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# Toxicity effect of *Annona reticulata* seed extract against bean aphid, *Aphis craccivora* Koch

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A B S T R A C T

Bean aphid is one of the major destructive pests to country bean in Bangladesh. Therefore, an investigation was carried out to evaluate the efficacy of *Annona reticulata* L. seed extract in six different solvents *viz.*, ethylalcohol, ethylacetate, acetone, chloroform, dichloromethane and n-hexane for their toxicity and repellent effects against bean aphid, *Aphis craccivora* Koch applied. Three different doses (0.25, 0.50 and 1.0 g $mL^{-1}$) with each solvent extract were used in the laboratory. The results revealed that *A. reticulata* seed extracts had direct toxic and repellent effects on bean aphid. The highest mortality was observed in the ethylalcohol extracts at 24 and 48 hours after treatments. Considering $LD_{50}$ values, it was observed that the extract in different solvents show toxicity in order of ethyl alcohol > n-hexane > chloroform > dichloromethane > ethyl acetate > acetone. Mortality percentages of all solvent extracts were directly proportional to the level of concentration and hours after treatment. The results also showed the repellency effect against *A. craccivora*. Among different doses, 1.0 mg $mL^{-1}$ had the highest insect mortality and repellency. The highest repellency was observed in ethyl alcohol extract (72.89%) and the lowest repellency was observed in acetone extracts (60.04%). All solvent extracts except acetone showed in the same repellency class *i.e.* class IV. Based on the mean repellency rate, it was found that all concentrations possess the same repellency class *i.e.* class IV.

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## INTRODUCTION

*Aphis craccivora* Koch (Homoptera: Aphididae) is one of the most destructive pests of the country bean, *Lablab purpureus* L., and it infests the leaves, twigs, inflorescences and the pods (Das 2002). The pods turn black due to thick colonies of the blackish-green *A. craccivora* and their severe damage to the plant. Both nymphs and adults of *A. craccivora* damage bean crops from vegetative to fruiting stages and may cause yield loss up to 100% in different species of legumes (Attia et al. 1986). When plants are heavily infested, leaf distortion and stunted growth commonly occur, and the end result is poor quality of fruits (York 1992). Furthermore, *A. craccivora* transmits about 20 non-persistent plant viruses including broad bean mosaic, Iranian strain viruses, etc., in many places in the world (Thottappilly and Rossel 1985). The indiscriminate and large-scale use of synthetic chemical pesticides to control this pest draws out in ecological imbalances, besides their toxic effects on other living organisms, including human beings. Hence, there is an urgent need for developing methods with ecofriendly standards (Rajappan et al. 2000). By 1971, the more than 6,000 of plant species were screened for insecticidal properties, and of those, more than 2,000 were reported to exhibit measurable to considerable activity (Jacobson 1975; Crosby 1971; Ahmed and Grainge 1982). Scientists are working to protect insect infestation by the indigenous plant materials and weeds. The use of locally available plants/weeds in the control of storage pests is an ancient technology in many parts of the world and is being used by our farmers also. The extracts of plant materials were unique in action and can be easily produced by farmers and small industries (Talukder and Howse 1994). The efficacy of *Azadirachta indica* Juss, (*neem*), *Calotropis procera* Aiton (*akanda*), *Polygonum hydropiper* L. (*biskatali*) and *Ipomoea sepiaria* Roxb. (*bankalmi*) on different

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crop pest has been reported by many workers (Vietmeyer 1992; Randhawa and Parmar 1993). Among these, *A. indica* was the most popular and widely used (Schmutterer and Singh 1995; Förster and Moser 2000; Basedow et al. 2003). Recently, Amin et al. (2000) also reported the use of *akanda*, *biskatali* and *neem* leaves as botanical insecticides against lesser grain borer.

Therefore, there is an utmost necessary of further research for uses of indigenous plant materials/weeds as botanical insecticides. Thus, due to the unique activity of indigenous plant materials/ weeds for pest control and also for greater interest of the farmers the present research was undertaken to study the effect of *Annona* seed extract as botanical insecticide against bean aphid to determine the mortality and the repellency as well as to assess the dose-mortality response on this pest.

## MATERIALS AND METHODS

The experiments were carried out to evaluate the efficacy of *Annona* seed crude extracts in different solvents at the laboratory of the department of entomology of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during from April 2015 to August 2015.

