

## INHIBITORY EFFECTS OF LEAF EXTRACTS ON MORPHOLOGY OF *PISUM SATIVUM* CV. ARIKIL

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### Abstract

Aqueous leaf extracts concentration of *Eucalyptus tereticornis* J.E. Smith., *Dalbergia sissoo* Roxb., *Azadirachta indica* A. Juss and *Tectona grandis* L.f. on radicle length and plant height in a dose-dependent manner. The leaf extract of *Tectona grandis* inhibited seed germination. The result of present investigation indicates that commonly used aqueous leaf extracts has significant inhibitory action on *P. sativum* cv. Arikil.

Since ancient times, plants, herbs and spices are important resources in traditional medicine (Akelere 1993). China, Indonesia and a number of Middle East countries to treat various infectious diseases (Zohara 2012, Fadeyi *et al.* 2013, Ngutaa *et al.* 2015). It is well known that the medicinal property of plant extracts is due to the phytochemical substances present in it (Prabhu *et al.* 2011). The World Health Organization estimates that up to 80% of the world's population depend on traditional medicinal system for some aspects of primary health care (Farnsworth *et al.* 1985).

In spite of their reflective therapeutic advantages, few constituents of medicinal plants are known to have inhibitory, mutagenic, toxic, carcinogenetic, clastogenic and teratogenic effects on lower animals (Gadano *et al.* 2006, Siddiqui 2014).

However, these plants may exert toxic effects on other plants as genetic constitution and metabolic pattern are different from animals. Therefore, a thorough experimental approach to understand the adverse effects of these plants is an urgent requirement. In present study, inhibitory effects of these medicinal plant leaf extracts on seed germination, radicle length and plant height of *P. sativum* var. *Arikil* were investigated.

The experiments were conducted at research laboratory of Bundelkhand University, Jhansi, Department of Botany, Uttar Pradesh, India, during August to October, 2009.

Fresh and clean leaves of *E. tereticornis*, *D. sissoo*, *A. indica* and *T. grandis* were collected locally from Jhansi, Uttar Pradesh, India. They were collected from different spots from campus of Bundelkhand University, Jhansi, India. The leaves were collected from the middle region of tree canopy, washed and shade-dried by spreading them on a clear laboratory table and were cut into smaller pieces. They were then blended by using blender and were taken in a 2000 ml reagent bottle. The leaves were powdered with the help of kitchen blender and different concentrations of extracts (12.5 g powdered leaf/l, 25 g powdered leaf/l, and 50 g powdered leaf/l, were prepared. The solutions were kept in water bath maintained at 45 - 55°C for 24 hrs. After 24 hrs, aqueous resultant extracts were filtered through sterile cotton followed by Whatman No.1 filter paper, and were taken in another 2000 ml bottle. They were sealed and stored in a refrigerator until further use. The filtrates of individual plant extracts were stored and used for treating the seeds of *P. sativum* var. *Arikil*.

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Healthy uniform grains of *P. sativum* var. Arikil ( $2n = 14$ ) were obtained from Agriculture Seed Store, Govt. of Uttar Pradesh, Jhansi, India. The seeds were surface sterilized with 1% sodium hypochlorite for 20 min and then rinsed with distilled water several times to remove excess of the chemical. The seeds were presoaked in distilled water for 3 hrs and then soaked in aqueous extracts of leaves of different medicinal plants for 6 hrs. The seeds from different treatment groups (30 each) were placed on moistened pre-sterilized cotton in Petri dishes (15 cm diameter) and incubated in a plant growth cabinet for 120 hrs in dark at a temperature of  $20 \pm 2^\circ\text{C}$ . Seeds in the control groups (CN) were treated with double distilled water. The seed germination was determined by observing radicle formation. The radicle length of germinated seeds were measured at every 24 hrs up to 120 hrs using a millimeter ruler and plant height were measured once the plants started flowering. Laboratory experiments in Petri dishes were done for seed germination and radicle length. Plant height experiment was done in pots. The entire experiments were repeated thrice under similar experimental conditions.

Data were compared by analysis of variance (ANOVA), using the STATVIEW 4.5 (abacus concept; Berkeley, USA) software package and difference were considered statistically significant at  $p < 0.05$ .

