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Antifeeding and repellent effect of plant extract *Salvia officinalis* against *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae)

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Abstract

In the present investigation, we have tested the antifeedant, insecticidal and repellent activities of different solvent extracts of ethyl acetate and ethanol in *Salvia officinalis* Linn (Lamiaceae) against the selected red flour beetle, *Tribolium castaneum* Herbs. (Coleoptera: Tenebrionidae).The research findings showed that the solvent extracts had many activities. However, the ethanol extract had the highest mortality compared to ethyl acetate of *Salvia officinalis*. Thus, the isolation and identification of the phytochemical group found in the selected plant's ethanol extract are now underway. This present study showed that chemical pesticides would gradually replace phytochemicals.

Keywords: *Salvia officinalis, Tribolium castaneum,* Antifeedant activity, Repellent activity, ethanol, and ethyl acetate.

1. 0 Introduction

In 2050, the global population is expected to exceed 9.1 billion, and food production is expected to increase by 70% to nourish this growing population (United Nations. World Population Prospects 2017). The seriousness of "food insecurity" highlights the enormous challenge of providing everyone with protected, nutrient-rich, and adequate food (WHO 2019). Unfortunately, increased agricultural production alone will not be enough to achieve the "zero hunger target" by 2030 or meet rising food demand.

Pre-harvest and post-harvest issues and insect infestation represent a significant restriction in optimal food production, resulting in massive grain losses. Before reaching the consumer, food grains undergo various processes, such as harvesting time, cleaning, drying, storage, processing, and transportation. It has been determined that food losses in the post-harvest chain start at harvest and continue until food marketing at the consumer's end (Hodges *et al*., 2011; Mesterházy *et a*l., 2020; Grolleaud, 2002). Technical limitations, including poor infrastructure, faulty packing, and inadequate inventory management systems, can also result in grain losses. Poring grains and food products is a significant problem worldwide, and insect pests cause controlled damage (Haq *et al*., 2005). Approximately 9% of stored grain yield is reduced in a favourable environment due to insect pests and mites (Fields, 2006).

Tribolium castaneum (Herbst) is the most serious pest of stored products known as the red flour beetle. It is included in the family Tenebrionidae, i.e. darkling beetles and red flour beetles, and is a common inhabitant of milled cereal products, stored flour, and fungus-infested grain (Park 1962; Beeman 2003; Campbell and Hagstrum 2002; McKay *et al.,* 2019). *T. castaneum* causes damage to flour mills and other locations where dried foods and cereal products are stored or processed. *T. castaneum* adult females lay eggs on flour, complete their life cycle, and deplete the nutritional quality of grains over time. The flour becomes contaminated with a pungent smell in severe infestations and loses nutritional and market value (Payne 1925; A.S 1976). *T. castaneum* contaminates food products through moulting and excretion in addition to direct feeding, making the product commercially undesirable. The grain may be rejected depending on the level of infestation. Infested grains can have a profound impact on human health as well as a negative impact on the environment.

The red flour beetle is quite possibly the main put-away grain bug that has been accounted for to harm an assortment of products such as grains (Atta *et al*., 2020), flour (Naseri *et al*., 2017), peas (Pretheep-Kumar *et al*., 2007), beans (Abdullahi *et al.,* 2018), nuts (Pires *et al*., 2017), dried fruits (Sarwar, 2015) and spices (Tripathi *et al*., 2009). Preventive and therapeutic control measures are used to control stored grain pests. Highly toxic synthetic pesticides have been used for many years among these. However, these chemicals have serious consequences for public health and the environment and insecticide resistance (Madkour *et al*., 2012).

Several studies assessed that leaves *S.officinalis* are the source of insecticidal compounds to identify that rich content of bioactive compound molecules such as flavonoids, alkaloids, polyphenols, and tannins (Iqbal *et al*., 2006; Sharma *et al*., 1998), shows the source for new biopesticides (Rajashekar *et al*., 2014). These botanical insecticides have several advantages over synthetic ones, including environmental safety, less hazard, cost-effectiveness, and easy availability (Gupta and Pathak, 2009; Tembo *et al*., 2018).

