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Efficacy of certain Indian spices against red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) in stored wheat

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ABSTRACT

Red flour beetle *Tribolium castaneum* (Herbst) is one of the major destructive pests of stored grain, emitting an unpleasant odor during severe infestation via secreting quinine. Heat treatment, fumigation, and insecticides have shown promising results against the *T. castaneum* infestation, but on the verge of insect resistance and severe issues to human health and the environment. Here, we have shown the efficacy of Indian spices viz, *Cinnamomum zeylanicum* (cinnamon), *Myristica fragrans (*nutmeg), *Piper nigrum* (black pepper), *Syzygium aromaticum* (clove), and *Trigonella foenum-graecum* (fenugreek) against *T. castaneum* infestation in stored wheat. Clove and black pepper exhibited the highest effectiveness causing 98.3% and 96.6% of mortality respectively. In the context of joint action, Cinnamon powder was taken as a standard, in a ratio of 1:1 with other spice powders. The objective was to assess whether these combinations exhibited synergistic or antagonistic effects. Among the different spice mixtures tested, the combination of cinnamon and clove showed 33.3% mortality within 24hr. All the spice combination results indicate an antagonistic relationship, indicating the absence of any synergistic effects. And in terms of repellency, the evaluation of different spices indicated that clove exhibited a significant repellent effect, with a remarkable 66.6% repellency rate within 12hr. So, the result shows this trend (mortality- Clove>Black pepper>Cinnamon>Nutmeg>Fenugreek) (Joint action- Cinnamon + Clove > Cinnamon + Black pepper > Cinnamon + Fenugreek>Cinnamon + Nutmeg) (Repellency- Clove>Black-pepper>Cinnamon>Nutmeg>Fenugreek) the following was an effort to find an alternative management strategy for pest control and shows that these spices possess components that impart insecticidal and repellence activity.

KEYWORDS: *Tribolium castaneum*, Indian spices, natural insecticides, eco-friendly pest control.

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most widely cultivated crops in the world(Madavi et al. 2022). India is the second largest producer of wheat in the world after China with millions of farmers growing it across the country(Kumar et al. 2020). According to the Ministry of Agriculture and Farmers Welfare, the total wheat production in India during the 2020-21 crop year (July-June) was estimated at around 106.24 million metric tons (Madavi et al. 2022). Post-harvest losses of wheat can occur due to various factors, out of which insect alone causes 5-10% damage(Kalita and Bhola 2021; Shakera Tun Noor Nova 2020; Ali et al. 2016). A variety of insect pests infest the stored food grain, among them *Tribolium castaneum* (Herbst) is the most widespread and destructive pest (Kumar et al. 2020). This has caused 60-70% postharvest losses in India (Ojianwuna and Enwemiwe 2021). This insect causes significant damage to wheat by grain loss, contamination, reduced germination, and a reasonable decrease in nutritional value(Sagheer et al. 2016).

Tribolium castaneum (Herbst) is a species of the family Tenebrionidae, the darkling beetle, also known as the red flour beetle(Kalita and Bhola 2021). It is a most widespread and destructive secondary pest of damaged food grain which affects the quantity as well as the quality of the commodity (Babarinde et al. 2018; Jaleel et al. 2015a)(Jaleel et al. 2015b) such as grain, flour, cereals, meal, crackers, beans, spices, dried pet food but the most preferred food is he milled grain product such as flour(Sagheer et al. 2016). Red flour beetles are small in size and have chewing mouthparts. These beetles are reddish brown, and their antennae are club-shaped with three segments. The adults can live for several months and during this time females lay hundreds of eggs, starting the cycle which includes egg, larva, pupa, and Adult (Kalita and Bhola 2021; Madavi et al. 2022) *Tribolium castaneum* feed on dust or exposed surface of the grain, both adult and larva damage the grain. The damage can be physical and nutritional and produces an unpleasant odor (Sagheer et al. 2016; Madavi et al. 2022; Mansour Ismail and Mohamed Amin Sleem 2021; Sundar et al. 2021a) (Sundar et al. 2021) Red flour beetles secrete a chemical that includes quinines, which affect product quality(Manonmani, Usha Rani, and Ramar 2017).

