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Tolerance to Allelopathy by Effective Microorganisms (EM) in Chickpea (*Cicer arietinum* L.)

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Abstract

Aqueous leaf extract of *Syzygium cumini* (L.) Skeels significantly reduced the root and shoot growth, pod yield and up of nitrogen and phosphorus in chickpea (*Cicer arietinum* L.). Effective Micro-organisms (EM) application provided sufficient relief to chickpea against allelopathic stress due to aqueous leaf extract. Consequently the root and shoot growth, pod and shoot nitrogen content of EM inoculated plants were significantly enhanced as compared to non-inoculated plants. However, adverse impact of extract on shoot phosphorus content remained unchanged by EM application.

Introduction

The phenomenon of allelopathy, where one plant exerts a detrimental influence on another through the production of germination and growth inhibiting substances has been widely reported (May and Ash, 1990; Hussain and Abidi, 1991; Kil and Yun, 1992; Noor and Khan, 1994). It is an important ecological process in vegetational composition (Muller, 1969) and agricultural sciences (Tukey, 1969). Chemicals involved in such interactions are phenols, glucosides, amino acids, alkaloids, terpenes and sugars (Naqvi, 1976; Harborne, 1977; Hussain and Abidi, 1991). These chemicals may be present in leaves, stems, roots, rhizomes, flowers, fruits, seeds and trichomes (Horsley, 1977).

Higa *et al.* (1984) introduced EM Technology of nature farming, which is based on the concept of using beneficial microorganisms found in natural conditions. EM stock solution consists of photosynthetic bacteria, *Azotobacter*, *Streptomyces* and *Lactobacillus* spp. They increase crop growth and yield by increasing photosynthesis, nitrogen fixation, controlling soil diseases and accelerating decomposition of lignin material in the soil (Hussain *et al.*, 1994). Experiments conducted on various crops of economic importance like wheat, maize, rice, pea, mungbean, potato etc. have shown good prospects for practical applications of EM (Ayub *et al.*, 1993; Chaudhary and Iqbal, 1993; Hussain *et al.*, 1993; Ibrahim *et al.*, 1993; Minami and Higa, 1994; Sangakkara and Higa, 1994). However, the earlier studies were conducted under normal field conditions and the studies regarding the use of EM under stressed conditions has not yet received any attention. The present paper describes the usefulness of EM in improving crop growth and yield, and uptake of nitrogen and phosphorus in chickpea (*Cicer arietinum* L.) under allelopathic stress caused by aqueous leaf extract of *Syzygium cumini* (L.) Skeels. *S. cumini* is a medium size tree grown in Pakistan for its shade, timber and fruits. The aqueous leaf extract of this tree exhibits allelopathic activity (Haneef, 1996).

Materials and Methods

Experiment was conducted in pots (25 cm diameter, 30 deep). Chickpea was grown in heat sterilized soil, amended with 1:1 mixture of green manure and farmyard manure 5 g/100 g of soil. The treatments applied were control, allelopathy and EM+allelopathy. Each treatment replicated thrice.

Aqueous leaf extract of *Syzygium cumini* was used to render the pot soil allelopathic. The leaf extract obtained by soaking 100 g of chopped leaves in 1000 g distilled water for 5 hours at 25°C and filtered. Each plot of the respective treatments was supplied with 100 ml of filtrate before sowing and further repeated with an interval of 3 weeks.

EM was applied at a 1:500 dilution one day before sowing at 500 ml/pot. Further applications were employed throughout the study period. EM stock solution obtained from Nature Farming Research Center, University of Agriculture, Faisalabad.

Six surface sterilized seeds of chickpea were sown in pot and finally 3 uniform plants were maintained. The treatment was in triplicate. Pots were placed in a netting chamber under natural conditions of temperature and light.

Plants were harvested after 45, 60 and 75 days of sowing. The following parameters were measured:

- i) Root and shoot length (cm)
- ii) Fresh and dry weight (g) of root and shoot
- iii) Number and fresh and dry weight (g) of pods
- iv) Shoot nitrogen (%) and phosphorus (ppm) content at 75 days growth.

The data was subjected to statistical analysis by the Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results

Roots of chickpea gave variable growth response under different treatments. Maximum and significantly grain

Table 1: Effect of EM (Effective Microorganisms) on root and shoot growth, and pod yield of chickpea under allelopathic stress caused by aqueous leaf extract of *Syzygium cumini*