Table 1 summarizes the effect of aqueous extracts of leaves of medicinal plants on seed germination in *P. sativum* var. Arikil. In control group, about 3.3% of seeds germinated after 24 hrs, which increased with incubation period to approximately 5.66, 6.66, 7.66 and 9.66% at 48, 72, 96 and 120 hrs, respectively.

**Table 1. Effect of various concentrations of leaf extracts on seed germination of *P. sativum* var. Arikil.**

Treatment groups	Time intervals (in hrs)				
	24	48	72	96	120
CN	$3.33 \pm 1.0$	$5.66 \pm 0.57$	$6.66 \pm 0.57$	$7.66 \pm 0.57$	$9.66 \pm 2.96$
<i>T. grandis</i> (g/l)					
12.5	$3.33 \pm 2.30$	$5.33 \pm 0.57$	$5.33 \pm 0.57^c$	$5.33 \pm 0.57^b$	$5.33 \pm 0.57^b$
25	$2.66 \pm 1.52$	$4.33 \pm 1.15^b$	$4.33 \pm 1.25^b$	$4.33 \pm 1.25^b$	$4.33 \pm 1.12^b$
50	$4.12 \pm 1.25$	$4.33 \pm 1.15^b$	$4.33 \pm 1.57^b$	$5.33 \pm 0.57^b$	$5.33 \pm 0.57^b$

a =  $p < 0.001$  highly significant; b =  $p < 0.01$  very significant; c =  $p < 0.05$  significant compared in respective group. CN = Control.

Whereas seeds treated with leaf extract of *T. grandis* exhibited lower seed germination in all concentrations compared with control group at respective incubation period. The highest percentage of germination observed was 5.33 in 12.5 g powdered leaf /l at 48 hrs, after that, seed germination was arrested in all incubation periods, which is significantly lower than control group ( $p < 0.01$ ).

In *P. sativum* var. Arikil seeds treated with leaf extracts of *A. indica*, *T. grandis*, *D. sissoo* and *E. tereticornis*. However, the radicle length significantly was reduced ( $p < 0.05$ ) in treated groups compared to control at respective time intervals. *P. sativum* var. Arikil seeds treated with *D. sissoo* leaf extract at 24 hrs, no radicle length was reported in all concentrations, however there were gradual increase in radicle length at 48, 72, 96 and 120 hrs, respectively. *T. grandis* leaf extract has the highest inhibitory effect at all-time intervals in all concentrations on radicle length compared to other extracts. However, in case of *A. indica* leaf extract, there was a gradual increase in radicle length at all-time intervals in all concentrations. In addition, *P. sativum* var.

Arikil seeds treated with *E. tereticornis* leaf extract, growth in radicle length gets arrested after 72 hrs in all the concentrations tested.

Fig. 1 shows inhibitory effect of aqueous extracts of leaves of four medicinal plants on plant height of *P. sativum* var. *Arikil*. Maximum plant height of *P. sativum* var. *Arikil* were observed in all the control groups ( $11.05 \pm 0.49$  cm) in *E. tereticornis* ( $8.55 \pm 1.42$  cm) in *D. sissoo* ( $11.40 \pm 1.67$  cm) in *A. indica* and ( $9.95 \pm 1.67$ ) in *T. grandis*.

**Table 2. Effects of leaf extract of different medicinal plants on radicle length in germinating seeds of *P. sativum* var. *Arikil*.**

