

Research Article

Evaluation of Some Selected Local Phytoextracts Against Wheat Aphid *Schizaphis graminum* Rondani (Hemiptera: Aphididae) under Laboratory and Field Conditions

Saleem Hussain¹, Muhammad Tayyab¹, Tauqir Anwar¹, Talha Nazir^{2*}, Muhammad Zeeshan Majeed³, Zohaib Asad^{2,4}, Muhammad Adnan³ and Tajwar Alam⁵

¹Pest Warning and Quality Control of Pesticides, Punjab Agriculture Department, Government of the Punjab, Sargodha 40100, Pakistan; ²Centre for Agriculture and Bioscience International (CABI), Rawalpindi 46000, Punjab, Pakistan; ³Department of Entomology, University of Sargodha, Sargodha 40100, Punjab, Pakistan; ⁴Department of Plant Pathology, Pir Mehr Ali Shab Arid Agriculture University Rawalpindi (PMAS-AAUR), Rawalpindi 46000, Punjab, Pakistan; ⁵Institute of Soil and Environmental Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

Abstract | Plant-derived insecticides have been focused for biorational pest management because these are relatively quickly biodegradable, cost-effective, less persistent and exhibit low mammalian toxicity than synthetic pesticides. This study assessed the toxicity potential of seven local plant species (*i.e.* parthenium *Parthenium hysterophorus* L., eucalyptus *Eucalyptus camaldulensis* Dehnh., milkweed *Calotropis gigantea* (L.) Dryand., bakain *Melia azedarach* L., neem *Azadirachta indica* A. Juss., tobacco *Nicotiana tabacum* L. and thorn apple *Datura stramonium* L.) against wheat aphid (*Schizaphis graminum* Rondani) under laboratory and field conditions. Using foliar spray method, toxicity bioassay was conducted with three different concentrations (*i.e.* 250, 500 and 1000 ppm) of methanolic plant extracts under controlled laboratory conditions, while three different concentrations (25, 50 and 75 g/L) of each botanical extract was tested in the field trial. Results showed a significant mortality and suppression of *S. graminum* individuals at 72 h after treatment in the laboratory and at 10th day post-treatment in the field. In laboratory bioassay, maximum aphid mortality was caused by the extracts of *D. stramonium* (92%), *A. indica* (84%), *N. tabacum* (78%) and *M. azedarach* (70%) at 72 h post-treatment at their highest concentration (1000 ppm). Results of field trial showed significant aphid mortality by *D. stramonium* (78%), *A. indica* (72%), *N. tabacum* (68%) and *M. azedarach* (62%) extracts noted for their highest concentration (75 g/L) at 10th day after treatment. Based on overall results of the study, the extracts of *D. stramonium*, *A. indica*, *N. tabacum* and *M. azedarach* are recommended to be considered for biorational management of *S. graminum*.

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***Correspondence** | Talha Nazir, Centre for Agriculture and Bioscience International (CABI), Rawalpindi 46000, Punjab, Pakistan; **Email:** talha23december@gmail.com

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Introduction

Wheat (*Triticum aestivum* L.) is the most economical and nutritious staple food around the world (Khan *et al.*, 2009). It is consumed by more than 35% world human population (Khakwani *et al.*, 2012). About 70% of the total population of Pakistan consume wheat grains, therefore it is imperative to improve its yield to feed growing population (FAO, 2019). Pakistan's average wheat yield is much less as compared to other wheat producing countries for which multiple factors are involved including inappropriate sowing schedule, insect pest and weed infestations, use of low yielding varieties and inappropriate use of fertilizers and irrigation water (Khan *et al.*, 2012; Memon *et al.*, 2013; John *et al.*, 2017; Yahya *et al.*, 2017).

Wheat crop in Pakistan is attacked and damaged by several insect pests but among these pests aphids (Hemiptera: Aphididae) are responsible for the foremost economic damage not only by their direct feeding on wheat plants but also by vectoring different plant diseases (Bukvayová *et al.*, 2006; Ali *et al.*, 2015). In Pakistan, the most abundant and prevailing species of wheat aphids include *Sitobion avenae* Fabricius, *Rhopalosiphum padi* Linnaeus and *Schizaphis graminum* Rondani (Shah *et al.*, 2006; Ahmad *et al.*, 2016; Shafique *et al.*, 2016). These aphid species result in 35–40% yield reduction by their direct feeding on plant sap and from 20 to 80% yield reduction indirectly by transmitting some fungal and viral diseases (Carvalho, 2006; Foster *et al.*, 2014). Up to 30% yield loss has been noted in an untreated experimental wheat field by *S. graminum* infestation (Ahmad *et al.*, 2016).

