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Studies on effect of sulphur oxidizing microorganism and humic acid on growth and yield of sunflower (*Helianthus annus* L.)

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Abstract
A field experiment was conducted in the *kharif* season of 2024-25 at the Department of Soil Science, College of Agriculture, Badnapur to study the effect of sulphur oxidizing microorganism and humic acid on growth and yield of sunflower (*Helianthus annus* L.). The experiment was laid out in a Randomized Block Design with six treatments: (T_1) RDF, (T_2) RDF + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*), (T_3) RDF + 5 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*), (T_4) RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (T. *thiooxidants*), (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*) and (T_6) 75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*) with four replications. The results indicated that the treatment (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* recorded the highest plant height at harvest (201.56 cm), ear head diameter at harvest (18.94 cm), leaf area plant^{-1} (29.57 dm^2), dry matter accumulation (136.71 g plant^{-1}), number of seeds ear head^{-1} (1254.1) and seed yield (2119.5 kg ha^{-1}).

Keywords: Growth, *Helianthus annus* L., humic acid, seed inoculation, sulphur oxidizing bacteria, sunflower, *T. thiooxidants* and yield

1. Introduction
Sunflower (*Helianthus annuus* L.) is a versatile oilseed crop valued for its short growing cycle, photo insensitivity, and adaptability to diverse agro-climatic conditions and soil types. Rising population, enhanced living standards, and increased demand from food processing industries have led to a considerable shortage of edible oils, compelling higher imports to fulfill domestic needs (Rauf, 2017) [22].

Sulphur (S), although absorbed in small amounts in organic forms, plays a vital role in plant growth and metabolism. Plants predominantly absorb sulphur as sulphate (SO_4^{2-}), which is made more available through the action of sulphur-oxidizing bacteria (SOB) such as Thiobacillus, Thiothrix, and Beggiatoa (Das *et al.*, 1996; Katyal *et al.*, 1997) [7, 12]. These bacteria oxidize reduced inorganic sulphur compounds to sulphate, enhancing sulphur bioavailability for plants, while also mitigating environmental hazards like hydrogen sulphide emissions (Vidyalakshmi *et al.*, 2009) [24]. Among SOB, *Thiobacillus thiooxidans*, an acidophilic autotroph, is widely used as a biological fertilizer to improve soil sulphur content and promote plant growth (Waksman and Joffe, 1922; Chaudhary, 2018) [25, 3].

Humic acid, a major component of humus, is increasingly employed in agriculture due to its beneficial effects on soil properties and plant health. It improves soil structure, water retention, nutrient availability, and root growth, which collectively boost fertilizer efficiency and crop yield (Dandge *et al.*, 2016) [6]. Humic acid also enhances micronutrient accessibility and microbial activity in the soil. Its sustained presence in the soil ecosystem makes it a valuable component in integrated nutrient management (Nalia and Sengupta, 2019) [14].

2. Materials and Methods
2.1 Experimental site
The field experiment was conducted during the *kharif* season of 2024-25 at the experimental

farm of the Department of Soil Science, College of Agriculture, Badnapur. The experimental field exhibited a clayey texture in its soil composition, with a measured pH level of 7.67, indicating a neutral acidity level. The sample exhibited organic carbon levels of 4.53 g kg^{-1} and available nitrogen measured at 148.73 kg N ha^{-1}. The analysis indicated available phosphorus measured at 15.48 kg P ha^{-1} and available potassium recorded at 548.40 kg K ha^{-1}. The crop received a total of 731.8 mm of rainfall throughout the duration of the experiment.

2.2 Experimental details

After completion of preparatory tillage operations, the experimental units were laid out as per plan. The experiment was laid out in randomized block design (RBD) with six (6) treatments each replicated four times. The treatment comprises (T_1) RDF, (T_2) RDF + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*), (T_3) RDF + 5 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*), (T_4) RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (T. *thiooxidants*), (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*) and (T_6) 75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*). Sunflower seed variety LSFH-171 was sown on July 18, 2024 by dibbling method as per randomly replicated plot having size 4.2 × 3 m^2 and 3.00 × 2.40 m^2 in gross and net plot respectively, maintained row to row spacing 60 cm and plant to plant 30 cm and using a seed rate of 5-6 kg ha^{-1}. Before sowing, the strain of sulphur oxidizing bacteria (*Thiobacillus thiooxidans*) was sourced from AICRP (All India Coordinated Research Project) VNMKV, Parbhani. The concentration of sulphur oxidizing bacteria (*Thiobacillus thiooxidans*) was measured at 1.04 x 10^6 cfu g^{-1} of biofertilizer. This measurement was obtained using the standard serial dilution plate count method with thiosulphate media, as outlined by Vidyalakshmi *et al.* (2009) [24]. Seed treatment was conducted using sulphur oxidizing biofertilizer at a rate of 100 ml 10 kg^{-1} seed. kg^{-1} of seeds.

