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Preparation of herbal extracts and evaluation of their efficacy against rice weevil (*Sitophilus oryzae* L., Curculionidae; Coleoptera)

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Abstract

The experiment was conducted to evaluate the efficacy of Neem, Kalmegh, Cinnamon, Black pepper and Clove extract along with Diatomaceous Earth (DE) against rice weevil (*Sitophilus oryzae* L.) in terms of percent mortality and repellency of adult weevil at different exposure period. Maximum adult weevil mortality (49.70 to 90.15%) was noticed after 3 weeks of treatment with 60 gm of Neem, Kalmegh and Clove along with 1 gm of Diatomaceous Earth. But in case of Cinnamon and Black pepper maximum mortality of adult weevil (85.57 to 100%) was found when these were used in 40gm dose in combination with DE. In the repellency study, it was found that the treatment composed of Clove and DE registered the best result after 1 week of treatment in repelling adult rice weevil (98.80%).

Keywords: Efficacy, Botanicals, Diatomaceous Earth, Rice weevil

1. Introduction

Worldwide, rice is an important staple food and it is regularly attacked by several insect-pests particularly at storage. India is one of the leading producers of rice in the world accounting 20 percent of the global rice production. It contributes 52 percent of total food grain production and 55 percent cereal production in India. West Bengal is the highest producer of rice among all the states of India and therefore, it is popularly known as "Rice Bowl of India". Storage pests are the major threat of rice and cause significant losses worldwide. It is estimated that 5-10% of the world's grain production is lost due to ravage of insect-pests [1]. According to the estimates of International Grain Council (IGC), the mean quantity losses of grains by the store grain pests in India are approximately 10% of the annual production, which is about 12 million tons per year [2].

Rice weevil, *Sitophilus oryzae* L. (Curculionidae; Coleoptera) is one of the most destructive pests of stored cereal grains worldwide and consequently it has been the subject of concern to all the grain handlers. It is a common pest of warm climate and attacks paddy, wheat, maize and almost all cereals and their products. Especially such type of insects is very active in warm and humid climate. India is considered to be the native land of this weevil. It is also one of the serious pests of commercial grain in transport train wagons, bullock carts and ships. Its young grub bore into the grains and feed inside the contents rendering them unfit for consumption. They are mainly controlled with synthetic chemicals like phosphine, organophosphates, and synthetic pyrethroids. However, the continuous and indiscriminate use of these chemicals has led to the emergence of insect populations with high levels of resistance, health hazard due to residual toxicity and environmental pollution. An alternative is the use of herbal insecticides which could solve these problems [3, 4].

In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides. Terrestrial plants are known to contain a rich source of bioactive metabolites which show antifeedant, repellent and toxic effect in a wide range of insects [5]. The diatomaceous earth (DE) is also considered to be as an alternative to the traditional insecticides. The DEs are natural in origin and composed of the fossils of diatoms that in general act as desiccants when applied on the cuticle of insects [6].

Therefore, the present studies were undertaken to evaluate the effectiveness of different plant extracts in combination with diatomaceous earth (DE) to protect rice grain from the rice weevil.

2. Materials and Methods

The experiment was conducted in the laboratory of the Dr. B.C. Roy College of Pharmacy & Allied Health Sciences, Durgapur, West Bengal during June and September of the year 2017. The stock cultures of adult rice weevil was maintained on rice grains in the laboratory in a controlled temperature, humidity and photo period of $27\pm 1^\circ\text{C}$, $65\pm 5(\%)$ and L16:D8, respectively.

2.1 Extraction from different plant species

The neem leaves, aerial parts of kalmegh, bark of cinnamon, black pepper seeds and clove buds were dried and macerated using domestic grinder. The powdered material was separately subjected to ethanol extraction with Soxhlet apparatus. Crude extracts were filtered through Whatman (No. 1) filter paper and concentrated [7]. The purity of the extract was examined with chromatography [8]. Then the lyophilised material was stored at room temperature for further use as an extract.

2.2 Adult weevil mortality test

Rice grain of 100 gm per treatment was mixed with different herbal extracts in perforated plastic containers and stirred for 10 minutes with a rotary shaker. Each treatment was replicated for four times. Then 20 unsexed adults (1-2 days old) of rice weevil were introduced in each container. After 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks dead insects were counted. The insects were considered dead if the appendages did not move when probed with a fine brush. Dead weevils were removed from the substrate after sieving with the sieve of 5 mm diameter holes. Per cent mortality was calculated using Abbott's correction formula for natural mortality in test and controls [9].

