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Effect of Different Plant Extracts on Tribolium Castaneum (Herbst) Insect

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Abstract:

Different extracts significantly affect the growth and development of the larvae, pupa and adult stages of the insect *Tribolium castaneum*. The most effective inhibitor of the insect is black pepper *(Piper nigrum)* followed by ginger (*Zingiber officinale)*, garlic (*Allium sativum)*, and then onion peels *(Allium cepa)*. As the concentration of the plant extracts in ethanol increases, there is an increase in the percentage of mortality of the insect under study an experimentation procedure. Further, the repellant activity, developmental stages and feeding activity also get hindered after adding the different plant extracts.

Introduction

Biological factors, including pests like birds, rats, fungi, and insects, have historically been the primary cause of both quantity and quality losses during grain storage. Insects generate the biggest problems in storage facilities because they provide ideal circumstances for the growth of rot organisms, mostly fungi (Osuji, 1985; Oke and Muniru, 2002). Effective conservation and maintenance of this core resource are critical for the survival of humanity because of the importance of stored grains and cereal products (Stejskal et al., 2015). Insect pests of stored grains are the leading source of quality and quantity losses to commodities worldwide, ranging from 10 to 40 per cent (Fields, 2006) (Lorini and Filho, 2004). The rust-red flour beetle, Tribolium castaneum (Herbst), is mostly to blame for this problem since it may reduce grain weight by as much as 40 per cent (Ajayi and Rahman, 2006)(Rees, 2007). It feeds on the seeds' endosperm, which causes the seed coats to harden and produce a musty odour (Keskin and Ozkaya, 2013). An effective control method is required to prevent the spoiling of these grains. Hence, the grains lose their viability as food, a planting medium, and a source of sprouts. Synthetic grain protectants are now used in the storing of grain. These substances have disastrous outcomes for the natural world and human health (Salem et al., 2007). In addition, these pesticides have been used so often and without discrimination against stored grain pests that the insects have become highly resistant to them. Natural agents that are just as efficient in controlling insects without endangering human health or lowering grain quality should be investigated (Mahdi and Rahman, 2009; Salem et al., 2007; Fields, 2006).

Being organic and natural, plant-based chemicals might be a starting point for such research. In recent research, some potential natural plant extracts display insecticidal activity in stored grain systems (Tatun et al., 2014) (Tripathi et al., 2009). Human societies have long used plants to deter insect pests from agricultural crops. Farmers all around the globe still employ this method, especially in places with limited access to synthetic pesticides and in organic farming, where plants are utilized either in the form of extracts, company plants, or the harvested plant itself. Insect pests have been the focus of several research on organic plant insecticides (Brian et al. 2020) (Pramoda et al. 2021), but less attention has been paid to the potential effectiveness of plant waste material. The research supports this view (Francis et al., 2001) (Dawit & Bekelle, 2010). Peels of onion, garlic, ginger, and long black pepper are the subject of the current investigation. Ginger

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(*Zingiber officinale*) belongs to the Zingiberaceae family. It's a blooming plant with a rhizome or root used in traditional medicine and cooking. Some genetic varieties among ginger plants may have originated in the tropical rainforests of the Indian subcontinent or Southern Asia (Ferguson and Everett, 1982). Some nations, including Nigeria, Taiwan, India, Jamaica, and Bangladesh, produce ginger extensively. Reports indicate that it is a successful plant in warm regions (Schauenberg P and Paris F, 1977). One of the most widely used plants in traditional medicine, ginger has a long history of usage across many cultures (Ahmad et al., 2019). For centuries, the Chinese have relied on ginger as a medicine for nausea, a digestive aid, and a treatment for rheumatism and bleeding. Baldness, snakebites, toothaches, and respiratory issues were reportedly treated with it (C., Duke and Ayensu, 1985). Some Africans think eating ginger regularly can help repel mosquitoes, whereas in Arabian medicine, ginger is considered an aphrodisiac (Qureshi et al., 1989). (C., Duke and Ayensu, 1985). It's often believed that ginger oil has powerful therapeutic properties. Sesquiterpenes, including bisapolene, zingiberol, and zingiberene are thought to be the primary active components in ginger oil.

