0971-4693/94 US $ 5.00
Allelopathy Journal 23 (2): 287-296 (2009) *International Allelopathy Foundation* 2009
Table: -, Figs : 9

Allelopathic effects of *Conyza canadesis* the germination and growth of wheat, sorghum, cucumber, rape and radish

XINGXIANG GAO , MEI LI*, ZONGJUN GAO , HONGJUN ZHANG [1] and ZUOWEN SUN[2]

Institute of Plant Protection,
Shandong Academy of Agricultural Sciences, Ji'nan 250100, China
E. Mail: limei9909@163.com

(Received in revised form : February 8, 2009)

ABSTRACT

The allelopathic effects of aqueous extract from *Conyza canadesis* were tested in Petri dishes in Laboratory and Greenhouse assays. The aqueous extract strongly inhibited the germination, germination speed and seedling growth of sorghum (*Sorghum vulgare* Pers.), cucumber (*Cucumis sativus* L.), wheat (*Triticum aestivum* L.), rape (*Brassica campestris* L.) and radish (*Raphanus sativus* L.). Rape and radish were more sensitive than other spp. *C. canadesis* released the allelochemicals mainly as root exudates. The extract inhibited the hypocotyl and root growth of endosperm-removed wheat and sorghum cultivated in darkness, but the inhibitory effect was drastic in sorghum than in endosperm-removed wheat. The aqueous extract decreased the photosynthesis activity but increased the MDA contents of cucumber and radish. Thus allelopathic effects of *C. canadesis* affects non-photosynthesis activity and MDA, but may also affect the photosynthesis activity.

Key words: Allelopathic effects, aqueous extracts, *Conyza canadesis* L., cucumber, radish, rape, root exudates, seed germination, seedling growth , shoot leachates, sorghum, wheat

INTRODUCTION

Allelopathy is the positive or negative effects of chemicals released by one plant sp on the growth or reproduction of others (1,12). Chemicals extracted from plant roots or shoots directly inhibits or stimulates the germination, growth and development of other plants (8,9). Allelochemicals also indirectly affects the other plants through the inhibition of microorganisms (nitrogen-fixing and nitrifying bacteria and ectomycorrhizae) (17). Allelopathic plants alters the plant population and even community dynamics (2,4,11). *Conyza canadesis* L. is major weed in China, often used as herbal medicine to inhibit bacterial growth and prevent diarrhoea in children (5). It also inhibits the growth of common mold. Its oil contains (16,20) limonene (28.0%), β-bisaolene (19.0%) and isofarnesen (7.5%). Its negative allelopathic effects have also been observed in the field conditions, but allelopathic studies are scanty.

This study aimed to determine (i). the allelopathic effects of *C. canadesis* L. on the seed germination and growth of crops [*Triticum aestivum* L. (wheat), *Sorghum vulgare* Pers. (sorghum), *Cucumis sativus* L. (cucumber), *Brassica campestris* L. (rape) and *Raphanus sativus* L. (radish)] and (ii). the mechanism of its allelopathic effects.

*Correspondence author, [1] Institute for Control of Agrochemicals, Ministry of Agriculture, Beijing 100026, China, [2] Plant Protection Research Station Shandong Province, Ji'nan 250100, China.

MATERIALS AND METHODS

Whole plants of *C. canadesis* L. were collected from the roadside in Ji'nan, Shandong Province, China in May 2005. Wheat, sorghum, cucumber, rape and radish seeds were obtained from local market. The plant samples were air dried in shade and ground into powder. An aqueous extract of 0.1 g·mL^{-1} from *C. canadensis* was obtained as under: 1000 g (dry weight) of powder from *C. canadesis* was soaked in 10 L distilled water for 72 h at room temperature (T = 20^{o}C). Then it was extracted for 20 min under ultrasonic power (500 W) and filtered through Whatman No. 2 filter paper. It was diluted with distilled water to get concentrations of 0.0125, 0.025, 0.05 and 0.1 g DW·mL^{-1} (dry weight of *C. canadesi*) and distilled water was used as control. Thirty seeds of each crop were placed in Petri dishes (9 cm dia) containing 10 mL *C. canadesis* aqueous extract with two sheets of filter paper and 4 test concentrations of extract were applied as per treatment. The Petri dishes were placed at 26 ± 1^{o}C in dark. Germination was measured daily and the seedling, root and hypocotyl length of test crops were measured 4 d after sowing. Distilled water was used as control.

