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Allelopathic effect of walnut leaf extracts on germinating grain amaranth (*Amaranthus hypocondriacus*) seeds in western Himalayan agri-silviculture system

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ABSTRACT The effects of walnut leaf extracts on grain amaranth (*Amaranthus hypocondriacus*) cv Prai seed germination and post-germination seedling growth were investigated. Walnut leaf extract was prepared by keeping the crushed dried leaves in distilled water. Seed germination, seedling elongation and weight were determined on the date of final count, however, other seedling vigour *i.e.* vigour 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 formulae. There was a significant reduction in seed germination and seedling vigour as an effect of treatment of walnut leaf extracts on grain amaranth. However, MGT and T_{50} increased as the leaf extract concentration increased and was found to be lowest in the control. It was found that seed germination and seedling vigour of grain amaranth were affected negatively by walnut leaf extracts in concentration dependent manner.

Key words: Allelopathy, walnut leaf extract, germination, seedling growth, grain amaranth

Grain amaranth (*Amaranthus hypocondriacus*) is an important pseudocereal grown extensively in mid and high hill areas. In high hill, it is mostly cultivated as a pure crop and it serves as bread and butter for the Garhwal Himalayan peasants. It has multiple utility and is grown both for grains and green leaves. The grains possess superior nutritional quality as compared to cereals. It is raised on marginal lands with little inputs thus offering great promise for an ecologically sustainable agriculture. Being a C_4 plant, grain amaranth is extremely energy-efficient.

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, it is toxic [1]. Roots, leaves and fruit hulls contain large quantities of a colourless, nontoxic compound, hydrojuglone, that when oxidized, is transformed into more toxic juglone [2]. The allelochemicals are known to affect germination, seedling growth, their further development and even grain setting of a number of plant species [3]. A few studies have been done on physiological action of juglone's inhibitory effect during seed germination and seedling growth [4]. Juglone inhibits plant growth by reducing photosynthesis and respiration [5 and 6], increasing oxidative stress, reducing chlorophyll content and some anatomical structures such as stomata, xylem vessel [7]. In north-west Himalayan part of India, tree-based intercropping *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 for intercropping [8]. 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, present study was undertaken to determine the effects of walnut leaf extracts on seed germination and seedling growth of grain amaranth.

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MATERIALS AND METHODS

Naturally fallen leaves of more than 10-years old trees were collected from the geographical location falling between 30°15′ north latitude and 78°30′ east longitude and at an altitude of 2,100 m asl from nearby area of Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand. The walnut trees younger than 7-years-old do not contain sufficient juglone to cause toxicity [10]. The leaves were collected in September 2011, since juglone content of walnut was found highest in matured leaves. The leaves were properly washed with distilled water for removing soil and dust, and then dried at 70°C in an oven for 24 hr. After drying, 100 g of crushed dried leaves were soaked in 1,000 ml of distilled water for 48 hr for preparing 100% concentration of stock solution. The filtrate was centrifuged and supernatant was decanted. The treatment consisted of five concentrations of aqueous leaf extracts (20, 40, 60, 80 and 100%).

The seeds were surface sterilized with 1% sodium hypochlorite. Hundred seeds of each replication of every treatment were placed separately in pre-sterilized Petri-dishes with two-fold filter paper at the bottom. The experiment was laid out in a completely randomized design with four replications. The 10 ml each of the control and five concentrations of walnut leaf extract were added in each Petri-dish on first day and 5 ml later on as and when required. The germination test was conducted as per ISTA [9] procedure. After germination count, 10 normal seedlings were randomly selected to measure root, shoot, and seedling length, fresh and dry weight. Seedling vigour index (SVI) I and II were derived by multiplying % germination with seedling length and dry weight of seedlings, respectively [12]. For speed of germination, an index was computed for each treatment by dividing number of normal seedlings produced each day by the corresponding day of counting. Relative Growth Index (RGI) was calculated as: RGI = (First count/final count) x 100 [13]. Mean daily germination (MDG) was derived as the total number of seeds germinated divided by number of days to complete germination. Mean germination time (MGT) was calculated as:

$$MGT = \Sigma (n \times d)/N$$

where n = number of seeds which germinated after each period of incubation in days d, and N = total number of seeds that emerged at the end of the test [10].

The time to 50% germination (T_{50}) was calculated using the following formula of [15] modified by [16] as under:

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

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 $n_i < N/2 < n_j$.

To determine statistical difference between treatments, variance analysis and least significant difference (LSD) tests were performed (0.05) [17].

