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EM AND VAM TECHNOLOGY IN PAKISTAN V: RESPONSE OF CHICKPEA (*CICER ARIETINUM* L.) TO CO-INOCULATION WITH EFFECTIVE MICROORGANISMS (EM) AND VA MYCORRHIZA UNDER ALLELOPATHIC STRESS

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Abstract

A pot experiment was conducted to study the tolerance induced by vesicular arbuscular mycorrhizal fungi (VAM) and effective microorganisms (EM) in chick pea (*Cicer arietinum* L.) against allelopathic stress caused by aqueous leaf extract of *Syzygium cumini* (L.) Skeels. The adverse impact due to aqueous leaf extract on plant vegetative and reproductive growth and shoot nitrogen content was significantly reduced by the application of EM and VAM. Co-inoculation of EM and VAM was found more effective than inoculation with either alone. However, reduction in shoot phosphorus content due to allelopathic stress could only be compensated by VAM inoculation alone. VAM colonization was stimulated by EM application.

Introduction

Allelopathy is an important ecological process in vegetational composition (Muller, 1969) and agricultural sciences (Tukey, 1969). Allelochemicals such as phenols, glycosides, amino acids, terpenes and sugars (Whittaker & Fenny, 1971; Harbone, 1977; Hussain & Abidi, 1991) are released from different parts of plants like leaves, stems, roots, rhizomes, trichomes, flowers, seeds and fruits (Kuti *et al.*, 1990). All basic plant processes such as hormonal balance, protein synthesis, respiration, photosynthesis, chlorophyll formation and plant water relations may be disturbed by allelochemicals (Yamane *et al.*, 1992).

Higa (1988) introduced EM (Effective Microorganisms) Technology of nature farming, which is based on the concept of using beneficial microorganisms found in natural conditions. EM is a mixture of 80 species of co-existing microorganisms. It has predominantly photosynthetic bacteria, *Lactobacillus* spp., yeast and actinomycetes (Hussain *et al.*, 1994). Experiments conducted in Japan, Pakistan, India, Sri Lanka, Malaysia, Thailand and in many other Asian countries have shown good prospects for practical application of EM (Imai & Higa, 1994; Sangakkara & Higa, 1994; Lin, 1994; Chawdhary *et al.*, 1994; Javaid *et al.*, 1995, 1997).

VA-mycorrhizae are obligate mutualistic symbionts and are ubiquitous in roots of vascular plants in nature (Gabor, 1992). These fungi impart many benefits to plants such as nutrient absorption, stimulation of growth regulating substances, increased rate of photosynthesis, osmotic adjustment under drought stress, enhancement of nitrogen fixation by symbiotic bacteria, increased resistance to soil pathogens and tolerance to environmental stresses (Bethlenfalvay & Linderman, 1992). Many studies in the literature report beneficial relationship between VA mycorrhiza and soil microorganisms such as phosphate solubilizing bacteria and fungi (Krone *et al.*, 1987; Gopalakrishna, 1980), free living nitrogen fixing bacteria (Brown & Carr, 1980) and hormone producing bacteria (Azcon *et al.*, 1978). The beneficial effects of co-inoculation of EM and VA mycorrhizae have also been observed in maize, tomato and chickpea (Bajwa & Jilani, 1994; Bajwa *et al.*, 1995; 1998) under normal conditions. However, the usefulness of such microbial interactions under stressed conditions is yet to be investigated. The present study was, therefore, undertaken to investigate the effect of co-inoculation of EM and VA mycorrhizal fungi on growth, yield and nutrient uptake in chickpea under allelopathic stress caused by aqueous leaf extract of *Syzygium cumini* (L.) Skeels. *S. cumini* is a medium size tree and is generally grown on or near the agricultural lands in Punjab (Pakistan). The fallen leaves of this tree are known to exhibit the allelopathic potential (Haneef, 1996).

