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Use of *Trichoderma Hamatum* Alone and In Combination with *Rhizobial* Isolates as Biofertilzer for Improving the Growth and Strength of Sunflower

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ABSTRACT

In the present pot experiment, *T.hamatum* alone and in combination with *rhizobial* isolates was investigated their effects on growth of sunflower besides determining its effect on organic and inorganic constituents in leaves of same plant. The observations showed that *T.hamatum* itself and *rhizobial* isolates *viz.,* JUR1, JUR2 and JUR4 significantly increased the lengths of both root & shoot and biomass as compared to control, same isolates in combination with *T.hamatum* were also found effective in promoting growth at 30th and 60th day of growth (p<0.05). Total chlorophyll content was found significantly increased by *T. hamatum*, JUR1, JUR3, fertilizer and fungicide in their respective groups where as *T.hamatum* with JUR3, JUR4 and fertilizer increased same parameter at 30th day (p<0.05). However, JUR1 alone and JUR4 with *T.hamatum* found to maintain the increase in chlorophyll content up to 60th day (p<0.05). Similarly total carbohydrate and crude protein contents of sunflower leaves were increased by *T.hamatum*, JUR1 and JUR4 individually and *T.hamatum* with JUR3 and JUR4 at 30th day though the increase in these parameters was observed at 60th day of growth by JUR4 alone and JUR4 with *T.hamatum* (p<0.05). *T.hamatum,* JUR1 and JUR4 individually and *T.hamatum* with JUR1, JUR3 and JUR4 significantly increased nitrogen content at both day intervals (p<0.05). *T. hamatum* alone and with JUR1, JUR3 at 30th day while with JUR4 at 60th day of growth significantly increased phosphorus content (p<0.05). The results concluded that *T.hamtum* alone and in combination with *rhizobial isolates* found effective not only increasing the growth of sunflower plant by improving its root-shoot length and biomass but also increasing the organic and inorganic content of same plant.
KEYWORDS: Biofertilizer, *Trichoderma hamatum, Rhizobial isolates,* Sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) belongs to the family *Compositae* and one of most important oilseed plants in the world and ranked second than soybean [1,2]. Though its oil is rich in saturated fat content but it considered low as compared to other vegetable oils [3]. During the last 3 decades, its cultivation is gradually increases globally and interestingly the climatic conditions of Pakistan is friendly compactable to the growth of sunflower [4]. However, its yield and production according to the consumer demand is very low in Pakistan for certain reasons including less availability of local varieties or hybrids, etc [5] and imported seeds always considered as a gateway of new fungal and insect pests/varieties.

As Pakistan is a developing country, more than 67% its total population is living in rural areas and their earning are mainly depends on agriculture [6]. In spite of this, there is always a gap reported between the productivity of crops and consumers' demand in our country that increases day by day. This gap could be fulfilled by maintaining the sustainable agriculture but there are number of threats available for it including improper irrigation system, poor farming practice that affects soil fertility which may leads to reduced crop productivity [7]. Normally fertilizers play an important role for increasing crop productivity and these provide essential plant nutrients such as nitrogen, phosphorus, boron, zinc, individually or in combination of two or three, according to the plant growth requirements [8]. Factors including non-availability of specific fertilizer on time, their continuously increasing prices and inappropriate application methods, etc again put some limitations in the use of fertilizers [9]. In addition, these also produced environmental and health harzads [10, 11]. In order to minimize the adverse effects of inorganic fertilizer, biotechnologists introduced fungi and growth promoting bacteria like *Rhizobium, Bradyrhizobium,* etc, as biofertilizers which can be inoculated in rhizospere (soil near the roots) where they enhanced the availability of vital nutrients from soil for plant and in turn increased their growth and productivity [12].