C. canadesi allelochemicals released in greenhouse

C. canadesis plants were transplanted in plastic pots (25 cm dia and depth). When the *C. canadesis* plants were 30 cm tall, the root exudates were obtained by irrigating each pot with 500 mL distilled water once in 3 days. The water leached from the bottom of each pot was collected as root exudates. Simultaneously, the shoot leachates were obtained by spraying the stem and leaves of *C. canadesis* with 200 mL distilled water per pot. The water droppings from the stems and leaves were collected as shoot leachates.

The seeds of rape, radish, cucumber and wheat were sown in plastic pots in loam soil in greenhouse and irrigated as needed with shoot leachates or root exudates. Distilled water was used as control. Seedling height and dry weights were measured at 15 d after sowing.

Photosynthesis activity

Wheat seeds were soaked in distilled water for 2 h and germinated in dark incubator at 26 ± 1 ^{o}C. The endosperm was removed when the wheat hypocotyls were 1-2 cm long and rinsed with water for 2 min. Ten healthy seedlings of same size were selected and placed in each beaker containing 10 mL *C. canadesis* aqueous extract. Four concentrations (0.0125, 0.025, 0.05 and 0.1 g DW·mL^{-1}) of extract were used and distilled water was used as control. The beakers were placed in an incubator (26 ± 1 ^{o}C for 10 h light and 14 h dark) during the study. Root and hypocotyl lengths were measured after 8 d.

Non-photosynthesis activity

Sorghum seeds were soaked in distilled water for 2 h and germinated at 26 ± 1 ^{o}C in dark. When sorghum hypocotyls were 1-2 mm long, 10 healthy seedlings of same size were selected and placed in each Petri dish (9 cm dia), containing 100 g river sand and irrigated with 20 mL aqueous extract. Four concentrations (0.0125, 0.025, 0.05 and 0.1 g DW·mL^{-1}) of extract were used, and distilled water was used as control. They were incubated at 26 ± 1^{o}C in the dark and Root and hypocotyl lengths were measured 36 h after sowing.

Malondialdehyde (MDA) content

Radish and cucumber seeds were soaked in distilled water for 2 h and germinated at 26 ± 1 ^{o}C in the dark. When hypocotyls were 1-2 mm long, 10 healthy seedlings of same size were selected and placed in each Petri dish (9 cm dia), containing clean 100 g river sand irrigated with 20 mL aqueous extract. Four concentrations (0.0125, 0.025, 0.05 and 0.1 g DW·mL^{-1}) of extract were assayed and distilled water was used as control. These were then incubated at 26 ± 1 ^{o}C in dark for 3 d. Then 10:14-h light : dark cycle was maintained in incubator and samples were collected 7 d after sowing.

The production of malondialdehyde (MDA) and the end product of lipid peroxidation, was estimated based on TBA activity as per Velikova *et al.* (15). The MDA content was measured by thiobarbituric acid (TBA) reaction. One g of each plant sample and 4 mL of 10% trichloroacetic acid (TCA) were homogenized. The homogenate was centrifuged at 1200 × g for 10 min and 2 mL of supernatant was added to 2 mL 0.62% TBA in 10% TCA. The mixture was incubated at 98 ^{o}C for 20 min. The reaction was stopped by ice. It was centrifuged at 1200 × g for 10 min, then supernatant was taken to measure its absorbance at 450, 532 and 600 nm.