### Collection and Rearing of Test Insect

The bean aphid (*Aphis craccivora*) was collected from the infested plants of fields and built up insect stocks in the laboratory. Then, the stock insects were reared in rectangular jars (14 cm × 10.5 cm × 30 cm), setting in a growth chamber. Fresh twigs were given time to time. The jars were covered with pieces of cloth and fastened with rubber bands to prevent insect's escape.

### Collection, Processing and Preparation of Test Plant Dust

The *A. reticulata* seed was collected. After that primarily it was air dried. Finally, the seeds were well dried in an oven at 40 $^{o}$C for six hours. Then, it was grinded into powdered form with the help of mortar. At last, the powdered dusts were stored in specimen jars under laboratory conditions. Botanical dust was prepared by pulverizing the dried part in a magnetic stirrer (mortar). A 60-mesh diameter sieve was used to obtain the fine dust.

### Preparation of Crude Extract

One hundred grams of *A. reticulata* seed powder was dissolved in 300 mL of different solvents (Ethyl alcohol, Ethyl acetate, Acetone, Chloroform, Dichloromethane, and *n*-hexane) separately, stirred for 30 minutes in a magnetic stirrer. The mixture was then allowed to stand for 72 hours and shaking several intervals. Then, the mixture was filtered through a filter paper (Whatman no. 42) and allowed to evaporate the solvents. Finally, hard different colour extracts were obtained. The extracts were preserved in tightly corked vials and stored in a refrigerator for experimental use.

### Preparation of Stock Solution

Different doses or concentrations [0 (control), 0.25, 0.5, and 0.1.0, mg mL$^{-1}$] were prepared by diluting the crude extracts of *A. reticulata* seed in different solvents prior to insect bioassay. Pilot trials or experiments were done to obtain the appropriate dose.

### Procedure for Toxicity and Repellency Test

*Treatment Combination*

Different concentrations of *A. reticulata* seed crude extract (mg mL$^{-1}$) in six different solvents were treated as individual treatment. Three replications were maintained for each of the treatment/concentration (1.0, 0.5 and 0.25 mg mL$^{-1}$) of the ata seed extracts in different solvents along with control. Ten insects (both nymphs and adults) were used in each treatment. Observations were made on insect mortality and repellency.

### Insect Bioassay Tests (Toxicity Test and Repellency Test)

*Insect Mortality test*

The toxicity test was conducted according to the method (residual film method) described by Busvine (1971). One milliliter (1.0 mL) solution of crude extracts of different concentration was applied to the Petri dishes (90 mm) using a pipette covering the whole area of the Petri dishes internally. The Petri dishes were then kept in air for a few minutes to evaporate the solvents. After that 10 insects per replication were released and each treatment was replicated thrice. In the control, Petri dishes were treated with the solvents only. Insect mortalities were recorded at 24 and 48 hours after treatment (HAT). Observed mortalities (*i.e.* original data) of the insects were corrected by Abbott's formula (1987) as follows: [P = {(P'-C)/ (100-C)} × 100], Where, P = Corrected mortality (%), P' = Observed mortality (%), and C = Controlled mortality (%).

*Insect Repellency Test*

The repellency test was conducted according to the method described by Talukder and Howse (1994). Petri dishes sized (90 mm diameter) filter papers (Whatman No. 40) were cut in two half and 1.0 mL of each solution of *A. reticulata* seed extract was applied to a half filter paper uniformly with a pipette. The treated half discs were then air-dried and attached with the untreated half with a cello-tape. Precautions were taken so that attachment is not interfere with the free movement of insect from one half to another but distance between the filter paper segments remained sufficient to prevent seepage of test samples from one half of circle to another. Each filter paper was then placed in a Petri dish and 10 insects were placed there. There were three replications for each concentration of plant extracts. Numbers of insects on each portion were counted at hourly intervals upto the 5$^{th}$ hour. The data were expressed as percentage repulsion (PR) by the following formula: [PR (%) = ($N_c$-50) × 2] Where, $N_c$ = the percentage of insects present in the control half. Positive (+) values expressed repellency and negative (-) values attractancy. The average values were then categorized according to the following scale (McDonald et al. 1970) (Table 1).

