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Research Article

PESTICIDAL ACTIVITY OF INDIAN MEDICINAL PLANTS AGAINST RED FLOUR BEETLE *TRIBOLIUM CASTANEUM* (HERBST, 1797) (COLEOPTERA: TENEBRIONIDAE)

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A B S T R A C T

Tribolium castaneum is a very important insect pest of food grains and stored grain products. These stored commodities are vulnerable to insects attack and the quality of food is deteriorated. It is necessary to conserve the stored food grains reserves so that the supply foods remain continuous and the prices of food grains and derived products remain stable. The present experiments were carried out in the ovicidal activity of Indian medicinal plants, *Plumbago zeylanica*, *Solanum xanthocarpum*, *Tribulus terrestris* and *Gloriosa superba* against *Tribolium castaneum*. The effects of these plant extracts on percent mortality were recorded at different concentration of plants extracts ranging from 50 to 800ppm. The maximum ovicidal activity was recorded in *Plumbago zeylanica* methanol extract and followed by ethyl acetate and chloroform respectively. Results also indicate a definite potential of these extracts towards incorporation of these extracts in pest management programs and towards optimizing food security through utilizing them as bio-pesticides.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* Herbst is a cosmopolitan pest which often destroys stored products especially wheat flour. It is also considered the most common species in the pest complex attacking stored wheat. Although it is considered a secondary pest, requiring prior infestation by an internal feeder, it can readily infest with other grains damaged during harvesting (Devi and Devi, 2015). In addition, larvae and adults feed on grain dust and broken grain, but not the undamaged whole grains and spend the entire life cycle outside the grain kernels (Karunakaran *et al.,* 2004). In severe infestation, the flour turns grayish and has a pungent, disagreeable odour- making it unsafe for human consumption. Furthermore, *T. castaneum* causes a substantial loss in storage due to its high reproductive potential (Prakash *et al.,* 1987). *T. castaneum* may also cause an allergic response (Alenko *et al.,* 2000). It is known that they spread diseases since they can breed throughout the year in the warm area.

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Tribolium castaneum is a very important insect pest of food grains and stored grain products. The Rust - red flour beetle is a common and most destructive pest of stored products and is cosmopolitan in distribution. Both the adults and grubs cause serious damage to some kinds of grains including broken grains, flour and dried fruits. This pest generally found in granaries, mills, warehouse, and stored grains, feeding on rice. Currently different kinds of preventive and curative control measures are practiced to get protection against this insect pest. Loss in weight and germination ability of grains is a severe problem, especially due to pitiable sanitation along with poor storage facilities that encourage stored pests attack, disease causing organisms and increase in temperature and humidity of the stored products (Phillips and Throne, 2010; Upadhyay and Ahmad, 2011; Semeao *et al.,* 2012; Padin *et al.*, 2013; Keskin and Ozkaya, 2013).

Management of these insect pests is severely dependent upon the use of synthetic insecticides. However, application of these synthetic commercial insecticides has led to several serious problems such as environmental deterioration due to chemical residues, insect resistance against these repeatedly used chemicals, deterioration of food grains due to residues and harmfulness of synthetic chemicals to the non-target

organisms in the surroundings (Zapata *et al.,* 2010; Perez *et al.,* 2010; Grünwald *et al.,* 2014). Chemical pesticides have been used for a long time, but have serious drawbacks (Sharaby, 1988), such as direct toxicity to beneficial insects, fishes and human (Munakata, 1977; Pimental *et al.,* 1981; Goodland *et al.,* 1985), pesticide induced resistance (Brown, 1968; Waiss *et al.,* 1981), health hazard (Bhaduri *et al.,* 1989) and increased environmental and social costs (Pimental *et al.,* 1980). Serious health impacts on humans and ecological changes has forced the researchers to find the new ways of stored grains insect pests management and diverting their attention towards the natural products use as insecticides such as the use of plant extracts as repellents (Rajendran and Sriranjini, 2008). In many countries, efforts are being made to minimize the use of harmful insecticides through the use of indigenous plant products, implementation of IPM approaches, use of bio-degradable products (Khattach and Hameed, 1986) and applying insect growth regulators (Metcalf, 1975) to protect stored grains. In many areas of the world locally available plant materials are widely used to protect stored product against damage by insect infestation (Goloband Webley, 1980; Talukder *et al.,* 1990). These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides. The present experiment was, undertaken to study the ovicidal activity of indian medicinal plants against red flour beetle, *Tribolium castaneum*.

