

## Effects of Walnut Leaf Extract on Seed Germination and Seedling Vigour of Toria (*Brassica rapa* L. var. toria)

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**ABSTRACT** The significant reduction in seed germination and seedling vigour were observed by the application of walnut leaf extracts in toria (*Brassica rapa* L. var. toria). However, MGT and  $T_{50}$  as lower values indicated for higher seed vigour, increased with the increased leaf extract concentration, and were lowest in the control. Since the seed germination and seedling vigour were affected negatively by walnut leaf extracts on concentration dependent, therefore, to reduce allelopathic effects on companion toria crop, farmers should remove all fallen walnut leaves.

**Key words:** Walnut leaf extract, seed germination, vigour, toria

Walnut (*Juglans regia* L.) is a large deciduous tree species distributed in the Himalayan range 1375-3350 m from Afghanistan (West) to Bhutan. It is a source of livelihood for the rural population owing to huge demand for fruit nut, aesthetic timber qualities, and rapid growth potential [1]. It occurs both in wild and cultivated habitats and can be found along the bunds of agricultural fields throughout the region. The fall of leaves in autumn (September-October), coincides with the sowing of important winter season crops. Presence of trees in agroforestry system results in the exposure of associated crops with allelochemicals of the fallen leaves which after decomposition leaches into the soil by winter rains and snowfall.

The allelo-chemicals are known to affect seed germination and seedling growth of plant species [2]. The chemical in walnut allelopathy is juglone (5-hydroxy-1, 4 naphthoquinone), reported in leaves, stem, hulls, inner bark and roots [3]. It may affect other plants either through root contact, leakage, decay of fallen leaves or when rain leaches and drips off juglone from leaves and branches on the plants [4]. There are no reports of allelopathic effects of walnut leaf extract on important winter season agricultural

crops, like toria, under agri-silviculture system. The present study was undertaken to assess the effects of walnut leaf extract on seed germination and seedling growth in toria crop.

### MATERIALS AND METHODS

#### *Leaf collection and extraction*

Naturally fallen leaves of more than ten-years old trees [younger than seven-years old do not contain sufficient juglone to cause toxicity, 5] were collected in late September 2008 from nearby Hill Campus, Ranichauri, Tehri Garhwal (2100 MSL, 30<sup>0</sup>15' N latitude and 78<sup>0</sup>30' E longitude). Leaves were properly washed and then dried. The 100 g of powdered leaves were soaked in 1000 ml of distilled water for 48 hr for preparing concentrated aqueous stock solution. The filtrate was centrifuged and supernatant was decanted. The treatments consisted of five dilutions of aqueous leaf extracts (20, 40, 60, 80 and 100%), whereas pure distilled water (0% leaf extract) was taken as the control.

#### *Experimental trials*

Seeds of toria (*Brassica rapa* var. toria cv. PRT 4) was procured from Department of Crop

Improvement, GBPUA&T, Hill Campus, Ranichauri, Tehri Garhwal (Uttarakhand) and used after surface sterilization with 1 per cent sodium hypochlorite. The 100 seeds each of PRT 4 were placed separately in pre-sterilized Petri dishes with filter paper for the experiment. The experiment was laid out in completely randomized design with four replications. The treatment contained 10 ml each of control and five concentrations of walnut leaf extracts (added in each Petri dish on first day, followed by 5 ml later as per need).

The material was placed in an incubator (20°C) until completion of experiment. The speed of germination, mean germination time (MGT) and relative growth index (RGI) were calculated as per reported methodology [6, 7]. Germination index (GI) was calculated as described in the Association of Official Seed Analysts [8]. However, seed germination was observed daily following AOSA rules [9]. The time to 50 per cent germination ( $T_{50}$ ) was calculated following Farooq *et al.* [10]. Germination energy is the inherent ability of any seed or seed lot that reflected the potential of germination at faster rate and was calculated as under:

$$\text{Germination energy (\%)} = \frac{\text{Germination before peak period including peak period}}{\text{Total number of seeds taken}} \times 100$$

Normal seedlings were recorded on date of final count and expressed as percentage [11]. Ten normal seedlings randomly selected were used to measure the root length, shoot length and seedling length. Pooled root and shoot part of seedlings were dried in an oven (85°C) until constant weight was attained. Seedling vigour index (SVI) I and II were derived by multiplying per cent germination with seedling length and dry weight of seedlings, respectively [12]. Data were analysed by following the method of Snedecor and Cochran [13].

## RESULTS AND DISCUSSION

The effects of walnut leaf extracts on seed germination characteristics, like germination per

cent, speed of germination, mean germination time,  $T_{50}$  value, relative growth index, germination index and germination energy have been presented in Table 1. There was significant gradual decrease in germination with the increase in concentration of walnut leaf extract. The maximum value (92.00%) was recorded in control. Application of 100 per cent leaf extract resulted in maximum reduction in seed germination (51.09%) as compared to the control.

Earlier establishment of crops in field leads to greater yield in less period and depends on speed of germination of seed. The impact of treatments on speed of germination was compared and the undiluted extract was found to be the most inhibitive. As the extract concentration decreases, the speed of germination increases in a concentration dependent manner. Hence, maximum speed of germination index (11.99) was recorded for the control. Significantly least germination speed index (4.29) was reflected for undiluted leaf extract (100%) treatment. Significant effect of walnut leaf extract was seen on the MGT and  $T_{50}$  of test crops at 5 per cent level of significance. The lowest MGT (4.21 days) was noted in the control (0%), whereas the maximum MGT (5.95 days) was recorded in undiluted extracts (100%).