Many scientific reports attest to ginger's many therapeutic benefits. Ginger's antioxidant characteristics make it effective in situations of ulcerogenesis, where it serves to protect stomach mucosa from a variety of ulcerogenic substances (Dugasani et al., 2010) "(Gull et al., 2012)" Since prostaglandin has been demonstrated to have a housekeeping and gastro-protective role by preserving stomach mucosal integrity (Ajith, Aswathy, & Hema, 2008), this has both positive and negative effects (Duarte et al., 2008). The strong antiemetic properties of ginger are shown by its ability to stimulate intestinal motility and block serotonin receptors. According to research (Dugasani et al., 2010), ginger may inhibit 5-hydroxytryptamine receptors in the gastrointestinal tract while simultaneously stimulating peripheral anti-cholinergic and anti-histaminic receptors (Gull et al., 2012). It has been shown that ginger has anti-inflammatory effects by blocking prostaglandin production and interfering with cytokine signaling (C., Duke and Ayensu, 1985). The volatile oils in ginger have been linked to the oil's scavenging properties, as revealed by many investigations (C., Duke and Ayensu, 1985). (Kumar et al., 2014). One additional findings revealed that A. *sativum* (garlic) and Z. *officinale* (ginger) were much more effective, resulting in 15 times greater mortality risk and 4 to 5 times reduction in grain weight losses when combined with rice grains (Ahmad et al., 2019). Another study reveals that during the storing process, cereal grains are vulnerable to a variety of insect pests.

The most dangerous pests of stored grains are T. *castaneum.* Adults and juveniles both eat the grains that have been saved for them. A mixture of acetone and deltamethrin was used to apply extracts of Datura *stramonium* and Zingiber *officinale* (Afsar Ali et al., 2020). Influence of herbal extracts Datura *stramonium* and Zingiber *officinale* were used with the new chemical pesticide Deltamethrin at doses of 0.01%, 0.02%, 0.03%, 0.04%, and 0.05%, and a control group was also included (Afsar Ali et al., 2020). The findings have strong statistical significance. The result displays the proportion of T. castaneum that died after being exposed for 72 hours. With a concentration of 0.05% after 72 hours, the mean death rate was 52%, but at a concentration of 0.01% after 24 hours, the mean mortality rate was just 3.45%. An analysis of variance (ANOVA) was used to determine the proportion of Tribolium *castaneum* deaths after 24, 48, and 72 hours following exposure to one of five treatments containing varying concentrations of Zingiber *officinale* (0.01%-0.05%) and one control group. Research showed that higher concentrations were associated with a higher risk of death. The average rate of death was 41.82 percent at a concentration of 0.05% after 72 hours, and it was 0.01 percent at a concentration of 0.01% after 24 hours (Afsar Ali et al., 2020). According to the findings of this research, several plant extracts have the potential to serve as effective insect-control agents. There was significant insecticidal action against T. confusum from S. *aromaticum*, T. *foenum*, and A. vulgaris. The research showed that plant extracts from M. *oleifera* and O. *majorana* may protect flour against T. *confusum* by raising the repellency percentage of the flour (Salim et al., 2019).

The significant death rates observed in the current research suggest that T. castaneum may be protected from further harm by using plant-derived pesticides such as leaf extracts from A. indica, H. *souveolens*, and M. *oleifera*. The largest mortality effects were seen with A. *indica*, making it the most efficient plant extract in this research; the least mortality effects were shown with M. *oleifera*, making it the least recommended. In its stead, though, you may utilize Hyptis *souveolens*, which, while not as potent as A. *indica*, nonetheless had the second-highest

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impact. Because of this, the results of this research imply that Azadirachta indica and H. souveolens are effective plant extracts for managing stored-grain pests, such as the red rust flour beetle, Tribolium *castaneum* (Medugu. A, 2021).