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves of indian medicinal plants, *Plumbago zeylanica* Linn. (Plumbaginaceae), *Solanum xanthocarpum* Linn. (Solanaceae), *Tribulus terrestris* Linn. (Lygophyllaceae) and *Gloriosa superba* Linn. (Liliaccac) were collected from Salem District, Tamilnadu, India. Then washed and shade dried. After shade drying, the leaves were powdered by using electronic blender. 100 grams of the dry powder were extracted in 500 ml of chloroform, ethyl acetate and methanol using soxhlet apparatus and the crude extracts were individually condensed with a rotary evaporator for 30 min at 45^0C and crude extract were stored in refrigerator for further studies. The extracts obtained were dissolved in the corresponding pure solvent until a 10% (w/v) stock solution was obtained.

Rearing of stored product pest T. castaneum

Stored product pest, *T. castaneum* was procured from the local market and godowns from the infested blackgram and have been continuously cultured on the same food source until the end of the experiments. The stock culture was maintained in the laboratory in the dark atmosphere at 28 ± 2^0C and 70 - 80% relative humidity. The eggs were collected on black filter papers by placing *T. castaneum* adults on the filter papers after 48 h (Ho *et al*., 1996).

Ovicidal activity of different solvent extract of selected plants

Ovicidal activity of selected plant extract: The eggs were exposed to different plant extracts individually on 9 cm petridish. 50 eggs (24 h old) were placed in each petridish and then the petridishes were kept in 650 ml jars with screwed lids. Different concentrations (20, 40, 60, 80, 100ppm) were applied on filter paper (Whatman No. 1), cut into 9 cm diameter, and were attached to the lower side of the lid of the jar. After evaporation of the solvent in about three minutes, the lids were closed tightly with the jars. The exposure period was 96 h. After exposure, petridishes were taken out of the jars and kept in the incubator at 28 ±2ºC. The final mortality counts were made after 11 days with the help of a hand lens. Unhatched eggs with black spots inside were considered and counted as dead. The data obtained from the present experiment was subjected to the following formula to derive the ovicidal activity of the selected plant extracts. Percentage mortality was calculated and data were corrected for natural mortality in controls using the Abbott (1925) formula. The corrected mortality was then subjected to probit analysis to estimate LC_{50} and LC_{90} values (Sokal and Rohlf 1973). Analysis of variance (ANOVA-Two Way) was used to determine the effect of solvent extract concentrations on ovicidal activity. Following a significant ANOVA, differences amongst means were established using Least Significant Difference (LSD) test at 0.05% level.

RESULTS

Ovicidal activity of P. zeylanica chloroform extract

Ovicidal activity of chloroform extract of *P. zeylanica* was tested with different concentrations (20 – 100 ppm) and the results pertaining to the experiments are shown in table 1. Perusal of the data indicates that the rate of mortality is increase with increase in concentration. Least ovicidal activity 2.17% was recorded at 20 ppm concentration and maximum insecticidal activity 89.20% was noted at 100 ppm concentration against the eggs of *T. castaneum*. It was observed that the LC_{50} value of 67.82 ppm with 61.32 – 70.33 ppm (LCL – UCL) and LC_{90} values of 102.54 ppm with 93.62 – 112.44 ppm (LCL- UCL) from the data.

