**Repellent and anti-feeding activity of *Cinnamomum tamala* essential oils *against* Angoumois grain moth *Sitotroga cerealella* (Olivier)**

**ABSTRACT-** Stored food faces severe damage due to infestation by insects. Theessential oils extracted from leaves and bark of *Cinnamomum tamala* (Indian bay leaf) by hydro distillation method was screened as fumigant for repellent and antifeedant activity against Angoumois grain moth *Sitotroga cerealella* in laboratory assay. Fumigation with different concentration of essential oils significantly (*P <* 0.01) showed repellent action with 100% repellency and antifeeding activity. Highest reduction in feeding deterrence index 76.19% and 80.30% observed in leaf and bark oils of *C. tamala* essential oils against *S. cerealella* in comparison to control groups. These studies showed strong insecticidal activity of both essential oils and its potential role as a fumigant against *S. cerealella*. From this study it is concluded that these essential oils have potential for application in Insect Pest Management programs for stored-grain insect pests.

**Key words-** Essential oil, *Sitotroga cerealella*, essential oils, repellent, antifeedant activity

1. **INTRODUCTION**

“Wheat (*Triticum aestivum* L.) is an essential cereal crop that serves as a primary food source for billions worldwide” (Pena et al. 2017). It occupies about 23.3 per cent of the gross cropped area of the country. Nevertheless, various insect pests often constrain wheat production to storage condition. During storage these insect pests damages the quality and quantity of stores products (Stathas et al. 2023). The quality and quantity of stored grains loss due to the feeding of insect on grains and production of their waste materials (Kalpana et al. 2022). “According to an estimate, the annual storage losses in India were estimated as 14 million tonnes of food grains worth $16,000 million every year. Out of this, food grain losses due to insects alone account for a monetary loss of $300 million. The stored grain pest, *Sitotroga cerealella* (Olivier), is also known as Angoumois grain moth is considered one of the main storage grain pests in Asian countries, where farmers usually use traditional storage structures” (Basavanjali et al. 2020; Mitu and Santi, 2022).

“For control and management of insect pestsmany chemical fumigants and contact synthetic insecticides like deltamethrin and malathion are commonly used to prevent the loss of stored products throughout the world. These pesticides like methyl bromide, aluminium phosphide are effective against insect infestation but their repeated use has disrupted environmental condition. Due to continuous use of these synthetic pesticides sometimes resulted in the development of pest resistance against it” (Elzen and Hardee, 2003; Benhalima et al. 2004; Islam and Talukdar, 2005).

“To overcome this adverse impact on environment and human beings alternatives of chemical pesticides are being searched. Therefore, it is urgent need to develop certain insecticides which should be ecologically safe, biodegradable and cause no toxicity to non-target animals. The Botanical products in form of plant extracts has shown to possess potential for development as new fumigants have high insecticidal activity against insect pests that check its infestation stage and they may have advantages over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability” (Isman, 2008; Mishra and Tripathi, 2011; Mishra, 2022).

*“Cinnamomum tamala* belonging to family Lauraceae is a tree native to Asian countries. It has aromatic leaves called tejpatta which are used for culinary and medicinal purposes. Historically, it is one of the oldest known and used spices. It is mainly used for flavouring food and in pharmaceutical preparation because of its hypoglycaemic, stimulant and carminative properties” (Hussain et al. 1980). The major chemical constituents present in the leaf oil are eugenol, α-phellandrene and β-phellandrene (Satyal et al., 2013). The other minor components of the oil include cis-caryophyllene, elixene, α- pinene, limonene and myrcene (Vishwam, 2015). *C. tamala* plants extracts have insecticidal, antibacterial and antimicrobial activities reported from previous researchers (Chaubey, 2016; Tiwari and Talrja, 2020; Parul et al. 2021))

Therefore, the purpose of present work was to investigate repellent and antifeedent activity of the plant extracts from Leaf and bark of *C. tamala* for testing insecticidal activity against lepidopteron pests under laboratory conditions.

1. **MATERIAL AND METHODS**

**2.1 Plant Collection**

Leaf and bark of *C. tamala* were collected from the local area of Madhepura district, Bihar situated in the Plains of River Koshi located in the Northeastern part of Bihar at latitude between 25°.34 to 26°.07' and longitude between 86°.19' to 87°.07' for insecticidal activity. The collected plant materials were air-dried at room temperature (20-25°C) for one week for extraction of essential oils.

