

**EFFECTS OF NEEM (AZADIRACHTA INDICA) SEED KERNELS
EXTRACT ON *TRIBOLIUM CASTANEUM* (Herbst) AN ANALYSIS.**

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Abstract

This article focuses on the effect of Neem (*Azadirachta indica*) Seed Kernel Extract on *Tribolium castaneum*. The insecticidal effect of neem has been demonstrated in various groups of harmful insects, have indicated as miraculous tree, dispensary of multifunctional crops and living pharmacies due to its multiple use. Neem is one of the botanical pesticides in general use and used in organic farming. Around the world today is widely used as an independent treatment or together with synthetic pesticides or entomopathogenic. The extract of neem is a powerful repellent, antifeedant, regulator of growth and dissuasion of the oviposition that affects more than 200 species of parasites. Almost all parts of the neem plant, namely, seeds, leaves, roots, bark, trunk and branches have been recognized for their medicinal and insecticidal properties. The most important component identified was the tetranortriterpenoids (limonoids) Azadiractina. Unlike most current insecticides available on the market, seed extracts appear to be non-toxic to humans and animals and are essentially non-phytotoxic. Many scientists have demonstrated the usefulness of neem and other plant products for the management of diseases and crop pests. At the moment, it is possible to see how neem trees grow successfully in 72 countries around the world in Asia, Africa, Australia, North, South and Central America.

1. OVERVIEW

Neem (*Azadirachta indica*), the versatile tree that has many good and useful qualities is native to India, from where it has spread to many Asian and African countries. Neem and its allelochemicals have a variety of effects on pests. While all parts of the tree repel insects, extracted seeds are exceptional repellents and feed deterrents for a wide spectrum of economically agricultural pests and insects. Seed extracts prevent the feeding of at least 25 species of crop pests, inhibit the growth and development of others and make others sterile to date, 140 active components have been identified that occur in different parts of the tree. The most important component identified was the tetranortriterpenoids (limonoids) Azadirachtin. Unlike most current insecticides available on the market, seed extracts appear to be non-toxic to humans and animals and are essentially non-phytotoxic.

The Neem is known as for *Azadirachta indica*, medicinal and pesticide properties, which has become a unique plant species that has potential antiparasitic properties among the various plant species known to mankind. It has been reported to have insecticidal, antifungal, antibacterial and antiviral properties. Neem belongs to the family Meliaceae contains about 1400 species, some of which are distinguished by having insecticidal characteristics. Because they contain triterpenoid limonoids (Akhtar et.al. 2008). This group or compounds have aroused much interest due to its high activity on the behavior and physiology of different species of phytophagous insects. About 18 secondary compounds have been identified in the neem seed extract, which finds azadirachtin in higher concentrations, which can range from 10 to 25 percent (Govindachari et. al., 2000). In addition to azadirachtin, there are other triterpenoids such as nimbin, melianol and selanin. These triterpenoids represent the total bioactivity of the neem seed; however, it is believed that 72% to 90% of biological activity is due to azadirachtin, which is the main active compound (Schmutterer, 1990).

Almost all parts of the neem plant, namely, seeds, leaves, roots, bark, trunk and branches have been recognized for their medicinal and insecticidal properties (Chaturvedi et al., 2003), which are environmentally friendly, and they have no side effects on the floor and on the ground. Neem insecticides are used to protect both food and income crops.

The insecticidal effect of neem has been demonstrated in different groups of insects, including Lapidoptera, Diptera, Coleoptera, species of homoptera and hemiptera (Sadre et al., 1983).

The extract of neem is a powerful repellent, antifeedant, regulator of growth and dissuasion of the oviposition that affects more than 200 species of parasites (Martincz-villar et.al. 2005). Neem raw material enrichment or purification have been including bioactive compounds, such as azadirachtin, behavior, growth and development, insect survival and storage of stored products (Mordue and Blackwell 1993, Pascaul, ct.al 1990, Singh 1993).

2. PHYSICO-CHEMICAL PROPERTIES OF NEEM SEED KERNEL EXTRACT

The neem tree has become very important since azadirachtin A (Fig. Aa, Ab) was isolated for the first time by Morgan from the seeds of neem seeds of *Azadirachta indica* A. Juss; (Neem). It is a highly oxidized tetranortriterpenoid triterpenoid substance with a decaline segment and a modified furan segment, which are joined by a single bond between C-8 and C-14. It is used as a natural biopesticide and is effective as an inhibitor of concomitant feeding and ecdysis against a broad spectrum of insect species at ppm levels. It represents about 0.2-0.8% of the seeds in weight and is accompanied by a series of other triterpenoids, such as Nimbin (Fig. Ac), which is said to possess biological activity, as a blood purifier, anti-inflammatory, antitumor activity, immunostimulant and bactericidal. In most of the neem extracts, the next most abundant triterpenoid product is salanine (Fig. Ad), which has some insect repellent properties and interrupts growth.

