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Suppressive Effects of Aqueous Extracts of *Azadirachta indica* Leaf on Some Initial Growth Parameters of Six Common Agricultural Crops

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Abstract: The paper presents the suppressive effect of different concentration of aqueous leaf extracts of Neem *(Azadirachta indica)* on some agricultural crops e.g. *Cicer arietinum* L., *Brassica juncea* (L.) Czern and Coss; *Cucumis sativus* L.; *Phaseolus mungo* L.; *Raphanus sativus* L.; and *Vigna unguiculata* (L.) Walp. The experiment was conducted in sterilized petridishes with a photoperiod of 24 hours on an average temperature of 29.5^{0}C. The result showed that aqueous leaf extract of *Azadirachta indica* caused significant inhibitory effect on germination, root and shoot elongation and development of lateral roots of receptor plants. The effect was proportional to the concentrations of the extracts and higher concentration had the stronger inhibitory effect whereas in some cases the lower concentration showed stimulatory effect. The study also revealed that inhibitory effect was much pronounced in root and lateral root development rather than shoot and germination.

Key words: *Azadirachta indica,* suppressive effect, germination, root and shoot length and aqueous extract

Introduction

Neem (*Azadirachta indica*), belongs to the family Meliaceae (Hooker, 1875), a moderate-sized to large, usually evergreen tree, with a fairly dense rounded crown (Tewari,1992), is a valuable multipurpose tree with religious, medical and social uses, since last 4000 years. It is native of Indo-Pakistan subcontinent (Cheneya and Knudsen, 1988) and planted particularly in the agroforestry practices in north-western part of Bangladesh (Quddus, 2001). Agroforestry species remain a part of the agro-ecosystem for a longer period and often produce large amount of litter. The accumulation of such litter on the soil under agroforestry system of farming does not only mean a nutrient enrichment, but can also have negative effects on the agricultural crops due to the release of the toxic substances. These toxic substances may be released by rain action or through decomposition of litter. Consequently, the release of allelochemicals into the soil inhibits seed germination and establishment of certain crops (Rice, 1979). Although much researches have been done on neem in different aspect such as manure and soil conditioner (Ahmed and Grainage, 1985; Ahmed and Grainage, 1986; National Research Council, 1992); Genetic improvement (Bisla and Beniwal, 1997); Uses (Onyewotu, 1985; Von Maydell, 1986; Nehra *et al*., 1987; Singh and Rai, 1989; Alam, 1990; Vandenbeldt, 1990; Hegde, 1991; Harsh *et al*., 1992; Gill and Deb Roy, 1993) but few researches have been done on the allelopathic aspect of neem (Hazra and Tripathi, 1989; Alam, 1990; Joshi and Prakash, 1992; Gill and Deb Roy, 1993). So the purpose of the present study was to elucidate the allelopathic potential of different concentration of *Azadirachta indica* leaf extracts on some common agricultural crops used in Bangladesh.

Materials and Methods

Azadirachta indica A. Juss, was considered as the donor plant and the receptor agricultural crops selected were Indian mustard (*Brassica juncea* (L.) Czern and Coss), Cucumber (*Cucumis sativus* L.), Black gram (*Phaseolus mungo* L.), Radish (*Raphanus sativus* L.) and Falen (*Vigna unguiculata* (L.) Walp.), Chickpea (*Cicer arietinum* L.). The aqueous extracts of were prepared from fresh leaf of *Azadirachta indica* plant. 100 gram of fresh senescent leaves of the species were soaked in 500 ml of distilled water and kept at room temperature. After 24 h the aqueous extract was filtered through the sieve and then some extracts were diluted to make the concentration of 10, 25, 50 and 75% and stored for seed treatment experiments. The following treatments were used in the experiment:

T_0 = Seeds of receptor plants grown in distil water only (Control)
T_1 = Seeds of receptor plants grown in leaf extracts of 10% concentration
T_2 = Seeds of receptor plants grown in leaf extracts of 25%concentration
T_3 = Seeds of receptor plants grown in leaf extracts of 50% concentration
T_4 = Seeds of receptor plants grown in leaf extracts of 75% concentration
T_5 = Seeds of receptor plants grown in leaf extracts of 100% concentration

Germination and growth records: The germination test was carried out in sterile petridishes of 12 cm in size placing a Whatman no.3 filter paper on petridishes. The extract of each concentration was added to each Petridish of respective treatment daily in such an amount just to allow the seed getting the favorable moisture for germination and growth. The control was treated with distil water only. 20 seeds of each agricultural crop were placed in the Petridish replicating five times. The petridishes were set in the analytical laboratory of the Institute of Forestry and Environmental Sciences, Chittagong University, Bangladesh at a room temperature ranging from 29-30^{0}c. The experiment extended over a period of ten days to allow the last seed germination and the measurement of the shoot and root length. The seed was considered as germinated when the radicle emerged and the germination was recorded daily. The results were determined by counting the number of germinated seeds, number of lateral roots and measuring the length of primary root and main shoot on 10th day of the experiment. The data were subjected to Analysis of Variance and Duncan's Multiple Range Test.

