International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

Insecticidal, ovicidal and repellent activities of different solvent extracts of *Rivina humilis* Linn. (Phytolaccaceae) against the selected stored grain pest, *Tribolium castaneum* Herbs. (Coleoptera : Tenebrionidae)

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Abstract

In the present investigation Insecticidal, ovicidal and repellent activities of different solvent extracts of *Rivina humilis* Linn (Phytolaccaceae) were tested against the selected stored grain pest, the red flour beetle, *Tribolium castaneum* Herbs. (Coleoptera : Tenebrionidae). The results obtained from the experiments are clearly indicated that the solvent extracts showed different range of activities, but, significant activity was recorded only with methanol extract of *Rivina humilis*. Thus, the isolation and identification of phytochemical group present in the methanol extract of the selected plant are underway. This present study confirmed that the phytochemical will gradually replace the chemical pesticides in the future.

Keywords: *Rivina humilis*, *Tribolium castaneum*, larvicidal activity, ovicidal activity, Repellent activity.

1.0 Introduction

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran, 2002) and insect contamination in food commodities is an important quality control problem of concern for food industries. Stored product insect control is currently based mainly on the use of two broad categories of insecticides: residual insecticides and fumigants. The development of resistance to these substances and the demands of consumers for residue free food, has lead researchers to evaluate the use of alternative control methods, which do not leave residues on the product and are generally safe for the environment. Insect pathogens, such as entomopathogenic fungi, bacteria, viruses, protozoa and nematodes, offer many advantages, such as high efficacy and compatibility with other IPM methods, and thus, are considered to be among the most promising alternatives to chemical-based insect control (Moore *et al* ., 2000).

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. The protection of stored grains from insect damage is currently dependent on synthetic pesticides. Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, carbamates and pyrethroids. There are problems in pesticide resistance and negative effects on non-target organisms including man and the environment. The use of organochlorine insecticides have been banned in developed countries and alternative methods of insect pest control are being investigated.

Fumigation is one of the major chemical methods to control stored-product insect infestations worldwide. Fumigation is the method of choice for many stored-grain managers because it is effective against all life stages, inexpensive, rapid and leaves minimal residues (van Someren Graver, 2004). Currently, phosphine and methyl bromide are the products most widely used (Bond,1984Lee *et al* ., 2004; Emekci, 2010). Carbon dioxide and sulfuryl fluoride are also registered for fumigation of stored grain in several countries. Methyl bromide has largely been phased out in developed countries, and it is slated to be phased out in the rest of the world by 2015, because it is an ozone depleting substance (Fields and White, 2002). Phosphine is not effective against some insect populations in India, Australia and Brazil, because of resistance (Bell and Wilson, 1995; Benhalima *et al* ., 2004).

Although effective fumigants and contact synthetic insecticides are available, there is global concern about their negative effects such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance and pesticide residues (Kostyukovsky *et al.*, 2002a). Currently national governments, food industries and exporters rely heavily on fumigation as a quick and effective tool for insect pest control in food commodities (Rajendran, 2001). Despite their significance in assuring quality, several fumigants have been withdrawn or discontinued on grounds of environmental safety, cost, carcinogenicity, ozone depletion and other factors (Rajendran, 2001; Shaaya and Kostyukovsky, 2006). Wand Khalis Ali *et al*., (2013) screened methanol extracts of six local plants *Anethum graveolens,Apium graveolens*, *Eucalyptus glauca*, *Malva parviflora*, *Mentha longifolia* and *Zingiber officinale* were studied for their toxicity effect on mortality of the last larval stage of *T. Confusum*. Farzana Parveen *et al*. (2013) evaluated the repellency of 5^{th} instars of *T. castaneum* caused by 5 doses of 4 fractions of the *Cedrela serrata* leaves extract after consecutive 1 h interval for 5 h by 2 methods. Their research conducted on *C. serrata* to isolate its active compound and to use it on commercial bases against *T. castaneum*.

