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Potential of *Tamarindus indica* and *Citrus sinensis* methanolic extracts in managing *Tribolium castaneum*

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Abstract

The study aimed to assess the larvicidal efficacy of *Tamarindus indica* and *Citrus sinensis* extracts against *Tribolium castaneum*, a significant pest of stored products. Adult stages of *T. castaneum* were collected and reared on pre-damaged rice grains under controlled conditions. Mature leaves of *T. indica* and *C. sinensis* were collected, shade-dried, powdered, and extracted using methanol. Stock solutions were prepared and diluted to concentrations of 62.5, 125, 250, and 500 ppm. Toxicity bioassays were conducted using clean plastic bottles, each containing 50 grams of wheat flour and 50 adults of T. castaneum. Filter papers soaked in test solutions were affixed to the bottle caps. Insect mortality was observed for four days, and data were analyzed using MS Excel for statistical and regression analysis.

Results indicated a significant increase in larval mortality with higher concentrations of both extracts. For *T. indica*, mortality rates ranged from 29.08% at 62.5 ppm to 85.71% at 500 ppm. *C. sinensis* extracts showed mortality rates from 22.96% at 62.5 ppm to 77.04% at 500 ppm. The study concluded that both *T. indica* and *C. sinensis* extracts are effective in controlling *T. castaneum*, with T. indica exhibiting higher efficacy.

Keywords: Tribolium castaneum, tamarindus indica, citrus sinensis, biopesticides, plant extracts

Introduction

The storage of food products and grains is a global challenge due to pests that damage crops (Panezai, *et al*., 2019) [15]. The red flour beetle, *Tribolium castaneum* (Herbst) (Figure 1), is a major secondary pest that affects a variety of stored products, including cereals, cereal products, cocoa beans, and dried fruits (Adarkwah, *et al*., 2010) [3]. It is considered a secondary pest because it typically infests grains or seeds already damaged in storage. The damage inflicted by *T. castaneum* starts with superficial scratches on the surface of the seeds. Over time, these scratches develop into galleries and more severe injuries that can alter the original shape of the seeds (Pires, *et al*., 2017) [16].

Fig 1: Wheat flour infested with *T. castaneum*

Flours from maize, millet, sorghum, wheat, and cassava, are susceptible to attack by *T. castaneum* at varying degrees as the pest can successfully colonize, feed and develop on the flours (Ehisianya, *et al*., 2022) [6]. Infestation of stored wheat by *T. castaneum* changes wheat quality, specifically increasing fat acidity (FAV) and decreasing seed germination and microfloral levels. Over time, FAV increases regardless of insect activity, but the presence of insects accelerates this increase by altering both the microflora composition and the cereal substrate. Seed germination also reduces with a more rapid loss of germination in insect-infested wheat due to the destruction of the germ by insect feeding (Lustig, *et al*., 1977) [12].

Cocoa is a vital crop that serves as a major source of income in some countries, but its production has been significantly affected by *T. castaneum*. It was estimated that up to 50% of cocoa beans could be damaged, resulting in an economic loss of 3.16 billion US dollars due to production loss (Jung, *et al*., 2020) [11]. *T. castaneum* can negatively impact both the quantity and quality of stored cocoa within just 30 days of infestation, with the severity of the impact increasing with higher population densities and longer post-infestation storage times (Abdullahi, *et al*., 2018) [2].

Plant extracts contain secondary metabolites that can protect plants from insect herbivores, pathogens, and vertebrate herbivores in various ways. Several plant extracts were found to possess toxic effects against *T. castaneum*. The powders of *Telferia occidentalis* (fluted pumpkin), *Zingiber officinale* (ginger), *Vitex grandifolia* (Vitex), and *Dracaena arborea* (dragon tree) at 15 g / 500 g of groundnut seeds concentration, showed protection against *T. castaneum* (Epidi & Odili, 2009) [8]. Panezai, *et al*. (2019) [15] showed that Rosemary (*Rosmarinus officinalis*) plant extracts caused 58.67% mortality in adults and 58.67% mortality was observed against the larvae of *T. castaneum*. Calneem® oil which contains approximately 0.3% azadirachtin, at the highest dosage of 3.0% Calneem® oil achieved at least 90% beetle mortality within 72 hours on grain, and 88% mortality on filter paper. Mortality rates were dose-dependent (Adarkwah, *et al*., 2010) [3].