Materials and Methods

Earthen pots of 25 cm diameter were filled with heat sterilized sandy loam soil amended with 1:1 mixture of sterilized green manure and farmyard manure @ 6% w/w. The treatments employed were control, aqueous leaf extract of *S. cumini* (ALES), ALES + EM, ALES + VAM and ALES + EM +VAM. Four seeds of chickpea were sown in each pot, which were thinned to two uniform seedlings after germination. Each treatment was replicated three times and the pots were randomized on a wire netting house bench.

The aqueous leaf extract of *S. cumini* was obtained by soaking thoroughly ground fresh leaves in distilled water @ 10% w/v for five hours at 25°C and filtered. The respective pots were supplemented with the filtrates @ 100 ml/pot, 2 days before sowing and 3 weeks after sowing. The mycorrhizal infested roots of *Medicago denticulata* were used as VAM inoculum. EM culture solution was obtained from Nature Farming Research Center, University of Agriculture, Faisalabad. This solution was applied to the soil of the respective pots at a dilution of 1:500 five days before sowing @ 300 ml / pot. To ensure the adequate population of microorganisms in the soil, pots were regularly supplemented with EM solution with 15 days intervals till the end of the experiment.

Plants were uprooted after 45, 60 and 75 days growth. Data for plant height, root, shoot and pod fresh and dry weight and pod number per plant were recorded. Nitrogen in shoot of 75 days old plants were estimated by Kjeldahl method (Bremner & Mulvaney, 1982) and phosphorus was determined by the method of Jackson (1962). The results were compared statistically by Dancan's Multiple Range Test (Steel & Torrie, 1980).

A part of fresh roots was cleared and stained following the procedure of Phillips & Hayman (1970) for VAM infection study. Extent of VAM infection was measured by slide length technique (Giovannetti & Mosse, 1980). Arbuscular and vesicular infections were quantified by counting the number of these structures per cm of root length.

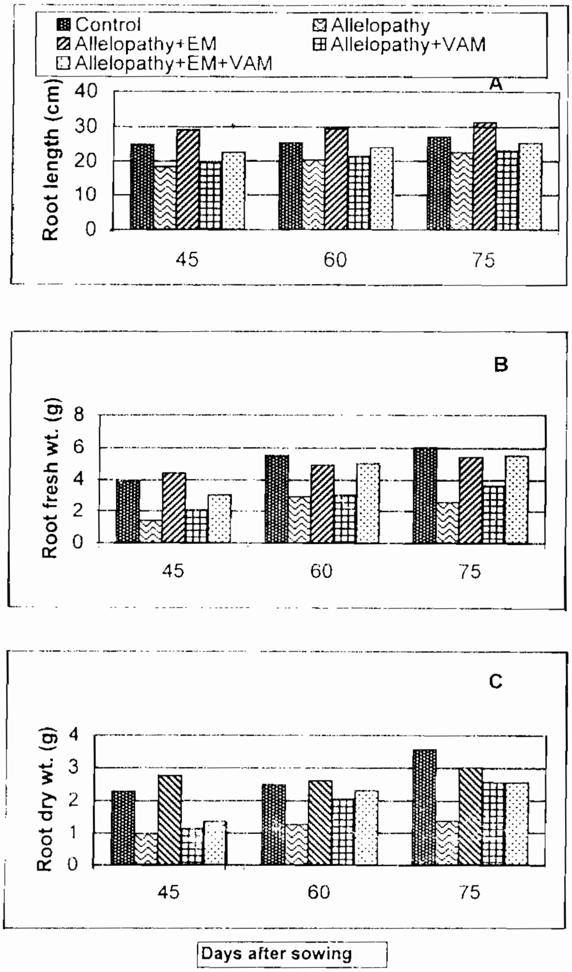

Fig.1.(A-C). Effect of co-inoculation of EM (effective microorganisms) and VA-mycorrhiza on root growth of chickpea under allelopathic stress caused by aqueous leaf extract of *S. cumini*.

