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INTERACTION EFFECT OF *Azotobacter* AND PHOSPHATE – SOLUBIALISING FUNGI ON SEED GERMINATION AND SEEDLING GROWTH OF SUNFLOWER

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SUMMARY

Five *Azotobacter* and seven phosphate- solubilising fungi were isolated from sunflower rhizosphere, identified, screened for their nitrogen fixation and phosphate solubilising efficiency. *Azotobacter* cultures did not inhibit any fungi; on the other hand, all fungal cultures suppressed the growth of *Azotobacter*, except three *Penicillium* cultures (H.F – 3, 4 and 5). *Azotobacter* cultures GA2 and GA3 increased seed germination but the other three did not. All *Azotobacter* cultures increased radicale/plumule length significantly except GA-1 where it reduced the radicle length by 13.6 percent over the control. All fungal cultures showed high deleterious effect on seed germination as well as on radicle/plumule growth as compared with the control. The combined growth of *Azotobacter* cultures (GA-1 and GA-3) with *Penicillium* (HF-4 & 5) and *Aspergillus* (GF-1 and 2) species increased the length of both radicle and plumule but the remaining culture combinations decreased the length of both radicle and plumule over the control.

Key words: Sunflower (*Helianthus annuus* L.), *Azotobacter*, phosphate, fungi, germination, seedling, growth.

INTRODUCTION

Azotobacter is an asymbiotic free-living nitrogen fixer and it is known for its efficiency to fix atmospheric nitrogen asymbiotically, besides the production of plant growth hormones and fungistatic substances (Brown and Burlingham, 1968; Marenka 1963; Minsshustin et al., 1969; Mallikarjunaiah, 1982; Shende et al., 1977).

Azotobacter-inoculated sunflower plants showed increased growth of shoot and tap root (Siegel and Schmidt, 1966) and higher yield of seeds (Obliswammi et al., 1976). The phosphate solubilising microorganisms play an important role in solubilising the insoluble form of phosphatic compounds to soluble form by the production of acids and/or enzymes directly contributing to an improved phosphorous nutrition of plants. Ralston and McBride (1976) noticed a beneficial effect of phosphate-dissolving bacteria on seedling growth of *Pinus resinosa* when the soil was enriched with insoluble calcium phosphate. Prinkhod'ko (1985) reported that a mixture of *Azotobacterium* and *Phosphobacterin* on tobacco had increased the number of flowering plants by six percent in a non-fertilized soil. Chia Tsuei Kung (1961) observed that the original microflora in the hog dung and urine were activated with the inoculation of *Azotobacter* and it was further noticed that the mixed inoculation with *Azotobacter* and *Aspergillus niger* of hog dung and

urine released phosphorous, three to five times greater in hog dung and urine over the uninoculated control. No inhibitory effect on each other was noticed when *A. chroococcum* and phosphate-solubilising bacteria were inoculated to sterilised soil and their population was better as compared with their separate cultivations. Mochalov (1966) and Godalakrishma Murthy et al., (1967) reported that when rice seed were treated with *Azotobacter* and phosphobacterium both organisms were established quickly in rhizosphere and multiplied. On the other hand, inoculation of *Helminathosporium oryzae* together with these organisms did not result in an increased population of the pathogen in rhizosphere. Stefan and Boi (1960) observed an increased uptake of phosphorous and nitrogen by sunflower plants when bacterial fertilizers were applied at sowing.

In view of the above-cited facts it was thought worthwhile to undertake a laboratory study in order to assess the effect of *Azotobacter* and phosphate-solubilising fungal cultures individually as well as together on seed germination and seedling growth of sunflower.

MATERIALS AND METHODS

Rhizosphere soil from sunflower plants was collected as per the procedure outlined by Dobereiner et al., (1972) during March 1982 from four different locations of GKVK and Hebbal farms, University of Agricultural Sciences, Bangalore -65. In each locality four places were selected randomly and plants were uprooted from respective places without damaging the root system. Excess soil was removed carefully and soil adhering to the root system was collected by tapping gently. The collected soil was pooled together and a portion of the soil sample was obtained; likewise, samples were obtained from the other three localities, collected and stored in a refrigerator for further use.