Table 1 Ovicidal activity of *P. zeylanica* extracts against *Tribolium castaneum* on *Vigna mungo*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		χ^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
Chloroform extract								
Control	00 ± 0.00[a]							
20	2.17 ± 0.44.[a]							
40	18.46 ±1.26[b]							
60	45.82±2.84[c]	67.82	61.32	70.33	102.54	93.62	112.44	2.322
80	69.54±3.58[d]							
100	89.20±4.60[e]							
Ethyl acetate extract								
Control	0.00 ± 0.00[a]							
20	6.88± 0.64[a]							
40	24.84± 1.80[b]							
60	52.62 ± 2.68[c]	60.22	54.20	64.68	91.94	86.22	99.48	1.531
80	73.66 ± 3.42[d]							
100	92.93 ± 4.26[e]							
Methanol extract								
Control	0.00 ± 0.00[a]							
20	11.58±0.63[a]							
40	34.55 ±1.46[b]							
60	55.89 ±2.60[c]	55.45	50.72	60.32	94.22	88.40	102.26	6.536
80	73.36 ±3.28[d]							
100	98.46 ±4.84[e]							

Values are Mean ± S.D of Five replications (n= 20 for each concentration). Values with different alphabet in the column are statically significant by DMRT at *P≤ 0.05.*

Ovicidal activity of P. zeylanica ethyl acetate extract

Ethyl acetate extract of *P. zeylanica* was tested with different concentrations against the eggs of *T. castaneum* and the results pertaining to the above experiments are shown in table 1. Perusal of the data clearly revealed that the rate of mortality is increase with increase in concentration. Eggs exposed to 20 ppm concentration of the extract shown minimum mortality 6.88% whereas, the maximum egg mortality 92.93% was noted at 100 ppm concentration. It was observed that 60.22 ppm with 54.20 – 64.68 ppm (LCL – UCL) and 91.94 ppm were calculated as LC_{50} and LC_{90} values respectively.

Ovicidal activity of P. zeylanica methanol extract

Minimum ovicidal activity of 11.58% and maximum ovicidal activity of 98.46% were recorded against the experimental group of eggs exposed to methanol extract of *P. zeylanica* at 20 and 100ppm concentrations respectively. The data pertaining to the above experiment have shown in table 1. Furthermore, eggs belongs to 40, 60 and 80 ppm of the experimental groups shown 33.64, 54.27 and 72.32% egg mortality respectively to the same extract. The LC_{50} value of 55.45 ppm and LC_{90} values of 94.22 ppm were calculated from the experimental data.

Ovicidal activity of S. xanthocarpum chloroform extract

Chloroform extract of *S. xanthocarpum* was tested with different concentration and the experimental data are given in table 2. Critical analyses of the data indicate that the rate of mortality is increase with increase in concentration. Lowest ovicidal activity of 2.28% was recorded at 20 ppm concentration contrarily highest ovicidal activity of 86.85% was recorded at 100 ppm concentration of chloroform extract of *S. xanthocarpum* (LC50 = 72.94 ppm ; LC90 = 109.22 and calculated chi-square value =3.341).

Table 2 Ovicidal activity of *S. xanthocarpum* extracts against *Tribolium castaneum* on *Vigna mungo*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		χ^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
Chloroform extract								
Control	0.00 ± 0.00[a]							
20	2.28± 0.44[a]							
40	19.64±1.96[b]	72.94	66.46	76.95	109.22	101.35	118.24	3.341
60	36.36±2.48[c]							
80	58.66±3.22[d]							
100	86.85±4.66[e]							
Ethyl acetate extract								
Control	0.00 ± 0.00[a]							
20	13.42± 0.54[a]							
40	27.82± 1.86[b]	60.40	54.46	61.47	93.55	88.23	103.55	2.330
60	53.64± 2.42[c]							
80	79.26± 3.48[d]							
100	97.44± 4.36[e]							
Methanol extract								
Control	0.00 ± 0.00[a]							
20	11.48±0.62[a]							
40	25.48±1.48[b]	58.44	53.22	64.58	97.30	90.66	105.90	1.571
60	55.74±2.76[c]							
80	74.80±3.86[d]							
100	93.26±4.24[e]							

Values are Mean ± S.D of Five replications (n= 20 for each concentration). Values with different alphabet in the column are statically significant by DMRT at *P≤ 0.05.*

