Indian J. Weed Sci. **36** (1 & 2) : 99-103 (2003)

Allelopathic Effect of Basil (*Ocymum sanctum*) Materials on the Germination of Certain Weed Seeds*

Shiv D. Sharma[1] and Megh Singh
Citrus Research and Education Center
University of Florida, IFAS, 700 Experiment Station Road, Lake Alfred, Florida, USA

ABSTRACT

Allelopathic effect of basil (**Tulsi**) (*Ocymum sanctum*) on the germination of some weed species was evaluated. The germination of radish, redroot pigweed, hairy beggarticks and guineagrass was completely inhibited with addition of 7.5 g basil leaf powder to 100 g of sand as compared to plants grown in sand alone or in a mixture of sand and sphagnum. The germination of seeds was significantly inihibited in redroot pigweed (13%) and hairy beggarticks (12%) when grown in 10% (w/v) basil leaf extract as compared to distilled water. Significantly lower germination of 58, 47 and 45% with basil stem+root extract (at 2.5% w/v) was recorded, respectively, in radish, redroot pigweed and hairy beggarticks. The length of radicle+root in case of redroot pigweed and hairy beggarticks was significantly lower in basil stem+root extract (2.5% w/v) than in distilled water and was significantly reduced further when the extract concentration was increased to 5% (w/v).

INTRODUCTION

Allelopathic interactions between plants play a crucial role in natural ecosystems (Rizvi *et al.*, 1992). The allelopathic potential of certain plant species has been exploited in biological control programmes, which can influence the growth and distribution of weed species. Due to the complex nature of the ecosystem and interaction of abiotic and biotic factors, it is very difficult to demonstrate allelopathy in field situations (Inderjit and Foy, 2000). The visible effects of allelochemicals on plant processes are only secondary signs of primary changes. Therefore, studies on the effects of allelochemicals on germination and/or growth are only the manifestation of primary effects occurring at the molecular level.

Bioassay is an integral procedure in allelopathy studies but the difficulty has been the lack of standardized bioassays, including incomplete information on the allelochemicals source, method of extraction, fractionation concentrations, and the absence of comparisons with known compounds. The most widely used bioassay to test for allelopathic activity is the inhibition (or sometimes stimulation) of seed germination. Properly conducted seed-germination bioassay has great value as they are simple, rapid, and require relatively small volumes (3-10 ml) of solution depending on the seed and substrate used for absorption. Their sensitivity varies according to test species and allelochemicals, and it is less than that of the other bioassays methods (Leather and Einhelling, 1985).

Basil, a native to warm climates belonging to mint family, is fragrant and aromatic and the sweet basil (*Ocimum basidium* L.) is used in cookery. Another species, Holy basil (*Ocymum sanctum* L.) is a tropical species found in eastern hemisphere. It has medicinal value and, therefore, it was interesting to examine, if basil has some allelopathic effects and hence its effect on the germination of certain

*Florida Agricultural Journal Series No. R-07715.
[1]CCSHAU Regional Research Station, Karnal-132 001 (Haryana), India.

broadleaf and grass weeds under controlled conditions.

MATERIALS AND METHODS

Basil plants were grown in one gallon size plastic pots in commercial potting medium [Metro-Mix 500 (Grace Sierra Company, USA) contained Canadian sphagnum peat moss, horticultural vermiculite, processed bark ash and composed pine bark], in a greenhouse under controlled temperature 25/16°C (±0.5%) day/night, 70% (±5%) relative humidity and normal daylight conditions. The plants were fertilized as required with Nutrileaf fertilizer containing 20 N-20 P_2O_5-20 K_2O. When required, fresh basil leaves were stripped off from the mature plants and utilized. The germination bioassays of weed seeds–radish (*Raphanus sativus* L.), redroot pigweed (*Amaranthus retroflexus* L.), hairy beggarticks (*Biden pilosa* L.) and guineagrass (*Panicum maximum* Jacq.) were conducted to determine the allelopathic effect of basil plant. Unless stated otherwise, 25 seeds of each weed were placed on a layer of two filter papers with the test solution in separate petri dishes. Test solution extracts were applied uniformly to the entire filter paper disk to avoid chromatographic separation of components because filter paper binds the components of extracts differentially, and the germination of seeds depends upon their proximity to the point of extract application (Muller *et al.*, 1964). Petri dishes were incubated in growth cabinets at 25°C (±0.5) temperature and 70% (±5) relative humidity. The germination was observed as the emergence of the radicle 2 mm beyond the seed coat and was scored over a period of time upto two weeks depending on species.