**Table 1.** Relationship between repellency classes for the repellency test

| Class | Repellency rate (%) | Class | Repellency rate (%) |
|---|---|---|---|
| 0 | > 0.01 – 0.1 | III | 40.1 – 60 |
| I | 0.1 – 20 | IV | 60.1 - 80 |
| II | 20.1 – 40 | V | 80.1 -100 |

### Statistical Analysis

The experiments were conducted using completely randomized design (CRD). The data obtained from the experiments were statistically analyzed by a Mathematical and Statistical (MSTAT-C) computer program. The means of the statistically significant parameters were further tested by DMRT. The probit analyses were done according to Finney (1947) and Busvine (1971) using probit analysis software {GW-BASIC (1987) version 3.22}developed by Microsoft Software Company to estimate $LD_{50}$ values with their 95% fiducial limits. Heterogeneity was tested by a chi-square test. The tabulated values of chi-square were determined using probit transformation table (Busvine 1980). In case repellency, data

(percent repulsion, PR) were analyzed using Analysis of Variance (ANOVA).

## RESULTS

### Effect of Solvent Extracts

The effect of six different solvent extracts on the mortality of bean aphid is presented in Table 2. The differences among the solvents extracts having the maximum and the minimum were found to be statistically significant. At 48 HAT, it was observed that the highest toxicity was recorded in the ethyl alcohol extract (74.2%) followed by n-hexane (55.8%), chloroform (52.5%), acetone (46.7%) and ethyl acetate (40.8%).

**Table 2.** Effect of different solvent extracts of *A. reticulata* seed on the mortality of *A. craccivora* Koch at different HATs

| Solvents used | Mortality (%) at two different HAT*s | |
|---|---|---|
| | 24 | 48 |
| Ethyl alcohol | 65.8 a | 74.2 a |
| Ethyl acetate | 15.0 c | 40.8 c |
| Acetone | 11.7 c | 46.7bc |
| Chloroform | 23.3bc | 52.5bc |
| Dichloromethane | 23.3b | 40.8bc |
| n-hexane | 26.7 b | 55.8b |
| $S_{\bar{x}}$ | 0.233 | 0.207 |
| *P* value | < 0.001 | |

*HAT = Hours after treatment; within column values followed by different letter(s) are significantly different at $P<0.05$ by DMRT.

### Effect of Doses

The highest mortality (73.3%) was observed at maximum concentration (1.0 mg $mL^{-1}$) of solvent extract at 48 HAT and lowest (16.8%) was found in control, and the highest mortality was 4.9 times more over control treatment. Statistical significance was found among all of the treatment doses ($p<0.001$). Also, it was observed that the mortality percentage was directly proportional to the level of concentrations (Table 3).

**Table 3.** Residual toxic effects of different concentrations of *A. reticulata* seed extract on *A. craccivora* Koch at different hours after treatments (HATs)

| Dose (mg $mL^{-1}$) | Mortality (%) at different exposure hours | |
|---|---|---|
| | 24 HAT* | 48 HAT |
| 1.00 | 47.2 a | 73.3 a |
| 0.50 | 33.3 b | 62.8 b |
| 0.25 | 26.7 b | 54.4 c |
| Control | 3.3 c | 16.8 d |
| $S_{\bar{x}}$ | 0.191 | 0.169 |
| *P* value | | < 0.001 |

*HAT = Hours after treatment; within a column values followed by same letter(s) did not differ significantly at $P<0.05$ by DMRT.

### Interaction Effects on the Mortality of Bean Aphid

The interaction results of mortality percentages of *A. craccivora* treated with *A. reticulata* seed extract in six different solvent with three different doses and different exposure hours are shown in Table 4. All the solvent extracts and doses showed significantly differed ($p<0.001$) aphid mortality. It was found that the mortality percentage was directly proportional to the level of concentrations of different solvent extracts. The orders of toxicity of six different solvent extracts (based on the mortality percentages at 48 hours after treatment and the highest concentration) were: ethyl alcohol > acetone > chloroform > dichloromethane > *n*-hexane > ethyl acetate (Table 4).

