

## ***In-vitro* Response of Cereal Aphid *Sitobion avenae* (Homoptera: Aphididae) against some Selected Botanical Extracts**

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### **Abstract**

Cereal aphid, *Sitobion avenae* (Homoptera: Aphididae), is one of the destructive pests of wheat and other crops around the globe. Irrational utilization of highly toxic synthetic chemicals against this particular pest has led to several issues of health and environment. In such case, there is a need to find out the control measures, which are less toxic and friendly to environment. The present study was aimed to evaluate the effectiveness of aqueous extracts of four indigenous plants, *i.e.* akk (*Calotropis procera*), lemon (*Citrus limon*), neem (*Azadirachta indica*) and dhatura (*Datura alba*), against *S. avenae* in laboratory conditions. Toxicity bioassays were conducted according to Completely Randomized Design using 2% aqueous extracts with three replications. Results showed a differential response of aphids to all botanical extracts. Aphid mortality increased with the exposure time with maximum values (*i.e.* 39.52, 31.75, 26.76 and 24.07% for *A. indica*, *C. procera*, *C. limon* and *D. alba* extracts, respectively) recorded at 72 h post-treatment. Similarly, *A. indica* extract exhibited minimum fecundity (2.67 offspring per adult) and adult body weight (340.09 µg) and maximum nymphal development time (9.32 days), followed by *C. procera*, *C. limon* and *D. alba*. Overall study results suggest that *A. indica* and *C. procera* extracts would be effective botanical options for combating aphid infestations on wheat and other cereal crops.

**Keywords:** *Sitobion avenae*, Toxicity bioassay, Botanical extracts, *Azadirachta indica*, *Calotropis procera*

Article History: **Received:** 20<sup>th</sup> February, 2020; **Revised:** 11<sup>th</sup> October, 2020 **Accepted:** 20<sup>th</sup> October 2020

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## Introduction

Wheat is an important cereal and staple crop fulfilling the food requirements of ever-growing world population. Pakistan is the 8th largest wheat producing country and contributes about 3.17% to the global wheat production (Qureshi et al., 2007). However, there is a considerable yield gap among Pakistan and other top wheat producing countries (Badar & Din, 2005). One of the limiting factors for wheat production in Pakistan is the infestation by aphids. Cereal aphids are recognized as major destructive pests of wheat (Steffey & Gray, 2012). Many studies have been conducted to estimate the yield losses due to wheat crop infestation by these aphids (Poehling et al., 2007).

Aphids affect the plant directly as well as indirectly. Direct wheat yield losses caused by the aphids would be as high as 35-40% usually occurred due to sap sucking, while indirect losses would vary among 20-80% caused by the transmission of various viral and fungal diseases by these aphids (Rossing et al., 1994; Aslam et al., 2005; Akhtar & Mujahid, 2006; Akhtar et al., 2010). In Indo-Pak regions, *Sitobion avenae* (Hemiptera: Aphididae) is a serious pest of wheat crop and is considered as the most dominant species of cereal aphids. It causes considerable yield loss in wheat crop of Pakistan (Khan et al., 2006; Aheer et al., 2008).

For the control of aphids and other insect pests in the field, synthetic insecticides are always the first choice by farmers. Farmers in Pakistan mostly rely on the blind and irrational use of various synthetic insecticides for the control of aphids on wheat crop. This increased and extensive use of hazardous and persistent synthetic insecticides is alarming for the human health and environmental quality (Iqbal, 2009). Moreover, elimination of natural enemies of insect pests and pest resistance to insecticides are also among the most contemporary issues of chemical pest control (Godfrey, 2000). This situation necessitates the development of alternates of conventional synthetic insecticides. For instance, phytochemicals and plant extracts have long been a subject of interest for researchers to establish these materials with least health and environmental effects and to reduce the non-target effects of synthetic insecticides (Dancewicz et al., 2011).

Botanicals are supposed to be used as a replacement of synthetic insecticides in order to reduce insect pest losses. These are usually environment friendly and also safe for the human health as compared to the synthetic insecticides. Many studies have demonstrated the effectiveness of various botanicals against a wide range of insect pests including aphids (Prakash & Rao, 2003; Mamoon-ur-Rashid, 2012; Biswas, 2013). In this direction, this study was aimed to determine the toxicity of 2% aqueous leaf extracts of some indigenous plant species, *i.e.* akk (*Calotropis procera*), lemon (*Citrus limon*), neem (*Azadirachta indica*) and dhatura (*Datura alba*), against *S. avenae* and their effect was evaluated on different life history parameters of aphids including adult body weight, fecundity, maturity rate and survival percentage.

