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Evaluation of a push-pull approach for *Trogoderma granarium* (Evert) using a novel dispensing system for repellents/attractants under laboratory conditions

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Abstract
The present study was conducted to know the efficacies of various attractants/repellants (*using Y- Tube olfactometer*) on larvae and adults stages of Khapra beetle. Eight essential oils of plants were tested. The result revealed that Citronella oil as the most potent attractant against the larvae (76.6%); Wheat germ oil and Sesame oil act as a same (73.3%) for adults, while the Almond oil showed (100%) repulsive effect for larvae and Coconut oil showed (86.6%) repulsive effect for adults. The essential oils can be organized in descending order according to the repellant effect for larvae: Almond oil> Camphor oil>Mustard oil>Tea tree oil>Coconut oil and for adults: Coconut oil>Almond oil>Camphor oil > Mustard oil > Tea tree oil. According to an attractive effect for larvae: Citronella oil>Sesame oil>Wheat germ oil and for adults: Sesame oil >Wheat germ oil >Citronella oil. The repulsive index (RI) and attractive index (AI) were calculated for both the stages of beetles. The responsiveness ($\chi 2$ = *goodness to fit*) against attractants/ repellants for finding out the significant values in respective to beetle were recorded. The study also confirmed that some oils such as Citronella oil causes toxicity in beetles due to strong fragrance. Hence, the information contained in this paper emphasized on the novel dispensing system as a pull – push strategy under laboratory conditions to identify the proper management practices in Integrated Pest Management (IPM).

Keywords: *Trogoderma granarium*, Attractants/Repellants, stored grains products, Y- tube olfactometer, essential oils, integrated pest management (IPM)

1. Introduction
Insect infestation is a major contributor to quality deterioration of durables (cereals, pulses, roots and tubers) stored in warm and humid climates. Apart from the detrimental economic impact, these losses pose a major threat to global food security and safety [1]. Khapra Beetle *Trogoderma granarium* Evert (Coleoptera: Dermestidae) is one of the most notorious primary insect pests of stored grains and causes direct and various indirect losses [2], [3]. It is a very serious pest under hot and dry conditions, from quarantine point of view, especially in western countries that are of strategic importance to India for exports of cereals, pulses, oilseeds, etc. If infestation is severe, the devastation is complete, reducing the grain to mere frass [4]. Its exuviae, shed skin and other body parts are carcinogenic to human beings. This pest is polyphagous by nature in India. Due to presence of this pest which was responsible to attracts the trade restriction implications. The US Government spent about $15millions for its eradication programme, when it was accidentally introduced into USA [5]. In India, many export shipments have suffered heavy losses due to detection of this pest in one or other forms. The various control measures such as physical, chemical, mechanical methods are being implemented to control them. The protection of stored grains from insect damage is currently dependent on synthetic pesticides such as fumigation with phosphine (ALP) ; methyl bromide or dusting with compounds like primiphos methyl and permethrin. But the use of methyl bromide is being restricted because of it has a potential to damage the ozone layer [6]. The wide spectrum use of pesticides causes adverse effects on target and non- target organisms including human beings. For stored products and quarantine uses, many alternatives were tested to replace methyl bromide and ALP fumigation. Hence, there is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, yet are effective, economical and convenient to use [7]. The use of plant products and essential oils are one of the important approaches in insect pest management programme.

They have many advantages over synthetic insecticides. Essential oils are volatile in nature and have natural complex secondary metabolites which are characterized by a strong odor and generally have a lower density than that of water [8], [9]. They have received a great deal of attention as pest control agents [10], [11]. These essential oils are volatile and can function as fumigants, and may also be applicable to the protection of stored products [12], [13]. Therefore, the present study was conducted to determine the efficacies of essential oils for management of various stages of Khapra beetle which add an alternative control against pesticides. It would be also useful in providing good scope for the further development of ecofriendly methods.

2. Materials and Methods

The present study was conducted during the period from September 2014 to December 2015. Samples of Khapra beetles were collected from the local ration shops of Rajendra Nagar, Hyderabad. Afterwards, the cultures were maintained in Stored Grain Laboratory of Plant Bio-security Division, National Institute of Plant Health Management, Hyderabad.

