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CONTROL OF *RHYZOPERTHA DOMINICA* BY VARIOUS SOLVENTS EXTRACTS OF FRUITS OF *ZANTHOXYULUM RHETSA* ROXB DC (RUTACEAE)

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ABSTRACT

Rhyzopertha dominica a lesser grain borer is a primary beetle pest of stored grain in many regions of the world. Fumigation as a pest control method plays an important role in control and management of infestation of stored commodities worldwide. The study was conducted to control the **Rhyzopertha dominica** in ***Triticum aestivum*** (L.) (Poaceae) under modified atmosphere storage. The seeds and carpel extracts as well as the oil of the fresh and dried carpels were tested against the stored grain pest *Rhyzopertha dominica* under laboratory conditions. Five different concentrations of carpel and seeds extracts of *Zanthoxyulum rhetsa* Roxb DC (Rutaceae) were tested for their efficacy. Carpels and seeds extract produced high incidence of mortality and reduced the rate of development resulting in significant reduction in their population exhibiting insecticidal activity and seed protective effect to a promising level and can be opted as good alternatives to chemical pesticides.

INTRODUCTION

Many farmers suffer heavy losses of stored grains due to insect pests. There are about 39 species of pests, which attack the stored grains and grain products. According to Food and Agriculture Organization, about 10-25% of the world harvested food is destroyed by rodent and insects pests (Anonymous, 1980). Intense use of insecticides and pesticides results in the evolution of resistant strains in addition to environmental contamination and various health hazards (Prakash & Rao, 2006; Rahman *et al*., 2009). Hence a safe method is to use the bioactive pesticides for protection of stored grains (Tapondjou *et al*., 2001; Boeke *et al*., 2004; Talukdar *et al*., 2004; Tooba *et al*., 2005; Epidi *et al*., 2008). A number of plant ingredients are known to suppress the feeding and breeding behaviour of insects unlike direct mortality IN insecticides (Jilani, 1984). Plant parts with insecticidal properties are used as insecticides all over the world as they are convenient, less expensive, highly effective and very safe for the humans and environment.

Medicinal plants such as *Azadirachta indica* (A. juss), *Cassia fistula* (L.), *Calotropis procera* (Ait), *Chrysanthenum coronarium* (L.) and *Lantana camara* (L.) show insecticidal activity, antifeedant, repellant and growth regulating properties against *Rhyzopertha dominica* (Fabr.) (Gautam *et al*., 2003; Deka & Singh, 2005; Singh & Singh, 2005; Hazan *et al*., 2006; Prakash & Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Sankari & Swamy, 2007). These are widely used as traditional stored grain protectants in powder form, crude mixtures or extracts due to their easy accessibility and biodegradable nature (Dwivedi& Garg, 2003). Many plants like *Annona squamosa, Lantana camara* (L.), *Cassia fistula, Azadirachta indica* (A. juss), and *Calotropis procera* have been proved to be lethal for various stored grain pests. (Tewari & Singh, 1978; Dwivedi & Garg, 2003; Dwivedi & Karsawara, 2003; Deka & Singh, 2005; Morya *et al*., 2010). Leaves of *Ocimum sanctum* (L.),*Vitex negundo* (L.), *Aegle marmelos* (L.) and *Lippia geminate* (L.) have been used in the protection of stored rice forms in rural India (Prakash & Rao, 2006). *Rhyzopertha dominica* is one of the most destructive pest of stored grains both in larval and adult stage. It infests other stored food items like Triticum, beaten rice (Poha), dry fruits etc., (Kuzumenov *et al*., 1984; Raju, 1984) (Edde, 2012). The adults are sturdy fliers, which fly from warehouse to warehouse, causing severe infestation and convert the stored grains to mere frass (Frenmore & Prakash, 1992). Hence, in the present study *Rhyzopertha dominica* is selected for testing the efficacy of *Zanthoxyulum rhetsa* Roxb DC (Rutaceae) on Triticum. Fumigation plays a key role in control and management of infestation stored commodities worldwide. Exposure of insects to toxic concentrations of atmospheric gases has been practiced for centuries and has been promoted

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in recent years as a biorational substitute for chemical fumigations (Sadeghi *et al*., 2011). *Zanthoxyulum rhetsa* Roxb DC (tirphal) is a member of the Ruteace family. The plant is endowned with the medicinal properties by which it pacifies vitiated vata, kapha, asthama, bronchitis, cardiac ailments, stomatitis, pyorrhea, hemorrhoids, diarrhea, arthritis, boils, ulcers, poison and traumatic eye injury. The plant is also used as an astringent, stimulant and digestive (fruit), aphrodisiac (bark), antiseptic and disinfectant (essential oil).