Spices are derived from natural sources and can be effective in controlling pests in stored grains, including wheat(Meena and Lal 2019). Spices are considered safe for the environment, unlike synthetic pesticides, which can have harmful effects on non-target organisms and the ecosystem, spices do not leave toxic residues that can contaminate the soil, water, and air(Farhana, Islam, and Islam 2006). Overuse of synthetic pesticides can lead to the development of resistance in pest populations. Using spices as an alternative can help prevent or delay resistance development. spices are generally considered safe for human consumption and do not pose a significant health risk if accidentally ingested(Padín et al. 2013).) Spices and their derived products are both biodegradable and can effectively combat the pest without harming beneficial insects(Ushasri et al. 2022)

PHYTOCHEMICALS OF DIFFERENT SPICES

S.no.	Spice	Botanical name	family	Part use	Chemical release	Reference
1-	Cinnamon	*Cinnamomum Zeylanicum*	Lauraceae	Bark	α -pinene, β-pinene, b-caryophyllene, amuurolene, γ-cadinene, δ-cadinene and amuurolol	(Farhana, Islam, and Islam 2006)
2-	Fenugreek	*Trigonella foenum-graecum*	Fabaceae	Seed	Trigonelline, choline, gentianine, carpaine and Saponins	(Farhana, Islam, and Islam 2006)
3-	Black pepper	*Piper nigrum*	Piperaceae	fruit	Piperine, piperidine, piperettine, β- pinene and α-pinene, δ-Iimonene and β-caryophyllene, linalool oxide and α-terpineol.	(Farhana, Islam, and Islam 2006)
4-	Nutmeg	*Myristica fragrans Houtt.*	Myristicaceae	fruit	Sabinene, myristicene, α-Pinene, β-Pinene and terpinen-4-ol.	(Farhana, Islam, and Islam 2006)
5-	Clove	*Syzygium aromaticum*	Myrtaceae	Flower bud	Eugenol, β-caryophyllene, eugenyl acetate, methyl eugenol, flavonoids, galloyltannins, phenolic acids, and tri-terpenes.	(Farhana, Islam, and Islam 2006)

MATERIALS AND METHODS

The study was conducted at 12ºC ± 5ºC and 94 ± 5% humidity. The experiment was a completely randomized design with 5 treatments and 10 replications of each; a control without any treatment was also included. This methodology is from (Saxena, 2016) though some changes are made.

Rearing of test insect

Tribolium castaneum was cultured using fresh wheat kernels as the substrate. The whole grain wheat, containing adult red flour beetle *Tribolium castaneum* was collected. The collected infected wheat was housed in a plastic jar covered with a lid to prevent adults' escape. To prepare the substrate, fresh wheat grains were washed with distilled water and dried in sunlight. Sterilized jars were taken and half filled with wheat. Each jar was then populated with a hundred beetles, (both male and female) and the mouth of the jar was covered with muslin cloth secured with a rubber band. After about two weeks of oviposition, alive adult and dead beetles were removed and wheat was left undisturbed with laden eggs until the emergence of the adult.

After 35-40 days new *Tribolium castaneum* emerges from the stored wheat. The newly emerge beetles were then separated. The emerging adults were used for the experiment.

Preparation of spice powders

Five spices were used viz, Bark of *Cinnamomum zeylanicum* (cinnamon), the fruit of *Myristica fragrans (*nutmeg), fruit of *Piper nigrum* (black pepper) flower bud of *Syzygium aromaticum* (clove), and the seed of *Trigonella foenum-graecum* (fenugreek).