Naima Iram *et al*. (2013) evaluated insecticidal action of two plant products and a synthetic insecticide on a major stored-product insect, *T. castaneum* (Herbst) . The plant species studied were, *Psidium guajava* and *Citrus reticulata*. Their results reported that all tested treatments had significant effects pertaining to all variables analyzed and ethanol extract was found to be remarkably more potent than powder form of same plant. Dichloromethane extract from *L. camara* and *C. procera* were evaluated for their repellent effect on *T. castaneum*. Both plant extracts exhibited a significant repellency. Their results indicated that *L. camara* showed more repellency against *T. castaneum* as compared to *C. procera* (Santa Kalita *et al*., 2013).

Shakarami Jahanshir (2013) evaluated the effects of four plant essential oils, *Mentha aquatica, Thymus daenensis*, *Myrtus communis* and *Artemisia hausskenechtii* against two adult flour weevils, *T. castaneum* and *T. confusum*. Their results concluded the all four essential oils possessed fumigant toxicity against the stored product insects under study. However, *M. aquatica,* due to its stronger fumigant toxicity, proved more effective in controlling storage grain pest.

Rhizomes of *Drynaria quercifolia* were evaluated for pesticidal and pest repellency activities against *T. castaneum*, using surface film method and filter paper disc method, respectively. Their results concluded that chloroform soluble fraction of rhizome of *D. quercifolia isuseful* in controlling *T. castaneum* (Alam Khan *et al*., 2014). Buxton *et al*., (2014) revealed the effect of the dry powders of the roots and leaves of *Ocimum canum*, *Zanthoxylum xanthoxyloides*, *Moringa oleifera* and *Securidaca longipedunculata* on the survival of *T. castaneum* in the laboratory. Their results suggested all the plant materials exhibited various levels of bio efficacies, with Z. *xanthoxyloides* and S. *longipedunculata* exhibiting the highest potency.

Karina *et al*., (2014) determined the repellent activity and toxicity of essential oils isolated from these plants against *T. castaneum*, using the area preference and contact toxicity methods. They conclude the repellent effect of *P. auritum* may be related to its safrole content, a known repellent and the evidence of Piper species could be used for development of repellents against *T. castaneum.*

Guruprasad *et al*., (2015) reported the repellent and insecticidal activity of different solvent extracts of *Clerodendron inerme* leaf against the *T. castaneum* , *Rhyzopertha dominica*, and *Callosobruchus chinensis* (L.). Their result indicated that the repellent activity was dose and extract dependent in different intervals of time and dose-mortality of the methanolic extract varied depending on the insect species. Ghada *et al*., (2015) studied the effect of two-plant extracts *viz*. *Ocimum basilicum* and *Rosmarinus officinalis* on the last larval and adult stages of *T. confusum*. Their results showed that the larval instar was highly susceptible to both plant extracts than the adult stages.

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of mosquito. The objective of this research was to evaluate the potentiality of different organic solvent extracts of *Rivina humilis* towards the control of selected stored grain pest, *Tribolium castaneum*.

2.0 Materials and Methods

2. 1 Insecticidal activity of different solvent extract of *Rivina humilis* against the adults of *Tribolium castaneum*

The fumigant toxicity of plant extract was tested as previously described (Huang et al., 1997). To determine the fumigant toxicity of selected plant extracts, 2 cm diameter filter papers (Whatman No.1) were impregnated with the tested doses of extracts. The impregnated filter paper was then attached to the screw caps of a 44 ml Plexiglas bottle. Caps were screwed tightly on the vials, each of which contained separately 20 adults (1-5 days old) insects. Mortality was recorded after 24 h of exposure. When no leg or antennal movements were observed, insects were considered dead. The mortality was calculated using the Abbott correction formula (Abbott, 1925). Five replicates of each control and treatment were set up. After 24 h, the insects were transferred to clean vials with culture media and kept in the incubators for end-point mortality determination. A second experiment was designed to assess 50% and 95% lethal doses. A series of dilutions was prepared to evaluate mortality of insects after an initial dose-setting experiment. Ten adult insects were put into 44 ml Plexiglas bottles with screw lids. Different plant extract amounts tested on *T. castaneum* were 20, 40, 60, 80 and 100 ppm respectively. Control insects were kept under the same conditions without any extract and each dose was replicated five times. The number of dead and alive insects in each bottle was counted 24 h after initial exposure. The mortality was evaluated by direct observation of the insects every hour till total mortality. Probit analysis (Finney, 1971) was used to estimate LC_{50} and LC_{90} values.