RESULTS AND DISCUSSION

The effect of walnut leaf extracts on seed germination and seedling vigour characteristics have been presented in table 1. There was a significant gradual decrease in germination at first and final count with increase in concentration of walnut leaf extracts. Highest value (31.50 and 62.75 %) was observed for the control, whereas application of 100% leaf extract resulted in lowest germination (2.75 and 13.00%). The result also depicted that each treatments of leaf extract concentration differed significantly from 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 was in conformity with the findings of Prasad *et al.* [18]. 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 effect on root, shoot and seedling length over the control (0%). However, results of 20% extract concentration were on par with the control, for shoot length. The maximum root, shoot and seedling length (4.49, 3.02 & 7.51 cm) was observed for the control, whereas lowest value (2.23, 1.36 and 3.71 cm) was measured for

Table 1. Allelopathic effect of walnut leaf extracts on seed germination and subsequent seedling growth of grain amaranth

Treatment	Germi-narion (%) at first count	Germi-narion (%) at 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	Speed of germi-nation index	Relative growth index (RGI)	Mean daily germi-nation (MDG)	Mean germi-nation time (MGT)	Time to 50% germi-nation (T_{50})
0%	31.50	62.75	4.49	3.02	7.51	0.160	0.063	471.17	3.92	12.46	50.20	7.18	5.59	4.03
20%	20.50	47.25	3.29	2.87	6.16	0.133	0.045	290.61	2.13	8.59	43.34	5.25	5.91	4.27
40%	13.75	36.50	3.26	1.50	4.75	0.125	0.040	173.40	1.47	6.35	37.64	4.06	6.12	4.59
60%	7.25	23.00	2.48	1.40	3.87	0.100	0.028	89.07	0.65	3.91	30.53	2.56	6.26	5.08
80%	5.00	18.75	2.46	1.37	3.82	0.095	0.025	71.55	0.47	3.05	26.65	2.15	6.43	5.16
100%	2.75	13.00	2.35	1.36	3.71	0.093	0.022	48.20	0.29	2.07	21.07	1.58	6.54	5.71
GM	13.45	33.54	3.05	1.91	4.96	0.117	0.037	90.66	1.48	6.07	34.90	3.79	6.13	4.80
SEm (±)	1.05	0.96	0.09	0.08	0.09	0.004	0.002	6.38	0.11	0.18	0.96	0.11	0.10	0.05
CD (0.05)	2.13	2.87	0.27	0.25	0.29	0.012	0.008	18.96	0.34	0.55	2.86	0.33	0.31	0.16
CV (%)	15.68	5.76	5.98	8.82	3.97	7.370	15.889	6.69	15.83	6.10	5.53	5.87	3.44	2.36

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 as well as seedling elongation. Similar results were also observed with juglone in cucumber [19], tomato and bean [20], corn [6] and wheat [21]. 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 (0.093 g) was observed for 100% concentration, whereas maximum seedling fresh weight of 0.160 g was observed for the control treatment. The maximum dry weight value of 0.063 g was recorded for the control, whereas least (LSD<0.05) value (0.022 g) was observed for maximum concentration of leaf extracts (100%). Vigour indices (germination % x seedling length) and (germination % x 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. Maximum values for vigour index I and II (471.17 and 3.92) were computed for the control, whereas least value (48.20 and 0.29) were calculated for 100% leaf extract concentration. In previous studies, it was determined that walnut leaf extracts decreased seed germination and seedling length along with seedling fresh and dry weight for various crops. This result was in close agreement with the findings in watermelon, tomato, garden crest, alfalfa, [22] and in cauliflower [18].

