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# Antifeedant and repellent effects of four botanical oils against fall armyworm, *Spodoptera frugiperda* on maize under laboratory condition

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is globally recognized as the most destructive invasive pest of maize (*Zea mays*), a leading cereal crop. This study was conducted in CRD design to evaluate the antifeedant and repellant effects of four botanical oils viz. neem, castor, black cumin, and sesame on the first, second and third instars larvae. Three concentrations (5.0, 7.5, and 10%) of these oils were tested with an untreated control, and a chemical Tween-20 was used as an emulsifier. The results indicated that among the treatments, 10% castor oil exhibited the highest repellency (93.33, 88.33, and 80.00%) against the first, second, and third instar larvae, respectively, whereas 5% sesame oil showed the lowest (56.67, 40.00, and 43.33%). Repellency decreased with longer exposure periods. The 10% castor oil treatment also resulted in the lowest leaf consumption (16.67 mm$^2$ and 77.67 mm$^2$) and the highest mortality (20.00% and 16.67%) against the second and third instar larvae after 24 hours of exposure. The highest consumption of leaf (238.00 mm$^2$ and 522.33 mm$^2$) was recorded against second, and third instar larvae, respectively after 24 hours of exposure in untreated control. Considering repellency and antifeedant efficacies, the castor oil appears to be a favorable option for integration with other components of Integrated Pest Management (IPM) package against the first, second, and third instar larvae of *S. frugiperda.*

## Introduction

Maize (*Zea mays* L.) is globally renowned as one of the most widely consumed commercial cereals, alongside rice and wheat, due to its high productivity and nutritional value (Lopes *et al.*, 2016). It plays essential roles in various industries including paper, bioplastics, beverages, textiles, and serves as feed

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for fish, livestock, and poultry, thus contributing to its growing popularity among growers (Rosegrant *et al*., 2009; Islam and Knan, 2021). Recent studies have highlighted that maize's profitability is higher compared to other cereals in Bangladesh (Hasan *et al*., 2017; Mottaleb *et al*., 2018; Adnan *et al*., 2021). However, the global maize production has indecently declining due to the invasion of the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Adhikari *et al*., 2020; Azad *et al*., 2020). The pest is native to South American regions but has spread to various countries including Bangladesh (Capinera, 2017; FAO, 2020; Ullah *et al*., 2023). It is a highly polyphagous pest and attacks more than 353 plant species posing 100% yield loss in cereal crops (Sisay *et al.*, 2019; Toepfer *et al*., 2021). Its larvae are voracious to young leaves, leading to symptoms like the "dead heart", reduced photosynthetic activity, and negative impacts on the reproductive parts of plants and resulting yield loss (Bateman *et al*., 2018; Kumela *et al*., 2019; Tefera *et al*., 2019).

Farmers often prefers to quick-fix solutions as the synthetic pesticides to control this notorious pest (Tumma and Chandrika, 2018). However, excessive pesticide use can have severe repercussions on human health, the environment, non-target organisms, and biodiversity (Mweke *et al.*, 2020; Wan *et al.*, 2021). Hence, researchers are worldwide now exploring alternative to manage this insect infestations sustainably (Isman, 2006). Botanicals especially plant oils have shown promising insecticidal properties against fall armyworm (Negrini *et al.*, 2019; Siddhartha *et al.*, 2019). They offer numerous benefits such as being selective, biodegradable, and less harmful, while also possessing repellent, attractant, antifeedant, and growth-inhibitory properties (Mahmood *et al.*, 2016; Hikal *et al.*, 2017; Stankovic *et al.*, 2020). The use of botanicals is gaining popularity in organic farming as safer alternatives for integrated pest management (Ahmad *et al.*, 2013; Misra, 2014; Neeraj *et al.*, 2017). For example, botanical oils and extracts including black cumin, neem, sesame and castor were reported to be effective in managing diverse insect pests (Yasmin *et al*., 2017; Barbosa *et al*., 2018). Further, numerous other studies by using oils from botanicals have also been implied (Liu *et al*., 2017; Hossain *et al*., 2021). Considering the merits of botanicals, this study aimed to find out the repellent efficacy of four botanical oils such as black cumin, castor, neem and sesame against fall armyworm.

