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RESEARCH ARTICLE

Potencies of *Justicia adhatoda* L. for its possible phytotoxic activity

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ABSTRACT

The phytotoxic effects of *Justicia adhatoda* L. were investigated on cauliflower, broccoli, tomato, foxtail millet and barnyard grass. The experiments were carried out under laboratory and in pot experiments. Six different aqueous methanol extract concentrations (control, 0.001, 0.003, 0.01, 0.03 and 0.1 g DW equivalent mL^{-1} extract) were tested in the laboratory and six aqueous extract concentrations (control, 1.0, 2.0, 3.0, 4.0 and 5.0 g DW mL^{-1} extract equivalent) were evaluated in the pot experiment. Results showed a reduction in germination and growth (shoot length, root length and biomass weight) at higher extract concentration compared to control. The leaf extracts from *J. adhatoda* showed that the foxtail millet and barnyard grass are germinating below 50 % both in the laboratory condition and in the pot experiment at their maximum concentration. When maximum extracts have been applied, we have found less than 0.5 cm of shoot and root of foxtail millet and barnyard grass. Maximum dry weight reduction was observed in foxtail millet and barnyard grass at the same concentration. The findings show that *J. adhatoda* may have phytotoxic potential and thus contains phytotoxins. Therefore, *J. adhatoda* can be used in sustainable crop production as a mulch or soil additive to suppress weeds.

Introduction

The favourable or deleterious biochemical relationship between plants and microorganisms is known as phytotoxicity or allelopathy. It implies any direct or indirect effect of one plant species on another plant species or micro-organism by producing secondary metabolites (1, 2).

The population of the world growing difficulties to improve the crop productivity. Weeds are the key obstacles to effective crop production. Globally, weeds caused greater yield losses (about 34%) to pests (3, 4). Farmers generally depend on synthetic herbicides for managing weeds in their crop areas. Synthetic herbicides often cause overwhelming adverse environmental and human health impacts (5).

Researchers are now involved in sustainable biological alternative strategies to minimise the environmental effect of synthetic agrochemicals (6, 7). Allelopathy or phytotoxicity can be a secure alternative approach in the agricultural ecosystem for sustainable weed management. Many medicinal plants have been acknowledged as phytotoxic plants. Several researchers have studied the allelopathic or phytotoxic nature of some plant species on a wide range of plants and weeds. (4, 8-11). Therefore, researchers are paying close attention to allelopathic or phytotoxic behaviour of plants as an alternative method for safe and ecological management of weeds (11-13).

Although a myriad of pharmacological properties of *J. adhatoda* L. has been investigated, but very little information on phytotoxic potentialities has been reported. The present attempt has been taken to evaluate the phytotoxic potentiality of *J. adhatoda* which will the possibility for selecting new candidates for phytotoxic plants. The aim of the study was

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therefore to evaluate the phytotoxic activity of *J. adhatoda*.

Materials and Methods

PART-A

The study was performed in the laboratory and in pot experiments in the Department of Agricultural Chemistry of Sher-e-Bangla Agricultural University (SAU), Bangladesh.

Condition of pot experiment

The temperature and relative humidity of the net house were recorded during the study period in the SAU weather station. The average minimum and maximum temperatures were respectively 15.4 °C to 38.20 °C, and the average relative minimum and maximum humidity were 30.30 and 80.20 %, respectively.

PART-B

Collection of Plant samples and test species

Leaves of *J. adhatoda* were collected from Sher-e-Bangla Agricultural University (SAU) Campus, Dhaka, Bangladesh (23° 77' N latitude and 90° 33' E longitude) during January 2017. The plant has been identified by Professor Asim Kumar Bhadra, Director of Landscape and Crop Conservation centre, Sher-e-Bangla Agricultural University (NCBI: txid141317). Five test species; three dicotyledonous: cauliflower (*Brassica oleracea*), broccoli (*Brassica oleracea* var. *italica*), tomato (*Solanum lycopersicum*) and two monocotyledonous: foxtail millet (*Setaria italica*) and barnyard grass (*Echinochloa cruss-gali* Beauv L.) were used in this experiment. The seeds of cauliflower, broccoli and tomato were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh whereas; foxtail millet and barnyard grass were bought from the local farmer's field of Japan. Cauliflower, broccoli and tomato seeds were chosen due to their known growth patterns and weed species were considered due to their detrimental effects on crops (4, 14-16).

