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Repellent and Toxicological Impact of Aqueous Extracts of Leaves of *Aegle Marmelos* and *Solanum Torvum* Plants against *Tribolium Castaneum* (Herbst)

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Abstract: Pests have been damaging large amount of stored grains. Plants with numerous active substances are used to protect crops. The use of natural products of plant origin slowly faded until recently when use of synthetic pesticides started threatening human health and environmental safety. In the present study, leaf extract of *Aegle marmelos* and *Solanum torvum* was isolated and tested their insecticidal activity against *Tribolium castaneum* (Herbst). The results showed that plants including *Aegle marmelos* and *Solanum torvum* leaf extracts have a high potency to control the pest due to the presence of phytochemical and bioactive components. The percentage mortality increased with increased time. The percentage mortality of *Aegle marmelos* is high in 20% concentration at 72 hours (36.6 ± 0.471). The percentage repellency of *Aegle marmelos* is high in 20% concentration at 72 hours (80 ± 0.471). The percentage mortality of *Solanum torvum* is high in 20% concentration at 72 hours (30 ± 0) and percentage repellency is high in 20% concentration at 72 hours (73.3 ± 0.471). On the basis of the result of present study these plants can be considered for the pesticide formulation that will be useful to control the pest.

Keywords: Plant extracts, *Triboilum castaneum*, Percentage repellency, Percentage mortality, Phytochemical analysis

I. INTRODUCTION

Since ancient time pests have been damaging and causing heavy losses to stored grains both quantitatively and qualitatively [1]. Plants are sources of numerous active substances that are used to protect crops. Currently, due to the limitations of using synthetic insecticides, plant products have attracted increasing attention as possible pesticides [2]. There is a need for plants that may provide potential alternatives to the currently used insect control agents as they constitute a rich source of bioactive molecules [3]. Moreover, these substances are usually low-toxic to humans, and, thus, some of them are even used in traditional cuisine, such as spices [4] and show low persistence, which limits residues in crops. Many stored plants and their parts contain oils or alkaloids that decrease insect feeding, which by itself reduces crop loss. A limited number of insects can cause significant loss of tobacco, due to the presence of alkaloids (nicotine) in stored plant organs [5].

The importance of botanical pesticides is attributed to their efficacy, biodegradability, varied modes of action, low toxicity as well as availability of source materials [6]. Botanical pesticides are derivatives of plants that repel, inhibit growth or kill pest [7]. Botanical pesticides are derived from plants belonging to different families and are either utilized as plant extracts, essential oils or both [8]. A total of 80 plants belonging to 39 families have been documented for their insecticidal and pesticidal potential. Of these, families with more number of species used as insecticide or pesticide are Fabaceae with 9 species; Lamiaceae with 8 species; Euphorbiaceae and Asteraceae with 5 species [9]. In the present study we investigated the insecticidal effects of plants *Aegle marmelos* and *Solanum torvum* against the pest *Tribolium castaneum*.

II. MATERIALS AND METHODS

A. Experimental Design

The experimental set up includes one control and three sets of experimental petridishes as triplicate. Petridishes marked at different concentration includes 5%, 10%, 15% and 20% of the extract. Each petridish contains a filter paper smeared with a particular concentrations of aqueous extract of plants except the control, which contain filter paper with distilled water. Equal number of pest (*Tribolium castaneum*) was introduced to each petridish. The experimental set up was maintained for 3 days for each concentration.

B. Collection of Insects

The red flour beetle (*Tribolium castaneum*) is a species of beetle belonging to the family Tenebrionidae, the darkling beetles. The red flour beetle is reddish-brown in colour and its antennae end in a three-segmented club [10]. The heterogeneous population of *Tribolium castaneum* was collected from the nearby godowns. The adult pest are separated from the collection through hand picking and sieving method as shown in Fig. 1.