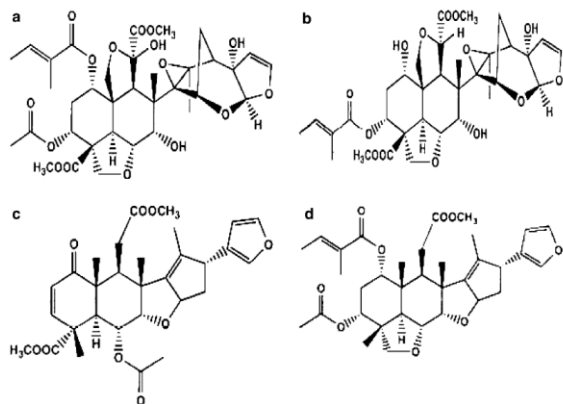


Fig. A. Chemical structure of Azadirachtin A (a); of Azadirachtin B (b); of Nimbin (c) and of Salannin (d)

3. *TRIBOLIUM CASTANEUM*(Herbst)

Taxonomic position: Insecta, Holometabola, Coleoptera, Tenebrionidae.

Common name: Red flour beetle.



Worldwide, but more common in warmer regions. This pest occurs in temperate areas, where it survives winters in protected places, especially with central heating.

It mainly infests seeds, cereals and other products, usually those that had already been injured by other pests or damaged during collection and storage. The affected product is contaminated by farces and the increase in humidity favors the molding. The economic losses consist of a reduction in the weight and quality of the product, difficulty in cooking, reduction in the marketing of infested products and an unpleasant smell. The presence of parasites can cause allergic reactions.

Tribolium castaneum has developed resistance to many common pesticides, whose intensity differs in different regions. The direct applications of a pyrethrin aerosol in the beetle resulted in a mortality of almost 90%, but lower if applied to the infested meal. The treatment of insect larvae with insect growth regulators (IGR) has resulted in poorly trained cockroaches or a greatly reduced adult emergency. The control was also obtained with a pyrethroid alone or in combination with an IGR and with spinetoram.

4. NEEM SEED KERNELS EXTRACT AGAINST *TRIBOLIUM CASTENEUM* (Herbst)

Before 1947, few synthetic insecticides used in crop protection were stomach poisons based on heavy metals such as lead and arsenic, which were killed only when eaten and were known as first generation insecticides. Some botanical extracts have also been used, such as rotenone and pyrethrum, which rapidly degrade in the environment. After the Second World War, varieties of artificially synthesized compounds were recognized. These were effective in killing insects with the simple physical contact known as second-generation insecticides, starting with DDT in 1947 and these often-killed natural enemies more efficiently than they killed a parasite, are known as broad-spectrum insecticides. The cost of application, the reappearance of parasites and the ability to develop resistance to insecticides increased indirectly.

Resistance may be defined as ability of a strain of insects to tolerate doses of an insecticide, which in the normal course would kill the majority of a population of the same species.

Phosphine resistant populations of *T. castaneum* were treated with methyl bromide to control infestation. But the use of methyl bromide is being restricted and will be phased out by 2015 because of its potential to damage the ozone layer. The problem of resistance to chemical insecticides in the insect pests increased manifolds.

5. METHOD AND DATA ANALYSIS

Testing the Bio efficacy of different Extracts

The bioefficacy study of different neem seed kernels extracts against *Tribolium castaneum* adults was carried out using F1 progeny. For the experiment, wheat grains (20 g) were taken in a bottle. Wheat was spiked with different concentrations i.e. 20,000, 15,000, 10,000, 5,000 and 1000 $\mu\text{g g}^{-1}$ of different neem seed kernels extracts using standard A and B (Table 1). There were three replications for each treatment and for control treatment, only wheat and acetone was used. The bottles were put in electric shaker for 5 minutes to enable thorough mixing of extract with wheat grains. Ten adults of same age were released into each bottle and mouth of bottle was covered with muslin. The observations of mortality of *Tribolium castaneum* adults were taken after every 24 hrs till complete or constant mortality was obtained. The observation of appearance of larvae and larval growth were also taken out for 15 days. The corrected percent mortality was calculated using Abbott's formula.

