Journal of Crop and Weed 8(1):12-16 (2012)

Seed germination and seedling growth of rice bean and grain amaranth as influenced by allelopathic effect of walnut [*Juglans regia* L.] leaf extracts under west Himalayan *agri-silvi* system

B. PRASAD, A. BAHUGUNA, S. TRIPATHI AND R. S. BALI

College of Forestry and Hill Agriculture
GB Pant University of Agriculture and Technology,
Hill Campus, Ranichauri- 249199, Tehri Garhwal, Uttarakhand

Received: 11.11.2012, Revised: 24.04.2012, Accepted : 21.05.2012

ABSTRACT

A laboratory experiment was conducted to observe the effect of Juglans regia L. leaf extracts on germination and subsequent seedling growth of ricebean (cv. PRR 1) and grain amaranth (cv. PRA 3) under West Himalayan agri-silvi system. Seven treatments comprised of distilled water (Control = 0), 2, 4, 6, 8, 10 and 12 g of leaf extracts per litre of distilled water 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 grain amaranth. 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 grain amaranth. However, ricebean seed showed a considerable tolerance against walnut leaf extract and no significant reduction was observed for germination up to 80% leaf extract concentration, while subsequent seedling growth i.e. root, shoot and seedling length, seedling fresh and dry weight along with vigour index I and II were inhibited on concentration dependent manner of walnut leaf extracts.

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

Walnut [*Juglans regia* L.], is a large deciduous tree distributed in the Indian Central Himalaya between 1375-3350 msl, extending in the west to Afghanistan and east to Bhutan. It occurs both as wild form as well as under cultivation along the risers of the field in western Himalayan traditional agri-silvicultural system. 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 Malik, 2002). The inhibitory effect of walnut on associated plant species is one of the allelopathy (Davis, 1928). The chemical responsible for walnut allelopathy is '*juglone*' (5-hydroxy-1, 4 naphthoquinone) (RietVeld, 1983; Jose, 2002). Hydro *juglone* is found in leaves, stem, hulls, inner bark and roots. When exposed to air or soil, compounds hydro *juglone* is oxidized into the allelochemical Juglone, which is highly toxic (Bertin *et al.*, 2003). *Juglone* can affect other plants either through root contact, leakage or decay in the soil, falling leaves, or when rain leaches and drips Juglone from leaves and branches on the plants (Appleton and Berrier, 2000). Small amounts may be injurious to sensitive plants turn brown, wilt and die. Walnut roots can extent Juglone in the soil well beyond the crown or drip line of the tree, affecting susceptible plants. A few studies have been done on 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 (Kocacaliskan and Terzi, 2001), increasing oxidative stress (Segura-Aguilar *et al.*, 1992), reducing chlorophyll content and some anatomical structures such as stomata, xylem vessel (Jose and Gillespie, 1998). 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 tree species. Its high value, aesthetic qualities, capacity for nut production, rapid growth potential and adaptability to management makes the species very suitable to intercropping (Thevathasan *et al.*, 1999). However, sometimes agricultural losses are observed due to improper selection of tree species with arable crops, which sometimes results in negative allelopathic interactions. In spite these, till date no attempt has been made to address the allelopathic effects of walnut leaf extracts on certain underutilized crops under North-West Himalayan agri-silvi system.

Therefore, the present study was carried out with the main aim to examine the allelopathic effects of walnut leaf extracts on seed germination and subsequent seedling growth of rice bean and grain amaranth.

MATERIALS AND METHODS

Naturally fallen leaves of more than ten-years old trees were collected in between $30^0$15' North latitude and $78^0$30' East longitude and at an altitude of 2100 m MSL from nearby area of GBPUA&T, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India under North-West Himalayan agri-silvi system. Leaves of more than ten-

Email: prasadbsst@gmail.com

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). The leaves were collected in the second fort night of September, 2011, since the *juglone* contents of walnut was found to be highest in the matured leaves. Collected leaves were properly washed with distilled water for removing the soil and dust, and then dried at 70^0C in an oven for 24 hrs. Dried leaves were crushed and made in powder form. Crushed dried leaves of 2, 4, 6, 8, 10 and 12g were soaked in 1000 ml of distilled water for 48 h to prepare aqueous leaf extracts of 2, 4, 6, 8, 10 and 12%. The filtrate was centrifuged and supernatant was decanted. Seeds of two underutilized crops *viz.*, ricebean (cv. PRR 1) and grain amaranth (cv. PRA 3) were procured from Department of Crop Improvement of the University.

