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Seed Germination and Seedling Growth of Wheat and Barley Influenced by the Allelopathic Effect of Walnut (*Juglans regia* L.) Leaf Extracts under Mid Hills of Uttarakhand Agri-Silvi System

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ABSTRACT

A laboratory experiment was conducted to observe the effect of *Juglans regia* L. leaf extracts on germination and subsequent seedling growth of wheat (cv. VL-907) and barley (cv. PRB-502) under West Himalayan agri-silvi system. Eleven treatments comprised of distilled water 0 (Control), 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% concentration of leaf extracts were employed. The effect of aqueous extracts was found inhibitive indicating a direct proportional relationship with concentration dependent manner on seed germination and subsequent seedling growth of wheat. Invariably, there was a decrease in root, shoot as well as seedling length, fresh and dry weight of seedling and vigour index I and II with increasing walnut leaf extracts concentration on wheat. However, barley seed showed a considerable resistance against walnut leaf extract and no significant reduction and trend was observed for seed germination and subsequent seedling growth i.e., fresh and dry weight, vigour index I and II for different concentration of leaf extracts.

Key words: Allelopathy, walnut leaf, germination, wheat, barley

INTRODUCTION

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment, (Reigosa *et al.*, 2013; Rice, 1984). Allelochemicals are present in many types of plants and are released into the environment by a variety of mechanism including decomposition of residues, volatilizations and root exudation. These chemicals are known to affect germination, growth, development, distribution and reproduction of a number of plant species (Inderjit and Mallik, 2002). However, the effect of these chemicals on other plants are known as allelopathy to be dependent on the concentration released into the soil/environment. Allelopathy involves a plant's secretion of biochemical materials into the environment to inhibit germination or growth of surrounding vegetation. Allelopathic effect of trees on agriculture crops are well documented (Kohli *et al.*, 2000).

The inhibitory effect of walnut on associated plant species is one of the oldest examples of allelopathy which produces a non-toxic colorless chemical called hydrojuglone. Hydrojuglone is

found in leaves, stem, fruit hulls, inner bark and roots. When exposed to the air or soil compounds, hydrojuglone is oxidized into the allelochemical juglone which is highly toxic (5-OH-1, 4 naphthoquinone), an allelo-chemicals, which is present in leaves, hulls and inner root bark (Bertin *et al.*, 2003). Rain washes juglone from the leaves and carries it into the soil. Thus, neighbouring plants of the walnut are affected by absorbing juglone through their roots (Rietveld, 1983). Walnut has been reported to be toxic to both herbaceous and woody plants (Funk *et al.*, 1979). Particular in West Himalayan, tree based intercropping systems have been used and walnut (*Juglans regia* L.) is one of the most common tree used for this practice. Its high value, aesthetic qualities, capacity for nut production, rapid growth potential and adoptability to management makes the species very suitable to agri-silvi system. However, some times agricultural losses are observed due to improper selection of tree species with arable crops which some times results in negative allelopathic interactions. Hence, an attempt was made to study the allelopathic effects of walnut leaf extracts on wheat and barley seed germination and subsequent seedling growth. 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).

MATERIALS AND METHODS

A laboratory experiment was conducted at College of Forestry and Hill Agriculture, Uttarakhand University of Horticulture and Forestry, Ranichauri Campus, Tehri Garhwal, Uttarakhand, India. Fresh fallen leaves of walnut (*Juglans regia* L.) were collected near by area of College of Forestry and Hill Agriculture, Ranichauri Campus in the first week of October, 2009 i.e., the right time of rabi crop sowing. Leaves were dried in daylight and mechanically crushed the dried leaves and made a fine powder. Later, fine powder was soaked in distilled water at 25+2°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 20°C refrigerator until required. The 100 g of crushed leaf powder was soaked in 1000 mL of distilled water for preparing 100% concentration. The treatment comprised of ten concentration of aqueous leaf extract (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) while pure distilled water (0%) was taken as control on wheat and barley variety VL-907 and PRB-502, respectively recommended for hills under West Himalayan agri-silvi system. One hundred seeds of each treatment of each variety 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 (CRD) with three replications. Ten milliliter distilled water (each of control) and 10 concentration of leaf extracts were added in each petri-dish on first day and 5 mL water or as 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 8th 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 eight days of the start of experiment. 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 according to Abdul-Baki and Anderson (1973). To determine the statistical differences among the treatments, least significant difference (LSD-0.05) values were indicated.

