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Research article

ROOTONIC WITH BIO-ZINC TO ACCELERATE *PENNISETUM GLAUCUM* SEED GERMINATION AND PLANT GROWTH

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ABSTRACT: Currently, commercializing the *Piriformospora indica* fungal formulation using magnesium sulphite as a carrier with the registered trade name "Rootonic". It enhances the plant growth, biotic and abiotic stress tolerance and also increases the accessibility of nutrients to plants. Biotic stress and nutritional deficiency i.e. zinc in soil are the two important factor for the deteriotion of grain quality. To consider these two problems, the study was done to check the effect of combination of Rootonic and Bio-Zinc on diseases causing isolate *Fusarium solani* in *P. glaucum*. *Fusarium* species not only reduces the quality but also affect the quantity and yield of grains in *P. glaucum*. Seeds were treated with *F. solani* and Rootonic at the time of sowing under green house condition. Seed germination and morphological parameters significantly increased in the treatment having combination of both Rootonic as well as Bio-Zinc. Rootonic or Bio-Zinc alone although promotes the plant growth but failed to suppress the pathogenicity caused by *F. solani*. These results suggest that novel combination of Rootonic and Bio-Zinc suppress the pathogenicity considerably caused by *F. solani* and at the same time promote the plant growth and had significant impact on chlorophyll, phenol and flavonoid content of *P. glaucum*.
Keywords: Antioxidant, Bio-Zinc (Zinc gluconate), *Fusarium solani* (*F. solani*), *Pennisetum glaucum* (*P. glaucum*), *Piriformospora indica* (*P. indica*), Rootonic

INTRODUCTION

Piriformospora indica, root endophytic fungus of order Sebacinales in the Basidiomycota colonizes number of plants and increases the plant growth, flowering at an early stage, higher yield and biotic and abiotic stress tolerance [60] and was formulated in talcum powder and branded as Rootonic [5]. *P. glaucum* is the popular staple cereal crop of tropical regions and important component of farming system in the arid and semi-arid regions. Plants act as a promising source of natural or phytochemical antioxidants like flavonoids and phenolic acids which helps in scavenging free radicals, prevention of ageing and various oxidative stress related diseases like cardiovascular and neurodegenerative diseases cancer [40,41,55]. Zinc is one of the essential elements in plants for many enzymatic reactions and plant metabolism [18, 39]. Zinc deficiency is a worldwide problem for crop production in many types of soil especially in calcareous soil [9, 21, 52]. Soil pH and organic matters [33, 43] are the two major factors for zinc deficiency. In many countries including India, Pakistan, Turkey and Iran, deficiency of zinc is reported as one of the global problem [2, 24]. About 50% of cereals grown in soil with low zinc availability are deficient in zinc [10, 20]. Zinc is important in early seed germination [1, 44] and seedling vigor [67]. During seed germination zinc is important in protein synthesis, cell elongation, membrane function [63], stress tolerance [8] and protection from pathogen attack [56, 51]. Deficiency of zinc results in decrease in crop yields and nutritional quality which also affects human health and physical development [24, 62]. *F. solani* causes one of the most economically diseases i.e. *Fusarium* stalk rot in *P. glaucum*. It produces toxins which remain in active form and contaminate the grains and its quality [19]. It is ubiquitous in soil and one of the most important pathogen of *P. glaucum* and impossible to control with the chemicals. Its severity is not always in the form of wilting and necrosis but also affects the process of photosynthesis [47] and decreases the plant growth and development [40].

MATERIALS AND METHODS

Experiment was carried out in green house at 28±2ºC and light intensity of 8000-10000 Lux and in cemented pots of 92 X35X32(cm).

Eight treatments were performed in triplicate manner:

a) Control b) *F. solani* c) Rootonic d) Bio-Zinc e) Rootonic + *F. solani* f) Bio-Zinc +*F. solani* g) Rootonic+ Bio-Zinc h) Rootonic+ Bio-Zinc +*F. solani*

Preparation of soil

Sandy clay soil of pH range 6.3 to 8.7 was obtained from Noida Uttar Pradesh, India mixed with mature farm yard manure (1:1).