Early establishment of crops in

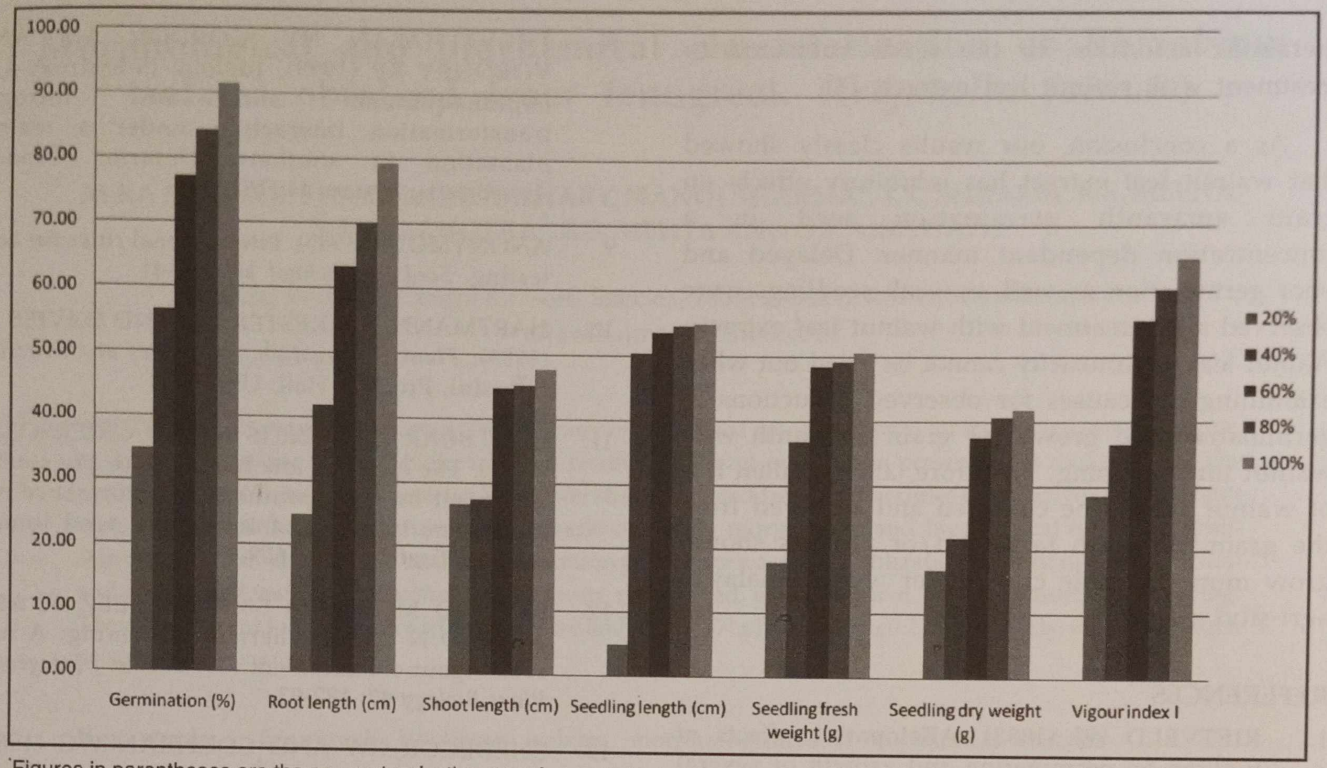

Fig. 1. Allelopathic effect of walnut leaf extracts on seed germination and seeling growth of grain amaranth

field leads to more yield in lesser duration and depends on speed of germination index of seed. With reference to effect of treatments on rate of germination of grain amaranth, undiluted extract was found to be the most inhibitive. As the extract concentration increases, the speed of germination decreases. Hence maximum speed of germination index (12.46) was noticed for the control, which was significantly more over all other treatments in grain amaranth. However, significantly least germination speed index (2.07) 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 (50.20 and 7.18) for RGI and MDG were calculated in the control (0%) treatment, whereas least value (21.07 and 1.58) was recorded in undiluted extracts. However, value for each treatment differed significantly with respect to RGI and MDG.

The response of grain amaranth to walnut leaf extract, for early germination, was recorded as indicated by lower values of mean germination time (MGT) and T_{50} (Table 1). A significant (LSD<0.05) effect of walnut leaf extracts was seen on MGT and T_{50} and lowest value (5.59 and 4.03 days) was recorded in the control (0%), whereas maximum MGT and T_{50} (6.54 and 5.71 days) was recorded for undiluted extracts (100%). However, results with respect to MGT of 20 and 40, 40 and 60, along with 60, 80 and 100% did not differ significantly. In case of T_{50}, each treatment differed significantly (LSD<0.05) from each other, except 60 and 80% treatment, which were statistically at par. Early and more uniform germination was observed in seeds for the 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 early and rapid germination, whereas RGI and MDG expressed germination spread over the time. These findings support the earlier work where lower germination rate and percentage were observed following treatment with walnut leaf extracts and juglone, for various plant species [19 and 1]. The delayed and unsynchronized germination might be attributed to interference in

metabolic activities, in the seeds subjected to treatment with walnut leaf extracts [4].

As a conclusion, our results clearly showed that walnut leaf extract has inhibitory effects on grain amaranth germination seed in a concentration dependent manner. Delayed and poor germination as well as weak seedlings were observed after treatment with walnut leaf extracts. Walnut leaf phytotoxicity cannot be ruled out when examining the causes for observed reductions in germination and growth of grain amaranth with walnut intercropping. Therefore, all the fallen leaf of walnut should be collected and removed from the grain amaranth farm and/or farmers should grow more tolerable crop under west Himalayan agri-silvi system.

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