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Biofertlizers are reported to be involved efficiently in carrying out practices for maintaining the sustainable agriculture [13,14]. Studies provide evidences that bacterial inoculation also promote plant growth by increasing N uptake and reducing the amount of fertilizer nitrogen that normally used [15]. *Rhizobium* sp. is well-reported for its ability for nitrogen fixation by creating symbiosis in roots nodules of leguminous plants and helped the plants to utilize atmospheric nitrogen for their healthy growth [16]. Increased in nitrogen contents of seeds, number of nodules and yield of different crops have been observed by using *rhizobium* inoculants with and without fertilizer in many experiments [15]. On the other hand, these inoculants are also advantageous for promoting the growth of non-leguminous plants by different effects induced in rhizosphere like hormone production, phosphate solubilization, controlling root-infecting pathogens [17]. These abilities make *rhizobial* inoculants as important biofertilizer for conducting sustainable agriculture [18]. *Rhizobia* are reported to produce various metabolites such as cytokinins, riboflavin, vitamins, etc and their invasion in roots of legume and non-legume plants, not only promotes an increase in plant growth but also significantly improves the plant health [19]. Similarly diazotrophic *rhizobacteria* also increase the vegetative growth of crop plants by interacting with their roots [20, 21].

Trichoderma species including *T.viride, T.harzianum, T.hamatum*, etc are one of the frequent colonizers of rhizosphere of many plants [12]. These species are well-reported for not only to restrict the growth of root-infecting pathogens and prohibit the occurrence of plant diseases (mycofungicide) but also for promoting growth of plants and thereby increase the yield (biofertlizer) [22]. Hence these are successfully used in greenhouse and fields for increasing plant production [23, 24]. The *Trichoderma* genus is reported to improve the growth of plants, increasing the half-life of seedling, plant height and weight and leaf area, etc [25]. These beneficial effects on plant growth in the presence of *Trichoderma* inoculants are reported due to the improvement in mineral uptake, decomposing organic matter, production of plant hormones, enzymes and antibiotics, etc [12]. This genus was found equally effective in stimulating the growth of both legume and non-legume plants like growth stimulation of *Phaseolus vulgaris* (bean) seedling was observed by rhizosphere competent and endophytic strains of *Trichoderma* [26] and *T. viride* induced growth promotion in cotton plants [27].

Therefore considering the importance of *Trichoderma* and *rhizobial* species as biofertilizer in maintaining the long-term fertility of soil which in turn induce the growth promotion in plant, the present study was designed to use *T. hamatum* alone and in combination of rhizobial isolates to improve the growth of sunflower by estimating its physical and biochemical parameters.

MATERIALS AND METHODS

Experimental Plant

Seeds of *Helianthus annuus* (sunflower) were purchased from Old vegetable market, Hyderabad, Pakistan.

Fertilizer and Fungicide

NPK (fertilizer) and carbendazim (fungicide) were purchased from dealer of Agrochemical, Old vegetative market, Karachi, Pakistan and were used as positive controls @ 2500ppm each of them.

Isolation of *Trichoderma hamatum* from Rhizoplane

Root samples of wild herb *Amaranthus viridis* (family: *Amarantheceae*) were collected and used to isolate *Trichoderma hamatum* from rhizoplane by using standard method [28]. In which roots were washed in running tap water, 1cm long root pieces from tap and lateral roots were cut and washed in sterilized distilled water. Then root pieces were transferred on plate containing potato dextrose agar (PDA) incorporated with penicillin (100,000unit/liter) and streptomycin (0.2g/liter) to inhibit the growth of gram-positive and negative bacteria. Petri plates were incubated for 5 days at 28°C. Grown fungi were identified by expert of Botany Department, University of Karachi, Karachi, Pakistan. Of which, *T. hamatum* was made separated, isolated pure and preserved on PDA slants for further use.

Table 1: Test microorganisms with code no and their host plants

S.No.	Test microorganism	Code No.	Host plant	Location
1.	*Trichoderma hamatum*	JUF1	*Amaranthus viridis*	North Nazimabad
2.	*Rhizobium sp.*	JUR1	*Trigonella foenum-graecum*	University of Karachi
3.	*Bradyrhizobium sp.*	JUR2	*Phaseolus unguiculata*	Memon Goth
4.	*Bradyrhizobium sp*	JUR3	*Vigna radiata*	Net house of JUW
5.	*Bradyrhizobium sp*	JUR4	*V.mungo*	Net house of JUW