Aims and Objectives:

To study the efficacy of selected plant peel extract on the various life-cycle aspects of the pest insect: (a) adult mortality (b) larva mortility (c) rate of development (d) anti-feedant effect of the extracts (e) repellant study in adults.

Materials and Methods:

The culture of *Tribolium castaneum* will be carried out in infested kernel of wheat. *Tribolium castaneum* will be cultured in a controlled environment room at 28 °C and 70% R.H. in the dark.

Theory:

Extraction Methods and Preparation of formulations:

Many extracts were made from the peels of the three plants chosen. Dried peels were employed for the purpose here.

Powder-suspension

Weighing 10g of powdered leaves, 10 ml of GD water will be added to create a stock solution of powder suspension, which will then be further diluted to concentrations of 50%, 25%, and 10%.

Extract-suspension

Several extracts will be made from the plants' peels. Garlic (*Allium sativum*), ginger (*Zingiber officinale*), and onion *(Allium cepa)*, skins will be air-dried in the shade for usage. The entire peppercorns from the black long pepper plant (Peepali) will be utilized. The soxhlet process, employing double-distilled water and ethanol as solvent, will be used to make extracts from dried, ground plant peels.

Experimental sets

Included in this group were one set that had been given a treatment consisting of watered-down extracts of the specified plants. There will be three different doses of each extract utilized to (i) test the insecticidal and repellent properties of Tribolium on sets that have been treated with ethanol extracts of all the chosen plants. (ii) To evaluate Tribolium's insecticidal and repellent properties, we will employ three dosage levels of each extract. Powder suspension of all plant material selected was applied to treated sets (iii). Tribolium's insecticidal and repellent activities will be studied using three dosage levels of each extract.

Adulticidal/Larvicidal effect:

To do this, we will wash, dry, and sterilize several small culture tubes. Each tube will contain 20 mL of wheat grain. By using a micropipette, the spice extract will be added to the wheat once the acetone has evaporated, and the two will be well mixed with a glass rod. The test insect population will consist of around twenty adults and ten larvae; after the release, the culture tube's aperture will be covered with a muslin cloth secured with rubber bands. Petroleum jelly will be put around the inside of the culture tube, just above the level at which the wheat is sitting, to prevent insects from crawling up the walls. Rates of mortality will be documented beginning with Day 1 of treatment. Several preliminary investigations are expected to be initiated to estimate a death rate of 10% to 90%. Each extract will be tested three times in a lab environment, with dosages ranging from 0.5 to 2.5 times the effective dose. Ten people and 20 grams of unprocessed wheat will be examined as a comparison.

Anti-feedant effect of the extracts:

With minor adjustments, the wheat wafer disc bioassay will be used to perform the antifeedant test (Paruch et al., 2001, Morimoto et al., 2006). These discs of wheat flour and water will be cooked at 80 degrees Celsius for one hour and used as the test food or substrate. Discs will measure 2.5 centimetres in diameter and weigh between 0.40 and 0.41 grams. After fasting for 36 hours, the test insects will be put into Petri dishes with wafer discs. Extract solvents of 30, 60, 90, or 120 mg/ml will be used to soak wafer discs. The treated discs will be air-dried at 30 degrees Celsius for a full 24 hours, then weighed and fed to adult Tribolium castaneum insect beetle. Fifty insects will be put in Petri dishes with the treated and weighted wafer discs and observed for 10 days. Acetone-treated wafers will be offered for the control of insect beetles. Weighing the discs before the experiment begins and again after the adults have fed them for ten days will be used to determine the results. For all plant extracts, we will do five replicates of each treatment. For this exercise, Ben

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Jannet et al methods will be used to determine the Feed deterrence index as a percentage (2000). Feed deterrent index (FDI) as a percentage = C-T/C+T 100 where C is the consumption of the untreated disc and T is the consumption of the treated disc.

Rate of Development (Days)

Today's development rate will be based on the typical time it takes for an insect to get from egg to adult.

