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Effect of micro organisms on physiological traits, growth and yield of sunflower under rainfed condition

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ABSTRACT

The research trial was conducted during 2017-18 at ARS, Kovilpatti, Tamil Nadu Agricultural University to know the effect of various bio-fertilizers on the growth, post-anthesis stress and yield of sunflower under dry land. The research trial was laid out in randomized block design with sixteen numbers of treatments, including single and different combination of silicate solubilizer, sulphur oxidizer, pink pigmented facultative methylotrophs and Vesicular Arbuscular Mychorrhizae and replicated thrice. The results revealed that, combined application of seed and soil application of silicate solubilizer and sulphur oxidizer + soil application of Vesicular ArbuscularMycorrhizae+pink pigmented facultative methylotrophs spray registered significantly higher plant height (172.7 cm), leaf area index (2.44), dry matter production (4120 kg ha^{-1}),number of seeds capitulum (1150), 1000-seed weight (55.7 g), yield (1990 kg ha^{-1}) and gross returns (59,700 ha^{-1}). The economic study revealed that higher net return of Rs. 31779 ha^{-1} and B: C ratio of 2.36 were recorded by a combination of silicate solubilizer + pink pigmented facultative methylotrophs spray. The relative leaf water content and soil moisture recorded at 65 DAS shows that higher leaf relative water content (77%) and available soil moisture (33.3%) were recorded by the combined application of seed and soil application of silicate solubilizer and with or without sulphur oxidizer+ soil application of Vesicular ArbuscularMycorrhizae+pink pigmented facultative methylotrophs spray.

Keywords: Pink pigmented facultative methylotrophs, silicate solubilizer, sulphur oxidizer, sunflower, VAM, yield

Sunflower is a major source of vegetable oil in the world. In India, sunflower is cultivated in 4.1 lakh ha and the production is 2840000 m.t with productivity of 709 kg ha^{-1} (Average of 2014-15 to 2018-19) (Anonymous, 2020). Sunflower is mostly cultivated under rainfed condition with least chemical fertilizer. Sunflower is an exhaustive crop and inadequate and imbalanced crop nutrition affects production. Drought is one of the major destructive environmental stress, which drastically reduces the crop yield than any other environmental stress. For guaranteed food security, crops must be escaped from the impact of drought and nutrient deficiency.

Microorganisms are well known mineralizer under different stress conditions hence ensure improved mineral uptake. Usage of biofertilizers is one of the important components of integrated nutrient management. Different colonies of microorganisms present in the soil, help the plants able to grow under unfavorable environmental conditions. Agriculture sector becomes sustainable because of different micro organism available in the soil (Kumar *et al.,* 2017; Kumar *et al*., 2016; Rana *et al*., 2018).

Pink Pigmented Facultative Methylotrophs (PPFMs) expel auxins and cytokinins that help the plants to tolerate water stress and influence germination and root growth (Doronina *et al*., 2002). Pattanashetti (2012) explained that growth of plants, chlorophyll content and tuber yield were augmented due to PPFMs treatment. PPFM spray along with mulching increased the yield of seed cotton because of better relative water content (RWC) and chlorophyll stability index (CSI) thereby enhanced translocation of photosynthates (Rajasekar *et al.,* 2016; Srinivasan and Aanathi, 2017; Kannan *et al.,* 2019). Osmo-protectants like sugars and alcohols are exuded from the surface of plants due to methylotrophs which protect the plants from dehydration and excessive radiations (Manish and Divjot 2019). Nysanth *et al.* (2019) found that, paddy growth and yield was significantly increased by application of PPFM isolates.

VAM application augments the plant growth by increasing the availability of essential plant nutrients like P, Zn, Cu and S. Suri *et al.* (2011) reported that inoculation of VAM along with 75% recommended P_2O_5 dose significantly increased available N and P status. Extra-radical hyphal mycelium was significantly higher in AMF-inoculated soil than uninoculated AMF soils (Yamsiyah *et al.,* 2018).