**2.2 Extraction of essential oils**

The air dried leaf and bark were grounded in mixer and subjected to hydrodistillation in 1L of distilled water in a Clevenger-type apparatus for 4 h in University Department of Zoology, B.N. Mandal University, Madhepura from January-2023 to December-2023. The essential oil was collected and dried over anhydrous sodium sulphate and stored in sealed eppendorff tubes at 4-5°C for the further experimental works.

**2.3 Insects rearing**

The rice moth, *S. cerealella* were collected from infested wheat grains (*Triticum aestivum*, variety 'hard red winter') in a local store house and reared in the laboratory in glass jar (30 cm height, 20 cm diameter). Insects were fed crushed rice grain mixed with 5% (w/w) and kept in a rearing room at a temperature of 28±1ºC, a relative humidity of 65±5% and a photoperiod of 12 h light: 12 h dark. Newly emerged adults (2-3 days old) were used in the bioassays.

**2.4 Repellent Activity**

Repellency assay was carried out in a Y-shaped glass tube (each arm with diameter of 2 cm and 10 cm length). Five gram of crushed rice mixed with different concentrations of *C. tamala*  leaf/bark oils was placed in one of the paired arms of the Y-tube (experimental arm) and the other arm contained only crushed rice (control arm). Twenty adult individuals of *S. cerealella* were released through the median arm of the Y-tube. The open ends of the tube were plugged with cotton wool and the experimental set up was left for three hours. After completion of the three hours, number of insects present in the experimental, control and median arms was counted. Each concentration was replicated six times.

Percent repellency (PR) was calculated using formula: **PR = [(NUT – NT/ NUT+NT] 100**

where NUT = number of insect in control arm and NT = number of insects in experimental arm.

**2.5 Antifeedant activity**

Antifeedant activity of *C. tamala* leaf and bark oils was tested in *S. cerealella* larvae. For this assay, food was prepared by crushing 10 gm of rice grains and mixing 5% (w/w) yeast powder in it. Now, food was mixed thoroughly with 0.3µl/gm, 0.6µl/gm and 1.2µl/gm *C. tamala* leaf and bark essential oils. Now ten 3rd instar *S. cerealella* larvae were placed on Whatman filter paper No. 1 and then placed at the bottom of glass petridish (10 cm in diameter and 1.0 cm in height). Now, 10 gm of food prepared was spread around the larvae, cover the petri dish and kept in laboratory conditions applied for insect rearing. After the end of the larval life, antifeedant activity was calculated using **AFA = [C-T/C] × 100**

where, C = consumption of food in control group, and T = consumption of food in treated group.

**2.6 Data analysis**

Correlation and linear regression analysis were conducted to define all concentration-response relationships (Sokal and Rohlf, 1973). Analysis of variance was performed to test the equality of regression coefficient (Sokal and Rohlf, 1973).

1. **RESULTS**

**3.1 Repellency**

Results of repellency assay indicated that essential oils of *C. tamala* leaf and bark were repellent to *S. cerealella* adults after fumigation by essential oils. The percent repellency (PR) was recorded 27.33%, 54.66%, 97.33% and 100% and 38.66%, 63.33% and 100% at 2, 4, 8 and 16µl of *C. tamala* leaf and bark essential oils respectively (Table-1) with preference index (-0.27,-0.54,-0.97,-1.0) against leaf and (-0.38,-0.63,-1.0,-1.0) against bark essential oils (Table 1).

**3.2 Antifeedant assay**

*C. tamala* leaf and bark oils reduced the consumption of food by *S. cerealella* larva as antifeedant activity was found to increase with increase in oil concentration. Consumption of food by 3rd instar larva was significantly reduced to 71.68, 44.87 and 23.80%, and 67.03, 39.39 and 19.70% in comparison to control condition (fumigated with acetone only) at 0.3, 0.6 and 1.2 µl/gm of *C. tamala* leaf and bark oils respectively (For *C. tamala* leaf oil F = 112.18; and for *C. tamala* bark oil F = 118.91; P<0.01; Table 2).