Distribution

Rhyzopertha dominica is thought to have originated from the Indian subcontinent, but now it has an cosmopolitan distribution. It is considered as one of the serious pest of stored products throughout the tropics. It is also found in temperate countries, due to its ability of prolonged flight or a result of the international trade in food products.

Identifying Characters

Rhyzopertha dominica, the lesser grain beetle, is a very small measuring about 3 mm, cylindrically shaped, dark brown and somewhat shinny in appearance, head is tucked underneath the prothorax concealed, abdomen is tapered, with the end of the elytra curved gradually.

Biology and Life Cycle

Eggs: The female lays 200 to 500 eggs in her lifetime. She either drops them loosely into the grain or lays each individually in cracks of grains. It has been observed that at higher temperature there is higher egg production.

Larvae: white with a dark head and prominent legs. They bore into grain kernels, taking advantage of any cracks in a kernel's exterior shell.

The development from egg to adult can be quite rapid at high temperature and high humidity, taking just 21 to 28 days.

Pupa: Pupation takes place within the kernel and exit holes are evident after the adult emerges.

Adult are quite long-lived and are strong fliers. The adults are often not that evident when inspection of infested grain as they eat into the inside grain kernels where they are not easily visible.

Damage

Damaged kernels are the ones that are the most frequently attacked by these beetles; however, the grain borers are capable of invading undamaged grain. They infest corn, wheat, barley, rice, wood and books.

Inspection and Management

The key to control of grain borers is to first identify the infested food product. When inspecting for the grain borers, focus on the areas where whole wheat, corn, etc are stored to a lesser extent, flour products. Check areas where spilled grain could accumulate such as on or under shelving, under pallets, in the rails or storage racks, and under grain conveyors. Routinely check the larger grain storage bins, the grain can be moved about to look for adult beetles. If the grain is heavily infested, it will be visibly evident. The lesser grain borer are most likely to be found in a home or a supermarket. If smaller packages or quantities of infested grain are infested the packages of grain also can be frozen for six days at $-18^{O}C$ to kill all life stages. Food packaging is one of the most important parts of food industry which is related with food security. It provides not only a method for transporting food safely, but also extends product's self-life via preventing from harmful bacteria, contamination and degradation (Chin, 2010).

Impact

R. dominica is one of the major pest of *Triticum aestivum* (Flinn *et al*., 2004) and *Oryza sativa* (Chanbang *et al*., 2008a, b) throughout the world. The larvae and adult both infest and produce frass and cause weight losses by feeding on grains. (Emery and Nayak, 2007).

The impact of *R. dominica* infestation are the loss in the quantity and quality of stored grain, (Sánchez-Mariñez *et al*., 1997) and the cost involved to prevent or control infestations of stored grains. (Cuperus *et al*., 1990; Anonymous, 1998).

Larvae consume both the germ and endosperm of wheat and rice during their development in grain and thus produce more frass than other stored grain pests. (Campbell and Sinha, 1976). *R. dominica* damages the grain, causing weight losses of up to 40%, compared to other stored grain pests. (Sittusuang and Imura, 1987). *R. dominica* feeding on seed germ reduces germination rates and the vigour of the grains. (Bashir, 2002).

Food production and nutritional value

R. dominica infestation of *Triticum aestivum*, *Zea mays* and *Sorghum bicolor* grains resulted in substantial changes in the elemental contents of calcium, phosphorus, zinc, iron, copper and manganese (Jood *et al*., 1992, Jood and Kapoor 1992). Single or mixed populations of *Trogoderma granarium* (Khapra beetle) and *R. dominica* resulted in substantial reductions in the various contents of total lipids, phospholipids, galactolipids and polar and nonpolar lipids of *Triticum aestivum*, *Zea mays* and *Sorghum bicolor* (Jood *et al*., 1996). *R. dominica* has also been reported to decrease the vitamin contents of grain; 75% level of infestation of cereal grains caused losses of 23 to 29% (thiamine), 13 to 18% (riboflavin) and 4 to 14% (niacin) (Jood and Kapoor, 1994).Chapatis or rotis prepared from flours prepared with 50% *R. dominica* and *T. granarium* tasted bitter (Jood *et al*., 1993). 75% infestation of grains causes a significant reduction in protein nitrogen and true protein contents (Jood and Kapoor, 1992).