JUW= Jinnah University for Women

Isolation of *Rhizobial* isolates from root nodules

Root samples of *Trigonella foenum-graecum, Phaseolus unguiculata, Vigna radiata* and *V.mungo* (family: *Fabaceae*) were collected and used to isolate *rhizobial* cultures by crushed-nodule method [28]. In which, roots were washed in running tap water to remove adhering soil particles. Select healthy pink, unbroken and firm nodules. Immerse the nodules in 0.1% $HgCl_2$ for 5 minutes for surface sterilization. Then nodules were washed with sterilized water thrice to remove extra micro-organism. Place the nodules in 70% ethyl alcohol for 3 minutes and washed them again with sterilized distilled water. Nodules were crushed in sterilized distilled water (1 mL) to make uniform suspension of rhizobium that referred as nodule extract. Make serial dilutions of nodule extract (1:10 to 1:10,000). Spread 0.5mL of each of last two highest dilutions on Yeast Extract Mannitol Agar (YEMA) plates and incubate at 28°C for 10 days. White gummy colonies of *rhizobia* were appeared within 4-7 days. The *rhizobial* isolates were sub-cultured, purified and tested for the ability to form nodules on their respective host plants [29]. The stock culture of pure and tested rhizobial isolates were maintained on YEMA slants, coded (Table 1) and stored at 4 to 8°C for further use in the present study.

Preparation of Conidial and cell inoculums of *T.hamatum* and *Rhizobial* isolates

Four petri plates containing five day old cultures of *T.hamatum* on PDA were blended with 40mL of distilled water (10mL/petri plate), then make its volume up to 50mL with the help of sterilized distilled water and considered it 1:10 dilution. Then its serial dilutions from 1:100 to 1:10,000 were made. Twenty five milliliters of highest dilution was used as inoculums after calculating number of conidia per mL or colony forming unit per mL with help of haemocytometer. Similar procedure was used to prepare cell inoculums of *rhizobial* isolates after calculating number of cells per mL.

Experimental design and procedure

The randomized complete block designed pot experiment was conducted in net house of Department of Botany, Jinnah University for Women, Nazimabad Karachi, Pakistan in 2010 to check the effects of *T.hamatum* alone and in combination with *rhizobial* isolates on the growth of sunflower plant. Seeds of sunflower were sown in pots filled with 2 kg soil each. After 5 days of germination, developing seedlings in each pot were initially inoculated with different treatments. Twenty five milliliters of suspension of each treatment (approximately 1.2 x 10^6 conidia/mL of *T.hamatum* and 1.9 x 10^8 cells/mL of *rhizobial* isolates) were used. Five replicates were used for each treatment. During the first few days after inoculation, care was taken in watering the plants to avoid the washing the inoculums out of the soil and watering was done on alternate days. Five plants of each treatment (1 plant/replicate/treatment) were uprooted at 30^{th} and 60^{th} day of growth to measure the selected physical and biochemical parameters.

Physical Parameters

Lengths of root & shoot and fresh weight of plants were measured at 30^{th} and 60^{th} day. Root length was measured from the point of attachment of the stem base to the tip of the adventitious root. Where as shoot length was measured from the stem base to the tip of the longest leaf stretched at each time intervals and plant fresh weight (biomass) was recorded through electrical balance.

Biochemical Analysis

Biochemical parameters were estimated in leaves of experimental plants and divided into organic (chlorophyll, carbohydrates and crude protein contents) and inorganic parameters (nitrogen and phosphorus). Total chlorophyll and total carbohydrate contents were determined by Arnon [30] and Anthrone methods [31] while crude protein by multiplying percent nitrogen value through 6.25 [32]. The percent nitrogen and phosphorus were estimated by methods described by Nesseler [33] and Allen *et al* [34].

Statistical analysis

Results of present pot experiment are expressed as mean ± SD (standard deviation). Data was analyzed by *One-way* ANOVA followed by Least significant difference (LSD) test by using SPSS 16 (version 4). The differences were considered significant at $p<0.05$ when treatments' mean compared with control.