**4. DISCUSSION**

Botanical products as natural pesticides in form of essential oils have a broad spectrum of activity against insect pests. As such, they have considerable potential as stored product protect ants and for pest management. Potential of plants essential oils as a source of insecticides has been reported with references to various pests (Huang et al. 2000; Batish et al. 2008; Cosimi et al. 2009; Nerio et al. 2009). *C. tamala* essential oil have insecticidal effects with repellent activity which ultimately may be reduced damage caused by the insect was observed in the present study. The leaf and bark of *C. tamala* essential oils decreased consumption of flour disk by *S. cereallela* larvae. The repellent and antifeedant activity of *C. tamala* (*Lauraceae*) oils have been reported previously against maize weevil, *Sitophilus Zeamais* (Chaubey and Kumar, 2023). Management Similar results have been shown by *Schinus molle, Alpinia conchigera, Zingiber zerumbet* and *Curcuma zedoaria* essential oils and eugenol in *Tribolium castaneum* and *Sitophilus oryzae* (Chaubey, 2012; Sithisut et al. 2011, Cardiet et al. 2012). The repellent and anti-feeding activity of the essential oils is caused due to volatile nature showing their low persistence in the environment and probable nurotoxicity. Several essential oils have been known for their neurotoxicity in insect pests (Chaubey, 2012; Rani, 2012; Kumar and Tiwari, 2018). These oils interfere with neuromodulator octopamine or GABA-gated chloride channels and cause disruption in its nervous system in insects (Hollingworth et al*.* 1984; Enan, 2005; Tong and Coats, 2012).

These oils reduced amylase and protease enzyme activities along with reduction in consumption index, relative consumption rate and relative growth rate (Ebadollahi et al. 2022). *C. tamala* leaf and bark oils reduce food consumption by larvae of *S. cereallela*. This reflects that essential oils reduce damaged of grains by feeding action, thus, reduces quantitative and qualitative losses. The damage to grains causes deterioration and contamination in damaged grains due to mibrobial action as well as reduction in protein and carbohydrates level.

1. **CONCLUSION:** *C. tamala* leaves and bark essential oils show repellent and antifeedant activities on *S. cereallela*. Since these two volatile oils are of botanical origin and are parts of human diet, these are safe for human when used in insecticide formulation. Their high fumigant actions show its low persistence in the environment, thus, elimination residual properties. Thus, these *C. tamala* leaves and bark essential oils as pesticides can used in developing eco-friendly insecticide formulation based on volatile organic chemicals may therefore possess more potential in the future because of insect resistance.

**Table 1: Repellent activity of *Cinnamomum tamala* leaf and bark essential oils against stored grain insect pest *Sitotroga cereallela***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Concentration**  **(vol:vol)** | **Leaf oil** | | **Bark oil** | | |
| **Mean % of insect**  **treated ±SE** | **(PI) Preference Index\*\*** | | **Mean % of insect**  **treated ±SE** | **(PI) Preference Index\*\*** |
| **2** | 27.33±1.01 | -0.27 | 38.66±1.17 | | -0.38 |
| **4** | 54.66±1.34 | -0.54 | 63.33±1.53 | | -0.63 |
| **8** | 97.33±0.19 | 0.97 | 100±0.00 | | -0.10 |
| **16** | 100±0.00 | -0.10 | 100±0.00 | | -0.10 |

Percent repellency (PR) was calculated using formula: PR = [(NUT – NT/ NUT+NT] 100, where NUT = number of insect in control arm and NT = number of insects in experimental arm.

**Table 2: Effect of *Cinnamomum tamala* leaf and bark essential oils on food consumption by larvae of *Sitotroga cereallela* insects**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Oil** | **Concentration**  **(µl/gm)** | **Food consumed (gm) (Mean±SE)** | **AFA** | **F-value\*\***  **(df=3,20)** |
| **Control** | - | 7.31±0.23 (100) | - | - |
| ***C. tamala*  leaf oil** | 0.3 | 5.24±0.37 (71.68) | 16.51 | 112.18 |
| 0.6 | 3.28±0.31 (44.87) | 55.12 |
| 1.2 | 1.74±0.21 (23.80) | 76.19 |
| ***C. tamala*  bark oil** | 0.3 | 4.90±0.39 (67.03) | 32.96 | 118.91 |
| 0.6 | 2.88±0.28 (39.39) | 60.60 |
| 1.2 | 1.44±0.19 (19.70) | 80.30 |

Six replicates were set for each concentration of essential oil and control, Values in parentheses indicate per cent change with respect to control taken as 100%, Antifeedant activity (AFA) = [C-T/C] × 100, Where, C = consumption of flour disc in control group, and T = consumption of flour disc in treated group, \*\*Significant at P<0.01

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that no generative AI technologies such as large language, models (Chat GPT, COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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