Crude alcoholic extracts of *Sonchus oleraceus*, *Ageratum conyzoides*, and *Ambrosia maritima* were identified as the most active against *T. castaneum*. LC 50 value for the extracts of *S. oleraceus* was 20 mg/ml after 36 hours, while it was 50 mg/ml after 96 hours for the extracts of *A. conyzoides*. However, the extracts of *A. maritima* showed an LC50 value of 20 mg/ml after 72 hours (EL-Kamali, 2009) [7]. At 1.5 g and 0.90 g / 60 g of wheat flour, black pepper, and neem seed powders showed mortalities of 65.2% and 69.4%, respectively, against *T. castaneum* under laboratory conditions. (Saeed, *et al*., 2016) [18].

The extracts of *Citrus sinensis* were proven to possess insecticidal properties against a variety of pests. Fruit peels and seeds of *C. sinensis* were efficient against the common pistachio psylla, *Agonoscena pistaciae* (Hemiptera: Aphalaridae), which is a significant pest of pistachio trees (Rouhani, *et al*., 2019) [17]. *C. sinensis* fruit peel and seed extracts showed varying effects against the third instar nymphs of *Paracoccus marginatus* (Gowtham, *et al*., 2019) [9].

The extracts of *Tamarindus indica were* also reported to exhibit pesticidal effects. The crude powder of *T. indica* seed exhibited the highest toxicity with a 96-hour LC50 of 156.39 mg/l, compared to T. indica bark (96h LC50 of 1104.74 mg/l) against the vector snail *Indoplanorbis exustus*, which serves as an intermediate host for liver flukes of the Fasciola species (Soni & Singh, 2015; 2017) [19, 20]. A mixture of crude and solvent extracts from *Curcuma amada* rhizome and *T. indica* leaves was tested against *Culex vishnui* larvae which resulted in the highest mortality rate (100%) at 100 ppm concentration (Burman, *et al*., 2019) [5].

Materials and methods

Insect rearing

Adult stages of *Tribolium castaneum* were collected from grocery shops in Sangareddy town, Telangana State, India. They were reared on pre-damaged grains of rice (*Oryza sativa*) under controlled conditions of 27 ± 1°C temperature and 55 ± 5% relative humidity in a growth chamber. Each three-liter jar was filled with 800 grams of wheat flour and damaged wheat grains, and approximately 150 beetles were introduced into each jar. The jars were covered with muslin cloth and secured with rubber bands to prevent beetle escape and contamination. The insects were kept in the chamber for two months for rearing. Adults of the first generation were transferred to new jars for egg collection, and these eggs were subsequently moved to fresh jars with damaged grains. Adult insects were then allocated to experimental and control jars for data collection.

Preparation of botanical extracts

Mature leaves of *Tamarindus indica* and *Citrus sinensis* were gathered from nearby villages in the Sangareddy District of Telangana State, India. The leaves were shade-dried for 14 days and then they were powdered using an electric blender. The prepared powders were stored in clean plastic jars until usage. The extracts were collected by soaking 100 grams of prepared powders in 250 ml of methanol for 3 days with frequent shaking and by filtering through Whatman filter papers No. 1. The filtrates were allowed to evaporate to get semi-solid extracts which were stored in the refrigerator at 4^0 C until usage.

Preparation of test solutions

Stock solutions were prepared by adding 10 ml of methanol and 990 ml of distilled water to 1 gm of the extract. By serial dilution, test solutions of 62.5 ppm, 125 ppm, 250 ppm, and 500 ppm were prepared.

Bioassays

For conducting toxicity bioassays, clean plastic bottles were used. 50 grams of Wheat flour were taken into each bottle along with 50 adults of *T. castaneum*. Filter papers of 5 cm diameter were soaked in the test solutions separately and airdried before they were fixed to the inside of the test bottle caps. Insect mortality was observed for 4 days. Insects that did not respond to touch with a brush were recorded as dead. The experimental design was a randomized setup with four replications. Statistical analysis was performed using MS Excel software. Regression analysis was performed.

