Journal of Environmental Biology **March 2008,** 29(2) 243-247 (2008)

Allelotoxicity of *Parthenium* leaf extracts on cytomorphological behaviour of sunflower (*Helianthus annuus*)

G. Kumar* and Neelam Gautam

Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad-211 002, India

(Received: October 17, 2006 ; Revised received: March 29, 2007 ; Re-revised received: April 18, 2007 ; Accepted: April 30, 2007)

Abstract: *In recent decades allelopathy has gained much attention in the sustainable agricultural systems. It is necessary to trace out the mechanism of action of allelochemicals of plants on other plants. Allelochemicals of different plants interact differently with each other. In the present context an attempt has been made to study the influence of allelochemicals released by Parthenium on the growth, morphology and cytology of Helianthus annuus, both being strong allelopathic plants. The lower concentration of decomposed Parthenium leaves showed enhancing effect while higher doses depicted suppressive effect on growth and morphology of Helianthus annuus. However, cytological studies of pollen mother cells (PMCs) revealed increase in abnormality percentage with increasing concentration of allelochemicals. This study suggests better understanding of allelochemicals interaction and their incorporation into the betterment of crop.*

Key words: *Allelopathy, Sunflower, Parthenium, Chromosomal aberrations*
PDF of full length paper is available with author ***(*kumar_girjesh@yahoo.com)***

Introduction

Existence of weeds in crop fields causes chemical competition which is referred as 'Allelopathy'. Allelopathic plants release allelochemicals which are secondary plant metabolites which affect the nearby plants. Due to the action of allelochemicals, a large number of physiological functions and biochemical reactions are affected, such as seed germination, cell division, cell elongation, membrane permeability and ion uptake (Ortega *et al*., 1988; Tomita-Yokotani *et al.,* 2005; Setia *et al*., 2007).

Intensive scientific research on the effect of weeds on crops, crops on weeds, crops on crops has only occurred over the past few decades. Several researchers have studied the impact of allelochemicals on different plants in crop and agroforestry systems, such as Fetene and Habtemariam (1995), Saxena and Sharma (1996), Prately (1989), Prately and Ingrey (1990), Rawat *et al.* (1998), Rizvi *et al.* (1999), Kruse *et al.* (2000). Verma and Rao (2006) showed the effect of extract of four weeds on germination, survival and protein content of varieties of *Glycine max.* These researches envisage the possibility of using allelochemicals to promote sustainable agriculture and improving the ecological environment. In the present text, *Parthenium* was selected for the experimentation as it is oftenly found in crop fields and is highly allelopathic. Sunflower itself imparts high allelopathic effects but still greatly affected by allelochemicals released by *Parthenium*.

In recent decades, many researchers, such as Einhellig (2002), Yang *et al*. (2002), Batish *et al*. (2002), Bogatek (2006), Setia *et al.* (2007) have reported effect of various allelochemicals of different plants on physiological and biochemical process but reports regarding effects of allelochemicals on cytology of plants are still scanty. So, in the present text an attempt has been made to evaluate the effect of obnoxious weed, *Parthenium hysterophorus* on cytology and morphology of a strong allelopathic plant, *Helianthus annuus*.

Materials and Methods

For the treatment, *Parthenium* leaves were chopped into pieces. Chopped pieces were weighed and mixed with soil in different quantities, *viz.* 50, 100, 200, 300 and 500 g. Chopped leaves in the pots were watered and left for decomposition for 1 week. After this, dried and healthy seeds of *H. annuus* were presoaked in water for 6hr and then sown in these pots. Plants when matured, morphological parameters were studied. Germination and survival percentages were also taken for the treated plants at different treatments. Morphological data were analyzed statistically in terms of standard error, standard deviation, variance and co-variance. The experiment was performed with 5 replicates and the LSD test at the 5% level was done for the morphological parameters. For germination, survival, pollen and ovule fertility, *t*-test was applied to determine the significant differences among the mean values of the treatments.