Repellant study in adult

There will be a need for a petri dish that is six inches in diameter. Each petri dish will be lined with Whatman filter paper no. 1 and divided in half. The paper will be sprayed on both sides, with acetone on one side and a concentrated solution of each spice extract on the other. After joining the treated and untreated sides of filter paper using transparent adhesive tape, the resulting whole will be placed in a Petri dish. Twenty adults of the test insect will be placed in the petri-dish's central well, and when the acetone has evaporated, a second petri-dish will be placed on top. All treatments will be performed three times, and the order in which they are performed will be determined randomly. Adults will be counted in both the treated and untreated areas after 1 hour. In addition to running control in parallel with the extracts, we will take three readings for each one.

Recording of observations

The observations pertaining to various aspects studied will be recorded as follows:

1. Adult mortality (percent)
2. Larval mortality (percent)
3. anti-feeding effect
4. rate of development
5. repellent study

Analysis:

Table: Adult mortality (%) of and Tribolium castaneum under different

treatments of various types of plant extracts

a. **Adult Insect Mortality:**

Table 1:

Plant	Extract in ethanol	Concentration (%)	% Mortality
Onion	peels	5%	5.12
Ginger	rhizomes	5%	13.01
Garlic	bulbs	5%	12.25
Black Pepper	seeds	5%	22.23
Control	----	0	0

Plant	Extract in aqueous solution	Concentration (%)	% Mortality
Onion	peels	5%	0.23
Ginger	rhizomes	5%	2.01
Garlic	bulbs	5%	1.52
Black Pepper	seeds	5%	2.67
Control	------	0	0

Table 2:

Plant	Extract in ethanol	Concentration (%)	% Mortality
Onion	peels	10%	7.12
Ginger	rhizomes	10%	14.01
Garlic	bulbs	10%	13.25
Black Pepper	seeds	10%	23.23

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Control	----	0	0

Plant	Extract in aqueous solution	Concentration (%)	% Mortality
Onion	peels	10%	0.78
Ginger	rhizomes	10%	2.48
Garlic	bulbs	10%	1.89
Black Pepper	seeds	10%	3.48
Control	----	0	0

Table 3:

Plant	Extract in ethanol	Concentration (%)	% Mortality
Onion	peels	25%	9.12
Ginger	rhizomes	25%	15.01
Garlic	bulbs	25%	14.25
Black Pepper	seeds	25%	25.23
Control	----	0	0
Plant	**Extract in aqueous solution**	**Concentration (%)**	**% Mortality**
Onion	peels	25%	0.95
Ginger	rhizomes	25%	3.57
Garlic	bulbs	25%	2.75
Black Pepper	seeds	25%	5.12
Control	----	0	0

Table 4:

Plant	Extract in ethanol	Concentration (%)	% Mortality
Onion	peels	50%	10.24
Ginger	rhizomes	50%	17.52
Garlic	bulbs	50%	15.64
Black Pepper	seeds	50%	26.52
Control	----	0	0

Plant	Extract in aqueous solution	Concentration (%)	% Mortality
Onion	peels	50%	1.05
Ginger	rhizomes	50%	4.32
Garlic	bulbs	50%	3.36
Black Pepper	seeds	50%	6.24
Control	----	0	0

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*The experiments were conducted in 3 replicates. The % mortality of the adult insect was maximum in the black pepper treated group, the next high effect was that of ginger, then garlic and the least effect was that in the onion peels group. A separate group was used as the control group of the insect without any treatment.

b. Larval mortality (percent) in 50% ethanol extract of different plant botanical extracts:

Table 5:

SNo.	Spices	Regression Equation	LD_{50}
1.	Onion	$\hat{Y}$ = 1.1191 + 0.01213X	37.586
2.	Ginger	$\hat{Y}$ = 0.9932 + 0.01621X	28.436
3.	Garlic	$\hat{Y}$ = 0.9861 + 0.01638X	30.21
4.	Black Pepper	$\hat{Y}$ = 0.8553 + 0.02226X	20.151

All experiments were conducted in 3 replicates.

