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Response of Bioinoculants to Early Seedling Growth in Sunflower (*Helianthus annuus*, L.)

Shyam Sundar Lakshman*[1], M.K. Ghodke[2]
[1]AICRP-Sunflower (ICAR-Indian Institute of Oilseeds Research), Nimpith, 24 Parganas (South), West Bengal, India. E-mail: lakshmanshyam_ss@yahoo.co.in
[2]Department of Genetics and Plant Breeding, Oilseeds Research Station, AICRP-Sunflower, Marathwada Agricultural University, Latur-413056, Maharashtra, India.
**Corresponding author*

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Abstract
The present study was carried out to evaluate the response of different bioagents/biofungicides and growth regulators on seed germination and early seedling growth of sunflower. Eleven (11) treatments were established for the present investigation. Higher germination was observed in T5, T6/T7, T10 and T11 treatments in contrast to other treatments. Treatments T7, T3 and T11 were found significantly better than all other treatments. Generally, root and shoot length increased with the advancement of growth stages. T4 showed higher number of secondary roots in comparison to all other treatments. The shoot length and root length in all the cases (irrespective of the cultivars: DRSH-1, DRSF-108, LSFH-171) were highly influenced by the bioinoculants and chemicals; however, influence of bioagents was found better than the chemicals. Similarly, the seedling weight in 96 hours after sowing and 144 hours after sowing in all the cultivars was reported higher than when the seeds were treated with bioinoculants, which reflected the efficacy of the bioinoculants compared to others.

Keywords
Sunflower; Bioinoculants; Early seedling; Root and shoot growth

Introduction

An experiment was carried out to evaluate the response of different bioinoculants and growth regulators on seed germination and early seedling growth of sunflower. Two hybrid varieties, viz. DRSH-1, LSFH-171, and one open-pollinated variety viz. DRSF-108, were used as the experimental material for the present investigation. Gibberellic acid (GA3) and 1-Naphthalene Acetic Acid (NAA) were used as growth regulators; whereas *Trichoderma viride*, *Pseudomonas fluorescens* and *Trichoderma harzianum* biofungicides were used as bioinoculants. Eleven treatments were established for investigation. In the case of seed germination, the percentage of seed germination was higher in bioagents treated seeds than in control seeds. Similarly, higher values of shoot and root lengths and fresh and dry weights of sunflower were recorded when the seeds were treated. *Pseudomonas fluorescens* treated seeds (T4) showed higher number of secondary roots in comparison to all other treatments. The overall results indicate that the growth of bioagent treated sunflower seedlings excelled over the untreated seedlings in terms of growth. All the bioagents either in combination or individually proved to be a boosters as biofertilizers/bio-stimulants, and are reported at par with the growth regulators in relation to seed germination and early seedling growth.

Materials and Methods

The sunflower seeds were sterilized with 2% mercuric chloride solution before treatment. After sterilization, the seeds were washed well with sterile distilled water. Twenty-five seeds were selected from each type and dressed well with the water. A control set up was also made by following the same conditions except the addition of bioinoculants. Ten seedlings were selected at random from each lot and the observations were made on the 2^{nd}, 4^{th} and 6^{th} day of planting. The seedlings were uprooted gently without causing any damage to the root and shoot systems. The shoot and root lengths were measured with a metric scale. The shoot and root fresh weights were determined using an electronic balance.

After every 12 hours the data on seed germination was recorded. Total number of germinated seeds were counted in all the treatments, at an interval of 12 hours after soaking, and recorded as emergence count per petri plate. The data on shoot and root length was recorded at 48, 96 and 144 hours, respectively, of seed soaking from 10 randomly selected seedlings. The seedling weight was recorded in 96 and 144 hours after soaking. The present study was conducted using following material:

Treatments:

T-1: *Trichoderma viride* [Strain-I: 20 g/l]
T-2: *Trichoderma viride* [Strain-II: 20 g/l]
T-3: *Trichoderma harzianum* [20 g/l]
T-4: *Pseudomonas fluorescens* [20 g/l]
T-5: *T. viride* [Strain-I (5.0 g/l) + P. *fluorescens* (5.0 g/l)]
T-6: T. viride [Strain-II (5.0 g/l) + P. *fluorescens* (5.0 g/l)]
T-7: Control (Double Distilled Water)
T-8: *T. harzianum* (5.0 g/l) + P. *fluorescens* (5.0 g/l)
T-9: Blitox (2.0 g/l)
T-10: Seed plus (Gibberellic acid - 10%) [2.0 g/l]