Fig. 1 Picture showing *Tribolium castaneum*

C. Collection of Plants

The plants used were *Aegle marmelos* and *Solanum torvum*. *Aegle marmelos* is a fruit-bearing tree indigenous to dry forests on hills and plains of central and southern India, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos and Cambodia. It is cultivated throughout India, as well as in Sri Lanka, northern Malaya, Java and in the Philippines. It is also popularly known as Bilva, Bilwa, Bel, or Beli fruit, Bengal quince, stone apple, and wood apple as shown in Fig. 2.. *Solanum torvum*, the turkey berry, devil's fig, pea eggplant, platebrush or susumber, is a bushy, erect and spiny perennial plant. The plant is usually 2 or 3 m in height and 2 cm in basal diameter, but may reach 5m in height and 8 cm in basal diameter. The shrub usually has a single stem at ground level, but it may branch on the lower stem. The stem bark is gray and nearly smooth with raised lenticels as shown in Fig. 3.

The leaves were used for the experiment. The plant leaves are collected from the local area of Palakkad district, Kerala. The plants were collected at the end of December. The plant was selected on the basis of their useful properties. The fresh leaves were collected and washed in the distilled water to remove all dirt then it was shade dried for two weeks. After complete drying, the leaves were powdered by using electric blender. They were pulverized into fine powder and sieved with fine mesh. The fine plant powder was kept in airtight container until required. Fifty grams of powder was needed to complete the experiment.



Fig. 2 Picture showing *Aegle marmelos*



Fig. 3 Picture showing *Solanum torvum*

D. Plant Extract Preparation

25 gm of powder is taken and mixed with 100ml of distilled water. The mixture was kept in a closed container for 24 hours with repeated agitation. After 24 hours the mixture was filtered by using filter paper. The extract was brownish in color. After the complete filtration, the extract was collected. The extract were kept in bottles until required. The bottles were refrigerated. The extract was the stock solution for the experiment. The two plant leaf extract (*Aegle marmelos* and *Solanum torvum*) was prepared in the same manner. From the stock solution 5%, 10%, 15% and 20% concentrations were made. Totally 12 petridishes and one control was required for the single concentration.

E. Bioassay for Percentage Mortality

To observe the mortality, different concentrations were applied on the filter paper and allowed to dry. Then it was placed in a petridish. Ten beetles were introduced into each petridish. The box was closed and tightly sealed by using cellotape. The petridishes were observed after 24, 48, and 72 hours and the dead beetles were counted. A control box was kept, where the filter paper was smeared with distilled water. Three set of petridishes (triplicate) was kept for different concentrations such as 5%, 10%, 15% and 20%. Mortality was observed.

F. Bioassay for Percentage Repellency

To observe the repellency, first, the filter paper was cut into two halves. One half was smeared with distilled water and the other half with different concentrations such as 5%, 10%, 15% and 20%. One control box was maintained for each hour. Both the halves were put into the petridish and ten beetles were introduced. The box was closed and sealed with cellotape. Beetles which stayed on filter paper smeared with distilled water was observed.

G. Phytochemical Analysis

The plant extract was assessed for the presence of alkaloids, flavonoids, amino acids, tannins, reducing sugars, steroids, phenols, saponins, phytosterols and cardiac glycosides by phytochemical analysis (screening) using standard methods as shown in Table I.

- 1) *Test for alkaloids:* 2ml of plant extract was treated with Wagner's reagent, Brown (or) Red color precipitate indicates the presence of alkaloids.
- 2) *Test for Flavonoids:* 0.5g of plant powder was added with 10 ml of distilled water, 3-5ml of dilute ammonia solution was added to the side of the test tube, then add 1ml of con. Sulphuric acid. Yellow color indicates the presence of flavonoids.
- 3) *Test for amino acids:* To 2-3 ml of purified plant extract add 2-5 drops of Ninhydrin solution was added. The samples were kept in boiling water bath for 1-2 minutes. Purple color indicates the presence of aminoacids.
- 4) *Test for tannins:* 2ml of purified plant extract was taken in a test tube and add 2 drops of 5% ferric chloride solution and the presence was indicated by yellow color.
- 5) *Test for reducing sugar:* To 2ml of purified extract add 2ml of Fehling's solution. The solution was kept in water bath at 40 degree Celsius. Formation of brick red precipitate at the bottom of the test tube indicates the presence of reducing sugars.
- 6) *Test for steroids:* 1ml of purified extract was placed in a test tube, 2ml of acetic anhydride followed by addition of 3-5 drops of chloroform. Then 2 drops of con Sulphuric acid were added along the side portion of the test tube drop by drop. Formation of blue or green color indicates the presence of steroids.
- 7) *Test for Phenols:* To 1ml of purified sample add 2ml of distilled water. Then 2-3 drops of 10% aqueous Ferric Chloride solution was added. The presence of phenol was indicated by the formation of blue or green color.
- 8) *Test for Saponins:* 2ml of purified plant extract was taken and added with 5 ml of distilled water. The mixture was vigorously shaken. The presence of saponins was indicated by the formation of persistent foam.
- 9) *Test for Phytosterols:* To 1 ml of purified plant extract add 2 ml of chloroform and few drops of acetic anhydride and then add equal volume of Con. sulphuric acid. Formation of bluish green color indicates the presence of phytosterols.
- 10) *Test for Cardiac Glycosides:* To 1 ml of plant extract add few drops of Con.sulphuric acid. The formation of red color indicates the presence of Cardiac glycosides.