PART-C

Extraction procedure

The leaves were washed with tap water, sun-dried and dried in an oven for 3 days at 60 °C. The dried leaves were powdered and held at 4 °C before extraction in aerated polybag in the refrigerator. 50 gm powder leaves were extracted with 80% (v/v) aqueous methanol for 72 hrs filtered through Whatman 1 filter paper. The residue was re-extracted for 24 hrs and purified again. The two filtrates were then combined and evaporated at 40 °C in rotary evaporator (HAHNVAPOR-HS-2005S, Korea). The filtrates were filled with methanol and distilled water and homogenised into 250 ml volumetric flasks by manual shaking. The ready stock solution was held at 4 °C in the fridge until the final test concentration was ready.

PART-D

Bioassay of Laboratory

An aliquot of leaves with final concentrations of the ml^{-1} laboratory of 0.001, 0.003, 0.01, and 0.1 gm DW of ml^{-1} extract dissolved in the 50 ml methanol and added in a filter layer of 6.0 cm Petri dish. Methanol extracts in filter paper were evaporated in the air-flow and then applied 1.3 ml of 0.05 % (V/V) aqueous solution (Tween 20) (polyoxyethylene sorbitan monolaurate). Twenty seeds from each test species were placed on filter paper in Petri plates. The control consisted of filter paper moistened with Tween 20 without leaf extracts. The Petri plate coated with aluminium foil and polyethene paper, were put in the growth chamber (RGX-250E, China) under 25 °C for 96 hrs with light intensity of 2,500 lux. The seeds that showed signs of emerging 1 mm radicle from the seed coat are considered to be germinated (15, 16). Germination of seeds was reported at 12 hr intervals for 96 hr until the germinated seed were constant. Showing short, dense and spiral hypocotyl seedlings and sunken primary root was considered in abnormally germinated seeds (14). During counting these forms of irregular or dead seedlings were removed. Germination percentage (%G) is measured as the number of seeds germinated in total days as a proportion of the number of seeds shown as a percentage in each procedure (15). Each experiment was replicated three times with the random design pattern. After 48 hr incubation, shoot and root dry weights of the seedlings were assessed. The growth bioassay experiment was performed with minor modifications with the similar pattern of our previous study (4).

PART-E

Bioassay of pot experiment

Final assay concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 g DW equivalent extract ml^{-1} for the net house was dissolved in the required volume of distilled water and added to the pot. Then, 20 seeds were placed in each pot (previously well prepared and filled with deep red brown terrace type soil). The control treatment was added only distilled water without any extracts of *J. adhatoda* which described the above section. The pots in the net house were arranged in a completely random design for 96 hrs. The progress of germination was inspected and data collected every 12 hrs and lasted up to 96 hrs. The seedlings were considered abnormally germinated seeds with small, dense and spiralling shoots and stunted primary root (14). The shoot and root weights of the seedlings were measured after 48 hrs of incubation. A growth bioassay experiment was performed with minor modifications according to our previous study (4, 16).

PART-F

Statistical analysis

Statistically, the data collected for various trial have been analysed to see the substantial difference between treatments. The average value of all parameters was estimated and Anova was calculated.

Significant differences between treatments were calculated with the Tukey's t-test ($P \leq 0.05$). Statistic 10 computer software was used for statistical analysis.

Results and Discussion

Effect of aqueous methanol and aqueous extracts of leaves obtained from *J. adhatoda* on germination

Wide range of variability on the percentage of germination of cauliflower, broccoli, tomato, foxtail millet and barnyard grass was found in the aqueous methanol and aqueous extracts of *J. adhatoda* leaves (Supplementary Fig. 1 & 2). With 0.1 g DW extract ml^{-1}, 30% of germination was seen in cauliflower and barnyard grass in the laboratory. In the context of a net house, 40% of germination was detected in cauliflower, while 30% of germination was detected in barnyard grass species with 5.0 g DW extract ml^{-1}. The maximum germination (90% to 100%) was observed at 0.001 g DW equivalent ml^{-1} extract in the laboratory conditions, while the least germination was observed at 1.0 gm DW equivalent extract ml^{-1} (Supplementary Fig. 1 & 2). The results indicated that the reduction trend of germination (%) was proportional to the applied extract concentrations that mean higher inhibitory effects were found at higher concentration and lower at a lower concentration. From those figures, it was also clear that the maximum value of germination (%) was observed at the control treatment compared to the rest of the treatments. Based on different treatment effects of five selected test plants, high phytotoxic effects were observed in the monocotyledonous plants (foxtail millet and barnyard grass) and comparatively low phytotoxic effects were shown in dicotyledonous plants (cauliflower, broccoli and tomato) in both laboratory and net house condition. On the other hand, among the dicotyledonous plant cauliflower showed much less effect followed by broccoli and tomato. The results indicated that the monocotyledonous test plants were more vulnerable than dicotyledonous species to *J. adhatoda* leaf extracts. The same pattern of findings was narrated by the researchers (4, 15, 1cf7).

