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PESTICIDAL ACTIVITY OF THE LEAVES OF *MANIHOT ESCULENTA* AGAINST THE PEST *TRIBOLIUM CASTANEUM*

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ABSTRACT

Manihot esculenta, Cassava also called, tapioca-root, kappa (predominantly in India) a woody shrub of the *Euphorbiacea*e family native to South America. In the present study reveals that, plant extracts of *Manihot esculenta* leaves in ethyl acetate solvent is highly toxic against adults of *Tribolium castaneum*. Higher doses and exposure time are required to achieve 100% mortality for the adults of *Tribolium castaneum*. The ethyl acetate extract of *Manihot esculenta* could be used as a potential grain protectant against *Tribolium castaneum*. The use of botanical materials as insecticides will benefit our agricultural sector. They are not only of low cost, but have no environmental impact in term of insecticidal hazard. Therefore, the findings of the current experiments strongly support the use and exploration of botanicals in pest management practices.

KEYWORDS: *Manihot esculenta*, *Tribolium castaneum*, Pesticidal activity, Disc method.

INTRODUCTION

Manihot esculenta, Cassava also called, tapioca-root, kappa (predominantly in India) a woody shrub of the *Euphorbiacea*e family native to South America, is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of food carbohydrates in the tropics, after maize and rice. Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people. It is one of the most drought-tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava. Cassava is a good source of carbohydrate, but a poor source of protein.

It is a half-woody or shrubby plant, 1.5 to 3 meters in height, growing from stout and fleshy roots. Leaves are alternate and smooth (except for some of the upper leaves, which are entire) and dividing to the base into three to seven narrow segments, 10 to 20 cm long. It contains calories, protein, fat, carbohydrate, calcium, phosphorus, iron, vitamin B and C and starch. While leaf contain vitamins A, B1 and also contain calcium, calories, phosphorus, protein, fat, carbohydrate and iron. While skin of stem contains tannins, enzymes, peroxidase and calcium oxalate. Cassava contains cyanoglycosides, linamarin, lotaustralin and methyl linamarin.

Its leaves are used for measles, small pox, chicken pox, and for skin rashes, leaf sap latex used for eye condition, used for rheumatism, fresh rhizome applied to ulcers, leaves used for fevers and headaches, colds, fever and to treat constipation, used in the treatment of ringworm, tumor, conjunctivitis, sores and abscesses.[1]

Amino acid in cassava leaves are also useful for wound healing in the skin, replacing damaged cells, bone health, helping us with good memories, and to help the body's metabolic system. Chlorophyll in the leaves serve as antioxidants and anticancer agent. Benefits of cassava leaves are very much; health can be supplemented with the intake of cassava leaves. Known cassava leaves have a number of important benefits such as suitable for those who are running the diet program. In addition, cassava leaves can also be used as medicine rheumatism, headaches, ulcers, cold medicine, diarrhea, overcome the worms, and increase stamina. It also used as a herbal medicine that can drink it with water directly.[2]

The genus *Manihot* is reported to contain the following toxins such as acetone, hydrocyanic acid, oxalic acid, saponin, and tryptophan.[3]

Tribolium castaneum (red flour beetle) is a species of beetle in the family *Tenebrionidae*, the darkling beetles. It is a worldwide pest of stored products, particularly food grains. The red flour beetle attacks

stored grain and other food products, including flour, cereals, pasta, biscuits, beans, and nuts, causing loss and damage. *Tribolium* can be a major pest in storage of grain based products, which cause severe damage to cereal grains throughout the world. This species has a long association with human stored food and has been found in association with a wide range of commodities including grain, peas, nuts and dried fruits. Control of stored product pest relies heavily on the use of synthetic insecticides and fumigants, which has lead to problems such as environmental disturbances, increasing cost of application and pest resistance to pesticides and lethal effects on non-target organisms, in addition to direct toxicity to users. Biological control may be an effective strategy for stored product pest management. Biological pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross resistance as well as offering new leads for design of target specific molecules.

The main significance of the study are it is an easy method to protect stored grain from *Tribolium castaneum*, biological pesticide does not cause any damage or pollution to the environment, it can be adopted by the farmers to protect storage grain in the storage facility, it will improve the agriculture field by maintaining food quality and identify the most effective one which could be used for further detailed study to control pest *Tribolium castaneum*. In our present investigation pesticidal activity of the leaves of *Manihot eusculenta* against the pest *Triboliumcastaneum* were studied.