Ovicidal activity of S. xanthocarpum ethyl acetate extract

Ovicidal activity of ethyl acetate extract of *S. xanthocarpum* was tested with the data obtained from the above experiment is shown in table 2. At 20 ppm concentration minimum egg mortality was recorded 13.42%. Besides, moderate egg mortality was noted against 40, 60 and 80 ppm concentrations i.e., 27.82, 53.64 and 79.26% respectively. The maximum ovicidal activity 97.44 % was recorded at 100 ppm concentration. (LC_{50}, 60.40 ppm; LC_{90}, 93.55 ppm with the calculated chi-square value of 2.330). The data obtained from 40-100ppm concentrations are statistically significant and are on par with that of control groups (DMRT, $p \leq 0.05$)

Ovicidal activity of S. xanthocarpum methanol extract

Ovicidal activity of methanol extract of *S. xanthocarpum* was tested against the eggs of *T. castaneum* and the results pertaining to the experiments are shown in table 2. Perusal of the data indicates that the rate of mortality is increase with increase in concentration. As it is evidenced from the table 20, 40, 60, 80 and 100ppm concentration of the plant extracts are responsible for the ovicidal effect of 11.48, 25.48, 55.74, 74.80 and 93.26% respectively with the LC_{50} value of 58.44ppm and LC_{90} values of 97.30 ppm.

Ovicidal activity of T. terrestris chloroform extract

Ovicidal actions of chloroform extract of *T. terrestris* against the eggs of *T. castaneum* are shown in table 3. Egg mortality of 1.86, 16.58, 31.52, 53.18 and 82.56% were recorded at 20, 40, 60, 80 and 100ppm concentrations. Statistical analyses of the data clearly revealed that they are highly significant since the values are on par with that of control (DMRT; $p \leq 0.05$)

Table 3 Ovicidal activity of *T. terrestris* extracts against *Tribolium castaneum* on *Vigna mungo*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		χ^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
Chloroform extract								
Control	0.00 ± 0.00[a]							
20	1.86± 0.38[a]							
40	16.58±1.44[b]	76.82	70.83	81.58	118.22	107.53	129.16	2.830
60	31.52±2.68[c]							
80	53.18±3.86[d]							
100	82.56±4.24[e]							
Ethyl acetate extract								
Control	0.00 ± 0.00[a]							
20	6.84± 0.40[a]							
40	23.46± 1.52[b]	63.68	56.33	68.29	99.51	91.63	109.21	3.288
60	46.55± 2.64[c]							
80	73.42± 3.86[d]							
100	92.78 ± 4.28[e]							
Methanol extract								
Control	0.00 ± 0.00[a]							
20	8.48 ±0.92[a]							
40	26.35 ±1.84[b]	62.22	58.48	65.39	99.72	90.55	109.60	3.437
60	42.64 ±2.68[c]							
80	76.92 ±3.92[d]							
100	94.64 ±4.38[e]							

Values are Mean ± S.D of Five replications (n= 20 for each concentration). Values with different alphabet in the column are statically significant by DMRT at *P≤ 0.05.*

Ovicidal activity of T. terrestris ethyl extract

Ethyl acetate extract of *S. xanthocarpum* was tested with different concentrations (20 – 100 ppm) and the results pertaining to the experiments are shown in table 3. Perusal of the data indicates that the rate of mortality is increase with increase in concentration. Lowest ovicidal activity 6.84% was recorded at 20 ppm concentration whilst, maximum egg mortality 92.78% was observed at 100 ppm concentration of the same extract. It was observed that the LC_{50} value of 63.38 ppm with and LC_{90} values of 99.51 ppm from the experiment.

Ovicidal activity of T. terrestris methanol extract

Methanol extract of *T. terrestris* leafs were tested with different concentration for its ovicidal activity against the eggs of *T. castaneum* and the results pertaining to the experiments are shown in table 3. Perusal of the data indicates that the rate of mortality is increase with increase in concentration. Ovicidal activity of 8.48% was recorded as minimum and 94.64% was recorded as maximum egg mortality at 20 ppm and 100 ppm concentrations respectively. The observed egg mortality of the above experiment shows statistical significance at $p \leq 0.05$, DMRT.