RESULTS

Physical parameters

Most of the treatments found to increase the root lengths of plants at 30^{th} and 60^{th} day as compared to control (Table 2). The maximum increased in root length was observed by JUR1 and JUR3 + JUF1 respectively as 15.5 and 47.7 cm at 30^{th} and 60^{th} day. Similarly, most of the treatments were found effective in increasing the shoot lengths of plants from 29.1 to 37.5 cm at 30^{th} day and from 44.3 to 53.6 cm at 60^{th} day as compared to their respective controls

(Table 2). Few treatments were also found to increase the fresh weight of plants as compared with their respective control at 30th and 60th day intervals.

Table 2: Effect of treatments on physical parameters of sunflower plant

		Physical parameters					
		30th day			60th day		
S. No.	**Treatments**	**Root length** (cm)	**Shoot length** (cm)	**Fresh weight** (gm)	**Root length** (cm)	**Shoot length** (cm)	**Fresh weight** (gm)
1.	Control	10.5± 0.86	26.5± 0.5	2.23±0.06	12.83±1.89	37.4±2.47	2.4±0.24
2.	JUF1	15.2± 2.69**b**	31.16± 2.75**b**	4.45±0.19**c**	30.16±2.46**a**	53.66±2.08**a**	5.05±1.08**c**
3.	JUR1	15.5± 0.86**a**	37.5± 0.5**a**	3.67±0.37	17.9±2.26	48.63±5.59**a**	3.67±0.37
4.	JUR2	14.6± 1.27**c**	31.83± 2.02**a**	3.63±0.66	21.1±6.46**d**	50.33±3.2**a**	3.63±0.66
5.	JUR3	12.16± 0.76	32± 2.64**a**	3.46±1.05	34.7±1.15**a**	53±3.6**a**	4.54±0.67**c**
6.	JUR4	14.16± 0.76**c**	31± 2.0b	3.41± 0.84	14.7±4.35	37.25±3.05	3.72±0.29
7.	FTZ	19.76± 2.8**a**	29.06± 0.45**d**	2.91±0.23	42±6.92**a**	54.66±0.57**a**	5.09±0.25**b**
8.	FGD	12.96± 4.6	30.36± 0.77**d**	2.66±0.13	25.3±5.5**c**	44.33±1.15**c**	3.73±1.04
9.	JUR1 + JUF1	14.73± 0.92**c**	26.33± 1.25	5.97±1.18**a**	21.3±3.21**d**	52.33±4.93**a**	4.21±0.1**d**
10.	JUR2 + JUF1	13.83±1.04**d**	28.33± 2.64	3.81±1.44**d**	19.93±2.4	48.16±2.02**a**	3.41±0.33
11.	JUR3 + JUF1	12.4± 0.85	35.66± 1.04**a**	3.62±0.94	47.66±4.93**a**	48.66±1.0**a**	4.45±0.19**c**
12.	JUR4 + JUF1	11.36± 0.77	35.83 ± 1.6**a**	5.05±1.09**b**	15.63±2.5	48.96±3.04**a**	3.79±0.98
13.	JUF1 + FTZ	13.5±1.77**d**	29.73± 2.18**d**	2.29±0.33	45±12.76**a**	51±3.46**a**	5.16±1.66**b**

FTZ = fertilizer, FGD = fungicide. **a** = $p < 0.0001$, **b** = $p < 0.001$, **c** = $p < 0.01$ and **d** = $p < 0.05$ (LSD) when compared with their respective control. Each value is the mean ± SD of 5 replicates.

Biochemical parameters

Organic parameters: Most of the treatments including JUF1, JUR1, JUR3, FTZ, FGD, JUR3+JUF1, JUR4+JUF1, JUF1+FTZ induced increase in total chlorophyll content at 30th day. Of which JUR1 and JUR4+JUF1 also maintained increase in the same content up to 60th day (Table 3). Few treatments including JUF1, JUR1, JUR4, JUR3+JUF1 and JUR4+JUF1 also increased the carbohydrate amount in experimental plants at 30th day where as JUR4 and JUR4+JUF1 increased carbohydrates at both day intervals (Table 3). Similarly, JUF1, JUR1, JUR4, JUR3+JUF1 and JUR4+JUF1 increased crude protein content in test plants, of which JUR4 and JUR4+JUF1 maintained increased in crude protein content upto 60th day (Table 3).