However, results with respect to MGT of 60 per cent and 80 per cent extracts did not differ significantly from each other. The same trend was also observed for days taken to 50 per cent germination ( $T_{50}$ ) and minimum  $T_{50}$  value (3.77 days) was recorded for the control, whereas significantly higher value of 5.79 days was recorded for undiluted leaf extracts (100%). Relative growth index (RGI) and germination index (GI) were significantly reduced by walnut leaf extracts containing juglone. The maximum RGI (90.27) was calculated for the control, whereas least value of 28.95 was recorded in undiluted extracts.

However, value of RGI for 60 and 80 per cent extracts concentration did not significantly differ from each other. Similarly, germination index (GI) was influenced significantly by the concentration of extracts. The highest germination index (29.90) was recorded in the control, whereas significantly

Table 1. Effects of walnut leaf extracts on seed germination and seed vigour parameters in toria

Treatment (leaf extract)	Germination (%)	Speed of germination index (SGI)	Mean germination time (MGT)	T <sub>50</sub> value	Relative growth index (RGI)	Germination index (GI)	Germination energy (GE)
20%	82.00 (10.87)	9.79	4.67	4.15	71.37	23.41	38.00
40%	75.00 (18.48)	8.14	5.11	4.84	51.45	18.41	28.50
60%	68.00 (26.09)	7.06	5.46	5.40	39.13	15.01	17.00
80%	55.00 (40.22)	5.54	5.48	5.30	38.96	12.15	11.00
100%	45.00 (51.09)	4.29	5.95	5.79	28.95	9.02	8.50
0% (Control)	92.00	11.99	4.21	3.77	90.27	29.90	54.50
SEm±	1.09	0.13	0.59	0.09	1.69	0.25	1.11
CD at 5%	3.26	0.41	0.17	0.25	5.03	0.75	3.30
CV (%)	3.15	3.56	2.31	3.57	6.35	2.84	8.47

Figures in parentheses are the per cent reduction values over the control

least value (9.02) was computed in undiluted extract (100%) treatment. A faster germination rate with high germination will facilitate early seedling establishment with good plant stand, as validated by germination energy (GE). The high GE (54.50%) was recorded for the control, whereas least value (8.50%) was calculated for undiluted treatments. The walnut leaf extracts containing juglone negatively influenced germination energy with increase in leaf extract concentration.

Present study clearly showed that walnut leaf extracts could inhibit toria seed germination. Walnut leaves containing juglone [14] is known to inhibit germination of various plant species [15, 16]. Similarly, in the present investigation toria seed germination is dependent on concentration of walnut leaf extracts. The reduced T<sub>50</sub> and MGT indicated earlier and rapid germination, whereas higher speed of germination, RGI and GI express the power of germination, *i.e.* germination spread over the time. These findings support the earlier work of Renolds in lettuce [15]. The delayed and unsynchronized germination might be attributed to interference in metabolic activities of the seeds by walnut leaf extract [17].

The reduction in root, shoot and seedling length with increasing concentration of walnut leaf extracts up to 100 per cent, except in treatment of 20 per cent concentration extract, showed higher shoot and seedling length over the control (Table 2). An inhibitory effect was noticed in fresh and dry weight of seedling with the increase in concentration from the control to 100 per cent and similar trend was observed in vigour index I & II (Table 2). Least fresh weight (0.43 g) was observed for 100 per cent concentration, whereas 0.83 g was observed for the control. The dry weight value of 0.084 g was much more as compared to 0.027 g observed at maximum concentration (100%).

In the present study, walnut leaf extracts containing juglone significantly inhibited all major growth parameters. Similar effects were observed with juglone in cucumber [18], tomato and bean [19], wheat and corn [20]. This may be attributed to inhibitive cell division due to walnut leaf extracts which has been reflected in the fresh weight, dry weight and vigour index (I & II) of seedling.

It may be concluded from the present study that juglone in walnut leaf extract has inhibitory

Table 2. Effects of walnut leaf extracts on seed and seedling vigour in toria

Treatment (leaf extract)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling vigour index (I)	Seedling vigour index (II)
20%	6.60	10.06	16.72	0.88	0.090	1371.37	7.30
40%	6.30	9.36	15.66	0.77	0.065	1175.03	4.98
60%	4.30	7.70	12.01	0.65	0.050	816.95	3.43
80%	3.79	6.09	9.89	0.56	0.035	543.73	1.96
100%	2.58	4.42	7.01	0.43	0.027	315.07	1.15
Control	6.34	9.62	15.97	0.83	0.084	1477.37	7.90
SEm±	0.91	0.10	0.16	0.01	0.0028	20.22	0.20
CD at 5%	0.27	0.31	0.49	0.04	0.008	60.08	0.61
CV (%)	3.66	2.69	2.57	4.12	9.62	4.25	9.23

effects on seed germination and seedling growth in toria, which is concentration dependent. Therefore, prior to farming toria crop, farmers should remove all fallen leaves of walnut.

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