For cytological analysis, young flowering buds from treated plants were fixed in Carnoy's fixative. Fixed buds were then preserved in 70% alcohol. Anther squashes were prepared in 2% acetocarmine (Belling, 1926). Sterility of pollen was studied by staining anthers in 1% acetocarmine-glycerine stain. Stained pollen grains were considered as fertile while unstained as sterile. Ovule / seed fertility was estimated by number of seeds per capitulum. Photomicrographs were taken from temporary preparations for study. Control plants were also examined under similar conditions for comparison with treated plants.

Results and Discussion

The treated plants exhibited several changes as compared to control sets. Cytological and morphological variations influenced

Table - 1: Germination, survival percentage and pollen and ovule fertility percentage at different treatments of *Parthenium* leaves

Treatment	Germination (%) + S.E.	Survival (%) + S.E.	Pollen fertility (%) + S.E.	Ovule fertility (%)+S.E.
Control	98.42 + 0.10	96.74 + 0.14	91.96 + 0.21	90.48 + 0.28
50 g	94.84 + 0.14	90.68 + 0.14	90.14 + 0.57	86.42 + 2.11
100 g	86.28 + 0.14*	80.64 + 0.12*	84.68 + 1.16**	82.40 + 0.90*
200 g	75.76 + 0.12	78.38 + 0.16*	80.18 + 0.96*	75.44 + 1.32**
300 g	70.46 + 0.16*	72.52 + 0.19*	74.38 + 1.41**	68.26 + 0.89*
500 g	61.92 + 0.22*	68.32 + 0.21*	65.20 + 2.07**	60.20 + 1.37**

* = Significant from control at 0.05 level (*t*-test)
** = Highly significant from control at 0.01 level (*t*-test)

by allelochemicals released by different quantities of decomposed leaves have been discussed below and tabulated in Table 1, 2 and 3.

Meiotic analysis: Control sets revealed completely normal meiotic stages. However, treated sets were remarkable with different chromosomal abnormalities like stickiness, scattering, precocious movement, secondary associations, laggards (Plate 1, Fig. 8), bridges, non-synchronous disjunction (Plate 1, Fig. 5), disturbed polarity. At diakinesis and metaphase – I, univalents (Plate 1, Fig. 4) and multivalents (Plate 1, Fig. 3) were recorded at considerable percentage at highest dose and were almost absent at lower doses of 50 g and 100 g. Multipolarity, micronuclei, fragmentation, unorientation *etc.* were rare in occurrence. Stickiness (Plate 1, Fig. 7, 9) was observed as most frequent aberration. Followed by it, scattering and precocious movement were also prevalent. Bridges (Plate 1, Fig. 9, 10 and 11) were also encountered at higher doses in very low percentage and were almost absent at lower doses. Total abnormality percentage exhibited a drastic change and recorded as very low (3.92%) at 50 g treatment while, it increased gradually at increasing concentration and recorded highest at 500 g (25.89%) treatment dose.

Meiotic studies showed that the allelochemicals released were effective in inducing various chromosomal abnormalities mostly of physiological type and very few clastogenic aberrations were encountered. This suggests the chromotoxicity of allelotoxins released by *Parthenium* which probably interferes with the normal cell division and led to these chromosomal aberrations. Chaniago *et al*. (2006) determined effect of *Amaranthus* and *Cyperus* weed extract on mitotic index of soybean at different concentration. Kumar *et al.* (2004), also reported effect of *Parthenium* extract on cytomorphology of *Trigonella*. Hegazy *et al.* (1990) and Ortega *et al*. (1988), also reported effects of allelochemicals on mitotic cells with increasing extract concentration.

Alongwith the increasing treatment doses with *Parthenium* leaves, the pollen and ovule fertilities were also affected greatly. A mild decrease in fertility was observed at lower doses which reduced noticeably at increasing doses (Table 1).