These spices were air dried for 5 days and then powdered in a grinder to their possible finest proportion. The powder was stored in a dry container with a tag mentioning the name of the spice for further use in the experiment. The mixture of Cinnamon extract and other spices was also prepared at a 1:1 w/w ratio for the experiment. So, four mixtures were prepared for demonstrating joint action against *Tribolium castaneum.* 100gm of wheat with 10gm of spice powder was taken for each treatment.

Bioassay 1: Study of the toxicological impact of spice powder on *Tribolium castaneum*

Small plastic glasses of diameter 70mm, and height 82 mm were washed and dried. Prepared 10gms spice powder mixed with 100 grams of wheat in each plastic glass. 20 adult red flour beetles were introduced in each glass and the mouth of the glass was covered with a muslin cloth secured by rubber bands. To prevent the beetle from crawling on the sides, petroleum jelly was applied inside each plastic glass just above the layer of wheat. To ensure proper identification of the spice-wheat mixtures, each container was labelled with the name of the spice used. The experiment involved monitoring the mortality rate by counting the number of dead red flour beetle after 12 hours, 24 hours, 36 hours, 48 hours, 62 hours, and 72 hours of exposure. To ensure the result's accuracy and provide different data for analysis, each spice-wheat extract was replicated in ten different sets. To establish a baseline for comparison, control having untreated 20 *Tribolium castaneum* in 100 grams of wheat was also replicated in ten different sets, runs in parallel.

$$\text{Mortality \%} = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insect} \times 100$$

Bioassay 2: Study of joint toxicity of spice powder on *Tribolium castaneum*

Another aspect of the experiment was joint action, which is conducted by different combinations of spices, to determine whether there is synergism or antagonism. Synergism also called potentiation occurs when the combined effect of two or more substances is greater than the sum of their individual effects while antagonism, occurs when the combined effect of two or more substances is less than the sum of their individual effects.

The experiment was done by using a mixture of cinnamon with other spices extract. Prepare different combinations of spices in equal parts. Four mixtures were prepared,

1- Cinnamon and Fenugreek
2- Cinnamon and Black pepper
3- Cinnamon and Nutmeg
4- Cinnamon and Clove

Separate plastic glasses were taken with each glass containing a specific combination of spices weighing 10 grams, along with 100 grams of wheat. To maintain proper identification, label each container with the type of spice combination it contains. Place 20 adult *Tribolium castaneum* in each glass then cover the mouth of the glass with muslin cloth. After a duration of 12 hours and 24 hours, the containers were opened, and the number of living and deceased *T. castaneum* was counted. Record and analyze the results to determine which spice combinations were most effective at repelling the insects.
The mortality percentage was calculated by using a formula,

$$\text{Mortality \%} = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insect} \times 100$$

Bioassay 3: Study of repellent action of different spice powders on *Tribolium castaneum*

A repellency assay was carried out by using a petri dish lined with filter paper. Each filter paper is marked into two halves. One-half of the paper is treated with dry dust of each spice and the other half is untreated. 20 adult *T. castaneum* were placed on each filter paper and covered by another petri dish to prevent the escape of the beetle. After about 12 hours insects in treated and untreated areas were counted respectively. For each spice, three replications were taken. For the calculation of the repellence percentage the following formula was used

$$\text{Repellency \%} = \frac{(NC-NT)}{(NC+NT)} \text{ x } 100$$

Where Nc- Number of insects in control (untreated) area
Nt- Number of insects on the treated area.

Statistical analysis-

The collected data were analyzed in a completely randomized design to find significant differences between the clove, black pepper, cinnamon, nutmeg, and fenugreek treatment. The analysis of variance (ANOVA) was applied. The test significance value is P<0.05. the calculation was performed by using IBM SPSS statistical ver. 27 software and graphs are plotted using the GraphPad Prism 9 software for Microsoft.

1

2

Fig 1: Powder form of different spices (Cinnamon, Fenugreek, Clove, Black pepper, and Nutmeg) 2: Experimental setup for mortality effect 3: Experimental setup to evaluate joint action 4: Stored wheat with *Tribolium castaneum* 5: Experimental setup for repellency rate.

