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INSECTICIDAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *ZANTHOXYLUM RHETSA* (ROXB.)DC. AGAINST *TRIBOLIUM CASTANEUM* HERBST.

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ABSTRACT

Tribolium castaneum Herbst a red flour borer beetle pest of stored grain in many regions of the world causing immense damage to food products including flour, cereals, pasta, biscuits, beans, and nuts. The insecticidal activity of different concentrations of carpel and seeds extracts of *Zanthoxyulum rhetsa* Roxb DC (Rutaceae) were tested for their efficacy using the Film residue method against a red flour beetle *Tribolium castaneum* Herbst. The results showed that the Methanolic extract of carpels *Zanthoxylum rhetsa* (Roxb) DC showed the highest (81%/1000ug/cm^2) toxic effect followed by the Petroleum and Chloroform (78%/1000ug/cm^2) and Aqueous extract (77%/1000ug/cm^2) of carpels *Zanthoxylum rhetsa* (Roxb.) DC. The Methanolic extract of seeds *Zanthoxylum rhetsa* (Roxb) DC showed the highest (75%/1000ug/cm^2) toxic effect followed by the Petroleum extract (73%/1000ug/cm^2) and Ethanolic extract (66%/1000ug/cm^2) of seeds *Zanthoxylum rhetsa* (Roxb.) DC. Among the solvents, the Methanol extracts showed more toxic effect than other extracts. Carpels and seeds extract of *Zanthoxylum rhetsa* (Roxb.)DC produced high incidence of mortality and reduced the rate of development resulting in reduction in their population exhibiting insecticidal activity to a promising level and can be opted as good alternatives to chemical pesticides.

KEYWORDS: insecticidal activity, stored pest, mortality, *Tribolium castaneum* Herbst, *Zanthoxyulum rhetsa* Roxb DC.

1. INTRODUCTION

Herbivorous insects are responsible for destroying one fifth of the world's total crop production annually. Protection of stored grain against insect pests is a major problem from the development of agriculture. Plant products have been successfully exploited as insecticides, insect repellents antifeedant and growth regulating properties (Dethier VG et. al, 1960, Schoonhoven, 1960, Mordue AJ, 1980). Higher plants are a rich source of novel natural substances that can be used to control insects which are environmental safe. (Arnason JT et. al, 1989). Insecticidal activity of many plant extracts or crude compounds against several stored grain pests has been demonstrated (Jilani G, 1989, Isman MB, 2000, Carlini CR, 2002) which are manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and eduction of fecundity and fertility. It has been reviewed the plants there is a strong connection between medicinal and pesticidal plants (Yang RZ, 1988). To minimize use of synthetic pesticides and to avoid pollution of the environment, recently natural antifeedant, deterrent and repellent substances have been searched (Lindgren BS, 1996, Klepzig KD, 1999, Govindachari TR, 2000). There is an urgent need to develop safe alternatives that are of convenient, less expensive, highly effective and very safe for the humans and environment.

Considerable efforts have been focused on plant derived materials as potentially commercial insecticides. The aim of our study is to evaluate the insecticidal activity of various extracts of carpels and seeds of *Zanthoxyulum rhetsa* Roxb DC.

Stored grain loss in weight and quality of products due to infestation of insects is a serious problem the agricultural sector is facing worldwide. It is estimated that stored grain loss of over 10% occur each year due to insect pests in particular *Tribolium castaneum* which is a major secondary pest of processed or damaged grains (Danahaye et al., 2007). *Tribolium castaneum* being both polyphagous and cosmopolitan, number of insecticides has been used for successful control (Islam and Talukdar, 2005). However the use of chemicals against insect pests of stored grains has become ineffective due to the development of resistance, in various strains of *T. castaneum* (Guedes et al., 1996; 1997) and hence a new trend is use of biopesticides for insect pest control in storage of cereals (Rizvi et al., 2001) which are environmental friendly convenient, less expensive, highly effective and very safe for the humans. Many indigenous plants have been used against insect pest of stored grains and other crops in Pakistan.Over 120 plants and plant products can be used for the control of stored

grain insect pests (Dales, 1996; Imtiaz et al., 1999). To minimize use of synthetic pesticides and to avoid pollution of the environment, natural antifeedant, deterrent and repellent substances have been searched for pest control during recent times (Lindgren BS, 1996, Klepzig KD, 1999, Govindachari TR, 2000). However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides.