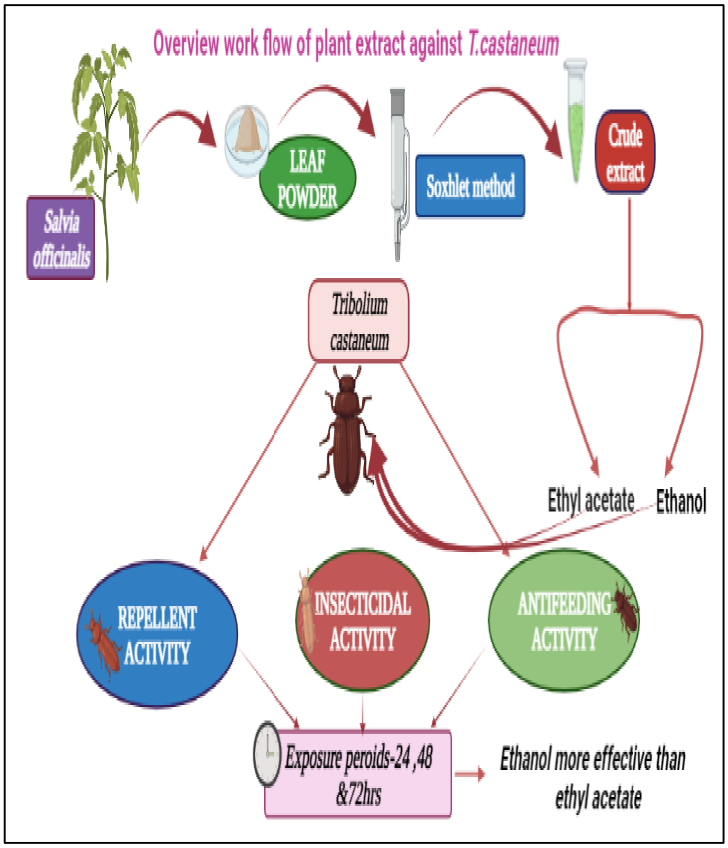

Fig1 Overview concept of work

The goal of this current study is to evaluate the effectiveness of *Salvia officinalis* ethanol and ethyl acetate extracts against the red flour beetle, *T. castaneum*, as well as to detect the antifeeding, insecticidal and repellent properties of various solvent extracts.

2.0 Materials and Methods

The research was conducted at the Department of Zoology in Government Arts College Nandanam. Insects were collected from the grains in the nearby local market. The insects are tested in a glass beaker under laboratory conditions. The beakers were closed with muslin cloth and tied with a rubber band to avoid the discharge of insects.

2.1 preparation of plant extract:

The fresh leaves of *S. officinalis* were collected from Kerala. Then, the leaves were dried shade at room temperature (28±1°C) for about 15 days. The dried leaves were powdered in the electric blender and stored in polyethene bags. The powder was packed in filter paper, and then the extract was extracted in the Soxhlet apparatus at 1:5 (plant powder: solvent) in ethyl acetate and ethanol solvent. After eight hours of extraction, the extract was kept in rotatory evaporation for 48 hrs. Then, extracts were stored at 4 °C in the refrigerator.

2.2 Rearing of *T.castaneum*:

T. castaneum was reared on sooji in our laboratory. The culture was maintained in the dark at 28 ± 2º C and 60 ± 5% RH. All adults used in the experiments were 1-10 days old. In the experiments, the day of death of the adult beetles was determined as the day the antennae and legs did not move upon gently disturbed using forceps.

2.3 Anti-feeding characteristic of crude extract against *T.castaneum*:

The probability of the antifeeding activity of the plant extracts was determined by Talukder & Howse (1993). Sooji flour was used as treated feed. The sooji flour was sprayed with two different solvent extracts, air-dried for 30 minutes, and then weighed 200 gmof sooji in each box after 20 weevils were placed on them. The experiment was carried out by two different methods choice method and no-choice methods. In this choice method, feed with the prospect between treated (T) and control(C). In this no-choice method feed without the prospect of treated (TT) samples. Sooji was sprayed with three various periods: 20, 40, and 60 seconds. After 2 days again sooji flour was reweighed. The experiment was designed in a randomized complete pattern with five replications. Finally, the weight loss of sooji was estimated and the amount of feed consumed (FC) was also calculated.

