Bharathy) at 28±2°C, 60-65 % RH and 16:8 light: dark photoperiod.

Plant materials and their preparations

The four plants materials, leaves of R. chalepensis, E. adenophorum. O. sanctum and rhizomes of Z. officinale were collected from the places around Gudalur. The Nilgiris, Tamilnadu, India and were ground to fine powder. Leaves and rhizomes were shade dried and powdered using an auto mixer and collected separately. The powdered materials (500

g) were then extracted twice with 1000 ml acetone at room temperature and filtered through Whatman No. 1 filter paper. The filtrate was concentrated to dryness by rotary evaporation at 55 °C. The yield of acetone extraction was 20.8, 17.2, 22.4 and 22.4 g for R. chalepensis, E. adenophorum, O. sanctum and Z. officinale, respectively.

Bioassay

All tests were conducted at 28±2°C and 60-65 % RH and continuous darkness. The powdered products (crude) were admixed with rice grains at 1, 2, 3, 4 and 5 g per 25 g grain in plastic dishes (8 cm dia.). Four replications of treated grains as well as untreated grains (control) were set up. Batches of twenty sexed insects from the laboratory culture were introduced into each plastic dish which was then capped with a piece of muslin cloth. The muslin allowed ventilation but prevented entry and exit of insects. The treatments were left for 21 days under ambient conditions of 28±2°C, 60-65 % RH and ensuring adequate ventilation. Mortality assessments were recorded on 1, 3, 5, 7, 14 and 21 days after treatment (DAT). The numbers of dead adult weevils, and those that showed no visible movement after 20 seconds were recorded. After 21 days of assessment, all adult weevils were removed from the plastic dishes for the emergence of F1 progeny. At the end of 21 days, cumulative data on percentage adult weevil mortality were corrected using Abbott's (1925) formula;

Corrected per cent Pt-Pc mortality (100 - P_c)

Where, Pt - Observed per cent mortality in treatment (%), Pc - Observed per cent mortality in untreated check (%). After the 21 day mortality counts, all adults (dead and alive) were removed and the glass pots were left in the incubators at the same conditions for an additional period of 45 days. Then, the plastic dishes were opened and the emerged individuals were counted to enumerate the progeny production. At the end of the 45-day observation, the extent of weevil damage was assessed using the exit-hole as a measure of damage to the grains. Grains riddled with exit-holes were counted; the percentage damage (PD) and weevil perforation index (WPI) to the grains were calculated using the methods of Adedire and Ajavi (1996) and Fatope et al. (1995), respectively.

A separate experiment was conducted to know the insecticidal property of acetone extracts of the plant powders against S. oryzae. A fixed quantity (1 kg) of rice grains was then sprayed with 100 ml of each solution, 0.5, 1.0, 2.0 and 3.0 % (w/v) prepared from crude extract using acetone. Also, there was 1 kg of grain was sprayed with acetone alone as control. From each combination, four samples of 50 g each were drawn out. Each sample was placed in a small glass pots (7 cm diameter and 8.5 cm height). Twenty five S. oryzae adults were introduced into each glass pot and then covered with nylon mesh secured with rubber bands. The pots were placed in incubators, at the 28 2_oc and 60-65%RF. Dead adults were counted 1, 2, 3, 5, 7, 14 and 21 days later. After the 21 day mortality count, all adults (dead and alive) were removed and glass pots were left in incubators at the same conditions for an additional period of 45 days. Then, the glass pots were opened and emerged individuals were counted to know the progeny production. At the end of 45-days the extent of weevil damage was assessed using the exit-holes. Percentage damage (PD) and weevil perforation index (WPI) were calculated.

In another experiment, the contact toxicity of the plant powders against test insect was determined by direct contact application. The crude extracts (acetone extracts) at concentrations of 100, 90, 80, 70 and 60 mg in 200 µl acetone were applied on to Whatman No.1 filter papers of size 4.25 cm diameter, which gave a dosage of 8.5, 7.65, 6.80, 5.95 and 5.10 mg/cm₂ respectively (Rajasekhar Reddy and Usharani, 2010), whereas the control received 200 µl acetone without any plant product. After drying each filter paper was placed in the bottom of a petridish (5 cm diameter x 1 cm) and then ten numbers of 2-5 day old, adults of S. oryzae, were released into the petridish and exposed to treatments. All the treatments were replicated ten times. Results on mortality were recorded after every 24 hrs. Less than 5% of test insects were in contact with the wall or lid of a petridish at a given time.