## Materials and Methods

The experiment was conducted at laboratory of Entomology department, Hajee Mohammad Danesh Science and Technology University (HSTU) during November 2021 to March 2022. The botanical oils of black cumin (*Nigella sativa*), castor (*Ricinus communis*), neem (*Azadirachta indica*) and sesame (*Sesamum indicum*) were evaluated against FAW larvae. Oils were collected from the local market. Three doses such as 10.0, 7.5, and 5.0% of each of the tested oils were prepared separately by diluting in water as solvent and emulsified by using one drop of tween-20 with the help of micro pipette (Dragon lap China, model: C40038142).

Mass culture of *S. frugiperda* was maintained in ambient laboratory conditions to obtain a large number of larvae for all experimental studies. Approximately 100 healthy of different instars of *S. frugiperda* were collected from an infested maize field in Kornai and Sadipur villages near the HSTU campus, Dinajpur. They were kept in plastic zipper bags (200mm × 175mm) and brought to the laboratory in ambient conditions for rearing. The larvae were identified as the frons has white inverted "Y" line on the head, and four dark warts in a square form on the dorsal surface of the eighth abdominal segment (Prasanna *et al.*, 2018). The larvae were placed individually into ventilated plastic boxes (6 L × 6 W × 4 H) (cm) with 15-30 days old maize leaves (Var. Dalia 4455, Tista Seeds Company Private Ltd., Bangladesh). The rearing boxes were cleaned daily and supplied with new fresh maize leaves. The pre-pupal stage was transferred to a plastic box (6 L × 6 W × 4 H) (cm) one third filled with soil for pupation. The pupae were collected and kept into the oviposition cage (40 L × 40 W ×40 H) (cm). Sterile cotton soaked in sugar solution and honey (mixed with water) was placed on a Petri dish inside the cage as a food source for newly emerged adults. Male and female also verified as described by Ganiger *et al*. (2018) and allowed for mating.

### *Repellency test*

Repellency effect of botanical oils against *S. frugiperda* larvae of first to third instars were carried out in Complete Randomized Design (CRD) following the method as described by Talukder and Howse (1995). Healthy young (20-30-days old) maize leaves were collected and cut into 6 cm discs and dipped separately of previously prepared in each concentration of each treatment. Another disc was dipped into water to serve as the untreated control. The treated leaves were then air-dried for 30 minutes and placed separately with untreated control in a Petri dish (120 mm D) provided the ventral surface of the leaves facing upward. One larva of each instar ($1^{st}$ to $3^{rd}$) was introduced in between the different treatments separately. Moist cotton was used at the cut end of the leaf petiole to prevent the leaves from drying-out during the experiment. Three replications with 10 larvae were released at the center of Petri dish for each concentration of treatment. Number of larvae on each treated and untreated leaves were counted at three-hour intervals up to $12^{th}$ hour. The data were expressed as percentage repulsion (PR) by the following formula: [PR (%) = (Nc-50) × 2], Where, Nc = the percentage of insects present in the control half, positive (+) values expressed repellency while negative (-) attractancy. The average values were then categorized according to the standard scale as described by McDonald *et al*. (1970). The repellency rates were categorized as >0.01-0.1, 0.1-20, 20.1-40, 40.1-60, 60.1-80, 80.1-100 for classes 0, I, II, III, IV and V, respectively.

### *Antifeedant test*

Only second and third instars larvae of *S. frugiperda* were used to evaluate the antifeedant effects of tested oils by leaf dipping method in CRD. Freshly collected young maize leaves (20-30-days old) were immersed in 5.0, 7.5 and 10% concentrations of each tested oil separately and allowed to air-dry for half an hour. Subsequently, the treated maize leaves were placed in ventilated plastic boxes (6 L × 6 W × 4 H cm). Same-aged *S. frugiperda* larvae from the stock culture were released individually into each plastic box using a fine camel hair brush to the each previously treated foods separately. The experiment was repeated three times for each concentration of treatments of each replication maintained 10 larvae. Leaves treated with water and an equal number of larvae were also maintained for untreated control. The leaf area consumed by each larva in the treated and untreated control were measured after 24 hours of exposure.

### *Statistical analysis*

The experimental data were analyzed by one way ANOVA in Completely Randomized Design (CRD) with Statistix 10 program and the mean were adjusted by Duncan's New Multiple Range Test (DMRT).