Economic impact

It is not easy to estimate the actual costs incurred in controlling of *R. dominica* because it is generally found in mixed population with other stored-product insect pests that cause damage. Two or more live 'grain-damaging' insects per kg of wheat resulted in an infested designation on the grain inspection certificate (FGIS, 1997). *R. dominica* produces insect-damaged kernels when the adults emerge from the kernels. If wheat contains more than produces insect-damaged kernels per 100 g it is designated as sample grade, which cannot be sold for human consumption and its market value drops dramatically (FGIS, 1997). Laboratory experiments have estimated that one *R. dominica* consumes 0.15 g of wheat in its life time (Campbell and Sinha, 1976; Storey *et al*., 1982). The cost of controlling storage pests *R. dominica* can be substantial.

Prevention and Control

Physical control

It involves the manipulation of the temperature, relative humidity, atmospheric composition, sanitation, ionizing radiation and the removal of the adult insects from the grain by sieving. (Jayas *et al*., 1995).

Sanitation

Thorough cleaning during grading operations, drying, cool storage and hermetically sealed packaging plays an effective role in conserving the seed viability and controllimg infestation by stored grain pests.

Grain packaging in airtight barrels aids in controlling *R. dominica*. (Garcia-Lara *et al*., 2013).Sieving is not equally effective for all species as several insect species, including *R. dominica*, spend most time of their life cycle remaining inside the grain or kernel which is over looked. Impacting the grain, by moving the grain using a pneumatic conveyer or dropping the grain onto a spinning, studded disc, aids in reducing R. *dominica* populations. Good sanitation, particularly the removal of spilt grain around storage facilities, is a preliminary step in reducing insect populations that can infest grain in storage.

Aeration and drying

Cool the grain with aeration fans, it gradually suppresses insect population growth in the storage period it is an effective non-chemical control. The Kansas State extension program has advocated early aeration starting from harvesting, using automatic fan controllers, as the best non-chemical insect suppression method which allows safe storage of grain for several months (Reed and Harner, 1997).

Moisture content (mc) of 25% is not uncommon in newly harvested grain in humid regions, but grains with 14% mc can be safely stored for 2-3 months. Reducing the moisture content of the grain reduces the number of eggs produced and the survival of offspring and adults. Hence ambient air drying, sun drying and mechanical drying help in reducing the moisture content. (Jones *et al*., 2012). In Asia, Africa, and Latin America grain drying is achieved by spreading a thin layer of grain in the sun, on the threshing floor or on rooftops.

Radiation

Radio-frequency heat treatment is used as a thermal method for the disinfection of post-harvest insect populations in agricultural commodities (Tang *et al*., 2000).

This method leaves no chemical residue and provides acceptable product quality with minimal environmental impacts (Wang *et al*., 2003). The efficiency of radio-frequency heat treatment against *R. dominica* both on the seed surface and inside the seed is good. (Janhang *et al*. 2005). Recently, a flameless catalytic infrared emitter was used to disinfest hard red winter wheat containing different life stages (eggs, larvae, pupae and 2-week-old adults) of *R. dominica* (Khamis *et al*., 2010). It is suggested that this technology is a promising tool for disinfestation of stored *Triticum aestivum*. (Khamis *et al*., 2010).