Calculations for various parameters

These calculations for various parameters were done as below:

$$\text{Germination speed (\%) over control} = \Sigma (Gt/D) / \Sigma (Gc/D) \times 100,$$

Where, Gt : Number of germinated seeds daily in treatment, Gc : Number of germinated seeds daily in control and D: Number of corresponding days

$$\text{Germination (\%)} = (\text{Number of seeds germinated / Total seeds number of}) \times 100$$

$$\text{MDA concentration } (\mu mol\cdot L^{-1}) = 6.45(D_{532} \text{ x } D_{600}) - 0.56D_{450}$$

$$\underset{(nmol\cdot g^{-1}FW)}{\text{MDA content}} = \underset{(\mu mol\cdot L^{-1})}{\text{MDA concentration}} \text{ x } \text{homogenate volume (mL)/FW(g) (fresh weight)}$$

Inhibition (%) = (C-T)/C × 100 (C is the control numerical and T is the treatment numerical)

Statistical analysis

All results are presented as the means ± standard error with three replications. All analyses were done in 3 replicates, and the results were calculated using a standard curve.

RESULTS

The germination speed of all tested crops decreased compared to control (Fig. 1). The degree of inhibition depended on the extract concentration. The germination speed decreased with the increasing concentrations of extracts. At the same concentration, the

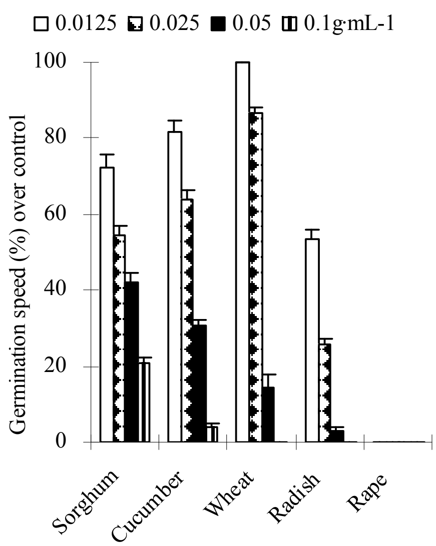

Figure 1. Effect of aqueous extract from *C. canadesis* on the relative seed germination speed of five crop plants

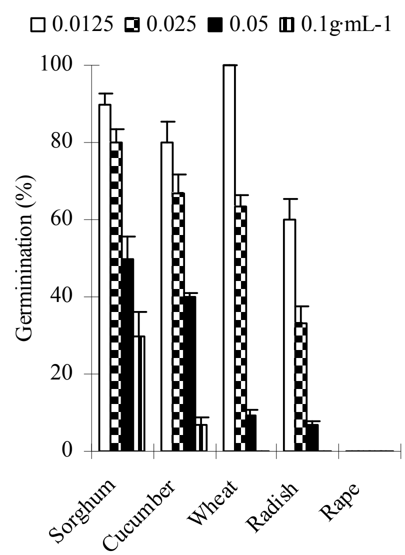

Figure 2. Effect of aqueous extract from *C. canadesis* on seed germination of five crop plants.

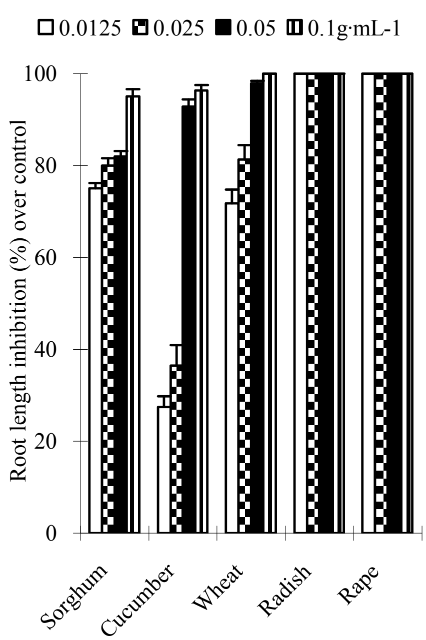

Figure 3. Effect of aqueous extract from *C. canadesis* on root length of five crop plants.