Treatments	Root			Shoot			Pod		
	Length (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
45 days after sowing									
Control	25c	4c	2,18c	36a	5,8a	3,2a	0	0	0
ALES	18,5d	1,2d	1 a	29c	3,2c	1,9c	0	0	0
EM	31,5a	6a	4,05a	34,5b	4,6b	2,6b	0	0	0
EM + ALES	29,5b	4,2b	4,75b	36,2a	5,8a	3,3a	0	0	0
60 days after sowing									
Control	26c	5,5b	2,5bc	40b	6,6b	3,9b	2c	0,3d	0,09d
ALES	20,1d	2,1d	1 ,3c	37d	5c	3c	8a	1,3b	0,35b
EM	36a	6,5a	4,5a	45a	8,3a	5a	8a	1,6a	0,41a
EM + ALES	30b	5c	2,9b	38c	6bc	3,6bc	5b	0,8c	0,22c
75 days after sowing									
Control	27c	6,1b	3,5b	46ab	7,1b	4,2b	5b	1,4b	0,28b
ALES	22d	2,3d	1,6c	33c	6c	3,8c	4c	0,6cd	0,19c
EM	37,5a	66,8a	4,5a	50a	8,7a	5,8a	4c	0,9c	0,23b
EM + ALES	31,5b	55,c	3,15 be	42b	7b	4,1b	7a	1,3a	0,35a

($P < 0.05$) root length was observed in EM alone treated plants. Plants inoculated with aqueous leaf extract of *Syzygium* (ALES) showed a significant reduction in root length than control. However, EM application significantly reduced the adverse effects of ALES at all the growth stages i.e. 45, 60 and 75 days after sowing. Root fresh and dry weight showed a trend similar to that of root length (Table 1).

The shoot growth in terms of length, and fresh and dry weight of shoot showed a persistent negative response to ALES application. The difference with control was significant ($P < 0.05$). EM application to ALES introduced plants increased the shoot growth significantly. Maximum shoot growth at all the growth stages was recorded in EM treated plants (Table 1).

Table 2: Effect of Effective Microorganisms (EM) on shoot phosphorus and Nitrogen contents of 75 days old chickpea plants, under allelopathic stress caused by aqueous leaf extract of *Syzygium cumini*.

Treatments	Phosphorus Content (ppm)	Nitrogen content (%)
Control	1,95	1,4
ALES	1 A	1,3
EM	2,1	1,8
EM + ALES	1,35	1,5

ALES - aqueous leaf extract of *Syzygium cumini*

After 60 days growth highest pod yield in terms of pod number, and fresh and dry biomass was recorded in plants inoculated either with EM or ALES followed by combined application of EM and ALES. All the treatments were

significantly superior to control ($P < 0.05$). However, after 75 days growth, combined inoculation of EM and ALES out yielded the other treatments (Table 1).

Shoot phosphorus contents were reduced significantly by the application of ALES whereas nitrogen contents were decreased non-significantly. Adverse effects of ALES on nitrogen uptake were significantly reduced by EM application whereas phosphorus contents remained unchanged (Table 2).

Discussion

Aqueous leaf extract of *S. cumini* reduced the growth of roots, shoots and pods. Shoot phosphorus and nitrogen contents were also adversely affected by the extract. EM application reverted the effect of extract on the growth of root, shoot and pods. Adverse effects of the extract on shoot nitrogen content were also reduced by the application of EM while the phosphorus content remained unchanged. Since roots were in direct contact with the soil, they showed abnormal growth in response to allelochemicals present in the aqueous leaf extract of *S. Cumin* resulting in reduced growth as compared to control. This reduction may be attributed to the reduced rate of cell division of the root cells due to the activity of the allelochemicals (Jensen and Welbourne, 1962; Bukolova, 1971). EM application reduced the adverse effects of the extract probably because of the yeast's activity present in the EM solution. Yeast is known to produce biogenic substances necessary for cell division (Hussain *et al.*, 1994). There is also a possibility that the allelochemicals in the extract could be decomposed by microbial activities or be neutralized by the substances produced by microorganisms.

The reduced shoot's N and P contents under allelopathic stress is comparable to the reduced K and N uptake by corn due to the presence of allelochemicals released from Agropyron response (Buchholtz, 1971). EM application significantly enhanced shoot N contents. Lactobacilli in EM solution accelerate the decomposition of organic substances, which are hard to decompose, and thus make the nutrients available to the plants (Hussain *et al.*, 1994). The adverse effects of leaf extract of *S. Cumini* on root growth and nutrient uptake resulted in the reduced shoot growth. EM application reduced this adverse effect and enhanced the shoot growth significantly, probably because of greater uptake of nutrients especially N through a better root system. Furthermore, photosynthetic bacteria in EM solution synthesize vitamins by effectively utilizing sunlight, and also synthesize glucose and amino acids, using secretions from the roots and substances produced when organic matter is decomposed. These substances promote the growth of the plant (Hussain *et al.*, 1994).

The present study reveals that EM has great potential in improving plant growth and yield, and enhancing nutrient uptake particularly nitrogen under allelopathic stress. However, the experiment was conducted in sterilized soil. Under normal field conditions the results may be different because of interactions between EM and soil indigenous microorganisms as indicated by Bajwa *et al.* (1995). Therefore, similar research work should be repeated under field conditions.

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References

Ayub, C.M., M.A. Hunjra, M.N. Malika and A.U. Malik, 1993. Nature farming profitability and sustainable development in vegetable production. Proceedings of the 1st National Seminar and Planning Meeting on Nature Farming, June 28, 1993, University of Agriculture, Faisalabad, Pakistan, pp: 76-82.