The objectives of this study are:

1. To evaluate the percent mortality of *Zanthoxylum rhetsa* (Roxb.)DC against *T.castaneum* after 24, 48 and 72 hour's interval.
2. To determine lethal time responses of *Zanthoxylum rhetsa* (Roxb.)DC against *T.castaneum*

1.1 Systematic Position

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Coleoptera
Family:	Tenebrionidae
Genus:	*Tribolium*
Species:	*T. castaneum*

1.2 Distribution

It is a cosmopolitan insect found worldwide. In India it is considered as serious stored grain pest, distributed all over the country damaging cereals and grains. This pest is found commonly in granaries, mills, warehouses etc.

1.3 Identification

The red flour beetles measure about 1/10 to 1/8 inch long and are flat, shiny, reddish - brown, and elongated in structure. Antennae are segmented, the last three segments at the tip of the antennae are abruptly larger than the preceding ones, forming a three – segmented club. It has curved sides. The eyes of the red flour beetle are separated by less than two eye diameters. Red flour beetles fly.

1.4 Life Cycle

T.castaneum breed in damaged grain, grain dust, high - moisture wheat kernels, flour, etc. The Female lays 300 to 400 eggs in a period of five to eight months (two to three eggs per

day).The eggs hatch into slender, cylindrical, white larvae tinged with yellow, within 5 to 12 days which varies from 22 to more than 100 days. The fully grown larvae transform to naked pupae, and emerge as adults in a week. The life cycle requires 7 to 12 weeks. The live span of adults is 3 years or more. The beetles prefer temperatures of 30° C to develop or breed.

1.5 Nature of Damage

Tribolium castaneum is found infesting stored products like seeds, grains, flour, dry fruits, nuts, oil cakes, dry museum specimens and stuffed animals. Neither the larva nor the adult can damage sound grains, but feed on grains which have been damaged by other insect pests. This is a serious pest of prepared cereal products and is found in flour mills. Heavy infestation, of the beetle turns flour greyish-yellow or develops red taints becomes mouldy and emits offensive pungent smell.

1.6 Treatment

The most simple and effective measure to control *Tribolium castaneum* is to locate the source of infestation and get rid of it. Sanitation is the best method to avoid any stored – product pests. Use of a strong suction vacuum cleaner to clean spilled foods from the cracks and crevices, behind, under the cupboard, shelves, or storage area will help in getting rid of the pest. Scrub the area with soap and hot water thoroughly dry it, cover with clean, fresh paper or foil before replacing with food. In the case of food factories, grain silos, bulk flour stores etc. fumigation may have to be considered.

2. MATERIALS AND METHODS

2.1 Sample collection

The fruits were collected from Sindhudurg Dist, Western Ghats of Maharashtra, India and authenticated at Blatter's herbaria, Mumbai (accession no. 9046 /2010).

Carpels were washed with deionized water and oven dried at 40^{o}c for 4 days and then subjected to grinding for powder formation. The powder was stored in air tight glass containers and used for further analysis.