In Pakistan, different types of synthetic pesticides are being employed for the management of aphid infestations on wheat and other crops (Shafique *et al.*, 2016). Anyhow many species of the aphids got resistant to several of these synthetic insecticides (Foster *et al.*, 2014; Shafique *et al.*, 2016). In case of wheat crop, only a small range of registered synthetic insecticides exist for aphid control which is ultimately resulting in lesser production of wheat (Carvalho, 2006). It is also predicted that ongoing climate changes are also favourable for aphid infestations on wheat crop which could reduce wheat production and impact negatively the world economy (Bezemer *et al.*, 1998; Van der Putten *et al.*, 2010; Simon *et al.*, 2021).

All these facts mentioned demand an alternative management strategy against wheat aphids to ensure future food security through sustainable wheat production. Moreover, excessive use of synthetic chemical insecticides has imparted adverse effects on human and animal health as well as on the environment (Duman and Mutlu, 2019; Mamay and Mutlu, 2019; Mutlu *et al.*, 2019; Nazir *et al.*, 2019; Teke and Mutlu, 2021).

Plant-derived pesticides, such as botanical extracts and essential oils, have been a promising biorational strategy for the management of insect pests (Isman, 2020). Botanical pesticides have some advantages over their synthetic counterparts such as these pesticides usually exhibit less mammalian toxicity and environmental persistency and are more environment-friendly than synthetic insecticides (da Silva *et al.*, 2017; Bedini *et al.*, 2020). Botanical pesticides exert lethal and sublethal effects on insect pests including mortality, repellency, ovipositioning deterrence, ovicidal effects, and reduced fertility and longevity (Isman, 2020). Extracts of many plant species have been evaluated and demonstrated effective against many insect pests including aphids (Nong *et al.*, 2015; Isman, 2020). To this end, this research work was intended to comparatively evaluate the toxicity potential of methanolic extracts of seven indigenous plant species against *S. graminum* aphids on wheat plants both under laboratory and field conditions.

Materials and Methods

Insect culture

Collection for mass rearing of wheat aphids (*S. graminum*) was made from wheat (variety Faisalabad-2008) field (32°08'21"N; 72°40'11"E) near Tehsil Sillanwali, District Sargodha, Punjab, Pakistan. Alate and apterous morphs of *S. graminum* are usually 1.2 to 2.0 mm long pale green to yellowish green individuals with a median dark green longitudinal line on abdominal notum (Figure 1). This aphid population was reared in the laboratory conditions at 25±2°C of temperature and 55–65% relative humidity under photoperiod of 16:8 L:D hours on the plants of same wheat variety grown in pots at College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan. During rearing, old infested plants were replaced with new ones at weekly intervals.



Figure 1: Apterous and alate morphs of wheat aphid *Schizaphis graminum* (Rondani, 1852).

Plants extracts

Seven plant species belonging to five botanical families (Table 1) were selected for the study and their fresh leaves were collected in August 2020 from Sargodha, Punjab, Pakistan. This selection of plant species was made on the basis of their wide availability in district Sargodha and on their literature-based insecticidal properties. For each plant species, one kilogram of healthy leaves was collected, shade-dried and grounded into powder using mortar and pestle in liquid nitrogen. The extraction of plant samples was done following procedure described by Khan *et al.* (2017). From each sample, five grams of powdered leaves were extracted with 100 mL of 95% methanol by keeping for 2 days in a thermostat water shaker at 35°C temperature. Then, these extracts were cooled down to room temperature (25°C) and were centrifuged for 15 min at 7000 rpm. Then the supernatant was acquired by using Whatman filter paper No. 1 with three repetitions for getting pure supernatant. Later on, this supernatant was passed by a Buchner funnel in the rotary evaporator at 30°C temperature. The process of evaporation of excessive solvent was completed by adjusting the volume of each plant extract to about 100 mL, and got each plant extract in resultant pellet. Each pellet (plant extract) was then mixed in a 1.0% aqueous solution of dimethyl sulfoxide (DMSO) with a final concentration of 50 mg/mL. The final products of all plant extracts were kept at 4°C temperature in air-

tight dark glass vials to be used for bioassays.