The potting medium in which basil nursery was raised in a tray considered as basil treated soil. A preliminary greenhouse experiment was conducted to examine the effect of basil treated soil on the germination of weed seeds planted in the Styrofoam cups containing the basil treated mix. The effect of basil treated mix on the germination of weed seeds was compared with their germination in normal potting mix. The seedlings were allowed to grow for five weeks.

The basil leaves stripped from mature plants were dried for a week to 10 days at room temperature inside a laboratory on the bench. The dried leaves were ground in a Wiley Sample Mill (mesh screen sizes of 1 mm) to make powder. Different amounts of basil powder e. g. 2.5, 5 and 7.5 g were weighed and thoroughly mixed in 100 g of sand kept in Styrofoam cups (16 oz) wihout any provision for drainage. Parallel check treatments were prepared by adding similar weight e. g. 2.5, 5 and 7.5 g of ground sphagnum moss to 100 g sand to provide the same amount of soil organic matter another check was kept as only sand. Fifteen seeds of the test weed species were placed 0.5 cm below the soil surface. Cups were watered as necessary and 20 ml of 1% (w/v) of Nutrileaf fertilizer (20 N-20 P_2O_5-20 K_2O) was added once every week. The cups were kept in the greenhouse under controlled conditions previously described. The germination and seedling growth were recorded every week for four weeks after treatment application. The plants were pulled from pots and length of shoot+roots was measured.

Fresh basil leaves were homogenized and liquified in distilled water using a Blender (Osterizer) and a concentration range of 0, 2.5, 5, 7.5, 10 and 12.5% (w/v) prepared. Extracts were filtered by vacuum filtration on a Buchner funnel with No. 2 Whatman filter paper. Twenty-five seeds of each tested weed species were placed on a layer of two filter papers in separate petri dishes (9.5 cm diameter) and 5 to 7 ml of extract was added. The petri dishes were kept in growth cabinets under conditions described above except that no light was provided. Radicle+root lengths were measured after 7 to 14 days depending on the species. Root length was included as it was difficult to separate it from radicle.

After picking the leaves from basil, the plants were uprooted from plastic pots and their roots

were washed in running water. Fresh stem and root's portions were homogenized and liquified in distilled water using a Blender and a concentration range of 0, 2.5, 5 and 7.5% (w/v) prepared. Extracts were filtered by vacuum filtration on a Buchner funnel with No. 2 Whatman filter paper. Seeds of each weed were placed in separate petri dishes together with 5-7 ml of test solution and petri dishes were incubated in darkness in the growth cabinet as mentioned earlier. Radicle+root lengths were measured after 7 to 14 days depending on the weed species. The average radicle+root length of germinated seed was used for statistical analysis.

The experiments were designed as a randomized complete block with four replications, and were repeated twice. The data from two experiments were combined to present the mean values after performing a test of homogeneity of the variance. Individual weed species were analyzed and the data were subjected to ANOVA after performing an arcsine transformation but are presented in the original form for clarity; the means were separated using Fisher's protected least significant difference test (LSD at $P \leq 0.05$). All statistical analyses were performed using the ARM statistical software programme (Gylling Data Management, Inc., SD).

RESULTS AND DISCUSSION

Effects of Basil Treated Soil on Germination

In the preliminary study, germination and young seedlings of some grassy and broadleaf weeds grown in basil treated potting mix were adversely affected as compared to when grown in normal potting mix (data not presented). Inhibition of germination of weed seeds grown and seedling growth was possibly because of the release of some phenolic compound (s) in the soil from basil and thus affecting the germination.