2.2 Extraction of the crude extracts from carpel and seeds powder.

The active components of the carpels and seeds *Zanthoxylum rhetsa* (Roxb.)DC were extracted using the cold extraction method (Farnsworth, N.R. 1988). Six different extraction solvents namely methanol, ethyl acetate, chloroform, ethanol, Petroleum Ether and distilled

water were used respectively. To 500ml each of pure methanol, ethyl acetate, chloroform, ethanol, Petroleum Ether and sterile distilled water were added 50g portions of the carpels and seeds in sterile conical flasks and allowed to soak at room temperature for 48 hours followed by placing on the cold shaker for 72 hours at 120 rpm was used to improve extraction of various phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump aided by a Whatman filter paper. Filtering was repeated thrice until the solution was clear. The filtrate was evaporated in a weighed porcelain dish and placed on a water bath for solvent evaporation. Drying was done to allow the calculation of the yield of the extraction process. The extraction efficiency was quantified by determining the weight of each of the extracts and the percentage yield was calculated as (weight of dry extracts in grams /initial dry plant extracts) × 100. A small proportion of dry extracts was stored for phyto-chemical analysis. The final filtrates were filter-sterilized by using what man's Filter paper. Sterile extracts obtained were stored separately in labeled, sterile capped bottles, in a refrigerator at 4°C before use during the insecticidal activity.

2.3. Insect bioassays

Test Insects

The red flour beetle, *T. castaneum* were cultured in Zoonosis Department, Haffkine Institute, Mass cultures were maintained in glass jars (1000ml),subcultures were maintained in beakers (500ml) with food medium and kept in an incubator at 30±0.5°C. A standard mixture of 19:1 ratio whole-wheat flour and powdered dry yeast (Khalequzzaman M et.al, 1994, Park T, 1948) was used as food medium throughout the experimental period.

2.4. Mortality tests Film residue method

(Busvine JR, 1971) was used to test the mortality of the adults of *T. castaneum*. The extracted materials of carpels and seeds methanol, carpels and seeds ethyl acetate, carpels and seeds chloroform, carpels and seeds Petroleum Ether and carpels and seeds methanol distilled water of carpels and seeds of ethanol were weighed and dissolved in acetone for dosing. For testing *T. castaneum*, mortality five doses 250 μg/cm^2, 500 μg/cm^2, 750 μg/cm^2, and 1000 μg/cm^{2}concentrations were used including control (water). Ten to fifteen day-old adults of *T. castaneum* was tested against varying concentrations carpels and seeds extracts of different solvents. The doses were prepared by mixing the product with 1 ml acetone/ water. After mixing properly and the liquid was dropped in a petri dish (9.5-cm diameter) and dried by fanning and finally in an oven at 40º., 20 adults of each species were released in each Petri

dish and for each dose studies were conducted in triplicate. The doses were calculated by measuring the weight of prepared product (µg) in 01 ml of water divided by the surface area of the petri dish and it was converted into µg/cm^2. Mortality was assessed after 24, 48 and 72 h of the treatment.

The calculation of mortality rate was corrected for control mortality according to Abbott's formula (Abbott WS, 1925).

Mc = (Mo- Mc/100- Me) X 100

Where,

Mo = Observed mortality rate of treated adults (%),

Me = mortality rate of control (%),

and Mc = corrected mortality rate (%)

The experiments were performed in the laboratory at 30ºC ± 0.5ºC.