**Table 4.** Interaction effect of solvents and doses at different exposure hours on the mortality of bean aphid, *A. craccivora* Koch

| Solvents used | Doses | | Mortality (%) at different time intervals | |
|---|---|---|---|---|
| | (mg $mL^{-1}$) | (mg $cm^{-2}$) | 24 HAT* | 48 HAT |
| Ethyl alcohol | 1.00 | 0.026 | 93.3 a | 96.7 a |
| | 0.50 | 0.013 | 86.7 a | 93.3 ab |
| | 0.25 | 0.006 | 83.3 a | 90.0 a-c |
| | Control | Control | 3.3 fg | 16.7 h |
| Ethyl acetate | 1.00 | 0.026 | 30.0 b-f | 66.7 b-f |
| | 0.50 | 0.013 | 20.0 d-g | 60.0 d-f |
| | 0.25 | 0.006 | 6.7 fg | 30.0 gh |
| | Control | Control | 3.3 fg | 16.7 h |
| Acetone | 1.00 | 0.026 | 16.7 e-g | 83.3 a-d |
| | 0.50 | 0.013 | 13.3 e-g | 66.7 b-f |
| | 0.25 | 0.006 | 13.3 e-g | 46.7 fg |
| | Control | Control | 3.3 fg | 16.7 h |
| Chloroform | 1.00 | 0.026 | 46.7 bc | 80.0 a-e |
| | 0.50 | 0.013 | 30.0 b-f | 66.7 b-f |
| | 0.25 | 0.006 | 16.7 e-g | 53.3 e-g |
| | Control | Control | 0.0 g | 16.7 h |
| Dichloromethane | 1.00 | 0.026 | 43.3 b-d | 76.7 a-e |
| | 0.50 | 0.013 | 36.7 b-e | 53.3 e-g |
| | 0.25 | 0.006 | 26.7 c-g | 53.3 e-g |
| | Control | Control | 6.7 fg | 16.7 h |
| n-hexane | 1.00 | 0.026 | 53.3 b | 76.7 a-e |
| | 0.50 | 0.013 | 30.0 b-f | 76.7 a-e |
| | 0.25 | 0.006 | 20.0 d-g | 63.3 c-f |
| | Control | Control | 6.7 fg | 16.7 h |
| $S_{\bar{x}}$ | | | 0.782 | 0.863 |
| *P* value | | | 0.333 | 0.001 |

*HAT = Hours after treatment; within a column values followed by same letter(s) did not differ significantly at $P<0.05$ by DMRT. Values were based on six different solvents, three concentrations, three replications of 10 insect each.

### Probit Analysis for Toxicity

The results of the probit analysis for the estimation of $LD_{50}$ values, 95% fiducial limits, regression equations and $\chi^2$ (chi square) values at 24 and 48 HAT for the mortality of *A. craccivora* are presented in Table 5. The calculated $LD_{50}$ values were 3.29E-04, and 2.33E-04 mg $cm^{-2}$ in ethyl alcohol extract after 24 and 48 hours exposure period, respectively; 0.05 and 1.65E-02 mg $cm^{-2}$ in ethyl acetate extract after the same hours of exposure period, respectively and in acetone extract the values were 99.88 and 9.39E-03 mg $cm^{-2}$ after the same exposure hours as well, respectively. The calculated $LD_{50}$ values in chloroform extract were 0.03 and 6.88E-03 mg $cm^{-2}$ after 24 and 48 hours after treatment and in dichloromethane extract the values were 5.38E-02 and 1.07E-02 mg $cm^{-2}$ after the same exposure hours, respectively; and in n-hexane extract the values were 2.80E-02 and 2.69E-03 mg $cm^{-2}$ after the same exposure period as well, respectively. Comparison of the $LD_{50}$ values at 48 HAT, it was observed that the *A. reticulata* seed extract in different solvents show toxicity in order of ethyl alcohol > *n*-hexane > chloroform > acetone > dichloromethane > ethyl acetate. From the probit analysis, it is clear that all the solvent extracts had more or less effective for controlling bean aphid but ethyl alcohol was the most effective.

### Repellent Effects on Bean Aphid

#### Solvent effect

The influence of different solvents extracts of *A. reticulata* seed on the mean repellency rate is presented in Table 6. Among the solvents ethyl alcohol extract showed the highest mean repellency effect (72.9%) followed by chloroform (71.5%), n-hexane (71.0%), dichloromethane (66.6%), ethyl acetate (64.3%), and acetone (60.0%). On the basis of the mean repellency rate, it was found that all solvents except acetone (repellency class III) were in the same repellency class *i.e.* class IV.