Ratio of germination and elongation were calculated as suggested by Rao and Kil (1986).

$$\text{Relative Germination Ratio (RGR)} = \frac{\text{Germination ratio of tested plant}}{\text{Germination ratio of control}} \times 100$$

$$\text{Relative Elongation Ratio (RER) of shoot} = \frac{\text{Mean shoot length of tested plant}}{\text{Mean shoot length of control}} \times 100$$

$$\text{Relative Elongation Ratio (RER) of root} = \frac{\text{Mean root length of tested plant}}{\text{Mean root length of control}} \times 100$$

Results

Germination: Table 1 shows the germination percentage of receptor plants. The study revealed that the leaf extracts significantly suppressed the germination and the severity of effect was proportional to the extract concentrations The highest inhibitory effect (-94.00%) was recorded in *R. sativus* at T_5 treatment while the lowest (-1.67%) was in *C. sativus* at T_3 treatment. Neither inhibitory nor stimulatory effect (0%) was observed in *P. mungo* at T_2 and T_4 treatment and in *B. juncea* at T_1 treatment. Maximum (103.44%) Relative Germination Ratio (RGR) was found in *P. mungo* at T_1 treatment and the minimum (6.0%) was in *R. sativus* at T_5 treatment (Fig. 1).

Shoot elongation (cm): The shoot elongations of different receptor crops are presented in Table 2.The higher concentration here also caused severe inhibition in comparison to control (T_0). The highest and lowest shoot elongation was recorded as 19.53 cm and 0.79 cm in *V. unguiculata* at T_0 treatment and in *B. juncea* at T_5 treatment respectively. T_4 treatment caused the highest (-92.19%) inhibition of shoot development in *C. arietinum* while T_1 treatment caused least inhibition (-8.14%) of the same of *V. unguiculata* at. Stimulatory effect was observed in *B. juncea* (+16.05%) and *R.. sativus* (+6.01%) at T_1 treatment. Maximum (116.05%) Relative Elongation Ratio (RER) of shoot was found in *B. juncea* at T_1 treatment and the minimum (7.81%) was in *C. arietinum* at T_4 treatment (Fig. 2).

Root elongation (cm): The root length of all the six bioassay species were greatly inhibited with the increasing of concentration of extract except *B. juncea* and *C. sativus* on which stimulatory effects of +18.68% and +6.97% was observed respectively at T_1 treatment (Table 3). The inhibitory effect was much more pronounced at T_5 and T_4 treatment. Among the survivors the highest inhibitory effect (-98.64%) was recorded from *R. sativus* at T_4 treatment and the lowest (-22.62%) recorded from *C. sativusat* T_2 treatment. Maximum (118.68%) Elongation Ratio (RER) of root was observed in *C. sativus* at T_1 treatment while among the survivors the minimum (1.36%) was in *R. sativus* at T_4 treatment (Fig. 3).

Number of lateral roots development: The study revealed that the number of lateral root development was significantly inhibited from the treatment T_2 to onwards (Table 4). T_5 and T_4 treatment Completely inhibited (-100%) the lateral root development in *B. juncea* and *R sativus* respectively. Only T_1 treatment had shown stimulatory effect on *B. juncea* (+21.39%) and *C. sativus* (+21.33%). Maximum number of lateral roots (35.67 nos.) was found in *C. sativus* followed by (29.20 nos.) in *V. unguiculata* at the control (T_0).

Discussion

Considering of germination and overall seedling growth, among the six receptor plants *R. sativus* exhibited more sensitive responses followed by *C. arietinum* and *V. unguiculata* to the aqueous extracts leaf extracts of *Azadirachta indica*. In contrast *C. sativus* exhibited less sensitive responses followed by *B. juncea* and *P. mungo*. These findings supports the results of Singh and Swami Rao and Reddy, (1984); Eyini *et al.* (1989); Alam, (1990); Joshi and Prakash, (1992), Nadal (1993) and Bora *et al.* (1999).