Hundred seeds of each treatment of each crop were placed separately in pre-sterilized petri-dishes with two fold filter paper at the bottom. Seeds were surface sterilized with 0.1% mercuric chloride solution. Petri-dishes were sterilized in hot air oven at 160^0C prior to start the experiment. The experiment was laid out in CRD with four replications. Ten ml distilled water each of control and six concentration of leaf extracts were added in each Petri-dish on first day and 5 ml later or as and when required. The petri-dishes were placed in an incubator at 25^0C temperature and the seeds germinated were counted daily on 8^{th} day for both crops. 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. Seedling dry weight was obtained after subjecting the 10 randomly selected seedlings in an oven at 80^0C 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 per cent with seedling dry weight (Abdul-Baki and Anderson, 1973). To determine the statistical difference between the treatments, variance analysis and least significant difference (LSD=0.05) tests were performed following the method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Allelopathic effect on rice bean

Results revealed that walnut leaf extracts application to germinating seeds did not significantly affect germination upto 80% concentration level, while the influence on seed germination for 100% and 120% concentration was found significantly negative, however, subsequent seedling growth also exhibited significant negative response for rice bean. The difference among the various treatments was found to be significant for the characters studied, *i.e.* root, shoot and seedling length, fresh and dry weight of seedling and also for vigour index I, II (Table 1). There was a non significant gradual decrease in the germination percentage with the increase in leaf extract concentrations. Maximum germination of 96% was found for control and minimum 90.33 per cent germination was observed for 120% aqueous leaf extract concentration. These results are also similar with the findings of Terzi *et al.* (2003) in muskmelon and Prasad and Prasad (2010) in barley. The root, shoot and seedling length were also negatively influenced by toxic effects of juglone with concentration dependent manner. The maximum root, shoot and seedling length (5.09, 4.82 and 9.91) cm respectively were recorded for untreated control, which were significantly different from other treatment, however, the significantly lowest value (wasnoticed for 120 concentration of leaf extract. While the root length (5.00 cm) for 20 treatment did not differ significantly with control. Here a clear observation was noticed that walnut leaf extract have greater negative influence on shoot length than root. 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 application. Such types of findings are also supported by Segura-Aguilar *et al.* (1992). As the leaf extract concentration increased, the seedling fresh and dry weight were also decreased on concentration dependent manner and the significantly maximum seedling fresh and dry weight (2.6 and 0.96) g were found for control while, minimum value (1.64 and 0.62) g were observed in 120% aqueous leaf extract concentration. However, the value of dry weight of seedling (0.89 g) after 2% concentration was at par with control. An inhibitory effect was also noticed on vigour index I & II with increase in leaf extracts concentration and the significantly greatest value (950.82 and 91.84) respectively were calculated also for control, while lowest values (440.22 and 56.01) were computed for 12% leaf extract concentration, however, the result of 2% & control for vigour index II did not differ significantly. Fresh and dry weight of seedling and in vigour index (I & II) with increase in leaf extracts concentration and significant differences for these characters could be due to expected variation in phytotoxin content juglone in different doses of leaf extract application. In several previous studies, it was determined that walnut leaf extracts decreased cucumber seedling fresh and dry weight (Kocacaliskan and Terzi, 2001; Terzi *et al.*, 2003).

Table 1: Allelopathic effect of walnut leaf extracts on ricebean

Treatments (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Vigour index I	Vigour index II
0	96.00	5.09	4.82	9.91	2.60	0.96	950.82	91.84
2	94.67 (1.39)	5.00 (1.77)	2.23 (53.73)	7.23 (27.04)	2.03 (21.92)	0.89 (7.29)	684.57 (28.00)	84.25 (8.26)
4	94.33 (1.74)	4.80 (5.70)	2.04 (57.68)	6.84 (30.98)	2.01 (22.69)	0.80 (16.67)	644.95 (32.17)	75.47 (17.82)
6	94.00 (2.08)	4.63 (9.04)	1.73 (64.11)	6.36 (35.82)	1.99 (23.46)	0.70 (27.08)	597.89 (37.12)	65.49 (28.69)
8	93.33 (2.78)	4.47 (12.18)	1.59 (67.01)	6.06 (38.85)	1.90 (26.92)	0.69 (28.13)	565.49 (40.53)	64.71 (29.54)
10	91.00 (5.21)	4.44 (12.77)	1.56 (67.63)	6.00 (39.46)	1.78 (31.54)	0.66 (31.25)	545.92 (42.58)	60.36 (34.28)
12	90.33 (5.91)	3.34 (34.38)	1.53 (68.26)	4.87 (50.86)	1.64 (36.92)	0.62 (35.42)	440.22 (53.70)	56.01 (39.01)
SEm (±)	**1.51**	**0.07**	**0.05**	**0.10**	**0.04**	**0.03**	**13.32**	**4.31**
LSD (0.05)	**4.60**	**0.22**	**0.18**	**0.31**	**0.13**	**0.11**	**40.42**	**13.07**