OBSERVATION

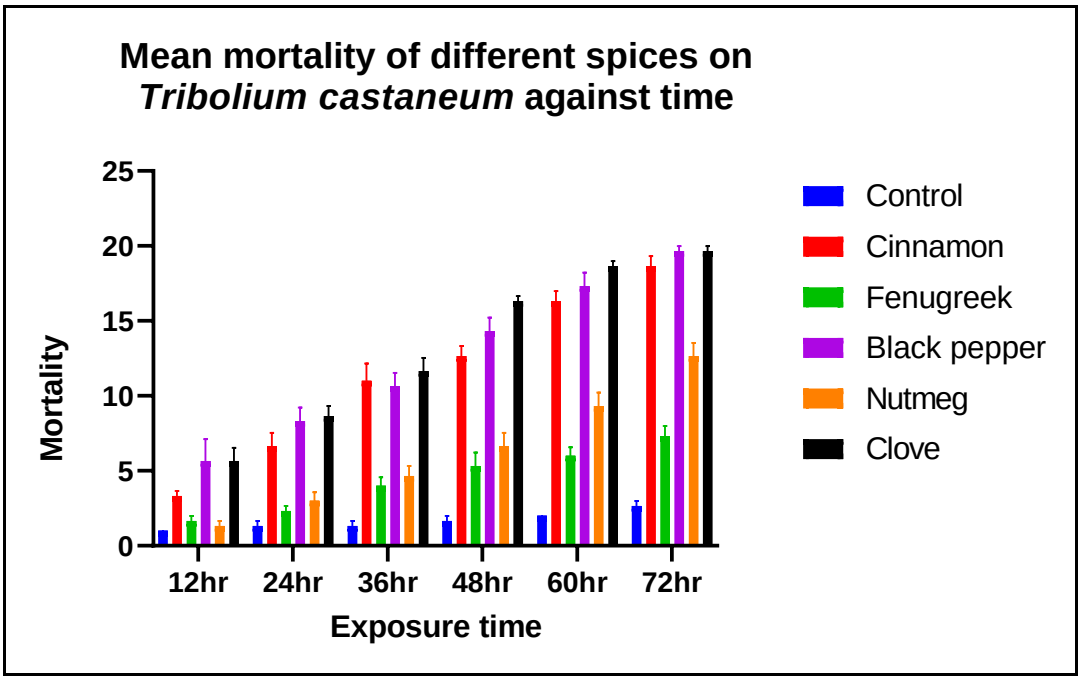
1- Mortality Effect

TABLE 1: Showing mean mortality effect of different spices on *Tribolium castaneum*

S.no.	Treatment	No. of beetle	Mortality (Mean ± S.E)					
			12hr	24hr	36hr	48hr	60hr	72hr
1-	Control	20	1.00 ± 0.00	1.33 ± 0.33	1.33 ± 0.33	1.66 ± 0.33	2.00 ± 0.00	2.66 ± 0.33
2-	Cinnamon	20	3.33 ± 0.33	6.66 ± 0.88	11.00 ± 1.15	12.66 ± 0.66	16.33 ± 0.66	18.66 ± 0.66
3-	Fenugreek	20	1.66 ± 0.33	2.33 ± 0.33	4.00 ± 0.57	5.33 ± 0.88	6.00 ± 0.57	7.33 ± 0.66
4-	Black pepper	20	5.66 ± 1.45	8.33 ± 0.88	10.66 ± 0.88	14.33 ± 0.88	17.33 ± 0.88	19.66 ± 0.33
5-	Nutmeg	20	1.33 ± 0.33	3.00 ± 0.57	4.66 ± 0.66	6.66 ± 0.88	9.33 ± 0.88	12.66 ± 0.88
6-	Clove	20	5.66 ± 0.88	8.66 ± 0.66	11.66 ± 0.88	16.33 ± 0.33	18.66 ± 0.33	19.66 ± 0.33