Laboratory bioassay

The bioassay was carried out against *S. graminum* under laboratory conditions to assess the efficacy of above mentioned plant extracts. Three different concentrations of each plant extract (*i.e.* 250, 500 and 1000 ppm) and one control were used as treatment. Seven-day old potted wheat (*T. aestivum*; variety Faisalabad-2008) plants were used for bioassay. After infesting wheat plants with the test insects, each wheat plant was sprayed with approximately 4 mL of each plant extract concentration using a manual atomizer. Plant extracts were sprayed directly on wheat plants. For the determination of insecticidal efficacy of these plant extracts, data regarding aphid mortality was taken with the help of a 3X magnifying hand lens after 24, 48 and 72 h post-treatment. For all treatments, five replications were maintained. Bioassay was conducted at controlled conditions (*i.e.* at 25±2°C temperature, 50-60% relative humidity and 16:8 h light: dark photoperiod).

Field experiment

For evaluating the efficacy of these plant extracts against *S. graminum*, a field trial was conducted at tehsil Sillanwali (Sargodha, Punjab, Pakistan) during the Rabi Season 2020-2021. Three concentrations (*i.e.* 25, 50 and 75 g/L) of all plant extracts were used along with one control plot. Wheat variety Faisalabad-2008 was sown for the application of these above-mentioned treatments. Plot size was 45 × 100 feet. For each plot, approximately 15 litre solution of each plant extract was sprayed using a Knapsack sprayer which was calibrated before use. Control plots were sprayed with water only. The data for aphid population reduction was noted after 4, 7 and 10 days post-application. For each treatment, three replications/blocks were maintained for each treatment. Uniform agronomic practices were adopted for all experimental plants.

Table 1: Plant species used in the study.

S. No.	Vernacular name	Scientific Name	Family
1	Gajar booti	<i>Parthenium hysterophorus</i> L.	Asteraceae
2	Sofeda	<i>Eucalyptus camaldulensis</i> Dehnh.	Myrtaceae
3	Akk	<i>Calotropis gigantean</i> (L.) Dryand.	Apocynaceae
4	Bakain	<i>Melia azedarach</i> L.	Meliaceae
5	Tobacco	<i>Nicotiana tabacum</i> L.	Solanaceae
6	Neem	<i>Azadirachta indica</i> A. Juss.	Meliaceae
7	Stinkweed	<i>Datura stramonium</i> L.	Solanaceae

Table 2: Factorial analysis of variance (ANOVA) comparison of the mortality of *S. graminum* individuals bioassayed against different plant extracts (See Figure 1).

Source	DF	SS	MS	F-value	*p-value
Concentration	3	1000.52	333.507	886.54	≤ 0.001
Time	2	889.16	444.581	1181.80	≤ 0.001
Treatment	6	122.06	20.343	54.08	≤ 0.001
Concentration × Time	6	424.06	70.676	187.87	≤ 0.001
Concentration × Treatment	18	50.10	2.783	7.40	≤ 0.001
Time × Treatment	12	36.17	3.014	8.01	≤ 0.001
Concentration × Time × Treatment	36	19.28	0.535	1.42	0.0599
Error	336	126.40	0.376		
Total	419	2667.74			
CV/GM		23.70/2.588			

* $p \leq 0.001$ (highly significant) and $p < 0.05$ (significant); three-way factorial analysis of variance (ANOVA) at $\alpha = 0.05$. DF: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares, F: F-statistic, CV: Coefficient of variation, GM: Grand mean.

Statistical analysis

Statistix® Software (V. 8.1; Analytical Software, Tallahassee, FL, USA) was used for the statistical interpretation of data. Three to five independent replications were run for all determinations and bioassays. Mean values of data are presented in figures along with standard error values. Factorial analysis of variance (ANOVA) was applied and the treatment means were further compared by Fisher’s least significant difference (LSD) post-hoc test at 5% standard probability level.