$FC = IW - [(FW \times IW)/FW]$

FC= feed consumed
IW= initial weight of sooji ; FW= final weight of sooji

The amount of feed consumed in two different tests (TT and CT) was calculated:

Absolute coefficient of deterrence $A = (TT - CT/TT + CT) \times 100$

Relative coefficient of deterrence $R = (C - T/C + T) \times 100$

The total coefficient of deterrence $T = A + R$

2.4 Repellent characteristic of crude extract against *T.castaneum*:

Five plastic boxes serve as the basis of the bioassay system (8cm 5cm). These five boxes are divided into four for treatment and one for control. Plastic tubes with a 12 cm tube connecting their lumens were used to connect the five boxes. After ten pairs of unsexed adults were introduced in the insect chamber box. The different concentrations of 200ppm, 400ppm, 600ppm, and 800ppm. Neem Azal was for the control treatment. Five replications were repeated.

After 24, 48, and 72 hrs, control and treated boxes were calculated by using Lwande's method

$$EPI = \frac{Nt - Nc}{Nt + Nc} X\ 100$$

Nc- Number of insects in control sample
Nt- Number of insects in treated sample

2.5 Insecticidal characteristic of crude extract against *T.castaneum*:

The insecticidal activity was performed on the newly emerged adults against *T.castaneum.* The Whatman no.1 filter paper was treated with different concentrations (100, 200, 300, and 400 ppm) of different plant extracts and was allowed to dry for 10 minutes. Then, the filter paper was attached to the cap of the lid internally and in each plastic jar, twenty adult weevils were introduced in each box. Control was treated with Neem Azal. The insecticidal activity was calculated by using Abbott's formula.

$$POD = \frac{Ts - Cs}{Cs} X100$$

POD: Percentage of damage
Ts: Number of insects in treated sample.
Cs: Number of insects in control.

3.0 Results

Table 3.1 Repellent activity of ethyl acetate extract of *S.officinalis* tested against *T.castaneum*

Concentration	Exposure periods (in Hrs)		
	24 Hrs	**48Hrs**	**72Hrs**
200 ppm	13.5± 0.42^{a}	21.2±1.47^{a}	27.6±0.46^{a}
400 ppm	33.8±0.79^{b}	40.2±0.55^{a}	52.3±0.84^{b}
600 ppm	60.7±1.12^{b}	64.3±0.71^{a}	68.7±1.33^{c}
800 ppm	79.2±0.97^{c}	82.8±0.69^{b}	94.7±0.88^{c}
Neem azal	100.0±0.00^{d}	100.0±0.00^{d}	100.0±0.00^{d}

Values expressed are mean mortality ± standard deviations of five replications (n=20). Values with a different alphabet in the column show statistical significance at P<0.01% level.

The repellent activity of *S. officinalis* was tested against the stored pest of *T.castaneum* and the data obtained in the experiment are shown in table 3.1 and figure 3.1. It was observed that the highest repellent activity in the maximum concentration 800ppm in 24hrs (79.2±0.97) 48hrs (82.8±0.69) and 72hrs (94.7±0.88). In the same way 600ppm concentration induces24hrs ((60.7±1.12) 48hrs (64.3±0.71) and 72hrs (68.7±1.33). Likewise, 24hrs (33.8±0.79) 48hrs (40.2±0.55) and 72hrs (52.8±0.84) and (13.5±0.42) 48hrs (21.2±1.47) and 72hrs (27.6±0.46) mortality were observed from the concentrations of400 and200ppm respectively.

