Environment and Ecology 40 (2) :294—299, April—June 2022
ISSN 0970-0420

Seed Germination and Seedling Growth of Cabbage (*Brassica oleracea* varta Capital) and Broccoli (*Brassica oleracea* var Italica) Influenced by the Allelopathic Effect of Walnut (*Juglans regia* L). Leaf Aqueous Extracts under Mid Hills of Uttarakhand Horti-Silvi System

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Received 3 January 2022, Accepted 19 February 2022, Published on 14 April 2022

ABSTRACT

Effect of different doses of aqueous extracts of walnut leaf was studied on germinating seeds and early seedling growth of cabbage (cv varun) and broccoli (cv. F1 Hybrid) recommended for mid hills under West Himalayan horti-silvi system. Eight treatments comprised of distilled water control (0%), 10%, 20%, 30%, 40%, 50%, 60% and 70% concentration of leaf extracts were treated. The effect of aqueous extracts was found inhibitive, indicate a direct proportional relationship with concentration dependent manner on seed germination and subsequent seedling growth of cabbage and broccoli. The cabbage and broccoli varieties exhibited extent of phytotoxicity at 70% extracts application in comparison to untreated control. Invariably there was a decrease in first count, germination, seedling root and shoot length, seedling fresh weight, dry weight, vigour index I and II with increasing aqueous extracts concentration on germinating cabbage and broccoli.

Keywords Allelopathic, Germination, Growth, Walnut leaf, Cabbage, Broccoli.

INTRODUCTION

Walnut (*Juglans regia* L.) is a large deciduous fruit tree with long fragrant leaves, distributed in the Himalayas between 1375-3350 m. asl, extending in the west to Afganistan and east to Bhutan (Bahuguna *et al.* 2013). In the Himalayas, the walnut is one of the first species to lose its leaves, tree becoming leafless from September to October i.e. the right time of *rabi* crop sowing in hills (Dua *et al*. 2007). Presence of trees in horti/agri-silvi system results in direct exposure of associated crop to continuous release of chemicals. These chemicals influence local environment, germination and growth of plants. The effects of these chemicals on other plants are known as allelopathy to be dependent on the concentration released into the soil / environment (Tharayil *et al*. 2006, Tharayil *et al*. 2008, Kaur *et al.* 2009). Allelopathy involves

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a plant's secretion of biochemical materials into the environment to inhibit germination or growth of surrounding vegetation. Allelopathic effects of trees on agriculture crops are well documented. (Tripathi *et al.*1996; Kohli *et al.* 2000). Allelochemical released by trees inhibit seed germination (Singh and Bawa 1982), reduce plant growth through inhibitive cell division (Baker 1996), reduce mineral uptake, increase or decrease respiration, inhibit protein and haemoglobin synthesis (Rice 1974). Walnut are often found growing on landscape site and when certain other landscape plants are planted near or under this shade tree, they tend to retard germination, yellow, wilt and die. The chemical responsible for walnut allelopathy is juglone (Willis 2000).

A colourless nontoxic reduced form called harmless hydro-juglone is abundant, especially in leaves, fruit hulls, stem and roots of walnut. When exposed to air or oxidizing substances, hydrojuglone is oxidized to its toxic form, juglone (Dana and Lerner 1990; 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). In previous studies, effects of juglone and walnut leaf extracts on various plant species were investigated (Einhelling, 1986, Hejl *et al*. 1993, Kocacaliskan, Terzi 2001). Therefore, it is important to select tolerant varieties of cabbage and broccoli for planting in areas adjacent to walnut trees. To the best of our knowledge, no research has been reported on the effect of walnut leaf extracts on germinating cabbage and broccoli. It is hypothesized that in a walnut based horti-silvi inter cropping system, juglone released from walnut trees could be an inhibitor to the germination and subsequent seedling growth of cabbage and broccoli close to the walnut trees. Thus, the objective of this work was to test this hypothesis in a laboratory experiment.