A consistent application of 30 kg P_2O_5 and 40 kg K_2O ha^{-1} was administered using single super phosphate and muriate of potash as the basal dose at rates of 100% and 75%, respectively, in accordance with the treatment combinations for all plots. Nitrogen was applied at a rate of 60 kg ha^{-1} using urea at the time of sowing. Humic acid granules were applied at rates of 5, 10, and 15 kg ha^{-1} during sowing as a basal application, in accordance with the specified treatment combinations.

Five plants from each plot were randomly selected and used for recording biometric observations, the growth parameters *viz*. plant height (cm), ear head diameter (cm) and leaf area (dm^2). Yield attributing parameters *viz*. number of seeds $earhead^{-1}$, seed yield (kg ha^{-1}) and dry matter accumulation $plant^{-1}$ (g), were recorded at harvest stage. Economic analysis of different treatments were also observed during the experiment. The data collected from the above observation were analysed statistically by the procedure prescribed by Panse and Sukhatme (1985) [17].

3. Results and Discussion

3.1 Effect of sulphur oxidizing microorganism and humic acid application on growth attributes of sunflower

3.1.1 Plant height at harvest

The height of the sunflower plant was statistically examined at harvest stage of the crop and was found significantly affected which has been displayed in table 1. The maximum plant height at harvest stage was recorded in (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (201.56 cm) which was found statistically at par with treatment (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (197.65 cm) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*) (195.88 cm). However, lowest plant height was recorded in (T_1) RDF (183.25 cm).

The observed effects can be attributed to the application of humic acid, which had significantly modified soil properties such as aggregation, aeration, permeability, and water-holding capacity. The presence of humic acid enhances microbial activity, leading to increased rates of organic matter mineralization and nutrient solubilization. The increase in microbial activity is linked to enhanced availability of macro and micronutrients, which meets the nutrient requirements of fast growing crops. Kumar *et al.* (2016) [13] observed a notable increase in the height of sunflower plants, measuring 129.67 cm, following the application of RDF combined with humic acid at a rate of 15 kg per hectare. Seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* might also have affected the plant height as described by Pidurkar *et al*. (2022) [19], who documented an increase in plant height, measuring 60.0 cm, resulting from the application of sulphur oxidizing bacteria which might have influenced the native sulphur pool in the soil. This conversion to SO_4 forms enhanced the availability of sulphur, promoting optimal plant growth and resulting in increased plant height. Pujar *et al*. (2014) [20] also reported comparable findings in their study on sunflower, where the maximum plant height of 195 cm was achieved through the application of sulphur at rates of 20 and 30 kg ha^{-1}, in combination with sulphur oxidizing biofertilizes.

3.1.2 Ear head diameter at harvest

The data pertaining to ear head diameter of sunflower recorded at harvest stage was found significantly different and presented is in table 1. The treatment (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (18.94 cm) recorded highest ear head diameter which was found at par with treatment (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*) (18.05 cm) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (17.78 cm) whereas treatment T_1 (RDF) (15.68 cm) recorded minimum head diameter at harvest.

The increase in ear head diameter observed under treatment T_5 (RDF + 15 kg humic acid ha^{-1} + seed inoculation of *T. thiooxidants*) is likely due to the combined effects of humic acid and sulphur oxidizing bacteria, which enhanced the physical and biological properties of the soil. Humic acid improves nutrient retention and uptake by enhancing soil aeration and cation exchange capacity. Meanwhile, *T. thiooxidants* facilitate sulphur mineralisation, which increases sulphate availability. This process contributes to enhanced plant metabolism, transpiration, and dry matter accumulation, ultimately resulting in a greater number of filled seeds and larger flower heads. AL-Abody *et al*. (2021) [1] reported an increase in head diameter associated with the application of humic acid in sunflower cultivation. Choudhary *et al.* (2019) [4] reported an increase in head diameter in sunflower as a result of applying 45 kg ha^{-1} of sulphur in conjunction with a sulphur oxidizing biofertilizer.