Abbott's correction formula:

$$\text{Corrected (\%)} = \left(1 - \frac{n \text{ in T after treatment}}{N \text{ in Co after treatment}}\right) \times 100$$

n= Insect population; T= Treated; Co= Control

Table 1: Effect of neem (*Azadirachta indica*) extract + DE on adult rice weevil after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks of treatment.

Dose of extract + DE	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Neem 10 mg + 1 g DE	10.04 *(18.46) ^f	20.24 (26.74) ^f	40.30 (39.41) ^c	50.22 (45.13) ^f	54.95 (47.84) ^c	65.08 (53.78) ^c
Neem 20 mg + 1 g DE	15.26 (22.99) ^e	30.14 (33.29) ^e	50.27 (45.16) ^d	55.28 (48.04) ^e	60.26 (50.92) ^d	70.66 (57.20) ^d
Neem 30 mg + 1 g DE	25.37 (30.24) ^d	35.20 (36.39) ^d	55.25 (48.01) ^c	60.11 (50.84) ^d	64.82 (53.62) ^b	70.20 (56.92) ^d
Neem 40 mg + 1 g DE	40.35 (39.43) ^c	44.80 (42.01) ^c	60.24 (50.91) ^a	65.10 (53.79) ^c	65.14 (53.82) ^b	79.62 (59.75) ^c
Neem 60 mg + 1 g DE	50.42 (45.24) ^b	49.81 (44.89) ^b	60.14 (50.88) ^a	70.58 (57.15) ^b	74.71 (59.81) ^c	85.11 (63.30) ^b
Control	0 (0) ^a	0 (0) ^a	0 (0) ^b	0 (0) ^a	1.3 (6.54) ^a	2.77 (9.58) ^a
S _{Em} (±)	0.249	0.189	0.165	0.165	0.168	0.215
CD (at 5%)	0.739	0.561	0.491	0.490	0.499	0.640
CV (%)	1.91	1.24	0.85	0.78	0.74	0.85

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

Table 2: Effect of kalmegh (*Andrographis paniculata*) extract + DE on adult rice weevil after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks of treatment.

Dose of extract + DE	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Kalmegh 10 mg + 1 g DE	30.19 *(33.33) ^f	35.34 (36.47) ^f	45.08 (42.18) ^f	50.06 (45.03) ^f	60.07 (50.81) ^f	70.15 (56.88) ^d
Kalmegh 20 mg + 1 g DE	40.59 (39.58) ^e	45.02 (42.14) ^e	49.95 (44.97) ^e	55.21 (47.99) ^e	65.06 (53.76) ^e	85.25 (67.42) ^b
Kalmegh 30 mg + 1 g DE	44.73 (41.97) ^d	50.47 (45.27) ^d	54.86 (47.79) ^d	60.41 (51.01) ^d	70.17 (56.89) ^d	85.17 (67.34) ^b
Kalmegh 40 mg + 1 g DE	50.11 (45.06) ^c	55.45 (48.13) ^c	60.11 (50.83) ^c	65.10 (53.79) ^c	79.86 (63.34) ^c	90.23 (71.79) ^a

2.3 Repellency test

For evaluation of repellency effect of *Azadirachta indica*, *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Andrographis paniculata*, *Piper nigrum* extracts and Diatomaceous Earth (DE) against rice weevil cup bioassay technique were followed. A quantity of 100 g of rice grain was treated with each of *Azadirachta indica*, *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Andrographis paniculata*, *Piper nigrum* extracts and Diatomaceous Earth at the concentrations of 10mg, 20mg, 30mg, 40mg and 60mg and 1 g, respectively. The treated rice grain was placed in a covered perforated (3 mm diameter hole) plastic container (9 cm high and 7 cm in diameter) and (20) twenty unsexed individuals of the weevil (1-2 days old) were placed on the treated rice in the centre of the perforated container. The experiment was conducted at $27\pm 1^\circ\text{C}$ temperature, $65\pm 5\%$ R.H. and L16:D8 day length and each treatment were repeated four times. The repellency of the extracts was measured in terms of the percentage of insects moved out of the container, away from the treated rice. All the insects which were present in the container after 24, 48 and 96 h and 1, 2 and 3 weeks of introduction were counted and considered at subsequent observation [10, 11].

