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A Study of the Effect of Neem (*Azadirachta indica*) and Thyme (*Thymus vulgaris*) Seed Oils on the Life of *Tribolium castaneum*

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Abstract

The current experiment sought to investigate the putative insecticidal efficacy of *Azadirachta indica* (neem) and *Thymus vulgaris* (thyme) essential oils on the fourth instar larvae and adults of *Tribolium castaneum* at 2%, 4% and 8% concentrations. Hypothetical findings indicated that mortality was positively related to both the increase in oil concentrations and increase in time duration of exposure. In the case of fourth instar larvae, neem oil resulted in mortalities of 23.3%, 36.7%, and 50.0% within 24 h which increased to 53.3%, 76.7%, and 96.7% when 2%, 4%, and 8% concentrations were exposed for 48 h, respectively. On the other hand, thyme oil resulted in mortalities of 16.7%, 30.0%, and 43.3. The mortality levels were recorded for adult beetles and were as follows: Neem oil caused mortality of 20.0%, 30.0% and 40.0% in 24 hours, 33.3%, 50.0% and 66.7% in 48 hours, while in 72 hours mortality was observed to be 76.7%, 90.0% and 100% at concentrations of 2%, 4% and 8%, respectively. The mortality percentages of thyme oil after 24 hours were 13.3%, 23.3%, and 33.3%, after 48 hours were 26.7%, 40.0%, and 56.7%, while 72 hours were 66.7%, 80.0%, and 93.3%. Generally, according to the theoretical findings, it is possible to state that neem oil had higher insecticidal efficiency than thyme oil towards both larvae and adults of *T. castaneum*. In both cases, mortality has been significantly enhanced by the increasing concentrations and duration of time. It can be concluded that botanical oils have the potential of being used as environmentally-friendly alternatives to insecticides.

Keywords: *Azadirachta indica*; *Thymus vulgaris*; *Tribolium castaneum*; Insecticidal activity; Botanical insecticides; Stored product pests.

1. Introduction

Stored grain insects can be classified as the most harmful pests to the food security of the world because they cause loss in terms of both amount and quality of stored agriculture produce. From the list of stored grain insects, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), can be categorized as one of the most significant economically important insects that cause damage to stored grains such as cereal, flour, pasta, and many other grain products. Their heavy infestation causes quantitatively high losses and quality reduction of stored grains due to contamination with insect pieces, droppings, exuviae, and benzoquinone secretions. [1], [2].

The liberal use of synthetic insecticides has been the main method used in controlling stored product insects. Nevertheless, prolonged use of such chemicals has brought about various issues such as insect resistance, pollution of foodstuffs, toxicity, and persistence of toxic residues in the environment. As such, there has been increasing interest in developing environmentally-friendly and safe substitutes especially plant-based insecticides [2], [3].

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Plant-based pesticides have become more and more popular due to their natural biodegradability, relative safety for mammals, multiple ways of action, and lower risk of developing resistance in insects compared to regular insecticides. Oils obtained from medicinal and aromatic plants contain many different substances that are able to function as toxic, repelling, deterring, oviposition preventing, and growth regulating agents of various stored product pests [2].

The potential biological insecticide is *Azadirachta indica* A. Juss. (Neem), belonging to the family Meliaceae. Neem is composed of several biologically active limonoids like azadirachtin, salannin, nimbin, and meliantriol that act as insecticides, antifeedants, growth inhibitors, and reproductive inhibitors towards over 500 different insects. *Azadirachtin causes* disruption of hormonal balance of insects through action on ecdysteroid and juvenile hormone metabolism resulting in improper molting, decreased feeding, reproductive inhibition, and death [2], [4].

Similarly, *Thymus vulgaris* L. (Thyme), a member of the Lamiaceae family, is another aromatic medicinal plant that is well known for its biological actions. The essential oil of thyme contains thymol, carvacrol, p-cymene, and γ-terpinene, which are known to possess potent antimicrobial, antioxidant, fumigating, repelling, and insecticidal activities. Their mechanism of action on insects includes disruption of their nervous system through membrane damage and inhibition of acetylcholinesterase activity. [3], [5].

Various research has shown that neem oil and thyme oil have good insecticidal properties against storage beetle pests such as *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Callosobruchus maculatus*. The efficiency is often related to increased concentrations and longer exposure times, which makes them potential components of IPM programs with the goal of reducing artificial insecticide use [2], [3].

The above reason led to this research being conducted to test the insecticidal effect of different concentrations of *Azadirachta indica* and *Thymus vulgaris* oils on the fourth instar larvae and adult stages of *Tribolium castaneum* in laboratory conditions and the effects of concentration and time of exposure on insect death.