## **Materials and methods**

### **Insect Culture**

Field collected clones of aphids (*S. avenae*) were reared in glass cages (20×20×30 cm) at controlled temperature 20±10°C and relative humidity 50±10% in order to acclimatize them with the laboratory conditions. Fresh wheat (*Triticum aestivum*) seedlings were grown in small earthen pots and were used as host for aphid rearing purpose. Active and healthy aphid individuals were used in bioassays.

### **Preparation and application of plant extracts**

Aqueous leaf extracts of the plants were prepared following previously described protocol (Odey, 2012). About 200 g fresh leaves of each treatment plant were placed for about 6 to 9 days for shade drying and in an hot air oven at 40°C for about 24 h for complete drying. These dried leaves were separately grounded to fine powder. Aqueous solution of botanicals was prepared by mixing 20 g of each plant leaf powder in 100 ml of water. Then, these solutions were placed in a shaker at 40°C for about 12 h for their homogenized mixing. After that, two times filtration of each botanical was done by using common filter paper (Whatman No. 1) to get the maximum refined form of each botanical. Three replications of each treatment were evaluated including a control treatment. For the comparison studies of different biological parameters, 20 freshly laid aphid individuals were released on each potted wheat plant and were allowed for one day to synchronize with the new plants. Single dose (2%) of each botanical was prepared and applied uniformly on the plant with the help of a hand sprayer.

### **Data recording**

Data regarding the mortality of exposed aphid individuals were recorded after 12, 24, 48 and 72 hours of application. Furthermore, the biological parameters of aphids, *i.e.* adult body weight, fecundity, maturity and survival rate, were also determined. Collected data was subjected to One-Way ANOVA under complete randomized design (CRD) for statistical interpretation of study parameters.

### **Fecundity and maturity rate (Days to reach maturity)**

Twenty 1<sup>st</sup> instar aphids were released on potted wheat plants with three replications of each botanical. After application of botanicals, aphids were allowed to survive and at maturity the data regarding fecundity was noted for three days for each botanical. In control treatment, the plants containing aphids were sprayed only with water. For calculating the maturity rate, data was recorded for three days when the aphids started to mature.

### Adult body weight

For calculation of adult body weight, a parallel experiment was conducted with four different plant extracts on potted wheat plants containing aphids with three replications of each botanical. When the aphids reached to maturity they were weighted. The standard used for recording adult body weight was  $\mu\text{g}$  (micro gram). The data was recorded for three days against each botanical and control treatment.

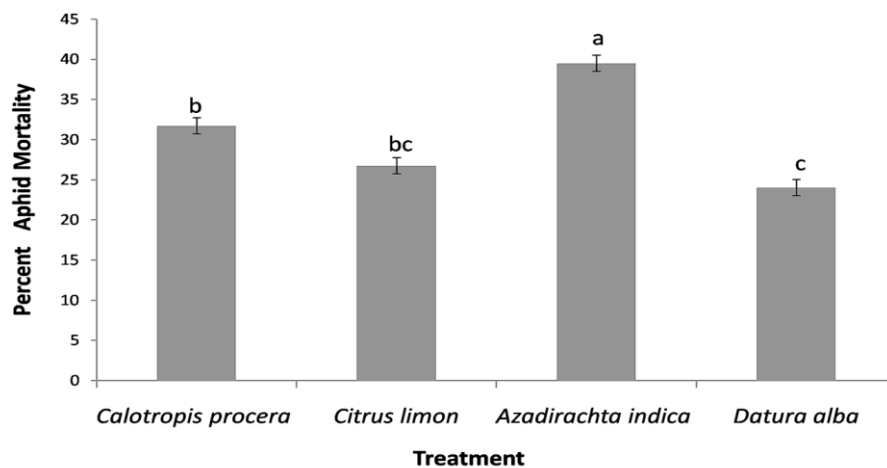
### Survival

Survival analysis was also conducted by subjecting the data to Kaplan-Meier survival analysis to draw a survival plot of *S. avenae* against four different botanical treatments.