Sm = Standard error, Statistical Significance Level P = 0.05. The linear regression analysis graph was calculated and interpreted. Further probit analysis was performed. The result of the probit analysis for ginger is given below as follows:

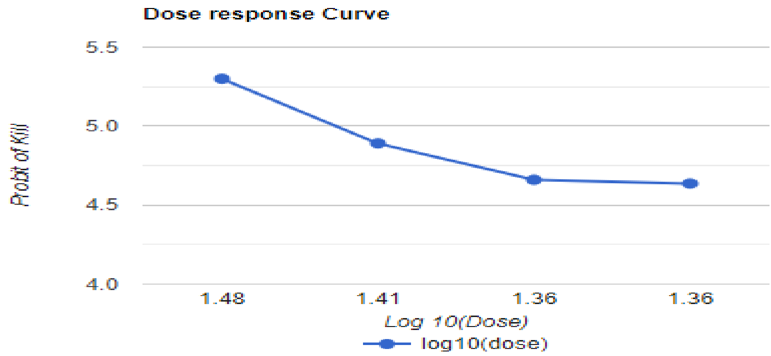

Figure 1: Representing the probit analysis for ginger plant extract.

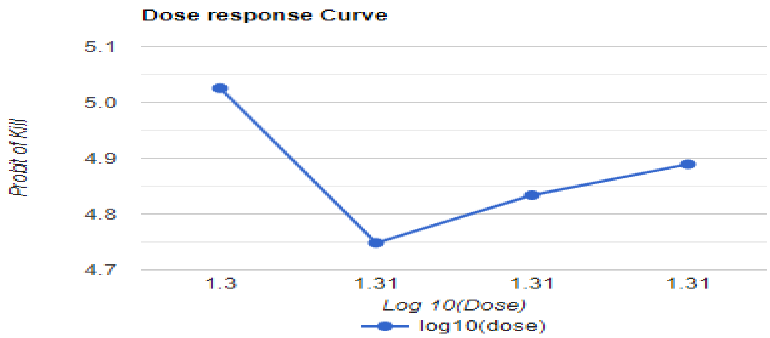

Figure 2: Representing the probit analysis for onion plant extract.

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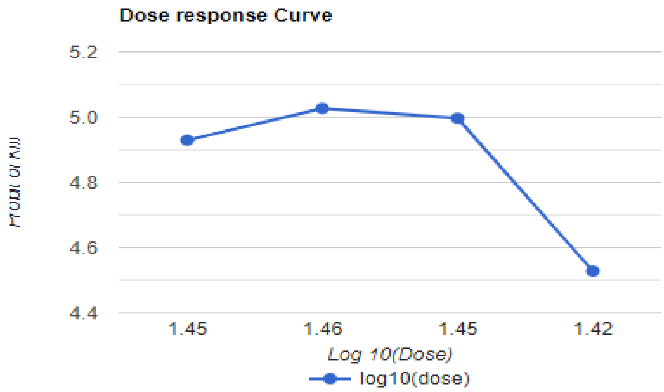

Figure 3: Representing the probit analysis for garlic plant extract

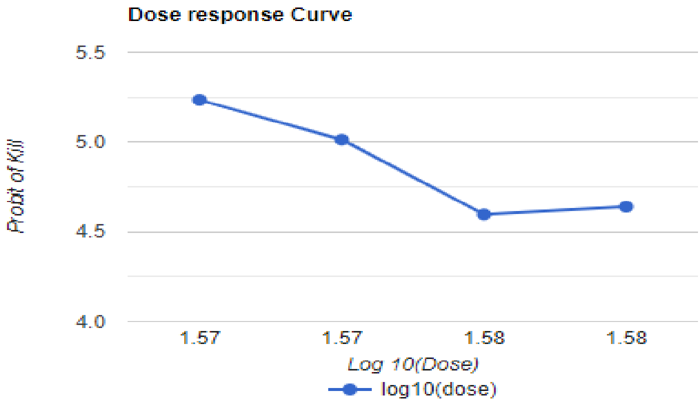

Figure 4: Representing the probit analysis for black pepper plant extract

c. **Rate of development starting from the 2nd instar stage of the larvae of the insect to the full insect development as analyzed experimentally:**

