Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
3(1):7-11
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

Efficacy of *Azadirachta indica* leaf, stem and bark extracts on seedling related traits of *Phaseolus vulgaris* L.

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ABSTRACT

In the current investigation an attempt has been made to appraise the efficacy of neem leaf, stem and bark aqueous extract on germination and morphological parameters of Phaseolus vulgaris. The extract of neem leaf, stem and bark showed both inhibitory and stimulatory activity on germination, shoot length, root length, seed vigour and tolerance index of bean seedlings. All the above parameters were found to be decreased with increase in the concentration of aqueous leaf, stem and bark extracts except in 5% concentration when compared to control. The maximum and minimum value was observed for all these parameters in 5% and 25% concentration respectively except for percent toxicity and dry weight. The toxicity was very insignificant in 5% and maximum dry weight observed in 25% concentration when compared to control. The result of the present study implies both inhibitory and stimulatory effects of neem leaf, stem and bark extracts, that may be due to the presence of some allelochemicals.

Key words: Neem, Phaseolus, vigour, germination.

INTRODUCTION

Agriculture is the backbone of Indian economy. About 65% of Indian population depends directly on agriculture. Agriculture derives its importance from the fact that it has vital supply and demand links with the manufacturing sector. During the past five years agriculture sector has witnessed spectacular advances in the production and productivity of food grains, oilseeds, commercial crops, fruits, vegetables, food grains, poultry and dairy. The fertility of soil is an important factor determining fertilizer requirements as well as the level of crop production that can be obtained (Batish, 2001). Due to increasing the number of herbicide resistant weeds and environmental concerns about the safety of synthetic herbicides, considerable effort has been put into designing alternative weed management strategies and reducing dependence on synthetic herbicides (Teerarak et al., 2010). The purpose of the present study is to reduce the chemical use and focus on the importance of bio-pesticides usage. Allelopathy is one of the promising strategy, which can be put to good use in several ways in agro ecosystem (Kamal and Bano, 2009). Allelopathy is a phenomenon where a plant species chemically interfere with the germination, growth and development of other plant species and has been known for over 2000 years. Compounds with allelopathic activity are present in many plants and in many plant organs including leaves, stems, fruits and buds (Mahall and Callaway, 1991; Indrajit, 1996 and Ashrafi et al., 2007). There are many plants which are traditionally used for their medicinal value that showed some allelopathic effect, like Basil (*Ocimum basilicum* L.) on some crops (Verma et al., 2012), Eucalyptus on some seed plants

(Lisanework & Michelen, 1993). Keeping the above in view, the present work was undertaken to study the allelopathic efficacy of *Azadirachta indica* A. Juss Linn. on the germination and morphological parameters of bean seeds.

The neem tree is a tropical evergreen tree native to India. Neem is known as "the village pharmacy" because of its healing versatility, and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. *Phaseolus vulgaris* L., the common bean also known as string bean, field bean, flageolet bean, French bean, garden bean, haricot bean. French bean is a herbaceous annual plant grown worldwide for its edible fruit, either the dry seed or the unripe pod, both of which are referred to as beans.

MATERIALS AND METHODS

Collection of plant materials and seed samples:
Fresh and healthy plant materials like leaves, stem and bark of neem tree were collected from the various places of Manasagangotri, campus. The certified seed samples of Common beans variety Selection -9 was procured from Annadaatha agro kendra, Mysore.

Preparation of aqueous extracts
The leaves, stem and bark were shade dried for about two-three weeks and made into a fine powder using grinder. 5, 10, 15, 20 and 25 grams of the powder was added to the conical flask containing 100ml of water. The conical flasks were kept in a rotatory shaker for about 24 hours. It was filtered through filter paper or muslin cloth. The extract was stored at 40^0C in dark bottles or in a refrigerator to reduce the allelochemicals degradation for the further usage.

Bioassay of seed germination studies
Seeds were sown in triplicates in plastic cups and filled with soil. To each plastic cups add 5ml of aqueous extract concentrations ranging from 5%, 10%, 15%, 20% and 25% and distilled water is taken as control. The same procedure is followed for remaining trials. The obtained results were calculated statistically.

Germination and morphological studies
The germination and morphological parameters were carried out following standard methods- Germination percentage and Root and shoot length (ISTA, 1985), Vigour index (Abdul-Baki and Anderson, 1973), Tolerance index (Turner and Marshal, 1972), Percentage of phytotoxicity (Chion and Muller, 1972) and Fresh weight and dry weight (Agarwal, 1994).

RESULTS

The effect of aqueous extracts of neem leaf, stem and bark on the germination percentage, vigour index, percent toxicity, root and shoot lengths, fresh and dry weight seedling of bean variety was investigated (Table 1). The percentage of germination was found to be decreased with increase in concentration of leaf, stem and bark extract except in 5% concentration, where the percentage of germination was found to be increased when compared to all other concentration along with control. The maximum and minimum percentage of germination in neem leaf, stem and bark extract was recorded in 5% and 25% concentration (98.69%, 95.94%, 94.09% and 55.29%, 52.07 and 47.11%) respectively.