Morphological analysis: Morphological studies clearly revealed that allelochemicals released by *Parthenium* pose inhibitory effect to some extent on some growth parameters. However, some allelochemicals may interact with allelochemicals of sunflower and may behave differently and lead to different morphological variations which need proper understanding and application. *Parthenium* consists of a variety of allelochemicals, 'Parthenin' being the chief and the major cause of all inhibitory effect on other plants.

A dose based reduction in germination percentage and a slight reduction in survival percentage were observed in the treated sets (Table 1). Significant values were observed at higher doses after t-test analysis (Table 1). *Parthenium* caused inhibition in the germination and survival percentages which suggests the toxic and inhibitory behaviour of 'Parthenin' which is the principal component of growth inhibitors. Similar reduction in germination and survival has been documented by Narwal (1994), Oudhia *et al.* (1998) and Kumar *et al.* (2004). Zackrisson and Nilsson (1992), suggested that low doses and short exposure times of seeds to leachates have strong negative effects on germination and early root development. Jadhav *et al.* (1995), also showed the effect of *Casuarina* leaf litter leachates on germination and seedling growth of rice and cowpea.

Various morphological changes induced at different treatments of *Parthenium* leaves have been tabulated in Table 3. Data regarding plant height showed an increase in plant height at lower doses upto 100 g which reduced gradually at higher dose and recorded as lowest (88.34 cm) at highest dose of 500 g. However, other growth parameters, such as stem diameter, leaf length and breadth, head diameter also rendered increase in size at lower doses while decreased at higher doses. These parameters also exhibited an exciting and unusual variation from general trend *i. e.*, all these were found to increase drastically in size at highest dose of 500 g.

Increase in morphological parameters at lower doses suggests stimulatory effect of some allelochemicals in lower amount. The probable cause of this is not clear but as sunflower also imparts allelopathic effects it might have suppressed the allelopathic effect of *Parthenium* at lower doses. Similar results have been presented by Yang *et al.* (2002). It is also well known that compost made from chopped leaves is a good source of nutrients because allelochemicals present, also act as a growth regulator. Despite of this, at higher doses allelopathic compounds present in *Parthenium* might have affected the growth parameters and reduction in photosynthesis subsequently resulting in reduced morphology due to its high amount.

Table - 2: Various meiotic abnormalities induced through allelochemicals released by *Parthenium*

Treatment	Total no. of PMC_s scored	Total no. of ABN. cells	Metaphasic abnormalities							Anaphasic abnormalities						Total abnormality percentage
			UN	MV	ST	SC	PM	SA	OTH	LG	ST	BG	NS	DP	OTH	
Control	2542	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50 g	2678	105	-	-	0.56	0.45	0.41	0.34	0.30	0.41	0.45	-	0.37	0.37	0.26	3.92
100 g	2548	198	-	-	1.22	1.02	0.94	0.61	0.53	0.90	0.98	-	0.73	0.69	0.45	8.06
200 g	2550	367	0.47	0.20	2.20	1.88	1.57	0.94	0.78	1.41	1.76	0.2	1.18	1.10	1.71	14.39
300 g	2645	560	0.60	0.38	2.95	2.72	2.34	1.40	1.32	2.12	2.57	0.30	1.81	1.59	1.10	21.17
500 g	2634	682	0.68	0.46	3.34	3.04	2.73	2.05	1.78	2.58	2.89	0.38	2.35	2.28	1.33	25.89

ST = Stickness, PM = Precocious movement, UN = Univalents, SA = Secondary association, DP = Disturbed polarity, OTH = Other abnormalities, MV = Multivalent, LG = Laggard, BG = Bridge, SC = Scattering, NS = Non-synchronous division, PMCs = Pollen mother cells, ABN = Abnormal

Table - 3: Various morphological changes induced at different treatments of *Parthenium* leaves