Table 1 Spiking of wheat at different concentrations using different neem seed kernels extracts using standard A (2, 00,000 $\mu\text{g g}^{-1}$) and B (20,000 $\mu\text{g g}^{-1}$) of the respective test extracts

Sr. No	Spiking level ($\mu\text{g g}^{-1}$)	Weight of wheat grains taken (g)	Volume of standard used (mL)	Volume of acetone used (mL)
1	20,000	20	2.0 (A)	0.0
2	15,000	20	1.5 (A)	0.5
3	10,000	20	1.0 (A)	1.0
4	5,000	20	0.5 (A)	1.5
5	1,000	20	1.0 (A)	1.0
6	Control	20	-	2.0

Bioefficacy of ethanol extract against *Tribolium castaneum*

The control sample showed no mortality upto 9th day of application. The mortality of 10 per cent was observed on 10th day, which was remained constant throughout the experiment. The

subsequent corrected percent mortality of ethanol extract obtained using Abbott's formula against *Triboliumcastaneum* is shown in and Fig 1.

It can be concluded that 20,000, 52 15,000 and 10,000 $\mu\text{g g}^{-1}$ concentrations were efficient in controlling the infestation in 7, 9 and 19 days respectively. The best concentration was 20,000 $\mu\text{g g}^{-1}$ as complete mortality of *Triboliumcastaneum* adults was observed just on 7th day of application. Moreover, no larval growth was observed in this concentration

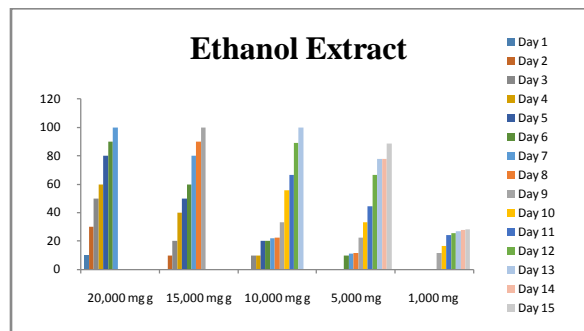


Figure 1 Corrected percentage mortality of *Triboliumcastaneum* with ethanol extract Bioefficacy of chloroform extract against *Triboliumcastaneum*

The control sample showed no mortality upto 9th day of application. The mortality of 10 per cent was observed on 10th day, which was remained constant throughout the experiment. The subsequent corrected percent mortality of chloroform extract obtained using Abbott's formula against *Triboliumcastaneum* adults is shown in Fig 2. It was found that at 20,000, 15,000 and 10,000 $\mu\text{g g}^{-1}$ concentrations the corrected per cent mortality increases with increase in time of application till the complete mortality was obtained whereas at 5,000 and 1000 $\mu\text{g g}^{-1}$ concentrations the corrected per cent mortality remain constant or increases slowly in the beginning, increases rapidly in the middle and then became constant afterward.

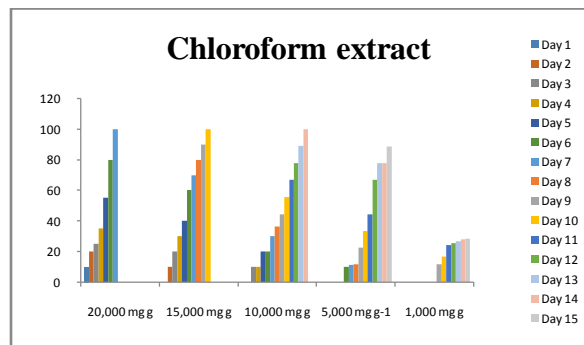


Figure 2 Corrected percentage mortality of *Triboliumcastaneum* with chloroform extract

Bioefficacy of hexane extract against *Triboliumcastaneum*

The control sample showed no mortality upto 9th day of application. The mortality of 10 per cent was observed on 10th day which was remained constant throughout the experiment. The subsequent results of corrected percent mortality rate obtained using Abbott's formula as a result of the treatment of hexane extract against *Triboliumcastaneum* are given in Fig 3. These data showed that the corrected percent mortality increased with increase in concentration and time of application of the extract. The concentration of 20,000 $\mu\text{g g}^{-1}$ was most effective whereas 1000 $\mu\text{g g}^{-1}$ was least effective where no mortality was observed even after 45 days of application.

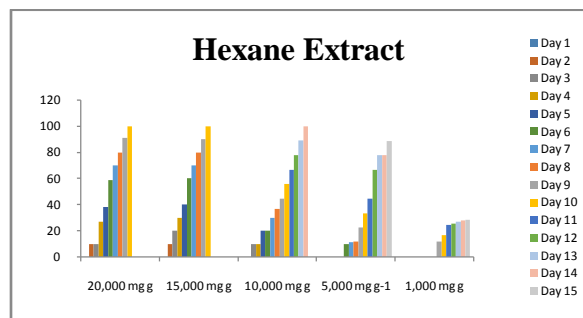


Figure 3 Corrected percentage mortality of *Triboliumcastaneum* with hexane extract.