Results

Plants grown under the allelopathic stress of aqueous leaf extract of *Syzygium* (ALES) exhibited a significant (P = 0.05) decrease in root and shoot growth at all the three growth stages viz., 45, 60 and 75 days after sowing (DAS). The adverse impact was more pronounced in roots than in shoots. In shoot adverse effect was more severe during early growth stage i.e., 45 DAS. EM and VAM applications significantly (P = 0.05) reduced the adverse impact of ALES, individually or in combination. It is evident from shoot growth that co-inoculation of EM and VAM improved stress tolerance ability of the plant more as compared to either alone inoculation (Fig. 1 & 2).

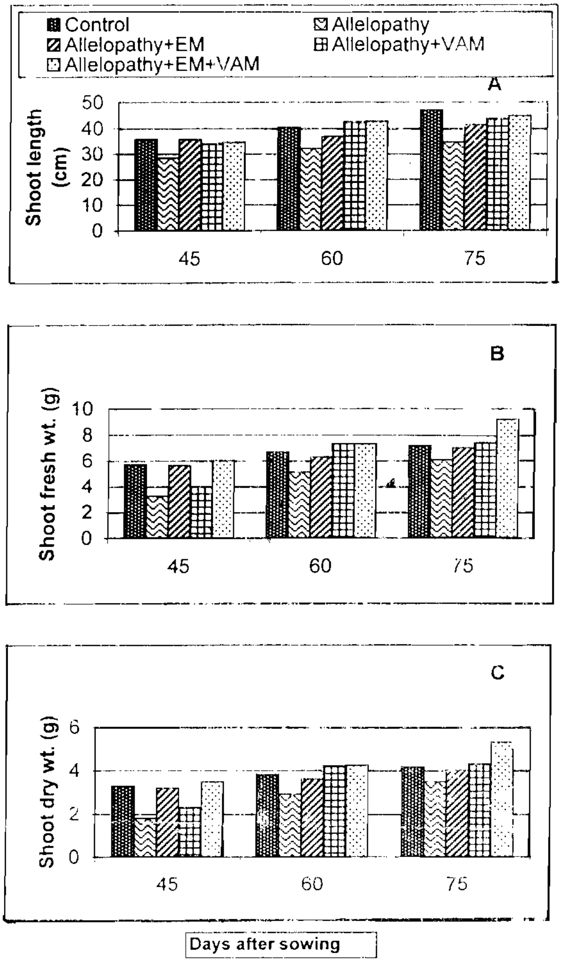

Fig.2. (A-C). Effect of co-inoculation of EM (effective microorganisms) and VA mycorrhiza on shoot growth of chickpea under allelopathic stress caused by aqueous leaf extract of *S. cumini*

A significant increase in number, fresh and dry weight of pods was observed in ALES introduced plants as compared to control, 60 DAS. Co-inoculation of EM and VAM further enhanced the pod yield at this growth stage. However, 75 DAS pod number and dry weight was markedly reduced by ALES application. Both EM and VAM application, either alone or combined, markedly suppressed the adverse impact of allelopathic stress and increased the pod yield significantly (Fig. 3).

ALES application negatively affected the P uptake, consequently reduced the shoot P content significantly (P = 0.05). Mycorrhizal inoculation improved the P nutrition under stressed conditions while no significant variation was evident in EM and EM +VAM inoculated plants. ALES also negatively affected nitrogen content but the