Basil Leaf Powder Bioassay

The germination of test weed seeds was significantly inhibited by the presence of basil leaf powder in sand as compared to when growth in sand+sphagnum or sand alone (Table 1). The germination of radish seed was 82% in sand, $\geq$ 92% in sphagnum+sand; it was reduced to 67% in sand that contained 2.5 g of basil powder and to only $\leq$ 8% when basil powder was increased to 5 g or higher. Significantly lower germination of $\leq$ 3, $\leq$5 and $\leq$ 7%, respectively, in redroot pigweed, hairy beggarticks and guineagrass was obtained when

Table 1. Effect of basil powder on the germination of weed seeds and shoot+root length four weeks after planting

Treatment	Radish		Redroot pigweed		Hairy beggarticks		Guineagrass	
	Germination (%)	Shoot+ root length (cm)	Germination (%)	Shoot+ root length (cm)	Germination (%)	Shoot+ root length (cm)	Germination (%)	Shoot+ root length (cm)
Control*	82a	6.9b	43a	3.5c	17ab	4.3d	13abc	8.8cd
Sphagnum (2.5 g)**	93a	8.1a	23b	5.8b	20ab	5.5c	20ab	9.5c
Sphagnum (5.0 g)	92a	8.7a	32ab	6.4a	25a	6.3b	15abc	11.5b
Sphagnum (7.5 g)	93a	8.9a	33ab	6.6a	18ab	7.0a	23a	12.4a
Basil (2.5 g)**	67b	5.8c	03c	3.1c	05ab	2.8e	07bc	8.3d
Basil (5.0 g)	08c	5.5c	00c	0.0d	05ab	2.6e	03c	5.3e
Basil (7.5 g)	02c	5.3c	00c	0.0d	00b	0.0f	00c	0.0f
LSD (P=0.05)	13	0.8	12	0.5	13	0.4	11	0.8

*Only sand without organic matter; **Sphagnum/basil powder was mixed in 100 g of sand.
Values with similar letters did not differ significantly according to Fisher's least significant test (LSD) (P=0.05).

basil powder (2.5 to 7.5 g) was added to sand, as compared to when grown in sand with or without sphagnum moss (Table 1). This significant reduction in germination of test species could be the result of the presence and/or release of phenolic compounds from basil leaf powder. The shoot height of all weed seedlings, grown in sand only, was shorter than grown in sand+sphagnum treatments and it was significantly less when basil powder was added to sand (data not presented). The length of a shoot+root was significantly lower in a radish (≤5.8 cm), redroot pigweed (≤3.1 cm), hairy beggarticks (≤2.8 cm), and guineagrass (≤8.3 cm) grown in sand containing basil powder (2.5 to 7.5 g) as compared to either only and or sand+sphagnum moss (Table 1). Seedlings of redroot pigweed, hairy beggarticks and guineagrass showed signs of chlorosis (whitish) possibly not absorbing any nutrients into the plant system during 3rd and 4th week of growth. Later seedlings were wilted and then dead after 3rd week in the treatments which contained ≥5 g of basil powder. These effects of basil powder were possibly due to the release of allelochemicals after decaying (Chou and Patrick, 1976).

Fresh Basil Leaf Extract Bioassay

There was no germination of guineagrass seed in either the test solution of fresh basil leaf extract or distilled water (check). Although the germination of radish seed was reduced when incubated in different concentrations of basil test solution, there was no significant difference between the check and test solutions (Table 2). The germination of seeds of redroot pigweed and hairy beggarticks was significantly reduced when these were incubated in different basil test solutions. Lowest germination of redroot pigweed (13%) and hairy beggarticks (12%) was recorded in 10% basil treatment (Singh *et al.*, 1989). In this study, the length of radicle+root was inhibited by the presence of basil extract in all test species but the difference between the treatments was not significant except for guineagrass where no germination was recorded (Table 2). The reasons for lack of germination in guineagrass remained unknown.

