



Allelopathic Effects of Walnut Leaf Extracts on Plant Growth and Nodulation of Pea

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ABSTRACT

Aqueous leaf leachate of walnut was evaluated for allelopathic effects on plant growth and nodulation of vegetable pea under poly house condition at KVK, Pithoragarh. Walnut leaf extracts with 20, 40, 60, 80 and 100% concentrations along with control were used in pot from seed germination to eve of the pod setting in vegetable pea. Plant growth parameters viz; plant height, root length, fresh and dry weight as well as collar diameter were significantly reduced as the concentration of leachates increased. Number of secondary roots, nodules per plant and size of nodule were also statistically decreased.

Keywords: Extract, Leaf, Pea, Plant height, Root length, Walnut.

INTRODUCTION

In North-West Himalayan region of India tree based intercropping has been in practice since ages and walnut is one of the most common trees species of horticulture based agro-forestry systems. Walnut (*Juglans regia* L.) have allelopathic effects on certain plants and toxic principle of leaf extract is Juglone (5-OH-1, 4 naphthoquinone), which is present in leaves, hulls and inner root bark. It has been reported to be toxic to certain herbaceous and woody plants (Funk *et al*, 1979; Rietveld, 1983). This species occurs wild as well as under cultivation along the risers of agriculture fields throughout the Western Himalayan region. Presence of trees in the agro-forestry system results in the exposure of associated crops due to release of allelo-chemicals from the fallen leaves which after decomposition leaches into the soil. It is one of the first species to shed its leaves and trees become leafless from September to October which coincides with the seed sowing of the most of the *Rabi* crops. The economy of hill peasants is largely depends on produces obtained from traditional agro-forestry systems. Their bread and butter as well as other family expenses are met out through cultivating vegetable pea as a cash crop. Poor growth, development and nodulation of vegetable pea (*Pisum sativum* L.) have generally been observed under walnut based agroforestry system and farmers faces low productivity alongwith poor fertility. In spite of the above fact, no attempt has been made to address the allelopathic

effects of walnut on growth and nodulation of vegetable pea.

MATERIALS AND METHODS

Naturally fallen leaves of walnut tree were collected in the month of September from more than ten years old trees because walnut trees younger than seven years old does not contain sufficient Juglone to cause toxicity (Piedrahita, 1984). The study area lies between 30°15' N latitude and 78°30' E longitude at an altitude of 1950 m at KVK, Gaina, Pithoragarh (GBPUA&T, Pantnagar). Collected leaves were brought to the laboratory and properly washed with distilled water and kept in laboratory for drying. After drying they were grinded and finally converted into powder form. 20, 40, 60, 80 and 100 g of leaf powder were soaked in 1 litre of distilled water for 48 h, stirred in between filtered and solution of 20, 40, 60, 80 and 100 per cent concentration of aqueous leaf extract were prepared for the experiment.

For plant growth and nodulation studies, 20 seeds of vegetable pea were sown in plastic pot having soil, sand and FYM in equal proportions (Airi *et al*, 1998). Five such pots were used for a single replication. The experiment was conducted in CRD and replicated four times under glass house condition during 2021 and again repeated in 2022 season. Throughout the duration of the experiment, $20 \pm 2^{\circ}\text{C}$ temperature was maintained. After 12 days of seed sowing 10 seedlings were remain in every pot for proper growth of seedling

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Table 1. Allelopathy effects of walnut leaf extracts on plant growth and nodulation of vegetable pea cv. PS 1100

Character	Plant height (cm)			Collar diameter (mm)			Root length (cm)			Number of secondary roots		
Treatment	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Control	27.37	29.35	28.36	2.88	3.00	2.94	21.97	22.55	22.26	42.25	39.95	41.10
20%	22.37	24.00	22.88 (19.32)*	2.45	2.70	2.57 (12.59)*	18.60	17.95	18.27 (17.92)*	34.37	32.70	33.53 (18.42)*
40%	20.02	22.10	21.06 (25.74)*	2.11	2.20	2.15 (26.87)*	16.67	16.52	16.60 (25.43)*	28.62	26.72	27.67 (32.68)*
60%	18.85	20.15	19.50 (31.24)*	1.81	1.92	1.86 (36.73)*	16.27	16.17	16.22 (27.13)*	22.65	21.20	21.92 (46.67)*
80%	18.07	19.00	18.53 (34.66)*	1.51	1.67	1.59 (45.92)*	15.57	15.37	15.47 (30.50)*	19.52	20.02	19.77 (51.90)*
100%	16.55	17.05	16.80 (40.76)*	1.32	1.42	1.37 (53.40)*	15.32	14.65	14.98 (32.70)*	13.12	12.30	12.71 (69.08)*
SEM+	0.16	0.20	0.16	0.02	0.02	0.20	0.14	0.11	0.09	0.22	0.27	0.19
CD(P=0.05)	0.49	0.61	0.50	0.08	0.06	0.07	0.41	0.35	0.27	0.65	0.80	0.57
CV (%)	1.60	1.89	1.58	2.54	2.97	2.43	1.62	1.38	1.07	1.66	2.13	1.49