The response of S. oryzae to the fumigant action of the crude extracts was investigated according to the method of Kim and Ahn (2001). Filter paper discs (4.25 cm dia) treated with 8.5 mg/cm2 of the compounds, were placed at the bottom of a polyethylene cup (4.5 cm diameter × 8.6 cm), and a diet cup containing adults (3.8 cm dia x 4 cm) was placed into the polyethylene cup. This prevented direct contact of the test adults with the test compound. Groups of 10 adults were placed in diet cups covered with a 60-mesh cloth. Each polyethylene cup was then sealed with a lid. The insects were exposed for 3 days. Mortality counts were made every 24hrs. All

treatments were replicated ten times.

Results and Discussion

The data on toxicity bioassay of the plant powders on adults of *S.oryzae* are presented in Table 1. Adult mortality significantly increased with increase in concentration and days of exposure. Mortality of *S. oryzae* adults started one day after treatment on wards in all the treatments. On 3 DAT significantly 2.50 to 38.75 per cent mortality was observed

Plant	Dose			Per cent morta	lity of S.oryzae*		
	(g) / 25 g	1 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT
		0.00	2.50	10.20	28.85	45.11	64.0
	1	(0.32)	(6.46)i	(18.11) _i	(32.44) _{gh}	(42.16) _{hij}	(53.27)
		1.25	8.75	24.71	38.15	57.19	73.8
	2	(3.23) _{ef}	(17.05) _h	(29.71) _{hi}	(38.07) _{fg}	(49.18) _{ghi}	(60.00)
Ruta		3.75	17.50	27.20	48.68	82.71	87.8
chalepensis	3	(9.69) _{cde}	(24.67) _{fg}	(31.37) _{ghi}	(44.23) _{bcd}	(66.10) _{de}	(72.25)
		8.75	22.50	30.88	59.02	85.01	94.2
	4	(17.05) _{abc}	(28.27) _{def}	(33.61) _{e-h}	(50.24) _{bc}	(70.09) _{cd}	(78.14)
		11.25	22.50	39.07	69.91	100.00	100.0
	5	(19.51) _{ab}	(28.27) _{def}	(38.66) _{⊳e}	(57.06)a	(89.96)a	(89.96)
		0.00	2.50	12.91	36.76	57.05	65.4
	1	(0.32)	(6.46)i	(20.99) _j	(37.26) _{fg}	(49.11) _{ghi}	(54.22)
		1.25	12.50	34.98	51.32	78.76	89.2
	2	(3.23) _{ef}	(20.60) _{gh}	(36.22)∝ _g	(45.74) _{cd}	(62.59) _{de}	(71.03)
Eupatorium		5.00	23.75	37.54	68.32	95.91	100.0
adenophorum	3	(11.06) _{cd}	(29.13) _{cde}	(37.74) _{c-f}	(55.93)a	(81.78)ab	(89.96)
		11.25	30.00	41.36	80.02	97.50	100.0
	4	(19.51) _{ab}	(33.16)bc	(39.98) _{bcd}	(63.76)a	(85.36)a	(89.96)
		15.00	32.50	51.91	92.30	100.00	100.0
	5	(22.63) _a	(34.73) _{ab}	(46.08)a	(76.60)a	(89.96)a	(89.96)
		2.50	20.00	32.54	42.24	53.44	59.1
	1	(6.46) _{def}	(26.47) _{ef}	(34.75) _{e-h}	(40.50) _g	(46.99) _{ghi}	(50.34)
		2.50	25.00	37.68	54.02	62.81	70.0
	2	(6.46) _{def}	(29.93) _{cde}	(37.85) _{c-f}	(47.29)ef	(52.42) _{fg}	(56.89)
Ocimum		6.25	32.50	42.68	57.77	72.04	81.9
sanctum	3	(14.29) _{bc}	(34.73) _{ab}	(40.75) _{bc}	(49.49) _{def}	(58.15) _{ef}	(65.47)
		8.75	30.00	47.81	60.20	89.17	100.0
	4	(17.05) _{abc}	(33.16)bc	(43.73) _{ab}	(51.01)₀	(71.12) _{bc}	(89.96)
		6.25	38.75	55.38	78.63	100.00	100.0
	5	(14.29) _{bc}	(38.46)a	(48.14)a	(63.60)a	(89.96)a	(89.96)
		2.50	13.75	21.86	28.92	38.73	53.3
	1	(6.46) _{def}	(21.69) ₉	(27.67)	(32.41) ^h	(38.44) _j	(46.95)
		2.50	17.50	28.52	36.89	45.53	62.0
	2	(6.46) _{def}	(24.67) _{fg}	(32.22) _{ghi}	(37.36) _{gh}	(42.41) _{ij}	(52.11)
Zingiber		5.00	22.50	29.70	40.63	57.39	65.7
officinale	3	(11.06) _{cd}	(28.27) _{def}	(32.94) _{fgh}	(39.49) ₁₉	(49.33) _{ghi}	(54.50)
		6.25	21.25	33.73	42.10	58.57	69.9
	4	(14.29) _{bc}	(27.41) _{def}	(35.49) _{d-g}	(40.43) _{efg}	(49.93) _{fgh}	(56.82)
		5.00	27.50	34.91	40.64	62.60	88.7
	5	(11.06)cd	(31.59) _{bcd}	(36.13)₀ _g	(39.54) _{ef}	(52.34) _{fg}	(73.60)
p=0.05)		7.54	4.40	5.04	0.04	7.00	
CD (0.05)		7.54	4.46	5.21	8.24	7.88	9.2
SEd		3.77	2.23	2.60	4.12	3.94	4.6