Preparation of fungal culture

F. solani culture was incubated in potato dextrose broth on shaking condition of 100 rpm at 28±2°C for 10 days and then biomass was separated from the culture filterate in sterile condition. Spores and mycelia were checked under the microscope (Motic BA310).

Soil treatment

Autoclaved soil were mixed with Bio-Zinc in the amount of 0.01g which was obtained from Prathista Industries Ltd. Hyderabad, India were thoroughly mixed with 100g of soil.

Procurement and surface sterilization of seeds

P. glaucum RAJ 121 seeds obtained from National Seed Corporation, IARI, New Delhi, India, were soaked overnight in sterile distilled water. For surface sterilization, seeds were treated with 15 min in 95% concentrated ethanol followed by treatment with 4% sodium hypochlorite (NaOCl) solution and then washed with sterile distilled water [6].

Surface coating of seeds

Surface sterilized seeds were mixed alone with 2-3g of fresh Rootonic powder or *F. solani* alone or in combination in which live propagules were 10^9 by direct mixing and control seeds were treated with equal quantity of sterile talcum powder. Seventy seeds were placed in each pot. All experiments were done in triplicates.

Analysis of growth parameters

Each pot having 70 plants of *P. glaucum* were analysed for different growth parameters like shoot length, number of leaves, leaf width, surface area of leaves, fresh and dry weight of shoot of treated as well as control plants were measured after 10, 20 and 30 days. Plant length was measured and fresh as well as dry weight of shoot (g) was recorded after drying in oven at 60°C for 48 hrs.

Root colonization

For root colonization, 10 root samples were taken randomly from colonized *P. glaucum* plants. Root samples were washed under tap water, treated with 10% KOH solution for 20 min, followed by acidification with 1M HCl for 15 min and washed with distilled water and finally stained with lactophenol blue for 1-2 hrs [14,46]. Root colonization was observed under light microscope (100X) and distribution of chlamydospores were taken as an index of colonization [61].

Chlorophyll estimation

Fresh leaves were harvested, washed, blot dried and then homogenised 1g of leaves in 10ml of chilled 80% acetone in chilled pestle mortar. Primary acetone extract mainly contain chloroplast pigments and then extract was centrifuged at 15,000 rpm for 15min at 4°C. Supernatant was taken out in fresh tube and its volume raised to 10ml by 80% acetone. The extract produced was then subjected to measure the absorbance at 645nm to 655nm respectively for Chl a and Chl b. Content of Chl a, b and total Chl (mg/g fresh weight) was calculated using the equation given by [64]. Three replicates were taken for each treatment.

Quantitative phytochemicals screening

Methanolic extract

For extraction of antioxidant compounds like phenol and flavonoids from leaves it was first oven dried for overnight and then 1g of leaf sample homogenised in 10 ml of methanol (1:10g/ml). Centrifuged the mixture at 10,000 rpm for 10 min at 4°C. Collected the supernatant and again centrifuged the pellet for 10 min. Recollected the supernatant, pooled and kept it at 4°C for further use. Three extracts were prepared for each treatment.

Determination of total phenol content

Total phenolic concentration was estimated by Folin-Ciocalteu colorimetric method [36]. A methanolic plant extract (0.5 ml of 1:10g ml^{-1}) was then oxidized with 5 ml Folin Ciocalteu reagent (1:10 diluted with distilled water) followed by neutralization with 4ml of 1M aqueous Na_2CO_3.

The mixtures were then incubated for 15 min and then absorbance was recorded at 765 nm. The standard curve was prepared by using different dilutions of gallic acid in methanol: water (50:50, v/v). Results were expressed in terms of GAE (gallic acid equivalent) mg g^{-1} of dry mass. All experiments were performed in triplicates.