Table 6:

SNo.	Spices	% Larval Emergence ± Standard. Error	% Pupal Emergence ± Standard. Error	% Adult Emergence ± Standard. Error
1.	Onion	51.60 ± 4.37	24.51 ± 2.92	9.24 ± 2.85
2.	Ginger	58.60 ± 5.37	20.51 ± 3.80	8.14 ± 1.85
3.	Garlic	57.40 ± 6.37	23.51 ± 4.80	7.24 ± 0.78
4.	Black Pepper	61.50 ± 5.37	19.42 ± 3.28	5.16 ± 1.75

d. **Anti-feedant effect of the extracts table is given below as follows:**

Once the insects consumed the wheat-stacked disc filter paper, the anti-feedant effect activity was determined every five days for ten days. Results from a one-way analysis of variance (SPSS) were presented as means standard error (SE), with means within each column followed by various letters denoting statistically significant differences ($P < 0.05$).

Table 7:

SNo.	Spices	Antifeedant index (%)
1.	Onion	55.0±3.9
2.	Ginger	64.0±4.5
3.	Garlic	62.0±3.9
4.	Black Pepper	75.23± 2.8

e. Repellent activity of the insect in the performed experiment:

Table 8:

SNo.	Plant extract	concentration	Repellant activity (%) after 1 hour
1	Onion	0.25%	1.24±1.5
2	Ginger	0.25%	7.25±3.5
3	Garlic	0.25%	5.25±4.7
4	Black Pepper	0.25%	10.0±3.9

Results:

As indicated in Figure 1, there is a probit analysis of the effect of ginger on the insect with LD_{50} calculation. As the log dose curve of the plant extract increases, the probit analysis line follows a decreasing slant line pattern. Figure 2 indicates the effect of onion on insect growth with LD_{50} analysis and probit kill rate graphical analysis. The line slope decreases as the log dose curve value increases for onion extract. Figure 3 indicates the effect of probit analysis growth curve for the insect after the addition of garlic extract. Figure 4 indicates the effect of adding black pepper on the insect in the form of probit analysis curve.

The results as indicated in Table 6 signify the emergence rate of pupa from the insect's egg after treatment with different plant extracts like onion, ginger, garlic and black pepper. Further, the data given in Table 6 indicates the emergence and development of the adult insect after transitioning through the different stages of development starting from the egg stage and hatching from it. After the hatching stage, the pupa stage, larvae stage and the final adult insect stage are formed. The effect of the development was observed in the insect after adding the different plant extracts like onion ginger, garlic and black pepper.

One way ANOVA analysis (SPSS software) of the antifeedant activity was calculated and the results revealed that the H0 hypothesis can be rejected as there is no effect of adding a plant extract on the antifeedant index percentage value of the insects in this conducted study. The H1 hypothesis is that the addition of the plant extracts has a significant effect on the anti-feeding activity values of the insect on the wheat flour discs (Table 7). The results indicate that the p-value is less so the Ho is rejected, whereas H1 is accepted. As the p value is 4.2705 x 10-12, which is small so the H1 hypothesis is accepted. According to the ANOVA analysis (SPSS software), the F-value equals 1508.263 which are beyond the 95% region of the experimental analysis curve acceptance region. The value of difference between the different groups of the plant-treated extracts is large with F-value equal to 18.31 so there is variance in the 99.7% region of the graph.

T-test analysis:

The t-test is rejected for the Ho hypothesis as the p-value score is less. The Ho hypothesis is that there is no effect of the addition of the plant extracts on the movement of the insect on the petri-plate. The H1 hypothesis is that the addition of the plant extracts affects the movement and the ability of the insect to repel on the petri-plate after 1 hour of the addition of the plant extracts. However, the H1 test is accepted in the case of onion addition in comparison to the addition of black pepper as the p-value score for t-test is 4.511 x 10-10 ($p < 0.0005$) (Table 8). The T-test value for the comparison of the repellent activity in onion in comparison to black pepper is 72.82 which are outside the 95% region of the graph curve region.