Inorganic parameters: Statistically significant increase in percent nitrogen was obtained after 30th day of growth in test plants treated with JUF1, JUR1, JUR4, JUR3+JUF1 and JUR4+JUF3 where as only JUR4, JUR1+JUF1 and JUR4+JUF1 showed significant increase in the same parameter at both day intervals (Table 4). Percent phosphorus content of experimental plants was significantly increased with the treatments of JUF1, JUR1, JUR4, JUR1+JUF1 and JUR3+JUF1 at 30th day of growth. Whereas statistically significant increase in phosphorus content was observed in test plants at 60th day of their growth by few treatments including JUF1, JUR2, JUR4 and JUR4+JUF1 (Table 4).

DISCUSSION

Biofertilizer is a term applies to live formulations of microorganisms used for agriculture which provide benefits upon application to seed and root by increasing growth and yield of plants or increasing the fertility of soil [14]. Out of several microrooganisms, fungal species belong to a genus *Trichoderma* and *Rhizobium* species belong to growth promoting *rhizobacteria* group are well-known biofertilizer [16, 22]. *Trichoderma* species are non-pathogenic, saprophytes and common inhabitants of soil especially rhizosphere (soil adhering to root surface) [12] and have been reported to be useful for several crop plants not only for promoting their growth by increasing the availability of nutrients through their biological activities but also protecting them from diseases by making pathogen-free environment around roots [22]. On the other hand *Rhizobium species* are well-known for their ability of nitrogen fixation through symbiotic association with roots of specific legume plants and affect the growth and yield of crop plants [16]. However they are also reported to be beneficial for non-legume plants [17]. Several studies

provide evidences that *rhizobium* species can induced not only increased in germination and seedling emergence but also improve the growth and yield of various cereal and non-cereal crops [15, 35].

In the present pot experiment, *T.hamatum* was used alone and in combination with *rhizobial* isolates to investigate their effect on growth of sunflower and organic & inorganic constituents in leaves of same plant. The plant growth was measured in terms of lengths of root & shoot and fresh biomass of whole plant. The results indicated that most of the treatments promote the growth of experimental plant at two growth intervals more than control ($p<0.05$). Of which *T.hamatum* itself found effective in increasing the lengths of both root & shoot and biomass. Out of *rhizobial* isolates, JUR1, JUR2 and JUR4 were found more efficient in promoting the growth of plant and fresh biomass in their respective groups as compared to JUR3. Interestingly the growth promoting effect of four of these *rhizobial* isolates were appeared more prominent in increasing the shoot length as compared the fertilizer NPK @ 2500 ppm at both 30^{th} and 60^{th} day of growth of sunflower ($p<0.05$). Similarly fungicide was found active in increasing the shoot length of experimental plants as compared to fertilizer NPK which is meant to increase the plant growth. However NPK was observed more effective in promoting the plant growth and biomass at 2^{nd} interval of uprooting. In combine treatments of *T.hamatum* with *rhizobial* isolates, again same isolates including JUR1, JUR2 and JUR4 found effective in increasing the plant growth at both intervals of growth ($p<0.05$). The same statistically significant increase was also observed by *T.hamatum* in combination with fertilizer. The growth promoting effects observed by *T.hamatum* alone and in combination of *rhizobial* isolates was confirmed its ability to produce antibiotics in rhizosphere that restrict the growth of microorganisms that have detrimental effects on plant growth [22]. It was evident that seed treatments with *T.hamatum* protect both seeds and seedlings of radish and pea from infections of *Rhizoctonia solani* and *Pythium spp* [36]. Besides producing antibiotics, *Trichoderma* species are also reported to control root-infecting fungi through mycoparasitism, competition for space and nutrients, and fungal cell wall degrading enzymes [37]. In addition these are reported to have plant growth stimulating effects by improving the soil condition [22]. This disease-free environment may be found effective for *rhizobial* isolates to promote growth of sunflower asymbiotically in the present study. Studies showed that *Rhizobium* strains have also plant growth promoting effects on non-legume plants and it was due to their abilities of producing of plant hormones that help to promote growth and yield of non-leguminous plants in response to seed or root inoculation [17]. *Rhizobial* inoculation improves the seed germination, seedling emergence, growth and development of lowland rice variety MR219 [38].