Note: * Percent reduction over control are given in parenthesis

Character	Fresh weight/plant (g)			Dry weight /plant (mg)			No. of nodules/plant			Size of nodules(mm)		
Treatment	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
Control	4.35	5.62	4.98	603.75	783.75	693.75	21.72	23.30	22.51	1.89	1.90	1.89
20%	3.04	4.03	3.53 (29.12)*	547.25	681.50	614.37 (11.44)*	18.70	16.72	17.71 (21.32)*	1.86	1.85	1.85 (2.11)*
40%	2.45	3.27	2.86 (42.57)*	421.00	577.00	499.00 (28.07)*	13.12	14.45	13.78 (38.78)*	1.46	1.54	1.50 (20.63)*
60%	2.24	2.95	2.59 (47.99)*	360.50	472.75	416.62 (39.95)*	10.30	11.82	11.06 (50.87)*	1.37	1.44	1.40 (25.93)*
80%	1.20	2.22	1.70 (65.86)*	280.25	381.25	330.75 (52.32)*	8.52	9.47	9.00 (60.02)*	1.25	1.29	1.27 (32.80)*
100%	1.06	1.19	1.12 (77.51)*	215.75	289.25	252.50 (63.60)*	6.82	7.60	7.21 (67.97)*	1.10	1.15	1.12 (40.74)*
SEM+	0.04	0.06	0.03	5.14	6.99	4.20	0.26	0.22	0.17	0.02	0.02	0.02
CD(P=0.05)	0.11	0.17	0.11	15.27	20.76	12.49	0.38	0.79	0.52	0.06	0.05	0.07
CV (%)	3.21	3.62	2.75	2.54	2.62	1.79	1.94	3.82	2.58	2.97	2.39	3.06

Note: * Percent reduction over control are given in parenthesis

$$= (4.98-3.53)/4.98 \times 100 = 29.12$$

$$= (4.98-2.86)/4.98 \times 100 = 42.57$$

$$= (4.98-2.59)/4.98 \times 100 = 47.99$$

and further investigations. Different concentration of leaf leachates were used at five days interval as a source of moisture to the seedlings from initial stage upto eve of the pod setting stage, whereas, distilled water was used in control treatment.

Ten survived plants from each treatment and replication were measured for plant height and collar diameter after 55 days of sowing. After the eve of the pod setting, the whole pot was kept in a water tank for 24 h to remove the roots and nodules without damaging them. Observations were recorded for root length, number of secondary roots, number of nodules per plant and size of nodules with the help of digital Vernier calliper. The plants were then shade dried and fresh

weight of all the plants was recorded. They are then dried in an oven at 80°C till constant weight to record dry weight.

Pooled data were also made by averaging the observations of two years of respective replication and statistical difference between the treatments variance analysis and critical difference (P=0.05) were performed by following the method of Snedecor and Cochran (1989). However, per cent reduction was calculated over control.

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RESULTS AND DISCUSSION

The allelopathic effects of walnut leaf extracts on plant growth and nodulation of vegetable pea under controlled conditions is presented in Table 1. Application of walnut leaf extracts significantly affected the plant growth and nodulation of vegetable pea in both the consecutive years. With an increase in the concentration of leaf extract there was a gradual reduction in plant growth and nodulation due to higher content of Juglone. There was significant decrease in plant height and collar diameter with an increase in walnut leaf extract concentration and maximum pooled value 28.36 cm and 2.94 mm, respectively were observed for control, while application of 100% leaf leachates showed maximum reduction of 40.76 and 53.40 per cent for plant height and collar diameter respectively, as compared to control (Table 1). The similar trend of plant growth retardation with the increase in the concentration of walnut leaf extracts were also observed in black alder seedling and strawberry plants (Neave and Dawson, 1989; Ercisli *et al*, 2005). Juglone exhibited significant inhibitory effect on the growth and physiology of hydroponically grown corn and soybean seedling including shoot and root relative growth rates, leaf photosynthesis, transpiration, stomatal conductance, leaf and root respiration (Jose and Gillespie, 1998).

