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## Allelopathic Possessions of Festering Walnut Leaf on Radish (*Raphanus sativus* L.) Seed Germination and Sprout Growth in Uttarakhand Himalaya

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**Abstract:** Phytotoxic effects of aqueous extracts of walnut leaf was studied on germinating seeds and early seedling growth of radish (*Raphanus sativus* L. cv. pusa chetki) under western Himalayan horti-silvi system. Radish seeds were treated with five treatments comprised of distilled water (Control), 40, 60, 80 and 100% concentration of leaf extracts. The effect of aqueous extracts was found inhibitive with concentration dependent manner on seed germination and subsequent seedling growth. The variety exhibited extent of phytotoxicity at 100% extracts application in comparison to untreated control. Invariably there was a decrease in first count, germination, seedling root and shoot length, seedling fresh and dry weight with increasing aqueous extracts concentration on germinating radish (psusa Chetki). Present investigation shows that the tree species have allelopathic potential and contain water-soluble substances. Seed germination, seedling elongation and weights were determined on date of final count, however, other seedling vigor i.e. vigor index, speed of germination index, Relative Growth Index (RGI), Mean Daily Germination (MDG), Mean Germination Time (MGT) and time to 50% germination ( $T_{50}$ ) were calculated as per their respective formula. The significant reduction in seed germination and seedling vigor were observed of walnut leaf extracts on radish. However, MGT and  $T_{50}$  is indicated as lower value for higher vigor were increased as the leaf extract concentration increased and found significantly lowest in control for radish. It was found that seed germination and seedling vigor of radish were affected negatively by walnut leaf extracts in concentration dependent manner.

**Key words:** Allelopathy, walnut leaf extract, germination, seedling growth, radish

### INTRODUCTION

Walnut (*Juglans regia* L.), is a large deciduous tree species distributed in the Indian Central Himalaya between 1375-3350 m asl, extending in the west to Afghanistan and east to Bhutan. This tree species occurs both as wild form as well as under cultivation along the risers of the agricultural fields throughout the region. It is one of the first species to shed its leaves and becomes leafless from September to October which coincides with the sowing of important winter season crops. Presence of trees in the agro-forestry system results in the exposure of associated crops due to release of allelo-chemicals of the fallen leaves which after decomposition leaches into the soil by winter rains and snowfall. These allelo-chemicals are known to affect seed germination and seedling growth of a number of plant species (Inderjit and Mallik, 2002). Walnut toxicity is linked during the attendance of a powerful naphthoquinone, juglone (5-hydroxy-1, 4- naphthoquinone) along within living tissues, juglone is usually establish into a abridged

harmless shape but while uncovered to atmosphere it become oxidized and therefore, noxious (Rietveld, 1983). Leaves, roots and fruit hulls surround big amount of a nontoxic compound, hydrojuglone, colorless, so as to while oxidized, is transformed into the further noxious juglone (Segura-Aguilar *et al.*, 1992). Juglone inhibit plant development by reducing photosynthesis and respiration (Jose and Gillespie, 1998; Kocacaliskan and Terzi, 2001), increasing oxidative stress, reducing chlorophyll content and anatomical structures like stomata and xylem vessel (Jose and Gillespie, 1998).

In the entire central Indian Himalayan region, tree based inter cropping i.e., agri-silvi system have been in practice since ages and walnut is one of the most common trees species. This species is a source of livelihood for rural population due to its high value nut production, aesthetic qualities, rapid growth potential and adaptability to management makes this species very suitable to intercropping (Thevathasans *et al.*, 1998). In spite of the above, till date no attempt has been made to address the allelopathic effects of walnut leaf extracts on important

winter agricultural crop like radish under agri-silvi system. Therefore, the present study was undertaken to assess the effects of walnut leaf extracts on seed germination and seedling growth of radish crop.

## MATERIALS AND METHODS

**Leaf collection and obtaining extracts:** The fallen leaves of more than ten-year old trees were collected from near by area of research station. The research was conducted at Govind Ballabh Pant University of Agriculture and Technology, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India that is situated at an altitude of 2100 m asl and lies between 30°15' N latitude and 78°30' E longitude. Leaves of more than ten-year old walnut trees were used for obtaining the extract because walnut trees younger than seven-years old do not contain sufficient juglone to cause toxicity (Prataviera *et al.*, 1983; Piedrahita, 1984). Collected leaves were brought to the laboratory immediately and properly washed with distilled water for removing the soil and dust and then dried at 70°C in an oven for 24 h. After drying 100 g of crushed dried leaves were soaked in 1000 mL of distilled water for 48 h for preparing 100% concentration of stock solution. The filtrate was centrifuged and supernatant was decanted. The treatment consisted of four concentrations of aqueous leaf extracts (40, 60, 80 and 100%) while pure distilled water (0%) was taken as control.