Table 3.2 Repellent activity of ethanol extract of *S.officinalis* tested against *T.castaneum*

Concentration	Exposure periods (in Hrs)		
	24 Hrs	**48Hrs**	**72Hrs**
200 ppm	17.7± 0.56^{a}	19.3±1.26^{a}	22.6±0.38^{a}
400 ppm	31.4±0.99^{b}	47.4±0.85^{a}	55.3±0.64^{b}
600 ppm	66.1±1.31^{b}	61.3±0.92^{a}	67.9±1.43^{c}
800 ppm	84.5±1.87^{c}	89.9±1.04^{b}	96.2±1.86^{c}
Neem azal	100.0±0.00^{d}	100.0±0.00^{d}	100.0±0.00^{d}

Values expressed are mean mortality ± standard deviations of five replications (n=20). Values with a different alphabet in the column show statistical significance at P<0.01% level.

The repellent activity of *S. officinalis* was tested against the stored pest of *T. castaneum* and the data obtained in the experiment are shown in table 3.2 and Figure 3.2. It was observed that the highest repellent activity in the maximum concentration 800ppm in 24hrs (84.5±1.87) 48hrs (89.9±1.04) and 72hrs (96.2±1.86).In the same way 600ppm concentration induces 24hrs ((66.1±1.31) 48hrs (61.3±0.92) & 72hrs (67.9±1.43). Likewise (31.4±0.99) 48hrs (47.4±0.85) & 72hrs (55.3±0.64) and (17.7±0.56) 48hrs (19.3±1.26) & 72hrs (22.6±0.38) mortality were observed from the concentrations of 400 and 200ppm respectively.

Figure 3.1 Repellent activity of ethyl acetate extract of *S.officinalis* tested against *T.castaneum*

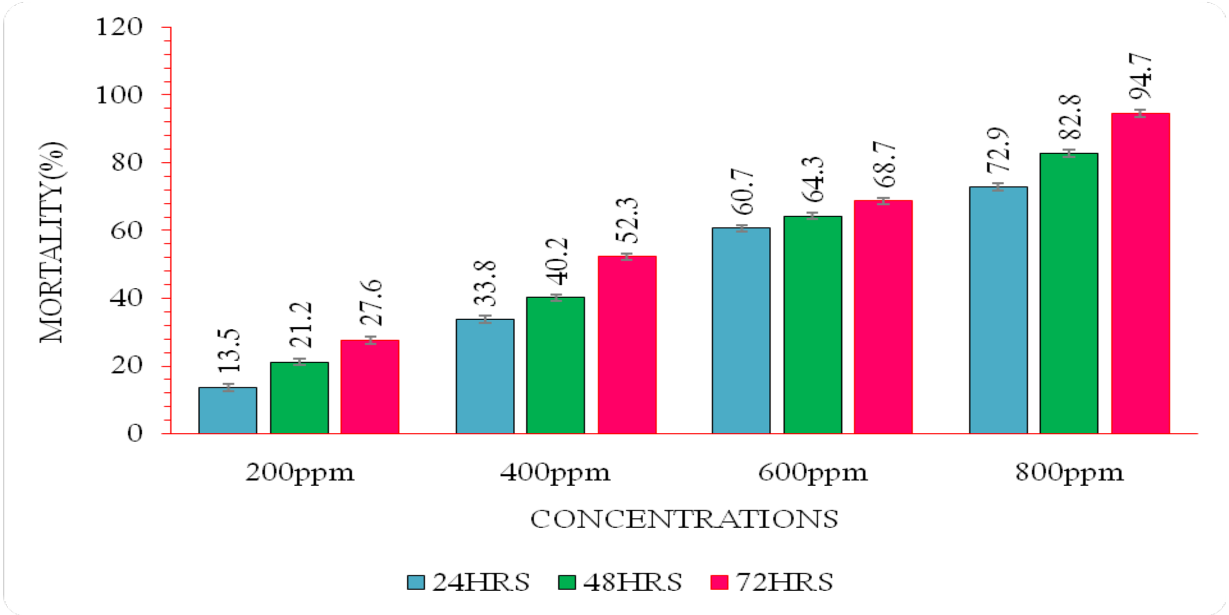

Figure 3.2 Repellent activity of ethanol extract of *S.officinalis* tested against *T.castaneum*