T-11: Sudha germinaid (NAA 1%; Growth regulator) [1.0 g/l]
(Population density - 2 x 109 (c.f.u./gram), Microbial adjuvant - 2%, Microbial media residue inert ingredient - 95-97%)

The experiment was conducted in petri plates in accordance of the methods ascertained by Dubois *et al.* (1956) and Horborne (1973). 50 seeds were placed on the filter paper within the petri plate. 5 ml of liquid solution of each treatment was applied on the filter paper at every 24 hours intervals. After every 12 hours, the data on seed germination was recorded. Total number of germinated seeds were counted in all the treatments at the interval period of 12 hours after soaking and recorded as emergence count per petri plate. The data on shoot and root length was recorded at 96 and 144 hours of seed soaking from 10 randomly selected seedlings. The seedling weight was recorded in 96 and 144 hours after soaking.

For growth study, height of 10 randomly selected seedlings from each treatment and each replication was measured with a meter scale from the ground level to the tip of the shoot (shoot length) and mean height was calculated from each treatment. The seedling weight (g) was measured in 96 and 144 hours after sowing in similar fashion. Root lengths of 10 randomly selected seedlings from each treatment were measured with a meter scale.

Results and Discussion

The results indicated that the growth of *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* or their combination treated sunflower seedlings excelled over the untreated ones.

The germination percentage was influenced by different treatments (Table 1; Plate 1). Result showed that the maximum number of seedling emergence was reported in T1 (94%) and T8 treatment (96%), which contains bioagents (*T. viride*, Strain-I, 10.0 g/l & 5.0 g/l) and was at par with T9 (Blitox, 2.0 g/l) and T11 (Sudha germinaid growth regulator, 1.0 g/l). In all the treatments, the germination percentage was significantly higher than the control. The results indicate that the bioagents like *T. viride*, *T. harzianum* and *P. fluorescens* have similar type of influence on seed germination like growth regulator and chemicals. The present study reveals that *T. viride, P. fluorescens* and *T. harzianum* and their combinations have significant role on seed germination as well as early seedling growth. All the bioagents either in combination or individually proved to be boosters as biofertilizers/bio-stimulants and are reported at par with the growth regulators in relation to seed germination and early seedling growth.

The seed germination experiment revealed that irrespective of all the sunflower cultivars, i.e. DRSH-1, DRSF-108, LSFH-171, the percentage of germination of seeds was highest in sunflower seeds treated by *Trichoderma harzianum* in combination with *Pseudomonas fluorescens* (5.0 g/l of water + 5.0 g/l of water) followed by seeds treated by *Trichoderma harzianum* (10.0 g/l of water) and Sudha germinaid (growth regulator, 1.0 g/l of water), seeds treated by Gibberellic acid with concentration of 10% (2.0 g/l of water) and Blitox (2.0 g/l of water), respectively. The fungal growth was reported checked best when the seeds treated by Blitox (no infection) followed by *Pseudomonas fluorescens* (<5% infection), *P. fluorescens* combined with *Trichoderma harzianum* (<10% infection), respectively. The inoculation of the bioinoculants in all the cultivars showed a considerable increase in the seed germination than the other cultivars treated under same experimental conditions. The reason for this may be the

tremendous pressure developed inside the seeds, which is responsible for breaking of the seed coat quickly (Sifton, 1959). This pressure may be induced by phyto-hormones especially auxin, indole acetic acid (IAA), cytokinin and gibberellic acid (GA) like substances secreted by *Trichoderma harzianum* or *P. fluorescens* or *Trichoderma viride.* The findings show proximity with the results of Okon (1985) and Okon and Kapulnik (1986) in case of a biofertilizer, *Azospirillum*, in rice. The observations made on 2nd, 4th and 6th days of sowing revealed that irrespective of all the sunflower cultivars, i.e. DRSH-1, DRSF-108, LSFH-171, the bioinoculums treated seeds had higher early seedling development than the control. The seedlings from these particular bioinoculants treated seeds had longer shoot and root lengths than the untreated ones. From this experimental finding, it may be concluded that the seed dressing by these bioinoculants induces the production of plant growth promoting substances and leads to the increase of shoot and root length (Table 1, 2 & 3). Secretion of plant growth hormones by *Azospirillum* was reported in several cereals and grasses (Balasubramaniam and Kumar, 1987; Bashan and Holgain, 1995). This also reflects a specific capability of the host plant to attract the bacteria and modify the rhizosphere, and/or to respond to some bacterial activity and benefit from it (Bottini *et al*., 1989). The fresh and dry weights of root and shoot system of ridge gourd and ash gourd were also found to be increased to a considerable extent in *Trichoderma harzianum* in combination with *Pseudomonas fluorescens* (5.0 g/l + 5.0 g/l) followed by *Trichoderma harzianum* (10.0 g/l of water) treated seedlings (Table 1, 2 & 3). T4 (*Pseudomonas fluorescens* treated seeds) showed higher number of secondary roots in comparison to all other treatments. This may be due to the formation and development of numerous root branching, root hairs and primary and secondary lateral roots, which increase the nutrient uptake capacity of roots (Gopalswamy and Vidhyasekaran, 1988a &1988b; Hartmann, Mahavir and Kligmaller, 1983).