*Data represented in the tables are the mean of three out of ten replications.
*Test significance level is P <0.05

Graph 1: Showing Mean mortality of different spices on *Tribolium castaenum* against time



2- Joint Action

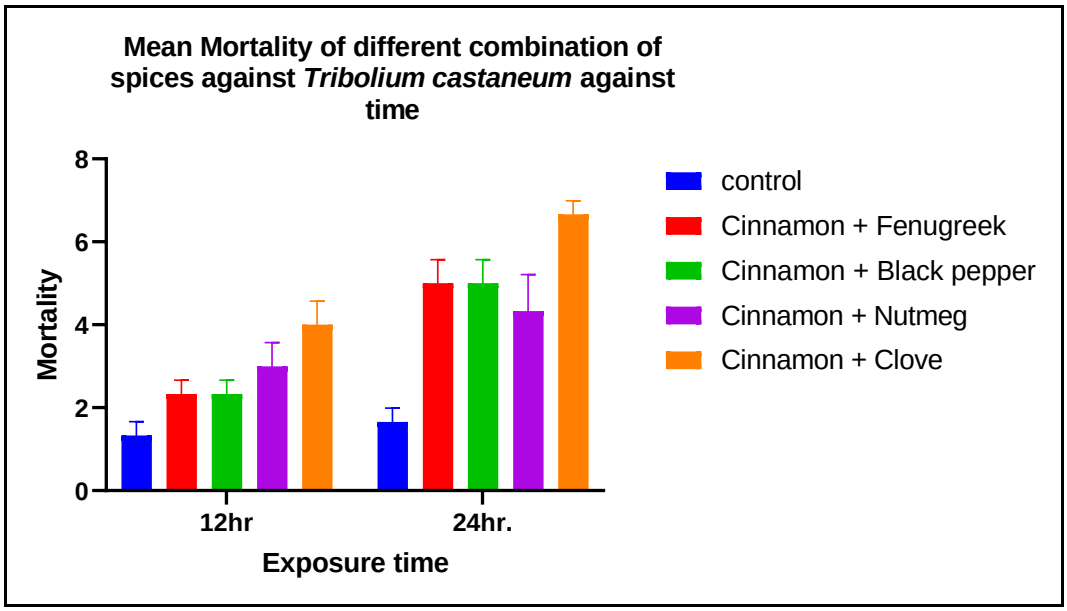
Table 2: Showing mean mortality caused by different spices mixed with cinnamon against *Tribolium Castaneum*

S.no.	Treatment	No. of beetle	Mortality (Mean ± S.E)		Type of joint action
			12hr	24hr	
1	Control	20	1.33 ± 0.33	1.66 ± 0.33	
2	Cinnamon + Fenugreek	20	2.33 ± 0.33	5.00 ± 0.57	Antagonism
3	Cinnamon + Black pepper	20	2.33 ± 0.33	5.00 ± 0.57	Antagonism
4	Cinnamon + Nutmeg	20	3.00 ± 0.57	4.33 ± 0.88	Antagonism
5	Cinnamon + Clove	20	4.00 ± 0.57	6.66 ± 0.33	Antagonism

*Three out of ten replication are considered.

* Test significance level is P<0.05.

Graph 2: Showing mean mortality of different spices mixed with Cinnamon on *Tribolium castaneum* against time