Treatment groups	Radicle length (cm)				
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
CN	$0.63 \pm 0.01$	$3.01 \pm 1.07$	$3.82 \pm 1.28$	$4.40 \pm 1.61$	$5.29 \pm 2.10$
<i>E. tereticornis</i> (g/l)					
12.5	$0.75 \pm 0.60$	$2.18 \pm 1.37$	$3.11 \pm 1.59$	$3.11 \pm 1.59$	$3.11 \pm 1.59^b$
25	$0.45 \pm 0.27$	$2.08 \pm 0.69$	$3.02 \pm 1.07$	$3.02 \pm 1.07$	$3.02 \pm 1.07^b$
50	$0.59 \pm 0.47$	$2.10 \pm 1.02$	$3.17 \pm 1.25$	$3.33 \pm 1.19$	$3.33 \pm 1.19^c$
<i>D. sissoo</i> (g/l)					
12.5	$0.00 \pm 0.00$	$1.91 \pm 0.87^c$	$2.43 \pm 0.92^c$	$3.68 \pm 1.07$	$5.19 \pm 1.24$
25	$0.00 \pm 0.00$	$1.68 \pm 0.86^b$	$2.88 \pm 1.02$	$3.77 \pm 1.34$	$4.79 \pm 1.93$
50	$0.00 \pm 0.00$	$1.16 \pm 0.85^b$	$1.89 \pm 1.19^b$	$2.49 \pm 1.39^b$	$3.03 \pm 1.42^c$
<i>A. indica</i> (g/l)					
12.5	$0.48 \pm 0.32$	$2.08 \pm 0.64^c$	$3.30 \pm 0.86$	$4.21 \pm 1.26$	$4.60 \pm 1.46$
25	$0.38 \pm 0.29$	$2.23 \pm 0.61$	$3.42 \pm 0.57$	$3.99 \pm 0.60$	$5.15 \pm 1.03$
50	$0.42 \pm 0.34$	$1.59 \pm 0.52^b$	$3.23 \pm 0.55$	$4.20 \pm 0.61$	$5.17 \pm 1.01$
<i>T. grandis</i> (g/l)					
12.5	$0.14 \pm 0.07^b$	$0.60 \pm 0.25^b$	$0.64 \pm 0.27^b$	$0.85 \pm 0.69^b$	$1.05 \pm 0.87^b$
25	$0.28 \pm 0.11^b$	$0.84 \pm 0.64^b$	$1.06 \pm 0.96^b$	$1.10 \pm 0.88^b$	$1.30 \pm 1.02^b$
50	$0.26 \pm 0.09^b$	$0.64 \pm 0.18^b$	$0.72 \pm 0.25^b$	$0.88 \pm 0.29^b$	$0.96 \pm 0.52^b$

a =  $p < 0.001$  highly significant; b =  $p < 0.01$  very significant; c =  $p < 0.05$  significant compared to control. CN = Control.

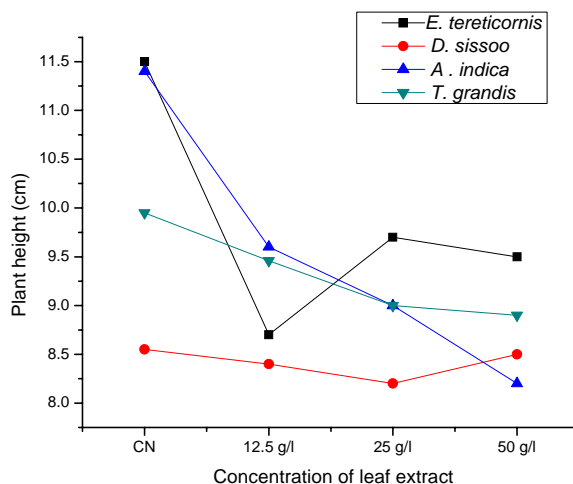


Fig. 1. Effects of leaf extract on plant height of *P. sativum* var. *Arikil*.

In case of *P. sativum* var. Arikil seeds treated with *D. sissoo* leaf extract, plant height decreases with increase in concentration ( $8.40 \pm 0.56$  cm) at 12.5 g powdered leaf/l and ( $8.20 \pm 1.11$  cm) at 25 g powdered leaf/l, respectively, but increase in plant height was reported ( $8.50 \pm 1.15$  cm) at 50 g powdered leaf/l. In addition, *P. sativum* var. Arikil seeds treated with *E. tereticornis* leaf extract, plant height decreases with increase in concentration ( $8.70 \pm 1.73$  cm) at 12.5 g powdered leaf/l and ( $9.50 \pm 1.74$  cm) at 50 g powdered leaf/l, respectively, but plant height increases at 25 g powdered leaf/l concentration which was  $9.70 \pm 1.13$  cm.

Leaf extracts of medicinal plants have a complex mixture of certain phyto-chemicals that may contain both mutagenic and antimutagenic properties. Previous investigations have reported that aqueous extracts of a few plant species contains phenolic compounds (Atoum *et al.* 2006) which inhibit seed germination and seedling growth of plants (Sangita *et al.* 2012).

Aqueous leaf extracts of medicinal plants containing phenolics and alkaloids might have lowered absorption of minerals, water and their translocation from roots to other plant parts (El-Khatib *et al.* 2004) with reduced photosynthesis, which might be one of the of reasons of shorter plant height. Many enzymatic functions important to plants are inhibited by the presence of phenolics and alkaloids in aqueous extracts (Rice 1984, Turk and Tawaha 2002).

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