Sl.no	Constituents	<i>Aegle marmelos</i>	<i>Solanum torvum</i>
1	Alkaloids	+	+
2	Flavonoids	-	-
3	Aminoacids	+	+
4	Tannins	-	-
5	Reducing sugars	+	-
6	Steroids	-	-
7	Phenols	+	-
8	Saponins	-	+
9	Phytosterols	+	-
10	Cardiac glycosides	+	-

“+” indicates presence and “-” indicates absence

Table I Phytochemical analysis of *Aegle marmelos* and *Solanum torvum*

H. Statistical Analysis

Results of the study are presented as mean \pm standard deviation per treatment group in triplicate. Significant differences between groups were determined by one way analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

A. Phytochemical Analysis

In the present study the phytochemical analysis of *Aegle marmelos* show the presence of alkaloids, aminoacids, reducing sugars, phenols, phytosterols and cardiac glycosides and the absence of flavonoids, tannins, steroids, saponins. The phytochemical analysis of *Solanum torvum* show the presence of Alkaloids, Aminoacids and saponins and the absence of flavonoids, tannins, reducing sugars, steroids, phenols, phytosterols, cardiac glycosides. Phytochemical screening of ethanolic extract of bael leaf has been shown to reveal the presence of alkaloids [11]. The alkaloid content was quantitatively estimated and was found in the range of 3.78 ± 0.15 to 16.08 ± 0.05 mg/g in different varieties of bael leaves [12]. Similarly, all the varieties exhibited good quantity of flavonoids and phenols starting from 10.40 ± 0.047 mg/mL and 5.80 ± 0.085 mg/mL, respectively [13]. The presence of medicinally important phytochemicals in the leaves of different varieties/accessions in aqueous and methanolic extracts of *Aegle marmelos*. Both the extracts revealed the presence of various phytochemicals such as tannins, saponins, flavonoids, alkaloids, terpenoids, carotenoids, cardiac glycosides, and reducing sugars in all the varieties and accessions while phlobatannins and anthocyanins were absent [12]. Earlier report on quantitative yield also revealed that *Aegle marmelos* contained highest quantity of alkaloids, flavonoids, and tannins as compared to other medicinal plants [14].

B. Percentage Mortality

In the present study the percentage mortality in *Aegle marmelos* is high at 72 hours for all the four concentrations (5%, 10%, 15%, and 20%). At 5% concentration, the percentage mortality is 13.3 ± 0.471 , it is 13.3 ± 0.471 for 10% concentration, 30 ± 0 for 15% concentration and 36.6 ± 0.471 for 20% concentration. The lowest percentage of mortality is seen at 24 hours 5% concentration (3.3 ± 0.471) as shown in Fig. 4. The percentage mortality in *Solanum torvum* is high at 72 hours for all the four concentrations (5%, 10%, 15%, and 20%). At 5% concentration, the percentage mortality is 13.3 ± 0.471 , 16.6 ± 0.471 for 10% concentration, 26.6 ± 0.471 for 15% concentration and 30 ± 0 for 20% concentration. The lowest percentage mortality is seen at 24 hours 5% concentration (3.3 ± 0.471) as shown in Fig. 5. The percentage mortality is high in *Aegle marmelos* than *Solanum torvum*. The analysis of variance for mortality of the *Aegle marmelos* leaves extract against *Tribolium castaneum* of different hours for different concentrations is significant at 5% level as shown in Table II. The analysis of variance for mortality of the *Solanum torvum* leaves extract against *Tribolium castaneum* of different hours for different concentrations is significant at 5% level as shown in Table III.