MATERIALS AND METHODS

Plant Material

Plant leaves of *Manihot esculenta* were collected from different areas of Thrissur district, Kerala state, India. The experimental pests were collected from the local market. The collected study materials were brought into the laboratory condition. Leaves were randomly collected from the plant rinsed with distilled water and shade dried. All the leaf materials were shade dried for 10 days. After drying the plant materials were powdered with the help of mixer blender. The leaf powders were stored in an air-tight glass bottle without any interference of humidity. Leaf powder was used for extraction in three different solvents. Solvents used for extraction are ethanol, petroleum ether and ethyl acetate. Fifty gram leaf powder was used for extraction against three solvents.[6] The adults of *Triboliumcastaneum* were collected from infested grains purchased from local market. And it was brought to the laboratory. The pest along with the grains was stored in a plastic container. Adult insects were sorted out after sieving the grains. The adults were used for subsequent experiment. They were maintained in containers throughout the study period. Small holes were provided on the plastic container for proper aeration.[7]

Extraction

50g of leaf powder was tightly packed with Whatman No: 40 filter paper. It was then carefully transferred into Soxhlet extractor for extraction. Petroleum ether, ethyl acetate and ethanol was used as the solvents for extraction. Extraction continued until the solvent changes to colourless. Extraction took about nine hours. Extract was stored at cold temperature.

Pesticidal activity

From each crude extract 15mg, 30mg and 50mg extracts were accurately weighed and serially dissolved in 1ml of acetone. Then it was made upto 100ml by using distilled water. Thus from each extract 150ppm,300ppm, and 500ppm concentration of test solutions were prepared. Test solutions of three different concentrations of each of the nine extracts were used for testing the *Triboliumcastaneum* in three time periods(24hr,48hr,72hr).[8] Lab studies have been carried out to ascertain pesticidal property of plant extract of *Manihot esculenta against Tribolium castaneum.*

Disc method

The methodology used for contact assay with impregnated filter paper was proposed Huang et al.[9] Sheets of filter paper (diameter 3cm) were impregnated with test solution (500ppm, 300ppm, 150ppm). One ml of each extract solution was spread with the help of pipette over the filter paper and placed into petri dishes, while control sheets were impregnated by without any plant extract. The filter paper discs impregnated with extracts were air dried until complete evaporation. Then they were placed into and 25 adults of *Tribolium castaneum* were released in each treatment and control dish, with three replicates per treatment and control plate. The plates were wrapped in plastic film to prevent escape of pest. Small holes were made on the plastic film for aeration. Mortality counts were taken after 24hr, 48hr, and 72hr intervals of treatment and expressed as percentages of mortality. Dead insects were counted, death being ascertained by the complete absence of movement.

Data analysis

The pests *Triboliumcastaneum* were collected. Only adults were taken for bioassay. Plant leaves were sorted and identified to species level. Plant extracts screened for pesticidal activity was made. Data were grouped in Table1. Pest mortality was measured in percentage and if the control mortality was ranged between 5-20%, it was corrected using Abbotts formula.[10]

Percentage of pest mortality can be calculated by using the formula.

$$Percentage\ mortality = \frac{Number\ of\ dead\ pest}{Total\ number\ of\ pest} \times 100$$

Standard deviations were calculated for three replicates and recorded for 24hr, 48hr and 72hr.

Graphs were based on time intervals (24hr, 48hr, and 72hr). Microsoft Excel package was used to prepare percentage mortality graphs against different concentrations of plant extract. Each graph compares the percentage of mortality of three solvents (petroleum ether, ethyl acetate, and ethanol).

RESULTS AND DISCUSSION

Pesticidal activity of *Manihot esculenta* against *Tribolium castaneum*

Pesticidal activity of the plant extract of *Manihot esculenta* in three different solvents (Petroleum ether, Ethyl acetate, Ethanol) was tested against adults of *Tribolium castaneum* at different concentrations (150ppm, 300ppm, 500ppm). The experiment showed a relation of pest mortality level among three time intervals (24hr, 48hr, 72hr).The results indicate variation in mortality among the plant extracts tested against the selected pest. The summary of results, in which the percentage of mortality of adults of *Tribolium castaneum* caused by different plant extracts are represented in Table1.