**Dose effect**

The repellency rate of different plant extracts at level of concentrations on bean aphid is presented in Table 7. It is observed from the table that the mean repellency rate was directly proportional with the doses. On the basis of the mean repellency rate, it was found that all concentrations possesses the same repellency class *i.e.* class IV.

**Interaction effect**

The interaction effects of solvent, dose and time are presented in Table 8. The highest mean repellency rate was found with n-hexane extract of *A. reticulata* seed at 1.00 mg $mL^{-1}$ dose level (76.0%) and possessed repellency class IV and the lowest rate was found with ethyl acetate extract at 0.25 mg $mL^{-1}$ dose level (46.7%) and showed repellency class III.

**Table 5.** $LD_{50}$, 95% fiducial limits, regression equations and $\chi 2$ values of *A. reticulata* seed extract in six different solvents treated on *A. craccivora* Koch.

| Solvents used | N* | Duration (hours) | $LD_{50}$ (mg $cm^{-2}$) | 95% fiducial limit | | Regression equations | $\chi 2$ value with 1 df |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| Ethyl alcohol | 450 | 24 | 3.29 E-04 | 6.51 E-07 | 0.17 | Y = 5.36 + 0.75 X | 0.21 |
| | 450 | 48 | 2.33 E-04 | 2.32 E-07 | 0.23 | Y = 5.52 + 0.83 X | 8.10 E-03 |
| Ethyl acetate | 450 | 24 | 0.05 | 1.92 E-02 | 0.13 | Y = 1.83 + 1.87 X | 0.44 |
| | 450 | 48 | 1.65 E-02 | 1.16 E-02 | 2.36 E-02 | Y = 2.71 + 1.88 X | 1.79 |
| Acetone | 450 | 24 | 99.88 | 5.61 E-16 | 1.78 E+19 | Y = 3.45 + 0.31 X | 8.47 E-02 |
| | 450 | 48 | 9.39 E-03 | 6.47 E-03 | 1.36 E-02 | Y = 3.21 + 1.84 X | 1.23 E-02 |
| Chloroform | 450 | 24 | 0.03 | 1.39 E-02 | 0.07 | Y = 2.95 + 1.38 X | 4.38 E-03 |
| | 450 | 48 | 6.89 E-03 | 3.55 E-03 | 1.34 E-02 | Y = 3.92 + 1.29 X | 3.98 E-02 |
| Dichloromethane | 450 | 24 | 5.38 E-02 | 7.60 E-03 | 0.38 | Y = 3.57 + 0.83 X | 6.54 E-02 |
| | 450 | 48 | 1.07 E-02 | 6.08 E-03 | 1.89 E-02 | Y = 3.86 + 1.10 X | 1.84 |
| n-hexane | 450 | 24 | 2.80 E-02 | 0.02 | 5.07 E-02 | Y = 2.52 + 1.71 X | 0.26 |
| | 450 | 48 | 2.69 E-03 | 2.05 E-04 | 3.53 E-02 | Y = 4.72 + 0.66 X | 0.36 |

N* Number of insects used in the experiment. Values were based on six different solvents, three concentrations, three replications of 10 insect each; $\chi 2$ = Goodness of fit; df = Degrees of freedom; $LD_{50}$ = Median lethal dose; Non-significant $\chi 2$ values indicate that all the equations fitted well with no heterogeneity

**Table 6.** Repellent effects of different solvents used in preparing different plant extracts on bean aphid *Aphis craccivora* Koch using treated filter paper at different HATs (Interaction of solvents and time)

| Solvents used | Repellency rate (%) at | | | | | Mean repellency (%)* | Repellency Class |
|---|---|---|---|---|---|---|---|
| | 1HAT | 2HAT | 3HAT | 4HAT | 5HAT | | |
| Ethyl alcohol | 66.7 a | 71.6 a | 80.4 a | 79.1a | 66.7 d | 72.9 | IV |
| Ethyl acetate | 45.8 cd | 57.3 bc | 62.7 b | 78.2 a | 77.3 bc | 64.3 | IV |
| Acetone | 40.9 d | 54.2 c | 65.8 b | 66.2 b | 73.3 cd | 60.0 | III |
| Chloroform | 60.0 b | 68.4 a | 72.0ab | 75.1 a | 81.8 ab | 71.5 | IV |
| Dichloromethane | 45.8 cd | 62.2 abc | 71.1ab | 74.2 ab | 79.6 bc | 66.6 | IV |
| n-hexane | 55.1 bc | 64.4 ab | 68.4 b | 79.6 a | 87.6 a | 71.0 | IV |
| $S_{\bar{x}}$ | 3.762 | 3.291 | 3.184 | 2.897 | 2.691 | - | - |
| *P* value | <0.001 | 0.002 | 0.003 | 0.013 | <0.001 | - | - |

* Indicate mean of means at different HATs within column values followed by same letter(s) did not differ significantly at $p<0.05$ by DMRT.