Chlorophyll content indicates the normal photosynthetic function of plant tissues through which it convert sunlight into high energy-producing compounds which are needed by plant for its regular metabolism. In the present study, out of all treatments used, *T. hamatum*, *rhizobial* isolates including JUR1, JUR3, fertilizer and fungicide individually in their respective groups induced significant increase in total chlorophyll content where as *T.hamatum* along with JUR3, JUR4 and fertilizer found effective in increasing the same parameter at 30^{th} day ($p<0.05$). However JUR1 alone and JUR4 in combination with *T.hamatum* were found to maintain the increase in total chlorophyll content up to 60^{th} day as compared to control ($p<0.05$). In case of carbohydrate and crude protein content of sunflower leaves, again *T.hamatum*, JUR1 and JUR4 individually and *T.hamatum* with JUR3 and JUR4 found effective increasing the carbohydrate and crude protein contents of experimental plants at 30^{th} day where as the increase in both these parameters was also observed at 60^{th} day of growth after giving treatment with JUR4 and JUR4 with *T.hamatum* ($p<0.05$). It has been reported Increased in chlorophyll content also linked to increase in total carbohydrate in plant tissues [39], the same theme was achieved in present study. On the other hand, increased protein content in growing parts of plant reflects the metabolic regulation associated with enhanced enzyme activity which helps plant to withstand environmental conditions [9] and to promote their growth.

Nitrogen content of experimental plants was significantly increased after treatments with *T.hamatum,* JUR1 and JUR4 individually and *T.hamatum* in combination with JUR3 and JUR4 at 30^{th} day of growth where as only JUR4 alone and *T.hamatum* with JUR1 and JUR4 showed significant increase in the same parameter at both day intervals ($p<0.05$). Treatments with fungicide decreased nitrogen content of sunflower at both intervals of growth and fertilizer after 60^{th} day showed inhibitory effect. Again *T. hamatum* alone found effective in increasing phosphorus content of sunflower and in combination with JUR1 and JUR3 at 30^{th} day while with JUR4 at 60^{th} day of growth. Similar significant increased in phosphorus content was also observed by JUR1, JUR2 and JUR4 individually in their respective groups ($p<0.05$). *Trichoderma species* are reported to improve mineral uptake, release mineral from soil and organic matter [22, 37] as same as *Rhizobium species* which are not only actively involved in mobilizing the N and P but also produced many growth promoting and health improving substances [16, 17]. Therefore together they are beneficial for the growth and improving the productivity of crop plants.

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Table 3: Effect of treatments on biochemical parameters of sunflower plant

		Biochemical parameters					
		30th day			60th day		
S. No.	Treatments	Total chlorophyll (mg/gm)	Total carbohydrate (mg/gm)	Crude protein (%)	Total chlorophyll (mg/gm)	Total carbohydrate (mg/gm)	Crude protein (%)
1.	Control	0.75 ± 0.20	375.73± 8.45	8.04 ± 0.13	1.47 ± 0.19	481.75 ± 80.94	10.28 ± 1.72
2.	JUF1	1.01 ± 0.22**d**	524.34± 44.78**d**	11.18 ± 0.94**d**	1.38 ± 0.29	546.78 ± 29.23	11.66 ± 0.62
3.	JUR1	1.31 ± 0.06**a**	557.54 ± 54.64**c**	11.89 ± 1.16**c**	2.43 ± 0.64**a**	633.33 ± 226.80	13.51 ± 4.83
4.	JUR2	0.89 ± 0.18	377.68± 31.70	8.05 ± 0.67	1.62 ± 0.14	619.6 ± 116.60	13.22 ± 2.48
5.	JUR3	1.34 ± 0.2**a**	442.83± 41.34	9.45 ± 0.88	1.7 ± 0.41	537.62± 39.25	11.47 ± 0.84
6.	JUR4	0.89 ± 0.03	694.51 ± 38.21**a**	15.33 ± 1.5**a**	2.85 ± 0.06**a**	867.8± 204.27**b**	18.52 ± 4.36**b**
7.	FTZ	1.27 ± 0.08**a**	397.14 ± 26.76	8.47 ± 0.56	1.18 ± 0.32	415.92 ± 62.79	8.87 ± 1.33
8.	FGD	1.34 ± 0.08**a**	356.96 ± 32.84	7.61 ± 0.70	1.36 ± 0.18	392.91± 177.81	8.38 ± 3.79
9.	JUR1 + JUF1	0.75 ± 0.04	473.28 ± 84.08	10.1 ± 1.79	1.45 ± 0.40	798.65 ± 215.14**c**	17.04 ± 4.61**c**
10.	JUR2 + JUF1	0.91 ± 0.07	434.35 ± 44.33	9.27 ± 0.94	1.45 ± 0.40	627.68 ± 194.83	13.38 ± 4.16
11.	JUR3 + JUF1	1.19 ± 0.12**b**	524.8 ± 55.98c	11.2 ± 1.19**d**	1.16 ± 0.14	491.37 ± 159.10	10.48 ± 3.39
12.	JUR4 + JUF1	1.36 ± 0.15**a**	644.67± 88.74**a**	13.75 ± 1.89**a**	2.85 ± 0.40**a**	866.88± 86.84**b**	18.5 ± 1.85**b**
13.	JUF1 + FTZ	1.37 ± 0.03**a**	475 ± 33.05	10.13 ± 0.70	1.31 ± 0.22	435.04 ± 57.96	9.28 ± 1.23