2.2 Ovicidal activity of different solvent extract of *Rivina humilis* against the eggs (24 h old) of *Tribolium castaneum*

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 were 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.

2.3 Repellent activity of different solvent extract of *Rivina humilis* against the adults of *Tribolium castaneum*

Repellency was assessed according to the method described by Xie *et al*. (1995) with some modifications. A bioassay system consisting of 4 glass jars (3 plant extract treatments, 1 negative control) was connected together at their rims by means of 10 cm nylon mesh tube. A 5 cm diameter circular hole was cut at the middle of the mesh for introduction of the test insects. Twenty nonsexual adult insects were introduced into the nylon mesh tube through the circular hole by means of 5 cm diameter funnel. Samples (100 g) of black gram was separately mixed with the individual extract and their mixture in the glass jars at concentrations of 50, 100, 200, 400 and 800ppm (0.5µl extract / sample grains) and kept at 28 ± 2ºC for 12 h so as to allow the solvent to evaporate completely. An appropriate amount of acetone was used as a negative control. Experiments were replicated five times. After 3 h, the contents of beetles at each treated or control diet was counted and the repellency (%) was calculated by following formula:

$$\text{Repellency (\%)} = \frac{C - E}{T} \text{X}100$$

Where,

C is the insect number in the negative control jar,
E is the insect number in extract treated jar and
T is the number of total insects.

3.0 Results

Insecticidal activity of different solvent extract of *R. humilis* tested against *T. castaneum* and the data pertaining to the experiments are shown in table 1. Lethal concentrations (LC_{50} and LC_{90}) of different fractions of *R. humilis* tested against *T. castaneum* are shown in table 5.29a. It was observed that LC_{50} values 267.75, 263.71, 260.03, 253.51 and 197.00ppm were estimated for hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts respectively. Their 95% confidence limits (LCL) ranged from 192.07, 118.17, 237.88, 230.75 and 165.49; UCL values of 329.88, 372.92, 281.05, 274.84 and 223.16 ppm respectively. Furthermore, the LC_{90} value for the data was found to be 497.7, 518.31, 476.08, 473.28 and 454.26ppm and their confidence limits (LCL) ranged from 412.36, 398.3, 442.16, 438.96 and 415.83ppm to (UCL) 703, 1068.02, 521, 518.85 and 507.54ppm. The calculated chi-square value of 8.411, 16.145, 7.144, 2.683 and 6.822 for hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts respectively (Table 2). Furthermore, the ovicidal activity and repellent activity of different solvent extracts of *Rivina humilis* against the eggs and adult *T. castaneum* were also tested and the results are depicted in the figures 1 &2.

Table 1. Insecticidal activity of different solvent extract of *Rivina humilis* tested against *Tribolium castaneum*

Concentrations Tested (ppm)	Insecticidal activity (%),				
	Hexane	**Diethyl ether**	**Dichloromethane**	**Ethyl acetate**	**Methanol**
100	24[a]	27 [a]	19 [a]	22 [a]	33 [a]
200	33[a]	38 [a]	33 [a]	34 [b]	52 [b]
300	49[b]	44 [ab]	64 [b]	60 [c]	68 [c]
400	76[c]	72 [c]	72 [b]	78 [d]	78 [c]
500	95[d]	96 [d]	96 [c]	95 [e]	98 [d]
Neemazal (100ppm)	100[d]	100[d]	100[d]	100[e]	100[d]