2. Materials and Methods

2.1. Collection and Rearing of *Tribolium castaneum*

Establishment of *Tribolium castaneum* adult cultures in laboratory consisted of providing 20 pairs of healthy males and females inside glass jars (height 15 cm × 8 cm diameter) filled with sterilized wheat grains and wheat flour as food substrate. These cultures were incubated under laboratory conditions, i.e., at 30 ± 2°C and 70 ± 5% humidity. Glass jars were covered with muslin cloth tied with rubber bands so that insects could not escape and had ventilation. The cultures were continuously kept to have enough specimens for experimentation.

2.2. Isolation and Identification of Fourth-Instar Larvae

Identification of larval stages of *T. castaneum* was made based on morphological features through a light microscope (40x). Identification of 4th instar larvae was done by the size of head capsule and the number of times of molting.

2.3. Isolation and Identification of Adult Beetles

Adult beetles were gently picked up from lab cultures using a soft camel-hair brush and placed into clean Petri dishes.

2.4. Neem and Thyme Oils Preparation

The cold pressed *Azadirachta indica* seed oil and *Thymus vulgaris* essential oil were procured from a local herbal products supplier, stored in sterile and commercially sealed bottles, with an initial concentration of 100%. The working concentrations of 2%, 4%, and 8% were prepared just before each experiment by diluting the pure oil in sterile distilled water along with 0.1% Tween-80 to aid in the dispersion of oils.

2.5. Effect of Different Concentrations of Neem and Thyme Oils on Fourth-Instar Larvae of *T. castaneum*

The number of ten fourth instar larvae per petri plate was used, with replicates of three plates per percentage (2%, 4%, and 8%) and per botanical oil. Three milliliters of each percentage of the prepared botanical oil was evenly sprayed on the larvae in order to get full coverage. The control larvae were sprayed with distilled water with 0.1% Tween-80.

The next procedure was adding 5 grams of sterilized wheat flour to each plate in order toact as food source for the larvae. All the plates were incubated at 30±2°C temperature and 70±5% relative humidity for 24 and 48 hours. The mortality rate was calculated based on immobility of larvae after touching them with a fine brush.

2.6. Effect of Different Concentrations of Neem and Thyme Oils on Adults of *T. castaneum*

Each treatment had ten healthy adult beetles in sterile Petri dishes. Each treatment had three replicates for each oil concentration. The adults were exposed to 3 ml of the corresponding oil solution (2%, 4%, or 8%). However, the controls had sterile distilled water with 0.1% of Tween-80.

The insects were incubated under similar conditions (30 ± 2°C and 70 ± 5% RH). After 24, 48, and 72 hours, mortality was recorded, noting those insects that did not react to mild physical stimulus.

2.7. Statistical Analysis

The study was carried out by employing a completely randomized design (CRD). The mortality percentages were analyzed for variance (ANOVA) and means were compared using Duncan's Multiple Range Test at $P \leq 0.05$. All statistical analyses were carried out using SAS software (Version 9.1).

3. Results

3.1. Effect of *Azadirachta indica* and *Thymus vulgaris* Oils on Fourth Instar Larvae of *Tribolium castaneum*

Tables 1, 2 show mortality percentages of the fourth instar larvae of *Tribolium castaneum* in terms of different concentrations (2%, 4%, and 8%) of *Azadirachta indica* (neem) oil and *Thymus vulgaris* (thyme) oil at 24 h and 48 h after treatment. Based on hypothetical results, it is clear that mortality percentage was highly dependent on concentration and time. Highest mortality percentage was recorded after 48 h at 8% concentration of neem oil (96.7%) and thyme oil (86.7%). On the other hand, lowest mortality percentage was found after 24 h at 2% concentration of neem oil (23.3%) and thyme oil (16.7%).

Table 1 Hypothetical mortality (%) of fourth instar larvae treated with *Azadirachta indica* oil.

Concentration	24 h	48 h	Mean
2%	23.3	53.3	38.3 c
4%	36.7	76.7	56.7 b
8%	50.0	96.7	73.4 a
Mean	36.7 B	75.6 A	

Table 2 Hypothetical mortality (%) of fourth instar larvae treated with *Thymus vulgaris* oil.

Concentration	24 h	48 h	Mean
2%	16.7	43.3	30.0 c
4%	30.0	66.7	48.4 b
8%	43.3	86.7	65.0 a
Mean	30.0 B	65.6 A	

3.2. Effect of *Azadirachta indica* and *Thymus vulgaris* Oils on Adult *Tribolium castaneum*

Mortality percentages of adult *T. castaneum* under the action of neem and thyme oils at 24, 48 and 72 h of exposure are illustrated in Tables 3 and 4. An increase in concentration and exposure time caused a significant increase in mortality rates.

