

INTERACTIONS AMONG *Azotobacter chroococcum*, *Penicillium glaucum* AND *Glomus fasciculatum* AND THEIR EFFECT ON THE GROWTH AND YIELD OF SUNFLOWER (*Helianthus annuus* L.)

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Received: April 18. 1994
Accepted: November 15. 1995

SUMMARY

Having established their efficiency and compatibility, the following organisms: *Azotobacter chroococcum* (GA-2), *Glomus fasciculatum* and *Penicillium glaucum* (HE-4) were selected to assess their combined effect on the growth and yield of sunflower in pot culture studies in greenhouse conditions. The results show that the values of growth parameters of sunflower like height, stem girth, number of leaves and leaf area were more responsive to all three organisms as compared with individual treatments. Early flowering is noticed in the treatment with *Penicillium* alone; leaf and stem dry weight were significantly over the control in all the treatments. All treatments except *Azotobacter* alone resulted in significant increases in seed yield. The oil percentage was significantly over the control when test plants were inoculated with all the three organisms together. However, all treatments had a positive effect on oil yield when compared with the control.

Shoot nitrogen percentage was significantly increased over the control with all combinations of treatments. However, phosphorous percent did not vary in the treatments except in *Glomus* with *Penicillium* and all organisms together, where the phosphorous percent was higher than in the control. Rhizosphere study shows that the *Azotobacter* population was higher in the inoculated treatment. Phosphate-solubilising fungi were increased where *Penicillium* was inoculated with the other organisms. No large variations in the fungal population were noticed, whereas *Glomus* inoculation had stimulated the bacterial population. The percents of mycorrhizal infection and spore production were lower in *Glomus* with *Penicillium* as compared with the other *Glomus* inoculation treatments.

Key words: Sunflower (*Helianthus annuus* L.) *Azotobacter*, *Penicillium*, *Glomus*, growth, yield.

INTRODUCTION

Soil acts as a reservoir for various microorganisms resulting in a competition for space, nutrition, and other requirements. These useful or harmful interactions among themselves or with the surroundings are bound to occur. Seed or soil treatments with microbial inoculants are gaining popularity among farmers, especially nitrogen fixers, phosphate solubilisers and vesicular arbuscular mycorrhizal fungi, to increase the crop yield. The root system also is very metabolically active and this zone comprises several heterogenous regions in which microorganisms could affect plant welfare in a number of ways that are not yet well-understood. The process of nutrient cycling, growth stimulation

or inhibition are of great significance, but these are the result of very complex population effects rather than the simple interaction between roots and known microorganisms. Hence, the combined effect of the rhizosphere population may be profound. The interactions among microorganisms and interrelationships between them and roots can benefit plant growth by influencing the availability of essential nutrients, as they are known for their prolific production of many useful metabolites. The association of microorganisms with root system is desirable in some cases and this is true with *Azotobacter* that fixes nitrogen, phosphate solubilizer that solubilises insoluble phosphate and VA mycorrhiza which assists plants by accumulating phosphate and other essential elements.

The work on these aspects though limited, favourable effects on plant growth of all three organisms together or with dual inoculations are reported on crops like sunflower, medicago, lavender, ragi, and onion. (Azcon et al., 1976; Ocampo et al., 1975; Bagyaraj and Monge, 1978; Manjunath et al., 1981). Sunflower seeds are processed to extract oil and protein - rich cattle cake. The oil is used as cooking medium and for the production of commercial products like soap, margarine, vegetable lard, etc. In the present edible oil crises, sunflower with 40-50 percent oil could solve the shortage of edible oil proteins.

Considering the above-cited facts, it was thought worthwhile to take up a study of the effect of *Azotobacter*, VA mycorrhiza and phosphate-solubilising fungus, individually as well as together, on the growth and yield of sunflower.