**Table 7.** Repellent effects of different concentrations on bean aphid *A. craccivora* Koch using treated filter paper at different HAT (Interaction of concentration and time)

| Conc. (mg $mL^{-1}$) | Repellency rate (%) | | | | | Mean repellency* (%) | Repellency Class |
|---|---|---|---|---|---|---|---|
| | 1HAT | 2HAT | 3HAT | 4HAT | 5HAT | | |
| 1.00 | 52.0 a | 63.1 a | 71.1 a | 78.2 a | 79.3 a | 68.8 | IV |
| 0.50 | 54.4 a | 65.6 a | 68.9 a | 74.2 a | 78.7 a | 68.4 | IV |
| 0.25 | 50.7 a | 60.4 a | 70.2 a | 73.8 a | 75.1 a | 66.0 | IV |
| $s_{\bar{x}}$ | 2.60 | 2.33 | 2.25 | 2.05 | 1.90 | - | - |
| *P* value | NS | 0.302 | NS | 0.243 | 0.244 | - | - |

* Indicate means of mean repellency at different HAT (Hours after treatment); NS= Not significant; within column values followed by different letter(s) are significantly different at $p<0.05$ by DMRT.

## DISCUSSION

Ethyl alcohol solvent extract of *A. reticulata* seed showed the highest mortality of *A. craccivora* which was 9.4 times more over control treatment. Similarly, the mortality percent recorded by Bhuyain et al. (2009, 2010) supported the present findings. The *biskatali* plant extracts to have a toxic, repellent and residual effect on progeny production against *T. castaneum* which is similar to the results of the present study. The present study of the investigation is also coincided with the findings of Das et al. (2008) who evaluated aphidicidal activity of hot and cold water extracts of some indigenous plants, *Azadirachta indica* Juss (*neem), Calotropis procera* (Aiton) (*akanda*), *Polygonum hydropiper* L. (*biskatali*) and *Ipomoea sepiaria* Roxb. (*bankalmi*), were tested against the bean aphid, *A.craccivora* Koch. Hot water extract of *P. hydropiper* and *A. indica* was found to be the most effective (87.6–94.5 and 80.5–89.6% mortality respectively, $p<0.01$) among all the extracts. Other hot and cold water extracts also appeared to be useful (59.5–87.5% mortality) as pesticides for *A. craccivora.* Kundu et al. (2007) investigated the toxic effect of biskatali plant extract in two solvents namely ethyl alcohol and chloroform against red flour beetle. They observed that plant extract in both the solvents were moderately toxic, strong repellent and had remarkable residual effect on progeny reduction against *T. castaneum*, which is similar to the results of the present study. Similar trend was also found with the findings of Sinchaisri et al. (1991) who evaluated forty nine ethanol crude extracts from 44 plant species were tested for the insecticidal activity on third instar diamond back moth larvae under laboratory conditions. Three plant extracts (20 mg $mL^{-1}$ conc.) showed high insecticidal activity (100% mortality of larvae): *Annona muricata* seeds, *Annona squamosa* seeds and *Stemona collinsae* roots.

**Table 8.** Repellency rate of *A. reticulata* seed extracts in six different solvents on bean aphid, *A. craccivora* Koch using treated filter paper (Interaction of solvent, dose and time)