Table 1: Response of Bioinoculants to Sunflower cv. DRSH-1

Treatments	*96 Hours*				*144 Hours*			
	Germi-nation %	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*	*Germi-nation %*	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*
T-1: *T. viride* [Strain-I, 20 g/l]	40.0 D	2.29 CD	3.1 CD	5.1 D	45.0 D	4.49 BC	5.0 D	9.6 DE
T-2: *T. viride* (Strain-II) (20 g/l)	35.0 E	2.14 D	3.5 B	5.6 BC	40.0 EF	4.31 DE	5.5 C	9.9 D
T-3: *T. harzianum* (20 g/l)	50.0 B	2.88 A	2.9 D	5.9 AB	54.0 AB	4.89 A	5.9 AB	10.9 AB
T-4: *P. fluorescens* (20 g/l)	45.0 C	2.12 DE	2.7 EF	4.2 E	50.0 BC	3.91 F	4.9 D	11.5 A
T-5: *T. viride* (Strain-I) + P. *fluorescens* (10=10 g/l)	45.0 C	2.54 B	3.8 A	4.9 D	46.0 CD	4.62 B	5.8 BC	8.9 F
T-6: *T. viride* (Strain-II) + *P. fluorescens* (10+10g/l)	35.0 E	2.57 B	3.2 C	5.0 B	52.0 A	4.67 AB	6.2 A	9.2 EF
T-7: *T. harzianum* (Strain-I) + *P. fluorescens* (10+10 g/l	55.0 A	2.12 DE	3.6 AB	5.9 A	57.0 A	4.55AB	5.9 B	10.9 B
T-8: Control	15 F	1.65 F	2.3 E	5.7 BC	21.0 G	4.26 E	4.0 E	6.8 H
T-9: Blitox (2 g/l)	40 D	2.31 CD	3.2 C	2.5 F	43.0 DE	3.65 G	5.6 BC	8.7 F

T-10: Seed plus (2 g/l)	46 C	2.4 BC	2.9 D	5.5 C	39.0 EF	4.45 BC	4.9 D	9.2 EF
T-11: Sudha germinaid (1 g/l)	50 B	2.57 B	3.6 AB	6.2 A	54.0 AB	4.67 AB	5.6 AB	10.5 BC
Ems (±)	0.82	0.019	0.05	0.05	1.48	0.04	0.17	0.17
L.S.D (P=0.05)	2.6	0.052	0.16	0.16	4.60	0.21	0.51	0.54
C.V. (%)	6.8	7.5	6.2	6.7	8.1	6.5	7.1	7.4