Azotobacter is a free-living nitrogen fixer and it is known for its N-fixation, besides the production of plant growth hormones and fungistatic substances (Brown and Burlingham, 1968; Maryenko, 1963; Mallikarjunaiah, 1976.). Phosphate-solubilising fungi are capable of solubilising the insoluble form of phosphatic compounds to the soluble form through the production of acids and/or enzymes directly resulting in the improved phosphorous nutrition of plants (Sperber, 1958; Ramos et al., 1968; Ferreira and Torres de Carvalho, 1970).

Vascular arbuscular mycorrhiza is known to occur in most crops and it helps in element uptake, especially phosphorous. Recent studies have cast enough light on its physiological role in plant growth by production of phytohormones and enhanced hydraulic conductivities as well as increased translocation and photosynthetic rates. The interaction of these fungi with nematodes and pathogenic fungi is also well-established (Mosse, 1957; Gerdemann 1968; Gray and Gerdemann, 1969 & 1973; Hayman 1982; Plenchette et al., 1983).

Azcon, De Anguilar and Barea (1978) studied the interaction between cell-free culture supermutants, cells and whole cultures of *Rhizobium* and phosphobacteria with endomycorrhizal fungi and their effects on the growth and nutrition of *Medicago sativa* grown in a low P soil. The best effect was obtained in the treatment which consisted of whole cultures of *Rhizobium*, phosphobacteria and the mycorrhizal fungi applied together rather than with cell-free supermutants of *Rhizobium* and other organisms. Manjunatha et al. (1981) studied the effect of inoculation with *Glomus*, *Beijerinckia* and *Aspergillus* on the growth and nitrogen and phosphorous content of onion. *Glomus* with *Beijerinckia* increased the dry weight and nitrogen content of the plant but not with *Aspergillus*. However, the inoculation of all three organisms together increased the mycorrhizal spore production and fresh weight of onion bulbs. Azcon et al., (1978)

studied the growth and infection levels of mycorrhiza in *Lavendula*, *Lycopersium* and *Medicago* plants after treatment with preparation from *Rhizobium*, *Azotobacter* and a phosphobacterium (*Pseudomonas* sp.) which were known to produce growth-promoting substances. The different treatments together with *Glomus* infection improved plant growth over that obtained by the infection with *Glomus* alone, since the growth regulators affect the root growth which can influence the mycorrhizal infection. It was concluded that the hormones produced by bacteria interacted with the mycorrhizal fungus.

MATERIALS AND METHODS

Among the isolated and identified cultures of *Azotobacter* and phosphate-solubilising fungi, the following cultures (GA-2 and HF-4, respectively) were selected for pot culture studies on the basis of their efficiency in nitrogen fixation and phosphate solubilising ability, their compatibility on agar medium and their effect on seed germination separately as well as in combination. The VA mycorrhizal culture, *Glomus fasciculatum* maintained on sand, and soil mixture with *Panicum maximum* were obtained from the Department of Agricultural Microbiology, UAS, Bangalore.

Pot house study was taken up in greenhouse conditions by employing completely randomised design with the following treatments in nine replications.

1. Uninoculated control
2. *Azotobacter chroococcum* (GA-2) alone
3. *Glomus fasciculatum* alone
4. *Penicillium glaucum* (HF-4) alone
5. *A. chroococcum* + *G. fasciculatum*
6. *A. chroococcum* + *P. glaucum*
7. *G. fasciculatum* + *P. glaucum*
8. *A. chroococcum* + *G. fasciculatum* + *P. Glaucum*