RESULTS AND DISCUSSION

Allelopathic effect on wheat: Walnut leaf extracts application to germinating seeds significantly affected germination and subsequent seedling growth in wheat. The difference among the various treatments were found to be significant for the character studied, i.e., germination, root, shoot and seedling length, fresh and dry weight of seedling and also for vigour index I, II and relative growth index (Table 1). There was a gradual decrease in the germination percentage with the increase in leaf extracts concentration. Maximum first count and germination percentage (final count) of 60.67 and 91.00 for 0% and minimum percent first and final count i.e., 19.33 and 66.33 was observed in 100% aqueous leaf extract concentration (Table 1). Thus there was an inhibitory effect on germination with increase in leaf extract concentration. In several previous studies, the seed germination of cucumber is affected negatively in a concentration dependent manner by walnut leaf extract (Orcutt and Nilsen, 2000). Maximum root length (14.55 cm), shoot length (14.67 cm) and seedling length (29.22 cm) were also recorded in control and minimum root length (3.59 cm), shoot length (4.71 cm) and seedling length (8.31 cm) were observed in 100% aqueous leaf extract concentration. Kocacaliskan and Terzi (2001) demonstrated that walnut leaf extracts inhibits germination and seedling growth of several plant species, such as water melon, tomato, garden crest and alfalfa. Similar results were indicated in earlier studies in cucumber (Terzi, 2008). The reduction in seedling growth by walnut leaf extracts is attributed to inhibitive cell division, increasing oxidative stresses and reduced mineral uptake at higher doses of leaf extract.

Fresh and dry weight of seedling and germination along with seedling growth and dry weight are reflected in terms of vigour index I and II, respectively were influenced also negatively by extracts of walnut leaf at concentration dependent manner (Table 2). The maximum seedling fresh

Table 1: Allelopathic effect of walnut leaf extracts on wheat (cv. VL-907) and barley (cv. PRB-502) seed germination

Treatment (%)	First Count (%)		Germination (%)		Root length (cm)		Shoot length (cm)		Seedling length (cm)	
	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
0	60.67	65.00	91.00	96.00	14.55	16.25	14.67	18.63	29.22	34.88
10	55.67	65.33	89.67	96.00	13.18	16.67	13.55	19.21	26.73	35.88
20	50.33	65.67	87.67	96.67	11.96	14.78	12.07	20.26	24.04	35.03
30	46.67	65.67	86.33	96.33	10.96	15.82	11.14	20.65	22.10	36.48
40	43.00	66.00	84.33	97.00	9.86	14.88	9.06	20.45	18.93	35.33
50	39.00	65.67	82.00	96.67	7.74	15.59	8.41	20.84	16.15	36.44
60	33.33	66.00	80.33	96.33	6.83	14.93	7.66	20.53	14.49	35.45
70	31.33	64.33	79.33	96.67	6.20	16.02	6.87	19.65	13.07	35.67
80	26.67	65.00	76.67	96.00	5.37	14.92	6.36	20.09	11.73	35.01
90	23.33	64.33	73.67	95.67	4.26	14.95	5.58	20.17	9.84	35.12
100	19.33	64.33	66.33	96.00	3.59	13.29	4.71	19.17	8.31	32.46
Gm	39.03	65.21	81.57	96.30	8.59	15.24	9.09	19.96	17.69	35.25
Sem (+)	0.71	0.47	0.49	0.46	0.34	0.69	0.24	0.40	0.38	0.76
CD (0.05)	2.10	2.38	1.44	2.35	1.00	2.03	0.71	2.18	1.13	2.24
CV	3.18	2.25	1.04	1.82	6.89	7.88	4.65	8.51	3.78	3.76

Table 2: Allelopathic effect of walnut leaf extracts on wheat (cv. VL-907) and barley (cv. PRB-502) subsequent seedling growth

Treatment (%)	Seedling F.W. (g)		Seedling D.W. (g)		Vigour index				Relative growth index	
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	Wheat	Barley	Wheat	Barley	I		II		Wheat	Barley
0	3.55	4.96	0.38	1.42	2658.60	3347.95	34.89	135.96	66.66	67.71
10	3.19	5.19	0.36	1.33	2396.80	3443.51	32.28	128.01	62.09	68.05
20	3.06	5.01	0.32	1.21	2106.94	3385.94	28.05	117.04	57.42	67.93
30	2.88	4.88	0.30	1.20	1908.11	3513.72	25.90	115.95	54.06	68.17
40	2.47	4.87	0.28	1.29	1596.06	3383.91	23.61	124.80	50.99	68.04
50	2.12	5.12	0.27	1.27	1324.46	3522.07	21.87	122.45	47.56	67.93
60	2.05	5.05	0.25	1.28	1164.19	3415.51	20.35	123.62	41.50	68.51
70	1.85	4.85	0.22	1.18	1036.90	3448.10	17.72	114.10	39.49	67.55
80	1.30	4.97	0.19	1.30	899.27	3362.06	14.82	124.38	34.77	67.71
90	1.09	5.09	0.17	1.14	724.45	3359.47	12.28	109.08	31.67	67.25
100	0.96	4.96	0.14	1.20	550.55	3116.18	9.51	115.17	29.15	67.02
Gm	2.22	4.99	0.26	1.25	1487.84	3390.76	21.93	120.96	46.85	67.80
Sem (+)	0.03	0.12	0.01	0.05	30.57	68.92	0.47	5.55	0.84	0.39
CD (0.05)	0.09	0.37	0.01	0.16	9.67	10.13	1.38	6.28	2.48	2.16
CV (%)	2.63	4.51	3.31	7.98	3.55	3.52	3.71	7.95	3.13	3.01