Total flavonoid content

Flavonoid concentration was determined by Aluminum chloride colorimetric method [11]. 0.5 ml of plant extract (1:10 g ml^{-1}) were separately mixed with 1.5 ml of methanol followed by treatment with 0.1 ml of 10% $AlCl_3$, and then mixed with 0.1 ml of 1M potassium acetate and the mixture was then diluted with 2.8 ml of distilled water. The mixture was then incubated at room temperature for 30 min and the absorbance was measured at 415 nm. Total flavonoid content was determined by preparing standard curve of quercetin at different concentrations in methanol g ml^{-1} and experiments were done in replication of three.

Statistical analysis

All data are represented as mean ± SD for at least three replicates. Analysis of variance (ANOVA) method was employed for carrying out statistical analysis of triplicate data collected of different experiments [54].The mean values were compared with Least Significant Difference (LSD) test at significance level of 1% [53].

RESULTS AND DISCUSSIONS

Zinc plays various important roles in plant metabolism by increasing the enzyme activity, synthesis of cytochrome, ribosomal stabilization [58], carbohydrate metabolism, membrane stability [2,12,15], protein synthesis, pollen formation and auxin synthesis [2,7,34,]. Zinc deficiency found in most of the regions of the world having high pH and high amount of $CaCO_3$ and phosphate which can fix the free zinc in soil and decrease its availability for plants [2, 3, 25]. Submerged soils are well known for zinc deficiency as it binds with free sulphides [38].

In the present study, we have used *P. glaucum* plants to show maximum resitibility against the pathogenecity of *F. solani* upon treatment with the combination of Rootonic and Bio-Zinc as compared to other treatments and control. *F. solani* is one of the biotic stress which affects the plant growth, development and yield. Here we have measured the important parameters related to plant growth, photosynthetic pigments and induction of metabolites during stress condition.

Deficiency of zinc in plants reflects in the development of abnormalities with visible symptoms like chlorosis, stunted growth, smaller leaves and spikelet sterility. It adversely affects the quality of grains and increases the susceptibility to fungal infection [8]. Zinc also affects the water uptake capacity in plants [15, 45, 57]. This deficiency can be corrected by application of zinc fertilizers but overdose of fertilizer can become toxic and harmful for plants. Traditionally, the requirement of zinc is being met by the application of inorganic fertilizer like zinc sulphate etc. Addition of Rootonic or Bio-Zinc alone did not make significant impact on seed germination. However combined application of Rootonic and Bio-Zinc improves the seed germination.

Seed germination

Germination of *P. glaucum* seeds were observed on every 12 hrs but the initiation of germination was observed after 36 hrs (consideration for germination was the emergence of radical). Shoot length of germinating seeds measured which were more than 1mm in length after 10 days of sowing. In all eight treatments number of germinating seeds increased continuously as well as length of shoots also increased subsequently.

Seventy seeds were sown in each pot having different treatments. After one week seed germination was observed in each pot and marked differences were seen in the germination as given in Table 1.

Table1. Seed germination observed after one week for different treatments in each pot

Treatments	Number of seed germinated (Mean ± S.D.)	Percentage enhancement of seed germination over *F. solani*
Control	41±1.0	11.36
F. solani	36.33±1.52	-
Rootonic	44±2.0	17.43
Bio Zinc	44±2.5	17.41
Rootonic +*F. solani*	43±3.0	15.51
Bio-Zinc+ *F. solani*	43.67±3.05	16.80
Rootonic+Bio-Zinc	52.67±2.51	31.02
Rootonic+ *F. solani* +Bio-Zinc	46±1.0	21.02

Combination of Bio-Zinc and Rootonic enhanced seed germination over *F. solani* alone treatment by 31.02% followed by treatment with Rootonic and Bio-Zinc**.** Zinc is important in seed germination for protein synthesis and resistance against pathogens [8, 51] and on other side *Fusarium* sp. is highly pathogenic and cause different diseases i.e., seed rot, wilt and seedling blight in number of crops [28] and therefore deteriotes the quality of seed germination.