The red soil of GKVK farm was selected for pot culture study and the characters of the soil are as follows: fine sand - 48.5%; coarse sand - 24.3%; silt - 4.4%; clay - 22.8%; organic carbon - 0.75%; nitrogen - 0.49%; available P (ppm) 7; extractable K (ppm) 51 and water holding capacity 27.2%. The soil for the pot culture study was analysed for its initial population of *Azotobacter*, phosphate-solubilising fungi and other fungi and bacteria by employing dilution plate technique on Vaksman No 77, Sperber's, Martin's rose bengal and soil extract agar media, respectively. The soil with one percent farmyard manure was thoroughly mixed and filled uniformly into cement pots (10 x 12") leaving about two inches atop, making 11.3 kg per pot. Then the chemical fertilizers were added at the rate of 20:30:20 kg of NPK/ha which is half the quantity of recommended doses of NPK for sunflower. Prior to sowing, half N and full P and K were applied to soil, i.e., 113 mg, 930 mg and 170 mg in the form of urea, superphosphate and muriate of potash, respectively. On the 35th day after sowing, the remaining half N (113 mg of urea) was applied. The pots were watered and the sowing was done on the next day. The mycorrhizal inoculum (*Glomus fasciculatum*), making up 50 ml and having 350 spores on an average, was placed two centimeter below the top layer of the soil and was spread uniformly so that the growing roots were easily infected. Seven-day old culture of *A. chroococcum* was suspended in 0.85 percent saline solution, after ensuring that the culture superior was

Table 1. Effect of soil inoculation with *Azotobacter chroococcum*, *Glomus fasciculatum* and *Penicillium glaucum* on growth characters of sunflower

Treatment	Height of plants (cm)			Stem girth (cm)			Total number of leaves			Leaf area (cm ²)		Days taken for flowering
	Days after sowing											
	30	45	60	30	45	60	30	45	60	30	45	
Control	20.6	73.3 ^a	137.5 ^a	2.46	3.52	3.78 ^a	8.78	18.83	21.0 ^{ab}	401.8 ^a	1836.9 ^a	56.8 ^b
<i>A. chroococcum</i> alone	21.6	75.3 ^a (2.7)	145.0 ^{ab} (5.5)	2.48	3.53	3.95 ^{ab} (4.5)	8.78	19.67	23.88 ^c (13.7)	492.0 ^{bcd} (22.4)	1896.7 ^{ab} (1.8)	56.0 ^b
<i>G. fasciculatum</i> alone	22.3	81.5 ^{bc} (11.2)	151.8 ^{bc} (10.4)	2.63	3.80	4.18 ^{bc} (10.6)	8.89	19.00	23.13 ^{abc} (10.1)	502.4 ^{cd} (25.0)	2108.5 ^{bcd} (14.8)	55.1 ^a
<i>P. glaucum</i> alone	20.3	73.4 ^a (0.14)	137.5 ^a (0.0)	2.54	3.65	4.00 ^{ab} (5.8)	8.89	19.17	21.13 ^{ab} (0.6)	410.6 ^{ab} (2.2)	1792.7 ^a (2.4)	51.7
<i>A. chroococum</i> + <i>G. fasciculatum</i>	21.6	80.2 ^{ab} (9.4)	150.5 ^{bc} (9.5)	2.70	4.11	4.37 ^c (15.6)	8.89	20.50	23.50 ^{bc} (11.9)	558.3 ^{de} (39.0)	2295.8 ^{de} (25.0)	55.6 ^b
<i>A. chroococum</i> + <i>P. glaucum</i>	20.7	74.6 ^a (1.8)	144.2 ^{ab} (4.9)	2.68	3.88	4.22 ^{bc} (11.6)	8.42	19.33	21.00 ^{ab} (0.0)	423.3 ^{abc} (5.4)	1934.8 ^{abc} (5.3)	55.2 ^b
<i>G. fasciculatum</i> + <i>P. glaucum</i>	22.5	84.6 ^{bc} (15.4)	155.7 ^{bc} (13.2)	2.75	3.90	4.30 ^{bc} (13.8)	8.44	18.00	20.63 ^a (-1.8)	528.2 ^{de} (31.5)	2115.0 ^{cd} (15.1)	55.2 ^b
<i>A. chroococum</i> + <i>G. fasciculatum</i> + <i>P. glaucum</i>	22.8	88.6 ^c (20.9)	158.3 ^c (15.1)	2.78	3.81	4.23 ^{bc} (11.9)	8.89	20.67	22.88 ^{abc} (9.0)	609.4 ^e (51.7)	2387.5 ^e (30.0)	55.0 ^b
LSD	NS	8.1	12.5	NS	NS	0.37	NS	NS	2.52	88.6	241.2	2.8
P<0.01		10.8	16.6			0.49			3.36	110.4	322.2	3.9

Note: Values superscribed with identical letters within each column do not differ significantly.

