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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (21): 3885-3889, 2007
ISSN 1028-8880

Effect of Aqueous Extract of *Azadirachta indica* (Neem) Leaves on Germination and Seedling Growth of *Vigna radiata* (L.)

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Abstract: This research was carried out to evaluate the allelopathic effect of leaves aqueous extract of *Azadirachta indica* on germination and seedling growth of *Vigna radiata*. Different methods were used to preparing leaves aqueous extract. Germination percentage and seedling growth of *V. radiata* significantly decreased gradually as the concentration of the aqueous leaves extracts of *A. indica* increased in comparison with water control. Severe toxicity was observed at high concentrations and moderate toxicity at low concentrations in comparison with water control. Aqueous leaves extract significantly inhibited root length more than shoot. These results indicated that some kind of inhibitor(s) was the responsible agent for the phytotoxic effect of *A. indica* on germination and seedling growth of *V. radiata*.

Key words: *Azadirachta indica*, *Vigna radiata*, aqueous extract, germination, seedling growth, root and shoot length

INTRODUCTION

Azadirachta indica (Neem) is a tree (Meliaceae) and it is growing in tropical and semi-tropical regions.

A. indica propagates readily from cuttings, tissue culture or seed. Seed propagation in nurseries followed by direct planting into the field is the accepted method to produce plantation stands quickly and efficiently (Jacobson, 1989). It is widely used as a shade tree in many areas because it tolerates a wide variety of field conditions (Koul, 1990; Schmutter, 1990). The tree tolerates heat up to 50°C and poor, shallow, even saline soils (Ascher, 1993).

All biologically active *A. indica* compounds are suspected to be derived from one parent compound, the tetracyclic triterpenoid (Ascher, 1993). It is generally accepted that this compound azadirachtin is responsible for the majority of biological effects observed in organisms exposed to Neem compounds (Mordue and Blackwell, 1993; Verkerk and Wright, 1993).

Biological activity of Neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. However, crude extract of different parts of Neem have been used as traditional medicine for the treatment of various diseases (Isman *et al*., 1990).

Neem is a fast growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. *Allelochemicals* might be produced by Neem leaves are released into the environment and subsequently influence the growth and development of neighboring plants. It is important to keep in mind that allelopathy involves the addition of a chemical compound(s) into the environment, while resource competition involves the removal or reduction of some factor(s) in the environment such as nutrients, water, or light (Verkerk and Wright, 1993).

There are hundreds of secondary metabolites in the plant kingdom and many are known to be *phytotoxic* (Einhellig, 2002). Allelopathic effects of these compounds are often observed to occur early in the plant life cycle, causing inhibition of seed germination and/or seedling growth. The compounds exhibit a wide range of mechanisms of action, from affects on DNA, photosynthetic and mitochondrial function, phytohormone activity, ion uptake and water balance. Interpretations of mechanisms of action are complicated by the fact that individual compound can have multiple phytotoxic effects (Einhellig, 2002).

One of the most studied aspects of allelopathy is the role of allelopathy in agriculture. Current research is focused on the effects of weeds on crops, crops on weeds (Pheng *et al*., 1999) and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural pesticides (a number of them are either commercially available or in the process of large-scale manufacture) to promote sustainable agriculture.

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However, like many allelopathy studies, it was based on artificial lab experiments and unwarranted extrapolations to natural ecosystems.

According to the all above information, it is important to study the effect of *A. indica* leaves aqueous extract on germination and seedling growth of some important crops such as *V. radiata* (L.) to know if the *A. indica* exhibits allelopathic effects on germination and seedling growth of *V. radiata* (L.). The allelochemical(s) of *A. indica* may be acts as germination and growth stimulator or inhibitor. It depends on the nature of compound(s).

MATERIALS AND METHODS

Plant material: Leaves of *A. indica* were picked from the campus of Higher College of Technology in Oman. Leaves were shade dried then ground and kept in glass jar at 5°C until use for extraction. Leaves aqueous extraction of *A. indica* were used in germination test of *V. radiata* and subsequent seedling growth.