3. Results and Discussion

3.1 Percent adult mortality of rice weevil

The efficacy of *Azadirachta indica* extracts and DE against adult rice weevil in different doses is indicated in table-1. Most of the treatment showed significant variation in adult weevil mortality in all observations. Considerable adult mortality of 45.24%, 44.89%, 50.88%, 57.15%, 59.81% and 63.30% was recorded in neem 60 mg + 1 g DE after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks, respectively. Highest adult mortality was found after 3 weeks of treatment in *A. indica* 60 mg+ 1 g DE. The insecticidal property of neem may possibly due to antifeedant and fumigation action which lead to death of storage insect pest. This finding corroborates with [12, 13] who reported significant insecticidal activity of neem extract against adult rice weevil in terms of adult mortality.

Kalmegh 60 mg + 1 g DE	60.25 (50.91) ^b	65.05 (53.76) ^b	70.44 (57.07) ^b	75.36 (60.24) ^b	84.78 (67.04) ^b	90.15 (71.71) ^a
Control	0 (0) ^a	0 (0) ^a	0 (0) ^b	2.09 (8.32) ^a	3.89 (11.38) ^a	2.77 (17.31) ^c
SEm (±)	0.175	0.135	0.161	0.148	0.158	0.280
CD (at 5%)	0.521	0.401	0.479	0.439	0.470	0.832
CV (%)	1.00	0.72	0.80	0.67	0.63	0.95

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

The insecticidal activity of Kalmegh (*A. paniculata*) extract and DE is presented in the table-2. Among all the treatments highest mortality of adult rice weevil was noticed from the treatment with *A. paniculata* 60 mg and 1 g DE after 3 weeks of application which was at par with 40 gm dose. Most of the observations from all the treatments including control treatment were significantly different in terms of adult weevil

mortality. The active ingredient viz, andrographolide is a bitter antifeedant substance present in the kalmegh might be responsible for adult mortality [14]. The observation in the present study is in agreement with [15], who reported 67.69-72.01% adult mortality of *Callosobruchus chinensis* with the crude extract of *Andrographis paniculata*.

Table 3: Effect of Cinnamon (*Cinnamomum zeylanicum*) extract + DE on adult rice weevil after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks of treatment.

Dose of extract + DE	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Cinnamon 10 mg + 1 g DE	19.71 *(26.36) ^f	24.79 (29.86) ^f	30.52 (33.53) ^f	34.37 (36.07) ^f	39.55 (38.96) ^f	44.80 (42.01) ^f
Cinnamon 20 mg + 1 g DE	29.69 (33.01) ^e	23.32 (33.25) ^e	34.95 (36.24) ^e	40.62 (39.59) ^e	44.87 (42.05) ^e	54.85 (47.78) ^e
Cinnamom 30 mg + 1 g DE	34.88 (36.20) ^d	40.13 (39.30) ^d	44.85 (42.04) ^d	49.95 (63.42) ^d	49.95 (44.97) ^d	69.80 (50.74) ^d
Cinnamom 40 mg + 1 g DE	40.55 (39.55) ^c	44.78 (42.0) ^c	50.39 (45.22) ^c	85.57 (67.67) ^c	55.00 (47.86) ^c	59.80 (56.66) ^c
Cinnamom 60 mg + 1 g DE	50.20 (45.11) ^b	84.67 (66.95) ^b	55.01 (47.87) ^b	65.62 (54.10) ^b	69.85 (56.69) ^b	64.95 (53.70) ^b
Control	0 (0) ^a	0 (0) ^a	0 (0) ^a	0 (0) ^a	5.71 (13.82) ^a	13.26 (21.35) ^a
SEm (±)	0.166	0.140	0.101	0.131	0.094	0.091
CD (at 5%)	0.492	0.417	0.30	0.388	0.28	0.270
CV (%)	1.10	0.80	0.59	0.60	0.46	0.90

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

When the rice grain was treated with cinnamon (*C. zeylanicum*) extract and DE, all the treatments showed better adult weevil mortality than the control treatment (Table-3). Highest adult rice weevil mortality (67.67%) was registered from the 40 mg cinnamon + 1 g DE after 1 weeks of treatment which is significantly different from other doses. The results in the present study are in the same line with that of [16], who reported adult mortality of *Callosobruchus maculatus* in black gram due to the insecticidal effect of cinnamon. The essential oil present in cinnamon possesses contact and fumigant toxicity against several stored product insects [17], which might be responsible for the adult rice weevil mortality.