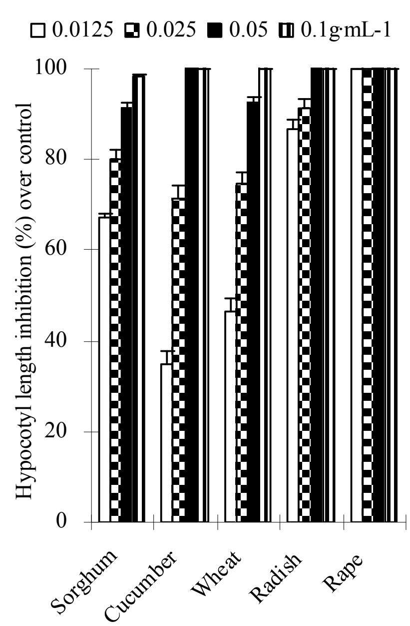

Figure 4. Effect of aqueous extract from *C. canadesis* on hypocotyl length of five crop plants.

different crops showed variable responses. At 0.0125 g·mL $^{-1}$, the germination speed (%) of wheat, sorghum, cucumber and radish over control was 100, 72.08, 81.82 and 53.25%, respectively. Rape seeds did not germinate at this concentration.

Aqueous extracts of *C. canadesis* strongly inhibited the seed germination of wheat, sorghum, cucumber, rape and radish (Fig. 2). The inhibitory effects increased with the increasing concentrations of extracts. Rape was the most sensitive specie in all tested crops, its seed germination was completely inhibition at all concentrations. At 0.05 g·mL^{-1}, the germination of wheat, sorghum, cucumber, rape and radish was 9.09, 50.00, 40.00, 0.0 and 6.67%, respectively (Fig. 1).

The radish roots growth was completely inhibited in all concentrations of aqueous extract. The wheat and sorghum roots were also strongly inhibited, followed by cucumber. At 0.025 g·mL $^{-1}$, the root length inhibition of rape, radish, wheat, sorghum, cucumber over control were 100, 100, 81.28, 80.00 and 36.46%, respectively.The roots growth in all crops were strongly inhibited at 0.05 and 0.1 g·mL $^{-1}$ (Fig. 3).

The rape hypocotyls were completely inhibited in all concentrations of aqueous extract. Those of radish and sorghum were also strongly inhibited, followed by wheat and cucumber. The hypocotyls length inhibition in radish, sorghum, wheat and cucumber at 0.025 g·mL^{-1} was 91.46, 80.25, 74.63 and 71.51% over control, respectively (Fig. 4).

Mode of release of *C. canadesis* allelochemicals

The mode of release of *C. canadesis* allelochemicals was tested in the greenhouse. As shown in Fig. 5, the inhibition of the seedling height and dry weight of four crops treated by root exudates was greater than those of shoot leachates. Take rape as example: the inhibition of seedling height and dry weight treated by root exudates were 57.14% and 61.35%, while the inhibition of those treated by shoot leachates were 14.29% and 29.46%. The inhibition of root exudates to recipient rape, radish and cucumber (57.14%, 53.59% and 33.71% in seedling height, 61.35%, 43.67% and 45.67% in dry weight) was higher than wheat (8.36%, 4.12% in seedling height and dry weight, respectively)**.** This result was similar to Petri dish results; rape was the most sensitive, followed by radish, cucumber and wheat.