Bajwa, R., A. Javaid and Z. Tasneem, 1995. Response of indigenous soil microflora to EM inoculation in Pakistan. *Biota*, 1: 73-79.

Bremner, J.M. and C.S. Mulvaney, 1982. Total Nitrogen. In: *Methods of Soil Analysis: Chemical and Microbiological Properties*, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). American Social Agronomy, Madison, WI., USA., pp: 595-624.

Buchholtz, K.P., 1971. The Influence of Allelopathy on Mineral Nutrition. In: *Biochemical Interactions among Plants*, United States National Committee for the International Biological Program (Eds.). National Academies, Washington, DC., pp: 86-89.

Bukolova, T.P., 1971. A Study of the Mechanism of Action of Water-Soluble Substances of Weeds on Cultivated Plants. In: *Physiological Biochemical Basis of Plant Interactions in Phytocenoses*, Volume 2, Gaidamak, V.M. (Ed.). Naukova Dumka, Kiev, pp: 66-69.

Chaudhary, M.S. and M. Iqbal, 1993. Soil fertility improvement with EM for vegetable crops. Proceedings of the 1st National Seminar and Planning Meeting on Nature Farming, June 28, 1993, University of Agriculture, Faisalabad, Pakistan, pp: 67.

Haneef, B., 1996. Role of EM and VAM in reclamation allelopathically affected soil. M.Sc. Thesis, Pu University, Lahore, Pakistan.

Harborne, J.B., 1977. *Introduction to Ecological Biochemistry*. Academic Press, New York, Pages: 224.

Higa, T., Y. Arakawa, T. Kimura and A. Suzuki, 1984. Studies on application of microorganisms in nature farming. I. Effect of effective microorganisms on the yield and quality of carrots and peanuts. A Special Presentation at Meeting of the Japanese Society for Horticultural Sciences, pp: 7.

Horsley, S.B., 1977. Allelopathic Interference among Plants. II. Physiological Modes of Action. In: *Proceedings Fourth North American Forest Biology Workshop*, Wilcox, H.E. and A.F. Hamer (Eds.). State University of New York, Syracuse, New York, pp: 93-136.

Hussain, F. and N. Abidi, 1991. Allelopathy exhibited by *Imperata cylindrical* (L.) P. Beauv. *Pak. J. Bot.*, 23: 15-25.

Hussain, T., G. Jilani, M. Yaseen and S.N. Akhtar, 1993. Prospects of nature farming with EM technology. Proceedings of the 1st National Seminar and Planning Meeting on Nature Farming, June 28, 1993, University of Agriculture, Faisalabad, Pakistan, pp: 41-49.

Hussain, T., R. Ahmad, G. Jilani and T. Higa, 1994. Applied EM technology. Nature Farming Research Center, University of Agriculture, Faisalabad, pp: 1-6.

Ibrahim, M., M.A. Avais, N. Ahmad and A. Khan, 1993. Effect of effective microorganisms (EM) on wheat. Proceedings of the 1st National Seminar and Planning Meeting on Nature Farming, June 28, 1993, University of Agriculture, Faisalabad, Pakistan, pp: 50-53.

Jackson, M.I., 1962. *Soil Chemical Analysis*. Constable and Co. Ltd., London, Pages: 497.

Jensen, T.E. and F. Welbourne, 1962. The cytotoxic effects of growth inhibitors on excised roots of faba and *Pisum sativum*. *Proc. S.D. Acad. Sci.*, 41: 136-136.

Kil, B.S. and K.W. Yun, 1992. Allelopathic effects of water extracts of *Artemisia princeps* var. *orientalis* on selected plant species. *J. Chem. Ecol.*, 18: 39-51.

May, E.F. and J.E. Ash, 1990. An assessment of the allelopathic potential of *Eucalyptus*. *Aust. J. Bot.*, 38: 245-254.

- Minami, T. and T. Higa, 1994. Kyusei nature farming in Japan: Effect of EM on the yield of paddy rice. Proceedings of the 2nd International Conference of Kyusei Nature Farming, October 7-11, 1991, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP., Brazil, pp: 97-102.
- Muller, C.H., 1969. Allelopathy as a factor in ecological process. *Vegetatio*, 18: 348-357.
- Naqvi, H.H., 1976. Some phytotoxic compounds in Italian ryegrass. *Pak. J. Bot.*, 8: 63-68.
- Noor, M. and M.A. Khan, 1994. Allelopathic potential of *Albizia samans* Merr. *Pak. J. Bot.*, 26: 139-147.
- Sanagakkara, U.R. and T. Higa, 1994. Effect of EM on nitrogen fixation by bush bean and mungbean. Proceedings of the 2nd International Conference of Kyusei Nature Farming, October 7-11, 1991, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP., Brazil, pp: 118-124.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 978-0070609259, Pages: 481.
- Tukey, H.B., 1969. Implications of allelopathy in agricultural plant science. *Bot. Rev.*, 35: 1-16.