The effect of different concentrations of neem leaf, stem and bark extract on vigor index and tolerance index showed significance difference when compared to control (Table-1). As the concentration of extract increased the VI decreased except in 5%, when compared to control. The maximum and minimum value of vigor index were recorded at 5% and 25% concentration (5116.37%, 4835.17%, 4411.48% and 631.93%, 681.56% and 566.13%) in neem leaf, stem and bark extract respectively, The Tolerance index of *P. vulgaris* seedlings showed significant increase in 5% concentration (163.92%, 156.72% and 151.42%) in neem leaf, stem and bark extract compared to control. Among three aqueous extracts, leaf extract was found to be more effective. Minimum value was recorded in 25% concentration i.e., 24.29%,

Table1. Germination percentage, vigourindex, tolerance index, percent toxicity, fresh weight, dry weight, plumule and radicle length of *Phaseolus vulgaris* seedlings under different concentrations of leaf, stem and bark aqueous extracts.

Extracts	Concentrations	Leaf extract	Vigour Index	Tolerance index	Percent toxicity	Fresh weight (g/plant)	Dry weight (g/plant)	Plumule length (cm)	Radicle length (cm)
Leaf extract	Control	96.086 ±0.58^{b}	4080.86±368.72^{b}	100.00±0.00^{b}	0.00± 0.00^{d}	2.62±0.049^{b}	0.12±0.035^{f}	18.75±0.47^{b}	23.45±5.08^{b}
	5	96.51±2.82b	5116.37±403.76^{a}	163.92±8.518^{a}	-15.807±5.786^{e}	3.61±0.179^{a}	0.14±0.037^{e}	21.72±1.35^{a}	31.52±4.64^{a}
	10	87.636±0.608^{c}	3375.12±234.72^{c}	120.06±7.190^{c}	10.42± 5.091^{d}	2.56±0.120^{c}	0.18±0.062^{d}	16.81±1.28^{c}	23.99±2.07^{c}
	15	76.36±0.438^{d}	2482.09±340.59^{d}	87.203±3.817^{d}	25.553±1.960^{c}	2.32±1.102^{d}	0.28±0.024^{c}	13.95±0.15^{d}	18.38±4.36^{d}
	20	64.27± 0.756^{e}	1841.60± 45.15^{e}	63.42±3.533^{e}	44.73 ±2.896^{b}	2.08±0.202^{e}	0.34±0.262^{b}	10.35±0.30^{e}	14.85±3.48^{e}
	25	55.29±0.770^{f}	631.93± 231.83^{f}	24.29±5.953^{f}	76.26 ±8.228^{a}	1.44±0.299^{f}	0.66±0.169^{a}	4.48±1.61^{f}	8.28±2.01^{f}
Stem extract	Control	94.21±0.384^{b}	3883.86±468.41^{b}	100.00±0.00^{b}	0.00±0.00^{d}	2.24±0.498^{b}	0.12±0.032^{f}	17.75±0.54^{b}	21.90±0.39^{b}
	5	95.94 ±0.582^{a}	4835.17±575.27^{a}	156.72±7.316^{a}	-22.886±6.810^{e}	3.14±0.632^{a}	0.15±0.0262^{e}	19.79±0.61^{a}	28.38±4.92^{a}
	10	85.10±0.213^{c}	3094.85±127.46^{c}	109.68±1.356^{c}	6.628±3.755^{d}	2.13±0.141^{c}	0.20±0.0244^{d}	13.92±0.40^{c}	20.1±3.09^{c}
	15	73.64±0.514^{d}	2479.19±53.02^{d}	85.33±4.326^{d}	25.61±2.966^{c}	1.93±0.191^{d}	0.25±0.0094^{c}	12.2±0.45^{d}	15.68±1.20^{d}
	20	61.41±1.165^{e}	1621.35±255.54^{e}	56.311±5.925^{e}	37.582±3.42^{b}	1.633±0.254^{e}	0.32±0.0216^{b}	9.74±0.85^{e}	13.23±0.74^{e}
	25	52.07±0.1959^{f}	682.56±83.75^{f}	21.84±3.757^{f}	71.386±9.454^{a}	1.366±0.147^{f}	0.66±0.124^{a}	2.22±0.39^{f}	7.37±1.29^{f}
Bark extract	Control	90.64± 0.423^{b}	3372.48±37.02^{b}	100.00±0.00^{b}	0.00±0.00^{d}	2.64±0.204^{b}	0.15±0.0081^{f}	13.60±0.40^{b}	19.7±0.65^{b}
	5	94.9 ±0.737^{a}	4411.48±22.02^{a}	151.42±14.86^{a}	-41.05±3.243^{e}	3.206±0.335^{a}	0.19±0.021^{e}	19.18±0.436^{a}	26.18±0.45^{a}
	10	81.22± 0.255^{c}	2732.32±308.22^{c}	106.30±2.192^{c}	7.49±3.829^{d}	2.413±0.239^{c}	0.25±0.0124^{d}	13.00±0.428^{c}	18.45±2.87^{c}
	15	70.84± 0.938^{d}	2129.42±116.22^{d}	78.46±8.05^{d}	9.42±2.084^{c}	1.96±0.047^{d}	0.30±0.016^{c}	9.316±0.82^{d}	15.22±0.54^{d}
	20	57.57± 0.566^{e}	1497.00±79.49^{e}	51.05±4.515^{e}	14.78±2.062^{b}	1.56±0.286^{e}	0.54 ±0.015^{b}	6.813±0.485^{e}	11.00±0.27^{e}
	25	47.11± 1.284^{f}	566.13±158.04^{f}	16.61±1.817^{f}	20.727±0.997^{a}	1.26±0.169^{f}	0.66±0.169^{a}	1.433±0.339^{f}	6.973±2.765^{f}