2.1. Insect Rearing

The Khapra beetles were maintained in round plastic bottles of 1 Kg capacity, half filled with whole wheat grains/ rice and their mouths covered with double folded muslin cloth held tight with the help of 4″ rubber bands around its neck. The wheat/rice was properly dried, cleaned and conditioned. The culture was allowed to breed for three generations. The controlled conditions maintained in the laboratory were 27± 8 ˚C temperature, 65±5% relative humidity and 12 h photoperiod.

2.2. Collection of Volatiles (Essential oils)

The various types of essential oils were used in these experiments such as

1. Edible oils: Sesame oil @ 1% (v/w) ; Mustard oil @ 1% (v/w); Coconut oil @ 1% (v/w); Wheat germ oil @ 1% (v/w).

2. Non- Edible oil: Citronella oil @ 1% (v/w); Camphor oil @ 1% (v/w); Almond oil @1% (v/w); Tea tree oil @ 1% (v/w).

The essential oils were procured from the local market of reputed shops. The preliminary studies were conducted to see the efficacy of essential oils on Khapra beetle.

2.3. Behavioral Bioassays using Y – Tube olfactometer [14]

This instrument was designed by author and constructed by DWARKA SCIENTIFICS, Hyderabad. The 'Y' tube consisting of two arms to which are fitted broad tube serving as a test chamber (Size 12.5 cm). Air was blown from the other side of the 'Y' tube using an aerator [both A and B arms (7.5 cm)]. The air flow can be regulated by valve situated in the release chamber. The behavior of the insects was video graphed using Canon Powershot ISI- 120. Approximately 10 larvae / adults of Khapra beetles were released into the test chamber. Aerator was connected to both the arms A (Control) and B (Contains the cotton soaked oils). The filtered air was passed continuously at medium speed. Readings were taken at every 1min, 15min, 30min, 45min, 1hr, 2hrs, 3hrs, 6hrs, 9hrs, 12hrs and 24hrs. Photographs were taken by Canon Powershot ISI- 120 Digicam. Each experiment was repeated three times and the results mentioned below are an average of three experiments.

The repellent activity of essential oils was recorded in terms of Percentage Effective Repellency (%ER = (Nc- N_T/Nc) x 100 where Nc and N_T are number of individuals in control and treatment arms of olfactometer**)** after different intervals of time. Similarly, attractant activity was also recorded using suitable formula: Percentage Effective Attractancy (%EA) = (N_T – N_C/ N_T) X 100**).** Based on the data collected as described below, the repulsive index [15] and attractive index (AI) for Khapra beetle were calculated. The formula is given as: RI (Repulsive Index) = (Nc - N_T)/ (Nc+N_T) x 100 where Nc = No. of insects in control and the N_T =No. of insects in treated. RI varying from – 100 (Total attractancy) + 100 (Total repellency) with 0 meaning no effect.

AI (Attractive Index) = (No. of insects responded to test materials – No. of insect responded to control) ÷ (No. of insect released – No. of insect responded to control).

The collected data analyzed by IBM SPSS-19 Statistical Software for $\chi 2$ (Chi square) *goodness–of-fit* for significance of response.

2.4. Statistical Analysis

All the experiments were carried out in triplicates. The raw data collected from the readings were transferred to an electronic format and converted into a spreadsheet layout (Microsoft excel, 2007). Graphs were generated from the spreadsheets. Repulsive Index and Attractive Index were calculated using suitable formulae as mentioned in above section. The data were also subjected to IBM SPSS-21 Statistical Software to analyze $\chi 2$ values (Chi square) *goodness–of-fit* for significance of response.

3. Results and Discussions

The efficacies of the eight essential oils were observed on the 3rd instar larvae and adults stages of the Khapra beetles (Table1). All the experiments were carried out in triplicates.