Results and Discussion

Insecticidal effect of plant extracts on S. graminum under laboratory conditions

Results of laboratory bioassay revealed that all seven extracts showed a substantial mortality of *S. graminum* ($F = 54.08, p < 0.001$; Table 2). Moreover, a significant impact of plant extract concentrations, exposure time intervals and of their interaction has been shown by factorial ANOVA on the mean *S. graminum* mortality ($F = 886.54, p < 0.001, F = 1181.80, p < 0.001$ and $F = 187.87, p < 0.001$, respectively; Table 2). Plant extracts of *D. stramonium* caused substantial mortality of *S. graminum* for all concentrations. Nonetheless, maximum mortality (92%) was noted at 72 h post-treatment at 1000 ppm concentration, while minimum mortality (46%) was noted at the lowest concentration of *D. stramonium* (i.e., 250 ppm) (Figure 2). The toxicity of *A. indica* extract against *S. graminum* revealed a related trend as in *D. stramonium*. Maximum mean mortality (84%) of *S. graminum* was noted at 72 h post-treatment through

the concentration of 1000 ppm, whereas the minimum mortality (44%) was noted by 250 ppm concentration (Figure 2). Maximum aphid mortality was noted at 72 h post-treatment by 1000 ppm concentration of the extracts of *M. azedarach* (70%), *C. gigantean* (66%), *E. camaldulensis* (60%) and *P. hysterophorus* (54%) and minimum mortality (36, 34, 26 and 22%) was noted at the lowest concentration (i.e. 250 ppm) after 72 h of treatment. In untreated (control) Petri dishes, mean *S. graminum* mortality remained about 5%.

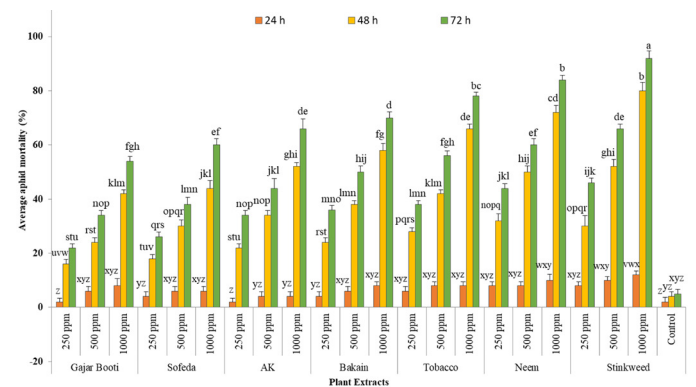


Figure 2: Percent mortality (mean ± SE; n = 5) of *S. graminum* by methanolic plant extracts recorded at different time intervals. Treatment columns bearing different lowercase letters are significantly different from other treatments (factorial ANOVA followed by LSD test at $\alpha = 0.05$) (See Table 2).

Efficacy of plant extracts against S. graminum under field conditions

Results of the field trial exhibited that all tested plant extracts showed considerable mortality of *S. graminum* individuals ($F = 96.03, p < 0.001$; Table 3). Moreover, factorial ANOVA showed a significant impact of all plant extract concentrations ($F = 585.02, p < 0.001$), time intervals ($F = 753.76, p < 0.001$) and

of their interaction ($F = 120.64, p < 0.001$) on mean aphid mortality (Table 3). Maximum mortality of *S. graminum* was noted by *D. stramonium* (78%) at the highest concentration of 75 g/L on 10th day post-treatment, while the lowest concentration (25 g/L) of *D. stramonium* caused minimum aphid mortality (52%) at 10th day post-treatment (Figure 3). Whereas maximum mortality of aphids was witnessed in *A. indica* (72%), *N. tabacum* (68%), *M. azedarach* (62%), *C. gigantean* (54%), *E. camaldulensis* (48%) and *P. hysterothorus* (40%) at their highest concentrations and the minimum mortality was noted by *A. indica* (44%), *N. tabacum* (36%), *M. azedarach* (28%), *C. gigantean* (22%), *E. camaldulensis* (18%) and *P. hysterothorus* (14%) at the lowest concentration (25 g/L) on 10th day post-treatment (Figure 3). Mean percent mortality of *S. graminum* was recorded by *D. stramonium* (66%), *A. indica* (64%), *N. tabacum* (54%), *M. azedarach* (50%), *C. gigantean* (42%), *E. camaldulensis* (34%) and *P. hysterothorus* (26%) by the higher concentration (75 g/L) and mean percent mortality of *S. graminum* was recorded in *D. stramonium* (50%), *A. indica* (46%), *N. tabacum* (38%), *M. azedarach* (34%), *C. gigantean* (30%), *E. camaldulensis* (24%) and *P. hysterothorus* (16%) at 50 g/L concentration on 7th day post-treatment (Figure 3). Mean mortality of *S. graminum* in the untreated (control) plots was about 6% after 10th day post-treatment.