Extract preparation

Method I: Water extract was prepared by pouring 300 mL autoclaved boiling distilled water on 50 g of dried powder of *A. indica* leaves. The mixture was allowed to stand for 24 h under laboratory conditions. The supernatant was taken from the mixture and centrifuged at 3000 rpm for 15 min, this would be full strength concentration (100%). Then it was kept in a refrigerator at 5°C until the time of testing. Series of dilutions were prepared from the stock solution (50, 25, 15, 10 and 5%) and were tested for their effects on germination and growth of *V. radiata*.

Method 2: Water extract was prepared by pouring 300 mL autoclaved boiling distilled water on 50 g of dried powder of *A. indica* leaves. The mixture was boiled for 5 min, then allowed to stand for 24 h under laboratory conditions as mentioned in method 1.

Method 3: Water extract was prepared by pouring 300 mL autoclaved boiling distilled water on 50 g of dried powder of *A. indica* leaves. The mixture was allowed to stand for 72 h under laboratory conditions. The supernatant was taken from the mixture and centrifuged at 3000 rpm for 15 min as mentioned in method 1.

Germination and seedling growth test: Twenty five seed of *V. radiata* were germinated in each Petri dish on filter paper with 10 mL of each concentrations of leaves aqueous extract of *A. indica* for the different methods as mentioned above. Distilled water was used as control. Three replicates were incubated in a randomized complete block design at 20°C in an incubator with fluorescent light. Germination criteria were the emergence of the radical through the pericarp. Germination percentages were recorded every day and total seedling length was measured after 3 days of incubation using five seedlings taken randomly from each dish.

Statistical analysis: ANOVA test was used to determine the level of significance within the *V. radiata* regarding the effect of *A. indica* seed aqueous extract on germination and seedling growth of *V. radiata*. Significance of differences was accepted when ($p \leq 0.05$).

RESULTS

Germination and seedling growth: Germination percentages of *V. radiata* (method 1) after all periods of imbibition were decreased as the concentration of the aqueous leaves extract of *A. indica* increased as shown in Fig. 1, but the inhibition of germination was more pronounced ($p \leq 0.05$) after first day of imbibition.

The seedling growth, either total seedling length (Fig. 2), root and shoot length of *V. radiata* (Fig. 3) also decreased with increasing concentration of aqueous leaves extract in comparison with water control.

Aqueous leaves extract of *A. indica* had significant ($p \leq 0.05$) toxic inhibitor(s) on root and shoot length of *V. radiata* but it had more toxicity on root length than shoot.

Germination percentages of *V. radiata* (method 2) were decreased significantly ($p \leq 0.05$) as the concentration of the aqueous leaves extract of *A. indica* increased after all periods of imbibition (Fig. 4). The inhibition of germination was more pronounced at higher concentration.

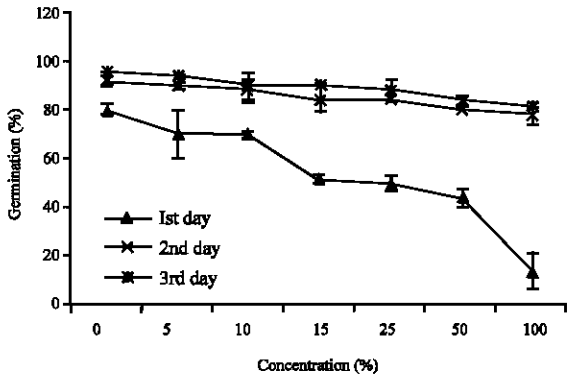

Fig. 1: Effect of different concentrations of *A. indica* leaves aqueous extract on germination percentages of *V. radiata* (standard error was shown)

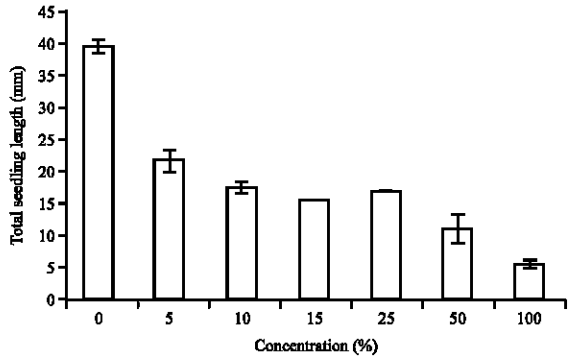

Fig. 2: Effect of different concentrations of *A. indica* leaves aqueous extract on total seedling length of *V. radiata* (standard error was shown)