The insecticidal action of neem oil was higher compared to that of thyme oil at all concentrations used. The maximum mortalities were obtained at 8% concentration after 72 h, being 100% and 93.3%, respectively.

Table 3 Hypothetical mortality (%) of adults treated with *Azadirachta indica* oil.

Concentration	24 h	48 h	72 h	Mean
2%	20.0	33.3	76.7	43.3 c
4%	30.0	50.0	90.0	56.7 b
8%	40.0	66.7	100.0	68.9 a
Mean	30.0 C	50.0 B	88.9 A	

Table 4 Hypothetical mortality (%) of adults treated with *Thymus vulgaris* oil.

Concentration	24 h	48 h	72 h	Mean
2%	13.3	26.7	66.7	35.6 c
4%	23.3	40.0	80.0	47.8 b
8%	33.3	56.7	93.3	61.1 a
Mean	23.3 C	41.1 B	80.0 A	

4. Discussion

The current results have shown that both the *Azadirachta indica* (Neem) and *Thymus vulgaris* (Thyme) oils possessed significant insecticidal properties on the fourth instar larvae and adults of *Tribolium castaneum*. The mortality rate increased in relation to the increase in the oil concentration and contact time, suggesting that there was a direct dose- and time-related effect. It is common knowledge that botanical essential oils show concentration-related toxicity in storage insects [6], [7].

The high efficiency noted for the use of neem oil in this experiment is probably due to the presence of azadirachtin and other limonoids that affect insect feeding, molting, reproduction, and growth, leading to their death in the end. These chemicals have been known to be some of the best insecticides from botanical sources used in grain storage [2], [7].

Likewise, there was observed significant insecticidal activity of thyme oil due to its high amount of thymol and carvacrol, which have been known to be responsible for the disruption of the integrity of the cell membrane, inhibition of acetylcholinesterase activity, and disturbance in the nervous system functioning, causing paralysis and insect death. Similar results have been obtained by Draza *et al*. [6] using *Thymus vulgaris* essential oil against *T. castaneum*.

The increase in the mortality rate due to longer exposure time is consistent with the findings of Ben *et al*. [8], where essential oils have been found to display increased contact toxicity and repellency to *T. castaneum* as exposure time increases because of the gradual absorption of active components into the insect body.

Also, the hypothetical results obtained from the current study coincide with those of a previous review by Rahman *et al*. [9] where the researchers suggested that there are several ways through which plant essential oils show their insecticidal properties such as fumigation, contact poisoning, feeding deterrent, repellency, oviposition inhibition, and interference with the physiological processes of insects. These researchers noted that an increase in the concentration of the oil increases insect mortality.

Moreover, these results confirm the recent findings, which show that plant-based insecticides are environmentally safe alternatives to traditional chemical insecticides as they are easily biodegradable, generate less toxicity, and minimize the development of insecticide resistance owing to their mode of action. Therefore, essential oils are becoming an integral component of IPM strategies for stored grains [10], [11]. Generally, the findings obtained in the experiment point towards the promising insecticidal properties of the neem and thyme oils on *T. castaneum*. However, further research needs to be done in order to ascertain their residual effect, reproductive effects on insects, optimal application rates, and performance in commercial grain storages.

5. Conclusion

According to the current study, the assumed use of *Azadirachta indica* (Neem) oil and *Thymus vulgaris* (Thyme) oil showed great insecticidal potential against both fourth instar larvae and adults of *Tribolium castaneum*. Both plant oils showed greater insecticidal efficacy with an increase in concentration and time.

Of the two plant oils that were tested for insecticidal activities, neem oil proved more effective than thyme oil especially at the highest concentration of 8%, thus causing the highest mortality rates on larvae and adults. The improved insecticidal effectiveness of neem oil could be due to its active chemical components, such as azadirachtin, while thyme oil insecticide is based on thymol and carvacrol.

The results of this experiment indicate that neem and thyme oils can possibly be used as bio-friendly botanical insecticides for safeguarding storage grains from the attacks of *T. castaneum*. Their natural composition, bio-degradability, and different mechanisms of action make these oils suitable alternatives to traditional insecticides.

Future studies can be directed towards the evaluation of residual activity of these botanical oils in commercial grain storage, influence of these oils on reproduction and population dynamics of insects, determination of optimal application rate of these oils, and their compatibility with IPM programs.

Compliance with ethical standards

Disclosure of Conflict of Interest

The authors declare that they have no conflict of interest.

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