(A) Larval Experiments

The 3rd instar larvae of Khapra beetle were chosen for experimental purpose. All the eight essential oils were tested against 3rd instar larvae of the Khapra beetles. Out of all, three of them *i.e.* Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7) showed good attraction against these larvae (Fig.1, Fig.2 & Fig.7). Coconut oil (A3), Tea tree oil (A6) and Mustard oil (A5) showed repulsion (Fig.4, Fig.6 & Fig.5) whereas Almond oil (A3) and Camphor oil (A8) act as a highly repellant against these larvae (Fig.3 & Fig.8). Larvae were showed 63.3%, 56.6% and 76.6% percentage attraction against Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7). The repulsive index (RI) was calculated 100 for Almond oil (A3), Coconut oil (A4), Mustard oil (A5), Tea tree oil (A6) and Camphor oil (A8). The attractive index (AI) was calculated 0.59, 0.56 and 0.73 for Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7). Citronella oil showed the repellant activity along with toxicity among these larvae (Table 2A).

(B) Adult Experiments

Both male and female adults were used for the experimental purpose. In case of adults, all the eight essential oils were tested against adult stages. Out of them, Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7) showed attraction against the adults (Fig.9, Fig.10 & Fig.15). Whereas Almond oil (A3), Coconut oil (A4), Mustard oil (A5), Tea tree oil (A6) and Camphor oil (A8) showed repulsion against adults (Fig.11, Fig.12, Fig.13, Fig.14 & Fig.16). Adults

showed 73.3%, 73.3% and 20% percentage attraction against Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7). Repulsive index was calculated are 100 for Almond oil (A3), Mustard oil (A5), Tea tree oil (A6) and Camphor oil (A8) except 79.3 for Coconut oil (A4) against adults. Attractive index (AI) was calculated as 0.73, 0.73 and 0.11 for Sesame oil (A1), Wheat germ oil (A2) and Citronella oil (A7) (Table 2B). As a result, it was implicit that Sesame oil, Wheat germ oil and Citronella oil showed good attraction for both larvae and adult stages of Khapra beetle. Apart from this, the Almond oil, Coconut oil, Mustard oil, Tea tree oil and Camphor oil are highly repulsive against larvae as well as adults. Out of all, only citronella oil creates the toxicity among the larvae

Table 1: Outcome of Behavioral bioassays showed the effect of various essential oils which act as an attractants/ repellents on larvae and adults stages of Khapra beetle.

Attractants/ Repellents	Attraction (Pull strategy)		Repulsion (Push strategy)	
	Adults	Larvae	Adults	Larvae
A1 - Sesame oil (1% v/w)	Attraction*	Attraction**	x	x
A2 -Wheat germ oil (1% v/w)	Attraction*	Attraction**	x	x
A3 - Almond oil (1% v/w)	x	x	Repulsion*	Repulsion*
A4 - Coconut oil (1% v/w)	x	x	Repulsion**	Repulsion**
A5- Mustard oil (1% v/w)	x	x	Repulsion#	Repulsion**
A6- Tea tree oil (1% v/w)	x	x	Repulsion*	Repulsion*
A7- Citronella oil (1% v/w)	Attraction#	Attraction**	x	x
A8 - Camphor oil (1% v/w)	x	x	Repulsion*	Repulsion*

Low Attraction; *Attraction ; **High Attraction; #Low Repulsion; *Repulsion; **High Repulsion

Effect of Essential oils (Edible and Non- Edible oils) on larvae of Khapra beetle