Treatment	Statistics	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf breadth (cm)	No. of nodes	Days to 50% flowering	Head diameter (cm)	No. of seeds/head	Seed weight (g)
Control	Mean	98.36±1.23^{d}	4.50±0.05^{c}	13.94±0.35^{c}	9.28±0.07^{d}	26.60±0.75^{c}	45.40±0.40^{d}	15.22±0.19^{c}	151.80±2.72^{a}	4.38±0.06^{a}
	S. D	2.75	0.10	0.78	0.16	1.67	0.89	0.43	6.10	0.13
	VAR	7.59	0.01	0.61	0.03	2.80	0.80	0.18	37.20	0.02
	C.V	2.80	2.22	5.59	1.77	6.29	1.97	2.80	4.02	2.98
50 g	Mean	107.4±1.63^{c}	4.58±0.06^{b}	14.10±0.37^{c}	9.34±0.09^{c}	27.40±0.98^{b}	46.20±0.58^{c}	16.16±0.34^{b}	154.60±3.21^{a}	4.28±0.06^{b}
	S. D.	3.65	0.13	0.83	0.21	2.19	1.30	0.76	7.20	0.13
	VAR	13.30	0.02	0.69	0.043	4.80	1.70	0.57	51.80	0.02
	C.V	3.40	2.85	5.87	2.22	8.00	2.82	4.68	4.66	3.05
100 g	Mean	121.52±1.63^{a}	4.72±0.06^{a}	14.84±0.42^{b}	9.46±0.09^{b}	28.40±1.03^{a}	46.80±0.58^{c}	16.46±0.36^{b}	160.60±4.40^{a}	4.22±0.07^{c}
	S. D	3.65	0.13	0.93	0.21	2.30	1.30	0.81	9.86	0.15
	VAR	13.33	0.02	0.87	0.04	5.30	1.70	0.66	97.30	0.02
	C.V	3.00	2.76	6.28	2.19	8.11	2.79	4.93	6.14	3.51
200 g	Mean	114.86±1.69^{b}	4.62±0.06^{b}	14.46±0.49^{bc}	9.42±0.10^{b}	27.80±1.02^{b}	47.20±0.73^{bc}	16.34±0.40^{b}	133.20±4.18^{b}	4.18±0.06^{c}
	S. D	3.79	0.13	1.09	0.22	2.28	1.64	0.89	9.36	0.13
	VAR	14.34	0.02	1.19	0.05	5.20	2.70	0.80	87.70	0.02
	C.V	3.30	2.82	7.54	2.30	8.20	3.48	5.47	7.03	3.12
300 g	Mean	94.00±1.68^{d}	4.32±0.06^{d}	13.60±0.43^{d}	9.24±0.09^{d}	26.40±1.03^{c}	47.60±0.93^{b}	14.06±0.32^{d}	124.60±4.18^{c}	4.08±0.07^{d}
	S. D	3.77	0.13	0.97	0.21	2.30	2.07	0.71	9.37	0.16
	VAR	14.22	0.02	0.94	0.04	5.30	4.30	0.50	87.80	0.03
	C.V	4.01	3.02	7.13	2.24	8.72	4.36	5.04	7.52	4.03
500 g	Mean	88.34±1.72^{e}	4.78±0.08^{a}	15.08±0.51^{a}	9.62±0.10^{a}	26.20±1.11^{c}	48.20±1.02^{a}	17.08±0.43^{a}	94.80±3.43^{d}	4.06±0.07^{d}
	S. D	3.84	0.18	1.14	0.22	2.49	2.28	0.95	7.69	0.17
	VAR	14.78	0.03	1.29	0.05	6.20	5.20	0.91	59.20	0.03
	C.V	4.35	3.74	7.54	2.25	9.50	4.73	5.59	8.12	4.12

Note: Mean values within a group followed by same letters are not significantly different at the 5% level of LSD test