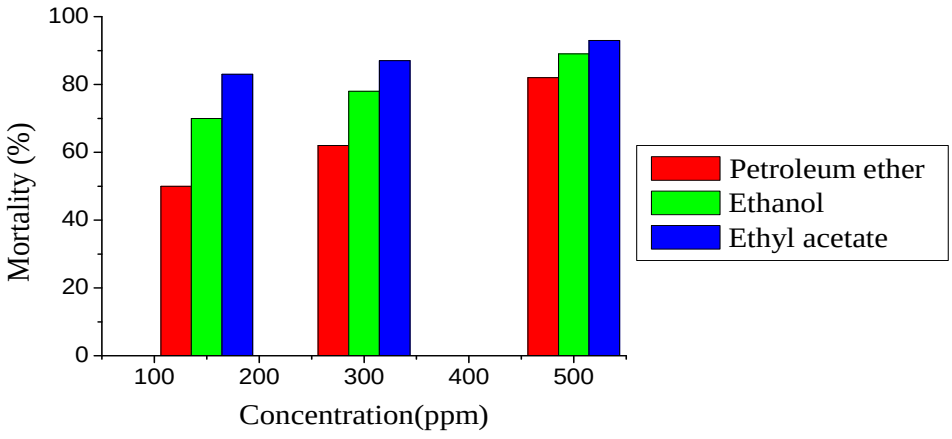
The results indicate that mortality values significantly increased depending on the increasing plant extract concentration and with time of exposure. As considering leaf extracts ethyl acetate medium is more effective than petroleum ether and ethanol. Studies were carried out with *Manihot esculenta* at the three concentrations in three different time interval. More over at 500 ppm concentration it gives above 95% mortality.

The leaf extract of *Manihot esculenta* in three solvents (ethanol, petroleum ether, ethyl acetate) at three time intervals (24hr., 48hr., 72hr.,) also at different concentrations (150 ppm, 300 ppm, 500 ppm) the more effective one is ethyl acetate extract of *Manihot esculenta* leaves. And most significant result is produced after 72hr treatment i.e. above 95 % mortality.

Table 1: Mortality percentage of *Tribolium castaneum* treated with plant extracts of *Manihot esculenta* by disc method.

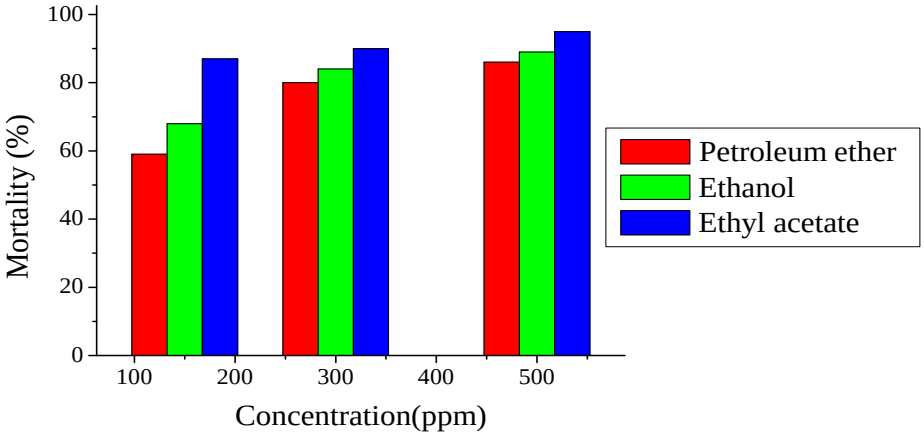
Plant (solvent)	Conc. (ppm)	No.of insects dead	Mortality (%)		
			24(hr) (%)	48(hr) (%)	72(hr) (%)
Manihot esculenta (Petroleum ether)	500	25	83±0.41	86±0.84	91±0.24
	300	25	70±0.30	80±0.45	88±0.32
	150	25	50±0.20	59±0.32	78±0.21
control	-	25	-	-	-
Manihot esculenta (Ethanol)	500	25	87±0.23	89±0.32	93±0.23
	300	25	78±0.14	84±0.41	87±0.21
	150	25	62±0.24	68±0.31	75±0.13
control	-	25	-	-	-
Manihot esculenta (Ethyl acetate)	500	25	93±0.0.32	95±0.30	97±0.30
	300	25	89±0.22	90±0.45	94±0.31
	150	25	82±0.20	87±0.21	92±0.20
control	-	25	-	-	-