| Solvents used | Dose (mg mL$^{-1}$) | Average repellency rate (%) at different time intervals | | | | | Mean repellency rate (%) | Repellency class |
|---|---|---|---|---|---|---|---|---|
| | | 1 HAT | 2 HAT | 3 HAT | 4 HAT | 5 HAT | | |
| Ethyl alcohol | 1.00 | 60.0 a-g | 60.0 a-g | 86.7abc | 80.0 a-d | 53.3 cde | 68.0 | IV |
| | 0.50 | 66.7 a-f | 73.3 a-e | 73.3 a-e | 66.7 a-e | 73.3 a-d | 70.6 | IV |
| | 0.25 | 40.0 c-h | 66.7 a-f | 46.7 c-e | 80.0 a-d | 73.3 a-d | 61.3 | IV |
| Ethyl acetate | 1.00 | 46.7 b-h | 46.7 c-g | 53.3 b-e | 80.0 a-d | 73.3 a-d | 60.0 | III |
| | 0.50 | 33.3 d-h | 40.0 d-g | 73.3 a-e | 66.7 a-e | 73.33 a-d | 57.3 | III |
| | 0.25 | 6.7 hi | 46.7 c-g | 33.3 e | 66.7 a-e | 80.0 a-d | 46.7 | III |
| Acetone | 1.00 | 26.7 e-h | 40.0 d-g | 60.0 a-e | 53.3 b-e | 73.3 a-d | 50.6 | III |
| | 0.50 | 33.3 d-h | 40.0 d-g | 53.3 b-e | 53.3 b-e | 73.3 a-d | 50.6 | III |
| | 0.25 | 40.0 c-h | 46.7 c-g | 40.0 de | 53.3 b-e | 60.0 b-e | 47.9 | III |
| Chloroform | 1.00 | 13.3 ghi | 66.7 a-f | 53.3 b-e | 73.3 a-e | 66.7 a-e | 54.6 | III |
| | 0.50 | 73.3 a-e | 33.3 efg | 46.7cde | 60.0 a-e | 66.7 a-e | 55.9 | III |
| | 0.25 | 66.7 a-f | 40.0 a-d | 53.3 b-e | 73.3 a-e | 53.3 cde | 57.3 | III |
| Dichloromethane | 1.00 | 73.3 a-e | 66.7 a-f | 80.0 a-d | 73.3 a-e | 80.0 a-d | 74.7 | IV |
| | 0.50 | 53.3 a-h | 73.3 a-e | 73.3 a-e | 86.7abc | 93.3 ab | 76.0 | IV |
| | 0.25 | 60.0 a-g | 73.3 a-e | 86.7abc | 80.0 a-d | 80.0 a-d | 76.0 | IV |
| n-hexane | 1.00 | 60.0 a-g | 66.7 a-f | 73.3 a-e | 86.7abc | 93.3 ab | 76.0 | IV |
| | 0.50 | 46.7 b-h | 60.0 a-g | 53.3 b-e | 80.0 a-d | 93.3 ab | 66.7 | IV |
| | 0.25 | 46.7 b-h | 40.0 d-g | 60.0 a-e | 60.0 a-e | 93.3 ab | 60.0 | III |
| $s_{\bar{x}}$ | | 14.57 | 12.75 | 12.33 | 11.22 | 10.42 | - | - |
| *P* value | | 0.043 | 0.016 | 0.033 | 0.047 | NS | - | - |

*Indicate means of mean repellency at different HAT (Hours after treatment); NS= Not significant; within column values followed by same letter(s) did not differ significantly at $p<0.05$ by DMRT.

The results of the present investigation agree with the findings of Kundu et al. (2007) who investigated the repellent effect of Biskatali plant extract in two solvents namely ethyl alcohol and chloroform against red flour beetle. They observed plant extract in chloroform solvent showed the highest repellency to *T. castaneum* than in ethyl alcohol solvent. The present study supported with the Talukder and Howse (1994, 1995) who reported that the seed extracts of *Aphanamixis polystachya* had strong repellent effects on red flour beetle and rice weevil.

## CONCLUSION

The present study indicates the potentiality of our local botanical (*A. reticulata* seed extracts) as insecticide against bean aphid. Much promising information was obtained from the present study. However, it has been observed from the results that the tested botanical products had more or less effective to repellent and direct toxicity compared to control. Higher concentrations of different solvent extracts have increased their effects. The experimental results indicate that 1.00 mg mL$^{-1}$, 0.50 mg mL$^{-1}$ and 0.25 mg mL$^{-1}$ of different solvents extracts are effective for bean aphid mortality and repellency. Among these concentrations, 1.00 mg mL$^{-1}$ extract in different solvents showed strong mortality and repellent effects. Finally, it can be concluded that these initial efficacy tests of the present experiment will be helpful to identify the potentials of botanical products. Further research may be conducted in future to ascertain the effect of plant extracts on reproductive strategy of aphid and to isolate the active principles that are present in the extracts.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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