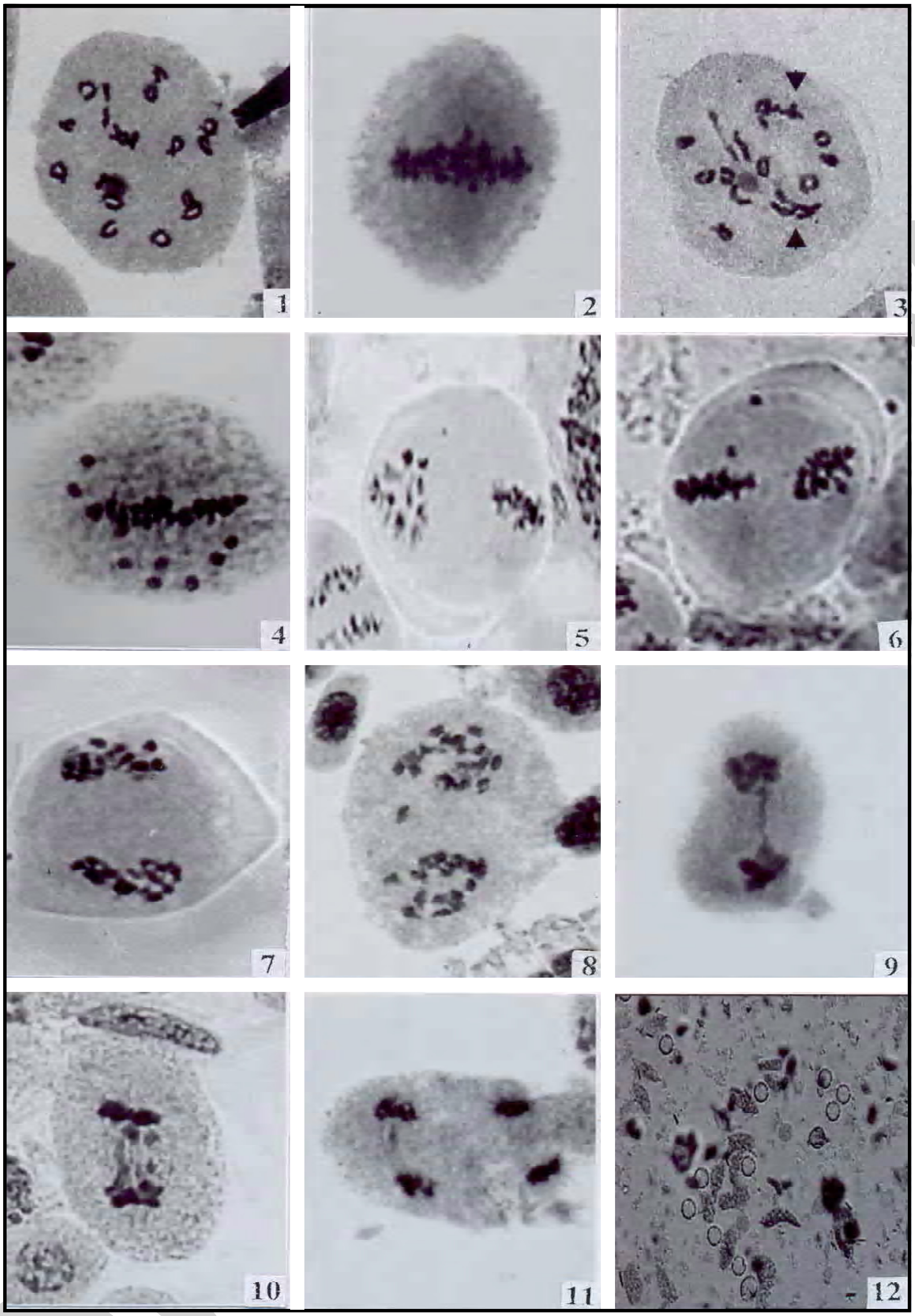

Plate 1 : Photograph of control and treated meiotic cells of *Helianthus*

Control -
Fig. 1: Diakinesis with 17 bivalents
Fig. 2: Normal Metaphase
Treated -
Fig. 3: Diakinesis with chain multivalents (shown by arrow head)
Fig. 4: Precocious movement with eight univalents at metaphase – I
Fig. 5: Non-synchronous division
Fig. 6: Precocious movement at metaphase – II
Fig. 7: Stickiness at anaphase – I
Fig. 8: Lagging chromosomes at anaphase – I
Fig. 9: Stickiness with bridge formation at anaphase – I
Fig. 10: Multiple bridges with stickiness of chromosomes at anaphase – I
Fig. 11: Bridge at telophase – II
Fig. 12: Sterile (unstained) and fertile (stained) pollen grains