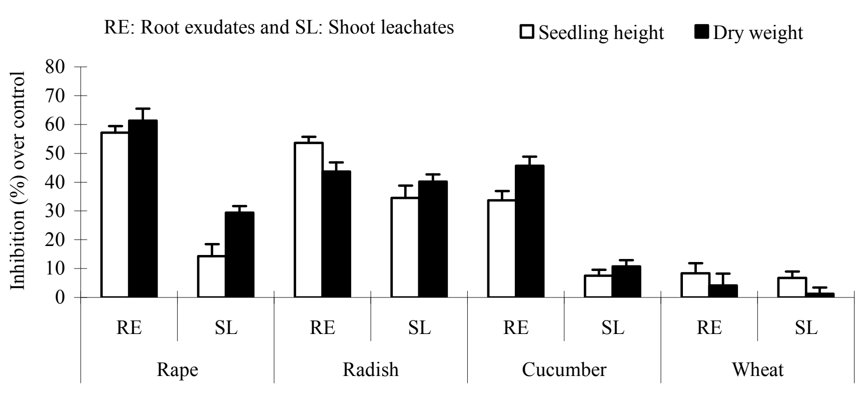

Figure 5. Effects of root exudates or shoot leachates of *C.canadesis* on seedling growth of receptor crops

Photosynthetic activity or non-photosynthetic activity

The inhibitory effects of extracts on the growth of endosperm-removed wheat were most inhibitory (Fig. 6). The highest concentration (0.1 g·mL$^{-1)}$, caused 57.38% and 82.21% inhibition rate in roots and hypocotyls of endosperm-removed wheat. At the same concentration, the inhibition was more in roots than hypocotyls.

The inhibitory effect was slightly weaker at lower concentration, but very strong at higher concentration i.e. was concentration dependent. The inhibition rate in sorghum roots and hypocotyls was 1.67% and 20.38% at 0.0125 g·mL^{-1} concentration and was 88.01% and 81.93% at 0.05 g·mL^{-1} concentration (Fig. 7). Thus the extract inhibited the growth of endosperm-removed wheat and sorghum and the inhibitory effect was drastic in sorghum than in endosperm-removed wheat.

Malondialdehyde (MDA) content

The *C. canadesis* extract increased the MDA content both in radish and cucumber with increasing concentrations of the extract (Figs. 8, 9). At 0, 0.0125, 0.025, 0.0375 and 0.05 g·mL^{-1} concentrations, the MDA contents were 3.07, 3.21, 4.95, 5.22 and 8.77 nmol·g^{-1}FW (fresh weight) in cucumber and 3.96, 4.22, 5.63, 6.08 and 12.64 nmol·g^{-1}FW in radish.

DISCUSSION

Allelopathy widely exists in nature and plays a vital role in crop production systems and pests (insects, nematodes, pathogene, weeds) management. In our study, the aqueous extracts of *C. canadesis* inhibited the seed germination speed, germination, and root and hypocotyl growth of five test crops. Nevertheless, inhibition was dependent on plant species. Rape proved to be the most sensitive spp. The differences in responses to plant allelochemicals depends on the receptor plant assayed (10), owing to variations in genetics and evolutionary histories.

The roots of wheat and radish were inhibited more strongly than hypocotyls at the same concentrations. These observations agree with the findings of other researchers, who reported that root length is the most sensitive and reliable response parameter to allelochemicals and is easy to measure (14,18). While the results of sorghum and cucumber were contrary, the hypocotyls were more sensitive than their roots. As roots and hypocotyls of rape were completely inhibited, the difference could not be observed. The *C. canadesis* allelochemicals variably influenced the different crops and different parts of same crop. The effect of aqueous extract was concentration dependent i.e. higher the concentration, the lower was the growth of seedlings as compared to control. According to Osvald (6), allelochemicals may stimulate or inhibit the plant growth at different concentrations. *C. canadesis* aqueous extract inhibited both the photosynthetic and non-photosynthesis activity, but the later was most affected. It also increased MDA content both in radish and cucumber, which might lead to membrane lipid damage, thereby leading to cell death and growth inhibition (3,13).