Figures represent means of four replications, each set up with 20 adults (n=80); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in parentheses are arcsine transformed values

among treatments. At the end of 14 DAT, 100 per cent mortality of the weevil was observed at 5 g application of plant powders except Z. officinale. The results further revealed that 4 g and 3 g application of E.adenophorum was on par with the higher concentrations of plant powder application, that recorded 97.50 and 95.91 per cent weevil mortality. There was 100 percent weevil mortality at the end of 21 DAT. O. sanctum @ 4 g recorded 95.91 per cent mortality and it was on par with R. chalepensis 4 g (94.28 %), E. adenophorum @ 2 g (89.22 %), Z. officinale 5 g (88.73 %) and *R.chalepensis* @ 3 g (87.81 %) on 21 DAT. Least weevil mortality was observed at 1 g of plant powder. The least mean per cent punctured grains after 7 weeks of storage was observed in the grains treated with E. adenophorum 5 g (1.62 %) and 4 g (2.52 %) followed by R.chalepensis

5 g (3.07 %) and O. sanctum 5 g (3.05 %) as against 75.55 per cent mean per cent punctured grains recorded in the untreated control grains (Table 2). All the treatments significantly reduced the mean per cent weight loss, compared to control grains (47.04 %). E. adenophorum @ 5 g recorded least weight loss (1.77 %). The number of emerged adults decreased with increase in concentration of the plant powder. Least emergence was observed in E. adenophorum @ 5 g treated grains (2.75) followed by E. adenophorum @ 4 g (5.75), R.chalepensis @ 5g (8.00) and O. sanctum (10.50). Whereas in untreated control 97.25 adults emerged. The weevil perforation index (WPI) indicated that E. adenophorum might possess some active principle to suppress the population of S. oryzae adults and its progeny than other plant species. The percent damage indicated the activity

of one plant material at different concentrations, while WPI compared the activity of different species of plant extracts used.

The acetonec extracts of *R. chalepensis, E. adenophorum, O. sanctum* and *Z. officinale* that showed toxic properties to *S.oryzae* were further examined. The use of plant extracts in the control

of stored insects is an ancient practice (Qi and Burkholder, 1981). The results of toxicity study of plant extracts against *S.oryzae* adults were presented in the Table 3. The mortality of *S.oryzae* adults on 1DAT ranged from 11.00 to 58.00 per cent in treatments. Highest mortality was observed in the grains treated with *E. adenophorum* 3 % (58.00 %) and it was on par with *R. chalepensis* 3 % (57.00

Table 2. Effects of plant powders on S.oryzae infestation in rice

		 Mean num 				
Plant	Dose g/25g	Mean % punctured grains	Mean % weight loss	emerged	WPI	
	1	11.41 (19.72) _{ijk}	8.27 (16.69)hi	29.25 (5.45)ki	13.28	
Ruta	2	9.54 (17.97) _{ghi}	6.59 (14.83) _{e-h}	24.25 (4.97)ij	11.38gh	
	3	6.17 (14.36)r	5.21 (13.18)⇔f	18.25 (4.33) _{gh}	7.65	
chalepensis	4	4.16 (11.71)cde	3.97 (11.47)bc	11.50 (3.46) _d	5.25cd	
	5	3.07 (10.04)bc	3.40 (10.59)₀	8.00 (2.91)c	3.96ab	
	1	9.10 (17.54)gh	7.69 (16.06)ghi	26.00 (5.15) _{jk}	10.91 _{gt}	
	2	5.23 (13.20) _{def}	5.70 (13.81) _{def}	22.50 (4.79)ij	6.55de	
Eupatorium	3	3.84 (11.27)cd	4.96 (12.85)cd	11.50 (3.46) _d	4.89bc	
adenophorum	4	2.52 (9.00)ab	3.03 (10.02)₀	5.75 (2.49) _b	3.27 at	
	5	1.62 (7.04)a	1.77 (7.62)₂	2.75 (1.79) ₄	2.10	
	1	16.19 (23.69)	11.73 (19.99) _j	35.00 (5.95)m	17.80	
. .	2	13.42 (21.46)×	8.74 (17.18)i	30.50 (5.57)	15.27	
Ocimum	3	10.87 (19.20)hij	6.24 (14.44) _{d-g}	22.50 (4.78)ij	12.74h	
sanctum	4	5.52 (13.52)ef	5.13 (13.07)cde	14.25 (3.83)ef	6.85₀	
	5	3.05 (10.00)bc	4.90 (12.75)cd	10.50 (3.31)d	3.94ab	
	1	30.68 (33.60)n	15.46 (23.09)	38.25 (6.22)m	29.11	
_ ,	2	23.47 (28.92)m	14.41 (22.24) _{kl}	30.00 (5.52)	23.86m	
Zingiber officinale	3	12.64 (20.79) _{jk}	12.63 (20.76) _{jk}	21.50 (4.69)hi	14.46 _#	
Unicinale	4	9.57 (17.94) _{ghi}	9.38 (17.81)i	15.75 (4.02) _{fg}	11.39gh	
	5	8.13 (16.52) ₉	6.77 (15.06) _{fgh}	12.75 (3.63) _{de}	9.86	
Control	0	74.55 (59.71) ₀	47.04 (43.28)m	97.25 (9.87)n	50.00	
p=0.05) CD (0.05)		2.15	1.88	0.37	1.93	
SEd (0.00)		1.07	0.94	0.18	0.97	