Controlled atmosphere

Reducing temperatures to below 34°C reduces the rate at which the population of *R. dominica* increases. *R. dominica* cannot complete its life cycle below 20°C. In temperate countries, grain temperatures can be reduced by forcing air from outside through the grain, especially in winter. Grain can also be cooled by aeration using refrigerated air. Commercial units are available for both types of cooling. Increasing grain temperature to above 34°C also reduces the rate at which the population of *R. dominica* increases. *R. dominica* is one of the most heat tolerant of all stored grain insect pests, it can be controlled by heating the grain to 65°C in 4 minutes, and rapidly cooling it to below 30°C. Heat disinfestation of grain has the potential for higher market acceptance. The manipulation of nitrogen, oxygen and carbon dioxide (CO_2) within storage structures controls insect infestations. The combination application of carbon dioxide (5-20%) with the fumigant ethyl formate significantly enhanced the effectiveness of the fumigant against *R. dominica* and other stored grain insect species (Haritos *et al*., 2006).

Inert dusts

Inert dusts such as ash, lime, clay, diatomaceous earth (DE) and silica aerogel have been used as a traditional method of insect control. (Glenn and Puterka, 2005). Most effective inert dusts are DE and silica aerogel. They dehydrates the insect body by both cuticle lipid absorption and abrasion (Quarles and Winn, 1996). DEs have low mammalian toxicity (Athanassiou *et al*., 2004), hence these formulations are used as effective insect pest control (Vayias *et al*., 2006).

Host plant resistance

There are substantial differences in the resistance of host varieties to *R. dominica* (Kishore, 1993; Cortez-Rocha *et al*., 1993), however the use of resistant varieties have not yet been exploited as a method of control. Resistant varieties often do not prevent insect infestations, but reduce the rate of infestations. However host resistance varieties would enable the crop to be stored for a longer period before extensive damage is caused by insect populations. Caution is needed with regard to the introduction of resistant varieties as a method of control of *R. dominica*; the insect may overcome host plant resistance, as it has developed resistance to a number of insecticides, and the development of further resistance management strategies would be required.

Botanical insecticides

Due to environmental concerns and insect pest resistance to conventional chemicals, interest in botanical insecticides has increased. (Golob *et al*., 1999; Isman, 2000). They are less harmful to the environment, generally less expensive, and can easily processed and used by farmers and small industries since they are eco- friendly. They can be used as powders, solvent extracts, essential oils and whole plants for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Weaver and Subramanyam, 2000). Powdered leaves of *Salvia officinalis* L. and *Artemisia absinthium* L. to *Triticum aestivum* grains was very effective in reducing population size and delaying development time of *R. dominica* (Klys, 2004). Various plant oils and solvent extracts are used in controlling stored grain insect infestations (Stoll, 2000; Shaaya *et al*., 2003; Moreira *et al*., 2007; Rozman *et al*., 2007; Rajendran and Sriranjini, 2008, Lee *et al*., 2004). The essential oils obtained from different plant species repel several insect pests and possess ovicidal and larvicidal properties. (Cetin *et al*., 2004). *R. dominica* is susceptible to hexane crude extract of *Ageratum conyzoides*, experiencing more than 88% mortality after 24 h of exposure (Moreira *et al*. 2007). Presently botanicals insecticides constitute only 1% of the world insecticide market (Rozman *et al*., 2007).

Biological control

Natural enemies to control *R. dominica* and other stored grain insects has been limited in developed countries because of the low tolerance (0-2 insects/kg grain) of insects in stored grain. (Brower *et al*., 1991).

Predators

There have been several laboratory studies on the use of predators of *R. dominica* (Brower *et al*., 1991). *Teretriosoma nigrescens* a histerid beetle found in Central America, feeds on *R. dominica*. (Markham *et al*., 1994). The cadelle *Tenebroides mauritanicus* also feeds *Rhyzopertha* and stored-product insect eggs (Bousquet, 1990).

Parasites and parasitoids

Most of the parasitoids that attack the primary beetle pests are in the families Pteromalidae and Bethylidae. *Choetospila elegans* is a small pteromalid wasp that attacks *R. dominica* and certain other coleopteran and lepidopeteran insect pests. The wasp normally parasitizes larvae that are feeding inside the grain. At 32°C, a wasp takes approximately 15 days to complete its development on *R. dominica*; the generation time of *C. elegans* is almost half that of *R. dominica.* In the presence of hosts, female wasps live for 10-20 days at 32°C. A single female *C. elegans* is capable of parasitizing up to six *R. dominica* per day. *Anisopteromalus calandrae*, is also effective at reducing *R. dominica* populations. (Mahal *et al*., 2005). *Acarophenax lacunatus is* egg parasitic mite significantly reduces the population of *R. dominica* (Faroni *et al*., 2000; Gonçalves *et al*., 2004).