Values are mean of five replications. Values in parentheses are angular transformations. Values with different alphabet in the column differ significantly at p<0.005% (ANOVA, DMRT)

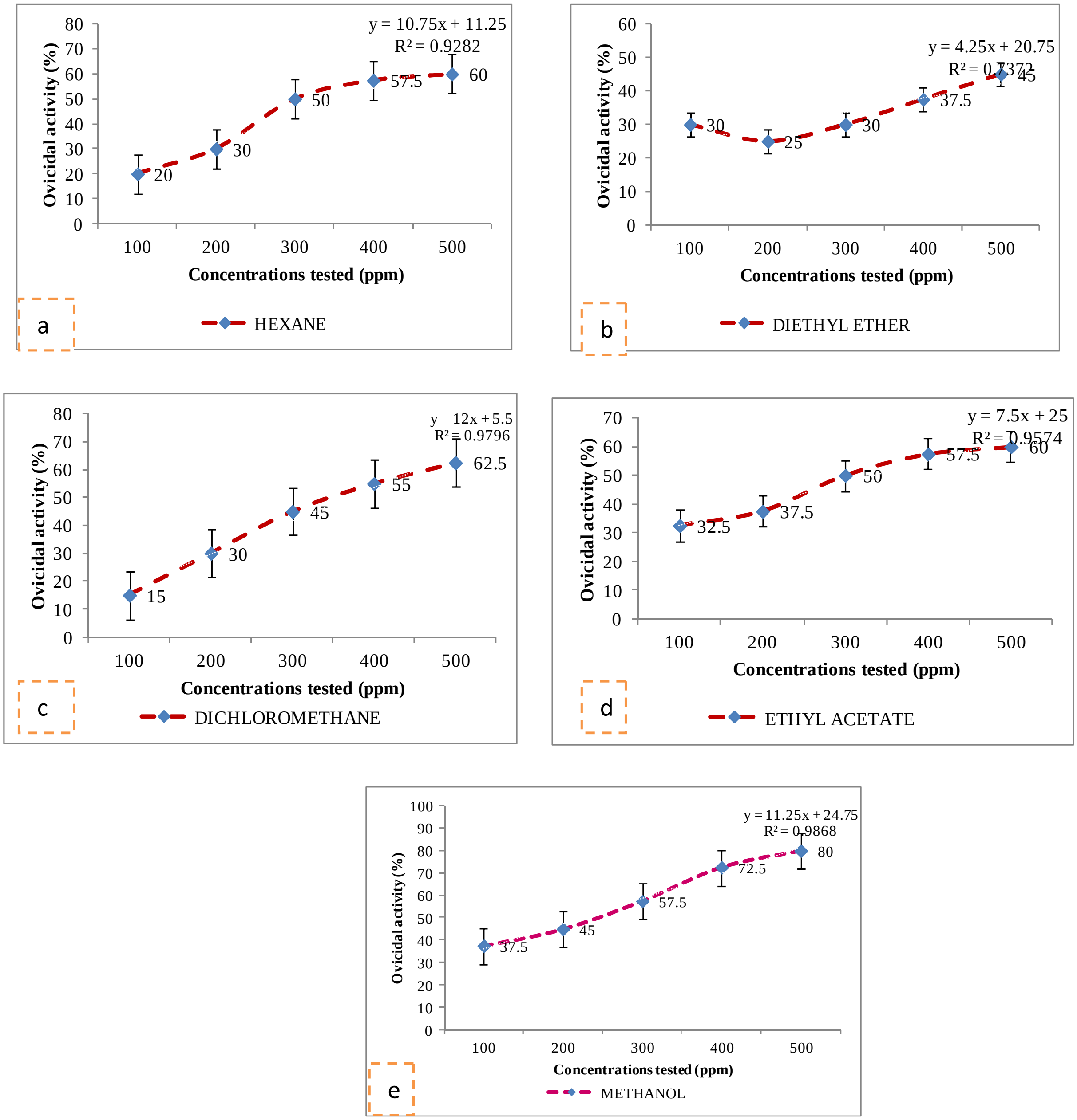
Table 2. Lethal concentrations of different solvent extract of *Rivina humilis* determined against *Tribolium castaneum*