CD: Critical difference, CV: Critical Variance, Sem: Standard Mean Error, GM: Grand Mean, F.W.: Fresh weight and D.W.: Dry weight

weight (3.55 g) and dry weight (0.38 g) were found for control (0%) and minimum fresh weight (0.96 g) and dry weight (0.14 g) observed in 100% aqueous leaf extract concentration and results of these parameters differed significantly among all other treatments. Vigour index (germination (%)×seedling length) and (germination (%)×dry weight of seedling) is a real reflection of seedling vigour of randomly selected 10 seedlings which were extremely reduced as the walnut aqueous leaf extracts concentration increased and vigour index I and II of germination with seedling length and dry weight of seedling. Therefore, these indexes were markedly inhibited by the walnut leaf extract and higher values (2658.60 and 34.89), respectively with control. While, least value (550.55 and 9.51) was calculated for 100% leaf extract concentration, respectively, however, the results among all treatments varied significantly. The gradual reduction in fresh weight of seedling as well as vigour index would be due to expected variation in phytotoxic contents i.e., juglone in different doses of leaf extract application. The reduction in seed germination and subsequent seedling growth are attributed to inhibitive physiological action to germination and further cell division at concentration dependent manner of walnut leaf extracts. The results agree with the findings of Kocacaliskan and Terzi (2001) in watermelon, tomato, garden cress and alfalfa.

Relative Growth Index (RGI) was significantly reduced by walnut leaf extracts and maximum value (66.66) for RGI were calculated in control (0%) Treatment while, least value (29.15) was recorded, respectively in undiluted extracts for wheat variety, however, the value for each and every treatment differed significantly with respect to RGI except the value was at par with 60 and 70% leaf extract concentration. Earlier and more uniform germination was observed in seeds for control as indicated by higher RGI and it express the power of germination i.e., germination spread over the time. These findings support the earlier work where retard germination rate and total germination were observed following walnut leaf extracts and juglone of various plant species (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; Willis, 2000).

Allelopathic effect on barley: No significant effect of walnut leaf extract on the percentage of barley seed germination at first count and at final count was recorded (Table 1). However, the subsequent seedling growth i.e., root, shoot and seedling length of barley were also not significantly differed with any concentration of walnut leaf extract treatment except the statistically lowest value 13.29 and 32.46 cm was observed for root and total seedling length, respectively with regards to 100% leaf extract concentration. The maximum root length of 16.67 cm for 10% aqueous leaf extract concentration while shoot length was found maximum 20.84 cm for 50% leaf extract concentration. Meanwhile, maximum seedling elongation 36.48 cm was measured for 30% leaf extract concentration. These results are also depicted close confirmation with findings of Terzi *et al.* (2003) in muskmelon.

The result of fresh and dry weight of seedlings across the different treatment of walnut leaf extract was found to be statistically similar, while the maximum (5.19 g) and minimum fresh weight (4.85 g) weighed for 10 and 70% leaf extract concentration treatment. The fact that barley seed germination and subsequent seedling growth i.e., root, shoot and seedling length, along with fresh and dry weight of seedling were not influenced by toxic effects of juglone indicates that there may be mechanisms for tolerance to juglone in barley seed coat. Some plants appear to have a protective capacity against oxidative stress from juglone by emitting enzymes that metabolize the compound to less toxic hydrojuglone (Segura-Aguilar *et al.*, 1992; Matvienko *et al.*, 2001; Terzi, 2008). With regards to vigour I and II (Table 2), the significantly maximum vigour index I (3522.07) was calculated for 50% aqueous leaf extract concentration and result at par with 30% treatment (3513.72) while non significant lowest value (3116.18) was noticed for 100% treatment. However, the product of seed germination and dry weight of seedlings i.e., vigour index II was computed significantly maximum (135.96) for untreated control while lowest index (109.08) was recorded for 90% aqueous leaf extract concentration. Table 1 also exhibited that no significant difference value with respect to relative growth index was observed across the different treatment of walnut leaf extracts and results were statistically similar to each other. Thus, there was as stimulatory effect on the product of percent germination and seedling length i.e., vigour index I upto 50% aqueous leaf extract, Rice (1984) also stated that allelochemicals in low concentration can stimulated the growth of certain plants. In previous studies, juglone's allelopathic effects on plants are generally toxic and beneficial on some cases (Whittaker and Feeny, 1971; Hale and Orcutt, 1987; Rizvi and Rizvi, 1992).

CONCLUSION

Our results revealed that the different concentration of aqueous leaf of walnut had inhibitory effects on seed germination, subsequent seedling growth along with growth indices at concentration dependent manner on wheat seeds, however, barley seeds showed an extent of tolerance with respect to germination and subsequent seedling growth against the walnut juglone and right be option in walnut intercropping under West Himalayan agri-silvi system as a rabi cereal.

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