Over the last few decades, cereal aphids (*S. avenae*, *S. graminum* and *R. padi*) have been very common wheat crop pests and pose a major threat to wheat production in Pakistan (Riaz et al., 2022). The use of eco-friendly insecticides is also a part of an integrated management strategy against wheat aphids for the reduction of economic losses (Shahzad et al.,

2013). For quick and efficient minimization of yield losses, several synthetic pesticides have been found effective (Shafique et al., 2016). But the chances for accumulation of toxic residual compounds in wheat grain make it a less desirable remedy for the management of wheat aphid, which is our staple food.

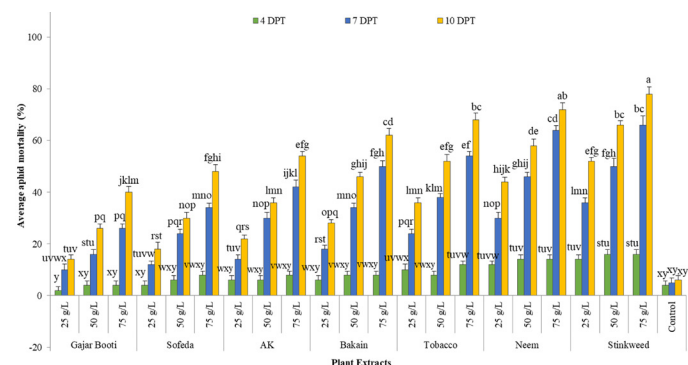


Figure 3: Percent mortality (mean ± SE; n = 5) of *S. graminum* by different plant extracts recorded at difference time intervals. DAT = days after treatment. Treatment columns bearing different lower-case letters are significantly different from other treatments (factorial ANOVA followed by LSD test at $\alpha = 0.05$) (See Table 3).

The development of biorational control methods such plant extracts have been a vital aspect of plant protection research. The application of botanical extracts and plant essential oils exhibit potential to control aphids (Iqbal et al., 2011; Farooq et al., 2016). The current research work demonstrated *in-vitro* and *in-situ* efficacy of seven local plant extracts against *S. graminum*. Results of the study have revealed that all plant extracts tested might be effective biopesticides for aphid management. Results of this work are corroborated by those of some previous studies demonstrating the efficacy of various plant extracts against wheat aphid *S. graminum* and some other species of aphid (Khan et al., 2017; Hafeez et al., 2021).

Table 3: Factorial analysis of variance (ANOVA) comparison of mortality of *S. graminum* treated with different concentrations of plant extracts (See Figure 2).

Source	DF	SS	MS	F-value	*P-value
Concentration	3	635.17	211.723	585.02	≤ 0.001
Time	2	545.58	272.788	753.76	≤ 0.001
Treatment	6	208.52	34.754	96.03	≤ 0.001
Concentration × Time	6	261.97	43.661	120.64	≤ 0.001
Concentration × Treatment	18	72.85	4.047	11.18	≤ 0.001
Time × Treatment	12	38.39	3.199	8.84	≤ 0.001
Concentration × Time × Treatment	36	18.07	0.502	1.39	0.0748
Error	336	121.60	0.362		
Total	419	1902.14			
CV/GM		25.55/2.3548			

In laboratory bioassay, resulted mortality of *S. graminum* was found time- and dose-dependent as it increased along with the concentrations of the plant extracts and along with the exposure time. Extracts of *D. stramonium*, *A. indica*, *N. tabacum*, *M. azedarach*, *C. gigantean*, *E. camaldulensis* and *P. hysterothorus* caused 92, 84, 78, 70, 66, 60 and 54% and 78, 72, 68, 62, 54, 48 and 40% mortality at 72 h and 10 days post treatment applications in laboratory and field experiments, respectively. These findings validate the conclusions of Roy *et al.* (2005) who reported maximum mortality (100%) by some botanical insecticides against *S. graminum*. Plant extracts are found to be a favourable alternative to conventional synthetic insecticides for the effectiveness against a wide range of agricultural insect pests. The results of a current research study are also in accordance with the results of Sohail *et al.* (2012) for suppressing the population of aphids with an application of plant extracts. Several experimental studies have reported the effectiveness of *A. indica* formulation against aphids (Tang *et al.*, 2002; Sallam *et al.*, 2009; Melesse and Singh, 2012). Our results are also in line with Esparza-Díaz *et al.* (2010) who have reported the effectiveness of *A. indica* (aqueous extract) with good insecticidal properties against *A. gossypii* after a time period of 72 h post-treatment. Formulations of *A. indica* show not only contact and systemic actions but also work as a growth inhibitor, toxicological repellent, sterilant and antifeedant against various insects (Gahukar, 2000).