MATERIALS AND METHOD

Leaves of more than ten years old walnut trees were used in obtaining the extracts because walnut trees younger than seven years old do not contain sufficient juglone to cause toxicity (Prataviera *et al.* 1983, Piedrahita 1984). The leaves of walnut were collected in the second week of August 2021, since the juglone content of walnut was found to be highest in the last week of July and the first week of August (Tekintas *et al.* 1988) and dried the leaves at 70^0C in an oven for 48 hrs. Later mechanically crushed the dried leaves and made fine powder then soaking in distilled water at room temperature for 48 hrs and mechanically stirred for one hour at end. The extracts thus obtained were filtered through Whatman No. 1 filter paper and stored in refrigerator until required. 100 g of crushed leaf powder were soaked in 1000 ml of distilled water for preparing 100% concentration of stock solution. Treatment consisted of seven concentration of aqueous leaf extract (10, 20, 30, 40, 50, 60 and 70 %) along with control on cabbage (cv varun) and broccoli (cv F1 Hybrid) recommended for hills under west Himalayan horti-silvi system. 50 seeds of each treatment were placed separately in pre-sterilized petridishes with two fold filter paper at the bottom. The experiment was laid out in CRD with four replications. 10 ml distilled water each of control and seven concentration of leaf leachate were added in each petridishes on first day and 5 ml later or as and when required. Seeds were surface sterilized with 0.1% mercuric chloride solution. Petridishes were sterilized in hot air oven at 160^0C prior to start the experiment. The petridishes were placed in an incubator at a temperature of 20^0C. The first count and seeds germinated were counted daily for 4 and 8 days respectively. Root length, shoot length and seedling fresh weights of ten randomly selected seedlings from each treatment were recorded after ten days of the start of experiment (Bahuguna *et al*. 2013). Seedling dry weight was measured after subjecting the samples in an air oven at 80 ^{0}C for 24 hrs and attained constant weight for three consecutive readings. Vigour index I was calculated as a product of germination and seedling length, however, vigour index II was worked out by multiplying germination per cent with seedling dry weight (Abdul-Baki and Anderson 1973).

RESULTS AND DISCUSSION

A. cabbage (*Brassica oleracea* var. Capitata)

The effect of walnut leaf extracts on seed germination and seedling vigour characteristics are presented in Table 1. There was significant gradual decrease in the germination percentage at first and final count

Table 1. Seed germination and seedling growth of cabbage (*Brassica oleracea* var Capitata) and broccoli (*Brassica oleracea* var Italica) influenced by the allelopathic effect of walnut (*Juglans regia* L.) leaf aqueous extracts under mid hills of Uttarakhand horti-silvi system. CD- Critical deference; CV- Critical variance; Sem- Stander mean frror.

Treatments	First count (%)	Germination/ final count (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Vigour index I	Vigour index II	Relative growth index (RGI)
Cabbage (*Brassica oleracea* var Capitata)										
0%	68.50	97.50	15.32	15.47	30.79	2.68	0.42	3002.03	40.95	70.26
10%	61.50	94.25	13.67	13.72	27.39	2.42	0.37	2581.51	34.87	65.25
20%	55.25	91.25	11.92	12.07	23.99	2.4	0.29	2189.09	26.46	60.55
30%	47.75	90.50	10.61	11.26	21.87	2.28	0.23	1979.24	20.82	52.76
40%	38.75	82.50	8.45	9.02	17.47	1.68	0.21	1441.28	17.33	46.97
50%	30.50	73.75	5.81	7.83	13.64	1.46	0.17	1005.95	12.54	41.36
60%	21.00	67.00	3.64	4.94	8.58	0.92	0.14	574.86	9.38	31.34
70%	18.50	64.75	2.21	2.95	5.16	0.67	0.09	334.11	5.83	28.57
CD@5%	3.83	2.70	1.23	0.96	1.94	0.63	0.11	186.63	13.97	3.69
CV	4.85	1.61	6.41	5.37	5.64	8.81	11.83	5.69	10.61	4.78
Sem	1.61	0.99	0.47	0.33	0.69	0.27	0.04	59.28	4.65	1.36
Gm	42.72	82.69	8.95	9.66	18.61	1.81	0.24	1638.51	21.02	49.63
Broccoli (*Brassica oleracea* var Italica)										
0%	65.00	96.75	15.57	16.08	31.65	2.29	0.49	3062.14	47.41	67.18
10%	58.75	93.50	12.93	13.34	26.27	2.03	0.44	2456.25	41.14	62.83
20%	52.75	90.50	11.18	11.60	22.78	2.01	0.35	2061.59	31.68	58.29
30%	44.25	89.75	9.87	10.90	20.77	2.09	0.28	1864.11	25.13	49.30
40%	35.00	81.75	8.71	8.67	17.38	1.49	0.26	1420.82	21.26	42.81
50%	27.75	72.25	5.07	7.49	12.56	1.27	0.22	907.46	15.90	38.41
60%	18.25	66.25	2.90	4.67	7.57	1.03	0.18	501.51	11.93	27.55
70%	15.75	63.50	2.47	2.63	5.10	0.88	0.13	323.85	8.26	24.80
CD@5%	3.20	2.07	1.11	0.89	1.89	0.59	0.14	181.33	13.38	3.88
CV	4.38	1.58	6.17	5.11	5.46	8.54	11.37	5.06	10.73	4.12
Sem	1.06	0.82	0.33	0.27	0.60	0.19	0.04	59.67	4.51	1.28
Gm	39.69	81.78	8.59	9.42	18.01	1.64	0.29	1574.71	25.34	46.40