*Figures represents means of four replications, each set up with 20 adults (n=80); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in parentheses are excise excise

%), *E. adenophorum* 2 % (53.00 %). Cent per cent mortality was observed in *E. adenophorum* 3 % and *R. chalepensis* 3 % on 3 DAT. *E. adenophorum* 2

% registered 100 per cent mortality of S. oryzae adults on 5 DAT followed by R. chalepensis 2 % and O. sanctum 3 % on 7 DAT. E. adenophorum 1 % and O. sanctum 2 % registered 100 percent S. oryzae adult mortality on 21 DAT where as Z. officinale 3 % registered 97.83 per cent S. oryzae adult mortality on 21 DAT. Hence the order of relative toxicity of plant extracts against S. oryzae adult was; E. adenophorum 3 % = R. chalepensis 3 % > E. adenophorum 2 % > R. chalepensis 2 % = 0. sanctum 3 % > E. adenophorum 1 % = 0. sanctum 2 % > Z. officinale 3 % = R. chalepensis 1 % ≥ E. adenophorum 0.5 % > Z. officinale 2 % = 0. sanctum 1 % = Z. officinale 1 % = R. chalepensis 0.5 % > 0. sanctum 0.5 % > Z. officinale 0.5 % (Table 3). Mean per cent damaged grains after 7 weeks incubation period ranged from 0.00 to 44.36 per cent (Table 4). There was nil damage in grains treated with E. adenophorum 3 % followed by E. adenophorum 2

% (0.27 %), R. chalepensis 3 % (1.06 %), O. sanctum 3 % (1.06 %), R. chalepensis 2 % (1.37 %), E. adenophorum 1 % (1.71 %) and O. sanctum 2 % (1.85 %) as against 44.36 per cent in the untreated control. Same trend was observed in the mean per cent weight loss also (Table 4). The number of emerged adults decreased with increase in concentration of extract. *E. adenophorum* had the least number of emerged adults (0.75) at 3 % concentration and it was on par with *E. adenophorum* 2 % (1.75) and *R. chalepensis* 3 % (1.50). There were 94.25 adults emerged from untreated control. The WPI indicated that *E. adenophorum*, having some active principle to suppress the population of *S. oryzae* adults and its progeny than other plant species (Table 4).

In the contact application method of acetonic extracts of *R. chalepensis*, *E. adenophorum*, *O. sanctum* and *Z. officinale*, the mortality rates depended on the type of plant extract, exposure time and dosage. At the dose of $8.5 \text{ mg/cm}_2 R$. *chalepensis* extract observed 51 per cent mortality and it was on par with *E. adenophorum* 8.5 mg/cm_2 (50.0 %) on 1DAT (Table 5) where as *Z. officinale* and *O. sanctum* extracts showed only 29.00 and 39.00 percent, respectively at the same dose and time. On 3 DAT, *E. adenophorum* extract showed 100 per cent mortality against *S. oryzae* adults, where as it