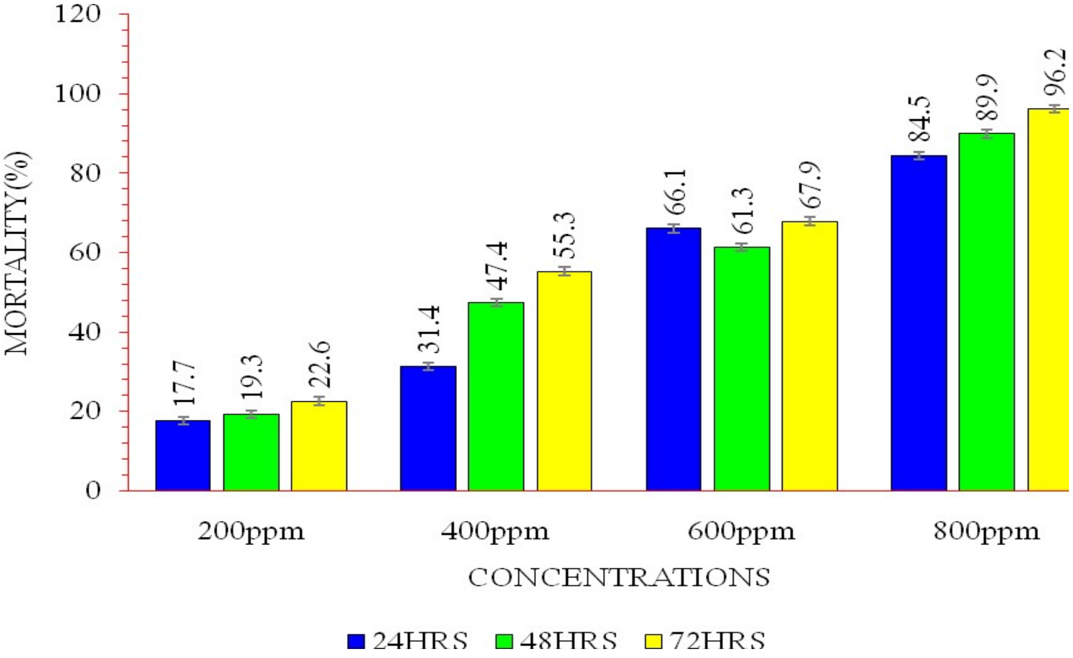

Table 3.3 Antifeeding deterrence coefficients of crude extract of *S.officinalis* tested against *T.castaneum*

Solvents	Exposure time (min)	Coefficients of deterrence			Efficacy of *S. officinalis*
		Absolute	Relative	Total	
Ethyl acetate	20	27.41	40.21	67.62c	+
	40	44.62	53.67	98.29bc	++
	60	57.01	84.46	141.47abc	+++
Ethanol	20	32.09	59.30	91.39c	+
	40	54.67	73.42	128.09bc	++
	60	78.41	97.87	176.28abc	+++

The analysis of the antifeedant effect of *S. officinalis* shows that ethanol extract was more significant than ethyl acetate. Similarly, results of antifeedant effect on *S. oryzae* and *T. castaneum* were observed by Talukder & Howse (1993, 1994) with four different extracts of *Aphanamixis polystachya*, acetone extract being the most significant with a total deterrence coefficient of 159.5. Valladares *et al*. (2003) also indicated an antifeedant effect of ethanol extract of senescent leaves of *Melia azedarach* on *S. oryzae*, with an antifeedant index of 100%.

