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Allelopathic Belongings of Dried Walnut Leaf on Seed Germination and Seedling Growth of Mustard (*Brassica campestris*) in Agri-silvi System of Uttarakhand Himalaya, India

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ABSTRACT

Phytotoxic effects of aqueous extracts of walnut leaf were studied on germinating seeds and early seedling growth of mustard (*Brassica campestris* cv. Karanti) under western Himalayan agri-silvi system. Five treatments comprised of distilled water (control), 40, 60, 80 and 100% concentration of leaf extracts were treated. 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 mustard. Present investigation shows that the tree species have allelopathic potential and contain water-soluble substances. They have inhibitory effects at higher concentrations but at low concentrations they have stimulatory effects. 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 mustard (*Brassica campestris*). It was found that seed germination and seedling vigor of mustard were affected negatively by walnut leaf extracts in concentration dependent manner.

Key words: Allelopathy, germination, mustard, seedling growth, walnut leaf extract

INTRODUCTION

Allelopathy refers to the chemical interactions that occur among plants and is mediated by the release of allelochemicals into the plant rhizosphere. Allelochemicals involved in this phenomenon, can be released through volatilization, leaching from leaves, degradation of plant residues and root exudation. It can be highly choosy in that they manipulate the enlargement of only one organism, or they can exhibit broad activity in influencing the growth of many species (Hale and Orcutt, 1987).

Mustard (*Brassica campestris*) is grown both in subtropical and tropical countries. In Asia, it is chiefly grown in China, India and Pakistan. India occupies the first position, both with regard to acreage and production of rape seed and mustard in the world and in India the *Brassica* crops occupy the second largest position after groundnut, with 3-5 million hectares, producing about 2 million tonnes of seed annually. The chief states producing them are Assam, Bihar, Uttar Pradesh, Haryana, Madhya Pradesh, Orissa, Punjab, Rajasthan and West Bengal (Rao, 2002). Walnut toxicity is associated with the presence of a potent napthoquinone, juglone (5-hydroxy-1, 4-napthoquinone). In living tissues, juglone is generally found in a reduced non-toxic form but when exposed to air it becomes oxidized and thus, toxic (Rietveld, 1983). Roots, leaves and fruit hulls contain large quantities of a colorless, nontoxic compound, hydrojuglone, that when oxidized, is transformed into the more toxic juglone (Segura-Aguilar *et al.*, 1992).

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 germination, seedling growth, their further development and even grain setting of a number of plant species (Inderjit and Mallik, 2002). A few studies have been done physiological action of juglone's inhibitory effect during seed germination and seedling growth (Terzi *et al.*, 2003). Juglone inhibits plant growth by reducing photosynthesis and respiration (Jose and Gillespie, 1998), increasing oxidative stress, reducing chlorophyll content and some anatomical structures such as stomata, xylem vessel (Hejl *et al.*, 1993). In North West Himalayan part of India, 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. Its high value, aesthetic qualities, capacity for nut production, rapid growth potential and adaptability to management makes the 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 certain pseudocereals under North-West Himalayan agri-silvi system. Therefore, the present study was undertaken to determine the effects of dried walnut leaf on seed germination and seedling growth of mustard.

MATERIALS AND METHODS

Collection of walnut leaves and preparation of extract: 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 (Piedrahita, 1984). Leaves were dried in open sunlight and mechanically crushed the dried leaves and made a fine powder. Later fine powder were soaked in distilled water at 25°C for 48 h and mechanically stirred for 1 h at end. The extracts thus obtained were filtered with Whatman No. 1 filter paper and stored in refrigerator until required. One hundred grams of crushed leaf powder were soaked in 1000 mL of distilled water for preparing 100% concentration of stock solution.

Experimental design: A laboratory experiment was conducted at Department of Crop Improvement, GBPUA and T, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India. Fresh fallen leaves of walnut (*Juglans regia* L.) were collected near by area of GBPUAT, Hill Campus, Ranichauri in the month of first week of October, 2010 i.e., the right time of rabi crop sowing. Treatment comprised of four concentration of aqueous leaf extract (40, 60, 80 and 100%) along with control on mustard variety karanti respectively, recommended for hills under West Himalayan Horti-silvi system. Hundred seeds of each treatment of each variety were placed separately in pre-sterilized petri-dishes with two fold filter papers at the bottom. The experiment was laid out in Complete Randomized Block Design with four replications. Twenty mL distilled water each of control and four concentration of leaf extracts were added in each Petri-dish on first day and 10 mL later or as and when required. Seeds were surface sterilized with 0.1% mercuric chloride solution. Petri-dishes were sterilized in hot air oven at 160°C prior to start the experiment. The petri-dishes were placed in an incubator at 20°C temperature. The seeds germinated were counted daily for 9 days. Root, shoot as well as seedling length and seedling fresh weight of ten randomly selected seedlings from each treatment of every replications were recorded after ten days of the start of experiment. Seedling dry weight was obtained after subjecting the 10 randomly selected seedlings in an oven at 80°C for 24 h. Vigour index I was calculated as a product of germination and seedling length, however, vigour index II was worked out by multiplying germination percent with seedling dry weight (Abdul-Baki and Anderson, 1973).