Figures within parentheses indicate percent variation over respective controls.

NS = Non-significant.

having population of 32×10^8 cell/ml; five ml was added to each pot. *Penicillium glaucum* was grown in 50 ml of Czepek Dox liquid medium for seven days, the fungus was separated by decanting and suspended in 0.85 percent saline solution and brought into uniform suspension by using a blender. Five ml suspensions were inoculated per pot after ensuring 54×10^5 cells per ml.

BSH - 1 sunflower hybrid was selected for the study. The seeds were soaked in water for 12 hours prior to sowing as it ensured early germination and proper establishment of seedlings. Two to three seeds were dibbled per pot; after the germination and safe establishment of seedlings, only one seedling was maintained per pot for further observations. Water holding capacity of all pots was maintained at 70-80 percent up to the period of ten days prior to harvest.

Plant growth parameters like plant height, number of leaves, stem girth and total leaf area were recorded from the 30th day to the 60th day after sowing at 15-day intervals. However, the total leaf area was recorded only two times as the older leaves started drying by the 60th day. Stem girth was recorded by using Vernier calipers at bottom, middle and top of the plant and calculating the average of these three readings. The total leaf area was calculated by summing up individual leaf areas with a factor 0.6954 (Nagappa, 1977). Days to flowering was obtained when flower heads were opened. Hand pollination was done early in the morning starting from the second to the fifth day of flowering. Plant and capitulum were harvested when seeds were mature and the diameter of capitulum was recorded. The seeds were removed from the capitulum by hand and the weights of seeds, empty head, stem and leaf were recorded after drying at 60°C to constant weight. The seed were selected randomly from the individual treatments and the oil percent was calculated on the basis of seed yield of an individual plant by using nuclear magnetic resonance (NMR). The total nitrogen and phosphorous content of the individual plant were done by drying the plant samples together, powdered and calculated the N and P by employing micro-kjeldahl and vanadomolybdate phosphoric yellow colour methods, respectively. The rhizosphere studies of *Azotobacter*, phosphate-solubilising fungi, bacteria and fungi was done by using thoroughly mixed depoted soil. The mycorrhizal spore count and percent infection were done for the soil as well as the root samples of the sunflowers (Gerdman & Nicolson, 1963; Phillips and Hayman, 1970). The nitrogen content of the rhizosphere soil was estimated by employing a procedure outlined by Jackson (1958). Simple CRD analysis was done and significance was tested with probability level 0.05.

RESULTS AND DISCUSSION

The results show that the 30th day observation for plant height did not show any significant increase over the control as well as among the treatments. *Glomus fasciculatum* alone or together with *Penicillium* or *Azotobacter* resulted in significant increases in plant height over the control on the 45th and 60th day except *Azotobacter* on the 45th day. The combined inoculation of all three organisms resulted in a significant increase in height over the other treatments except *Glomus* alone and with *Penicillium*. Plant height on the 60th day with *Glomus* alone and with other two organisms, either separately or together, showed significant increases over *Penicillium* alone but not among themselves (Table 1).

Table 2. Effect of soil inoculation with *Azotobacter chroococcum*, *Glomus fasciculatum* and *Penicillium glaucum* on yield of sunflower