Plant	D (0/) (Per cent r	mortality of S.ory	zae*				
Fidin	Dose (%) v/w	1 DAT	2 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT		
		27.00	43.88	54.71	67.10	77.04	81.10	86.05		
	0.5	(31.17) _{def}	(41.44) _{def}	(47.69) ^r	(55.02) ^r	(61.37).	(64.22) _{ef}	(68.21).		
		33.00	44.79	55.50	73.14	87.57	92.66	95.70		
Ruta	1	(35.02)cd	(41.97) _{cde}	(48.17) [,]	(58.85) _{ef}	(69.43) _{cd}	(74.37)cd	(79.73)∞		
chalepensis		44.00	53.00	71.19	91.66	100.00	100.00	100.00		
	2	(41.52)₀	(46.71)∝	(57.53)₀	(73.49)₅	(89.96)a	(89.96)a	(89.96)a		
		57.00	83.75	100.00	100.00	100.00	100.00	100.00		
	3	(49.03)a	(66.47)a	(89.96)a	(89.96)	(89.96)a	(89.96)a	(89.96)a		
		32.00	46.96	58.80	76.44	82.48	87.40	91.44		
	0.5	(34.31)cd	(43.22) _{cd}	(50.09) _{ef}	(61.05) _{de}	(65.41) _{de}	(69.30) _{de}	(75.21)∝		
		40.00	53.96	66.85	80.36	88.39	94.66	100.00		
Eupatorium	1	(39.18)bc	(47.28)₀	(54.97) _{cde}	(63.85)cd	(70.78)₀	(78.48) _{bc}	(89.96)a		
adenophorum		53.00	64.21	86.70	100.00	100.00	100.00	100.00		
	2	(46.73)a	(53.31)₅	(68.80) _b	(89.96)a	(89.96)a	(89.96)a	(89.96)a		
		58.00	85.75	100.00	100.00	100.00	100.00	100.00		
	3	(49.59)a	(68.05)a	(89.96)a	(89.96)a	(89.96)a	(89.96)a	(89.96)a		
		18.00	26.42	36.90	50.41	57.17	63.06	74.14		
	0.5	(24.93) ₉	(30.74),	(37.30) _g	(45.23) _g	(49.12) ₉	(52.60) _g	(59.50)		
		22.00	35.58	54.71	76.36	80.04	86.27	89.22		
Ocimum	1	(27.85)efg	(36.50) _{efg}	(47.69)	(60.92) _{de}	(63.65).	(68.49) _e	(71.31) _{de}		
sanctum		27.00	43.88	60.76	85.70	93.65	96.83	100.00		
	2	(31.10) _{def}	(41.44) _{def}	(51.22) _{def}	(67.97)。	(75.63)₀	(82.76) _b	(89.96)a		
		28.00	46.83	67.89	91.78	100.00	100.00	100.00		
	3	(31.91) _{de}	(43.15)∝	(55.57)∝d	(73.56)₅	(89.96)a	(89.96)a	(89.96)a		
		11.00	17.33	23.47	30.90	45.57	54.54	61.23		
	0.5	(19.17) _h	(24.49)h	(28.67)h	(33.73)h	(42.41) ^h	(47.61) _g	(51.50) _g		
		20.00	33.67	44.29	56.59	67.87	73.84	84.96		
Zingiber	1	(26.50) _{fg}	(35.41) ₉	(41.70) _g	(48.77) _g	(55.49)	(59.30)	(67.20) _e		
officinale		26.00	34.71	53.50	71.10	77.96	83.05	89.22		
	2	(30.56) _{def}	(36.07) _{fg}	(47.00) ^r	(57.60) _{ef}	(62.12) _e	(65.77) _e	(70.90) _{de}		
		32.00	47.83	60.71	75.18	91.74	94.74	97.83		
	3	(34.42)cd	(43.72) _{cd}	(51.18) _{def}	(60.15) _{de}	(73.50) _{bc}	(76.87)	(83.95)		
(p=0.05)		. ,		, ,	. ,	. ,	. ,	. ,		
CD (0.05)		5.11	5.80	5.23	4.45	4.75	5.37	5.81		
SEd		2.55	2.88	2.60	2.21	2.36	2.67	2.89		

Table 3. Effect of plant extracts on the mortality of S.oryzae in stored rice grains

*Figures represent means of four replications, each set up with 25 adults (n=100); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in

parentheses are arcsine transformed values

was only 88.67, 65.89 and 61.67 per cent in the case of *R. chalepensis, Z. officinale* and *O. sanctum*, respectively (Table 5). The results of the fumigation treatments of the crude extracts revealed that, *E. adenophorum* at 8.5 mg/cm₂ registered higher

mortality of *S. oryzae* adults (43.00 %) compared to *R. chalepensis* (26.00 %), *Z. officinale* (24.00 %) and *O. sanctum* (13.00 %) on 3 DAT (Table 6). Thus, volatile toxicants were not present in significant quantities in all the four plant extracts, however