In conducting toxicity bioassays, clean plastic bottles were utilized, each containing 50 grams of wheat flour and 50 adults of *T. castaneum*. Filter papers, each with a diameter of 5 cm, were soaked in the test solutions separately and then air-dried before being affixed to the inside of the bottle caps. The mortality of the insects was observed for four days, with insects that did not respond to touch with a brush being recorded as dead. The experimental design followed a randomized setup with four replications to ensure reliability and minimize bias. The corrected mortalities were obtained using Abbott's (1925) [1] formula. Data analysis was conducted using MS Excel software, where statistical analysis and regression analysis were performed.

Results & discussion

The results of the present study are given in Table No. 1. and Figure 2. In the toxicity bioassays with the extracts of *T. indica*, the results highlighted a significant increase in larval mortality with higher concentrations of the extracts. The mortality rate started at 29.08% when the larvae were exposed to a 62.5 ppm concentration. This mortality rate increased to 41.33% at a concentration of 125 ppm. As the concentration reached 250 ppm, the mortality rate climbed further to 63.78%. At the highest concentration tested, 500 ppm, the mortality rate peaked at an impressive 85.71%.

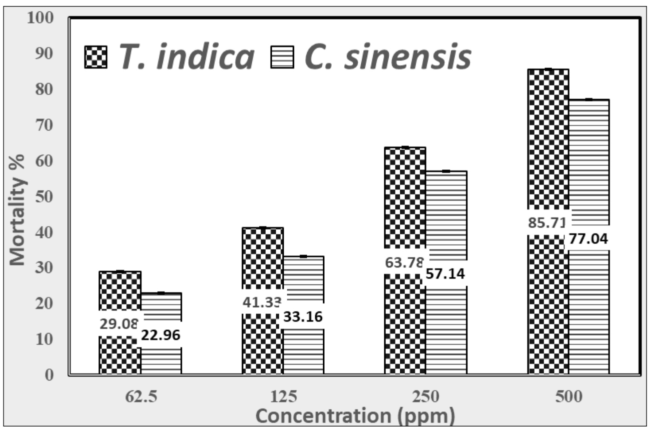

Fig 2: Results of Toxicity Bioassays of *T. indica* & *C. sinensis* against the adults of *T. castaneum*

Similarly, the extracts of Citrus sinensis also showed a notable concentration-dependent increase in larval mortality. At the lowest concentration of 62.5 ppm, the mortality rate was recorded at 22.96%. When the concentration was increased to 125 ppm, the mortality rate rose to 33.16%. At a concentration of 250 ppm, the mortality rate was observed to be 57.14%. The highest concentration of 500 ppm resulted in a mortality rate of 77.04%.

Table 1: Results of Toxicity Bioassays of *T. indica* & C. sinensis against the adults of *T. castaneum*

Tested Plant extracts	Mortality % against Test Conc. in PPM					LC 50 in PPM	Regression Equation	R² Value
	0	62.5	125	250	500			
T. indica	0.00 ± 0.46	29.08 ± 1.45	41.33 ± 1.81	63.78 ± 3.13	85.71± 3.66	195.08	y = 0.1269x + 25.244	0.9617
C. sinensis	0.00 ± 0.45	22.96 ± 1.69	33.16 ± 1.20	57.14 ± 2.96	77. 04 ± 2.80	254.19	y = 0.1227x + 18.811	0.9539

Coming to Regression analysis, for *T. indica* extracts (Figure 3), the regression equation derived was y=0.1269x+25.244 with an R^2 value of 0.9617. Similarly, for *C. sinensis* (Figure 4), the regression equation was determined to be y=0.1227x+18.811 with an R^2 value of 0.9539. The results demonstrate that both *T. indica* and *C. sinensis* extracts cause higher mortality rates as their concentrations increase. However, *T. indica* was more effective than *C. sinensis*, evidenced by higher mortality rates at each corresponding concentration. These findings suggest that *T. indica* could be a more powerful natural larvicidal agent, offering a promising alternative to synthetic pesticides for managing pest populations.