## Results

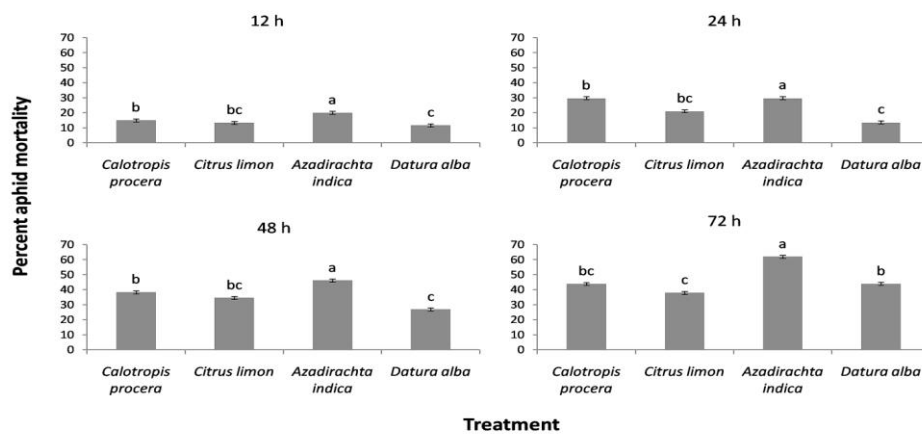
### Mortality of *S. avenae*

Comparative toxicity of selected plant extracts at 5% level of significance has been shown in Fig 1. Mean mortality of *S. avenae* differed significantly in all treatments (Fig. 1). Maximum mean mortality (39.52%) was observed in case of *A. indica* application. *C. procera* showed the mean mortality of 31.75%, followed by *C. limon* (26.76%) and *D. alba* (24.07%).

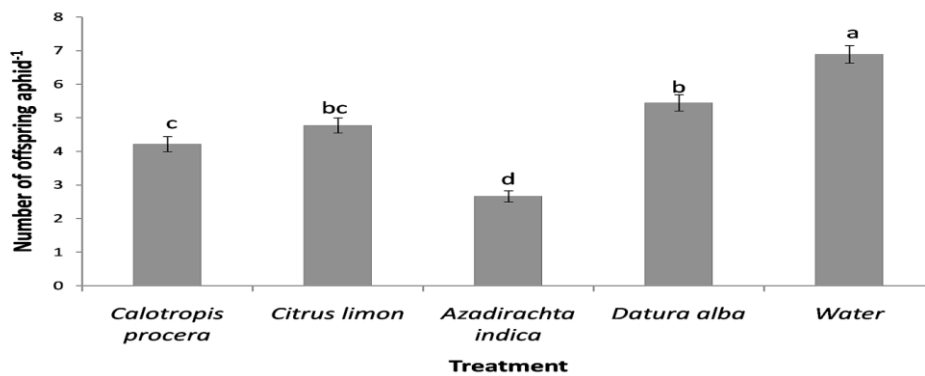


**Figure 1.** Percent mortality (mean  $\pm$  SE) of *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants.

Mean values for aphid mortality at different time intervals after the application of botanicals are given in Fig. 2. Results showed that the time interval had a significant effect on the mortality of aphids. With every passing time interval, a decrease in aphid population was observed. Mean mortality percentage was maximum (46.97%) after 72 h of botanicals application. After 48 h of treatment application, mean aphid mortality percentage was 36.52%, followed by the mortality percentages of 23.61% and 15.00% recorded at 24 and 12 h post-treatment, respectively.



**Figure 2.** Percent mortality (mean ± SE) of *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants recorded at different time intervals



**Figure 3.** Mean fecundity (mean ± SE) of *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants recorded at different time intervals

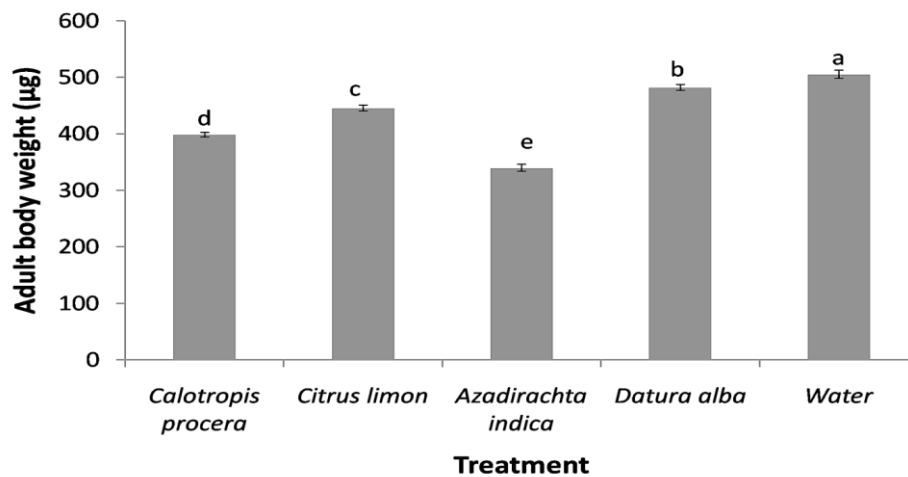
Results showed that maximum fecundity (6.89%) was observed in control treatment (Fig. 3). Mean fecundity caused by *D. alba* treatment was 5.44%, followed by *C. limon* (4.78%) and *C. procera* (4.22%). Minimum mean fecundity (2.67%) of *S. avenae* was observed for the application of *A. indica* leaf extract.