The main effects and interaction of various crude plant extracts and their doses are highly significant ($P < 0.05$) on percent mortality of *T. castaneum* at two different exposure intervals. The highest and lowest percent mortality ($55.83 \pm 2.17\%$ and $29.17 \pm 1.65\%$) was recorded in *Z. officinale* and *A. indica* treated wheat after 10 DEI, respectively, while no mortality was recorded in control treatment at both exposure intervals [15]. [16] observed that neem controlled up to 45.63% red flour beetle population after 168 h interval. [17] reported that $100 \mu\text{g/insect}$ controlled Red flour beetle population up to 53.13% after 72 h interval. Studies depict that 2.5% neem extract can control up to 45.63%. Reduction in mortality may be due to field strains exposure level in different countries. The result of the present study is similar to [18],[19],[20] who used neem as insecticide for control of insects.

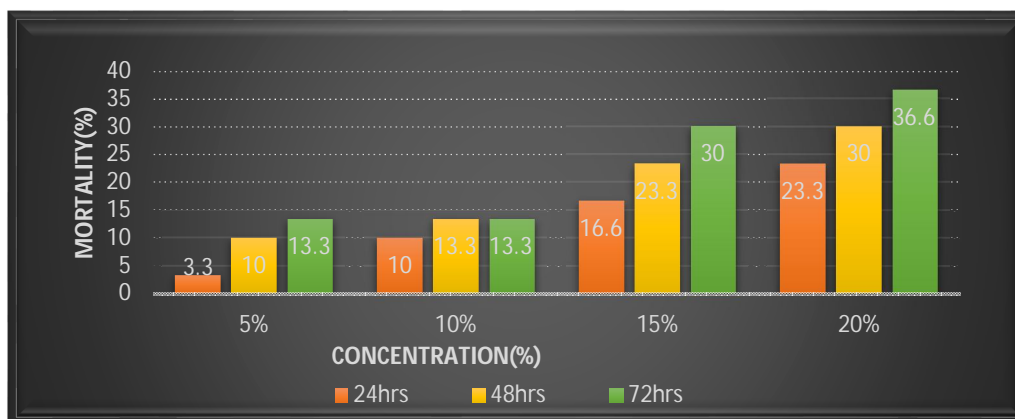


Fig. 4 Percentage mortality of *Tribolium castaneum* in *Aegle marmelos* aqueous extract

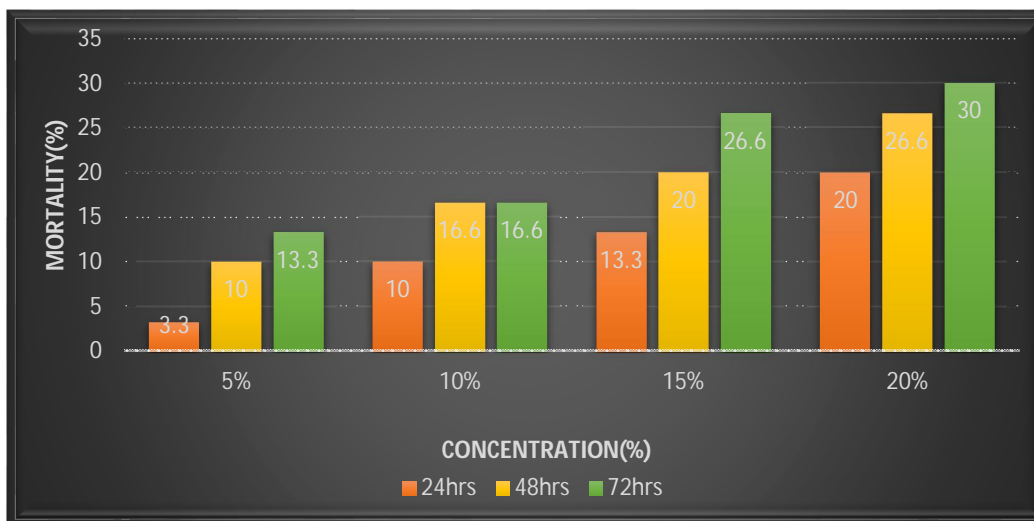


Fig. 5 Percentage mortality of *Tribolium castaneum* in *Solanum torvum* aqueous extract