**Experimental trials:** The seeds were surface sterilized with 1% sodium hypo chloride. Hundred seeds of each replication for every treatment were placed separately in pre sterilized Petri dishes with two fold filter paper at the bottom. The experiment was laid out in completely randomized design with four replications and 10 mL each of control and four concentrations of walnut leaf extract were added in each Petri dish on first day and 5 mL later on as and when required. The Petri dishes were placed in an incubator at a temperature of 20°C till completion of experiment. Normal seedling were recorded on date of final count for each of the respective crops and expressed as the percentage of seed which produced normal seedlings (ISTA, 1985). After the germination count, ten normal seedlings were randomly selected to measure the root length, shoot length and addition of shoot and root lengths gave seedling length. Root and shoot part of each seedling was dried separately in an oven at 85°C till a constant weight. Seedling Vigour Index (SVI) I and II were derived by multiplying% germination with seedling length and dry weight of seedlings, respectively (Abdul-Baki and Anderson, 1973). For speed of germination, when the seed has begun to germinate, checked daily and count from the test when they reach a pre-determined size till the date of

first germination to seed that were capable of producing a normal seedling. An index was computed for each treatment by dividing the number of normal seedling produces each day by corresponding day of counting. To determine the statistical difference between the treatments variance analysis and least significant difference (LSD = 0.05) tests performed following the method of Snedecor and Cochran (1989).

Relative Growth Index (RGI) was calculated as:

$$RGI = \frac{\text{First count}}{\text{Final count}} \times 100$$

(Brown and Mayer, 1986).

While Mean Germination Time (MGT) was calculated as:

$$MGT = \frac{\sum (n \times d)}{N}$$

where, n is number of seeds which germinated after each period of incubation in days d and N is total number of seeds emerged at the end of the test (Hartmann *et al.*, 1989).

While Speed of germination Index (GI) was calculated as described in the AOSA (1983) using the formula:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

However, germination was observed daily following AOSA (1990). The time to 50% germination ( $T_{50}$ ) was calculated to the following formula of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005) as under:

$$T_{50} = T_1 \frac{(N/2 - n_i)(t_j - t_i)}{n_j - n_i}$$

where, N is the final number of germination and  $n_i$  and  $n_j$  cumulative number of seeds germinated by adjacent counts at times  $t_i$  and  $t_j$  when  $n_i < N/2 < n_j$ .

While mean daily germination was calculated as:

$$MDG = \frac{\text{Final germination per cent}}{\text{Total No. of days in test}}$$

## RESULTS AND DISCUSSION

The effect of walnut leaf extracts on seed germination and seedling vigour characteristics have been presented in Table 1. There was significant decrease in the germination at first count (40.75%) and final count

Table 1: Allelopathic effect of walnut leaf extracts on seed germination and subsequent seedling growth of Radish (*Raphanus sativus* L.)

Characters							
Treatment	Germination (%) at first count	Germination (%) at final count	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
0%	40.75	73.00	8.28	5.08	13.36	1.592	0.173
40%	33.25	67.75	7.92	5.06	12.98	1.560	0.163
60%	27.75	65.50	7.62	5.04	12.66	1.530	0.160
80%	22.00	63.50	7.55	4.92	12.47	1.463	0.152
100%	14.00	49.25	6.03	4.64	10.66	1.435	0.145
GM	27.55	63.80	7.48	4.94	12.42	1.516	0.159
Sem (±)	1.80	0.40	0.02	0.02	0.03	0.014	0.005
CD (0.05)	5.45	1.20	0.06	0.06	0.09	0.045	0.014
CV (%)	13.12	1.25	0.54	0.87	0.49	1.960	5.930
Characters %							
Treatment	Vigour index I	Vigour index II	Speed of germination index	Relative growth index	Mean daily germination	Mean germination time	Time to 50% germination (T <sub>50</sub> )
0%	974.89	12.59	14.90	56.16	8.11	5.45	3.80
40%	879.55	11.01	13.08	45.60	7.47	5.69	4.40
60%	829.05	10.48	12.20	36.39	7.25	5.91	4.63
80%	791.84	9.68	11.01	27.65	7.03	6.20	4.84
100%	525.13	7.14	8.32	20.17	5.50	6.35	5.34
GM	800.09	10.17	11.90	36.99	7.07	5.92	4.60
Sem (±)	4.49	0.29	0.24	1.93	0.03	0.10	0.16
CD (0.05)	13.55	0.88	0.72	5.81	0.10	0.29	0.47
CV (%)	1.12	5.73	4.01	10.43	0.95	3.28	6.84