Mean ± SE followed by the same superscript are not statistically significant between the control and different concentrations of herbicide, when subjected to Tukey's mean range test ($p<0.05$)

21.84% and 16.61%in neem leaf, stem and bark extract respectively.

The mean value of phytotoxicity at different concentration of neem leaf, stem and bark extract were found to be significantly different (table 1). Phytotoxicity increased as the concentration of the extract increased, where as the percentage of the phytotoxicity was found to be very less in 5% concentration (-15.807%, -22.88% and -41.05%) in neem leaf, stem and bark extract respectively. Phytotoxicity increased from 10.424% to 76.266%, 6.628% to 71.386%, 7. 49% to 64.727% in 10% to 25% concentration of leaf, stem and bark extract respectively when compared to control. The root and shoot length decreased significantly as the concentration of leaf, stem and bark extract increased from 5% to 25% concentration (Table-1). The root length and shoot length of leaf, stem and bark extract was found to be decreased with increasing concentration of extract. Root and shoot length of leaf, stem and bark extract was maximum in 5% and minimum in 25% concentration (21.72 cm and 31.52 cm) (19.79cm and 28.38cm) and (4.48 and 8.28) respectively. The fresh weight and dry weight was significantly affected by different concentrations of extract when compared to control (Table 1). As the concentration of the extract increased the fresh weight decreased except at 5% concentration. The maximum and minimum value of fresh weight was recorded at 5% and 25% concentration in neem leaf, stem and bark extract (3.613g/plant-1.44g/plant, 3.14g/plant-1.36g/plant and 3.20g/plant-1.26g/plant) respectively. As the concentration increased the dry weight also increased from 5% to 25% concentration. However the dry weight showed significant difference when compared to control for the same. The dry weight increased from 5 -25% concentrations (0.14g/plant-0.66 g/plant, 0.15-0.66 g/plant and 0.19 g/plant-0.169 g/plant) in leaf, stem and bark extracts respectively.

DISCUSSION

Compounds with allelopathic activity are present in many plants and in many plant organs including leaves, stems, fruits and buds (Mahall and Callaway, 1991; Indrajit, 1996 and Ashrafi et al., 2007). Our results are similar with earlier results of (Al-Charchafchi et al., 2007) who reported that allelopathic effect of neem plant on many other plants especially during their germination and seedling growth. (Netsere and Mendesil, 2011) reported that the aqueous extracts of shoot, leaf and root of *Parthenium* weed exhibited allelopathic effect on soybean and haricot bean seed germination, shoot and root growth and dry matter of seedling. Our results are similar with the results of Shruthi et al., (2014) who reported aqueous extracts of Azadirachta indica leaf on seed germination, root and shoot growth, fresh and dry weight, phytotoxicity of green gram seedlings. There are many plants which are traditionally used for their medicinal value that show some allelopathic effect, like Basil (*Ocimum basilicum* L.) on some crops (Verma et al., 2012), Eucalyptus on some seed plants (Lisanework & Michelen, 1993). (Rejila et al., 2011) reported the allelopathic effect of leaf extract of *Jatropha curcas* on Green chilli and Sesame seeds. They observed abnormal growth in both shoot and seed germination and shoot length showed similar results on sesame. But more inhibition could be seen in higher concentration than in control and these abnormalities was due to the presence of allelochemicals like phenols and tannins.

Our results are in line with the earlier results of Kanaga et al., (2012) who reported suppressive effects of different concentrations of aqueous extracts of Azadirachta indica leaf on seed germination, root and shoot elongation and fresh and dry weight of cow pea and horse gram. From the present study, it can be concluded that aqueous extract of leaves, stem and bark of neem showed allelopathic effects on germination and morphological parameters of bean. The extract showed both negative and positive effect on all the parameters. The study revealed the allelopathic effect of aqueous neem leaf, stem

and bark extract might be due to the presence of allelochemicals.

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