Isolation of *Azotobacter* and phosphate-solubilising fungi was done by employing dilution plate technique on Waksmann No. 77 nitrogen-free medium and on Spereber's medium, respectively. After confirming the purity of individual cultures, five cultures of *Azotobacter* and seven cultures of phosphate-solubilising fungi were selected and maintained on agar plants of the respective media.

The identification of the five *Azotobacter* cultures was done with the help of Bergey Manual of Determinative Bacteriology 8th edition (1974) and some special diagnostic procedures as recommended by Voets and Dedkan (1966); phosphate-solubilising fungi (seven) were identified by the methods of Thom and Raper (1945), Gilman (1957) and Barnet (1962).

Nitrogen-fixing ability of *Azotobacter* was estimated by employing the micro-Kjeldhal method, calculating total nitrogen fixed per gram of carbon source utilised. The phosphate solubilising ability of the fungi was done by adding 10 percent sterilised solutions of 0.6 ml CaCl and 0.4 ml K_2HPO_4 to 20 ml of Sperber's medium cooled to 60 degrees centigrade and poured into sterilised petriplates and placing 0.5 cm Whatman No. 42 sterilised filter paper discs which were dipped in spore suspensions of respective fungi which were prepared in sterilised water. The area of clear zone was calculated by measuring the radius of the zone. Compatibility of the five *Azotobacter* and the seven phosphate-solubilising fungi was done by employint the cross streak method on Czapek Dox agar medium which favour good growth of both organisms.

Table 1. Identification of *Azotobacter* cultures and their nitrogen fixing ability

Azoto-bacter cultures	Utilisation of starch	Utilisation of mannitol	Mg N_2-fixed per gram of mannitol	Pigmentation	Growth characters (growth, elevation, margin)
HA-1	+	+	5.9	On fourth day Deep brown	Good growth, flat, entire, wrinkled at the edge of the colony
HA-2	+	+	5.9	On seventh day Pale brown	Moderate growth, flat, entire
GA-1	+	+	50.6	On third day Light brown	Moderate growth, flat, entire, wrinkled at the edge of the colony
GA-2	+	+	6.3	On third day Deep brown	Good growth, flat, entire, wrinkled at the edge of the colony
GA-3	+	+	5.8	On third day Deep brown	Good growth, flat, entire, wrinkled at the edge of the colony.
NS = Non-significant.					

Table 2. Phosphate-solubilising ability of different fungi on Sperber's medium

Phosphate-solubilising fungi	Area of clear zone (cm^2)
Aspergillus niger (HF-1)	26.9^b
Penicillium sp. (HF-2)	20.0^a
Penicillium sp.(HF-3)	22.0^a
Penicillium glaucum (HF-4)	49.00^e
Penicillium sp. (HF-5)	41.6^c
Aspergillus niger (GF-1)	39.4^c
Aspergillus niger (GF-2)	45.0^d
LSD ($P < 0.05$)	3.0
($P < 0.01$)	4.2
Note: Values superscribed with identicle letters do not differ significantly.	

Table 3. Interaction between *Azotobacter* cultures and phosphate-solubilising fungi

	Azotobacter chroococcum				
Phosphate-solubilising fungi	HA-1	HA-2	GA-1	GA-2	GA-3
Aspergillus niger (HF-1)	SGA	+	+	SGA	SGA
Penicillium sp. (HF-2)	SGA	SGA	SGA	SGA	SGA
Penicillium sp. (HF-3)	+	+	+	+	+
Penicillium glaucum sp. (HF-4)	+	+	+	+	+
Penicillium sp. (HF-5)	+	+	+	+	+
Aspergillus niger (GF-1)	+	+	+	SGA	+
Aspergillus niger (GF-2)	+	+	+	SGA	+
Note: SGA: Suppressed growth of *Azotobacter* (scant growth, mere observation).					
+: Combined growth of *Azotobacter* and fungus.					

The effect of *Azotobacter* and phosphate-solubilising fungi on seed germination was tested by soaking 100 seed of BSH-1 hybrid sunflower seeds for 12 hours in seven-day old cultures and then kept into a moist germinator. Per cent germination was calculated after a seven-day incubation period.

The combined effect of *Azotobacter* cultures and phosphate-solubilising fungi on germination of sunflower seeds was tested using 100 seeds. *Azotobacter* cultures were initially grown for ten days in 100 ml flasks containing Waksman No. 77 liquid medium which was fortified with 24 ml of Czapek Dox liquid medium following the growth of the fungus (Lakshmikumari et al., 1972). These flasks were further incubated for additional five days. The flask containing identical volumes of both media served as the control. The tests for seed germination as well as growth of radicle and plumule were followed as mentioned earlier.