Entomopathogens

Entomopathogenic fungi have found to effective against *R. dominica*. Its pathogenicity depends on variousphysical factors and biological The fungi possess the ability to infect the insects through cuticle (Boucias and Pendland, 1991; Thomas and Read, 2007). Recently, *Purpureocillium lilacinum* were the best in controlling target insect species (Barra *et al*., 2013). The combined controls of Isaria *fumosorosea* with enhanced diatomaceous earth and the plant extract bitter bar komycin (Riasat *et al*., 2013); *B. bassiana* and enhanced diatomaceous earth (Wakil *et al*., 2011); *B. bassiana* admixed with a diatomaceous earth formulation (Riasat *et al*., 2011); and *B. bassiana* with thiamethoxam and a diatomaceous earth formulation (Wakil *et al*., 2012). Could be an effective strategy to control *R. dominica* in stored wheat. *Bacillus thuringiensis* var. tenebrionis has been investigated for the control of *R. dominica* (Keever, 1994; Mummigatti *et al*., 1994, Mummigatti *et al*., 1994. Beegle, 1996). The combination of Cry3Aa protoxin and protease inhibitor (potato carboxypeptidase) resulted in the delayed development, increased mortality and the progeny suppression of *R. dominica* (Oppert *et al*., 2011).

Chemical control

The insecticidal efficacy of insecticides varies with the surfaces on which they are applied. Some insecticides degrade faster on concrete than on wood or any metal (Collins *et al*., 2000). Deltamethrin is more effective on plywood than on concrete against *R. dominica* and *Tribolium* spp. (Arthur, 1997). Insecticides such as Chlorpyrifos-methyl and pirimiphos-methyl are effective against most stored grain insect pests, but are relatively ineffective against *R. dominica*. *R. dominica* is the more susceptible to spinosad applied on *Triticum aestivumn* or *Zea mays* (Athanassiou *et al*., 2010b) than other stored grain pests. *R. dominica* mortality were extremely high on *Triticum aestivumn, Zea mays*, *Hordeum vulgare* and, *Oryza sativa* even when treated low levels of spinosad. (Vayias *et al*. 2009).

Insect growth regulators (IGR) take longer to control insect populations and are more expensive than other insecticides but show low toxicity to mammals. IGR's can be sprayed or dusted directly onto the grain, and they protect grain from infestation from two weeks to over a year. Methoprene a commercial formulated IGR labelled in the USA for direct use on to stored grains and is effective against *R. dominica* (Arthur, 2004). The combination of methoprene and Deltamethrin not only eliminats progeny production of *R. dominica* on stored rice but also provided adult control (Chanbang *et al*., 2007). IGRs have two juvenile hormone analogues, four chitin synthesis inhibitors. (Kavallieratos *et al*. 2012).

Neonicotinoids can be applied in different ways, such as foliar, soil drench, seed treatment and stem applications, and to various crops (Schulz *et al*., 2009). Insecticides such as Imidacloprid and indoxacarb control *R. dominica,* but they are not yet registered for use on stored grains (Daglish and Nayak, 2012).

Phosphine is used as a fumigant, to control insect infestations in stored commodities however it can become re-infested once the fumigant has dissipated. It is highly toxic to humans and should be handled with extreme care; usually it is applied to the grain as aluminium phosphide pellets or tablets, although magnesium phosphide is also available in some countries. Once treated with an insecticide, the grain often must be held for a certain period of time before it can be processed or used as animal feed. The period of protection depends upon the commodity treated, the temperature, grain moisture content and the insecticide used. For effective control the temperatures must be over 15°C; higher temperatures cause rapid control but faster degradation of the insecticide. High moisture content reduces the duration of protection.

MATERIALS AND METHODS

Samples of wheat grains: Local variety of wheat grains, *Triticum aestivum* (L.) (Poaceae) cultivated in this region, were collected from market, washed thoroughly with distilled water and dried properly in sunlight to remove moisture, if any, sieved to remove any dust particles and powder, before used for experiments

Sample collection of *Zanthoxyulum rhetsa* Roxb DC

The fruits were collected from Sindhudurg Dist, Western Ghats of Maharashtra, India and authenticated at Blatter's herbaria, Mumbai (accession no. 9046 /2010). Carpels and seeds were washed with deionized water and oven dried at $40^{o}c$ for 4 days later powdered. The powder was stored in air tight glass containers and used for further analysis.