Study of crop parameters: These seeds were further utilized under laboratory experiment to predict the relative storage potential and observations recorded on first count test, standard germination, seedling root length, seedling shoot length, seedling length, seedling fresh weight, seedling dry weight, seedling vigour index I and II, speed of germination, Relative Growth Index (RGI), Mean Germination Time (MGT), time to 50% germination (T_{50}) and mean daily germination were determined following standard methods:

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

Where:

n = Number of seeds which germinated after each period of incubation in days

N = Total number of seeds emerged at the end of the test (Hartmann *et al*., 1989)

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:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - ni\right)(tj\text{-}ti)}{nj - ni}$$

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 $n_i<N/2<n_j$.

Statistical analysis: To determine the statistical difference between the treatments variance analysis and Least Significant Difference (LSD) tests performed (0.05) following the method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of walnut leaf extracts on seed germination and seedling characteristics of mustard walnut leaf extracts application to germinating seeds significantly affected germination and subsequent seedling growth in mustard. The effect of walnut leaf extracts on seed germination and seedling vigour characteristics are presented in Table 1 reveals significant differences for treatments. There was significant gradual decrease in the germination percentage at first count

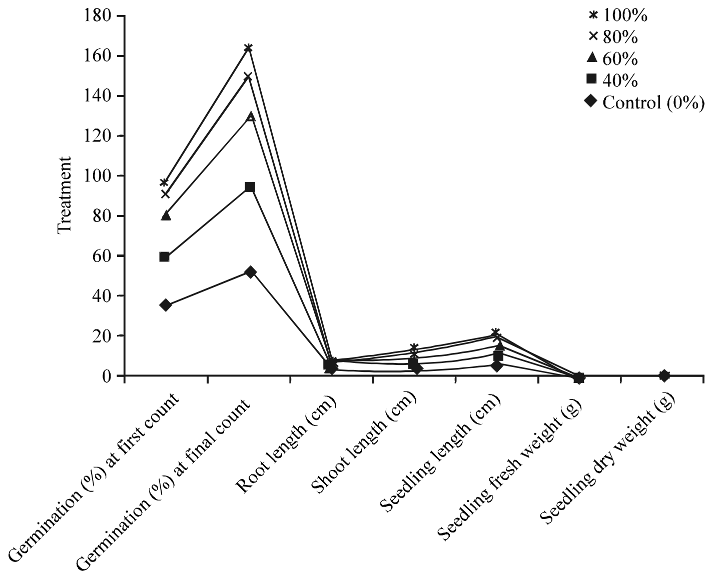

Fig. 1: Allelopathic effect of walnut leaf extracts on seed germination of mustard

Table 1: Allelopathic effect of walnut leaf extracts on seed germination of mustard

Characters	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)
Treatment							
0% (Control)	35.50	52.50	3.06	3.01	6.08	0.268	0.088
40%	25.00	41.75	1.86	2.96	4.81	0.220	0.085
60%	19.50	36.25	1.52	2.85	4.36	0.195	0.078
80%	10.50	19.75	1.25	2.46	3.71	0.180	0.075
100%	6.00	14.25	0.63	1.82	2.45	0.140	0.065
GM	19.30	32.90	1.66	2.61	4.28	0.200	0.077
Sem (±)	1.20	2.18	0.14	0.09	0.19	0.021	0.006
CD (0.05)	3.62	6.58	0.42	0.29	0.57	0.065	0.020
CV (%)	12.47	10.27	12.10	7.46	8.90	9.716	10.040

with the increase in walnut leaf extracts concentrations. The germination percentage at first and final count with the increase in walnut leaf extracts and higher value 35.50 and 52.50% was observed for control followed by 40% leaf extracts concentration 25.00 and 41.75%, however, lowest percent germination value of first and final count 6.00 and 14.25% was noted for 100% leaf extracts concentration and 100% concentration showed significant lower value over other treatments. 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 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 influence on root, shoot and seedling length over control (0%). The least seedling root, shoot and seedling length was observed in 100% treatment walnut leaf extracts treatment 0.63, 1.82 and 2.45 cm while, maximum root, shoot and seedling length was noted with control 3.06, 3.01 and 6.08 cm, respectively measured for control. 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. Kocacaliskan and Terzi (2001) demonstrated that both juglone and walnut leaf extracts inhibits germination and seedling growth of several plant species, such as wheat (Prasad *et al.*, 2011a), tomato and bean (Neave and Dawson, 1989), wheat and corn (Jose and Gillespie, 1998). 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). 100% walnut leaf extracts recorded minimum fresh weight (0.140 g) followed by 80% walnut leaf extracts (0.180 g) while maximum seedling fresh weight was found for control (0.268 g) differed significantly with other treatments. The significantly maximum dry weight value of 0.088 g was recorded in untreated control while significantly least (LSD<0.05) results (0.065 g) was observed at minimum concentration of leaf extracts (100%). Vigour index (Germination% × seedling length) and (Germination% × dry weight of seedling) is a real reflection of seedling vigour of seed/seed lot which were extremely reduced as the walnut aqueous leaf extracts concentration increased and statistically maximum value for vigour index I and II 319.36 and 4.61 were computed for untreated control over all other treatments while least value 35.02 and 0.91 were calculated also for 100% leaf extract concentration respectively. All leaf extracts concentration treatments showed significant effect over control. 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 watermelon, tomato, garden crest and alfalfa, Prasad *et al.* (2011b) in cauliflower.