Table 4. Effect of plant extracts of S.oryzae infestation in rice

Plants	Dose (%)	Mean % punctured grains.	Mean % weight loss.	Mean number of adult emerged.	WPI
	0.5	3.80 (11.23)fg	3.50 (10.77) _{hi}	14.00 (3.80) _{fg}	7.91₀
Ruta	1	3.17 (10.24)ef	2.40 (8.88)fg	7.75 (2.86)d	6.71 _{de}
chalepensis	2	1.37 (6.69)cd	0.36 (3.43) _{bc}	4.50 (2.22)b	2.97bc
	3	1.06 (5.90)c	0.12 (1.98)₀	1.50 (1.40)a	2.35b
	0.5	3.00 (9.96)ef	1.52 (7.06)₀	9.50 (3.16) _{de}	6.37d
Eupatorium	1	1.71 (7.50)d	0.88 (5.37)d	5.00 (2.33)bc	3.71c
adenophorum	2	0.27 (2.53) _b	0.16 (2.29) _b	1.75 (1.42)a	0.58a
	3	0.00 (0.29)a	0.00 (0.29)a	0.75 (1.10)a	0.00a
	0.5	5.40 (13.41)h	4.16 (11.75)ij	21.50 (4.68)h	10.84g
Ocimum	1	4.49 (12.20)gh	2.20 (8.51)efg	15.50 (3.99)g	9.20f
sanctum	2	1.85 (7.77)d	1.80 (7.69)ef	7.50 (2.82) _{cd}	3.98c
	3	1.06 (5.88)₀	0.60 (4.44) _{cd}	5.00 (2.33) _{bc}	2.35b
	0.5	10.24 (18.64)j	7.26 (15.61)ĸ	44.00 (6.66) _j	18.73i
Zingiber	1	7.84 (16.23)	4.82 (12.66)j	33.25 (5.81)i	14.98h
officinale	2	3.20 (10.26)ef	2.72 (9.46) _{gh}	14.75 (3.89)g	6.76de
	3	2.73 (9.48)₀	2.32 (8.73)fg	11.00 (3.38) _{ef}	5.84d
Control	0	44.36 (41.74) _k	40.70 (39.58)	94.25 (9.73) _k	50.00j
(p=0.05) CD (0.05)		1.29	1.66	0.49	1.25
SEd		0.65	0.82	0.24	0.62

*Figures represents means of four replications, each set up with 25 adults (n=100); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in parentheses are • / x + 0.5 transformed values, WPI – Weevil Perforation Index

E. adenophorum might possess higher amount of volatile toxicants than other plant extracts tested.

The trial showed that the leaf powders and acetonic extracts of *R. chalepensis, E. adenophorum, O. sanctum* and *Z. officinale* adequately protected paddy grains from *S.oryzae*, but the effectiveness was the determined by the characteristics of the plant,

dose and exposure interval. One of the findings of the current study was the dissimilar efficacy of botanical material among plant species so that; *E. adinophoram* and *R. chalepensis* were much more effective against *S. oryzae* on treated paddy grains than other species. This is consistent with the previous work of Enobakhare and Law-Ogbomo (2002) and Law-Ogbomo and Enobakhare (2007) using leaf powders

Table 5. Contact toxic	ty of	plant extracts against S.o.	yzae
------------------------	-------	-----------------------------	------

Plant	Dose (mg/cm2)	1 DAT	2 DAT	3 DAT
	5.1	23.00 (28.19) _{g-j}	38.00 (37.76) _{fgh}	44.11 (41.49)hi
D (-	5.95	28.00 (31.66) _{e-i}	37.00 (37.36) _{f-i}	49.44 (44.64) _{gh}
Ruta	6.8	32.00 (34.16)def	49.00 (44.38)de	61.67 (51.96)
chalepensis	7.65	42.00 (40.32)bc	61.00 (51.60)bc	76.33 (61.52)
	8.5	51.00 (45.56)a	69.00 (56.44)ab	88.67 (72.52)
	5.1	26.00 (30.48)f-j	45.00 (42.05)def	51.33 (45.76)g
	5.95	30.00 (32.95)efg	49.00 (44.41) _{de}	68.44 (56.35)d
Eupatorium	6.8	34.00 (35.34) _{de}	54.00 (47.31)cd	77.11 (62.08)
adenophorum	7.65	38.00 (37.87)cd	65.00 (54.00)b	93.78 (78.70)
	8.5	50.00 (44.96)ab	77.00 (61.99)a	100.00 (89.96)
	5.1	22.00 (27.73)hij	28.00 (31.66)ij	34.89 (36.08)j
a <i>i</i>	5.95	25.00 (29.88)f-j	38.00 (37.87) _{fgh}	42.11 (40.41) _h
Dcimum	6.8	27.00 (31.14) _{e-j}	41.00 (39.72)efg	50.44 (45.24)g
sanctum	7.65	27.00 (31.14) _{e-j}	45.00 (42.05)def	55.67 (48.37)r
	8.5	29.00 (32.32) _{e-h}	48.00 (43.80)de	61.67 (51.97)
	5.1	21.00 (26.92)ij	27.00 (30.84)j	28.78 (32.32)
7	5.95	21.00 (26.77) _j	31.00 (33.50)hij	35.67 (36.41)
Zingiber	6.8	23.00 (28.40) _{g·j}	32.00 (34.25) _{9'} j	40.22 (39.26)
officinale	7.65	27.00 (31.14) _{e-j}	34.00 (35.34) ₉ ,	51.33 (45.74) _g
	8.5	39.00 (38.54)cd	49.00 (44.38) _{de}	65.89 (54.50)
(p=0.05) CD (0.05)		4.86	5.72	5.93
SEd		2.46	2.90	3.00