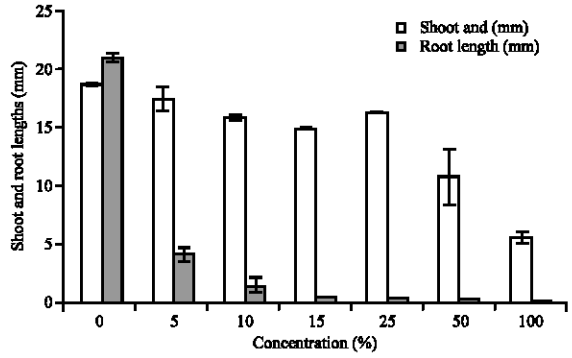

Fig. 3: Effect of different concentrations of *A. indica* leaves aqueous extract on shoot and root length of *V. radiate* (standard error was shown)

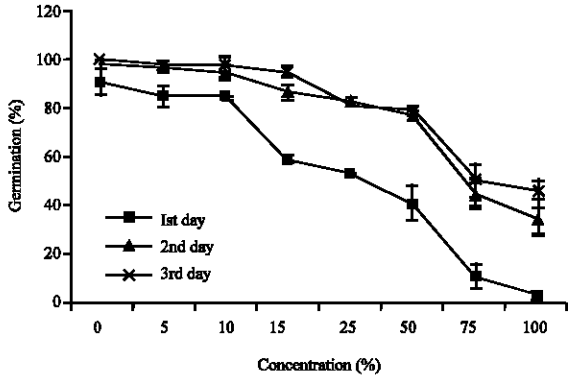

Fig. 4: Effect of different concentration of *A. indica* leaves aqueous extract on germination percentages of *V. radiata* (standard error was shown)

In this method, the percent of reduction in germination percentage was higher in comparison with method 1.

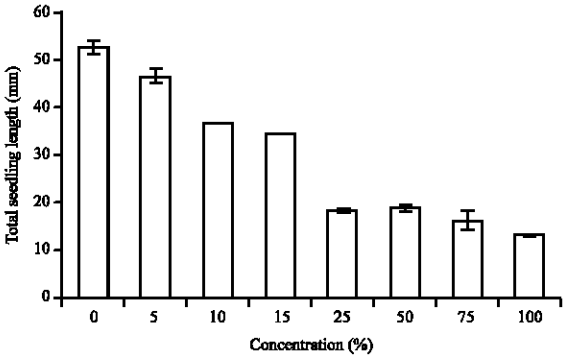

Fig 5: Effect of different concentrations of *A. indica* leaves aqueous extract on total seedling length of *V. radiata* (standard error was shown)

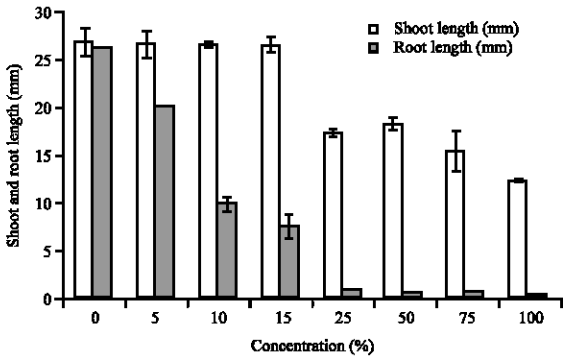

Fig. 6: Effect of different concentrations of *A. indica* leaves aqueous extract on shoot and root length of *V. radiata* (standard error was shown)

The total seedling length, root and shoot length of *V. radiata* also decreased with increasing concentration of aqueous leaves extract of *A. indica* (Fig. 5 and 6, respectively). Aqueous leaves extract of *A. indica* had significant toxic inhibitors on seedling growth of *V. radiata* but it had more toxicity on root length than shoot ($p \leq 0.05$).

Germination percentage of *V. radiata* (method 3) after the first day of imbibition was decreased significantly ($p \leq 0.05$) as the concentration of the aqueous leaf extract of *A. indica* increased (Fig. 7). While the inhibition of germination percentage of *V. radiata* after the second and third day of imbibition was indicated only at the higher concentrations.

Root, shoot and total seedling length of *V. radiata* also decreased with increasing concentration of leaves aqueous extract but the inhibitory effect on root length was higher than shoot as indicated in Fig. 8.