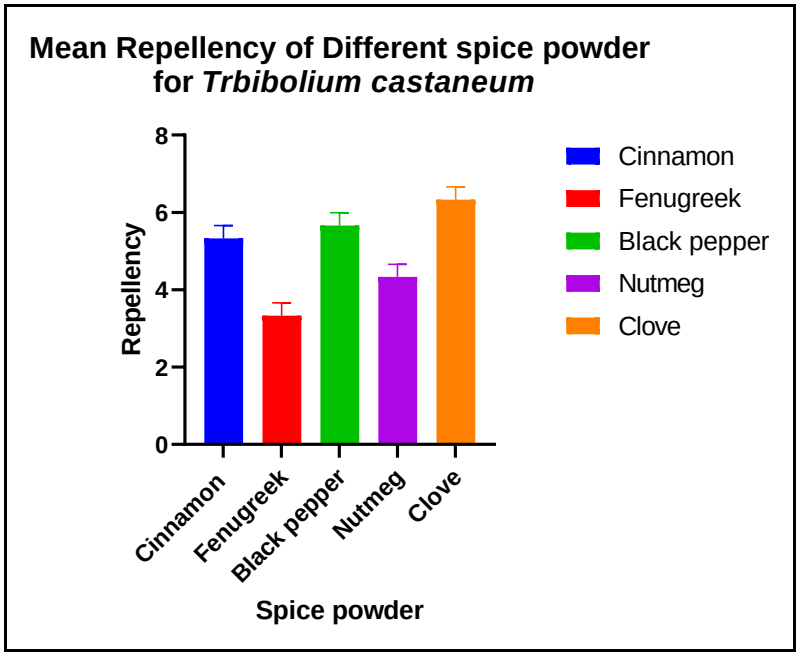


3- Repellence rate

Table3: Showing repellency data of spice against *Tribolium castaneum*

S.no.	Treatment	No. of beetle	Insects present in the untreated area			Insects present in the treated area			Mean ± S. E
			a	b	C	A	b	c	
1	Cinnamon	10	5	5	6	5	5	4	5.33 ±0.33
2	Fenugreek	10	3	3	4	7	7	6	3.33 ± 0.33
3	Black pepper	10	6	6	5	4	4	5	5.66 ± 0.33
4	Nutmeg	10	4	4	5	6	6	5	4.33 ± 0.33
5	Clove	10	7	6	6	3	4	4	6.33 ± 0.33

Graph 3: Showing Mean repellency of different spice powders for *Tribolium castaneum*



RESULT

In the current study, the efficacy of certain Indian spices against *Tribolium castaneum* was examined. The study investigates the mortality, the combined effect, and the repellent action of five different spices. Based on observation, *Syzygium aromaticum* (clove) and *Piper nigrum* (black pepper) exhibit the highest insecticidal properties and were found most effective against *Tribolium castaneum*, followed by *Cinnamomum zeylanicum* (cinnamon). *Myristica fragrans (*nutmeg) possess low potency than cinnamon. *Trigonella foenum-graecum* (fenugreek) was found to be the least effective.

Table 1 and graph 1 showed maximum mortality i.e., 98.3% was observed within 72hr of application in replications of Clove powder, followed by Black pepper and cinnamon showing 96.6% and 93.3% mortality respectively. All the other treatment shows a relatively low level of mortality with Nutmeg showing 63.3% mortality and Fenugreek showing 36.6% mortality. (Clove>Black pepper>Cinnamon>Nutmeg>Fenugreek). According to the result of ANOVA the F value differ significantly at different times of observation, the lowest was F=10.37 at the 12th hour while the highest was F=142.85 at the 72nd hour of observation.

In a joint action, the mortality count was recorded after 24hrs of treatment. The cinnamon powder was taken as a standard in a 1:1 ratio with other spices powder. Table 2 and graph 2 showed that a mixture of *Cinnamomum zeylanicum* and *Syzygium aromaticum* was the most toxic showing 33.3% mortality followed by a combination of *Cinnamomum zeylanicum* and *Piper nigrum* showing 25% mortality. The combination of *Cinnamomum zeylanicum* and *Myristica fragrans*, *Cinnamomum zeylanicum*, and *Trigonella foenum-graecum* was recorded as the least toxic showing 21.6% and 25% mortality respectively. (Cinnamon + Clove > Cinnamon + Black pepper > Cinnamon + Fenugreek>Cinnamon + Nutmeg). So, no synergistic behaviour of Cinnamon powder with

other spice powders has been recorded. All the combination results indicate antagonism. According to ANOVA result the F value post 12 and 24 hrs treatment was 9.93 and 4.83.