The result pertaining to speed of germination, relative growth index, mean daily germination, mean germination time and time to 50% germination as evident from data in Table 1 and Fig. 1 reveals that the treatments have significant influence on the speed of germination over control. 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 mustard, the undiluted extract was found to be the most inhibitive. As the extract concentration increases, the speed of germination decreases at concentration dependent manner. Hence maximum speed of germination index (11.60) was noticed for untreated control which was significantly greater over all other treatments in mustard. However, significantly least germination speed index (2.89) was reflected for undiluted extracts (100%) treatment.

Germination rate traits in terms of Relative Growth Index (RGI) and Mean Daily Germination (MDG) were significantly reduced by walnut leaf extracts containing juglone and maximum value 67.61 and 5.83 for RGI and MDG were calculated in control (0%) treatment while least value 43.24 and 1.58 was recorded, respectively in undiluted extracts, however, the value for each and every treatment differed significantly with respect to RGI and MDG. 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). The response of mustard to the walnut leaf extract for earlier germination was recorded as indicated by lower values of Mean Germinations Time (MGT) and T_{50} (Table 2 and Fig. 2). A significant (LSD<0.05) effect of walnut leaf extracts was seen on the

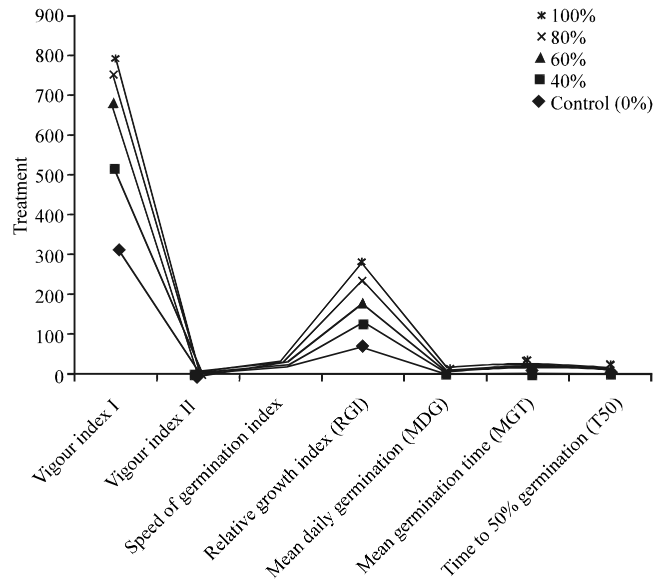

Fig. 2: Allelopathic effect of walnut leaf extracts on subequent seedling growth of mustard

Table 2: Allelopathic effect of walnut leaf extracts on subequent seedling growth of mustard

Characters	Vigour index	Vigour index II	Speed of germination index	RGI	MDG	MGT	(T_{50})
Treatment							
0 %	319.36	4.61	11.60	67.61	5.83	4.99	3.41
40 %	201.21	3.55	8.74	60.06	4.64	5.25	4.04
60 %	159.15	2.82	7.31	53.63	4.03	5.47	4.22
80 %	73.76	1.52	3.80	54.23	2.20	5.59	3.87
100 %	35.02	0.91	2.89	43.24	1.58	5.94	4.35
GM	157.69	2.67	6.86	55.75	3.65	5.44	3.97
Sem (+)	14.33	0.32	0.49	3.47	0.24	0.08	0.14
CD (0.05)	43.21	0.98	1.48	10.48	0.73	0.25	0.42
CV (%)	9.18	14.36	11.33	12.47	13.28	3.13	7.04

GM: Grand Mean, CD: Critical Difference, CV: Critical Value, Sem- SME, RGI: Relative growth index, MDG: Mean daily germination, MGT: Mean germination time

MGT and T_{50} and the lowest value 4.99 and 3.41 days was noted in the untreated control (0%) while maximum MGT and T_{50} 5.94 and 4.35 days was recorded respectively to undiluted extracts (100%) 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

The present study concluded that walnut leaf extracts has inhibitory effects on mustard 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. Although, allelopathic effects of walnut leaf extracts have been examined previously, no studies related to the effect of walnut leaf extract have been report for mustard to date. However, mustard seed exhibited extent of tolerance and might be option in walnut intercropping under West Himalaya agri-silvi system as a rabi crops. Therefore, walnut leaf phytotoxicity cannot be ruled out when examining the causes for observed reductions in germination and growth for mustard with walnut intercropping. Therefore, all the fallen leaf of walnut should be collected and removed away from the mustard farm and/or farmers may be grow more tolerable crop under West Himalayan agri-silvi system. A future study is necessary to get more detail information about walnut-mustard allelopathic relationships by using more mustard varieties.

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