Solvents Tested	LC_{50} (ppm)	95% Fiducial limits		LC_{90} (ppm)	95% Fiducial limits		χ^2
		LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)	
Hexane	267.75	192.07	329.88	497.7	412.36	703	8.411
Diethyl ether	263.71	118.17	372.92	518.31	398.3	1068.02	16.145
Dichloromethane	260.03	237.88	281.05	476.08	442.16	521	7.144
Ethyl acetate	253.51	230.75	274.84	473.28	438.96	518.85	2.683
Methanol	197.00	165.49	223.16	454.26	415.83	507.54	6.822

4.0 Discussion

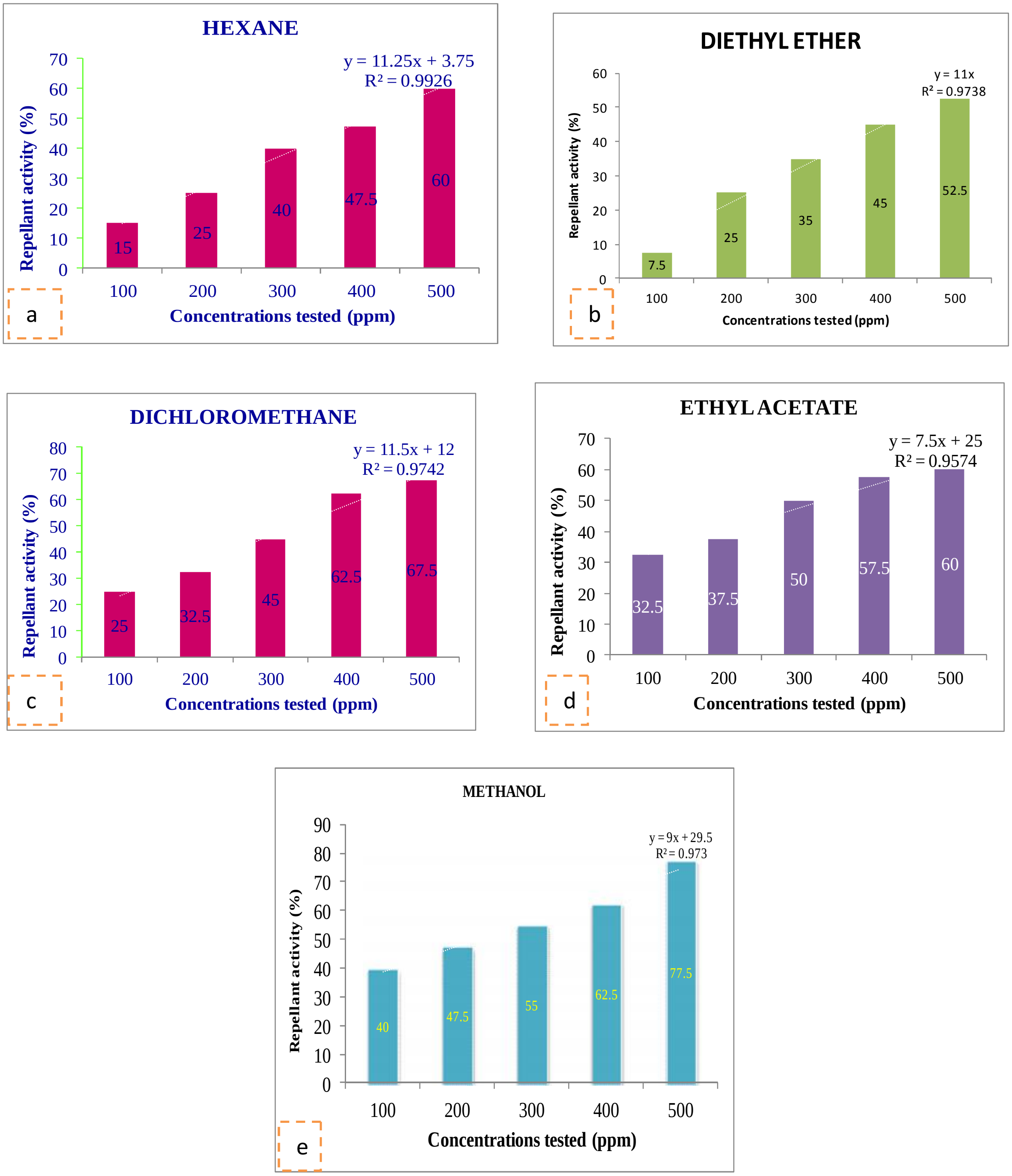
For thousands of years insects have been a problem associated with storing surplus dried food, with evidence from archaeological deposits (Solomon, 1965; Buckland, 1981) and written records of the storage and infestation of cereals stretching back to 3000 BC (Yasue, 1980; Beavis, 1988; Levinson and Levinson, 1994). There is a need to protect stored food from attack by insects because they can destroy large quantities, particularly during long-term storage and they can cause taint and contamination of grain with their excreta, cast skins and dead bodies (Pimentel, 1991; Van Lynden-van Nes *et al.*, 1996). Early attempts to control stored grain pests relied on methods such as mixing dry soil and wood ash with the grain causing lethal dehydration of insects, and the fumigant action of certain plant materials (Levinson and Levinson, 1998). In the tropics, especially in humid areas, pest infestation on stored foods is a more-or-less inevitable consequence of the adaptation of a set of pantropical (or even cosmopolitan) multivoltine pest species to the favourable and stable environment of food storage systems.