Bioefficacy of ethanol soluble hexane fraction against *Triboliumcastaneum*

As ethanol and chloroform extracts showed almost similar insecticidal activity and the hexane extract showed lower activity as compared to ethanol and chloroform extracts, so it was decided to carry out bioefficacy studies against *Triboliumcastaneum* using ethanol soluble and ethanol insoluble hexane fractions. The control sample showed no mortality upto 4th day of application. The mortality of 10 per cent was observed on 5th day, which was remained constant throughout the experiment. The subsequent data on adults corrected percent mortality obtained using Abbott's formula as a result of the treatment of ethanol soluble fraction against *Triboliumcastaneum* is summarized in Fig 4. These data showed that the corrected per cent mortality increased with increase in concentration of ethanol soluble hexane fraction. It was found that at 20,000 and 15,000 $\mu\text{g g}^{-1}$ concentrations the corrected per cent mortality increases with increase in time of application till the complete 61 mortality was obtained whereas at 10,000, 5,000 and 1000 $\mu\text{g g}^{-1}$ concentrations the corrected per cent mortality remain constant or increases slowly in the beginning, then increases rapidly in the middle only at 10,000 $\mu\text{g g}^{-1}$ and then became constant after 19, 30 and 22 days of exposure respectively. There was no mortality till 19 days at the exposure concentration of 1000 $\mu\text{g g}^{-1}$. Low Corrected per centage mortality of 11.1 was observed on 22nd days of application. This may be attributed to the presence of very low concentration of toxic compounds present at this concentration.

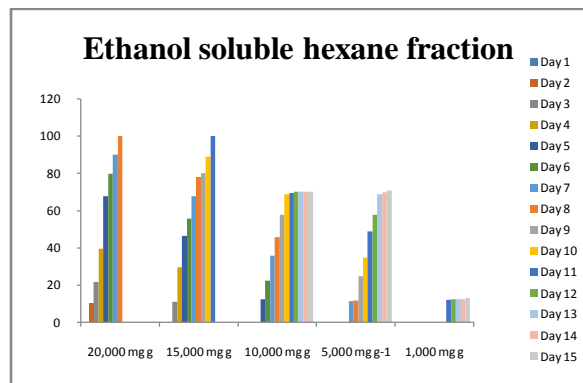


Figure 4 Corrected percentage mortality of *Tribolium castaneum* with ethanol soluble hexane fraction

6. CONCLUSION

We can conclude in the end on the basis of data obtained for adulticidal, larvicidal, residual efficacy and repellency, during the course of experimental research in the laboratory that pattern of toxicity of spice extracts against *T. castaneum* was observed. The spices were highly/significantly effective.

Our results show that *T. castaneum* can have a negative impact on the quantity and quality of cocoa stored within 30 days of the infestation, and the impact increases with increasing population density and subsequent storage time for infestation. The correlation between the factors was significant and positive. Multiple and simple linear regression analyzes were also significant ($P < 0.01$) and all equations were adapted to regression models and perfectly described the relationship between independent and dependent variables. Comparison of two fraction revealed that the ethanol soluble fraction has more insecticidal activity than ethanol insoluble fraction due to the presence active secondary metabolites. From the above study it was reported that ethanol insoluble hexane fraction showed negligible insecticidal activity against *Tribolium castaneum* even at higher concentrations.

To overcome the problem high doses of insecticides were applied. Generally chemical insecticides like melathion, fenitrothion, permethion, deltamethrin, cypermethrin have been used as grain protectant for stored grain. These are hazardous, being toxic to the flora and fauna of the ecosystem and also leading to abiotic and biotic environmental pollution. Insecticides were entering in the food chains and biomagnification took place at different trophic levels. Residues of insecticides left behind polluted the air, water and soil. This led to serious health problems in mankind.

The increased public awareness of human safety and environmental damage caused by insecticides has also diverted attention to the use of plant products. Plants produce secondary

metabolites, many of which have insecticidal properties, are an ecological alternative to synthetic insecticide products in the management of stored grain pests. Essential oils are potential alternatives to current grain fumigants and repellents due to their low toxicity to warm-blooded mammals and their high volatility. In addition to preventing qualitative and quantitative losses, they do not leave toxic residues in food grains.

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