Extraction of the crude extracts from carpel and seeds powder

The active components of the carpels and seeds were extracted using the cold extraction method (Farnsworth, N.R. 1988).

Table 1 Mortality percentage of red flour beetle, *R. dominica* 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 *Zanthoxyulum rhetsa R*oxb DC	250	120	30	35	40	35	29	29
	500		40	42	44	42	35	35
	750		42	45	48	45	38	38
	1000		45	46	50	47	39	39
	control		0	0	0	0	0	0
Aqueous extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	30	32	36	32	27	27
	500		35	38	40	37	31	31
	750		40	42	48	43	36	36
	1000		42	45	52	46	39	39
	control		0	0	0	0	0	0
Ethanolic extract of carpel of *Zanthoxyulum rhetsa R*oxb DC	250	120	45	47	50	47	39	39
	500		55	58	65	59	49	49
	750		60	64	68	64	53	53
	1000		65	68	75	69.	58	58
	control		0	0	0	0.00	0	0
Ethanolic extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	40	42	48	43	36	36
	500		42	48	50	46	39	39
	750		44	50	52	48	41	41
	1000		45	55	58	52	44	44
	control		0	0	0	0	0	0
Methanolic extract of carpel of *Zanthoxyulum rhetsa R*oxb DC	250	120	60	65	74	66	55	55
	500		75	48	80	67	56	56
	750		85	88	94	89	74	74
	1000		85	88	95	89	74	74
	control		0	0	0	0	0	0
Methanolic extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	55	62	65	60	51	51
	500		62	65	70	65	55	55
	750		65	66	72	67	56	56
	1000		70	75	80	75	63	63
	control		0	0	0	0	0	0
Ethyl acetate extract of carpel of *Zanthoxyulum rhetsa R*oxb DC	250	120	55	56	60	57	48	48
	500		56	60	65	60	50	50
	750		60	62	68	63	53	53
	1000		65	70	75	70	58	58
	control		0	0	0	0	0	0
Ethyl acetate extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	45	48	50	47	40	40
	500		55	58	60	57	48	48
	750		60	62	65	62	52	52
	1000		62	65	70	65	55	55
	control		0	0	0	0	0	0
Petroleum extract of carpel of *Zanthoxyulum rhetsa R*oxb DC	250	120	55	58	65	59	49	49
	500		60	65	70	65	54	54
	750		65	68	72	68	57	57
	1000		66	70	78	71	59	59
	control		0	0	0	0	0	0
Petroleum extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	50	52	54	52	43	43
	500		60	65	68	64	54	54
	750		65	70	72	69	58	58
	1000		75	78	80	77	65	65
	control		0	0	0	0	0	0
Chloroform extract of carpel of *Zanthoxyulum rhetsa R*oxb DC	250	120	55	58	64	59	49	49
	500		58	62	68	62	52	52
	750		60	65	75	66	56	56
	1000		70	75	82	75	63	63
	control		0	0	0	0	0	0
Chloroform extract of seeds of *Zanthoxyulum rhetsa R*oxb DC	250	120	55	62	65	60	51	51
	500		64	66	74	68	57	57
	750		70	75	78	74	62	62
	1000		71	82	88	80	67	67
	control		0	0	0	0	0	0

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. And further on a 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 through a 127c-1 filter funnel aided by a Whatman filter paper. Filtering was repeated thrice until the solution was clear. The filtrate was evaporated in a weighed procelian dish on a water bath. 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. The procedure was done separately for the six solvents used. A small proportion of dry extracts was stored for phyto-chemical analysis. For the preparation of dilutions of dry extracts for antibacterial assay, dry extracts were reconstituted by re-dissolving in $DMSO_4$ solvent. The final filtrates were filter-sterilized by using Whatman's Filter paper. Sterile extracts obtained were stored separately in labelled, sterile capped bottles, (Table 3) in a refrigerator at 4°C before use during the insecticidial activity.

Insect bioassays

Test Insects

The *R. dominica* 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.