3.1.3 Leaf area (dm^2)

According to data depicted in table 1. show significant difference in leaf area of sunflower. Treatment (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (29.57 dm^2) recorded larger leaf area which was found superior as compared to other treatments but was found statistically at par with treatment (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (27.46 dm^2) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (26.94 dm^2). The least leaf area was recorded in (T_1) RDF (24.08 dm^2).

The observed effects may be attributed to the soil application of humic acid, which has been shown to positively influence vegetative growth by enhancing photosynthetic activity and increasing leaf area. This outcome was accomplished by enhancing root activity, which subsequently leads to improved nutrient availability and uptake. The enhanced availability of nutrients promotes stronger leaf development, resulting in an expansion of leaf area. Padghan *et al.* (2018) [16] reported an increase in leaf area measuring 46.32 dm^2 at 85 days after sowing (DAS) and 70.06 dm^2 at 105 DAS, attributed to the application of 400 ppm humic acid and vermicompost in conjunction with 50 ppm NAA. Nalia and Sengupta (2019) [14] reported a maximum leaf area of 3.37 cm^2 resulting from a treatment that included soil application of humic acid at a rate of 15 litres per hectare, in conjunction with compost applied at 10 tonnes per hectare. Seed inoculation with sulphur oxidizing bacteria (*T. thiooxidants*) may also have enhanced availability of sulphur in the rhizosphere. This enhancement occurs through the microbial oxidation of native sulphur into sulphate (SO_4^{2-}), which is the form accessible to plants. Sulphur is essential for the synthesis of chlorophyll and the formation of proteins, contributing to robust vegetative growth and the increase of leaf surface area. Enhanced sulphur nutrition contributes to increased photosynthetic efficiency, resulting in larger and healthier leaves. Choudhary *et al.* (2017) [5] reported comparable results, indicating an increase in leaf area of 99.55 dm^2 following the application of 45 kg ha^{-1} of sulphur in conjunction with a sulphur oxidizing biofertilizer.

3.2 Effect of sulphur oxidizing microorganism and humic acid application on yield attributes of sunflower

3.2.1 Number of seeds earhead^{-1}

The data in table 2 revealed that the number of seeds earhead^{-1} was affected significantly by different treatments. Treatment (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (1254.1) recorded significantly maximum number of seeds earhead^{-1} compared to other treatments and was found at par with treatment (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (1223.71) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) 1182.25) whereas treatment (T_1) RDF (992.5) observed lowest number of seeds earhead^{-1} as compared to other treatments.

The findings align with the observations made by Patil *et al.* (2011) [18], who reported an increase in the number of pods plant^{-1}, specifically 85.00 for soybean and 55.21 for blackgram, following the application of potassium humate at a rate of 5 kg acre^{-1}. Waqas *et al.* (2014) [26] reported a significant increase in the number of pods plant^{-1} in mung bean, with a recorded average of 25.3 pods plant^{-1} resulting from the soil application of humic acid at a rate of 3 kg ha^{-1}. Sulphur is also essential for enhancing the vegetative structure, facilitating nutrient absorption, and contributing to strong sink strength through the development of reproductive structures and the production of assimilates. The incorporation of sulphur oxidizing bacteria ensures a favourable supply of nutrients, particularly sulphur in its bioavailable form, which is essential for optimal growth and yield related characteristics, including nitrogen assimilation by plants. Asadi Rahmani *et al.* (2018) [2] also reported comparable findings in canola as a result of the application of sulphur and *Thiobacillus*.

3.2.2 Seed yield (kg ha^{-1})

The data on seed yield of sunflower (Table 2) indicated that different treatments influenced seed yield of sunflower significantly. It was observed that the application (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (2119.5 kg ha^{-1}) recorded highest seed yield and was found at par with (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (2049.82 kg ha^{-1}) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (1963.13 kg ha^{-1}). However, the least seed yield was obtained with treatment (T_1) RDF (1722.98 kg ha^{-1}).

The observed increase in seed yield can be attributed to the synergistic effect of improved growth-contributing characteristics, which were clearly evident in the final output, specifically in seed and dry matter production. Nandini and Math (2019) [15] recorded similar results, indicating that the application of humic acid at a rate of 15 kg ha^{-1} enhanced the grain yield of blackgram. Rasool *et al.* (2015) [21] observed an increase in grain yield of rice, measuring 3233 kg ha^{-1}, with the application of humic acid at a rate of 25 kg ha^{-1}. Simultaneously, sulphur oxidizing bacteria transform elemental sulphur into plant accessible sulphate, thereby meeting the sulphur demand necessary for protein synthesis, enzyme activation, and reproductive development. Joseph *et al.* (2014) [10] also indicated that the yields of grain and straw, along with nitrogen uptake in canola and wheat, were significantly enhanced through the process of bacterization.