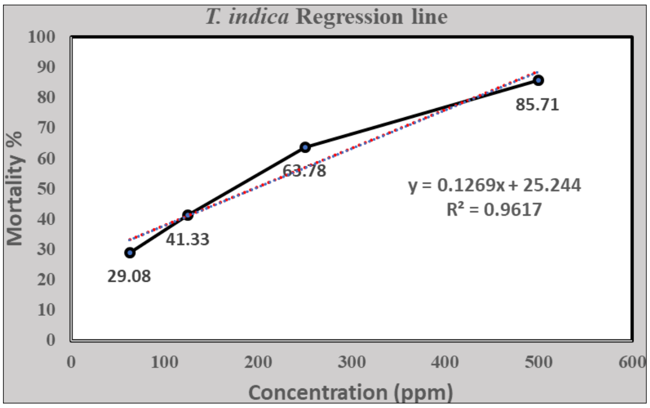

Fig 3: Regression analysis of Toxicity bioassay of *T. indica* extracts against the adults of *T. castaneum*

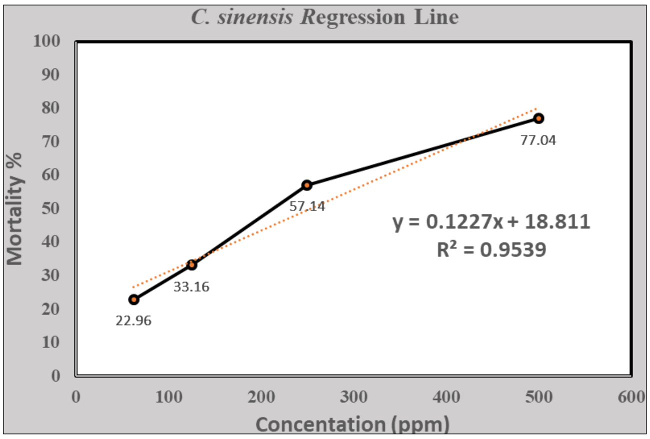

Fig 4: Regression analysis of Toxicity bioassay of *C. sinensis* leaf extracts against the adults of *T. castaneum*

The results of the present study are in line with that of previous studies. Fruit peels and seed extracts of *C. sinensis* have the highest mortality effect at 80 µl/ml concentration against *A. pistaciae*. The LC50 values were 62.04 µl/ml for C. sinensis peels, and 43.60 µl/ml for C. sinensis seeds. All extracts showed (Rouhani, *et al*., 2019) [17]. The efficacy of Ethanolic leaf extracts of *T. indica* reduced oviposition (9.7±0.03 and 3.3±0.25), egg viability (2.0±0.58 and 0.67±0.39), and adult emergence (1.00±0.00 and 0.33±0.19) of *Callosobruchus maculatus* at higher treatment levels (10.0% and 12.5% w/w) (Alkali & Abdullahi, 2017) [4].
Phytochemical screening of *T. indica* and *C. sinensis* revealed several bioactive compounds. The presence of alkaloids, glycosides, flavonoids, tannins, saponins, and triterpenoids in the extracts of *T. indica* (Osawota, *et al*., 2022) [14]. Ihemanma, *et al*. (2014) [10] reported the presence of reducing sugars, saponins, piperines, tannins, and flavonoids in *C. sinensis* peel extracts. Linoleic acid (36.6%), followed by α-linoleic acid (25.3%), oleic acid (17.8%), palmitic acid (9.7%), and stearic acid (3.3%) presence in the hexane extracts of Citrus seeds reported by Nunes, *et al*. (2015) [13]. In the present study extracts of both test plants *T. indica* and *C. sinensis* exhibited promising toxic effects against *T. castaneum*. The secondary metabolites present in these extracts might be responsible for these toxic effects.

Conclusion

The study highlights the effectiveness of *Tamarindus indica* and *Citrus sinensis* extracts in managing *Tribolium castaneum* infestations, revealing a clear dose-dependent increase in mortality rates. The extracts of *T. indica* were more potent compared to those of *C. sinensis*, highlighting their potential as effective natural larvicides. Regression analysis demonstrated strong correlations between extract concentration and mortality rates for both plants, with *T. indica* showing a slightly better fit. These findings support the use of plant-based extracts as an eco-friendly alternative to synthetic pesticides. The promising results pave the way for further research into the application of these extracts in real-world pest management scenarios.

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