Table 2: Response of Bioinoculants to Sunflower cv. DRSF-108

Treatments	*96 Hours*				*144 Hours*			
	Germi-nation %	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*	*Germi-nation %*	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*
T-1: *T. viride* [Strain-I, 20 g/l]	25.0 C	3.44 C	3.70 C	3.90 DE	28.0 E	4.54 B	6.10 C	6.20 D
T-2: *T. viride* (Strain-II) (20 g/l)	27.0 C	3.25 CD	3.40 D	3.60 EF	30.0 DE	4.35 BC	5.20 EF	5.40 F
T-3: *T. harzianum* (20 g/l)	30.0 C	4.20 AB	3.40 D	5.35 B	33.0 CD	5.60 A	6.10 C	5.90 F
T-4: *P. fluorescens* (20 g/l)	25.0 C	4.60 A	2.60 F	3.60 EF	28.0 E	5.60 A	5.20 EF	5.30 G
T-5: *T. viride* (Strain-I) + P. *fluorescens* (10=10 g/l)	40.0 B	3.10 CDE	3.30 DE	4.10 EF	48.0 B	5.50 B	6.30 B	8.20 B
T-6: *T. viride* (Strain-II) + *P. fluorescens* (10+10g/l)	45.0 AB	3.85 B	4.10 A	5.50 B	45.0 B	3.80 D	5.60 D	7.60 C
T-7: *T. harzianum* (Strain-I) + *P. fluorescens* (10+10 g/l	30.0 C	3.93 B	4.20 A	5.60 B	51.0 A	4.20 BCD	6.40 B	8.20 B
T-8: Control	15.0 D	2.60 F	2.20 G	3.00 FG	18.0 F	3.90 CD	4.00 G	3.80 H
T-9: Blitox (2 g/l)	28.0 C	2.80 EF	3.30 DE	4.60 C	33.0 CD	4.10 BCD	6.50 AB	6.10 DE
T-10: Seed plus (2 g/l)	40.0 B	3.30 CD	3.20 E	5.70 A	45.0 B	4.50 B	6.70 A	9.10 A
T-11: Sudha germinaid (1 g/l)	50.0 A	4.20 AB	3.90 B	5.30 B	53.0 A	5.10 B	6.50 AB	8.10 B
Ems (±)	1.12	0.031	0.028	0.052	1.33	0.052	0.12	0.16
L.S.D (P=0.05)	3.42	0.45	0.084	0.16	4.05	0.62	0.35	0.46
C.V. (%)	6.6	6.2	6.8	6.1	8.2	8.2	5.8	6.8

Table 3: Response of Bio-inoculants to Sunflower cv. LSFH-171

Treatments	*96 Hours*				*144 Hours*			
	Germi-nation %	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*	*Germi-nation %*	*Seedling wt. (g)*	*Shoot length (cm)*	*Root length (cm)*
T-1: *T. viride* [Strain-I, 20 g/l]	25.0 C	3.44 C	3.70 C	3.90 DE	28.0 E	4.54 C	6.10 C	6.20 D
T-2: *T. viride* (Strain-II) (20 g/l)	27.0 C	3.25 CD	3.40 D	3.60 EF	30.0 DE	4.35 C	5.20 EF	5.40 F
T-3: *T. harzianum* (20 g/l)	30.0 C	4.50 A	3.40 D	5.35 B	33.0 CD	5.60 A	6.20 BC	5.90 F
T-4: *P. fluorescens* (20 g/l)	25.0 C	4.60 A	2.60 F	3.60 EF	28.0 E	4.85 BC	5.20 EF	5.30 G

T-5: *T. viride* (Strain-I) + P. *fluorescens* (10=10 g/l)	40.0 B	3.10 E	3.30 DE	3.10 FG	52.0 A	5.20 AB	6.30 B	7.80 B
T-6: *T. viride* (Strain-II) + *P. fluorescens* (10+10g/l)	45.0 AB	4.60 A	3.40 D	3.60 EF	50.0 A	5.45 A	5.60 D	6.20 D
T-7: *T. harzianum* (Strain-I) + *P. fluorescens* (10+10 g/l	30.0 C	3.93 B	4.20 A	5.40 B	47.0 B	5.20 AB	6.40 A	8.20 A
T-8: Control	15.0 D	2.60 F	2.20 G	3.00 FG	18.0 F	3.90 CD	4.00 G	3.80 H
T-9: Blitox (2 g/l)	28.0 C	2.80 EF	3.30 DE	4.60 C	33.0 CD	4.10 BCD	6.50 AB	6.10 DE
T-10: Seed plus (2 g/l)	40.0 B	3.30 CD	3.20 E	6.70 A	45.0 B	4.50 B	6.20 B	9.10 A
T-11: Sudha germinaid (1 g/l)	50.0 A	4.50 A	3.90 B	6.30 A	55.0 A	5.48 A	6.70 A	8.30 B
Ems (±)	1.30	0.025	0.032	0.056	1.61	0.061	0.12	0.28
L.S.D (P=0.05)	4.60	0.075	0.097	0.47	5.12	0.36	0.61	0.46
C.V. (%)	7.4	6.7	7.5	6.4	7.8	8.5	6.6	7.2

The positive effect on the root system, increased root colonization and root proliferation are probably due to the growth hormones secreted by the bacteria or fungi. The increased nitrogen uptake from the soil might have correspondingly increased the biomass to some extent. The changes in root functions due to *Azospirillum* treatment in different wheat cultivars were also reported by Kapulnik *et al.* (1981). These growth enhancing effects are of interest because of their potential significance for yield increases in agronomic systems in which the use of fertilizers is the limiting factors for their development (Sarig *et al*., 1984). Net shoot, root and seedling weight was higher in bioinoculants treated seedlings than in control (Table 1, 2 & 3). It may be due to the absorption of nutrients from the growing media and stimulate the metabolism of photosynthesis. Photosynthetic activity plays an important role in the increase of leaf area leading to more biomass accumulation. The plants like *Digitaria decumbens*, *Panicum maximum* and *Pennisetum americanum* were subjected to *Azospirillum* inoculation and observed that the photosynthetic rate and dry matter contents were increased to a limited extent (Smith *et al*., 1976; Sarig *et al*., 1984).