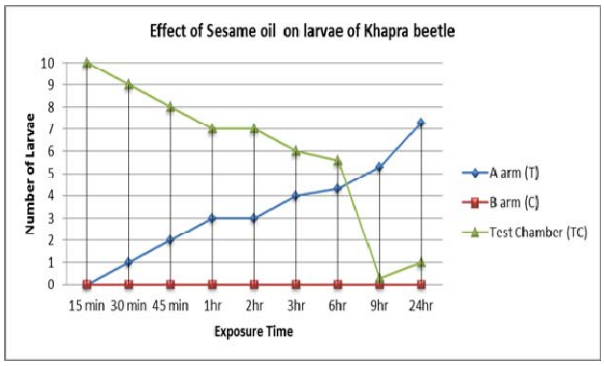

Fig 1: Effect of Sesame oil on larvae of Khapra beetle

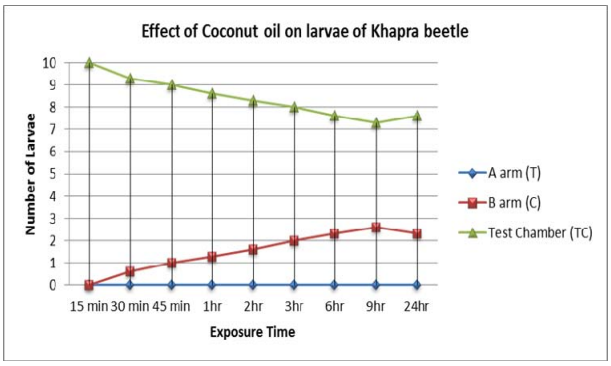

Fig 4: Effect of Coconut oil on larvae of Khapra beetle

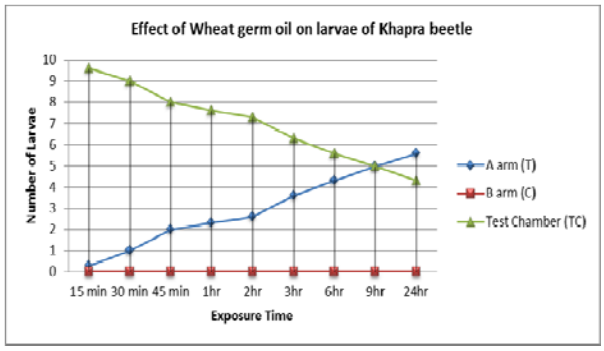

Fig 2: Effect of Wheat germ oil on larvae of Khapra beetle

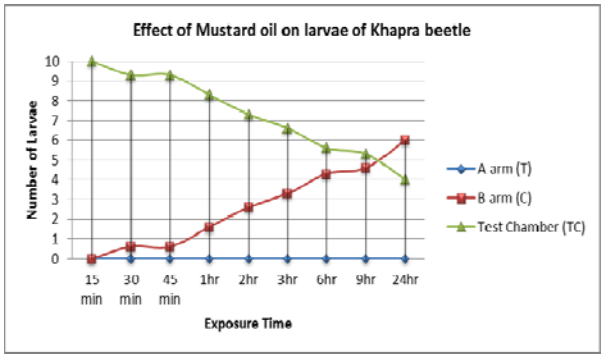

Fig 5: Effect of Mustard oil on larvae of Khapra beetle

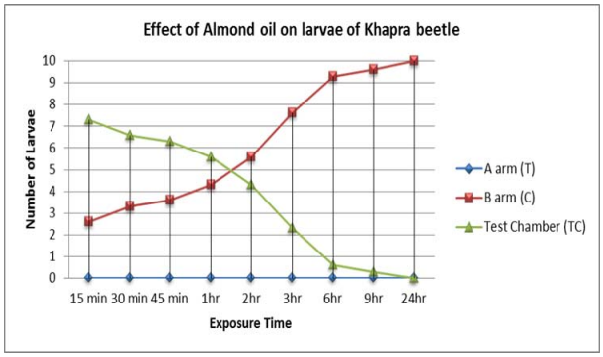

Fig 3: Effect of Almond oil on larvae of Khapra beetle

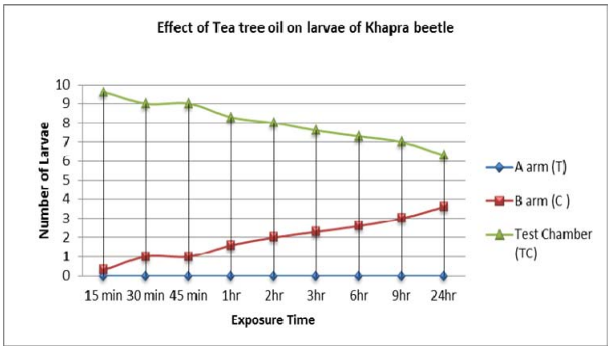

Fig 6: Effect of Tea Tree oil on larvae of Khapra beetle

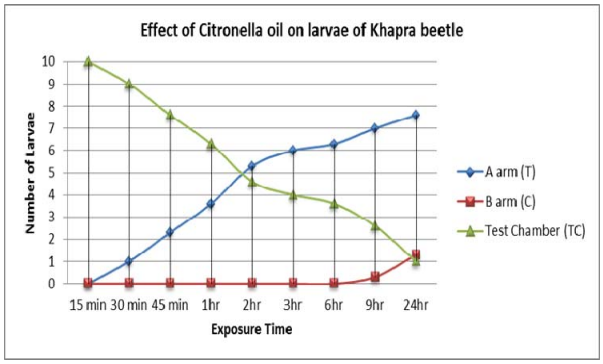

Fig 7: Effect of Citronella oil on larvae of Khapra beetle

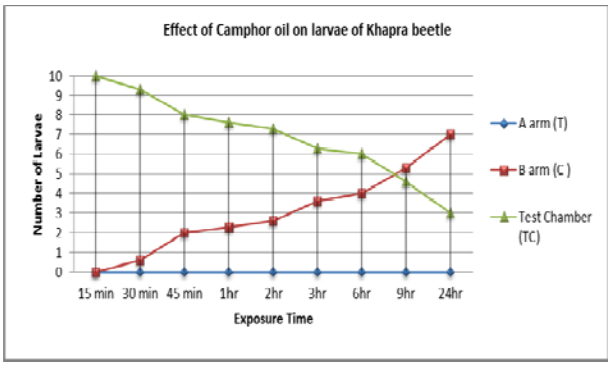

Fig 8: Effect of Camphor oil on larvae of Khapra beetle

Table 2: (A): Behavioural bioassay study using different types of essential oils (Edible and Non-Edible) against larvae of *Trogoderma granarium* Evert for chemical communication.