Mortality tests Film residue method (Busvine JR, 1971) was used to test the mortality of the adults of *R. dominica*. 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 beetle, mortality four doses were used including control (water). Ten to fifteen day-old adults of *R. dominica* was used at 250, 500, 750, 1000 $\mu g/cm^2$concentrations. The doses were prepared by mixing the requisite quantities of the product with 1 ml acetone/ water. After mixing properly and the liquid was dropped in a petri dish (9.5-cm diameter). After drying by fanning and finally in an oven at 40 ºC, 20 adults of each species were released in each Petri dish. For each dose three replications were taken. 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 $\mu g/cm^2$. 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,

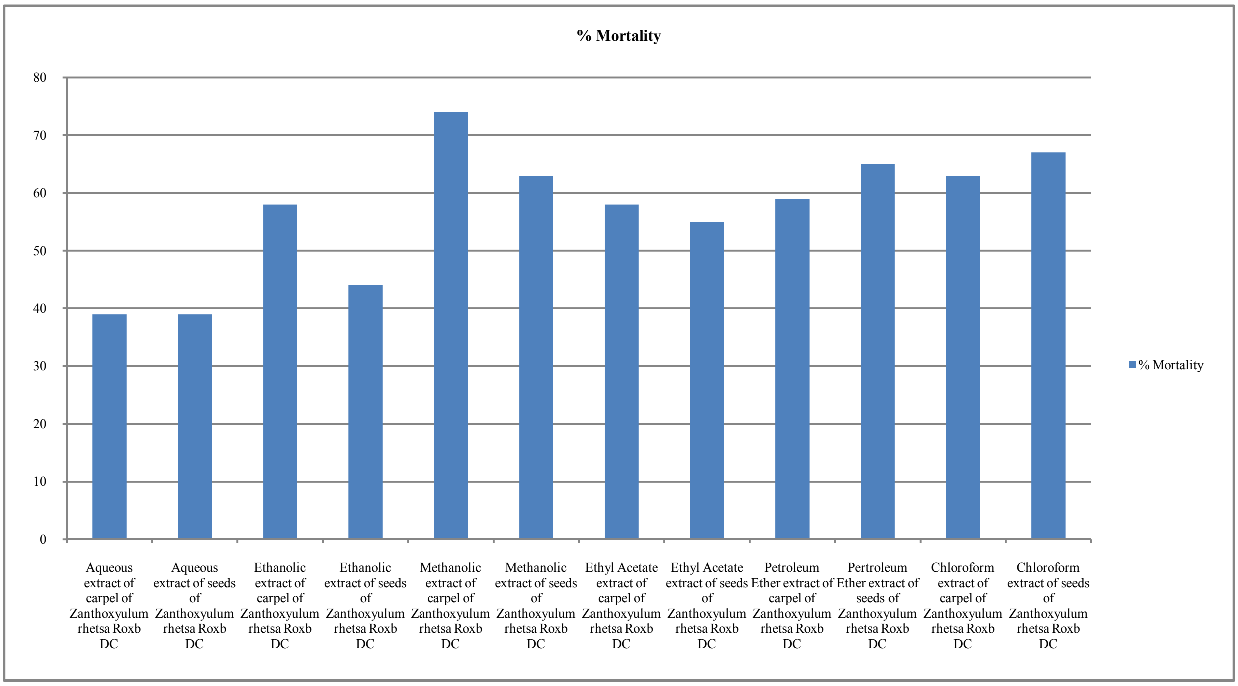
Mo = Observed mortality rate of treated adults (%),

Me = mortality rate of control (%),

and Mc = corrected mortality rate (%)

CONCLUSION

From the insecticidal activity results, it is observed that different solvent extracts of Zanthoxyulum rhetsa Roxb DC



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

Carpels and seeds are more or less effective for controlling red flour beetle. The Methanolic extract of carpels of *Zanthoxylum rhetsa* (Roxb.)DC showed the highest toxic effect 74 % followed by the Chloroform extract of seeds of *Zanthoxylum rhetsa* (Roxb.) DC 67%. This Plant is available throughout the country and the farmers may use this plant in their store houses for the management of stored grain pests. Further investigation on the identification of active ingredient from the carpel and seeds extracts, which is more effective than other extracts, is utmost needed.

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