### Adult body weight

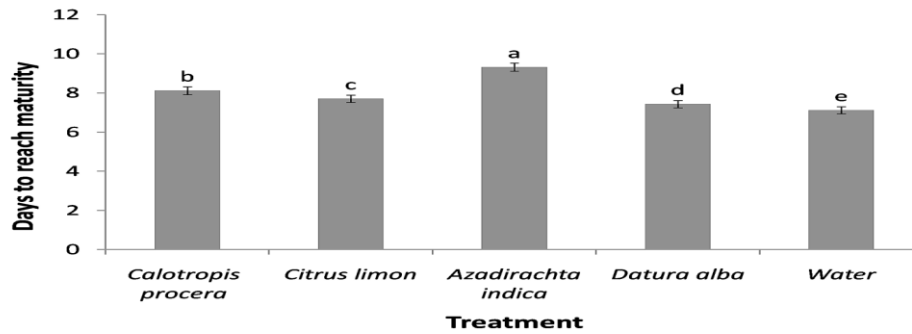
All the treatments had a significant effect on the adult body weight of aphids. Control treatment and *D. alba* showed the average adult body weight of 505.82 and 482.22  $\mu\text{g}$ , respectively (Fig. 4). Average adult body weight of 445.76 and 399.02  $\mu\text{g}$  was found on *C. limon* and *C. procera* treatments, respectively. *A. indica* leaf extract showed the minimum percent adult body weight of 340.09  $\mu\text{g}$ .

### Maturity time (days to reach maturity)

Maturity time (days to reach maturity) of cereal aphid, *S. avenae* against four different natural plant extract treatments is given in Fig. 5. There was a notable difference in the maturity time of aphids on *A. indica* (9.32 days) and water (7.12 days). There was subtle difference among the maturity times taken by aphids on *C. limon* and *D. alba* treated plants (Fig. 5).



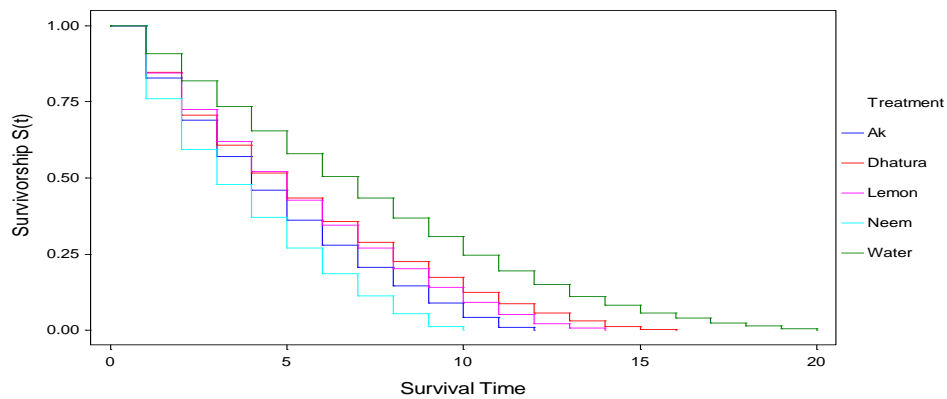
**Figure 4.** Adult body weight of *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants recorded at different time intervals



**Figure 5.** Days to reach maturity of *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants recorded at different time intervals

### Survival

Survival was calculated by releasing the 20 first instar nymphs of *S. avenae* on wheat plants. The aphids were allowed to stand for 24 h to synchronize with the plants. After the treatment application, data was recorded regarding the mortality of aphids at regular time intervals till their death. The obtained data was subjected to Kaplan-Meier survival analysis to draw a survival plot of *S. avenae* in response of different botanical extracts. Maximum survival of *S. avenae* was found in control treatment where aphids were able to survive for a maximum period of time as compared to all other treatments (Fig. 6). In other treatments, maximum aphid survival was found on *D. alba* treated plants, followed by *C. limon* and *C. procera*. The least survival of aphids was recorded on plants treated with the *A. indica* leaf extract (Fig. 6).