Figure 1. Ovicidal activity of different solvent extracts of *Rivina humilis* tested against the eggs (n=100) of *Tribolium castaneum*.



However, other stored-product pest scientists in the 1950s and 1960s alternated between optimism about the apparent efficacy and low cost of the new insecticides, and doubt about their useful lifespan due to resistance. For example, Parkin (1958) on the basis of research results from various sources in the mid-1950s provisionally recommended malathion for the control of stored-product pests. As they reported that in the mid-1970s `Malathion is without doubt the most widely used material in stored grain pest control, supporting this with case studies and evidence from both tropical and temperate countries. However, by as early as 1961, Parkin himself reported that strains of *Tribolium castaneum* on groundnuts in northern Nigeria were already showing signs of malathion resistance (Parkin *et al*., 1962), though the resistance problem was not widely acknowledged in stored products for another decade. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is common and most destructive pest throughout the world. It is generally found in granaries, mills, warehouses, feeding on wheat flour, atta, suji, rice (both husked and unhusked) etc. Neither larva nor adult can generally damage sound grains but they feed on those grains only, which have already been damaged by other pests. This pest has also been reported to attack the germ part (embryo portion) of the grain. Their presence in stored foods directly

Figure 2. Repellent activity of different solvent extracts of *Rivina humilis* tested against the *Tribolium castaneum* (n=40).



affects both the quantity and quality of the commodity.