The increased chlorophyll content could be correlated with the high level of photosynthesis. This might be due to uptake of more nitrogen from the growing media, and for these activities the working bacteria or fungi or artificial growth inoculants have found to be a great importance. Such experimental findings may be due to increasing level of protein content which may be due to the presence of kinetin that promotes the amino acid content which in turn helps in active protein synthesis (Tien, Gaskins and Hubbel, 1979). Similarly, these findings may be due to increasing level of sugar content in the leaves, or it might also be due to active role of bio/artificial inoculants in sugar metabolism (Watanabe, Cabrera and Barraquio, 1981).

Conclusion

From the present study, it can be concluded that the sunflower seeds tested in response to *Trichoderma harzianu, T. viride* alone or combined with *Pseudomonas fluorescens* inoculation showed high response in terms of seed germination, early shoot and root length, early seedling weight. It may be due to high phytomass and biomass accumulation, and they are attributed to the results of different enzymatic secretes by the bioinoculants on different physiological and

biochemical parameters. The beneficial effect of *Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* on sunflower cultivars varies itself depending upon the crop varieties, microbial strains, and genotypes X microbial strain interaction.

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References

Balasubramanian, A. and Kuamr, K. (1987). Performance of *Azospirillum* in irrigated and rain-fed upland rice. *IRRN*, 12: 43.

Bashan, Y. and Holgain, G. (1995). Inter-root movement of *Azospirillum brasilense* and subsequent root colonization of crop and weed seedlings in soil. *Microbial Ecology*, 29: 269-281.

Bottini, R., Fulchieri, M., Pearce, D. and Pharis, R.P. (1989). Identification of gibberellins A1, A2 and A3 in cultures of *Azospirillum lopoferum*. *Plant physiology*, 90: 45-47.

Dubois, M., Gills, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 26(3): 350-356.

Gopalswamy, G. and Vidhyasekaran, P. (1988a). Effect of *Azospirillum brasilense* on rice yield. *IRRN*, 12: 56-57.

Gopalswamy, G. and Vidhyasekaran, P. (1988b). Effect of *Azospirillum lipoferum* inoculation and inorganic nitrogen on wetland rice. *Oryza*, 26: 378-380.

Hartmann, A., Mahavir, S. and Kligmaller, W. (1983). Isolation and characterization of *Azospirillum* mutants excreting high amounts of Indole acetic acid. *Can. J. Microbiol.*, 29: 916-922.

Horborne, J.B. (1973). Phytochemical methods. A guide to modern techniques of plant analysis. pp.277. London: Chapman and Hall.

Kapulnik, Y., Sarig, S., Nur, I., Okon, Y., Kiel, J. and Henis, Y. (1981). Yield increases in summer cereal crops of Israel in fields inoculated with *Azospirillum*. *Expl. Agric.*, 17: 179-187.

Okon, Y. (1985). *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotech.*, 3: 223-228.

Okon, Y. and Kapulnik, Y. (1986). Development and function of Azospirillum inoculated roots. *Plant and Soil*, 90: 3-16.

Sarig, S., Kapulnik, Y., Nur, I. and Okon, Y. (1984). Response of non-irrigated sorghum bicolor to *Azospirillum* inoculation. *Expl. Agric.*, 20: 59-66.

Sifton, H.B. (1959). The germination of light sensitive seeds of *Typha angustata*. *Can. J. Bot*., 37: 719-741.

Smith, R.L., Bouton, R.H., Schank, S.C., Quessenberry, K.S., Tyer, M.E., Milam, J.R., Garkins, M.H. and Little, R. (1976). Nitrogen fixation in grasses inoculated with *Azospirillum brasilense*, *Curr. Sci.*, 48: 133.

Tien, T.M., Gaskins, M.H and Hubbel, D.H. (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of pearl millet, *Appl. and Environ. Microbiol.*, 37: 1016-1024.

Watanabe, I., Cabrera, D. and Barraquio, W.L. (1981). Contribution of basal portion of shoot to nitrogen fixation associated with wetland rice. *Plant and Soil*, 59: 391-398.