*Figures represent means of ten replications, each set up with 10 adults (n=100); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in parentheses are arcsine transformed values

of Ocimum gratissimum and Vernonia amygdalina to control *S. zeamais* in stored maize grains and *S.oryzae* in stored paddy grains, respectively. The insecticidal effect of leaf powders was attributed to one or more of the following: fumigation effect, repelling, stomach poison effecting case where the weevils feed on admixed grains, mechanical action, starvation or desiccation (Sharaby, 1988; Dales, 1996). Acetonic extracts of the peels of sweet orange, *Citrus sinensis* (L.) and sour orange, *Citrus aurantium* (L.), were effective against S. *oryzae*, *R. dominica* and *T. castaneum* (Rajasekharreddy and Usharani, 2010). Govindan and Nelson (2009) suggested *Azadirachta indica* A. Juss. and *Alpinia officinarum* Hance. for the management of *S. oryzae*.

The leaf powders and crude extracts of plants impared oviposition through lower number of F_1 emerging adult than the untreated control. The progeny development in the untreated control was higher than the treated grains. This illustrated the magnitude of the insect multiplication and damage that occur in unprotected seeds. The decrease in F_1 emergence in the treated grains could be resulted from increased adult mortality, ovicidal and larvicidal properties of the tested leaf powders and crude extracts. The ovicidal and larvicidal properties

Table 6. Fumigant	toxicity	of	plant	extracts
against S.oryzae				

	Dose			
Plants	(mg/cm2)	1 DAT	2 DAT	3 DAT
Ruta chalepensis	8.50	6.00 (11.06) _{ab}	14.00 (21.68)a	26.00 (30.54) _b
Eupatorium adenophorum	8.50	9.00 (16.58)a	18.00 (24.93) _a	43.00 (40.95) _a
Ocimum sanctum	8.50	0.00 (0.29)	2.00 (3.69)	13.00 (17.63)。
Zingiber officinale	8.50	3.00 (5.53)bc	13.00 (20.87) _a	24.00 (29.21)
(p=0.05) CD (0.05)		6.37	4.61	6.32
SEd		3.14	2.27	3.12

*Figures represent means of ten replications, each set up with 10 adults (n=100); In a column means followed by a common letter are not significantly different by DMRT (P=0.05); Values in parentheses are arcsine transformed values

could have arisen from impairing respiration through blockage of spiracle there by resulting in suffocation (Dales, 1996). All tested leaf powders had lower percentage weight loss and punctured grain than the untreated grains. The reduction could be attributed to high adult weevil mortality, reduction in F_1 adult emergence and certain nutritional inhibitors in the leaf powders and crude extracts of the plants (Dales, 1996) in the treated grains. Females of *S. oryzae* laid their eggs on the external part of the kernel (Golebiowska, 1969) and it was likely that newly hatched larvae were exposed to botanicals before entering the kernel. From the progeny production emergence of adults from all control samples indicated that tested insects were capable of effective oviposition and that prevention of progeny emergence was exclusively due to treatment. Thus extracts of the plants either suppressed oviposition are killed the larvae hatching from eggs laid in the medium culture. These results suggest that there may be different compounds in extracts possessing different bioactivities. Hojat Khoshnoud et al., (2008) showed that complete suppression (100 per cent reduction) of progeny production (F₁) was observed in the ethanol extracts of Verbascum cheiranthifolium Boiss and Verbascum speciosum Schard treated wheat than in control even at the lowest dose. Results showed that E. adinophoram extracts possesed some fumigant action against S.oryzae. Rajendran and Sriranjini (2008) studied the plant essential oils and their constituents as fumigants against S. oryzae.

However, the results indicated that higher concentrations of these plant extracts for a relatively short periods were much more effective than lower concentrations for a long period. One of the basic characteristics of an effective grain protectent is the ability to reduce progeny production in treated grains Progeny production on treated commodity was inhibited completely in all the doses, indicating that even if oviposition occurred before death, the activity of botanical extract during the first moult of larvae was satisfactory.