Effect of the aqueous methanol and aqueous extracts obtained from leaves of J. adhatoda on the shoot and root length (cm)

Effects of aqueous methanol and aqueous leaf extracts from *J. adhatoda* on the length of the shoot and root of all test plants are shown in Supplementary fig. 3 & 4. The lowest inhibition of shoot length was found in cauliflower in all treatments compared to control. The shoot length values were 1.69, 1.56, 1.37, 1.03 and 0.90 cm, respectively with concentrations of the corresponding extract of 0.001, 0.003, 0.01, 0.03 and 0.1 gm DW in laboratory conditions. On the other hand, the maximum inhibitions of shoot length were observed in barnyard grass and the values were 1.51, 1.08, 0.92, 0.80, 0.52 and 0.25 cm at 0.001, 0.003, 0.01, 0.03 and 0.1 gm DW equivalent extract ml^{-1} concentrations, respectively in the laboratory condition (Supplementary Fig. 3). Our findings were similar with previous our report (15). Shoot and root lengths were found to decrease with increasing treatment concentration in all test species. The inhibitory effect of the extracts on test plants may be a range of mechanisms such as lower mitotic activity in growing parts of the plant, lower ion absorption rate, inhibition of photosynthetic respiration and malfunctions of enzyme activity (18, 19). The root lengths of 2.58, 2.45, 2.42, 2.31 and 2.22 cm were found in cauliflower, broccoli, tomato, foxtail millet and barnyard grass respectively whereas, root lengths of 1.81, 1.72, 1.70, 0.39 and 0.37cm were observed with 0.1 gm DW e extract mL-1in the laboratory condition (Supplementary Fig. 3). Some previous study (4, 10, 17) have also found a common pattern of inhibitory trends. Besides, maximum inhibition of the root and shoot length was observed in a concentration of 5.0 gm DW extract equivalent ml^{-1}, while the lowest inhibition was reported at a concentration of 1.0 gm DW equivalent ml^{-1} at a net house state. We found the higher inhibition in the monocotyledonous species than dicotyledonous and root inhibition was the most pronounced than shoot inhibition in the net house. We found a similar trend of findings that were described by the researchers (20).

Analysis of the correlation between *J. adhatoda* extract concentration and shoot and root growth were strong negative associations for all the test species and the Pearson correlation coefficient in the laboratory condition ranged between −0.792 to −0.948 ($p<0.01$). Correlation analysis between *J. adhatoda* extract concentration and shoot and root growth represented the strong negative correlation in all the test species, and the Pearson correlation coefficient varied from −0.792 to −0.948 ($p<0.01$) in the laboratory condition. On the other hand, *J. adhatoda* also had a rather good negative association extract concentration of shoot and root growth and the Pearson correlation coefficient in the net house environment ranged from −0.943 to −0.991 ($p<0.01$) (Supplementary Table 1 & 2). Our findings were confirmed by the similar findings described by the researchers (19).

Effect of the aqueous methanol and aqueous extracts of leaves obtained from J. adhatoda on dry weight

The effect of aqueous methanol extracts leaves obtained from *J. adhatoda* on dry weight (DW) of five selective plants are seen in Supplementary fig. 5. Dry weight inhibition has been enhanced by increased extract concentration. The control treatment showed 0.051, 0.049, 0.048, 0.046 and 0.044 gm DW in the laboratory and 0.048, 0.045, 0.045, 0.043 and 0.041 gm DW in the net house for cauliflower, broccoli, tomato, foxtail millet and barnyard grass, respectively. The highest dry weight decrease of all test species was recorded at 0.1 and 5.0 gm DW equivalent extracts ml^{-1}, respectively. (Supplementary Fig. 5). The concentrations of leaves extract obtained from *J. adhatoda* inhibited the dry weight of all monocotyledonous species. Similar findings were found by the researchers (16, 21).

Conclusion

Aqueous methanol and aqueous leaf extracts from *J. adhatoda* had an inhibitory effect on the germination, shoot length, root length and dry weight of five test plants, including two weeds. The results of our study indicated that this medicinal plant has phytotoxic potential and may have phytotoxins. Thus, this species may also be used for the isolation and detection of the active substances to replace synthetic herbicides which could be used to integrate weed control in sustainable farming systems.

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Authors' contributions

For conceptualisation: MSIK and BKS conducted the experiments: AK and MAH; systematic analysis: MAH and RB; drafting and preparation of original draft: AK reading, reviewing and editing: MAH, NB, MAI, MTIC and AK supervision: The final manuscript was read and accepted by all contributors.

Conflict of interests

Authors do not have any conflict of interests to declare.

Supplementary files

Supplementary Fig. 1–5
Supplementary Table 1, 2

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