Effect on growth parameters

Analysis of different parameters of plant growth is an important property to study the plants behaviour against biotic stress i.e., pathogenecity of *F. solani*, because it induces stomatal closening, reduce the capacity of photosynthesis and drastically alter the nutrient balance in plants.Thus, its immediate negative effect on plant is stunted growth, lower biomass and decline in chlorophyll concentration.

We measured the shoot length, number of leaves, leaf width, surface area of leaves, fresh and dry weight of shoots of Rootonic and Bio-Zinc treated plants as compared with the control and *F. solani* treated plants had significant increament in growth rate. This novel combination enhanced the rate of seed germination and also showed the positive effect on shoot length and plant growth compared with other treatments (Figure1).

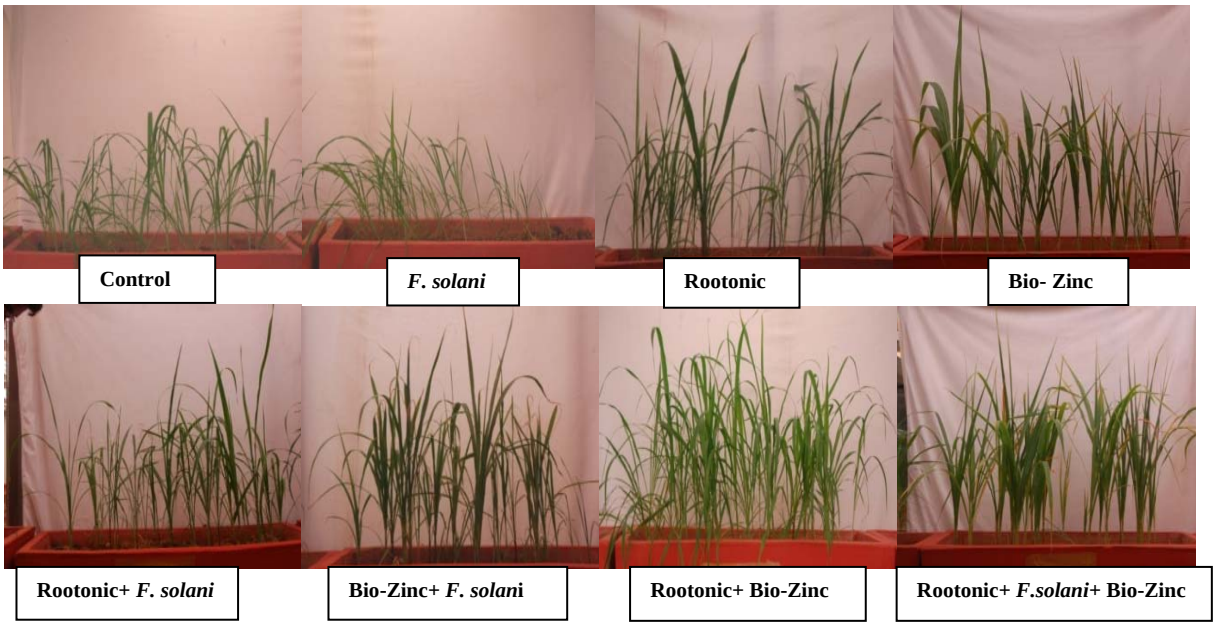

Figure-1. Effect of Rootonic, Bio-Zinc, *F. solani* and different combination treatments on growth parameters of *P. glaucum* plants at 60 days after sowing

Observations of different growth parameters of plants

Among all diseases, fungal diseases are mainly responsible for low crop productivity. *P. glaucum* is mainly affected by seed borne pathogens which cause reduction at initial and foliage stage of germination. *Fusarium semitectum, Aspergillus flavus* and *A. niger* deteriotes the quantity and quality of floral parts which in turn decrease the grain yield at maturity level [65]. Pathologists suggested that global yield losses of *P. glaucum* is 45%, 9%, 32%, 3%, due to downy mildew, smuts, striga, rusts, respectively. [17]. The survival of *Fusarium* sp. chlyamydospores in soils is up to many years even there is no host and it is difficult to eradicate [22,42].