with the increase in walnut leaf extracts and higher value (68.50 and 97.50 %) was observed for control, while application of 70% leaf extract resulted significantly lowest per cent germination (18.50 and 64.75 %) 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 Orcutt and Nilsen (2000). Reduction in root, shoot and seedling length across increasing concentration of walnut leaf extracts up to 70% was noticed. Each treatment of walnut leaf extract had significant influence on root, shoot and seedling length over control (0%). The maximum root, shoot and seedling length of 15.32, 15.47 and 30.79 cm was observed for control, while lowest value of 2.21, 2.95 and 5.16 cm was measured for 70% treatment respectively. 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 inhibit germination and seedling growth of several plant species such as watermelon, tomato, garden crest and cucumber (Tekintas *et al.* 1988), for tomato and bean (Neave and Dawson 1989), wheat and barley (Prasad *et al*. 2013), wheat (Bahuguna *et al*.2013 Prasad *et al.* 2011). An inhibitory effect was noticed in the fresh and dry weight of seedling

with the increase in leaf extract concentration from control to 70% and same trend was calculated in terms of vigour index I and II (Table 1). Least fresh weight (0.67 g) was observed for 70% concentration, while maximum seedling fresh weight of 2.68 g was observed from control treatment. The significantly maximum dry weight value of 0.42 g was recorded in untreated control, while significantly least (LSD <0.05) results (0.09 g) was observed at minimum concentration of leaf extracts (70%). 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 (3002.03 and 40.95) was computed for untreated control over all other treatments, while least value (334.11 and 5.83) was calculated also for 70% 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 in close agreement with the findings of Kocacaliskan and Terzi (2001) in watermelon, tomato, garden crest and alfalfa, Prasad *et al*. (2011) in cauliflower, radish (Bahuguna *et al.* 2013).

Germination rate traits in terms of relative growth index (RGI) was significantly reduced by walnut leaf extracts containing juglone and maximum value (70.26) for RGI were calculated in control (0%) treatment, while least value (28.57) was recorded respectively in undiluted extracts, however, the value for each and every treatment differed significantly with respect to RGI. RGI 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 (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).

B. Broccoli (*Brassica Oliracea* Var. Italica)

The effect of walnut leaf extracts on seed germination and seedling vigour characteristics are presented in Table1. There was significant gradual decrease in the germination percentage at first and final count with the increase in walnut leaf extracts and higher value (65.00 and 96.75%) was observed for control, while application of 70% leaf extract resulted significantly lowest per cent germination (15.75 and 63.50%) 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 Orcutt and Nilsen (2000). Reduction in root, shoot and seedling length across increasing concentration of walnut leaf extracts up to 70% was noticed. Each treatment of walnut leaf extract had significant influence on root, shoot and seedling length over control (0%). The maximum root, shoot and seedling length of 15.57, 16.08 and 31.65 cm was observed for control, while lowest value of 2.47, 2.63 and 5.10 cm was measured for 70% treatment respectively. 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 inhibit germination and seedling growth of several plant species such as watermelon, tomato, garden crest and cucumber (Tekintas *et al.*1988), for tomato and bean (Neave and Dawson 1989), wheat and barley (Prasad *et al.* 2013), wheat (Bahuguna et al., 2013 and Prasad *et al.* 2011), radish (Bahuguna *et al*. 2013). An inhibitory effect was noticed in the fresh and dry weight of seedling with the increase in leaf extract concentration from control to 70% and same trend was calculated in terms of vigour index I and II (Table 1). Least fresh weight (0.88 g) was observed for 70% concentration, while maximum seedling fresh weight of 2.29 g was observed from control treatment. The significantly maximum dry weight value of 0.49 g was recorded in untreated control, while significantly least (LSD <0.05) results (0.13 g) was observed at minimum concentration of

leaf extracts (70%). 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 (3062.14 and 47.41) was computed for untreated control over all other treatments, while least value (323.85 and 8.26) was calculated also for 70% 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 in close agreement with the findings of Kocacaliskan and Terzi (2001) in watermelon, tomato, garden crest and alfalfa, Prasad *et al*. (2011) in cauliflower.

Germination rate traits in terms of relative growth index (RGI) was significantly reduced by walnut leaf extracts containing juglone and maximum value (67.18) for RGI were calculated in control (0%) treatment, while least value (24.80) was recorded respectively in undiluted extracts, however, the value for each and every treatment differed significantly with respect to RGI. RGI 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 (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 aqueous leaf extracts of walnut had inhibitory effects on germinating cabbage and broccoli. Although allelopathic effects of walnut leaf extracts have been examined previously, no studies related to the effect of walnut leaf extract have been reported for cabbage and broccoli to date. However, cabbage and broccoli seed exhibited extent of tolerance and might be option in walnut intercropping under West Himalaya horti-silvi system as a *rabi* vegetable and cereal. Therefore, walnut leaf extracts phytotoxicity cannot be ruled out when examining the cause for observed reduction in seed germination and growth in cabbage and broccoli under walnut intercropping horti-silvi system. A future study is necessary to get more detail information about walnut- cabbage and broccoli allelopathic relationships by using more cabbage and broccoli varieties.

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