RESULTS AND DISCUSSION

Of the five *Azotobacter* cultures and seven phosphate-solubilising fungi isolated from rhizosphere soil of sunflower, five were identified as *Azotobacter chroococcum* and labelled as HA-1, HA-2, GA-1, GA-2 and GA-3, three fungal cultures were identified as *Aspergillus niger* (HF-1, HF-2, HF-3) and the rest belonged to *Penicillium* species of which HF-4 was *P. glaucum* (HFF-5, GA-1 and GF-2). Nitrogen fixed per gram of carbon utilised was comparatively low among the isolates and it varied from 5.6 to 6.3 mg. Significant variation among the isolates in solubilising tricalcium phosphate was noticed as indicated by the area of clear zone around their growth. The area of solubilising ranged from 20^2 to 49^2 cm on the third day. *Penicillium glaucum* (HF-4) showed the maximum solubilising area followed by *Aspergillus niger* (GF-2), with significant difference, and the others showed comparatively low capacity (Tables 1 and 2).

The *Chroococcum* cultures did not show any inhibitory effect on any of the tested fungi; on the other hand, three cultures of *Aspergillus niger* (HF-1, GF-1 and GF-2) and one culture of *Penicillium* (HF-2) could suppress the growth of all *Azotobacter* cultures. The remaining cultures could grow together with all the Azotobacter cultures (Table 3). *Azotobacter chroococcum* (GA-2 and GA-3) increased the germination percent by 3.3 and 1.7, respectively, over the control, the remaining cultures reduced the same by 2.3, 2.3 and 3.0, respectively. All cultures increased the radicle length significantly except for GA-1, which reduced it; however, there was no significant difference noticed among the treatments (Table 4). The four phosphate-solubilising fungi tested showed a highly deleterious effect on seed germination (reduction ranges from 5-6%) as well as on the length of radicle and plumule in relation to the control (Table 5).

The combined growth of GA-2 and GA-3 *Azotobacter* cultures with three cultures of *Penicillium* (HF-4, HF-5 and GE-1) and one culture of *Aspergillus niger* (GF-2) showed an increase in the length of both radicle and plumule, by 0.4 to 18.9 percent respectively, over the control. However, the *Azotobacter* cultures HA-2 and GA-3 with *A. niger* (GF-2) and *P. glaucum* HF-4, respectively, showed an increased length of the radicle, by 10.9 and 0.9 percent, respectively, over the control, but not the length of the plumule. On the other hand, the remaining culture combinations showed decreases in the length of both, the radicle and the plumule, by 2.1 to 27.8 and 7.7 to 34.9

Table 4. Effect of seed treatment with *Azotobacter* cultures on germination and seedling growth of sunflower

Treatment	Germination percentage	Radicle length (cm)	Plumule length (cm)
Control (uninoculated broth)	89.67	11.08[a]	2.65[a]
Azobacter chroococcum (HA-1)	87.33 (-2.6)	15.03[b] (35.6)	3.33[c] (25.7)
A. chroococcum (HA-2)	87.33 (-2.6)	14.90[b] (34.5)	3.44[c] (29.8)
A. chroococcum (GA-1)	86.67 (-3.3)	9.57[a] (-13.6)	2.81[a] (6.0)
A. chroococcum (GA-2)	93.00 (3.7)	15.1[b] (36.3)	3.24[bc] (22.3)
A. chroococcum (GA-3)	91.33 (1.9)	14.48[b] (30.7)	2.93[ab] (10.6)
LSD (P < 0.05)		1.56	0.39
(P< 0.01)		2.15	0.54
Note: Values superscribed with identical letters within each column do not differ significantly. Figures within parentheses indicate per cent variation over respective controls.			