Larvae released in the Experiment setup				**Percentage Repellency (%)**	**Percentage Attraction (%)**	**Repulsive Index (RI)**	**Attractive Index (AI)**	**χ2 value & Significance to response**
Species	**Essential oils**	**Total number of insects released (10 x R_1, R_2, R_3)**	**Total number of insect responded within 24 hrs.**					
Trogoderma granarium Everts	A1- Sesame oil	30	19(A)	10%	63.3%	-72.7	0.59	χ^2= 8.640a, *S = 0.003
	A2 -Wheat germ oil	30	17(A)	-	56.6%	-	0.56	χ^2= 5.167a *S = 0.023
	A3 - Almond oil	30	30(B)	100%	-	100	-	NA
	A4 - Coconut oil	30	9(B)	30%	-	100	-	χ^2= 4.800a *S= 0.028
	A5 - Mustard oil	30	18(B)	60%	-	100	-	χ^2= 5000a *S = 0.025
	A6 - Tea Tree oil	30	11(B)	36.6%	-	100	-	χ^2=4.887a *S = 0.027
	A7 – Citronella oil	30	23 (A)	-	76.6%	-	0.73	χ^2= 4.149a *S =0.042
	A8 – Camphor oil	30	21(B)	70%	-	100	-	χ^2= 6.563a *S = 0.010

*(A) = Arm A = Attraction; *(B) = Arm B = Repulsion; χ2 value (Chi- square) using SPSS 21; df =1; *S = Significant value, NA = Not Applicable