Table 3.4 Insecticidal activity of ethyl acetate extract of *S.officinalis* tested against *T.castaneum*

Conc	Exposure periods (in Hrs)		
	24 Hrs	**48 Hrs**	**72 Hrs**
200 ppm	15.7± 0.32^{a}	18.2±0.67^{a}	20.5±0.58^{a}
400 ppm	29.8±0.86^{b}	31.9±0.95^{a}	42.7±0.91^{b}
600 ppm	58.5±0.62^{b}	64.2±1.41^{a}	78.1±1.42^{c}
800 ppm	89.2±1.97^{c}	92.3±1.89^{b}	97.0±0.88^{c}
Neem azal	100.0±0.00 ^{d}		
Values expressed are mean mortality ± standard deviations of five replications (n=20). Values with the different alphabet in the column show statistical significance at P<0.01% level			

The insecticidal activity of *S. officinalis* was tested against the stored pest of *T. castaneum,* and the data obtained in the experiment are shown in Table 3.4 and Figure 3.3. It was observed that the highest repellent activity in the maximum concentration 800ppm in 24hrs (89.2±1.97), 48hrs (92.9±1.89), and 72hrs (97.0±0.88).Similarly 600ppm concentration induces24hrs (58.5.5±0.62) 48hrs (64.2±1.89)&72hrs (78.1±1.42). In the same manner 24hrs (29.8±0.86) 48hrs (31.9±0.95) & 72hrs (42.7±0.91) and (15.7±0.32) 48hrs (18.2±0.67) & 72hrs (20.5±0.58) mortality were observed from the concentrations of400 and200ppm respectively

Table 3.5 Insecticidal activity of ethanol extract of *S.officinalis* tested against *T.castaneum*

Conc	Exposure periods (in Hrs)		
	24 Hrs	**48Hrs**	**72Hrs**
200 ppm	16.5± 0.26^{a}	19.9±0.22^{a}	22.6±0.63^{a}
400 ppm	31.4±0.89^{b}	43.6±0.55^{a}	62.4±0.97^{b}
600 ppm	67.5±1.06^{b}	74.7±0.86^{a}	88.7±129^{c}
800 ppm	89.8±1.57^{c}	92.3±1.49^{b}	98.5±1.88^{c}
Neem azal	100.0±0.00 ^{d}		
Values expressed are mean mortality ± standard deviations of five replications (n=20). Values with the different alphabet in the column show statistical significance at P<0.01% level			

The insecticidal activity of *S. officinalis* was tested against the stored pest of *T. castaneum,* and the data obtained in the experiment are shown in Table 3.5 and Figure 3.4. It was observed that the highest repellent activity in the maximum concentration 800ppm in 24hrs (89.8±1.57), 48hrs (92.3±1.04), and 72hrs (98.5±1.88). Similarly, 600ppm concentration induces24hrs (67.5±1.06) 48hrs (74.7±0.86) & 72hrs (88.7±1.29). In the same manner (31.4±0.89) 48hrs (43.6±0.55) & 72hrs (62.4±0.97) and (16.5±0.26) 48hrs (19.9±0.22) & 72hrs (22.6±0.63) mortality were observed from the concentrations of400 and200ppm respectively

Insect culture **Insecticidal activity**

Repellent activity **Anti-feeding activity**

Fig 2. Represent the lab activity

4.0 Discussion

Protecting crops from insects is one of the most critical global issues. Insects were commonly controlled with artificial insecticides, whose toxicity puts the health of agricultural workers, animals, and food consumers at risk. Because of the detrimental impacts on human health and their negligible costs and environmental consequences, plant-based pesticides have grown in popularity. This article discusses the anti-insect properties of beneficial plant substances (such as essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids) and their importance as a substitute for chemicals used in insect removal in a variety of ways, such as growth retardants, chemosterilants, repellents, antifeedants, toxicants, and attractants. Therefore, using botanical insecticides as an integrated insect management program can prominently reduce the use of artificial insecticides (Wafaa *et al.*, (2017).

Atta *et al.,* (2020) assessed that the greatest mortality was reached with the highest dose of crude extract of *Z. officinale* at 10 days exposure interval. The findings of this study supported the use of *Z. officinale* because of its poisonous and repellent properties, which ultimately improve the grain quality of stored grains, in lowering the population of *T. castaneum.*

Many plant species produce a wide range of chemical compounds that can be toxic, deterring, or repellant to insect pests. Some of these substances are also toxic to plants, so they are kept in particular organs like seeds and flowers. These substances are specially designed to combat pest insects that feed on plants. (Shojaaddini *et al*., 2008). Monoterpenes could be one of the most effective and safest alternatives to artificial insecticides (Popovic *et al*., 2013). Monoterpenes can enter the insect's respiratory system and rapidly interact with physiological functions. Similar to the effects of pyrethroid insecticides, these substances can also directly stimulate as neurotoxic substances, affecting acetylcholinesterase activity and causing hyperactivity, convulsions, and tremors.