This study suggests that acetonic extracts of *E.* adinophoram and *R.* chelapensis possesse toxic principles with significant insecticidal effect and could be a potential grain protectant against *S.* oryzae. Today, the environmental safety of an insecticide is of paramount importance. Consequently, substances alternative to chemical pesticides, which are safer to our natural sources and threaten our future, can be found. In addition, cheaper pesticides can be obtained and environmental pollution will be gradually decrease.

References

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. *Econ. Entomol.*, 18: 265 267.
- Adedire, C.O. and Ajayi, T.S. 1996. Assessment of insecticidal properties of some plants as grain protectants against the maize weevil, *Sitophilus zeamais* (Mots). *Nigerian J.Entomol.* **13**: 93 – 101.
- Arnason, J.T., Philogene, B.J.R. and Morand, P. 1989. Insecticides of plants origin. *American Chemical Society Symposium Series*. 387: Washington.
- Best, G.A. and Ruthven A.D. 1995. Pesticides developments, impacts and controls. *Royal Society* of Chemistry, UK. 254p.
- Dales, M.J., 1996. A review of plant materials used for control using pests of stored product. *NRI Bulletin* 65, Chatham, UK, 84p.
- Enobakhare, D.A. and Law-Ogbomo, K.E. 2002. Efficacy of leaf powders *Ocimum gratissimum* and *Vernonia*

amygdalina as grain protectants against Sitophilus zeamais Mots. in three varieties of maize. J.Agric. For. Fish., **3:** 22-27

- Fatope, M.O., Mann, A. and Takeda, Y. 1995. Cowpea weevil bioassay: A simple pre-screen for plants with grain protectant effects. *Internat J. Pest Mgmt.*, **41**: 84–86.
- Golebiowska, Z., 1969. The feeding and fecundity of Sitophilus granarius L., Sitophilus oryzae L. (Coleoptera: Curculionidae) and Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae) in wheat grain. J. Stored Prod. Res., 5: 143-155
- Govindan, K. and Nelson, S.J. 2009. Insecticidal activity of twenty plant powders on mortality, adult emergence of *Sitophilus oryzae* L. and grain weight loss in paddy. *J. Biopesticides.*, 2: 169 – 172.
- Han, M.K., Kim, S.I. and Ahn, Y.J. 2006. Insecticidal and antifeedant activities of medicinal plant extracts against *Attagenus unicolor japonicus* (Coleoptera: Dermestidae). J. Stored Prod. Res., **42:** 15 - 22.
- Hojat Khoshnoud, Mahdi Ghiyasi, Reza Amirnia, Shiva Sadig Fard, Mehdi Tajbakhsh, Hojat Salehzadeh, and Parisa Alahyary. 2008. The potential of using insecticidal properties of medicinal plants against insect pests. *Pakistan J. Biol. Sci.*, **11**: 1-5
- Kim, D.H. and Ahn, Y.J. 2001. Contact and fumigant activities of constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. *Pest Manage. Sci.*, 57: 301 - 306.
- Law-Ogbomo K.E. and Enobakhare, D.A. 2007. The use of plant powders of Ocimum gratissimum and Vernonia amygdalina for the management of Sitophilus oryzae L. in stored rice. J.Entomol., 4: 253-257.
- Park, I.K., Lee, S.G., Choib, D.H., Park, J.D. and Ahna, Y.J. 2003. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus* oryzae (L.) J. Stored Prod. Res., **39**: 375–384.
- Phillips, T.W. and Thorne, J.E. 2010. Biorational Approaches to Managing Stored-Product Insects: *Annu. Rev. Entomol.*, **55**: 375-397
- Qi, I.T. and Burkholder, W.E. 1981. Protection of stored wheat from the granary weevil by vegetable oils. *Econ Entomol.*, 74: 502-505.
- Rajasekharreddy, P. and Usharani, P. 2010. Toxic properties of certain botanical extracts against three major stored product pests. *J. Biopesticides*, **3**: 586 - 589.
- Rajendran, S. and Sriranjini, V. 2008. Plant products as fumigants for stored product insect control. J. Stored Prod. Res., 44: 126 – 135.
- Sharaby, A. 1988. Evaluation of some murataceae: Plant leaves as protectants against infestation by *Sitophilus oryzae* L. and *Sitophilus granarius* L. *Insect Sci. Applic.*, **9:** 405 – 468.
- Usha Rani, P. and Udaya Lakshmi, S. 2007. Evaluation of *Tagetes erecta* essential oil, floral and leaf extracts as grain protectants: efficacy against four species of stored product pests. *Uttar Pradesh J. Zoo.*, **27:** 159 - 169.
- Wakefield, M.E. 2006. Factors affecting storage insect susceptibility to the entomopathogenic fungus Beauveria bassiana. In: 9th International Working Conference on Stored Product Protection. 885-862p.

Received: October 15, 2012; Accepted: January 18, 2013