Effect of Essential oils (Edible and Non- Edible) on adults of Khapra beetle

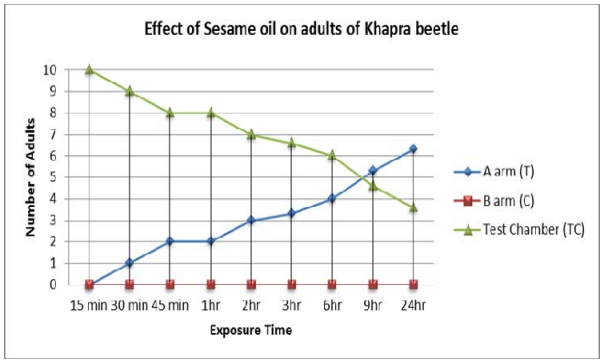

Fig 9: Effect of Sesame oil on adults of Khapra beetle

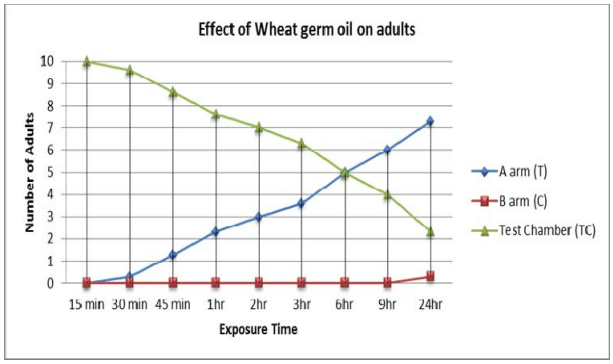

Fig 10: Effect of Wheat germ oil on adults of Khapra beetle

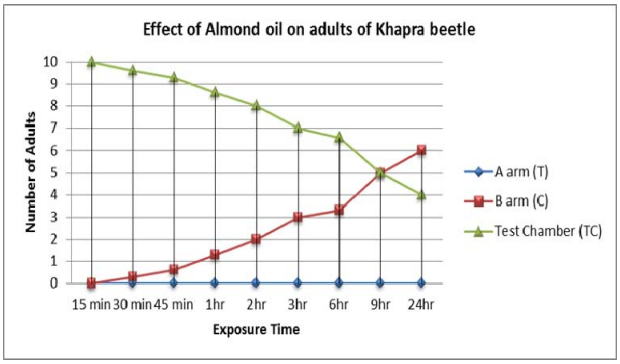

Fig 11: Effect of Almond oil on adults of Khapra beetle

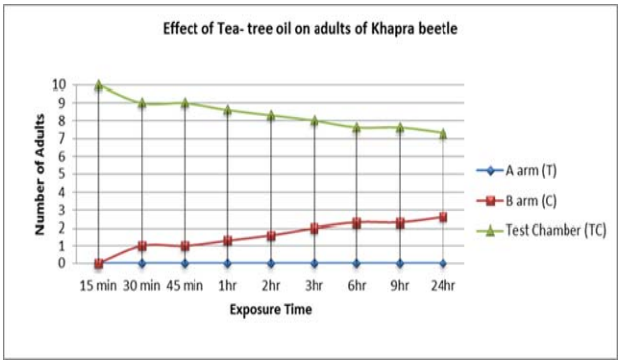

Fig 14: Effect of Tea- tree oil on adults of Khapra beetle

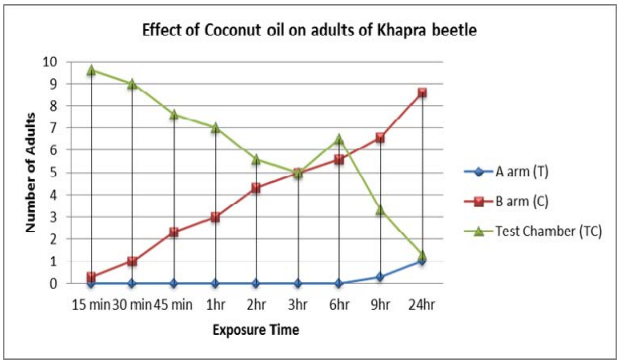

Fig 12: Effect of Coconut oil on adults of Khapra beetle

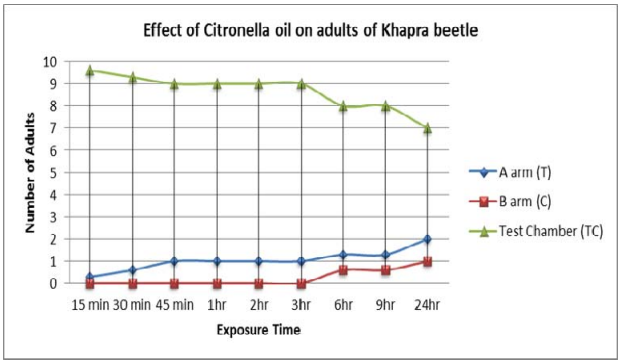

Fig 15: Effect of Citronella oil on adults of Khapra beetle

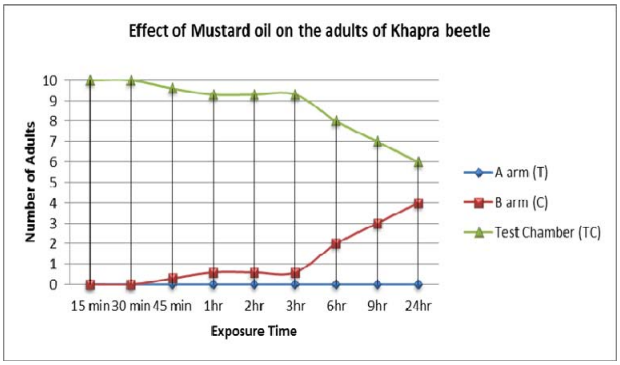

Fig 13: Effect of Mustard oil on adults of Khapra beetle

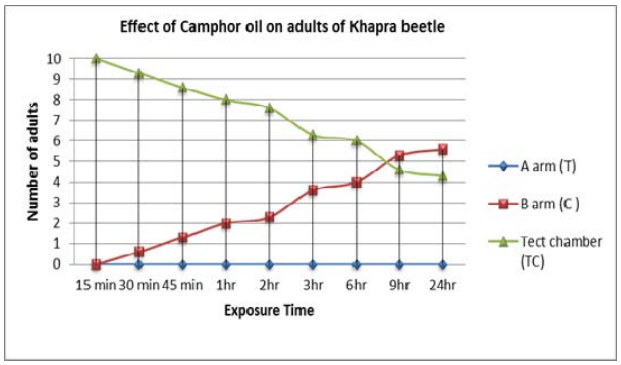

Fig 16: Effect of Camphor oil on adults of Khapra beetle

Table 2(B): Behavioural bioassay study using different types of essential oils (Edible and Non-Edible) against the adults stages of *Trogoderma granarium* Evert for chemical communication

Adults released in the Experiment set up				Percentage Repellency (%)	Percentage Attraction (%)	Repulsive Index (RI)	Attractive Index (AI)	χ2 value & Significance to response
Species	**Essential oils**	**Total number of insects released (10 x R_1, R_2, R_3)**	**Total number of insect responded within 24 hrs.**					
Trogoderma granarium Everts	A1- Sesame oil	30	22(A)	-	73.3%	-	0.73	$\chi^2 = 8.571$a *S =0.003
	A2 -Wheat germ oil	30	22(A)	3.3%	73.3%	-91.3	0.72	$\chi^2 = 10.995$a *S =0.001
	A3 -Almond oil	30	18(B)	60%	-	100	-	$\chi^2 = 5000$a *S= 0.025
	A4 - Coconut oil	30	26(B)	86.6%	-	79.3	-	$\chi^2 = 5400$a *S = 0.020