FTZ = fertilizer, FGD = fungicide. **a** = $p < 0.0001$, **b** = $p < 0.001$, **c** = $p < 0.01$ and **d** = $p < 0.05$ (LSD) when compared with their respective control
Each value is the mean ± SD (standard deviation) of 5 replicates.

Table 4: Effect of treatments on inorganic constituents of sunflower plant

		Inorganic parameters			
		30th day		60th day	
S. No.	Treatments	Nitrogen (%)	Phosphorus (%)	Nitrogen (%)	Phosphorus (%)
1.	Control	1.28 ± 0.02	0.08 ± 0.00	1.64 ± 0.27	0.09 ± 0.00
2.	JUF1	1.79 ± 0.15**d**	0.16 ± 0.08**c**	1.86 ± 0.10	0.27 ± 0.07**a**
3.	JUR1	1.9 ± 0.18**c**	0.15 ± 0.07**d**	2.16 ± 0.77	0.17 ± 0.06
4.	JUR2	1.28 ± 0.11	0.1 ± 0.00	2.11 ± 0.40	0.19 ± 0.13**c**
5.	JUR3	1.51 ± 0.14	0.12 ± 0.03	1.83 ± 0.13	0. 13 ± 0.03
6.	JUR4	2.45 ± 0.24**a**	0.18 ± 0.08**b**	2.96 ± 0.69**b**	0.23 ± 0.11**b**
7.	FTZ	1.35 ± 0.08	0.12 ± 0.03	1.42 ± 0.21	0.1 ± 0.03
8.	FGD	1.21 ± 0.11	0.08 ± 0.00	1.34 ± 0.60	0.09 ± 0.00
9.	JUR1 + JUF1	1.61 ± 0.28	0.14 ± 0.01**d**	2.72 ± 0.73**c**	0.16 ± 0.01
10.	JUR2 + JUF1	1.48 ± 0.15	0.09 ± 0.00	2.14 ± 0.66	0.12 ± 0.02
11.	JUR3 + JUF1	1.79 ± 0.19**d**	0.14 ± 0.00**d**	1.67 ± 0.54	0.15 ± 0.00
12.	JUR4 + JUF1	2.2 ± 0.30**a**	0.17 ± 0.00**c**	2.96 ± 0.29**b**	0.32 ± 0.02**a**
13.	JUF1 + FTZ	1.62 ± 0.11	0.1 ± 0.3	1.48 ± 0.19	0.12 ± 0.04

FTZ = fertilizer, FGD = fungicide. **a** = $p < 0.0001$, **b** = $p < 0.001$, **c** = $p < 0.01$ and **d** = $p < 0.05$ (LSD) when compared with their respective control
Each value is the mean ± SD of 5 replicates.