Ovicidal activity of R. apiculata chloroform extract

Ovicidal actions of chloroform extract of *R. apiculata* against the eggs of *T. castaneum* are shown in table 4. Perusal of the data indicates that the rate of mortality is increase with increase in concentration. It has been observed that 4.28 % egg mortality was recorded at 20 ppm concentration. Maximum ovicidal activity 83.75% was noted at 100 ppm concentration with the LC_{50} value of 73.54 ppm and LC_{90} values of 113.40 ppm. These data are statistically significant with the control eggs.

Table 4 Ovicidal activity of *R. apiculata* extracts against *Tribolium castaneum* on *Vigna mungo*

Concentrations (ppm)	Mortality	LC_{50} (ppm)	95 % Fiducial Limit		LC_{90} (ppm)	95 % Fiducial Limit		χ^2
			LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
Chloroform extract								
Control	0.00 ± 0.00[a]							
20	4.28± 0.68[a]							
40	18.59 ±1.70[b]	73.54	60.62	78.55	113.40	108.98	122.44	2.640
60	34.28±2.92[c]							
80	56.86±3.58[d]							
100	83.75±4.36[e]							
Ethyl acetate extract								
Control	0.00 ± 0.00[a]							
20	7.32± 0.62[a]							
40	22.70± 1.44[b]	63.58	59.77	66.97	99.32	93.51	108.72	3.280
60	44.62± 2.68[c]							
80	75.26± 3.84[d]							
100	93.84 ± 4.62[e]							
Methanol extract								
Control	0.00 ± 0.00[a]							
20	11.58 ±0.72[a]							
40	24.82 ±1.40[b]	62.54	52.78	65.44	98.23	90.62	106.39	3.218
60	46.34 ±2.88[c]							
80	72.98 ±3.66[d]							
100	95.82±4.74[e]							

Values are Mean ± S.D of Five replications (n= 20 for each concentration). Values with different alphabet in the column are statically significant by DMRT at $P \leq 0.05$.

Ovicidal activity of R. apiculata ethyl acetate extract

Ovicidal activity of ethyl acetate extract of *R. apiculata* against the eggs of T. castaneum was tested with different concentrations. The outcome of the results showing great deal of statistical difference among the test group of egg over the control as it is evidenced from the table 4. Generally highest concentration of the extract brings out maximum effects and the trend tends to decrease with the decrease in the concentration of the extract. It is apparently evident from the table 4 ($p \leq 0.05$)

Ovicidal activity of R. apiculata methanol

Methanol extract of *R. apiculata* was tested with different concentration (20, 40, 60, 80 and 100 ppm) and the results pertaining to the experiments are shown in table 4. Perusal of the data clearly indicates that the rate of mortality is increase with increase in the concentration of the extract. Lowest Ovicidal activity 11.58% was recorded at 20 ppm concentration, contrarily; maximum egg mortality 95.82% was noted at 100 ppm concentration with the LC_{50} value of 62.54 ppm and LC_{90} values of 98.23 ppm from the experiment.