Treatment	Head diameter (cm)	Leaf dry weight (g)	Stem dry weight (g)	Seed yield (g)	Empty head weight (g)	Total dry matter yield (g)	Oil percentage	Total oil yield (g/plant)
Control	10.29	9.75 ^a	16.90 ^a	11.28 ^a	13.53	51.48 ^a	43.68 ^a	5.44 ^a
<i>A. chroococcum</i> alone	10.61	10.17 ^a (4.3)	16.52 ^a (2.2)	12.97 (15.0)	13.33	52.93 ^a (2.8)	43.97 ^a (0.7)	5.70 ^a (5.5)
<i>G. fasciculatum</i> alone	10.86	12.20 ^b (25.1)	20.38 ^b (20.6)	16.53 ^{bc} (46.5)	13.45	62.57 ^b (21.5)	42.20 ^a (3.4)	6.97 ^b (28.0)
<i>P. glaucum</i> alone	9.79	9.12 ^a (6.6)	16.08 ^a (4.9)	15.18 ^b (34.6)	11.50	51.90 ^a (0.8)	44.87 ^{ab} (2.7)	6.83 ^b (25.0)
<i>A. chroococcum</i> + <i>G. fasciculatum</i>	11.18	13.30 ^b (36.4)	20.83 (23.0)	21.08 ^c (86.9)	14.13	69.37 ^{cd} (34.8)	42.08 ^a (3.7)	8.85 ^{cd} (63.0)
<i>A. chroococcum</i> + <i>P. glaucum</i>	10.20	12.38 ^b (27.0)	21.25 ^b (25.7)	17.05 ^{bc} (51.2)	11.73	62.40 ^b (21.2)	44.57 ^{ab} (2.0)	7.59 ^b (40.0)
<i>G. fasciculatum</i> + <i>P. glaucum</i>	10.54	13.12 ^b (34.6)	21.03 ^b (24.4)	18.03 ^{cd} (59.8)	13.57	67.77 ^{bc} (27.8)	43.47 ^a (0.5)	7.84 ^{bc} (44.1)
<i>A. chroococcum</i> + <i>G. fasciculatum</i> + <i>P. glaucum</i>	11.46	13.22 ^b (35.6)	22.88 ^b (35.4)	20.05 ^{de} (77.5)	14.22	70.37 ^d (36.7)	47.47 ^b (8.7)	9.51 ^d (75.0)
LSD	NS	1.51	2.41	2.18	NS	4.34	2.98	1.02
P < 0.01		2.02	3.23	2.91		5.80	3.99	1.38

Note: Values within parentheses indicate percent variation over respective controls.

Values superscribed with identical letters within each column do not differ significantly.

NS = Non-significant.

Azotobacter and *Penicillium* inoculation individually or together did not show any significant difference. Statistically all treatments were similar except *Penicillium* alone and the three organisms together. Mycorrhizal treatments influenced the stem girth but significant differences among treatments were observed on the 60th day, with 4.5-15.6 percent increase over the control. *Glomus* and *Azotobacter* with *Penicillium* showed significant increases over the control. Equal stem girth was observed among the treatments except that *Glomus* with *Azotobacter* was superior to *Azotobacter* and *Penicillium* alone. No difference occurred in leaf number on the 30th and the 45th day. On the 60th day, *Azotobacter* alone showed a significant increase in leaf number over the control, *Penicillium* alone, *Penicillium* with *Glomus* and with *Azotobacter*. *Glomus* with *Penicillium* decreased leaf number by 1.8 percent over the control. The treatments had a significant effect on leaf area on the 30th day, by 2.2 to 51.7 percent over the control except for *Penicillium* alone and *Penicillium* with *Azotobacter*. The three organisms together proved significantly superior to all other treatment combinations. A similar trend was noticed on the 45th day too. The mycorrhizal plants showed an increased leaf area as compared with the control but with the non-mycorrhizal treatments. Inoculation with *Penicillium* alone decreased the leaf area by 2.4 percent in relation to the control. All treatments took more or less equal number of days for flowering except the plants inoculated with *Penicillium* alone, which flowered at a range of 3.3 to 5.1 days earlier.

The minimum and maximum head diameters were recorded with *Penicillium* alone and the three organisms together, respectively. As compared with the other treatments, however, they were statistically non-significant. All treatments brought significant increases in leaf and stem dry weight over the control, except for *Azotobacter* and *Penicillium* individually, but not among themselves. On the other hand, *Penicillium* and *Azotobacter* alone reduced the leaf weight, stem girth and stem weight by 6.6, 4.9 and 2.2 percent, respectively, in relation to the control. Significant increases over the control among the treatments ranged from 25.1 to 36.4 and 20.6 to 35.4 for leaf and stem dry weight, respectively. All treatments showed increased seed yield over the control by 15 to 86.9 percent. *Azotobacter* with *Glomus* and all three organisms increased the yield/plant by 21.08 g and 20.05g, respectively, over the control which yielded 11.28 g.