Table 2: Allelopathic effect of walnut leaf extracts on grain amaranth

Treatments (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Vigour index I	Vigour index II
0	63.67	4.54	2.99	7.53	0.13	0.043	479.41	2.76
2	48.00 (24.61)	3.27 (27.97)	2.85 (4.68)	6.12 (18.73)	0.12 (7.69)	0.040 (6.98)	293.92 (38.69)	1.92 (30.43)
4	37.33 (41.37)	3.24 (28.63)	1.47 (50.84)	4.71 (37.45)	0.11 (15.38)	0.033 (23.26)	175.84 (63.32)	1.24 (55.07)
6	22.33 (64.93)	2.46 (45.81)	1.40 (53.18)	3.86 (48.74)	0.09 (30.77)	0.023 (46.51)	86.28 (82.00)	0.52 (81.16)
8	17.00 (73.30)	2.40 (47.14)	1.35 (54.85)	3.75 (50.20)	0.08 (38.46)	0.020 (53.49)	63.81 (86.69)	0.34 (87.68)
10	11.33 (82.21)	2.25 (50.44)	1.31 (56.19)	3.57 (52.59)	0.08 (38.46)	0.017 (60.47)	40.42 (91.57)	0.19 (93.12)
12	10.67 (83.24)	1.60 (64.76)	0.92 (69.23)	2.52 (66.53)	0.06 (53.85)	0.010 (76.74)	26.88 (94.39)	0.11 (96.01)
SEm (±)	**0.47**	**0.13**	**0.11**	**0.15**	**0.04**	**0.002**	**6.45**	**0.10**
LSD (0.05)	**1.42**	**0.39**	**0.35**	**0.46**	**0.01**	**0.007**	**19.57**	**0.31**

Note: Figures in parentheses are the percent reduction over control

Vigor index (I & II) is a multiple criterion of germination with seedling length and dry weight of seedling. Therefore, these indiceswere markedly inhibited by the walnut leaf extract (Prasad *et al.,* 2011) reported interference of juglone in normal growth of pea seedlings.

Allelopathic effect on grain amaranth

The effect of walnut leaf extracts on seed germination and seedling vigour characteristics like root, shoot and seedling length, fresh & dry weight of seedling along with vigour index I & II have been presented in table-2. There was significant gradual decrease in the germination percentage with the increase in walnut leaf extracts and higher value (63.67) was observed for control, while application of 12% leaf extract resulted significantly lowest germination (10.67%). The result also depicted that each treatments of leaf extract concentration differed significantly to each other with respect to germination except 100% and 120% of walnut leaf extract. 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 12% was noticed. Each treatment of walnut leaf extract had significant negative influence on root, shoot and seedling length over control, however, the result of 2% extracts concentration was at par with control for shoot length. The maximum root, shoot and seedling length (4.54, 2.99 and 7.53) cm was observed for control, while, statistically lowest value (1.6, 0.92 and 2.52cm) respectively were measured for 12% 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 results were also observed with juglone in cucumber (Tekintas, *et al.*, 1988), tomato and bean (Neave and Dawson, 1989), wheat and corn (Jose and Gillespie, 1998), wheat (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 12% and same trend was calculated in terms of vigour index I & II (Table 2). Least fresh weight (0.06 g) was observed for 12% concentration, while maximum seedling fresh weight of 0.13 g was observed from control treatment. The dry weight value of 0.043 g was recorded in untreated control, while significantly least results (0.01 g) was observed at maximum concentration of leaf extracts (12%); however, the result of 2% treatment (0.12 and 0.04) g was at par with control for both fresh and dry weight. Vigour index (Germination % x seedling length) and (Germination % x dry weight of seedling) is a real reflection of seedling vigour of seed or seed lot which were extremely reduced as the walnut aqueous leaf extracts concentration increased and statistically maximum value for vigour index I and II (479.41 and 2.76) were computed for untreated control over all other treatments, while least value (26.88 and 0.11) were calculated also for 12% 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 criterion of germination with seedling length and dry weight of seedling. Therefore, these indices 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 as well as of Prasad *et al.* (2011) in cauliflower.