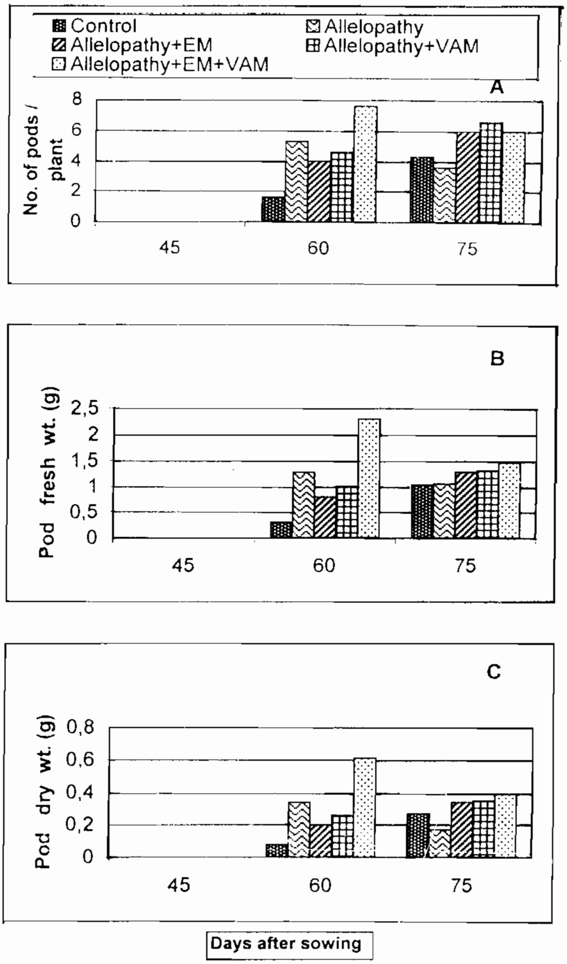

Fig.3. (A-C). Effect of co-inoculation of EM (effective microorganisms) and VA-mycorrhiza on pod yield of chickpea under allelopathic stress caused by aqueous leaf extract of *S. cumini*.

affect was less pronounced as compared to P content. Both EM and VAM enhanced the N uptake significantly. Maximum N content was recorded in co-inoculated plants (Fig.4).

Under allelopathic stress the EM greatly supported the establishment of mycorrhiza. The extent of infection and number of arbuscules and vesicles were significantly greater in EM+VAM than VAM inoculated plants (Fig. 5).

Discussion

Since roots were in direct contact with allelochemicals present in aqueous leaf extract of *Syzygium* (ALES), they were more severely affected than shoots. In earlier

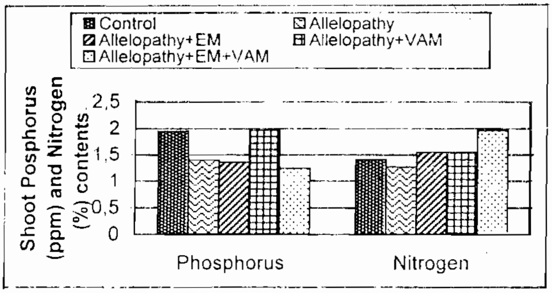

Fig.4. Effect of co-inoculation of EM (effective microorganisms) and VA-mycorrhiza on shoot phosphorus and nitrogen contents of chickpea under allelopathic stress caused by aqueous leaf extract of *S. cumini*.

studies similar affects of allelochemicals have been attributed to the reduced rate of root cell division and cell elongation (Muller, 1965; Buckolova, 1971). The reduced shoot N and P contents indicate the decreased absorption of these elements under allelopathic stress. Many earlier workers (Buchholtz, 1971; Glass, 1975; Kolesnichenico & Aleikina, 1976) have reported similar inhibition in nutrient uptake. In the present study the reduced root growth coupled with reduced nutrient absorption due to allelopathic stress resulted in the reduced shoot growth. The ALES application favoured the pod formation up to 60 days growth. However, at later growth stage pod yield was adversely affected by ALES application due to pre-mature fall of fruits.