According to Pugazhvendan *et al*. (2009), stored food grains suffer severe damage from insect infestation. The losses range from 5 to 30% of total agricultural output worldwide. *T. castaneum* has been discovered in various commodities, including cereals, flour, peas, nuts, dried figs, and spices. It infests groundnut kernels and pods as well. Due to its high reproductive potential, this insect causes significant loss in storage and can breed all year in warm areas. Synthetic pesticides are discouraged due to their adverse effects on human health and the environment. The maximum repellent activity was noticed in *T.purprea* powder, with EPI values of -0.11 and -0.56 at 1 and 6 hours against *T. castaneum*, respectively.

Likewise, Nujira Tatun *et al*. (2014) studied the extracts of *R. communis* and *C. papaya*, which were controlled in nutrition, improved larval mortality, and extended the intervals of the larval and pupal periods. The ratios of pupated and emerging adults and the amount of F1 offspring were decreased by feeding them. Also, Mostafa *et al.,* 2012 showed that four plant extracts revealed a strong to moderate toxicity at different concentrations on red flour beetle. *Cucumis sativus* extracts have the highest mortality among leaf extracts, whereas *Psidium guajava* extract showed the lowest mortality rate (50%).

Extracts of *Fumeria indicia, Viola odorata,* and *Linium statism* at various percentage doses were applied for their significant effects on insect mortality and population growth rate. After the application of revealed that *V. odorata* extract significantly displayed higher insect mortality, lower population growth rate levels, and the least reduction in grain weight (Waqar Islam and others, 2016).

Similarly, the repellent activity of clove, coriander, neem, and mint extracts against red flour beetle destroys storage grains and other crops. The findings of this investigation demonstrated that these plant extracts have successful insect-repelling properties. It concluded that these plants are natural sources of repellent material and are a potential source of natural/biological insect repellents (Abid Ramsha *et al*., 2019)—the efficacy of several medicinal plant extracts to kill *T. castaneum*, which infests stored goods. According to the findings, *A. sativum* (garlic) and *Z. Officinale* (ginger) were more active, causing a 15 times increased adult death rate and a 4 to 5 times reduction in grain weight losses. Surprisingly, either the lowest or highest doses showed improved control. The findings of this study suggest the use of botanicals for pest management of stored merchandise (Faheem Ahmed *et al*., 2019).

Likewise, plant oil has a high potential to be used as a bio-pesticide in managing *the T. castaneum* pest (Jeyasankar and Cheeaiyan 2016). Additionally, Agarwal and Agasha (2020) analyzed the microbiome of a crucial red flour beetle, a widespread pest of stored cereals. The beetle was a complete flour lifecycle, including habitat, food, and microbes. Finally, under various tested conditions, the microbiome was identified as not essential for survival and growth. So, it was concluded that *Tribolium castaneum* closely mimicked the host species' natural niche.

Previously, researchers Shamjana and Grace 2022 studied *T. castaneum* resistance mechanisms and used bioassays and genetic and biochemical techniques. The use of whole-genome and gene expression analysis sequencing channels provided a more extensive collection of gene resources for future research into *T. castaneum* resistance mechanisms, whichinclude 1) target site insensitivity, 2) metabolic resistance, and 3) Less cuticular penetration. These various resistance mechanisms have been identified to act in layers to deteriorate the insecticides faster before they release their toxic effect, as shown in Fig 3.