As a conclusion results clearly revealed that aqueous leaf extracts of walnut has inhibitory effects on germinating grain amaranth and ricebean at concentration dependent manner, while for grain amaranth, seed germination and subsequent seedling growth were markedly affected and observed as susceptible against juglone. However, ricebean seed exhibited extent of tolerance and might be option in walnut intercropping under West Himalayan agri-silvi system as a *kharif* crops.

REFERENCES

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigour determination by multiple criterion. *Crop Sci.,* **13**: 630-33.

Appleton, B. and Berrier, R. 2000. *Tree for problem landscape sites- The walnut tree: Allelopathic effects and tolerant plants,* Virginia Tech., pub No. 430-021. Virginia State University.

Bertin, C., Yang, X. and Weston, L. A. 2003. The role of root exudates and allelochemical in the rhizosphere. *Plant Soil,* **256**: 67-83.

Davis, E. F. 1928. The toxic principle of *Juglans nigra* as identified with synthetic juglone and its toxic effects on tomato and alfalfa plants, *American. J. Bot.,* **15**: 620.

Inderjit and Malik, A. U. 2002. *Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems.* Birkhauser-Verlag, Berlin, pp. 272.

Jose, S. 2002. Black walnut allelopathy: Current state of science. *In: Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems* (Ed. Malik, I.), Birkhauser-verlag AG, Basal.

Jose, S. and Gillespie, A. R. 1998. Allelopathy in black walnut (*Juglans nigra* L.) alley cropping: II. Effect of juglone on hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) growth and physiology. *Plant and Soil*, **203**: 199-05.

Kocacaliskan, I. and Terzi, I. 2001. Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. *J. Hort. Sci. Biotech.*, **76**: 436-40.

Neave, I. A. and Dawson, J.O. 1989. Juglone reduces growth, nitrogenase activity and root respiration of actinorhizal back older seedlings. *J. Chem. Ecol.*, **15**: 1823-36.

Orcutt, D. M. and Nilsen, E. T. 2000. *Physiology of plants under stress. Soil and Biotic Factors*. Wiley, New York.

Piedrahita, O. 1984. Black walnut toxicity. *Factsheet*, **11**: 7-8.

Prasad, B. and Prasad, S. 2010. Allelopathic effects of walnut (*Juglans regia* L.) leaf extracts on wheat and barley seed germination and seedling growth under West Himalayan agri-silvi system. *Env. Ecol.*, **28**: 610-13.

Prasad, B., Lavania, S. K. and Sah, V. K. 2011. Bioassay study on effect of walnut leaf extracts on seed germination and seedling vigour of cauliflower (*Brassica oleracea* var. *botrytis*). *Indian J. Agro-forestry*, **13**: 103-06.

Prasad, B., Prasad, R. and Sah, V. K. 2011. Effects of aqueous extracts of walnut (*Juglans regia* L.) leaf on germinating wheat (*Triticum aestivum* L.) in West Himalayan agri-silvi system. *J. Non Timper For. Prod.*, **18**: 31-34.

Prasad, B., Sah, V. K. and Lavania, S. K. 2011. Allelopathic effects of walnut leaf extracts on growth and nodulation of vegetable pea (*Pisum sativam* L. cv. Arkel). *J. Non Timber For. Prod.*, **18**: 119-22.

Rietveld, W. J. 1983. Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. *J. Chem. Ecol.*, **9**: 295-08.

Segura-Aguilar, J. I., Hakman and Rydstrom, J. 1992. The effects of OH 1-4-nephthoquinone on Norway spruce seeds during germination. *Pl. Physiol.*, **100**: 1955-61.

Snedecor, G.W. and Cochran, W.G. 1989. *Statistical Methods*. 8th Eds. East-West Press, New Delhi.

Tekintas, E., Tanrisever, A. and Mendilcioglu, K. 1988. Cevizlerde (*Juglans regia* L.) juglone izolasyonu ve juglon iceriginin yillik degisimi. Ege universitesi, *Ziraat Fakultesi Dergisi* **25**: 214-25.

Terzi, I., Kocacaliskan, I., Benilogu, O. and Salak, K. 2003. Effects of juglone on growth of muskmelon seedlings with respect to physiological and anatomical parameters. *Biol. Pl.*, **47**: 317-19.

Thevathasan, N. V., Gordon, A. M. and Voroney, R. P. 1999. Juglone (5-hydroxy-1, 4 naphthoquinone) and soil nitrogen transformation interaction under a walnut plantation in southern Ontario. Canada. *Agro For. Syst.*, **44**: 151-62.