**Figure 6.** Survival analysis of cereal aphid, *Sitobion avenae* (Homoptera: Aphididae) by 2% aqueous botanical extracts of different plants recorded at different time intervals

## Discussion

In case of mortality of cereal aphid, *S. avenae* (Homoptera: Aphididae), *A. indica* (neem) treatment was found to be most effective after a time interval of 72 h. Similar findings are reported by Aziz *et al.* (2013) that extracts from different neem products were highly effective against aphids and caused their maximum mortality. Neem leaf extract (NLE) was found to be more effective after imidacloprid and neem seed oils and caused significant mortality. Regarding different biological features of cereal aphid, *S. avenae*, it was found that in case of all parameters *i.e.* fecundity, maturity rate, adult body weight and survival; *A. indica* had significant effect than other botanical extracts. These results can be compared with Mordue & Nisbet (2000). They described that neem based products are very effective in reducing certain insect pest population and producing toxic effects. Antifeedant and physiological effects of neem have also been described by many studies. Our results are in line with those of Singh *et al.* (2012) who tested the leaf extracts of different plant species against aphids for their repellency properties. It was found that the extract of *A. indica* was the most repellent against aphids.

The obtained results are also in accordance with Kraiss & Cullen (2008) who evaluated the growth regulatory effect of *azadirachtin* against the fecundity, development and survivorship of aphids. The results showed that about 80% mortality of aphid nymphs was caused by *A. indica* extract. It was also found that the aphids which survived the application got an arrested development and took more time to reach maturity as compared to the normal time period.

Our results are also according to the findings of Pavela *et al.* (2004) who tested the effect of *azadirachtin* on cabbage aphids. Results were evaluated in the form of mortality, fecundity and developmental period. It was found that mortality was increased as the concentration was increased. The fecundity of aphids was also reduced after *azadirachtin* application. Results can also be compared with Singh *et al.* (2012) who tested different leaf extracts against aphids for their repellency properties. It was found that the extract of *A. indica* was most repellent against aphids.

Ali *et al.* (2010) also tested some botanicals against aphids. Among the tested botanicals, Indian neem was found to be most effective in controlling insects. Results regarding the effects of neem can also be compared with Lowery & Isman (1994) who described that 50% mortality of 2<sup>nd</sup> instar aphids from nine different species was caused by the lethal concentrations of *A. indica*. Growth regulatory effects were also exhibited by *A. indica* against aphid species.

Similar findings were presented by Santos *et al.* (2004) who evaluated *A. indica* against aphids. It was found that at high concentrations the mortality was highest and developmental period and reproduction were also affected to some extent. Results obtained in this research are also in comparison with Sohail *et al.* (2012) who tested the efficacy of three different extracts against aphids using a concentration of 2%. It was



found that the mortality caused by *A. indica* at 2% was about 68% suggesting the utilization of *A. indica* as potential botanical control option against this pest.

### Conclusion

This laboratory study was aimed to evaluate the response of cereal aphid, *S. avenae* against some selected indigenous plant extracts. Results demonstrated that 2% extract of *A. indica* leaves was most effective against aphid individuals causing maximum mortality (39.520%) followed by *C. procera* (31.751%), *C. limon* (26.764%) and *D. alba* (24.066%). Selected botanical extracts were also found regulating the biology of aphids regarding their survival, maturity rate, fecundity and their body weight. Overall study results conclude that the aqueous leaf extract of *A. indica* can be effectively utilized against *S. avenae* which is a potential pest of wheat crop. Furthermore, such botanicals with potential anti-insect activities can be synthesized at broad level for future utilization in Pakistan.

### Statement of conflict of interest

Authors have declared no conflict of interest regarding the publication of this article.

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#### **Citation of Article**

- Khalid, M.R., Aqueel, M.A., Majeed, M.Z. & Raza, M.A. (2020). *In-vitro* Response of Cereal Aphid, *Sitobion avenae* (Homoptera: Aphididae) against some Selected Botanical Extracts. *Journal of Agriculture and Food*, 1, 1–12.