Mycorrhizal inoculation reduced the adverse effect of allelopathic stress, consequently vegetative and reproductive growth and nutrient uptake was enhanced. Javaid & Bajwa (1999) observed similar effects of VA mycorrhizae on root and shoot growth of maize under allelopathic stress caused by leaves of *Melia azedarach*. There is possibility that VAM fungi may produce such chemicals, which can neutralize the effect of allelochemicals responsible for inhibition of root cell mitosis and growth. Stimulation of hormonal activity or enhanced water uptake may be involved in the growth responses to VAM inoculation (Schultz *et al.*, 1979). The enhanced nutrient uptake by mycorrhizal plants may be attributed to the reduced distance that nutrients must diffuse to plant roots (Rhodes & Gerdeman, 1975). Mycorrhizal hyphae may chemically modify the availability of nutrients for uptake by plants (Powell & Daniel, 1978). In the present study the better shoot growth of mycorrhizal than non-mycorrhizal plants under allelopathic stress could be attributed to both relief of stress on root development and increased uptake of nutrients.

EM application reduced the adverse impact of allelopathic extract probably because of activity of yeast present in the 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 the microorganisms. Furthermore, lactobacilli in EM solution accelerate the decomposition of organic substances, which are hard to decompose thus make the nutrients available to plants and hence increase

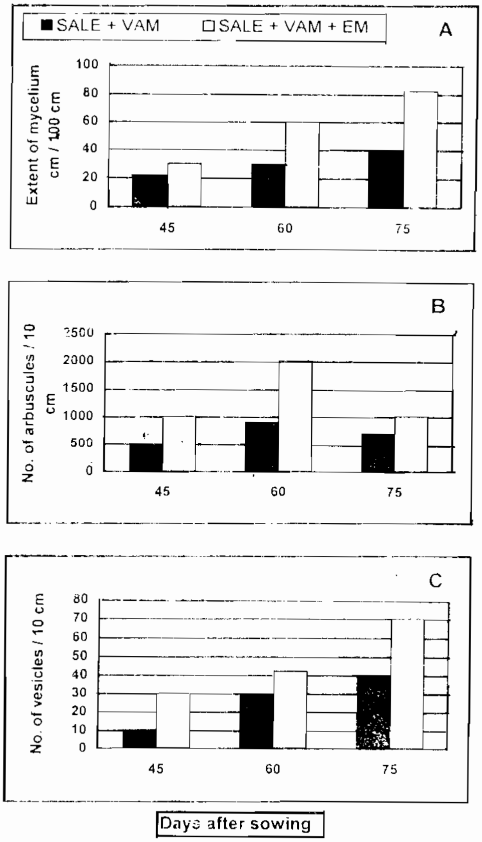

Fig.5. (A-C). Effect of EM on VA mycorrhizal development in chickpea under allelopathic stress caused by aqueous leaf extract of *S. cumini*.
SALE: *S. cumini* aqueous leaf extract

the plant growth (Hussain *et al.*, 1994). Moreover, 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 (Hussain *et al.*, 1994) These substances could be responsible to promote the growth of the plant under the described phenomenon.

Nitrogen uptake was higher in plants co-inoculated with EM and VAM as compared to those inoculated with either alone while phosphorus uptake could not be improved by co-inoculation under the stressed conditions. Bajwa *et al.*, (1998), however, observed increased uptake of both nitrogen and phosphorus by co-inoculated chickpea plants under normal (non-allelopathic) conditions. EM stimulated mycorrhizal colonization in co-inoculated plants. It is thought that soil microorganisms produce such compounds that increase root cell permeability (Barber & Martin, 1976), thereby enhancing the rate of root exudation (Bowen, 1990), which in turn, would stimulate

VAM fungal mycelia in the rhizosphere and might facilitate root penetration by the fungus (Azcon-Aguilar & Barea, 1987). Plant hormones, as produced by soil microorganisms, are also known to stimulate VAM development (Azcon *et al.*, 1978). The enhanced mycorrhizal development resulted in greater uptake of nutrients particularly nitrogen. This enhanced nutrient uptake coupled with accumulative effects of EM and VAM made the plant to be able to tolerate the allelopathic stress to a greater extent. Consequently, the co-inoculated plants showed a better vegetative and reproductive growth than non-inoculated plants or inoculated with either alone.

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