The possible mechanism of sudden increase in some growth parameters at highest dose is difficult to trace out because the action on physiological target site for all allelochemicals is not common and certain. These allelochemicals may act synergistically or antagonistically on the other plants. Studies regarding allelopathic effect of plants on other plants and their probable mechanisms are still scanty and in continuation. So, it would be complicated and challenging to mark out the appropriate cause of such results. However, this might be due to some interaction between allelochemicals produced by sunflower and *Parthenium* and their synergistic or antagonistic interaction at different concentrations of allelochemicals on some morphological parameters. Allelopathic effect of sunflower extract on mustard seed germination and seedling growth has been demonstrated by Bogatek (2006).

Days to 50% flowering was also found to be delayed at higher doses while early flowering was observed at lower doses as compared to control. Plants at 500 g dose were found to bloom lately as compared to all other doses. Counting of number of seeds/head exhibited an increase at lower doses upto 100 g as compared to control but reduced at higher doses. The seed weight of the treated sets was much reduced and the maximum seed weight was registered as 4.28 g at 50 g treatment dose of *Parthenium* leaves. An increase in total number of seeds/head at lower doses can be attributed to increase in head diameter. Although head diameter was largest at highest dose, still yield was lowest which might be attributed to decrease in pollen and ovule fertility at higher doses. Significant mean values of pollen and ovule fertility have been shown in Table 1.

Despite the fact that *Parthenium* is considered a toxic plant, industrial uses are reported in the literatures (Sastry and Kavathekar, 1990). From the above study enhancement in quantitative characters was observed which leads to the conclusion that the allelochemicals released may be exploited for the betterment of the crop plants. Also, understanding of mechanism, identification and isolation of allelochemicals may provide a basis for development of growth regulators and natural pesticides to boost up production in sustainable agriculture.

Acknowledgments

One of the author (N. G.) is thankful to CSIR for financial assistance in form of SRF. Sincere thanks are also due to other laboratory members for their support.

References

Batish, D. R., P. Tung, H. P. Singh and R. K. Kohli: Phytotoxicity of sunflower residues against some summer season crops. *J. Agron. Crop Sci.,* **188**, 19 (2002).

Belling, J.: The iron- aceto- carmine method of firing and staining chromosomes. *Bil. Bull.,* **150**, 160-162 (1926).

Bogatek, R., A. Gniazdowska, W. Zakrzewska, K. Oracz and S.W. Gawro Dski: Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biologia Plantarum,* **50**, 156-155 (2006).

Chaniago, I., Acram Taji and Robin Jessop: Assessment of possible allelopathic interactions between soybean (*Glycine max*) and *Amaranthus powellii* and *Cyperus rotundus* using *in vitro* systems. 13th Australian Agronomy Conference, Regional Institute Ltd. Publishers, Perth, WA (2006)

Einhellig, F.A.: The physiology of allelochemical action: Clues and views. *In*: Allelopathy, from molecules to ecosystems *(Eds.*: M.J. Reigosa and N. Pedrol). Science Publishers, Enfield, New Hampshire (2002).

Fetene, M. and S. Habtemariam: Investigations on allelopathic properties of coffee (*Coffea arabica*.) leaves, pulp and tree canopy soil. *J. Sci. Sinet, Ethiopian,* **18**, 51-65 (1995).

Hegazy, L., K.S. Mansour and N.F. Abdel-Hady: Allelopathic and autotoxic effects of *Anastatica hierochuntica*. *J. Chem. Ecol.,* **16**, 2183-2193 (1990).