It is depicted from the table-4 that black pepper (*P. nigrum*) and DE had insecticidal action in controlling the rice weevil. However, some mortality of adult weevil was also noticed in the control treatment after 1, 2 and 3 weeks of treatment might be due to time lapse. In this experiment, after 3 weeks of treatment 89.79% and 90% mortality was recorded in *P. nigrum* 40 mg + 1 g DE and *P. nigrum* 60 mg + 1 g DE, respectively, which is statistically at par. The result is also in same line with [11], who found that black pepper was highly effective in controlling the pulse beetle, *Callosobruchus maculatus*. This toxicity was attributable to the presence of piperine in black pepper [18].

Table 4: Effect of Black pepper (*Piper nigrum*) extract + DE on adult rice weevil after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks of application.

Dose of extract + DE	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Black pepper 10 mg + 1 g DE	50.07 *(45.04) ^f	60.02 (50.78) ^f	65.32 (53.92) ^f	69.94 (56.75) ^f	75.25 (60.15) ^f	80.14 (63.54) ^e
Black pepper 20 mg + 1 g DE	53.52 (47.02) ^e	64.90 (53.65) ^e	70.32 (56.94) ^e	75.28 (60.18) ^e	80.29 (63.65) ^e	85.08 (67.27) ^d
Black pepper 30 mg + 1 g DE	65.19 (53.85) ^d	70.05 (56.82) ^d	75.16 (60.11) ^d	79.98 (63.42) ^d	85.26 (67.42) ^d	95.08 (77.19) ^c
Black pepper 40 mg + 1 g DE	70.13 (56.87) ^c	79.91 (63.37) ^c	85.32 (67.47) ^c	84.74 (67.01) ^c	95.21 (77.38) ^c	100 (89.79) ^b
Black pepper 60 mg + 1 g DE	75.21 (60.14) ^b	85.16 (67.34) ^b	94.78 (76.80) ^b	95.01 (77.10) ^b	99.91 (89.15) ^b	100 (90.00) ^b
Control	0 (0) ^a	0 (0) ^a	0 (0) ^a	2.48 (9.07) ^a	14.16 (22.10) ^a	17.29 (24.57) ^a
SEm (±)	0.359	0.166	0.201	0.222	0.424	0.185
CD (at 5%)	1.067	0.493	0.597	0.659	1.261	0.549
CV (%)	1.64	0.68	0.77	0.80	1.34	0.54

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

Table 5: Effect of Clove (*Syzygium aromaticum*) extract + DE on adult rice weevil after 24h, 48h, 96h, 1 week, 2 weeks and 3 weeks of application.

Dose of extract + DE	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Clove10 mg + 1 g DE	13.30 *(21.62) ^f	14.20 (22.25) ^f	18.60 (25.40) ^f	20.10 (26.42) ^f	25.80 (30.20) ^f	28.80 (32.15) ^f
Clove20 mg + 1 g DE	16.60 (23.86) ^e	15.88 (23.14) ^e	19.80 (26.06) ^e	25.60 (30.08) ^e	27.90 (31.61) ^e	31.70 (34.20) ^e
Clove30 mg + 1 g DE	26.60 (31.39) ^d	18.66 (25.35) ^d	22.50 (28.43) ^d	27.80 (31.55) ^d	32.60 (34.59) ^d	34.60 (35.85) ^d
Clove40 mg + 1 g DE	30.00 (33.61) ^c	25.50 (30.16) ^c	28.60 (32.12) ^c	33.90 (35.35) ^c	38.70 (38.25) ^c	38.90 (38.28) ^c
Clove60 mg + 1 g DE	36.60 (37.24) ^b	33.30 (35.26) ^b	36.70 (36.99) ^b	41.50 (39.90) ^b	48.40 (44.04) ^b	49.70 (44.45) ^b
Control	0 (0) ^a	0 (0) ^a	0 (0) ^a	3.38 (10.59) ^a	12.39 (20.60) ^a	18.34 (25.35) ^a
SEm (±)	0.195	0.116	0.154	0.121	0.106	0.131
CD (at 5%)	0.58	0.344	0.456	0.36	0.314	0.388
CV (%)	1.59	1.02	1.24	0.84	0.64	0.75

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

After treating the rice grain with clove (*S. aromaticum*) and DE in each treatment and observation significant adult mortality was recorded than the control treatment (Table-5). Highest adult weevil mortality was recorded from 60 mg *S. aromaticum* + 1 g DE, which is statistically different from other doses. The result is in conformity with ^[19], who observed that clove powder was most effective against pulse beetle. The essential oil present in the clove having eugenol might be responsible for the insecticidal property of the clove ^[20].