Table 5. Effect of phosphate-solubilising fungi on seed germination and seedling growth of sunflower

Treatment	Germination percentage	Radicle length (cm)	Plumule length (cm)
Control (uninoculated broth)	89.75	14.55[c]	3.43[c]
Penicillium glaucum (HF-4)	5.75	2.65[b]	1.33[ab]
Penicillium (HF-5)	5.00	1.59[a]	1.20[a]
Aspergillus niger (GF-1)	6.00	2.75[b]	1.60[b]
Aspergillus niger (GF-2)	5.75	3.34[b]	1.65[b]
LSD (P < 0.05)		0.82	0.40
(P < 0.01)		1.11	0.56
Note: Values superscribed with identical letters within each column do not differ significantly.			

All five *Azotobacter chroococcum* cultures showed a comparatively lower nitrogen fixation than the average capacity of *Azotobacter* to fix around 10 mg (Goswami, 1976). *Azotobacter chroococcum* cultures did not have any inhibitory effect on the tested fungi; on the other hand, *Penicillium* (HF)3 suppressed the growth of all *Azotobacter* cultures. *Aspergillus niger* (HF-1) and the other *Aspergillus* cultures suppressed the growth of three cultures (HA-1, GA-2, GA-3) and one (GA-2) culture of *Azotobacter,* respectively. However, the remaining culture combinations express a combined growth. Azotobacter inoculation had less influence on percent seed germination but the growth of the radicle and plumule was stimulated. This indicates that the growth of the radicle and plumule must have been influenced by the growth-promoting substances produced by *Azotobacter* cultures (Debrvina, 1970; Ishwaran, 1976). All the test fungi inhibited seed germination as well as seedling growth. This may be due to a dense proliferation and development of the fungal mycelium on seeds which might have affected the seeds physically (Lynch and Pryn, 1977) or there might have been a competition between seed and fungus for oxygen (Lynch, 1978). Neither seeds nor fungi grow under anaerobic conditions, a reduction in the supply of oxygen increases the carbon requirement of the fungus, which in turn induces more soluble carbon compounds for fungal growth to be exuded by the seed indirectly making the seed more vulnerable to fungal colonisation. In addition to this, phytotoxic compounds might have been produced by the fungi (Anilkumar and Urs, 1975), as observed by Mishustin and Shilnkova (1971) and Mallikarjunaiah and Bhide

Table 6. Effect of combined growth of *Azotobacter* cultures and phosphate-solubilising fungi on germination and seedling growth of sunflower

Treatment	Germination percentage	Radicle length (cm)	Plumule length (cm)
Control (uninoculated combined broth)	77.5	14.47	4.52
A. chroococcum (HA-1)			
+*P. glaucum* (HF-4)	88.0 (13.5)	11.88** (-17.9)	2.94** (-34.9)
+*Penicillium sp.* (HF-5)	85.5 (10.3)	14.16 (-2.1)	3.61* (-20.1)
+*A. niger* (GF-1)	89.5 (15.5)	13.64 (-5.7)	3.86 (-14.6)
+*A. niger* (GF-2)	81.5 (5.2)	13.11 (-9.4)	3.36* (-25.7)
A. chroococcum (HA-2)			
+*P. glaucum* (HF-4)	80.0 (3.2)	12.62* (-12.8)	3.25** (-28.1)
+*Penicillium sp.* (HF-5)	84.0 (8.4)	11.20** (-22.6)	3.00** (-33.6)
+*A. niger* (GF-1)	84.5 (9.0)	12.74 (-12.0)	3.13** (-30.8)
+*A. niger* (GF-2)	85.5 (10.3)	16.05 (+10.9)	4.32 (-4.4)
A. chroococcum (GA-1)			
+*P. glaucum* (HF-4)	81.4 (5.2)	11.85** (-18.1)	4.17 (-7.7)
+*Penicillium sp.* (HF-5)	77.5 (0.0)	13.62 (-5.9)	3.86 (-14.6)
+*A. niger* (GF-1)	79.0 (1.9)	10.45** (-27.8)	3.51* (-22.3)
+*A. niger* (GF-2)	77.5 (0.0)	13.39 (-7.5)	3.37* (-25.4)
A. chroococcum (GA-2)			
+*P. glaucum* (HF-4)	80.5 (3.9)	15.60 (+7.8)	4.70 (+4.0)
+*Penicillium sp.* (HF-5)	79.5 (2.6)	15.55 (+7.5)	4.72 (+4.4)
A. chroococcum (GA-3)			
+*P. glaucum* (HF-4)	85.5 (10.3)	14.60 (+0.9)	4.06 (-10.2)
+*Penicillium sp.* (HF-4)	86.0 (11.0)	11.98** (-17.2)	3.29** (-27.2)
+*A. niger* (GF-1)	80.5 (3.9)	17.21** (+18.9)	5.28 (+16.8)
+*A. niger* (GF-2)	78.0 (0.6)	15.17 (+4.8)	4.54 (+0.4)
LSD ($P < 0.05$)		1.83	0.90
($P < 0.01$)		2.48	1.22

*Statistically significant at $P < 0.05$.
**Statistically significant at $P < 0.01$.
Figures within parentheses indicate per cent variation from respective controls.