3.2.3 Dry matter accumulation plant^{-1} (g)

According to the results based on dry matter accumulation plant^{-1} of sunflower (Table 2), effect of different treatments was found significantly effective. It was observed that the application of (T_5) RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (136.71 g) recorded highest dry matter among all the treatments but was found at par with (T_4) (RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (134.74 g) and (T_6) (75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants*) (131.46 g). The lowest dry matter was also found in treatment (T_1) RDF (117.51 g).

The observed increase in dry matter accumulation plant^{-1} may be attributes to auxin-like growth-promoting properties of humic acid. The enhancement in dry matter production is linked to an increase in plant height, a greater number of leaves plant^{-1}, and an expansion in leaf area plant^{-1}. Nalia and Sengupta (2019) [14] reported the highest dry matter accumulation of 486.061 g at 105 days after sowing, attributed to the application of humic acid at a rate of 15 litres ha^{-1}, in conjunction with compost at 10 t ha^{-1} in pigeonpea. Suryanwanshi (2021) also found maximum stover yield recorded in treatment RDF + Humic acid @ 10 kg ha^{-1} + VAM @ 5 kg ha^{-1} (3170.58 kg ha^{-1}). Kapase *et al.* (2013) [11] reported aligned findings, noting a significant increase in maximum dry matter resulting from the application of humic acid via vermiwash. Sulphur-oxidizing bacteria may also have interacted with native sulphur, converting it to SO_4, thereby enhancing the availability of sulphur for optimal growth and associated attributes linked to increased dry matter. The findings align with those of Choudhary *et al.* (2017) [5], who reported an increase in dry matter resulting from the application of 45 kg ha^{-1} of sulphur in conjunction with a sulphur-oxidizing biofertilizer in sunflower cultivation.

Table 1: Effect of sulphur oxidizing microorganism and humic acid application on plant height of sunflower

Treatment	Plant height (cm) at harvest	Ear head diameter (cm) at harvest	Leaf area $plant^{-1}$ (dm^2)
T_1: RDF	183.25	15.68	24.08
T_2: RDF + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	188.06	16.03	25.21
T_3: RDF + 5 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	191.25	16.7	25.78
T_4: RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (T. *thiooxidants*)	197.65	18.05	27.46
T_5: RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	201.56	18.94	29.57
T_6: 75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	195.88	17.78	26.94
S.E. (m) (±)	3.39	0.61	1.04
C.D. @ 5%	10.21	1.85	3.18

Table 2: Effect of sulphur oxidizing microorganism and humic acid application on number of seeds $earhead^{-1}$ and yield (kg ha^{-1}) of sunflower

Treatment	Number of seeds $earhead^{-1}$	Yield (kg ha^{-1})	Dry matter accumulation $plant^{-1}$ (g)
T_1: RDF	992.5	1722.98	117.51
T_2: RDF + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	1051.26	1786.01	119.49
T_3: RDF + 5 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	1118.37	1870.78	124.96
T_4: RDF + 10 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (T. *thiooxidants*)	1223.71	2049.82	134.74
T_5: RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	1254.1	2119.5	136.71
T_6: 75% RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria (*T. thiooxidants*)	1182.25	1963.13	131.46
S.E. (m) (±)	44.97	53.93	3.68
C.D. @ 5%	135.51	162.54	11.18

4. Conclusion

It can be concluded that the application of RDF + 15 kg humic acid ha^{-1} + seed inoculation of sulphur oxidizing bacteria *T. thiooxidants* (T_5) was the most effective treatment for sunflower cultivation. This treatment significantly improved key growth parameters along with yield attributes of sunflower. The beneficial effects can be attributed to the synergistic action of humic acid enhancing soil structure, nutrient solubilisation, and microbial activity and sulphur oxidizing bacteria, which increased sulphate availability and nutrient uptake. These combined amendments fostered vigorous vegetative growth, improved reproductive development, and ensured optimal nutrient assimilation, ultimately resulting in higher productivity and profitability in sunflower production.

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