The mean of different treatments were compared for plant growth attributes i.e., shoot length, number of leaves, leaf width, surface area of leaves, fresh weight and dry weight. On different time interval the plants treated with combination of Rootonic and Bio-Zinc showed significant increase for shoot length, number of leaves, surface area of leaves, fresh and dry weight than the other treatments at 1% level of significance. But, leaf width showed no significant difference between control and treated plants on 10^{th} day. The combinational treatment of Rootonic and Bio-Zinc showed the increase in leaf width significantly among other treatments at 20^{th} and 30^{th} days (Table 2).

Table 2. Different growth parameters a) Shoot length b) Number of leaves c) Leaf width d) Surface area of leaves e) Fresh weight f) Dry weight of treated and control plants

Morphological parameters	TREATMENTS								
	Days	**Control**	***F. solani***	**Rootonic**	**Bio Zinc**	**Rootonic + *F. solani***	**Bio-Zinc+ *F. solani***	**Rootonic+ Bio-Zinc**	**Rootonic+ *F.solani* +Bio-Zinc**
Shoot length (cm)	10th	2.10±1[a]	2.0±0.34[a]	4.24±1[b]	3.19±2[b]	3.11±1[b]	3.53±2[b]	6.23±1[c]	3.77±2[b]
	20th	16.37±1.24[a]	12.48±1[a]	24.47±1.9[b]	19.56±1.3[a]	14.54±2[a]	15.45±0.67[a]	27.99±0.9[c]	17.45±3[a]
	30th	25.21±1[a]	22.24±0.24[b]	28.1±3[a]	26.43±2[a]	25.10±0.45[a]	26.23±0.25[a]	32.44±0.2[c]	28.02±0.68[a]
Number of leaves	10th	1.5±0.5[a]	1.21±0.25[a]	2.64±0.45[b]	1.46±0.57[a]	1.84±0.59[b]	2.31±0.25[b]	3.33±0.34[c]	2.53±0.34[b]
	20th	3.53±0.45[a]	2.69±0.36[a]	3.99±0.18[b]	4.02±0.56[b]	3.62±0.67[a]	3.61±0.27[b]	4.57±0.5[c]	3.83±0.36[b]
	30th	4.14±0.15[a]	3.88±0.16[a]	5.22±0.28[b]	5.69±0.39[b]	4.45±0.25[a]	4.83±0.5[a]	6.71±0.24[c]	5.07±0.25[a]
Leaf width (cm)	10th	0.06±0.002[a]	0.2±0.002[a]	0.57±0.19[b]	0.50±0.024[b]	0.24±0.036[b]	0.3±0.02[a]	0.72±0.05[b]	0.35±0.05[b]
	20th	0.34±0.02[a]	0.23±0.012[a]	0.64±0.15[a]	0.56±0.027[a]	0.33±0.05[a]	0.4±0.05[a]	0.82±0.03[b]	0.62±0.08[a]
	30th	0.50±0.09[a]	0.39±0.15[a]	1.13±0.05[a]	1.4±0.05[a]	0.55±0.02[a]	0.62±0.06[a]	1.57±0.05[b]	0.83±0.02[a]
Surface area of leaves (cm^2)	10th	0.07±0.02[a]	0.04±0.001[a]	3.97±0.36[c]	2.98±0.78[b]	0.17±0.02[a]	0.18±0.05[a]	4.98±0.05[d]	1.59±0.04[e]
	20th	5.34±0.35[a]	4.34±2.5[a]	14.80±0.92[c]	12.55±0.29[b]	8.65±2[a]	9.33±1[b]	16.95±0.36[c]	11.2±0.36[b]
	30th	11.63±2[a]	9.56±1.9[a]	34.59±1[c]	27.6±2[b]	12.57±0.9[a]	14.69±0.5[a]	35.66±2[d]	15.76±2[a]
Fresh weight of shoots (gm)	10th	5.35±1[a]	3.19±1.5[b]	5.69±0.4[a]	6.48±2[a]	4.1±2[b]	4.05±2.34[b]	10.45±1.75[c]	5.7±2[a]
	20th	7.27±1.5[a]	5.94±0.79[b]	10.84±1[a]	9.04±0.5[a]	6.47±0.7[b]	7.01±1[b]	17.45±1[c]	12.3±1.39[a]
	30th	10.29±0.28[a]	8.3±2[b]	14.28±2[a]	13.24±1.78[a]	11.4±1.28[a]	12.4±0.74[a]	25.55±0.5[c]	18.3±1.5[a]
Dry weight of shoots (gm)	10th	0.27±0.01[a]	0.15±0.04[a]	1.5±0.25[b]	1.2±0.05[b]	1.2±0.005[b]	1.02±0.6[b]	2.67±1[c]	1.75±0.7[b]
	20th	1.5±0.05[a]	0.57±0.05[a]	3.02±0.5[b]	2.38±1[b]	2.69±0.5[a]	2.04±0.58[a]	5.47±0.75[c]	3.83±0.75[b]
	30th	3.2±1[a]	2.01±0.18[a]	6.25±0.78[b]	5.2±0.5[b]	4.95±1[b]	4.29±1.26[b]	10.34±0.5[c]	7.37±1[b]