However, the results of our findings are also different from some other similar studies. This could be because of the differential response of the targeted insect pest species against a specific plant extract and the varied toxicity of the plant extracts. Earlier studies have shown that plant extracts of many medicinal plant species have different levels of efficacy against different pest species. Likewise, the determination of the toxicity of seven different phytoextracts against four different insect pests revealed that aphids were highly susceptible to all plant extracts which were tested in the study causing 100 % aphid mortality after 24 h of application (Khan *et al.*, 2017).

In another study, plant extracts from Asteraceae family were tested against cabbage aphid *Brevicoryne brassicae* and were found non-toxic to predatory beetle *Coccinella undecimpunctata* and *C. carnea* (Farag and Ismasil, 1999). Similarly, while using plant extracts of nine Ghanaian plants, it was observed that aphid *B.*

brassicae was effectively controlled with plant extracts as done by the conventional pesticides e.g. emamectin benzoate, and these plant extracts were proven less harmful to ladybird beetles (Amoabeng *et al.*, 2013). According to another research, it is also believed that products based on plants give effective efficacy against insect pests but they also harm some non-target and beneficial insects including natural enemies and pollinators (Czaja *et al.*, 2015; Vivekanandhan *et al.*, 2018). Furthermore, some field studies showed that plant extracts were effective against aphids but were harmless to natural enemies of aphids (Abdel-Rahman *et al.*, 2019).

It is well-known that anti-insect and toxicological properties of plant-based pesticides are due to the existence of some secondary plant metabolites for example alkaloids, flavonoids, terpenoids and quinines (Isman, 2020). According to an estimation, approximately about 9,000 different flavonoid compounds are present in plants. These compounds play several vital functions in plants such as they reduce digestibility, decrease nutritional value and work as toxins or anti-feeding agents for insects (Mierziak *et al.*, 2014). The flavonoids are usually cytotoxic and effect on the growth, development and behaviour of phytophagous insect pests (Simmonds, 2003).

Conclusions and Recommendations

This study in brief has demonstrated the insecticidal efficacy of seven local plant extracts, particularly of *A. indica*, *D. stramonium* and *N. tabacum* against wheat aphid *S. graminum* causing 54–92% and 40–78% mortality under laboratory and field conditions, respectively. Therefore, in view of the aforementioned concerns of synthetic insecticides, these plant extracts are recommended to be incorporated in future integrated management of wheat aphid *S. graminum* as *biorational pest control tools*.

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Novelty Statement

This study demonstrated the comparative toxicity

potential of methanolic extracts of seven local plant species against cereal aphid *Schizaphis graminum* and has showed the effectiveness of neem (*A. indica*), thorn apple (*D. stramonium*) and tobacco (*N. tabacum*) extracts against aphids both under laboratory and field conditions.

Author's Contribution

SH performed experiments and prepared first draft. MT helped in data collection in laboratory and field. TA conducted the field study and collected data. TN performed statistical analyses and prepared results. MZM conceived the research idea and technically proofread the draft. TA helped in conduction of field experiment. MA helped in conduction and data collection of laboratory bioassay. ZA performed statistical analyses and prepared results.

List of abbreviations

Kg: kilogram; L:D: light and dark; mL: millilitre; Rpm: revolutions per minute; mg: milligram; DMSO: dimethyl sulfoxide; ppm: parts per million; h: hours; DAT: days after treatment; CV: coefficient of variation; DF: degree of freedom; F: F-statistic; GM: grand mean; MS: mean sum of squares; SS: sum of squares.

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Conflict of interest

The authors have declared no conflict of interest.

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