	A5 - Mustard oil	30	12(B)	40%	-	100	-	$\chi^2 = 5000$a *S = 0.025
	A6 - Tea tree oil	30	9(B)	30%	-	100	-	$\chi^2 = 8.688$a *S = 0.003
	A7- Citronella oil	30	6(A)	-	20%	-	0.11	$\chi^2 = 3.589$a *S = 0.058
	A8 - Camphor oil	30	17(B)	56.6%	-	100	-	$\chi^2 = 5.167$a *S =0.023

*(A) = Arm A = Attraction; *(B) = Arm B = Repulsion; χ2 value (Chi- square) using SPSS 21; df =1; *S = Significant value

The essential oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties [16]. The numerous vegetable oils can be used as protective additives. An advantage is that they are easy to apply. This information suggested that management interventions should be focused against reproducing adult females more to prevent the multiplication and spread of this pest. Therefore, behavioural bioassays were done by using larval and adult stages to determine the effectiveness of these essential oils. Obeng-Ofori and Reichmuth [17] used plant oils (such as coconut, sunflower, sesame and mustard) at 10 and 5 ml/kg alone and in combination with 1, 8 cineole, eugenol or camphor at 0.5, 1.0 and 5.0 ml or mg/kg against *Sitophilus granarius* (L.), *S. zeamais* (Mots.).Similarly in present studies various essential oils, *i.e.* Sesame, Wheat germ, Coconut, Mustard, Almond, Citronella, Camphor and Tea tree @ 1% (v/w) were used to check the efficacies as attractants/ repellants against 3rd instar larvae and adults of the *Trogoderma granarium* (Evert). All the oils were effective at varying period of time. Sesame oil, Wheat germ oil and Citronella oil showed attraction against larvae and adults of Khapra beetle whereas the other oils: Almond oil, Coconut oil, Mustard oil, Tea tree oil and Camphor oil showed repulsion effect against beetles.