(73.00%) at control, however, the lowest per cent germination of first count (14.00%) and final count (73.00%) at 100% leaf extract application respectively. The result also depicted that each treatments of leaf extract concentration were differed significantly to each other with respect to germination for both first and final count. Thus there was an inhibitory effect on germination with increase in leaf extract concentration. This is in conformity with the findings of other workers (Orcutt and Nilsen, 2000). Reduction in root, shoot and seedling length across increasing concentration of walnut leaf extracts up to 100% was noticed. Each treatment of walnut leaf extract had significant negative influence on root, shoot and seedling length over control (0%), however, the result of 40% extracts concentration was at par with control for shoot length. The maximum root, shoot and seedling length 8.28, 5.08 and 13.36 cm was observed for control while, lowest value 6.03, 4.64 and 10.66 cm, respectively measured for 100% treatment. The reduction in seedling growth may be attributed to inhibitive cell division due to walnut leaf extracts. In the present study, walnut leaf extracts containing juglone significantly prevented root, shoot and as well as seedling elongation. Similar observations were also noted with juglone in tomato and bean (Neave and Dawson, 1989), wheat and corn (Jose and Gillespie, 1998), wheat (Prasad *et al.*, 2011a). An inhibitory effect was noticed in the fresh and dry weight of seedling with the increase in leaf extract concentration from control to 100% and same trend was calculated in terms of vigour index I and II (Table 1). Least fresh weight (1.435 g) was observed for 100% concentration, while

maximum seedling fresh weight of 1.592 g was observed from control treatment. The significantly maximum dry weight value of 0.173 g was recorded in untreated control, while significantly least (LSD<0.05) results (0.145 g) was observed at maximum concentration of leaf extracts (100%). Vigour index I (Germination%×seedling length) and vigour index II (Germination%×dry weight of seedling) is a real reflection of seedling vigour of seeds/seed lot which were extremely reduced as the walnut aqueous leaf extracts concentration increased. Statistically maximum value for vigour index I and II (974.89 and 12.59) were computed for untreated control over all other treatments, while least value (525.13 and 7.14) were calculated also for 100% leaf extract concentration, respectively. In several previous studies, it was determined that walnut leaf extracts decreased seed germination, seedling length along with seedling fresh and dry weight for various crops. Vigour index (I and II) is a multiple criteria of germination with seedling length and dry weight of seedling. Therefore, these indexes were markedly inhibited by the walnut leaf extract. This result is close agreement with the findings of Kocacaliskan and Terzi (2001) in water melon, tomato, garden crest and alfalfa (Prasad *et al.*, 2011b) in cauliflower.

Earlier establishment of crops in field leads to harvest greater yield in lesser period and depends on speed of germination index of seed. When we compared the impact of treatments on speed of germination of radish, the undiluted extract was found to be the most inhibitive. As the extract concentration increases, the rate of germination decreases at concentration dependent

manner. Hence, maximum rate of speed of germination index (14.89) was noticed for untreated control, which was significantly greater over all other treatments in grain amaranth. However, significantly least germination speed index (8.26) was reflected for undiluted extracts (100%) treatment.

Germination rates in terms of Relative Growth Index (RGI) and Mean Daily Germination (MDG) were significantly reduced by walnut leaf extracts containing juglone. Maximum value (56.01 and 8.11) for RGI and MDG were calculated in control (0%) treatment, while least value (20.40 and 5.47) was recorded, respectively.

The response of grain amaranth to the walnut leaf extract for earlier germination was recorded as indicated by lower values of Mean Germinations Time (MGT) and  $T_{50}$  (Table 1). A significant ( $LSD < 0.05$ ) effect of walnut leaf extracts was seen on the MGT and  $T_{50}$  and the lowest value (5.45 and 3.80) days was noted in the untreated control (0%), while maximum MGT and  $T_{50}$  (6.37 and 5.30) days was recorded to undiluted extracts (100%). However, the results with respect to MGT of 40 and 60%, along with 60, 80 and 100% did not differ significantly.

While,  $T_{50}$  in grain amaranth each treatments maintained difference significantly ( $LSD < 0.05$ ) to each other except the result of 60 and 80% treatment was statistically at par. Earlier and more uniform germination was observed in seeds for control as indicated by lesser MGT and  $T_{50}$  and higher speed of germination, RGI and MDG (Table 1). The reduced  $T_{50}$  and MGT indicated earlier and rapid germination while, higher speed of germination, RGI and MDG express the power of germination i.e., germination spread over the time. These findings support the earlier work where retard germination rate and percentage were observed following walnut leaf extracts and juglone of various plant species (Reynolds, 1987; Rietveld, 1983). The delayed and unsynchronized germination might be attributed to interfere metabolic activities in the walnut leaf extracts subjected seeds (Terzi *et al.*, 2003).

### CONCLUSION

As a conclusion, our results clearly revealed that walnut leaf extracts has inhibitory effects on radish germinating seed in a concentration dependent manner. Delayed and poor germination as well as weak seedlings attributed to walnut leaf extracts exhibited to interfere metabolic activities of germinating seed. Therefore walnut leaf phytotoxicity cannot be ruled out when examining the causes for observed reductions in germination and growth for radish with walnut intercropping. Therefore, all

the fallen leaf of walnut should be collected and removed away from the radish farm and/or farmers may be growing more tolerable crop under Western Himalayan agri-silvi system.

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