*values are mean percentage of three replication of experiment with 25 insects, ±SD



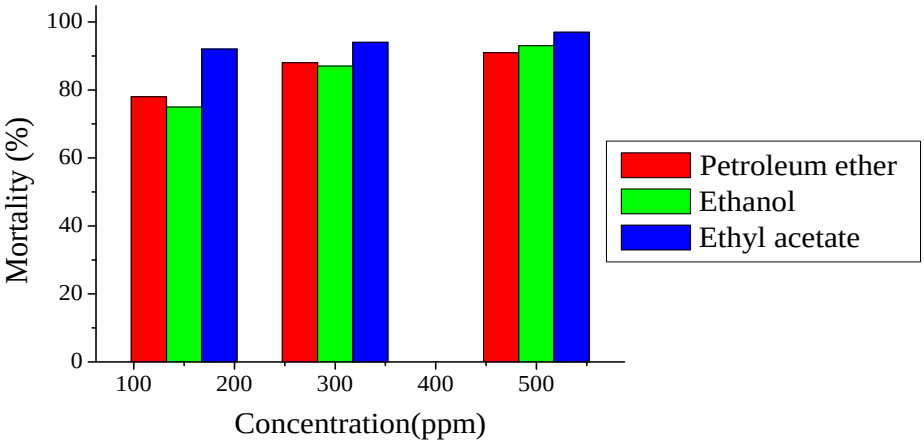
Graph 1: Mortality percentage after 24hr treatment of *Tribolium castaneum* with *Manihot esculenta.*

After 24 hours treatment ethyl acetate extract shows higher mortality and petroleum ether extract shows lesser mortality. Whereas ethanol extract shows medium mortality shown by Graph.1.*Manihot esculenta* (ethylacetate) > *Manihot esculenta* (ethanol) > *Manihot esculenta (*petroleum ether)



Graph 2: Mortality percentage after 48 hr treatment of *Tribolium castaneum* with *Manihot esculenta.*

Ethyl acetate extract shows higher mortality. Whereas ethanol and petroleum ether extract shows similar mortality rates after 48 hour treatment given in Graph 2. *Manihot esculenta* (ethyl acetate) > *Manihot esculenta* (ethanol) = *Manihot esculenta* (petroleum ether).



Graph 3: Mortality percentage after 72 hr treatment of *Tribolium castaneum* with *Manihot esculenta.*

After 72 hours treatment ethyl acetate extract attain 100%mortality shown in Graph 3. Whereas ethanol and petroleum ether extract shows accurately similar results at 150ppm and 300 ppm. *Manihot esculenta* (ethyl acetate) > *Manihot esculenta*(ethanol) =*Manihotesculenta (*petroleum ether).

From the analysis of above three graphs, it shows that ethyl acetate extract of *Manihot esculenta* produce higher pesticidal activity than ethanol and petroleum ether extract. Mortality value increases with concentration of extract and also with time of exposure.

CONCLUSION

The current study strongly recommends the need of exploring botanical derivatives as pest control agents. Even though there are differences in concentration of extracts, all solvent extracts could act as a toxic agent to prevent the proliferation of the test animal.

The ethyl acetate extract of *Manihot esculenta* produce higher pesticidal activity than ethanol and petroleum ether extract. Mortality value increases with concentration of extract and also with time of exposure. The plants which process pesticidal activity could be used as a potential grain protectant against *Tribolium castaneum*. The use of botanical materials as insecticides will benefit our agricultural sector. They are not only of

low cost, but have no environmental impact in term of insecticidal hazard. Therefore, the findings of the current experiments strongly support the use and exploration of botanicals in pest management practices.

The results of pesticidal activity of a number of higher plants based on traditional knowledge strongly indicate that plants are endowed with pesticidal property that can be harnessed cheaply for use in agriculture and related fields. The need to use plant-based products arises from the fact that the synthetic pesticides are harmful to humans, and the entire ecosystem due to high toxicity and persistence. Also, they are too expensive for the poor farmers in the developing countries of the world. On the other hand, plant- based products are cheap and bio-degradable and are therefore environmentally friendly. However, an agricultural programme that depends essentially on plant-based materials must be backed-up by a vigorous research programme into new plant sources.

Therefore, the leaf extract a potentially useful agent to control *Tribolium castaneum*, has to be better explored by doing detailed chemical analysis of the active compounds present in it and by understanding the mechanism involved in action.

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