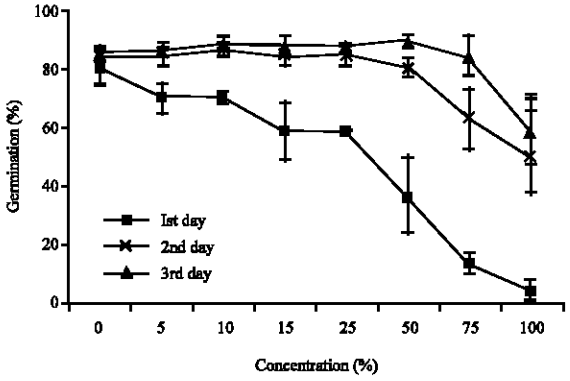

Fig. 7: Effect of different concentration of *A. indica* leaves aqueous extract on germination percentage of *V. radiata* (standard error was shown)

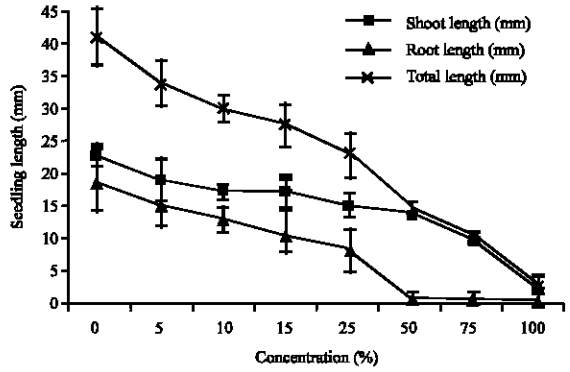

Fig. 8: Effect of different concentration of *A. indica* leaves aqueous extract on seedling growth of *V. radiata* (standard error was shown)

DISCUSSION

Leaves aqueous extract of the *A. indica* exerted a typical effect on germination and seedling growth of *V. radiata*. High toxicity at high extract concentration and low toxicity at low concentration. Thereby *A. indica* leaves extract may contain some phytotoxic substance(s) that inhibit germination and seedling growth of *V. radiata*. Previous investigation reported that aqueous extract of some plant species contains phenolics or other toxic substances (Belicova *et al*., 2001; Atoum *et al*., 2006; Modallal and Al-Charchafchi, 2006). These phenolics inhibit the germination and seedling growth of some plant species (Blum *et al*., 1984; Al-Charchafchi *et al*., 1987), by their interference with indol acetic acid metabolism, or synthesis of protein and ions uptake by plants (Castro *et al*., 1984). Therefore, some soluble allelochemicals such as phenolics might be released from *A. indica* leaves to the environment which had germination and growth inhibitory effect on new seeding of *V. radiata* or other plant species.

The results of the present investigation in method 1, method 2 and method 3 revealed that leaves aqueous extract of *A. indica* capable of inhibiting germination and seedling growth of *V. radiata*. This might be due to some soluble phenolic(s) or other phytotoxic substances release to the environment which had inhibitory effect on seed germination and subsequent seedling growth of *V. radiata*.

The inhibition on germination and seedling growth of *V. radiata* was more pronounced at the first day in comparison with that of second and third day of imbibition. This might be due to release chemical compound(s) from *V. radiata* seeds to the environment and make interference with leaves aqueous extract of *A. indica*. This interference might be acted as synergic or antagonistic effects on germination and seedling growth of *V. radiata*.

The results of present investigation in method 1, method 2 and method 3 revealed that leaves aqueous extracts of *A. indica* capable of inhibiting root length more than shoot length of *V. radiata*. This might be due to the direct contact between the root and phytotoxic compounds of the leaves aqueous extracts which in turn may inhibit cell division (Rietjens and Alink, 2003) which is highly active at meristimatic tissue of the growing root tip.

The present study concluded that the secondary metabolic compounds (either phenolics, saponins, alkaloids or others) probably produced by leaves of *A. indica* and affected as inhibitory action against germination and seedling growth of *V. radiata*.

This research needs further investigation to determine the chemical component(s) of *A. indica* leaves aqueous extract by using thin layer chromatography or High Performance Liquid Chromatography, then test their effects on germination and seedling growth of *V. radiata*.

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