Short Communication

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Sulphur is an important essential nutrient for oil seed crops which increases yield, oil content and protein content. Sulphur nutrition also promotes the efficiency of both nitrogenous and phosphorus fertilizers and other micronutrients uptake (Shrivastava *et al.,*2018). Phosphorus and silicon are available in the earth's crust abundantantly, but nutrient requirement of crops are generally met through the applied fertilizer. Insoluble phosphate and silicate are solubilized by Silicate Solubilizing Bacteria (SSB) and also it alleviates toxicity of heavy metal in crops and increases plant growth promoting attributes (Arjun *et al.,* 2020).There is an urgent need to alleviate drought stress and economize the cultivation cost of rainfed farmers. Hence, this research was undertaken to find out the consequence of different microbial organisms on the morphological traits, post anthesis stress management, yield and economics of sunflower under rainfed conditions.

The research experiment was conducted during 2017-18 at Agricultural Research Station ($8^0$48′ and $9^0$20′ North latitude and $78^0$25′ east longitude at 90 MSL), Kovilpatti, TNAU, Tamil Nadu, India to study the influences of biofertilizers and microorganisms on the performance of sunflower hybrid under rainfed conditions. The treatments were as follows, T_1 - Control, T_2- Seed and soil application of silicate solubiliser, T_3 - Seed and soil application of sulphur oxidizer, T_4 - Seed and soil application of silicate solubiliser and sulphur oxidizer, T_5 - Soil application of Vesicular Arbuscular Mycorrhizae (VAM), T_6 - Pink pigmented Facultative Methylotrophs (PPFM) spray on 50 and 65 DAS, T_7 - Soil application of VAM+PPFM spray on 50 and 65 DAS, T_8- Seed and soil application of silicate solubiliser + soil application of VAM, T_9 - Seed and soil application of sulphur oxidizer+soil application of VAM, T_{10} - Seed and soil application of silicate solubiliser+PPFM spray on 50 and 65 DAS, T_{11} - Seed and soil application of sulphur oxidizer+PPFM spray on 50 and 65 DAS, T_{12}- Seed and soil application of silicate solubiliser and sulphur oxidizer+ soil application of VAM, T_{13} - Seed and soil application of silicate solubiliser and sulphur oxidizer+PPFM spray on 50 and 65 DAS, T_{14} - Seed and soil application of silicate solubiliser+soil application of VAM+PPFM spray on 50 and 65 DAS, T_{15} - Seed and soil application of sulphur oxidizer+soil application of VAM+PPFM spray on 50 and 65 DAS, T_{16} - Seed and soil application of silicate solubiliser and sulphur oxidizer+soil application of VAM+PPFM spray on 50 and 65 DAS. The research experiment was conducted in randomized block design (RBD) and replicated thrice. The texture of soil was clay and structure was sub angular blocky having. EC:0.22 dSm^{-1}, pH: 8.1, available N: 156 kg ha^{-1}, available P: 13.1 kg ha^{-1} and available K: 360 kg ha^{-1}. Sunflower hybrid CO-2 was sown in the spacing of 60×30 cm during the end of October as per the treatment schedule.

Inorganic fertilizers @ 40: 50: 40 NPK kg $ha^{-1}$was applied as urea, Di-ammonium Phosphate (DAP) and Muriate of Potash (MOP). The crop was raised under rainfed condition. Data on plant height, LAI, Dry Matter Production (DMP), capitulum diameter (cm), capitulum weight (g), filled seeds/ capitulum, seed weight/ capitulum, seed density (g $100ml^{-1}$), test weight (1000 seed) and sunflower seed yield were recorded replication wise. The sunflower yield (seed) from the net plot area was weighed as kg $plot^{-1}$ after drying and presented in kilogram $hectare^{-1}$. The data were subjected to statistical analysis as suggested by Gomez and Gomez (1984). Growth and yield attributes and yield were statistically analyzed using AGRES computer software. Treatment means were compared using a partition of the sum of squares. Canopy temperature was directly measured with the help of an infrared thermometer and presented in Table 2.