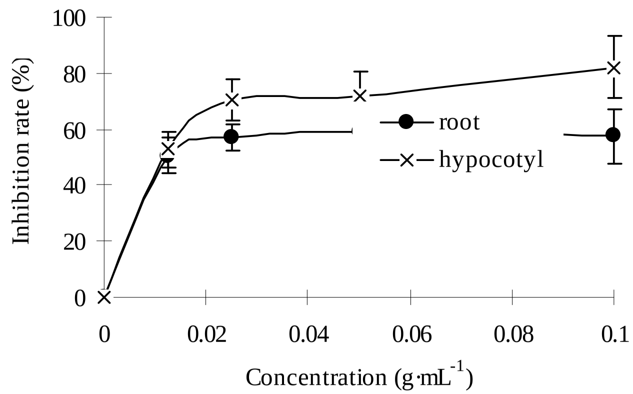

Figure 6. Photosynthesis activity of the extract by endosperm-removed wheat seedling growth

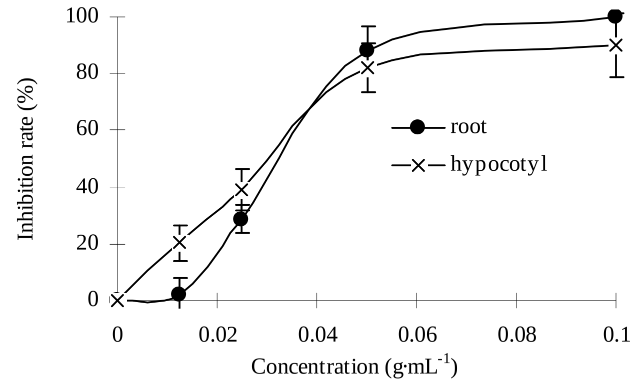

Figure 7. Non-photosynthesis activity of the extract by sorghum seedling growth

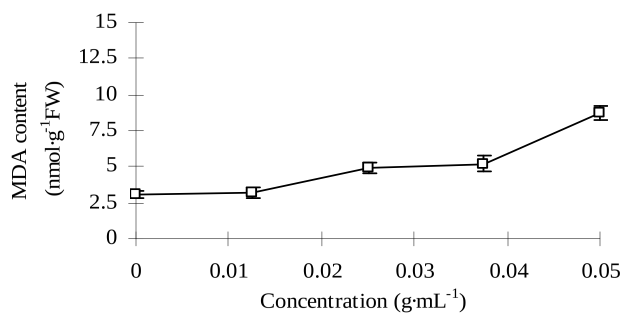

Figure 8. Effects of aqueous extract from *C. canadesis* on malondialdehyde (MDA) content in radish

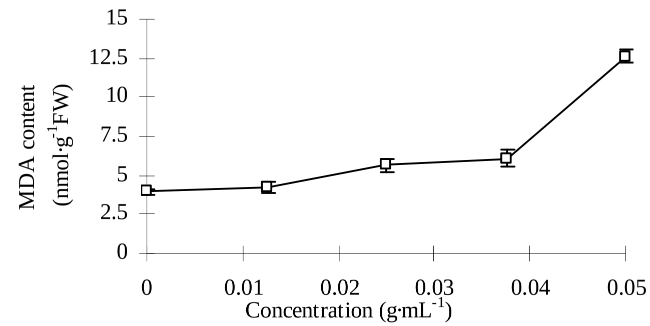

Figure 9. Effects of aqueous extract from *C. canadesis* on malondialdehyde (MDA) content in cucumber

Greenhouse assays showed that *C. canadesis* released the allelochemicals mainly as root exudates. In natural conditions, plants possibly release the allelochemicals into the agricultural ecosystem through volatilization, leaching of stems and leaves, decomposition of residues or root exudates (7,19). Further research is needed to (i). identify the allelochemicals involved, (ii). to understand the mechanism of *C. canadesis* allelopathy and (iii). identification of allelochemicals from *C. canadesis*. The bioactive natural products then could be used for weed control as environmentally friendly herbicides.

ACKNOWLEDGEMENTS

This research was supported by the National 863 High-Tech R&D Program of China (NO. 2006AA10A214), the National Key Project of Scientific and Technical Supporting Programs funded by the Ministry of Science & Technology of China during the 11th Five-Year Plan (NO. 2006BAD08A09), the High-Tech Innovation Foundation of SAAS (2006YCX018) and the Youth Foundation of SAAS (2005YQ025). Thanks to Dr. Chaoxian Zhang from the Institute for Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China for reviewing the manuscript.