(Small letter indicates statistical difference among different treatments)

Root colonization

Rootonic colonization was observed after 20 days of inocualtion. The control plant showed no colonization (Figure 2) wheras Rootonic treated plants showed the chlamydospores of *P. indica* in the roots of *P. glaucum* and microconidial spores were observed in plants infected with *F. solani* under 40 X after staining with lactophenol cotton blue stain [26]. Plants treated with the combination of Rootonic and Bio-Zinc showed the enhanced frequency of root colonization of *P.indica* spores.

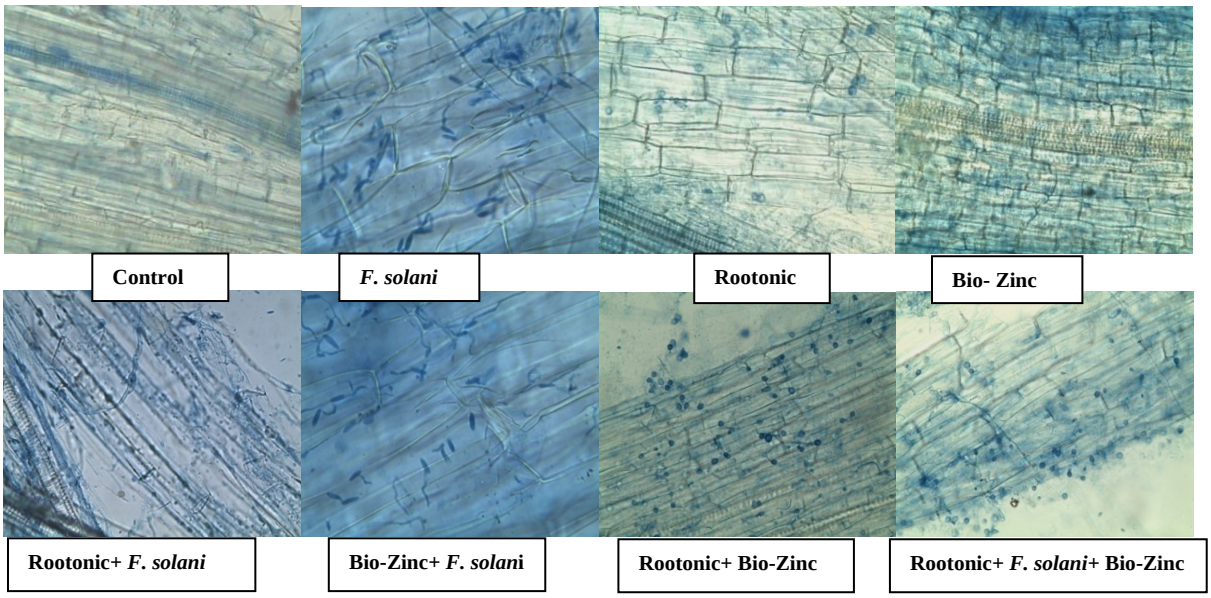

Figure 2. Root colonization of *P. glaucum* roots with different treatments under light microscope (40X)