Table 1: Germination percent of receptor agricultural crops to distil water (T_0) and different concentrations of *A. indica* extracts (T_1-T_5). Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in comparison to control (T_0)

	Agricultural crops					
Treatment	*C. arietinum*	*R. sativus*	*V. unguiculata*	*C. sativus*	*B. juncea*	*P. mungo*
T_0	85.00a*	83.33a	95.00a	100.00a	98.33a	96.67a
T_1	76.67a (-9.8)	85.00a (+2.00)	90.00a (-5.26)	93.33abc (-6.67)	98.33a (0)	100.00a (+3.44)
T_2	83.33a (-1.95)	63.33b (-24.00)	80.00a (-15.79)	91.67abc (-8.33)	95.0a (-3.39)	96.67a (0)
T_3	55.00b (-35.29)	10.00c (-88)	50.00b (-47.37)	98.33ab (-1.67)	66.67ab (-32.20)	95.00a (-1.73)
T_4	50.00b (-41.18)	8.33c (-90)	45.00b (-52.63)	90.00bc (-10)	73.33ab (-25.42)	96.67a (0)
T_5	51.67b (-39.21)	5.00c (-94)	41.67b (-56.14)	88.33c (-11.67)	45.00b (-54.24)	98.33a (+1.71)

*values in the columns followed by the same letter(s) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test (DMRT)

Table 2: Shoot elongation (cm) of receptor agricultural crops to distil water (T_0) and different concentrations of *Azadirachta indica* extracts (T_1-T_5). Values in the parenthesis indicate the inhibitory (-) or stimulatory (+) effects in comparison to control (T_0)

	Agricultural crops					
Treatment	*C. arietinum*	*R. sativus*	*V. unguiculata*	*C.sativus*	*B. juncea*	*P. mungo*
T_0	11.52a*	7.16ab	19.53a	11.44a	3.24a	16.61a
T_1	2.98b (-74.13)	7.59a (+6.0)	17.94a (-8.14)	10.49a (-8.30)	3.76a (+16.05)	10.95b(-34.08)
T_2	3.02b (-73.78)	5.99b (-16.34)	9.61b (-50.79)	5.81bc (-49.21)	2.97a (-8.33)	8.29bc (-50.09)
T_3	2.53b (-78.03)	1.11c (-84.50)	6.82b (-65.08)	8.85ab (-22.64)	1.87b (-42.28)	5.40cd(-67.49)
T_4	0.90b (-92.19)	1.00c (-86.03)	7.03b (-64.00)	3.42c (-70.10)	1.29bc (-60.19)	2.09d (-87.42)
T_5	1.09b (-90.54)	1.07c (-85.05)	5.45b (-72.09)	3.56c (-68.88)	0.79c (-75.62)	3.42d (-79.41)

* values in the columns followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's Multiple Range Test (DMRT)

Table 3: Root elongation (cm) of receptor agricultural crops to distil water (T_0) and different concentrations of *Azadirachta indica* extracts (T_1-T_5). Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in comparison to control

	Agricultural crops					
Treatment	*C. arietinum*	*R. sativus*	*V. unguiculata*	*C. sativus*	*B. juncea*	*P. mungo*
T_0	10.95a*	14.69a	14.23a	8.62ab	5.74a	8.41a
T_1	2.77b (-74.70)	11.29ab (-23.14)	4.01b (-71.82)	10.23a (+18.68)	6.14a (+6.97)	2.67bc (-68.25)
T_2	5.47b (-50.05)	9.35b (-36.35)	2.3b (-83.84)	6.67b (-22.62)	4.25ab (-25.96)	3.67b (-56.36)
T_3	1.32b (-87.94)	0.70c (-95.23)	1.41b (-90.09)	2.79c (-67.63)	1.98bc (-65.50)	1.83cd (-78.24)
T_4	1.00b (-90.86)	0.2c (-98.64)	1.14b (-91.94)	1.67c (-80.63)	0.84c (-85.37)	0.59d (-92.98)
T_5	1.07b (-90.22)	0.57c (-96.11)	0.84b (-94.10)	1.21c (-85.96)	0.38c (-93.38)	0.76d (-90.96)

*- values in the columns followed by the same letter(s) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test (DMRT)

Table 4: Number of lateral roots developed in receptor agricultural crops to distil water (T_0) and different concentrations of *Azadirachta indica* extracts (T_1-T_5). Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in comparison to control (T_0)