Water status of the plant was assessed by measuring the Relative Water Content (RWC) in leaf tissues. Relative leaf water content was measured by the procedure as follows. With the help of sharp razor blade, the base of the leaf was cut and it was weighed immediately to know the Fresh Mass (FM). The weight of fresh mass should be more than 0.5 g as suggested by Clausen and Kozlowski (1965). Then the leaves were floated in the closed petri dish containing distilled water. Mean while for getting Turgid Mass (TM) samples were periodically weighed until constant weight obtained. Before taking turgid weight, the leaf samples were wiped gently with the help of tissue paper. The petri dishes were kept in dim light (around 20 µmol m^{-2} s^{-1}) under normal room temperature. After taking turgid weight, the leaf samples were kept at 80^oC for 48 hours in an hot air oven for getting dry mass (DM) (Catsky, 1974; Turner, 1981). The RWC was calculated by substituting different values like FM, TM, and DM in the following equation:

RWC (%) = [(FM - DM)/ (TM - DM)] * 100.

Increased plant height (172.7 cm),LAI (2.44) and DMP (4120 kg ha^{-1}) were recorded by seed treatment along with soil application of Silicate solubilizer and Sulphur oxidizer + soil application of VAM+PPFM spray (Table 1). Hyphal network formed by AM fungi in the plant roots significantly increased the roots access to large soil surface area thereby enhancing the plant growth (Bowles *et al.* 2016). Nautiyal *et. al* (2013) found that, *Bacillus amyloliquefaciens* (NBRISN13 -SN13) inoculation on paddy plants increased growth of plants and tolerance to salt (NaCl 200 mM). It was found that, inoculation with sulphur-oxidizing bacteria

Table 1: Effect of treatment on growth attributes, yield attributes and yield of sunflower

Treatments	Plant height (cm)	Leaf Aear Index	Dry Matter Production (kg ha^{-1})	Capitulum diameter (cm)	Capitulum weight (g)	Number of filled seeds capitulum^{-1}	Weight density (g 100 ml^{-1})	1000 seed weight (g)	Yield (kg ha^{-1})
T_1- Control	154.4	1.60	3420	12.2	77.0	900	38.6	49.2	1580
T_2- Seed and SA of Silitcate solubilizer	157.2	1.73	3600	12.8	86.4	950	40.6	50.5	1680
T_3 - Seed and SA of Sulphur oxidiser	158.1	1.63	3410	12.1	78.0	910	38.7	49.1	1560
T_4 - Seed and SA of T_2 and T_3	158.4	1.74	3580	12.9	87.1	955	40.7	50.8	1700
T_5 - SA of VAM	161.8	2.10	3760	13.4	91.5	1070	42.7	51.4	1740
T_6 - PPFM spray	155.4	1.69	3700	12.9	90.5	1085	43.6	51.9	1780
T_7 - SA of VAM+PPFM spray	162.5	2.30	3830	13.5	93.1	1100	44.4	52.9	1810
T_8 - T_2+SA of VAM	166.6	2.37	3800	13.7	90.3	1120	43.2	51.7	1780
T_9 - T_3+SA of VAM	166.8	2.12	3730	13.5	91.4	1060	42.8	51.5	1760
T_{10} - T_3+PPFM spray	162.7	1.71	3790	13.7	93.3	1135	44.2	52.2	1840
T_{11} - T_3+PPFM spray	161.0	1.70	3710	13.0	90.2	1090	43.5	51.8	1800
T_{12} - T_2 and T_3+SA of VAM	168.3	2.30	3870	13.8	90.5	1115	43.1	51.6	1760
T_{13} - T_2 and T_3+PPFM spray	167.5	1.72	3810	13.8	93.2	1130	44.30	52.4	1830
T_{14} - T_2+ SA of VAM+PPFM spray	169.4	2.43	4100	14.4	101.0	1140	45.5	55.6	1985
T_{15}- T_3+ SA of VAM+PPFM spray	170.5	2.33	3850	13.5	92.9	1110	44.30	53.0	1830
T_{16}- T_2 and T_3+SA of VAM+PPFM Spray	172.7	2.44	4120	14.5	101.1	1150	45.6	55.7	1990
SEm (±)	**5.9**	**0.11**	**188**	**0.7**	**4.5**	**53**	**2.1**	**2.6**	**89**
LSD (0.05)	**NS**	**0.22**	**383**	**1.4**	**9.2**	**108**	**4.3**	**NS**	**182**

Note : SA is short form of Soil Application

Table 2: Effect of treatment on canopy temperature, RWC, soil moisture and economics of sunflower