Name of the plant	Hours	F	P value	F critical
<i>Aegle marmelos</i>	24hr	8.888	0.006	4.066
	48hr	15.16	0.001	4.066
	72hr	16.88	0.0008	4.066

(p<0.05 is significant)

Table II Analysis of variance for mortality at 24, 48, 72 hours for different concentrations of *Aegle marmelos* leaves extract against *Tribolium castaneum*

Name of the plant	Hours	F	P value	F critical
<i>Solanum torvum</i>	24hr	8.666	0.006	4.066
	48hr	8.666	0.006	4.066
	72hr	7.555	0.010	4.066

(p<0.05 is significant)

Table III Analysis of variance for mortality at 24, 48, 72 hours for different concentrations of *Solanum torvum* leaves extract against *Tribolium castaneum*

C. Percentage Repellency

The present study revealed that the percentage repellency in *Aegle marmelos* is high at 72 hours for all the four concentrations. At 5% concentration, the percentage repellency is 53.3 ± 0.471 , 63.3 ± 0.471 for 10% concentration, 73.3 ± 0 for 15% concentration and 80 ± 0.471 for 20% concentration. The lowest percentage of repellency is seen at 24 hours 5% concentration (43.3 ± 0.471) as shown in Fig. 6. The percentage repellency in *Solanum torvum* is high at 72 hours for all the four concentrations. At 5% concentration, the percentage repellency is 46.6 ± 0.471 , 60 ± 0 for 10% concentration, 66.6 ± 0 for 15% concentration and 73.3 ± 0.471 for 20% concentration. The lowest percentage repellency is seen at 24 hours 5% concentration (3.3 ± 0.471) as shown in Fig. 7. The percentage repellency is high in *Aegle marmelos* than *Solanum torvum*. The analysis of variance for repellency of the *Aegle marmelos* leaves extract against *Tribolium castaneum* of different hours for different concentrations is significant at 5% level as shown in Table IV. The analysis of variance for repellency of the *Solanum torvum* leaves extract against *Tribolium castaneum* of different hours for different concentrations is significant at 5% level as shown in Table V.

[21] showed that azadirachtin and neem concentrates repelled three insects in stored products, *Cryptolestes ferrugineus*, *S. oryzae* and *T. castaneum*, using a product which contained 98% AZA and the three neem extracts. In a choice test, filter paper strips treated with C200 lg/cm² of neem oil repelled *T. castaneum* adults and in a food preference chamber fewer adults settled in grains treated with 100 ppm of neem oil [22]. Although, the AZA content was very low in our study, it was found to cause overall repellency ranging from 52 to 88% for *T. castaneum* adults compared to the control.

[23] has investigated percent repellency against adult and larval stages of *T. confusum* with different concentration during the exposure period. . In case of *E. gluaca* for adult stage the highest repellency value was 100% with concentration 2.5% at the third hour. It was mainly due to main component present in essential oils of Eucalyptus species are 1-8 cineole which showed different insecticidal properties against insect pests.