(1985). In this study, increased germination was recorded in the combined growth of *Azotobacter* and fungi over the control. But the radicle and plumule length in general were decreased and this is not in accordance with the result of Mishustin and Shilnkova (1971). It seems that the fungus present together with *Azotobacter* had expressed its normal growth only after seed germination and then spread over the radicle and plumule, thus resulting in impaired seedling growth.

Note:

The following parameters were constant for all tests in case of *Azotobacter* and fungi unless stated otherwise: I) the tests were carried out in triplicate sets; II) C. P. grade chemicals were used; III) incubation temperature was 28-30 degrees centigrade; IV) inoculations were made from two- and five-day old cultures of *Azotobacter* and fungi, respectively. Seven-day old cultures were used for all studies.

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EFECTO DE LA INTERACCION DE *Azotobacter* Y HONGOS SOLUBILIZADORES DE FOSFATO SOBRE GERMINACION Y CRECIMIENTO DE GIRASOL

RESUMEN:

Cinco *Azotobacter* y siete hongos solubilizadores de fosfato fueron aislados de la rizosfera de girasol, identificados y seleccionados por su fijación de nitrógeno y eficiencia de solubilización de fosfato. Los cultivos de *Azotobacter* no inhibieron ningn hongo y por otra parte todos los cultivos de hongos suprimieron el crecimiento de Azotobacter, excepto tres cultivos de *Penicillium* (HF-3, 4 y 5). Los cultivos de *Azotobacter* GA-2 y GA-3 incrementaron la germinación de semilla pero no los otros tres. Todos los cultivos de *Azotobacter* incrementaron significativamente la longitud radícula y tallo excepto GA-1 que redujo la longitud de la radícula en un 13.6 por cien sobre el control. Todos los cultivos de hongos mostraron un efecto deletereo sobre la germinación de la semilla así como en el crecimiento de la radícula/tallo en comparación con el control. El crecimiento combinado de cultivos de *Azotobacter* (GA-1 y GA-3) con *Penicillium* HF-4, 5 y *Aspergillus* (GF-1 y 2) incrementó la longitud de la radícula y tallo pero las restantes combinaciones de cultivos decreció con longitud de ambos, radícula y tallo, respecto al control.

EFFET DE L'INTERACTION *Azotobacter* X CHAMPIGNONS SOLUBILISANT LE PHOSPHATE SUR LA GERMINATION DE GRAINES ET LA CROISSANCE DE PLANTULES DE TOURNESOL

RÉSUMÉ:

Cinq souches d'*Azotobacter* et sept champignons solubilisant le phosphate ont été isolés sur la rhizosphére de tournesol, identifiés puis testés quant à leur efficacité à fixer l'azote et solubiliser le phosphate. Les cultures d'*Azotobacter* n'inhibaient aucun des champignons étudiés, par contre tous les isolats de champignons bloqués la croissance d'*Azotobacter* excepté trois isolats de *Penicillium* (H.F. - 3, 4 et 5). Seuies les souches d'*Azotobacter* GA-2 et GA-3 ont stimulé la germination des graines. Mis a part GA-1 qui provoque une réduction de 13.6% par rapport au témoin, les autres cultures d'*Azotobacter* ont augmenté de faon significative la longueur de la radicule et des radicelles. Toutes les cultures fongiques ont exprimé un effet délétere sur la germination des graines et la longueur de la radicule et des radicelles par rapport au témoin non traité. Les associations regroupant les souches d'*Azotobacter* GA-1 et GA-3 avec les isolats HF-4 et 5 de *Penicillium* et les isolats GF-1 et 2 d'*Aspergillus* augmentent le développement radiculaire et radicellaire contrairement aux autres combinaisons qui ont provoqué une réduction de ces deux paramétres.