According to the analysis of variance (ANOVA), Clove powder exhibited the highest level of repellency, with a significant percentage of 60%. This was followed by black pepper and cinnamon, which showed a repellency percentage of 56.6% and 53.3% respectively. Nutmeg and fenugreek were the least repellents towards *Tribolium castaneum* as shown in Table 3 and Graph 3.

Discussion

The findings of this study demonstrate the highest mortality rate of *Tribolium castaneum* was observed in the treatment of Clove powder (98.3%) and black pepper (96.6%). Parallel work in a similar observation (Devi and Devi 2013) found that treatment with clove powder caused the highest mortality of 100% which stand true for this experiment as the highest mortality rate for the replica treated with clove powder is 98.3%. The result is also supported by a study conducted by (Abo-El-Saad et al. 2011) which showed that clove's insecticidal property is due to some bioactive components. These results confirm (Mahdi and Rahman 1970) observed the Insecticidal effect of some spices on *C. maculatus*. Therefore, it can be stated that clove and Black pepper can be implemented as a biopesticide for managing *Tribolium castaneum.* Black pepper and cinnamon also show significant mortality effects of 96.6% and 93.3% respectively. This result is supported by the study conducted by (R K Upadhyay and Ahmad 2011) where Black pepper significantly reduces the survival of adult and larvae *Tribolium castaneum*. Another experiment by (Ravi Kant Upadhyay, Kant, and Jaiswal 2007)examined the inhibitory activities of Black pepper again red flour beetle that reduces larval transformation into pupae (Onoja 2015)also found that black pepper is efficacious against pests. Other treatments showed comparatively less mortality effect as nutmeg showed 63.3% mortality while fenugreek showed a 36.6% mortality effect. Fenugreek was found to be least effective against *Tribolium castaneum*, this result is supported by (Saxena, 2016)A study conducted by (Devi and Devi 2013) screened various types of spices against red flour beetle and mentioned that nutmeg causes 51.63% mortality. Though the exact cause of mortality in the pest is not fully understood, maybe the presence of bioactive compounds could be responsible for this effect.

The results of joint action reveal that the mixture of *Cinnamomum zeylanicum* and *Syzygium aromatica* extract was the most toxic and the combination of *Cinnamomum zeylanicum* and *Trigonella foenum-graecum* was recorded as the least toxic. However, no synergistic behaviour of the extract of cinnamon with other spice extracts has been reported. All the combination results indicate antagonism. The same pattern of experiment done by (Authors, Haryadi, and Rahayu, n.d.) reported that a mixture of acetone extracts of black pepper and nutmeg in ratios 0.1 and 0.2% reduced the number of F1 offspring and prolonged the development period in *Sitophilus zeamais.*

In the present investigation cinnamon, clove, nutmeg, black pepper, and fenugreek exhibited different trends in repellency. After being agitated, the insects attempted to move toward the untreated filter paper area. The study showed the highest repellency of 60% in clove powder against *T. castaneum*. This was followed by Black pepper and cinnamon showed 56.6% and 53.3% repellency. Contrary to our findings a study (Shayesteh and Ashouri 2010)showed cinnamon powder was most repellent to adults 92.5% and black pepper 58.75%. Nutmeg and fenugreek powder were the least repellents towards *T. castaneum.*

Conclusion

This research paper demonstrates the effectiveness of Indian spices against *Tribolium castaneum*. The best treatment in terms of repellency and mortality was Clove and Black paper powder. The results indicate that these spices possess potent bioactive compounds that exhibit insecticidal properties against the *Tribolium castaneum.* Further investigation is needed to identify the specific bioactive components linked to the aforementioned spices. These spices have the potential to be used as an effective natural alternative to synthetic pesticides for the management of *Tribolium castaneum* infestations. However, further studies are warranted to elucidate the precise mechanisms of action and optimize application methods for maximum efficacy.

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