REFERENCES

1. Callaway, R.M. and Aschehoug, E.T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290:** 521–523.
2. Escudero, A., Albert, M.J. and Pitta, J.M. (2000). Inhibitory effects of *Artemesia herba-alba* on the germination of *Helianthemum squamatum*. *Plant Ecology* **148**: 71-80.
3. Lin, C.C. and Kao, C.H. (2000). Effect of NaCl stress on H_2O_2 metabolism in rice leaves. *Plant Growth Regulation* **30:** 151-155.
4. Mo, M., Q, X.F. and Zhang, X. (2005). Allelopathy of aqueous extract of Lactarius hatsudake on several crops and barnyard grass (*Echinichloa crus-galli* L.) In: Proceedings, IV World Congress on Allelopathy; Wagga, NSW, Australia; pp., 247-251.
5. Mozaffarian, V. (1996). *A Dictionary of Iranian Plant Names*. Farhang Moaser, Tehran.
6. Osvald, H. (1950). Antagonism between plants. In: *Proceedings, 7th International Botanical Congress*; Stockholm, Sweden; Almqvist Wiksell, Stockholm; pp., 167-171.
7. Peng, S.L., Wen, J. and Guo, Q.F. (2004). Mechanism and active variety of allelochemical. *Acta Botanica Sinica* **46:** 757-766.
8. Putman, A.R. and Tang, C.S. (1986). *The Science of Allelopathy*. John Wiley and Sons, New York, USA.
9. Rice, E.L. (1984). *Allelopathy*. 2nd ed. Academic Press, New York, USA.
10. Richardson, D.R. and Williamson, G.B. (1988). Allelopathic effects of shrubs of the sand pine scrub on pines and grasses of the sandhills. *Forest Science* **34**: 592-605.
11. Saxena, A., Singh, D.V. and Joshi, N.L. (1996). Autotoxic effects of pearl millet aqueous extracts on seed germination and seedling growth. *Journal of Arid Environments* **33**:255-260.
12. Schenk, H.J., Callaway, R.M. and Mahall, B.E. (1999). Spatial root segregation: Are plants territorial? *Advance in Ecological Research* **28:** 146-180.
13. Shah, K., Kumar, R.G., Verma, S. and Dubey, R.S. (2001). Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* **161**: 1135-1144.
14. Turk, M.A. and Tawaha, A.M. (2002). Inhibitory effects of aqueous extracts of black mustard on germination and growth of lentil. *Pakistan Journal of Agronomy* **1**: 28-30.
15. Velikova, V., Yordanov, I. and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants, protective role of exogenous polyamines. *Plant Science* **151**: 59-66.

16. Rustaiyan, A., Parvis, A. A., Moradalizadeh, M., Masoudi, S. and Ameri, N. (2004) Volatile constituents of three compositae herbs: *Anthemis altissima* L. var. altissima, *Conyza Canadensis* (L.) Cronq. and *Grantina aucheri* Boiss. Growing wild in Iran. *Journal of Essential Oil Research* **16**: 579-581.
17. Walker, J.F., Miller, J.T., Lei, S., Semones, E. and Nilsen, B.D. (1999). Suppression of ectomycorrhizae on canopy tree seedlings in *Rhododendron maximum* L. (Ericaceae) thickets in the southern Appalachians. *Mycorrhiza* **9**: 49-56.
18. Williams, M.M., Mortensen, D.A. and Doran, J.W. (1998). Assessment of weed and crop fitness in cover crop residues for integrated weed management. *Weed Science* **46**: 595-605.
19. Zeng, R.S. (1999). Review on bioassay methods for allelopathy research. *Chinese Journal of Applied Ecology*, **10:** 123-126.
20. Ogg, A.G., Stern, D.J., Molyneux, R.J., and Teramishi, R. (1975). Chemical constituents of Horseweed oil. *Food Additives* **6:** 195-198.