Photosynthetic pigment content

Photosynthesis is an important process for growth and overall development of green plants and can be drastically affected by pathogenecity of *F. solani*. In this study we mainly focussed on pigments of chlorophyll which plays a crucial role in photosynthesis and phytoprotection. The concentrations of chl a, chl b and total chlorophyll of all treated plants were measured and it was found that Rootonic and Bio-Zinc treated plants increased the pigment content than other treatments.

It was observed that this novel combination increases the content of photosynthetic pigments (Chl a, Chl b and total chlorophyll) compared to other treatments and control (Figure 3). The major impact of pathogenecity of *F. solani* is the degradation of photosynthetic pigments because of chlorosis. As a result color of leaves becomes brownish in color and stunted growth [65]. The concentration of pigments are found to be maximum in plants treated with the combination of Rootonic and Bio-Zinc than others.

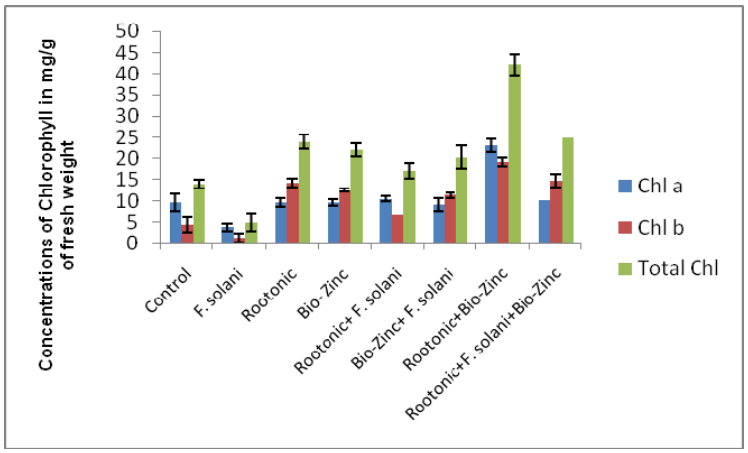

Figure 3. Photosynthetic pigment contents of different treatments

Total phenolic and flavonoid content

Polyphenols or phenolics are one of the important secondary metabolites that present ubiquitously in plants and its products contains high amount of antioxidants [49]. Previous work documented that phenolic compounds are the categories of antioxidant agents which help in the termination of free radicals [50]. On another side flavonoid also showed the antioxidant activity due to their scavenging and chelating process for free radicals [29, 48]. Antioxidant mainly contains the hydroxyl group that's why it is used as free radical scavengers in plants [13, 68].

For preparation of plant extract, methanol was used instead of ethanol because methanol is most effective in dissolving the active compounds i.e. saponins, tannins and anthocyanins in plant cells [59]. Antioxidant activity of plant extract is mainly depending upon the types of solvent used because every compound has different polarity with different rates of antioxidant potential [31]. Most of the parts of plants are rich in phenolic acids and its demand increases in food industry because it retards oxidative lipid degradation and enhance the nutritional and quality of food [27].