## Results and Discussion

### *Repellent effect of tested oils against first to third instar larvae*

The repellent effects of botanical oils and doses differed significantly among the treatments against the first, second and third instars larvae of *S. frugiperda* (Tables 1-3). Results revealed that repellency rate decreased with progress times. Among the treatments, significantly ($p < 0.01$, $F = 1.39$, $df = 11$) the highest repellency (93.33%) was observed at 10% dose of castor oil belongs to class V while the lowest (56.67%) at 5% concentration of sesame oil fits to class III. Similarly, among the treatments of tested oils, doses and times of intervals, statistically ($p < 0.01$, $F = 0.46$, $df = 11$) the highest repellency (88.33%) was observed at 10% castor oil lined in class V while the lowest (40.00%) noted at 5% concentration of sesame oil fitted in class II (Table 2). Likewise, the interaction repellent effects of applied oils, doses and times of intervals, significantly ($p < 0.01$, $F = 0.19$, $df = 11$) the highest

**Table 1. Repellent effect of botanical oils and doses against first instar larvae of *S. frugiperda* at different hours after treatment (DATs) (Interaction of botanical oils, doses and times)**

| Treatments (oils) | Doses (%) | Repellency (%) at different HATs | | | | Mean (%) repellency | Repellent classes |
|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | | |
| Neem | 5 | 93.33 ab | 86.67 ab | 66.67 cd | 40.00 c-f | 71.67 c | IV |
| | 7.5 | 100.0 a | 100.0 a | 86.67 abc | 60.00 abc | 86.67 ab | V |
| | 10 | 100.0 a | 100.0 a | 100.0 a | 66.67 ab | 91.67 ab | V |
| Castor | 5 | 100.0 a | 100.0 a | 80.00 abc | 53.33 a-d | 83.33 ab | V |
| | 7.5 | 100.0 a | 100.0 a | 93.33 ab | 66.67 ab | 90.00 ab | V |
| | 10 | 100.0 a | 100.0 a | 100.0 a | 73.33 a | 93.33 a | V |
| Black cumin | 5 | 86.67 bc | 80.00 bc | 73.33 bcd | 26.67 ef | 66.67 cd | IV |
| | 7.5 | 100.0 a | 80.00 bc | 66.67 cd | 40.00 c-f | 71.67 c | IV |
| | 10 | 100.0 a | 100.0 a | 86.67 abc | 60.00 abc | 86.67 ab | V |
| Sesame | 5 | 80.00 c | 73.33 bc | 53.33 d | 20.00 fg | 56.67 d | III |
| | 7.5 | 100.0 a | 66.67 c | 53.33 d | 33.33 def | 63.33 cd | IV |
| | 10 | 100.0 a | 100.0 a | 80.00 abc | 46.67 b-e | 81.67 b | V |
| CV % | - | 4.88 | 11.04 | 17.55 | 29.72 | 8.14 | |

CV = Coefficient of variation, HATs = Hours after treatment, within column mean values followed by different letter(s) are significantly different by DMRT at 5% level of probability

**Table 2. Repellent effect of botanical oils with different doses against second instar larvae of *S. frugiperda* at different hours after treatment (Interaction of botanical oils, doses and times)**

| Treatments (oils) | Doses (%) | Repellency (%) at different HATs | | | | Mean (%) repellency | Repellent classes |
|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | | |
| Neem | 5 | 93.33 a | 60.00 b-e | 60.00 abc | 53.33 ab | 66.67 cd | IV |
| | 7.5 | 93.33 a | 80.00 abc | 53.33 bcd | 33.33 bcd | 65.00 cde | IV |
| | 10 | 100.0 a | 93.33 a | 80.00 ab | 53.33 ab | 81.67 ab | V |
| Castor | 5 | 100.0 a | 73.33 a-d | 60.00 abc | 53.33 ab | 71.67 bc | IV |
| | 7.5 | 100.0 a | 86.67 ab | 73.33 ab | 40.00 bcd | 75.00 abc | IV |
| | 10 | 100.0 a | 100.0 a | 86.67 a | 66.67 a | 88.33 a | V |
| Black cumin | 5 | 80.00 ab | 53.33 cde | 53.33 bcd | 33.33 bcd | 55.00 de | III |
| | 7.5 | 93.33 a | 60.00 b-e | 33.33 cd | 40.00 bcd | 56.67 de | III |
| | 10 | 100.0 a | 93.33 a | 60.00 abc | 46.67 abc | 75.00 abc | IV |
| Sesame | 5 | 73.33 b | 40.00 e | 26.67 de | 20.00 de | 40.00 f | II |
| | 7.5 | 93.33 a | 46.67 de | 40.00 cd | 26.67 cd | 51.67 ef | III |
| | 10 | 93.33 a | 80.00 abc | 60.00 abc | 40.00 bcd | 68.33 bcd | IV |
| CV % | - | 12.37 | 25.28 | 33.46 | 32.55 | 13.31 | |