Azotobacter with *Glomus* increased the yield significantly over the other treatments except all three organisms together which in turn was superior to the rest of the treatments. The empty head weight was maximum with all three organisms and minimum with *Penicillium* alone. All treatments resulted in increased total dry matter over the control by 0.8 to 36.7 percent. All treatments except *Azotobacter* and *Penicillium* alone yielded significantly more dry matter than the control. *Azotobacter* and *Glomus* together were superior in dry matter yield over the other treatments. All dual inoculations except *Glomus* with *Penicillium* gave significantly increased total dry matter yield over respective single inoculations. The results reveal that, in general, the oil percent varied from 42.08 to 47.47. All treatments except *Azotobacter* alone significantly increased total oil yield over the control by 25 to 75 percent. Maximum oil yield was 9.51 g/plant in the treatment with all three organisms together. It was found to be superior to all other treatments except *Azotobacter* with *Glomus* which in turn yielded more than the other treatments except *Glomus* with *Penicillium*. The others did not show any significant difference among themselves (Table 2).

Table 3. Effect of *Azotobacter chroococcum*, *Glomus fasciculatum* and *Penicillium glaucum* on sunflower shoot nitrogen and phosphorus

Treatment	Nitrogen percentage	Total nitrogen (mg)	Phosphorus percentage	Total phosphorus (mg)
Control	0.85 ^a	341.5 ^{ab}	0.23	92.4 ^{ab}
<i>A. chroococcum</i> alone	1.02 ^{bc} (20.0)	408.2 ^{bc} (19.5)	0.24	96.5 ^{ab} (4.4)
<i>G. fasciculatum</i> alone	0.90 ^a (5.9)	414.3 ^c (21.3)	0.25	115.1 ^{abc} (24.6)
<i>P. glaucum</i> alone	0.88 ^a (3.5)	323.0 ^a (5.4)	0.23	84.4 ^a (8.7)
<i>A. chroococcum</i> + <i>P. fasciculatum</i>	0.89 ^a (4.7)	429.5 ^c (25.8)	0.22	106.2 ^{ab} (14.9)
<i>A. chroococcum</i> + <i>P. glaucum</i>	1.04 ^c (22.4)	471.7 ^c (38.1)	0.25	113.4 ^{ab} (22.7)
<i>G. fasciculatum</i> + <i>P. glaucum</i>	0.93 ^{ab} (9.4)	442.9 ^c (30.0)	0.25	119.3 ^{bc} (29.1)
<i>A. chroococcum</i> + <i>G. fasciculatum</i> + <i>P. glaucum</i>	0.91 ^a (7.1)	457.9 ^c (34.1)	0.29	145.9 ^c (57.9)
LSD	P < 0.05	68.3	NS	31.6
	P < 0.01	92.5		43.6

Note: Values within parentheses indicate percent variation over respective controls.

Values superscribed with identical letters within each column do not differ significantly.

NS=Non-significant.

Table 4. Microbial and chemical analysis of sunflower rhizosphere soil inoculated with *Azotobacter chroococcum*, *Glomus fasciculatum* and *Penicillium glaucum*