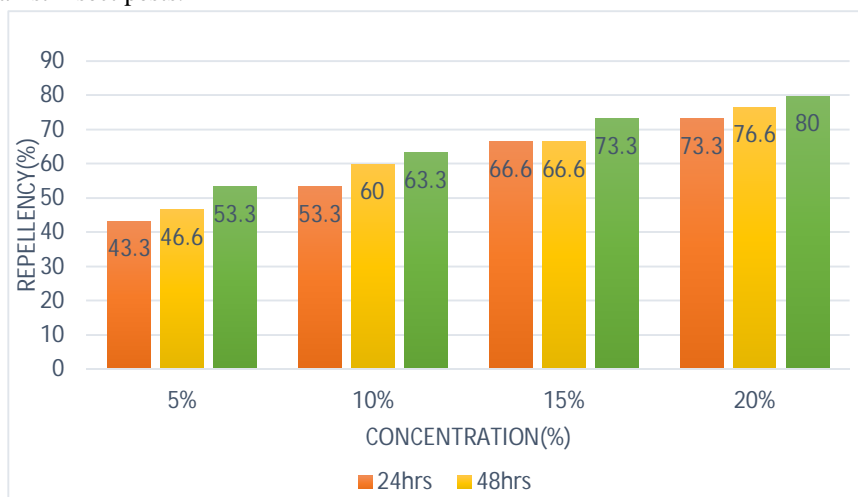


Fig. 6 Percentage repellency of *Tribolium castaneum* in *Aegle marmelos* aqueous extract

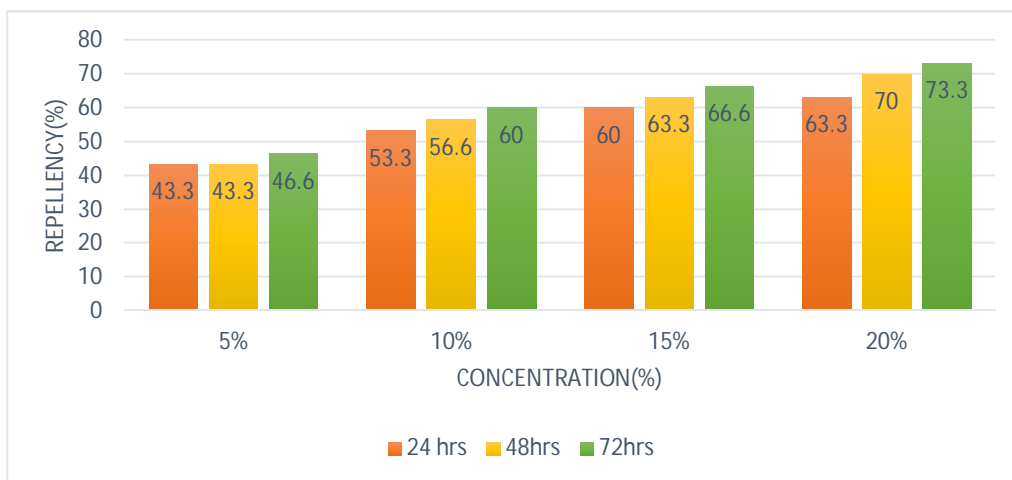


Fig. 7 Percentage repellency of *Tribolium castaneum* in *Solanum torvum* aqueous extract

Name of the plant	Hours	F	P value	F critical
<i>Aegle marmelos</i>	24hr	7.583	0.010	4.066
	48hr	19.55	0.0004	4.066
	72hr	19.33	0.0005	4.066

(p<0.05 is significant)

Table IV Analysis of variance for repellency at 24, 48, 72 hours for different concentrations of *Aegle marmelos* leaves extract against *Tribolium castaneum*

Name of the plant	Hours	F	P value	F critical
<i>Solanum torvum</i>	24hr	18.66	0.0005	4.066
	48hr	9.285	0.0055	4.066
	72hr	24.33	0.0002	4.066

($p < 0.05$ is significant)

Table V Analysis of variance for repellency at 24, 48, 72 hours for different concentrations of *Solanum torvum* leaves extract against *Tribolium castaneum*

IV. CONCLUSION

The plants including *Aegle marmelos* and *Solanum torvum* leaf extracts have a high potency to control the pest due to the presence of phytochemical and bioactive components. On the basis of above findings, percentage mortality and percentage repellency is due to the presence of bioactive compounds present in the plant extract so it is effective against the pest. The selected plants such as *Aegle marmelos* and *Solanum torvum* has a repellency and toxic effect against the stored grain pest *Tribolium castaneum*.

On the basis of the result of present study it can be concluded that these plants can be considered for the pesticide formulation that will be useful to control the pest. It is very effective in pest management. They could provide valuable alternatives to the synthetic insecticides.

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