Similarly the other comparative tests were conducted for comparing the t-test values of garlic

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with black pepper and ginger with black pepper. The H_o hypothesis is rejected in this study whereas the H1 hypothesis is accepted for the t-test comparison of ginger's repellent activity percentage values compared to black pepper. The T-value for this distribution analysis is 22.78 and this point is not present in the 95% acceptance region of the distribution curve. Further, the t-test repellent activity comparison was made between the plant extract garlic and black pepper (Table 8). The H_o value is rejected for comparing garlic with black pepper as the p-value is less. However, the H1 value is accepted for the comparison of garlic with black pepper t-test experimentation. The T-value for this analysis is 26.91 and the p-value is 1.738x 10-7 ($p< 0.0001$) which is small and hence significant. The plant extracts in ethanol showed more activity in comparison to the control aqueous plant extract preparation studies for the repellent activity experimentation assays (Table 8).

F-test analysis:

Further F-test analysis was performed for the different plant extracts upon addition to checking the repellent activity significance values. The F-test at 95% confidence level interval was conducted for the other plant extracts to draw a comparison with the black pepper values. The p-value for comparing ginger and black pepper in the F-test is 0.2848 so the Ho hypothesis cannot be rejected. The F-value for this effect is 0.25 with S1/S2 value is 0.5 in the 95% confidence level interval of the graph curve. The 95% confidence interval σ12/ σ22 value is [0.01619, 3.8598]. Hence the H_1 hypothesis is rejected. Similar results are obtained by comparing the F-test values for onion peel and black pepper addition in the repellent experiments. The next set of experiments was conducted for the F-test after adding garlic and black pepper in the repellent assays. In this experiment, the p-value is high, so the HO test cannot be rejected and H_1 test is rejected. The p-value is 0.8052 with F-value as 0.7337 which supports the H_o hypothesis. The 95% confidence interval values are in the range [0.06477; 15.4392]. The S1/S2 value is=0.86 and the σ12/σ22 range value is [0.04752, 11.3284].

Discussion:

One previous study revealed a major effect of adding different plant extracts on the insect Triboleum castaneum (Ahmad et al., 2019). In this study it was revealed that the addition of ginger and garlic extracts causes a tremendous reduction in the growth and causes mortality of the insect. The experiment revealed that this insect causes a significant reduction in the amount of wheat by consuming it whereas the addition of plant extracts causes retardation in the activity of the insect (Ahmad et al., 2019). In our experiments, similar results are obtained as the insect adult, larvae and pupa development and mortality increases after adding different plant extracts. Further, the repellant activity and antifeedant effect also increase after adding the other plant extracts. The LD50 dose of the different plant extracts also has a significant negative effect on the growth of the insects in the culture medium (Table 1).

Further, as the plant extract's concentration increases, the insect's mortality rate increases (Table 1, 2, 3, 4). As given in Table 5, there is an effect of the LD_{50} levels on the insect as the most potent plant extract is black pepper with the least LD_{50} value whereas the highest LD_{50} value is of onion extract and thus onion is the least potent inhibitor of the insect. Ginger is the next potent plant extract as obtained in the studies whereas garlic has lesser potent action as an insect inhibitor than ginger (Table 5). Further, there is retardation and inhibition in the insect, larvae, pupa and adult activity after adding the plant extracts (Table 6). Additionally, the antifeedant and repellant activity of the insect increases as the plant extracts are added to the insect feed in the experimentation procedure (Table 7, 8).

Conclusion:

Thus, the experimental results depict a more potent effect of adding black pepper on the growth of the insect through the different stages of development of the Tribolium *castaneum.* The impact of adding ginger, garlic and onion on insect growth is lesser than the effect of adding black pepper on the growth of the insect.

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