3. RESULTS AND DISCUSSION

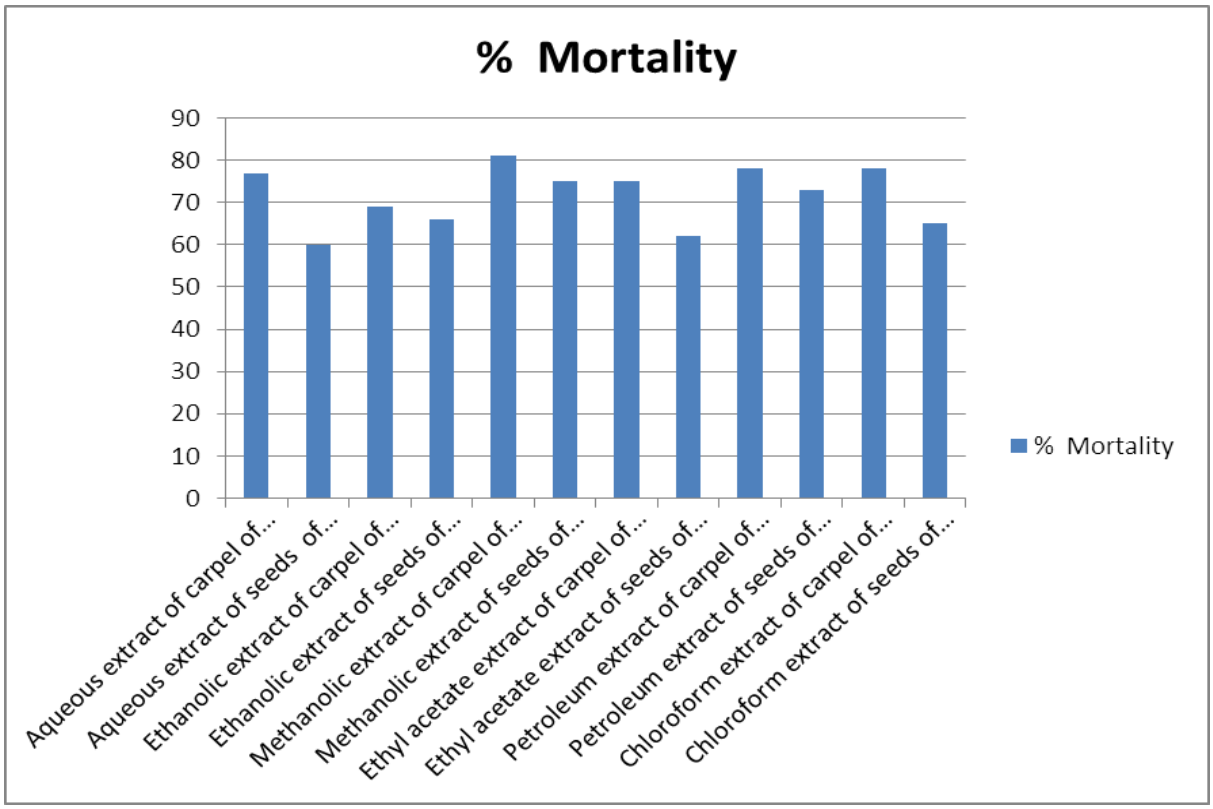
Extracts of carpels and seeds *Zanthoxylum rhetsa* (Roxb.)DC at different concentration with solvents such as methanol, ethyl acetate, chloroform, Methanol, Petroleum Ether and water were evaluated against *T. castaneum* by using the method of residual film technique revealed toxicity to *T. castaneum*. The % mortality of *T. castaneum* after 24, 48 and 72 hours at all doses 250, 500, 750, 1000 µg/cm^2 respectively revealed the potential of various extracts of carpels and seeds of *Zanthoxylum rhetsa* (Roxb.)DC as a potential insecticide against *T. castaneum*. The percentages of mortality calculated by using Abbott's formula (Table-1) demonstrates the insecticidal activity results. It is observed that different solvent extracts of *Zanthoxylum rhetsa* (Roxb.) DC would be more or less effective for controlling *T. castaneum*. The Methanolic extract of carpels *Zanthoxylum rhetsa* (Roxb) DC showed the highest (81%/1000ug/cm^2) toxic effect followed by the Petroleum and Chloroform (78%/1000ug/cm^2) and aqueous extract (77%/1000ug/cm^2) of *carpels Zanthoxylum rhetsa* (Roxb.) DC. The Methanolic extract of seeds *Zanthoxylum rhetsa* (Roxb) DC showed the highest (75%/1000ug/cm^2) toxic effect followed by the Petroleum extract seeds (73%/1000ug/cm^2) and Ethanolic extract (66%/1000ug/cm^2) of seeds *Zanthoxylum rhetsa* (Roxb.) DC. Zanthoxylum *rhetsa* (Roxb) DC is available throughout the country and the farmers may use this plant in their store houses for the management of stored grain pests *T. castaneum*. Further investigation on the identification of active ingredient from the carpel and

seed extracts, is more effective in identifying the active plant ingredient .The order of toxicity of the different extracts on red flour beetle, *T. castaneum* are shown in Graph 1.