Average of two years root length and number of secondary roots per plant were also significantly reduced on concentration dependent manner and maximum root length (22.26 cm) and number of secondary roots per plant (41.10) were counted for control treatment and minimum value of 14.98 cm and 12.71 respectively, was with the application of 100% walnut leaf extracts. The reduction in root length by walnut leaf juice on muskmelon and cucumber seedling was already demonstrated by Terzi (2008). The two year pooled data revealed that fresh and dry weight of pea plants were drastically affected as the leaf extract concentration increased and highest fresh and dry weight per plant (4.98 g and 693.75 mg) was in control and significantly lowest value (1.12 g and 252.50 mg) respectively, were observed in 100% leachate treatment. Similar findings also revealed in the reduction in various growth parameters (Seedling elongation, fresh and dry masses) and protein content of *Cucumis melo* during post germinative treatment by juglone (Terzi, 2009).

Number and size of nodules were also influenced negatively by walnut leaf extracts, depending on the concentration. The maximum

reduction 67.97 per cent for number of nodules per plant and 40.74 per cent for size of nodules were observed for 100% walnut leaf extracts over control (22.51 and 1.89 mm), respectively. While, size of nodules was not negatively influenced with 20% extracts, however, results of 60% (1.40 mm) and 80% (1.34 mm) were at par to each other. According to Mallik (2000) juglone also affect on nodulation in legumes. Dawson and Seymour (1983) revealed that a concentration of 10^{-3} M juglone absolutely inhibited the growth of *in vitro* *Frankia* isolate from root nodules of red alder and of *Rhizobium japonicum* strain 71. The similar results were also reported by Christoph and Dawson (1985) that nodulation of black alder in an amended prairie soil inoculated with a *Frankia* isolate from red alder was significantly decreased by the addition of aqueous suspension of 10^{-3} M and 10^{-4} M juglone. An interesting observation in this study was the blackening of root tip in the vegetable pea during plant growth by the application of walnut leaf extracts. However, the reason is unknown but might be implicated with root sensitivity to juglone (Orcutt and Nilsen, 2000).

Results showed that walnut leaf extracts impairs both vegetative growth and nodulation in vegetable pea. Since we did not examine the effect of walnut leaf extract on plant metabolism such as photosynthesis, enzymes and hormones and evolves to endogenous solute like carbohydrates, plant growth regulators and enzymes in plants. In addition, walnut leaf extract has a negative effect on plant nutrient element uptake from growth media (Ercisli *et al*, 2005). Therefore, as far as possible walnut leaves should be removed from the fields prior to sowing of vegetable pea with walnut as a companion crop to reduce phytotoxic effects.

REFERENCES

- Airi S, Rawat RS, Samant SS and Dhar U (1998). Treatments to improve germination of four multipurpose trees of central sub Himalaya. *Seed Sci and Technol* **26**: 347-354.
- Christoph S and Dwason JO (1985). Effect of Jugone on growth *in vitro* of *Frankia* isolates and nodulation of *Alnus glutinosa* in soil. *Pl and Soil* **87**(1):79-89.
- Dawson JO and Seymour PE (1983). Effects of Juglone concentration on growth *in vitro* of *Frankia* Ar13 and *Rhizobium Japonicum* strain 71. *J Chem Ecol* **9**: 1175-1183.

- Ercisli S, Esitken A, Turkkal C and Orhan E (2005). The allelopathic effects of juglone and walnut leaf extracts on yield, growth, chemical and PNE compositions of strawberry cv. Fern. *Pl Soil Env* **51**(6): 283-287.
- Funk DT, Case PJ, Rietveld WJ and Phares RE (1979). Effect of jugone on the growth of coniferous seedlings. *Forest Sci* **25**: 452-454.
- Jose S and Gillespie AR (1998). Allelopathy in black walnut (*Juglans regia* L.) alley cropping: II. Effects of juglone on hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L.Merr) growth and physiology. *Pl and Soil* **203**: 199-205.
- Mallik M A B (2000). Methods to evaluate allelopathic effects on nodulating bacteria and nodulation in legumes. *Allelopathy J* **7**(2): 197-213.
- Neave IA and Dawson JO (1989). Juglone reduces growth, nitrogenase activity and root respiration of actinorhizal black alder seedlings. *J Chem Ecol* **15**(Sci 1823-1836).
- Orcutt DM and Nilsen ET (2000). Physiology of plants under stress. Soil and biotic factors, Wiley, New York.
- Piedrahita O (1984). Black walnut toxicity. Ministry of Agriculture and Food, Ontario, Canada. Order No. 84-050.
- Rietveld WJ (1983). Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. *J Chem Ecol* **9**: 295-308.
- Snedecor GW and Cochran WG (1989). Statistical methods 8th Eds. East-West Press, New Delhi.
- Terzi I (2008). Allelopathic effects of juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. *African J Biotechnol* **7**: 1870-1874.
- Terzi I (2009). Allelopathic effects of juglone and walnut leaf and fruit hull extracts on seed germination and seedling growth in muskmelon and cucumber. *Asian J Chemistry* **21**: 1846-1848.

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