DISCUSSION

In the present study, ovicidal activity of *Plumbago zeylanica*, *Solanum xanthocarpum*, *Tribulus terrestris* and *Gloriosa superba* against *Tribolium castaneum*. The ovicidal activity of tested plant extracts on percent mortality was recorded at different concentration ranging from 50 to 800ppm. The maximum ovicidal activity was recorded in *Plumbago zeylanica* methanol extract and followed by ethyl acetate and chloroform respectively. This was similar to the study by Mondal and Khalequzzaman (2009) who observed that contact effect of cinnamon had greater toxicity effect against T. castaneum larva LC_{50} = 0.074 mg cm^{-2}. The three tested essential oils, cinnamon and cardamom had a significantly higher toxicity against adult, larva, and egg on *Tribolium castaneum.* This was in agreement with findings by Wang *et al.,* (2014). As similar to our results, Tunç *et al.,* (2000) found that essential oil from rosemary and eucalyptus had provided 90% toxic effect on eggs of *T. confusum* at 98.5 µL/L concentration of 72-h exposure time, while vapour of aniseed essential oil had a high ovicidal activity by a mortality of 90%. The different toxicity of essential oil from oregano against eggs of *T. confusum* would appear to be attributed to the difference of the species of oregano tested reported by Muller-Riebau *et al.,* (1996, 1997). Habib *et al.,* (2011) demonstrated that the leaf and seed extracts of *Datura stramonium* caused contact toxicity to different life stages of *T. castaneum*. Chayengia *et al.,* (2010) who evaluated efficacy of volatile oils, powders, ethanol extracts and water extracts of *Polygonum hydropiper, Psidium guajava, Zingiber officinala, Curcuma longa, Pogostemon cablin, Citrus reticulate, Oxalis debilis, Ipomoea aquatic, Eichhornia crassipe* and *Acontium ferox* against *S. oryzae*. They found that volatile oil of *C. reticulate* resulted in 100% mortality after 24 hours of exposure followed by *Curcuma longa* (90%). Among powder treatments, *C. reticulate* attained 66.7% mortality of adult weevils after 72 hours of exposure. This indicated that *C. reticulata* might be equally potent for controlling *S. oryzae* and *T. castaneum* under storage. Similar observations were recorded to the present observation; these effects were found to be directly proportional to concentration to which eggs were exposed. Many plant extracts and essential oils are known to possess ovicidal activity against various insect pests (Isman, 2000, Choi *et al.,* 2003). Ovicidal effect of garlic essential oil (Ho *et al.,* 1996) and nutmeg (Huang *et al.,* 1997) has been reported against *T. castaneum*. Ho et al. (1995) reported that hexane extracts of star anise were ovicidal to *T. castaneum*. Moreover, it is well known that rosemary oil has ovicidal activity against other insects such as *T. confusum* 107 and *Ephestia kuehniella* Zeller (Tunc *et al.,* 2000). Deka *et al.,* (1998b) reported that aqueous extract of *Azadirachta indica, Clerodendron inerme, Pongamia pinnata, Melia azadirach, Polygonum orientale, Lantana camara, Adhatoda vasica* and *Cassia tora* have ovicidal activity against *Helopeltis theivora*. Yasodha and Natarajan (2007) reported 63% ovicidal action against *Leucinodes orbonalis* when eggs were exposed to the extracts of kernels of *Azadirachta indica* and dried powder of *Acorus calamus*.

Shantibala and Singh (2006) also reported reduced hatching of pea pod borer, *Lampides boeticus* after treatment with some plant extracts. The hexane extract of *Azadirachta indica* and acetone extract of *Acorus calamus, Xanthium strumarium* and *Polygonum hydropiper* were found to possess higher ovicidal activity compared to other solvent extracts from the same plant (Sarmah, 2010). Roy *et al.,* (2009) in *Helopeltis theivora* when sprayed with methanol, acetone or petroleum ether extracts of *Clerodendron infortunatum* on freshly laid eggs. They further reported that methanol, acetone and petroleum ether extracts of *C. infortunatum* caused more or less same egg mortality under laboratory condition. Ovicidal activity is only apparent when the target system begins to develop (Michaelides and Wright, 1997). Alternatively, changes in the permeability of the chorion and/or vitelline membrane may occur during embryogenesis and may facilitate the diffusion of vapours into eggs so that vital physiological and biochemical processes are affected (Gurusubramanian and Krishna, 1996).

CONCLUSION

This present study concluded that different solvents extracts of selected Indian medicinal possess toxic principles with insecticidal effect and could be potential grain protectants against *T. castaneum.* Among the tested plants *Plumbago zeylanica* leaf extracts showed the highest toxic effect against *Tribolium castaneum.* Therefore, leaves extracts of *Plumbago zeylanica* may be recommended as cheap, easily available at farm level, eco-friendly with low mammalian toxicity and a good alternative to synthetic insecticides.

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