Table 1: Mortality percentage of red flour beetle, *T. castaneum* treated with different plant extracts by Film residue method.

Name of the Plants	Concentration (µg/cm^2)	No of Insect used	No of Insect dead			Total No of Insects dead	% of Average Mortality	% Corrected Mortality
			24 hrs	48 hrs	72 hrs			
Aqueous extract of carpel of *Zanthoxylum rhetsa* Roxb DC	250	120	40	55	60	51	42	42
	500		60	70	74	68	56	56
	750		72	86	90	82	68	68
	1000		87	95	98	93	77	77
	control		0	0	0	0	0	0
Aqueous extract of seeds of *Zanthoxylum rhetsa* Roxb DC	250	120	30	35	40	35	29	29
	500		42	45	50	45	38	38
	750		55	68	70	64	53	53
	1000		70	72	75	72	60	60
	control		0	0	0	0	0	0
Ethanolic extract of carpel of *Zanthoxylum rhetsa* Roxb DC	250	120	50	55	58	54	45	45
	500		60	70	85	71	59	59
	750		70	75	89	78	65	65
	1000		75	80	94	83	69	69
	control		0	0	0	0	0	0
Ethanolic extract of seeds of *Zanthoxylum rhetsa* Roxb DC	250	120	40	45	49	44	37	37
	500		55	60	62	59	49	49
	750		60	70	75	68	56	56
	1000		75	80	85	80	66	66
	control		0	0	0	0	0	0
Methanolic extract of carpel of *Zanthoxylum rhetsa* Roxb DC	250	120	60	75	80	71	59	59
	500		75	85	90	83	69	69
	750		85	89	95	89	74	74
	1000		95	98	99	97	81	81
	control		0	0	0	0	0	0
Methanolic extract of seeds of *Zanthoxylum rhetsa* Roxb DC	250	120	55	62	68	61	51	51
	500		65	70	80	71	59	59
	750		74	84	88	82	68	68
	1000		85	90	95	90	75	75
	control		0	0	0	0	0	0
Ethyl acetate extract of carpel of *Zanthoxylum rhetsa* Roxb DC	250	120	55	58	60	57	48	48
	500		65	70	75	70	58	58
	750		80	85	87	84	70	70
	1000		88	90	95	91	75	75
	control		0	0	0	0	0	0
Ethyl acetate extract of seeds of *Zanthoxylum*	250	120	45	50	55	50	41	41
	500		55	60	65	60	50	50
	750		65	70	75	70	58	58

rhetsa Roxb DC	1000		70	75	80	75	62	62
	control		0	0	0	0	0	0
Petroleum extract of carpel of *Zanthoxyulum rhetsa* Roxb DC	250	120	70	75	79	74	62	62
	500		75	80	85	80	66	66
	750		85	86	90	87	72	72
	1000		90	95	98	94.	78	78
	control		0	0	0	0	0	0
Petroleum extract of seeds of *Zanthoxyulum rhetsa* Roxb DC	250	120	65	67	70	67	56	56
	500		72	75	80	75	63	63
	750		82	85	87	84	70	70
	1000		85	88	90	87	73	73
	control		0	0	0	0	0	0
Chloroform extract of carpel of *Zanthoxyulum rhetsa* Roxb DC	250	120	68	70	74	70	58	58
	500		75	78	82	78	65	65
	750		85	87	90	87	72	72
	1000		90	95	99	94	78	78
	control		0	0	0	0	0	0
Chloroform extract of seeds of *Zanthoxyulum rhetsa* Roxb DC	250	120	55	60	65	60	50	50
	500		60	62	68	63	52	52
	750		70	75	78	74	61	61
	1000		75	79	83	79	65	65
	control		0	0	0	0	0	0



Graph 1: Mortality percentage of red flour beetle, *T. castaneum* treated with different plant extracts by Film residue method.

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