CV = Coefficient of variation, HATs = Hours after treatment, within column mean values followed by different letter(s) are significantly different by DMRT at 5% level of probability repellency (80.00%) was observed at 10% of castor oil belongs to class IV while the lowest (43.33%) at 5% of sesame oil and stand in class III (Table 3).

Present results revealed that the tested oils had profound insecticidal effects as repellent and anti-feedant against the first, second, and third instar larvae of *S. frugiperda.* Parallel results were cited by Wang *et al.* (2022) who found that of lavender, frankincense, and tea tree essential oils strongly repelled effects against fall armyworm larvae. Among them, tea tree essential oil showed an average dwell time of 0 seconds at concentrations of 0.5% and 2.0%, while rosemary essential oil had an average dwell time of 0 seconds at a concentration of 0.2%. This suggests that tea tree and rosemary essential oils exhibited the most effective repellent activities against armyworm larvae. Furthermore, Braga *et al.* (2020) reported that the metabolites present in *A. indica* influenced the repellent activity at different stages of *S. frugiperda,* which aligns with the results of the present study. These findings are also comparable with Shu *et al.* (2021) who observed that azadirachtin, a neem based botanicals acted as growth inhibition and mortality against the larvae of *S. frugiperda*.

***Antifeedant effect of oils against second and third instar larvae:*** The antifeedant effects of tested oils differed significantly on second ($p < 0.01$, $F = 4.5$, $df = 12$) and third ($p < 0.01$, $F = 3.5$, $df = 12$) instar larvae of *S. frugiperda* (Table 4). Due to the treatments, the highest mortality (20.00%) was found in 10% castor oil which provided the lowest consumed leaf (16.67 $mm^2$) area ($p < 0.01$, $F = 1037.7$, $df = 12$) at 24 hours of exposure. In the untreated control, there was no mortality observed but the highest leaf area consumed (238.00 $mm^2$) was recorded. Again, the highest mortality (16.67%) was observed in 10% castor oil but those showed the lowest consumed leaf area (77.67 $mm^2$) at 24 hours after treatments. However, no mortality was recorded in the untreated control treatments but showed the highest consumed leaf area (522.33 $mm^2$).

The results of the findings investigated that the application of botanical oils to as food medium on leaves had a notable impact on the food consumption of both second and third instar larvae of *S. frugiperda.* These results are with parallel findings reported by Cruz *et al.* (2017), and Da Camara *et al.* (2022). Wang

**Table 3. Repellent effect of botanical oils with different doses against third instar larvae of *S. frugiperda* at different hours after treatment (Interaction of botanical oils, doses and times)**

| Treatments (oils) | Doses (%) | Repellency (%) at different HATs | | | | Mean (%) repellency | Repellency classes |
|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | | |
| Neem | 5 | 80.00 a | 60.00 bcd | 46.67 a-d | 20.00 c | 51.67 de | III |
| | 7.5 | 86.67 a | 80.00 abc | 53.33 a-d | 33.33 abc | 63.33 bcd | IV |
| | 10 | 100.0 a | 86.67 ab | 66.67 ab | 46.67 ab | 75.00 ab | IV |
| Castor | 5 | 93.33 a | 66.6 a-d | 53.33 a-d | 40.00 abc | 63.33 bcd | IV |
| | 7.5 | 93.33 a | 86.67 ab | 66.67 ab | 40.00 abc | 71.67 abc | IV |
| | 10 | 100.0 a | 93.33 a | 73.33 a | 53.33 a | 80.00 a | IV |
| Black cumin | 5 | 73.33 a | 53.33 cd | 33.33 cd | 26.67 bc | 46.67 e | III |
| | 7.5 | 86.67 a | 66.67 a-d | 40.00 bcd | 33.33 abc | 56.67 cde | III |
| | 10 | 100.0 a | 86.67 ab | 60.00 abc | 40.00 abc | 71.67 abc | IV |
| Sesame | 5 | 73.33 a | 53.33 cd | 26.67 d | 20.00 c | 43.33 e | III |
| | 7.5 | 80.00 a | 46.67 d | 40.00 bcd | 26.67 bc | 48.33 de | III |
| | 10 | 93.33 a | 73.3 a-d | 53.33 a-d | 33.33 abc | 63.33 bcd | IV |
| CV (%) | - | 18.87 | 24.80 | 31.95 | 34.89 | 15.15 | |