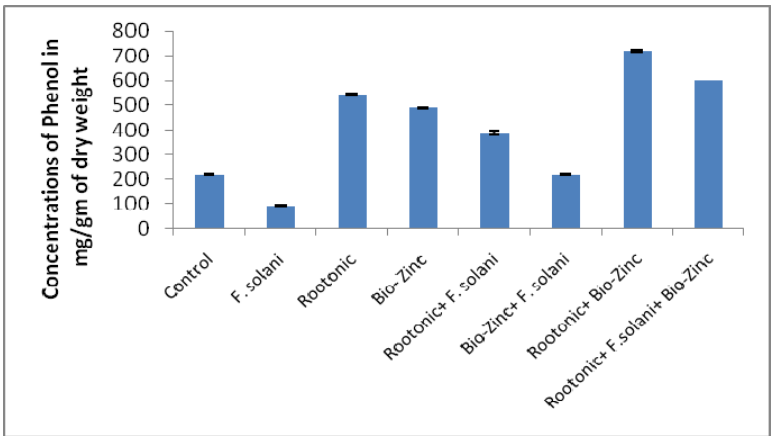

Figure 4. Effect of different treatments on total phenol content of *P. glaucum*

Figure 4 showed the total phenol content in the leaves of *P. glaucum*. It was determined on 30th day by Folin Ciocalteu reagent and calculated by gallic acid equivalent (equation of standard curve: y= 0.005x+ 0.061, R^2=0.995). The concentration of phenol in *P. glaucum* leaves varied from 93.12 to 721.47mg g^{-1} and highest phenol content were found in the plants which was treated with combination of Rootonic and Bio-Zinc.
Total flavonoid concentration of the leaves extract were determined with the help of Aluminium chloride calorimetric method in terms of quercetin equivalent (equation of standard curve:y= 0.040x-0.024, R^2=0.988) and its concentration ranged from 20.61to 81.12 mg g^{-1} (Figure 5) with maximum concentration of flavonoids in treatment of mixture of Rootonic and Bio-Zinc treated plants.

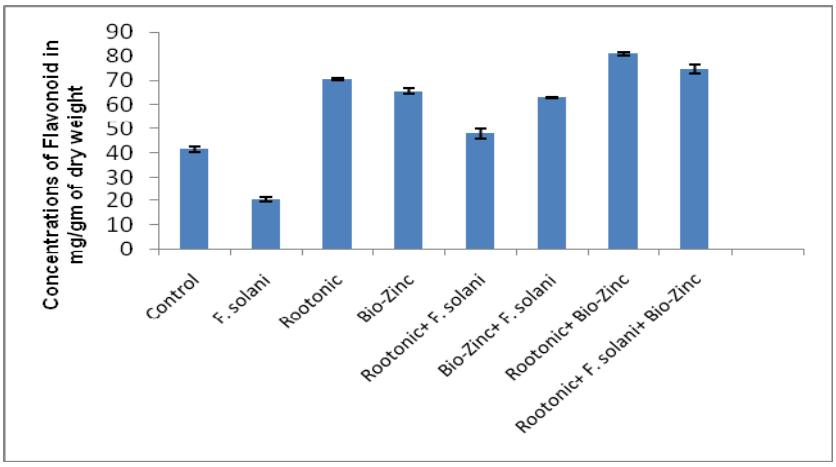

Figure 5. Effect of different treatments on total flavonoid content of *P. glaucum*

Flavonoids help in the modification of eicosanoid biosynthesis and have antiviral and anti- carcinogenic properties [16, 37]. Phenolic compound has its health benefits as it fights against cancer. Feluric acid is bound phenolic form and it is present in *P. glaucum* emphasizes its major role as an anti-carcinogenic [23, 30, 66].
Phenolic compounds plays an important role in defense mechanism against microbial pathogens and predators based on their toxicity and repellence to microbes and insects. These compounds are also reported as phytoalexins, phytoanticipins and modulator of plant defense genes and pathogenicity [4, 35]. It is one of the important precursors for lignin synthesis [32] which act as a barrier and increase the resistance against *F. solani* in host plants. Incorporation of phenolic compounds is an effective management to control *Fusarium* sp. in plants. Mazid et al. [35] reported accumulation of phenols is important for plant resistance against pathogens.

CONCLUSIONS

Present study reflects the combined application of Rootonic and Bio-Zinc had significant effect on seed germination, plant growth and contents of antioxidants in *P. glaucum*. These important parameters are associated with plant yield. Therefore, this preliminary study recommends further investigation.
In future application of Bio-Zinc with Rootonic promotes the zinc uptake and its accumulation in grains. Agronomic bio-fortification of this combination would be economical and useful strategy to solve the deficiency of zinc in different types of soils and also in humans globally and effectively.

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