Jadhav, B.B. and D.G. Gaynar: Effect of *Casuarina equisetifolia* leaf litter leachates on germination and seedling growth of rice and cowpea. *J. Allelopathy,* **2**, 105-108 (1995).

Kruse, M., M. Strandberg and B. Strandberg: Ecological effects of allelopathic plants - A review. National Environmental Research Institute - NERI Technical Report No. 315. Silkeborg, Denmark (2000).

Kumar, R., V. Sharma, R. Saikia and G. Kumar: Allelopathic effects of *Parthenium hysterophorus* leaves on morphology and cytology of *Trigonella foenum-graecum* L. *Res. Crops,* **5**, 246-251 (2004).

Narwal, S.S.: Allelopathy in crop production. Scientific Publishers. Jodhpur. pp. 111-112 (1994).

Ortega, R.C., A.L. Anaya and L. Ramos: Effects of allelopathic compounds of corn pollen on respiration and cell division of watermelon. *J. Chem. Ecol.*, **14**, 71-86 (1988).

Oudhia, P. and R.S. Tripathi: Allelopathic effects of *Parthenium hysterophorus* L. on kodo, mustard and problematic weeds. *In*: Proceeding First International Conference on *Parthenium* Management, Society for *Parthenium* management, 6-8 October. Vol. II. Publishers UAS, Dharwad, 2, 136-139 (1998).

Pratley, J.E.: Silvergrass residue effects on wheat. Proceedings of the 5th Australian Agronomy Conference, Regional Institute Ltd. Publishers, Perth. pp. 472 (1989).

Pratley, J.E. and J.D. Ingrey: Silvergrass allelopathy on crop and pasture species. Proceedings of the 9th Weed Conference, Regional Institute Ltd. Publishers, Adelaide. pp. 436-439 (1990).

Rawat, M.S., M. Geeta, P. Devi, R.K. Joshi, C.B. Pande, G. Pant and D. Prasad: Plant growth inhibitors (proanthocyanidins) from *Prunus armeniaca*. *Biochem. System. Ecol.,* **26**, 13-23 (1998).

Rizvi, S.J.H., M. Tahir, V. Rizvi, R.K. Kohli and A. Ansari: Allelopathic Interactions in Agroforestry Systems. *Crit. Rev. Plant Sci.,* **18**, 773-779 (1999).

Sastry, C.S. and K.Y. Kavathekar: Plants for Reclamation of Wastelands. Publications and Information Directorate, CSIR, New Delhi (1990).

Saxena, A. and A.K. Sharma: Allelopathic potential of *Acacia tortilis* in agroforestry systems of arid regions. *J. Allelopathy,* **3**, 81-84 (1996).

Setia, N., D.R. Batish, H.P. Singh and R.K. Kohli: Phytotoxicity of volatile oil from *Eucalyptus citriodora* against some weedy species. *J. Environ. Biol.,* **28**, 63-66 (2007).

Tomita-Yokotani, K., Takako Kato, Hirofumi Hashimoto and Masamichi Yamashita: Response of allelochemicals under pseudo-microgravity in sunflower plant (*Helianthus annuus* L. cv. Taiyo). *Biol. Sci. Space,* **19**, 143-147 (2005).

Verma, M. and P.B. Rao: Allelopathic effect of four weed species extracts on germination, growth and protein in different varieties of *Glycine max* (L.) Merrill. *J. Environ. Biol.*, **27**, 571-577 (2006).

Yang, Chi-Ming, Chyoung - Ni Lee and Chang-Hung Chou: Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: I. Inhibition of supply-orientation. *Bot. Bull. Acad. Sin.,* **43**, 299-304 (2002).

Zackrisson, O. and M.C. Nilsson: Allelopathic effects by *Empetrum hermaphroditum* on seed germination of two boreal tree species. *Can. J. For. Res.*, **22**, 1310-1319 (1992).