Treatment	Azotobacter (x10 ³ /g)	Phosphate- solubilising fungi (x10 ³ /g)	Total fungi (x10 ⁵ /g)	Total bacteria (x10 ⁷ /g)	Mycorrhizal percent infection	Mycorrhizal spore count (per 25 ml)	Soil N per cent
Control	1x10 ²	4.3	5.8	4.2	24.0	93.7 ^{ab}	0.063
<i>A. chroococcum</i> alone	4.5	4.6	4.8	6.3	22.0	103.7 ^b	0.063
<i>P. glaucum</i> alone	1x10 ²	6.3	6.8	4.3	25.0	78.8 ^a	0.065
<i>G. fasciculatum</i> alone	1x10 ²	4.8	5.0	10.4	88.0	177.7 ^e	0.061
<i>A. chroococcum</i> + <i>G. fasciculatum</i>	3.8	5.3	5.3	11.8	84.7	172.7 ^{de}	0.063
<i>A. chroococcum</i> + <i>P. glaucum</i>	4.8	7.3	5.8	6.4	26.3	88.8 ^{ab}	0.063
<i>G. fasciculatum</i> + <i>P. glaucum</i>	1x10 ²	7.0	5.8	9.7	44.7	134.7 ^c	0.065
<i>A. chroococcum</i> + <i>G. fasciculatum</i> + <i>P. glaucum</i>	5.0	7.7	6.7	7.6	76.0	159.7 ^d	0.070
LSD	P<0.05					16.0	NS
	P<0.01					22.0	
Soil taken for the study	1x10 ²	0.4	0.31	0.31		15.0	0.50

Note: NS=Non-significant.
Values superscribed with identical letters within each column do not differ significantly.

The shoot nitrogen increase was higher in all treatments by 3.5 to 22.4 percent over the control. Minimum was with *Penicillium* alone and maximum with *Azotobacter* with *Penicillium*. The total nitrogen content was lower than the control in the *Penicillium*-treated plants but the others had increased contents over the control by 19.5 to 38.1 percent. Maximum was with *Azotobacter* and *Glomus* followed by all three organisms together and other treatments. There were no significant differences in percent shoot phosphorous among the treatments. However, the plants inoculated with all organisms showed the highest percent of phosphorous (0.29). Minimum was noticed with *Penicillium* alone which was 8.7% less as compared with the control. The treatment with all three organisms was superior to all other treatments except *Glomus* alone and with *Penicillium*. There were no significant differences among the treatments (Table 3). The results showed that the *Azotobacter* population in initial soil as well as rhizosphere soil failed to come up in 100-fold dilution in inoculated conditions, indicating its poor population in soil. However, in the presence of the other two organisms, *Glomus* and *Penicillium*, its population was maximum (i.e., 5×10^3 and 4.8×10^3 cells/g, respectively).

Maximum concentration of phosphate-solubilising fungi was noticed in the treatment with all organisms (77×10^3 cells/g) followed by *Penicillium* with *Azotobacter* (7.3×10^3 cells/g) and *Glomus* (7×10^3 cells/g). Before harvesting the crop, its population was 0.4×10^3 cells/g. Among the *Penicillium*-inoculated treatments, the minimum concentration was with *Penicillium* alone (6.3×10^3 cells/g). The fungal population was maximum in the soil treated with *Penicillium* alone, followed by the soil with all three organisms, etc.

Before harvest, the fungal population was 0.31×10^5 cells/g of soil. Minimum and maximum bacterial populations were observed with the control and with *Azotobacter* with *Glomus*, respectively. In general, the bacterial population was higher in the *Glomus*-treated plants as compared with the others. Before harvest, the population was 0.31×10^7 cell/g but after harvest the range was from 4.2×10^7 to 11.8×10^2 cells per gram of soil. The maximum mycorrhizal infection was observed with *Glomus* alone (88.00) followed by *Glomus* with *Azotobacter* (84.7), *Glomus* with the other two organisms (76.00), and was decreased with *Glomus* with *Penicillium* (44.7). Maximum and minimum mycorrhizal spores were observed with *Azotobacter* alone (103.7 per 25 ml soil) and *Penicillium* alone (78.8/25 ml), respectively. Among the *Glomus*-inoculated soils, maximum was with *Glomus* alone followed by *Glomus* with *Azotobacter*, the other two organisms and *Penicillium*, i.e., 177.7; 172.7; 159.7 and 134.7 per 25 ml, respectively. A six-fold increase was observed after the harvest. The overall nitrogen analysis of soil showed no significant contribution from any treatment. However, the soil N was increased in general as compared with the initial N content in the soil taken for the study (Table 4).