The present result supports the finding of David *et al*. (1988), who showed the repellent activity of *Vitex negundo* against several species of stored products pests. Talukder and Howse (1993) reported strong repellent effect of *A. polystachya* on *T. castaneum*. Hussain *et al*. (1995) also reported that the extract of *P. hydropiper* and *Annona squamosa* had repellent effect against adult *T. castaneum*. Residual effect of Bishkatali plant extracts in chloroform and ethyl alcohol against *T. castaneum* are reported .The efficacy of Bishkatali extract as food protectant against the red flour beetle has evaluated by comparing the numbers of F1 progeny. Amin *et al*. (2000) found the inhibition activity of Akanda, Bishkatali, Neem extracts against the lesser grain borer. Talukder and Howse (1995) stated that the ground leaves, bark and seeds of *A. polystrachya* provided protection of wheat flour by reducing F1 progeny of *T. castaneum.*

Present investigation results have been confirmed by the work of Chander *et al*. (1999) who evaluated the acetone extract of the Sweet flag (*A. calamus*), in the laboratory, as a repellent, on the jute fabric against *T. castaneum*. The concentrations exhibited repellency, even after three months of aging, at room temperature. Also, Chander *et al.* (2000) evaluated the insect repellents, including local plants, like, Sweet flag rhizomes and 'Neem' formulations at 1% level, as bag treatments, for the control of storage insects, under the warehouse conditions. Both showed a good repellence against the test insects, even after three months. Also, Chandel *et al.* (2001) observed the effects of rhizomes of Sweet flag (*A. calamus*) on the grubs and adults of *T. castaneum*, after an oral administration through food, for a prolong exposure period and periodical observations over 125 days, revealed a phago-deterrent activity of the botanicals tested.

The concentrations have a significant effect upon the mortality of the insect and have a positive correlation, with it. Conclusions of present investigation research have an agreement with the work of Susha and Karnavar (1993), who studied the effect of Azadirachtin, as *vitellogenic oocyte* development in *T. granarium*, which reduced the *vitellogenic count*, when topically applied at the rate of 0.5 or 1.0g/ pupa.

Findings of current experiments correlate with the research work of Saxena *et al*. (1989), who found that *A. indica* had shown antifeedant effects against different insect pests of the stored products. Also, Hou *et al*. (2004) compared two known repellents of stored-product insects, viz., DEET and Neem, with the protein-enriched pea flpresent investigation, defatted protein enriched pea flpresent investigation, and pea protein-extract for their efficacy, in reducing penetration and invasion by several common stored-product insects, like, *S. oryzae*, *T. castaneum*, *Cryptolestes ferrugineus* and *Oryzaephilus surinamensis*. The methods of preparation for the pea-extracts affected the penetration of *S. oryzae*. The number of *S. oryzae, T. castaneum, C. ferrugineus* and *O. surinamensis* that pierced through the paper envelopes of the stored wheat treated with DEET, was reduced by 99%, 86%, 97% and 91%, respectively, as compared to those of the control.

Moreover, Hou *et al*. (2004) compared two known repellents of stored-product insects, *viz*., DEET and Neem with the protein-enriched pea for their efficacy, in reducing the penetration and invasion by several common stored-product insects, like, *S. oryzae*, *T. castaneum*, *Cryptolestes ferrugineus* and *Oryzaephilus surinamensis*. The way of preparation for the pea-extracts affected the penetration of *S. oryzae*. The number of *S. oryzae*, *T. castaneum*, *C. ferrugineus* and *O. surinamensis* that pierced through the wheat containing paper envelopes, treated with DEET, was reduced by 99%, 86%, 97% and 91%, respectively, as compared to the control. The protein-enriched pea did not prevent the insects to pierce through envelopes.

The present findings confirmed that the solvent extracts of *Rivina humilis* possessed certain group of phytochemicals and they are responsible for the biological activities against the selected stored product pest, *T. Castaneum.* Further, studies on the isolation and identification of phytochemical compounds will throw more light on the control of stored product pest using phytopesticides in the near future.

Acknowledgments

Authors are gratefully acknowledged to Professor and Head, Department of Zoology The Principal, Govt. Arts College (Autonomous) Nandanam for their support and laboratory facilities provided. The authors are also thankful to University Grants Commission (UGC, New Delhi; Ref. No. 42-583/2013 (SR) for their financial assistance.

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