Generally, larvae of Khapra beetle preferred the highly protein food. They attack grain by gnawing at various parts of kernel, and usually begin their attack at some weak place in the pericarp or seed coat of the wheat grain. Barak [18] identified that sesame oil was more attractive to larvae than wheat germ oil. The wheat germ oil is also considered as highly proteinaceous which acts as a larval food attractant. These essential oils are useful as grain protectants and fumigants. Gharsan [19] identified the repellent effect of 6 plant essential oils against Khapra beetle larvae. The studies of Padin *et al.* [20] reported similar results with camphor oil while conducting the studies on the repellency factor on blowflies. Batool & Mallah [21] identified the effectiveness of the vegetable oils and insecticides mixtures, against the larvae of the Khapra beetle. The results revealed that the vegetable oils exhibited different synergistic, potential and antagonistic effects. In 2001, the toxicity, repellency, and inhibition induced by grounded leaves, leaf extracts, and essential oil against *Sitophilus oryzae* and *Tribolium castaneum* was investigated [22].

The various research experiment protocols were used to know the efficacy of attractants/repellants on stored grain pests. The mode/s of action, appropriate dosages and duration of efficacy of oils has been investigated by various workers on different storage pests [23]. The presence of secondary compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, gives plant materials or their extracts for their anti-insect activity. Secondary compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, and antifeedants or modify oviposition [24]. They may retard or accelerate development or interfere with the life cycle of the insect in other ways [25]. In addition to action against adult insects, vegetable oils are generally reported to exert ovicidal action [26]. It was suggested that egg mortality was caused by the physical properties of the oil coating, blocking respiration, rather than by a specific chemical effect.

The present theme of studies thus revolves around the importance of food grain storage and the havoc caused by storage pests. Among them, the status of *Trogoderma granarium* (Evert) was given as notorious pest which causes difficulty in its control. Therefore, it would be a necessity to have an alternate management protocol in the scheme of Integrated Pest Management. The use of locally available plants avoids the need to establish complex mechanisms for pesticide distribution; the community can collect or grow the plants itself. The use of plant materials for storage protection is sustainable, can be continuously propagated year after year, biodegradable and do not have any negative impact on the environment.

Integrated Pest Management (IPM) is socially acceptable, environmentally responsible and economically practical Crop protection. The usage of essential oils in this manner evaluated shall form an important and integral protocol in the holistic scheme of IPM. These oils used in the present studies are safe, non-hazardous, eco-friendly, easily available, handy, easy to use, economical and sustainable besides being found effective. Hence, an integrated approach is required for the control of this pest and is essential for maintaining the quality of grains during storage as well as in the production [27]. In many cases small improvements in storage methods may lead to much better protection of stored product and thus to less loss. A good storage building is one thing, good safety measures is the another aspect. According to De Groot [28] however, good storage practices combined with good hygiene, adequate drying and all other safety measures will not always be effective in preventing storage losses. Although controlling physical, chemical and mechanical factors to a large extent can check the degree of deterioration, spoilage of grains, maximum efforts are required to control the spoilage factors of grains, such as insects and mites, rodents, birds and microflora. The growing awareness about environmental issues as well as health hazards due to the synthetic pesticides and associated problems of pests' resistance are important to be known among people. Therefore, modern concept of insect growth is being developed thereby in term of controlling their population. Natural chemicals are environmentally safer than classical insecticides. For this reason various researchers and users are trying to use plant volatiles/ organic pesticides/ biopesticides for the grain storage purpose.

4. Conclusion

Trogoderma granarium (Evert) has great economic importance due to the capability to cause huge loss of stored grain products. The various control tactics are being implemented to control them. One of the promising alternative approaches will be the use of pheromones and other attractants. The present study was focused on to check the efficacies of eight essential oils whether they are act as a repellant or attractant on Khapra beetle. The results revealed that Sesame oil, Wheat germ oil and Citronella oil showed good attraction for both larvae and adult stages of Khapra beetle. On the other hand, Almond oil, Coconut oil, Mustard oil, Tea tree oil and Camphor oil are act as repellants against larvae as well as adults. Out of all, only citronella oil creates the toxicity among the larvae. Overall, it was a good research and would add a complete package to Integrated Pest Management (IPM). It would also useful in providing good scope for the further development of ecofriendly methods for *Trogoderma granarium* (Evert) control.

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