Table 2. Effect of fresh basil leaf extract on the germination of weed seeds and on radicle+primary root length

Treatment	Radish		Redroot pigweed		Hairy beggarticks		Guineagrass	
	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)
Control*	87a	3.0a	57a	2.5a	67a	1.4a	0	0
Basil (2.5%)**	60a	2.0ab	47a	2.4a	40b	0.9a	0	0
Basil (5.0%)	65a	1.6a	17b	2.1a	25c	0.5a	0	0
Basil (7.5%)	75a	2.6ab	15b	0.6a	23c	0.6a	0	0
Basil (10%)	68a	2.4ab	13b	0.8a	12c	0.6a	0	0
LSD (P=0.05)	18	1.0	16	1.5	11	0.7	0	0

*Only distilled water; **Extract prepared in distilled water from fresh basil leaf.
Values with similar letters did not differ significantly according to Fisher' least significant test (LSD) (P=0.05).

Fresh Stem+Root Extract Bioassay

Test solutions extracted from fresh basil stem+roots inhibited the germination of different weed species (Waller *et al.*, 1986). The germination of radish seeds was 87% in check solution (distilled water), while it was significantly reduced to 58% in 2.5% (w/v) basil test solution (Table 3). The germination of radish seeds was further

reduced with higher concentration of basil test solution but the reduction was not significant. The germination rates of redroot pigweed and hairy beggarticks seeds were 70 and 64% in check solution and reduced to 47 and 45% in 2.5% (w/v) basil extract, respectively. The germination was further reduced significantly with the increase in basil test solution concentration. The lowest value of germination in redroot pigweed was 33% and in hairy beggarticks 24% in 7.5% (w/v) basil test solution. There was no germination of guineagrass seed in either of the solution, check or test. Similarly, the length of radicle+root was significantly lower in basil test solutions than in the check solution in case of redroot pigweed and hairy beggarticks only (Table 3).

Table 3. Effect of fresh basil stem+root extract on the germination of weed seeds and on radicle+primary root length

Treatment	Radish		Redroot pigweed		Hairy beggarticks		Guineagrass	
	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)	Germination (%)	Radicle+ root length (cm)
Control*	87a	2.9a	70a	2.6a	64a	1.6a	0	0
Basil (2.5%)**	58b	2.2b	47ab	1.9b	45b	1.1b	0	0
Basil (5.0%)	47b	1.5b	53ab	1.2c	35c	0.6c	0	0
Basil (7.5%)	42b	1.5b	33b	0.9c	24d	0.4c	0	0
LSD (P=0.05)	15	0.6	20	0.5	9	0.2	0	0

*Only distilled water; **Extract prepared in distilled water from fresh basil stem and roots.
Values with similar letters did not differ significantly according to Fisher's least significant test (LSD) (P=0.05).

Further research will be required to identify the presence of allelochemicals in basil residues and different extracts to confirm their inhibitory effect on the biological activities of the test plants.

REFERENCES

Chou, C. H. and Z. A. Patrick, 1976. Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. *J. Chem. Ecol.* **2** : 369-387.

Inderjit and C. L. Foy, 2000. Allelopathy : Past achievements and future approach. *Abstr. Weed Sci. Soc. Am.* **40** : 123-124.

Leather, G. R. and F. A. Einhelling, 1985. Mechanisms of allelopathic action in bioassay. In : *The Chemistry of Allelopathy*, A. C. Thompson (ed.). Washington, DC : American Chemical Society. pp. 197-205.

Muller, C. H., W. H. Muller and B. L. Haines, 1964. Volatile growth inhibitors produced by shrubs. *Science* **143** : 471-473.

Rizvi, S. J. H., H. Haque, V. K. Singh and V. Rizvi, 1992. A discipline called allelopathy, pp. 1-8. In : *Allelopathy : Basics and Applied Aspects*, S. J. H. Rizvi and V. Rizvi (eds.). Chapman and Hall, London.

Singh, M., R. V. Tamma and H. N. Nigg, 1989. HPLC identification of allelopathic compounds from *Lantana camara. J. Chem. Ecol.* **15** : 81-89.

Waller, G. R., D. Kumari, J. Friedman, N. Friedman and C. H. Chou, 1986. Caffeine autotoxicity in *Coffea arabica* L. In : *The Science of Allelopathy*, A. R. Putnam and C. S. Tang (eds.). Wiley-Interscience, New York.