3.2 Percent repellency of rice weevil

From repellency experiment on adult rice weevil (Table-6), it is revealed that all the herbal extracts in combination with DE as well as the mixture of extracts and DE had a significant effect on the insect. Treatment composed of clove and DE showed the best result in repelling the adult weevil among all the treatments in all observations except only after 24 hours of treatment with neem extract + DE. In terms of repellency the treatments are ranked as *S. aromaticum*+ DE > *P. nigrum*+ DE > *C. zeylanicum*+ DE > *A. paniculata*+ DE > *A. indica*+ DE.

Table 6: Repellency of different herbal extracts in combination with DE against adult rice weevil.

Herbal extract + DE (In 70%:30% ratio)	Mean adult weevil mortality (%)					
	24h	48h	96h	1 week	2 weeks	3 weeks
Neem + DE	12.11 *(20.37) ^c	18.70 (25.60) ^d	30.40 (33.45) ^b	49.90 (44.91) ^b	74.20 (59.45) ^b	80.10 (63.51) ^b
Kalmegh + DE	10.81 (19.20) ^d	14.10 (22.03) ^c	32.20 (34.56) ^c	53.30 (48.02) ^c	78.20 (62.15) ^c	85.00 (67.14) ^c
Cinnamon + DE	10.60 (18.99) ^c	19.40 (26.12) ^c	44.00 (41.51) ^e	66.70 (54.74) ^d	80.20 (63.56) ^d	95.00 (77.02) ^d
Black pepper + DE	12.00 (20.30) ^e	14.00 (21.77) ^b	37.10 (37.51) ^d	78.10 (62.08) ^e	91.20 (72.71) ^e	96.50 (79.19) ^e
Clove + DE	9.10 (17.55) ^b	25.40 (30.24) ^c	44.00 (41.57) ^e	98.80 (83.71) ^f	94.90 (76.92) ^f	97.80 (81.48) ^f
Mixture of botanicals + DE	12.30 (22.49) ^f	39.80 (39.11) ^g	79.90 (62.83) ^f	94.90 (85.46) ^g	98.50 (82.90) ^g	99.90 (87.93) ^g
Control	0 (0) ^a	0 (0) ^a	0 (0) ^a	8.61 (17.06) ^a	17.83 (24.97) ^a	23.39 (28.92) ^a
SEm (±)	0.025	0.079	0.067	0.034	0.023	0.066
CD (at 5%)	0.074	0.233	0.198	0.100	0.067	0.194
CV (%)	0.301	0.673	0.375	0.121	0.072	0.190

*Figures in the parenthesis are the angular transformed values. Data with same alphabets are statistically at par and different alphabets are significantly different (DMRT test)

Besides, better result was also found in mixture of different herbal extract than individual extract. Clove + DE registered highest repellent action against adult rice weevil which is in conformity with ^[21] who reported strong repellent activity (100%) against first instar nymph of the cockroach. Black pepper and cinnamon in combination with DE also performed better next to clove in terms of repellency. Similarly ^[22] also reported black pepper and cinnamon as a potent repellent to adult rice weevil.

4. Conclusion

From this experiment, it can be concluded that the herbal extracts of neem, kalmegh and clove @ 60gm in combination with DE 1gm was mostly effective to kill 49.70 to 90.15% adult rice weevil after 3 weeks of treatment. Whereas, cinnamon and black pepper registered maximum mortality in 40gm dose. In the repellency study, it was found that the treatment composed of clove and DE registered the best result in repelling the adult rice weevil. Thus it can be concluded that the insecticidal activities of the herbal extracts are satisfactory and therefore the resource poor farmers can use these botanicals to control rice weevil. Furthermore, the use

of botanical pesticides against different insect-pests reduces the environmental pollution and other hazards.

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