Treatments	Canopy temperature difference		Leaf RWC (%) at 65 DAS	Soil moisture (%) at 65 DAS		Cost of cultivation Rs. ha^{-1}	Gross return Rs. ha^{-1}	Net return Rs. ha^{-1}	BCR
	Bud initiation	Peak flowering		15 cm	30 cm				
T_1- Control	-1.2	-0.9	62	27.2	31.0	22621	47400	24779	2.10
T_2- Seed and SA of Silicate solubilizer	-2.2	-1.8	66	27.8	31.6	22721	50400	27679	2.22
T_3 - Seed and SA of Sulphur oxidiser	-1.2	-1	63	27.3	31.1	22621	46800	24179	2.07
T_4 - Seed and SA of Silicate solubilizer and Sulphur oxidiser	-2.5	-2.1	65	27.9	31.7	22721	51000	28279	2.24
T_5 - SA of VAM	-2.6	-2.2	68	27.6	32.2	33721	52200	18479	1.55
T_6 - PPFM spray	-2.9	-2.6	70	27.5	31.4	23221	53400	30179	2.30
T_7 - SA of VAM+PPFM spray	-3.4	-3.1	74	28.7	32.5	34421	54300	19879	1.58
T_8 - Seed and SA of Silicate solubilizer+SA of VAM	-3.6	-3.2	70	28.9	32.7	33921	53400	19479	1.57
T_9 - Seed and SA of Sulphur oxidiser+ SA of VAM	-2.2	-1.8	67	27.2	32.1	33721	52800	19079	1.57
T_{10} -Seed and SA of Silicate solubilizer+ PPFM spray	-3.6	-3.3	73	28.1	32.0	23421	55200	31779	2.36
T_{11} - Seed and SA of Sulphur oxidiser+ PPFM spray	-2.9	-2.4	71	27.4	31.6	23221	54000	30779	2.33
T_{12} -Seed and SA of Silicate solubilizer and Sulphur oxidiser+SA of VAM	-3.8	-3.1	70	29	32.9	33921	52800	18879	1.56
T_{13} -Seed and SA of Silicate solubilizer and Sulphur oxidiser+PPFM spray	-3.9	-3.4	72	28	32.2	23421	54900	31479	2.34
T_{14} -Seed and SA of Silicate solubilizer+ SA of VAM+PPFM spray	-4.5	-4	77	29.3	33.2	34621	59550	24929	1.72
T_{15} - Seed and SA of Sulphuroxidiser + SA of VAM+PPFM spray	-3.1	-2.8	74	28.5	32.4	34421	54900	20479	1.59
T_{16} - Seed and SA of Silicate solubilizer and Sulphur oxidiser+SA of VAM+ PPFM Spray	-4.3	-4	77	29.2	33.3	34621	59700	25079	1.72

Note : SA is short form of Soil Application

(*Thiobacillus*) increased growth of plants and dry matter of onion when compared to those plants grown without inoculation. Nysanth *et al.* (2019) found that, PPFM application significantly improved growth, dry matter production and paddy grain yield. Hence combined application of all microbial organisms significantly increased the growth attributes of crops.

The yield attributes *viz.,*capitulum diameter (14.5 cm), capitulum weight (101.1 g), number of filled seeds capitulum^{-1}(1150 nos.), seed density (45.6 g 100 ml^{-1}), and seed test weight (55.7 g) were significantly higher in the combined application of seed and soil application of Silicate solubilizer and Sulphur oxidizer+soil application of VAM + PPFM Spray (Table 1).

Inoculation with VAM fungi increased chlorophyll content, higher photosynthetic rate, nitrate reductase and glutamine synthetase activity in leaves and maintained it even at the later stage of growth (90 DAS) thereby improved the growth and yield (5.4 g plant^{-1}) in wheat (Panwar, 1999). Santi *et al.* (2018) observed that, oil palm seedlings treated with bio-silica showed better root growth under drought and also improved the leaf Chlorophyll Content (CC) 20% than control. Based on two years of study, Chaudhary *et al.* (2019) found that, mustard seeds inoculated with Silicate Solubilizing Bacteria (SSB) oxidized the sulphide compounds to easily absorbable form (sulphate) by plants. Better absorption of sulphate by plants resulted in enhanced mustard growth parameters *viz*., height, dry matter production, number of siliquae, weight of seed, oil content, leaves protein content and viable rhizospheric bacterial count. Pattanashetti (2012) reported that Coleus plant treated with PPFMs showed increased plant height, stem girth, CC, DMP, LAI and tuber yield.