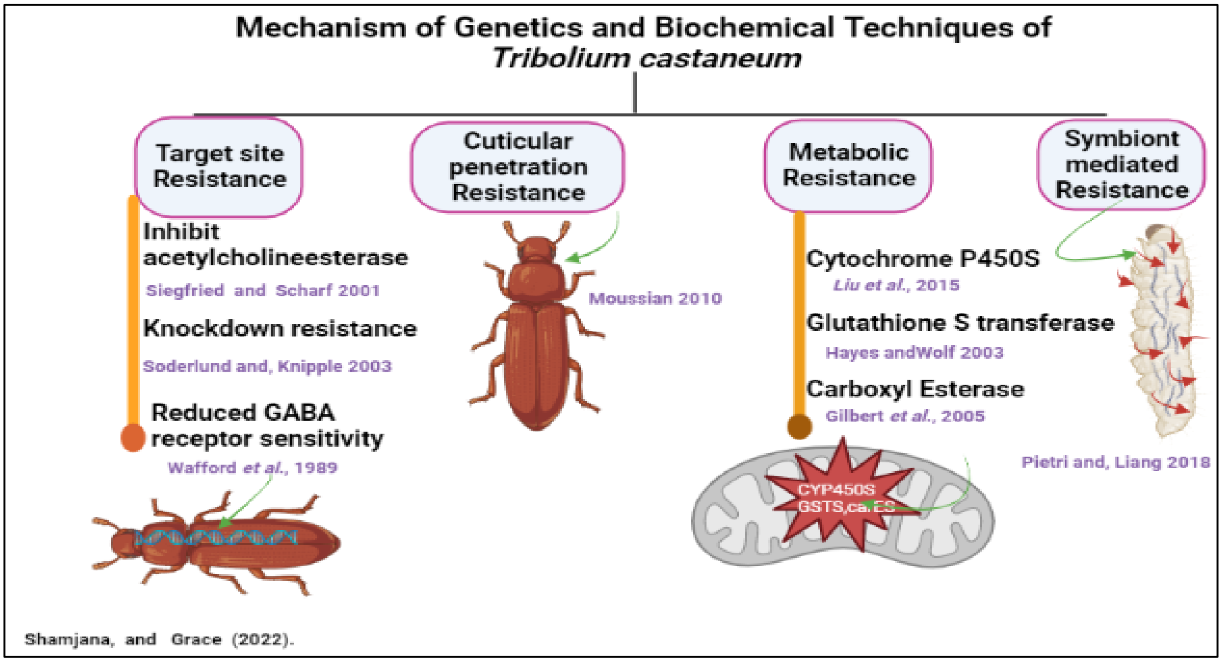

Figure-3-Mechanism of crude extracts against *T.castaneum*

Because of their persistent nature, these chemicals pose a significant environmental risk due to their increased risk of neurotoxic, carcinogenic, teratogenic, and genotoxicity effects in non-target animals, chronic residual toxicity, ability to cause hormonal imbalance, and hematotoxicity. (Khatter 2011). However, there is currently one alternative for protecting stored grains from insect damage. Plants are a good source of pest control compounds. The plant kingdom can be a rich source of chemicals with the potential for development as effective pest control agents.

Hence, it concluded that the effect of solvent extract of *S. officinalis* was tested on the stored beetles-*T. castaneum* and it was observed that the toxicity of solvent leaf extract was influenced by the solvent as well as the aromatic fragrance of the plant. The highest toxic effect was observed in the ethanol of *S. officinalis*. The highest mortality percentage was found to be directly proportional to the level of doses of plant extract. The powder of these botanicals may have also disrupted the respiratory activities of *T. castaneum,* leading to asphyxiation and death.

Conclusion

T. castaneum is one of the most prevalent and frequent pests in red flour and wheat flour in various agricultural products. Farmers who store their belongings in containers will suffer a significant loss. For the study, two different solvents were used to extract the plant S. officinalis: ethanol and ethyl acetate. *S. officinalis* extracts from various solvents were tested at various concentrations, and their potency was observed and calculated. The best efficiency was found in the ethanol extracts from this seed, which have a high polarity index.

The current analysis indicates that plant-based substances are easily degradable and do not affect humans, animals, or the environment. So, *S. officinalis* could be used as the best and most potent natural agent to control the stored red flour pest of *Tribolium castaneum*.

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