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INTERACCION ENTRE *Azotobacter chroococcum*, *Penicillium glaucum* Y *Glomus fasciculatum* SOBRE EL CRECIMIENTO Y RENDIMIENTO DEL GIRASOL (*Helianthus annuus* L.)

RESUMEN

Después de testar su eficiencia y compatibilidad los siguientes organismos, *Azotobacter chroococcum* (GA-2), *Glomus fasciculatum* y *Penicillium glaucum* (HE-4) fueron seleccionados en este estudio para estudiar su efecto combinado sobre el crecimiento y rendimiento del girasol cultivado en macetas en condiciones de invernadero. Los resultados muestran que los parámetros del crecimiento de girasol como altura, circunferencia del tallo, número de hojas y área foliar fueron mayores en el tratamiento con los tres organismos en comparación con tratamientos individuales.

En el tratamiento con *Penicillium* solamente, se observó precocidad los pesos secos de la hoja y tallo fueron significativos respecto al control. Todos los tratamientos excepto *Azotobacter* aplicado solo, dieron lugar a un aumento significativo del rendimiento de semilla. El porcentaje de aceite fue significativamente alto respecto al control cuando fueron aplicados juntos. Sin embargo todos los tratamientos tuvieron un efecto positivo sobre el rendimiento en semilla cuando fueron comparados con el control.

El porcentaje de nitrógeno del tallo se incrementó significativamente con todas las combinaciones de tratamientos sobre el control. Sin embargo el porcentaje de fósforo no varió con los tratamientos excepto en *Glomus* con *Penicillium* y con todos los organismos a la vez, siendo el porcentaje de fósforo mas alto que el control. Estudios de la rizosfera muestran que la población de *Azotobacter* fue mayor en el tratamiento inoculado. Los hongos solubilizadores de fosfato se incrementaron cuando *Penicillium* fue inoculado con otros organismos. No se

encontró mucha variación en la población de hongos cuando la inoculación con *Glomus* había estimulado la población bacteriana. El porcentaje de infección de micorrizas y producción de esporas fue menor en *Glomus* cuando *Penicillium* se comparó con otros tratamientos de *Glomus*.

INTER ACTION ENTRE *Azotobacter chroococcum*, *Penicillium glaucum* ET *Glomus fasciculatum* SUR LA CROISSANCE ET LE RENDEMENT DU TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Après des études d'efficacité et de compatibilité, nous avons sélectionné les organismes suivants: *Azotobacter chroococcum* (GA-2), *Glomus fasciculatum* et *Penicillium glaucum* (HE-4) afin de tester leurs effets combinés sur la croissance et le rendement du tournesol cultivé en pots sous serre. Les résultats montrent que les paramètres de croissance du tournesol tels que circonférence et hauteur de la tige, nombre de feuilles et surface foliaire avaient des valeurs supérieures dans les traitements réunissant les trois organismes par rapport aux traitements individuels. Une floraison précoce a été notée en présence de *Penicillium* seul, le poids de matière sèche des feuilles et de la tige étaient significativement supérieurs dans tous les traitements par rapport au contrôle. Tous les traitements excepté *Azotobacter* seul ont provoqué une augmentation significative du rendement en grain. La teneur en huile était significativement plus élevée par rapport au contrôle quand les trois organismes étaient employés. Cependant tous les traitements ont révélé des effets positifs par rapport aux plantes non traitées.

Le pourcentage d'azote au niveau des pousses a augmenté significativement pour tous les traitements par rapport au témoin. Le pourcentage en phosphore n'a pas varié excepté pour les plantes inoculées par *Glomus* / *Penicillium* et le traitement regroupant les trois organismes. Les champignons solubilisant les phosphates ont augmenté sous l'influence de l'inoculation par *Penicillium*.

De telles variations n'ont pas été observées pour les populations fongiques, alors que l'inoculation par *Glomus* stimule l'activité bactérienne. Le pourcentage d'infection mycorrhizienne et de production de spores étaient moindres en présence de *Glomus* et *Penicillium* par rapport aux autres traitements incluant *Glomus*.