Significantly higher sunflower seed yield of 1990 kg ha^{-1}was registered by the seed and soil application of silicate solubilizer and sulphur oxidizer + soil application of VAM + PPFM Spray (Table 1). Anandham *et al.* (2007) found that sulphur oxidizer inoculation increased the available form of sulphur from 7.4 to 8.43 kg ha^{-1} in soil and thereby increased the groundnut oil content. Soil application of 25 t fly ash ha^{-1} + silicate solubilizing bacteria (SSB) + FYM registered 16.3 per cent higher yield (3710 kg ha^{-1}) than control (Pedda *et al*., 2016). Kannan *et al.* (2019) found that application of *Prosopis* biochar @ 5 t ha^{-1} + mulching with crop residue @ 5 t ha^{-1} and PPFM foliar spray @ 500 ml ha^{-1} on 75 and 90 DAS increased seed cotton yield by 59% and 61 % during summer and winter respectively over control due to increased RLWC, proline accumulation in leaf and CSI. Cucumber seedlings inoculated with AM fungi significantly increased the availability of macro and micronutrients which augmented the photosynthate productions thereby higher DMP (Chen *et al*., 2017). Consequently, combined application of PPFM, VAM, silicate solubilizer and sulphur oxidizer have undoubtedly increased the yield of sunflower under rainfed conditions.

The canopy temperature recorded at bud initiation and peak flowering stages showed negative value in all treatments indicating that there was no stress at this stages as the rainfall during the period were sufficient enough to keep the soil with more available soil moisture. The relative leaf water content and soil moisture recorded at 65 DAS showed that higher RWC (77%) and ASM (33.3%) were recorded by the combined application of seed and soil application of silicate solubilizer and with or without sulphur oxidizer + soil application of VAM + PPFM Spray (Table 2). VAM inoculation significantly increased the leaf surface area and also antioxidant enzymes like super dismutase and catalase, proline and water content, hydrogen peroxide and carotenoids in leaves as well as in roots (Rafia and Moin, 2019). PPFM spray in paddy significantly enhanced the plant physiological parameters *viz*., cell membrane stability, chlorophyll and proline content and also grain yield (46.30g hill^{-1}) over control (grain yield of 33.65g hill^{-1}) (Nysanth *et al*. 2019).

Liu *et al.* (2015) found that Si application in sorghum increased the hydraulic and stomatal conductance, RWC and transpiration under osmotic stress. Root length and its surface area decide the plant nutrient uptake. If root surface area is high, the uptake of diffusible ions also high because of more exposed sites for uptake. However, the treatment which received PPFM spray invariable of other bio-fertilizer combination recorded higher relative water content percentage compared to other treatments (Table 2). Kannan *et al.*(2019) found that higher RWC, CSI, less proline content and higher seed cotton yield was recorded by combined effect of irrigation at IW/CPE to 0.8 + *Prosopis* biochar @ 5 t ha^{-1}+ mulching with crop residue @5 t ha^{-1} + spraying of PPFM @ 500 ml ha^{-1} on 75 and 90 DAS.

Higher gross return of Rs. 59,700 ha^{-1}was recorded by the combined application of seed and soil application of silicate solubilizer and sulphur oxidizer+soil application of VAM+PPFM Spray. The higher net return of Rs. 31779 ha^{-1} and B: C ratio of 2.36 were recorded by combined application of seed and soil application of silicate solubilizer+PPFM spray(Table 2) due to the low cost of bio fertilizer and high cost of VAM fertilizer.

CONCLUSION

The combined application of seed and soil application of silicate solubilizer and sulphur oxidiser + soil application of Vesicular Arbuscular Mycorrhizae

(VAM) + PinkPigmented Facultative Methylotrophs (PPFM) was found best in influencing higher yield and gross return. However combined application of seed and soil application of silicate solubilizer + Pink-Pigmented Facultative Methylotrophs (PPFM) spray gave highest net return and B:C under rainfed condition

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