	Agricultural crops					
Treatment	*C. arietinum*	*R. sativus*	*V. unguiculata*	*C. sativus*	*B. juncea*	*P. mungo*
T_0	11.55a*	29.07a	29.20a	29.4b	5.33ab	12.67a
T_1	5.17ab (-55.24)	17.40b (-40.14)	18.93b (-35.17)	35.67a (+21.33)	6.47a (+21.39)	10.13ab (-20.05)
T_2	6.12ab (-47.01)	19.6b (-32.57)	10.67c (63.46)	23.53c (-19.97)	4.20abc (-21.20)	12.27a (-3.16)
T_3	3.10b (-73.16)	1.00c (-96.56)	7.80c (-73.29)	16.8d (-42.86)	2.93bcd (-45.03)	7.8bc (-38.44)
T_4	2.33b (-79.83)	0.00c (-100)	5.78c (-80.21)	11.53e (-60.78)	1.53cd (-71.29)	3.93d (-68.98)
T_5	3.67b (-68.23)	2.00c -(93.12)	3.24c (-88.90)	10.80e (-63.27)	0.00d (-100)	6.87c (45.78)

*values in the columns followed by the same letter(s) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test (DMRT)

In almost all cases, all the concentration especially from 25% and onwards significantly reduced the germination and growth of the seedlings. The overall effect was more pronounced at higher concentration where the survivors exhibited poor growth with grayish in color. Many seedlings lost their ability to develop normally as a result of reduced radicle elongation and root necrosis. So, it may be concluded that the inhibitory effect of plant extracts dependent very much on their concentration. Similar observation was also found by Ballester *et al.* (1982), Rai and Triputhi (1984), Rizvi and Rizvi (1987), Chou and Leu (1992) and Daniel (1999). The study also revealed that development roots and lateral roots were severely impeded compared to germination and shoot growth. Even complete inhibition on the development of lateral roots was also occurred. These results correlated with findings of Meissner *et al.* (1982); Chou and Kuo, (1989); Zackrisson and Nilsson (1992). Those studies found that radicle growth was more sensitive and responds more strongly to small variation in toxin concentration. It means the buried portion of plants is much sensitive than the aerial portion to the allelochemicals. The adverse effect of *Azadirachta indica* on the germination and growth of tested crops might be attributed to the phytotoxic

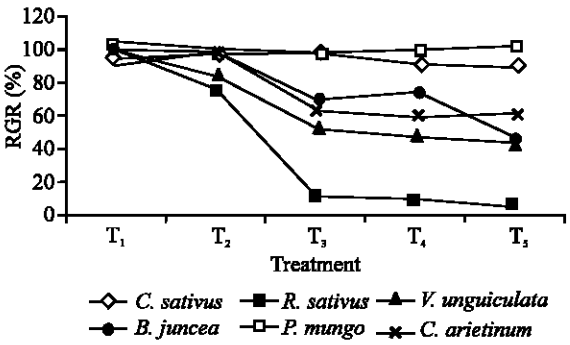

Fig. 1: Relative germination ratio of bioassay species grown in petridishes at different concentrations of *Azadirachta indica* extracts

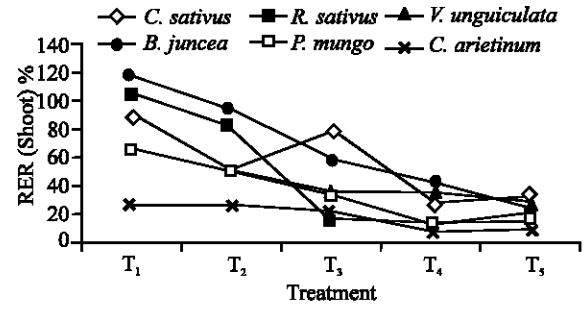

Fig. 2: Relative elongation ratio of shoot of bioassay species grown in petridishes at different concentrations of *Azadirachta indica* extracts

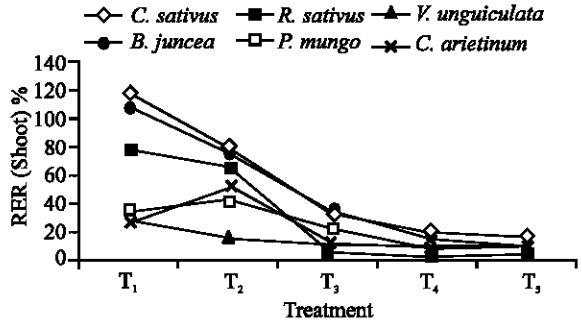

Fig. 3: Relative elongation ratio of root of bioassay species grown in petridishes at different concentrations of *Azadirachta indica* extracts

chemicals released from the leaves. Though laboratory bioassays are of great importance to single out the allelopathic effect extensive field study is recommended while proposing the tree as an associated species for large scale agroforestry plantations.

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