CV = Coefficient of variation, HATs = Hours after treatment, within column mean values followed by different letter(s) are significantly different by DMRT at 5% level of probability

**Table 4. Effect of botanical oils on the mortality and food consumption against 2nd and 3rd instar larvae of *S. frugiperda* after 24 hours of treatments**

| Treatments (oils) | Doses (%) | 2nd instar | | 3rd instar | |
|---|---|---|---|---|---|
| | | Mortality rate (%) | Consumed leaf area ($mm^2$) | Mortality rate (%) | Consumed leaf area ($mm^2$) |
| Neem | 5.0 | 10.00 c | 44.00 e | 10.00 b | 125.33 d |
| | 7.5 | 13.33 bc | 25.00 ghi | 10.00 b | 89.33 hi |
| | 10 | 16.67 ab | 21.00 ijk | 13.33 ab | 80.33 kl |
| Castor | 5.0 | 13.33 bc | 27.33 gh | 10.00 b | 93.00 gh |
| | 7.5 | 16.67 ab | 19.33 jk | 13.33 ab | 83.0 0 jk |
| | 10 | 20.00 a | 16.67 k | 16.67 a | 77.67 l |
| Black cumin | 5.0 | 10.00 c | 69.33 c | 10.00 b | 136.00 c |
| | 7.5 | 10.00 c | 37.00 f | 10.00 b | 100.67 f |
| | 10 | 13.33 bc | 24.00 hij | 13.33 ab | 86.00 ij |
| Sesame | 5.0 | 10.00 c | 86.67 b | 10.00 b | 148.7 b |
| | 7.5 | 10.00 c | 57.33 d | 10.00 b | 115.00 e |
| | 10 | 13.33 bc | 30.33 g | 13.33 ab | 96.67 fg |
| Control | - | 0.00 d | 238.00 a | 0.00 c | 522.33 a |
| CV (%) | - | 32.56 | 5.94 | 33.23 | 1.85 |

CV = Coefficient of variation, within column mean values followed by different letter(s) are significantly different by LSD at 5% level of probability

*et al.* (2022) found that different concentrations (2.0%, 0.5%, and 0.2%) of lavender and citronella essential oils had a promising impact on armyworm larvae, resulting in 100.0% antifeedant rate and causing mortality. Similarly, basil, peppermint, and rosemary essential oils also achieved 100% antifeedant rates and mortality while applied at concentrations of 2.0% and 0.5%. Braga *et al.* (2020) reported that the presence of metabolites in *Azadirachta indica* influenced the antifeedant activity at various stages of *S. frugiperda.* Sousa *et al.* (2018) concluded that turmeric, clove, and palmarosa plant oils exhibited inhibitory effects on the feeding activity of first and second instars of *S. frugiperda* caterpillars. Cruz *et al.* (2017) observed that essential oils from *Syzygium aromaticum* (L.) and *Eucalyptus citriodora* (Hook.) had harmful effects on third-instar larvae of *S. frugiperda.* Furthermore, the oils of *S. aromaticum, Citrus aurantium* (L.), and *C. limon* were found to exhibit antifeedant activity on *S. frugiperda* according to Da Camara *et al.* (2022). Result from this study of newer biopesticides of neem, castor, blackcumin and sesame convey the sustainable essence against early instars caterpillars of *S. frugiperda*.

## Conclusion

It is concluded that the botanicals oils of neem, black cumin, castor and sesame used in the present study had direct toxic effect against the early instars' caterpillars of *S. frugiperda*. So, farmers can use these botanicals as insecticides and it could benefit our agricultural sector because of antifeedant and repellent effect against fall armyworm. Among the four tested botanicals castor oil exhibited the highest levels of antifeedant and repellency against 1st, 2nd and 3rd instars larvae of FAW.

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