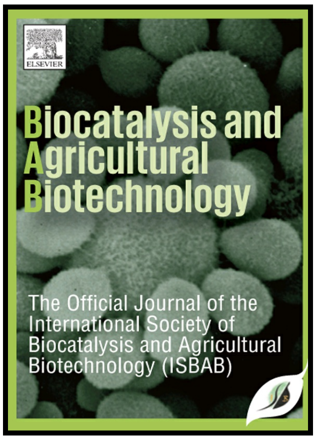

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EFFECT OF *PARTHENIUM* BASED VERMICOMPOST AND ZINC OXIDE NANOPARTICLES ON GROWTH AND YIELD OF *ARACHIS HYPOGAEA* L. IN ZINC DEFICIENT SOIL

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Abstract

Zinc is an important micro nutrient and is vital for germination, chlorophyll production, pollen function and fertilization. An investigation was carried out to explain the effect of *Parthenium* based vermicompost and nanoparticles (zinc oxide nanoparticles) on the growth profile and yield of *Arachis hypogaea* L. in zinc deficient soil. *Parthenium* mediated zinc oxide nanoparticle and zinc sulphate treated seeds were cultivated in zinc deficient soil. Presence of zinc oxide nanoparticles in the treated seeds were confirmed through atomic absorption spectroscopy. Various growth and yield related parameters, including fresh weight, dry weight, shoot length, root length, chlorophyll content, total free phenols, reducing sugar and total soluble sugar, number of pods etc. was positively affected by the nanoparticle treatment. Chlorophyll as well as total free phenols, reducing sugar and total soluble sugar levels were increased up to 300 ppm of zinc oxide nanoparticles treatments. Zinc oxide nanoparticle treatment increased the number of pods per plant when compared to control treatment. Best increase in pods and grains yield was recorded at 300 ppm of zinc oxide nanoparticle treatment. Zinc oxide nanoparticles were used as fertilizer for zinc deficient soil.

The uses of nanoparticles in agriculture may be enhancing the crop production in deficient soil.

Keywords: *Arachis hypogaea, Parthenium,* growth and yield, zinc oxide nanoparticle, zinc deficient soil

1. Introduction

Parthenium hysterophorus L. (family Asteraceae) is a poisonous, pernicious and aggressive weed. It is known to badly affect crop production, biodiversity, animal husbandry, human health and even ecosystem integrity (Rao, 1956; Kohli and Batish, 1994). It has some water soluble allelopathic chemicals such as phenolic acids and Parthenin—a sesquiterpene lactone of pseudoguanolide nature in various parts of the weed (Picman and Picman, 1984). It can be used as a soil supplement after removal of harmful allelochemicals. Adoption of vermicomposting technology constitutes an essential component of organic farming. Large number of weed plants that grow at an alarming rate and spread very fast in the cultivated lands, pastures, grasslands and forests are a good source of organic matter (Sharma *et al.,* 2004). Vermicompost is a sustainable source of macro and micro nutrients and plant-growth regulating materials (humic acids and plant growth regulators like auxins, gibberellins and cytokinins) which are responsible for increased plant growth and yield of many crops (Krishnamoorthy and Vajrabhiah, 1986; Senesi et al. 1992; Atiyeh et al. 2000; Atiyeh et al. 2002).

Effect of nanoparticles on crop plants is an emerging area of research that needs to be meticulously explored. In the recent past, engineered nanoparticles have received particular attention as potential candidates for increasing crop yield (Scrinis and Lyons, 2007; Barik et al. 2008; Arora et al. 2012). Some researchers reported the influences of chemically synthesized nano particles (gold, silver and iron) on crops by foliar spray. They concluded that nano particles treated crops showed a considerable increase in chlorophyll, protein, ascorbic acid, pod and grain weight (An et al. 2008; Roghayyeh et al. 2010; Nair et al. 2010). The effects of phyto mediated nanoparticles on crops have not been reported. In green chemistry method, the use of plants and microbes for the synthesis of metal oxide nanoparticles did attract the attention of researchers for having a rapid, in expensive, eco-friendly and a one-step method for the biosynthesis process (Kowshik et al., 2003).

In India, zinc is the fourth most important yield-limiting nutrient after macro nutrients (N, P and K). In India alone, 50% of the soils in which groundnut is grown show zinc deficiency, which is causing significant yield loss (Singh, 1999). Singh et al. (2004) and Gopala Gowda et al. (1994) reported that pod yield significantly increased by the application of zinc sulphate and zinc oxide. Zinc is vital for germination, chlorophyll production, pollen function, fertilization (Pandey et al. 2006; Cakmak, 2008) and biomass production (Kaya and Higgs, 2002).

The present work was focused to study the combined effects of *Parthenium* mediated zinc oxide nanoparticles treated (*Arachis hypogaea* L.) groundnut seed’s growth, development and final yield in zinc deficient soil with application of *Parthenium* based vermicompost.

2. Materials and methodology

All analytical grade chemicals (solvents and reagents) were purchased from Sigma-Aldrich chemicals, India. Deionised water was used for synthesis of nanoparticles and all experimental analysis. Laboratory glass wares were soaked in acid cleaning solution overnight and washed thoroughly in tap and deionised water.

2.1 Production of vermicompost

The vermicompost was prepared from compost of *P. hysterophorus* mixed with cow dung in 40%: 60% (w/w) ratio by employing *Eudrilus eugeniae* (incubated for 45 days) as described by Rajiv et al. (2013). Vermicompost samples were collected for analysis of nutritional status.

2.2 Synthesis and characterization of zinc oxide nanoparticles and nanosuspension

Green synthesized zinc oxide nanoparticles were synthesized through the reduction of zinc nitrate by leaf extract, which act as a capping agent, as described by Rajiv et al. (2012). *Parthenium* mediated zinc oxide nanoparticles were characterized with the help of X-ray diffraction (XRD) (Perkin-Elmer) and transmission electron microscopy (TEM- Model JSM 6390LV, JOEL, USA). Different concentration of zinc oxide nano suspensions (100, 200, 300 and 400 ppm) and zinc sulphate were prepared, according to Prasad et al. (2012).

2.3 Seed collection and treatment

Seeds of *A. hypogaea* L. (Co-6) were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The seeds were washed in a mild detergent and soaked in different concentration of nano suspension and zinc sulphate solution overnight at room temperature. Sample seeds were collected for zinc estimation by Atomic adsorption spectroscopy (AAS- Shimadzu AA-700).

2.4 Pot culture experiment

Pot culture experiments were conducted during December 2012- April 2013 at Karpagam University, Coimbatore (11˚16’N; 76˚58’E), Tamil Nadu. Zinc deficient soil was collected from Karur Dist., Tamil Nadu, India and physical and nutritional levels are as shown in Table 1. The soil (10 kg) was filled in earthen pots (18 cm x 36 cm). Vermicompost dosages and NPK (Nitrogen, Phosphorus and Potassium) were designed for the present study as per the recommendations of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, for red soil. The vermicompost and NPK (17:35:50) were mixed. After 15 days, the treated seeds were sown in the pots, as per the recommended agronomic practices. The treatments details are shown in Table 2. At 35 DAS and 70 DAS, 100 ppm of *Parthenium* mediated zinc oxide nanoparticle (T_2), 200 ppm of *Parthenium* mediated zinc oxide nanoparticle (T_3), 300 ppm of *Parthenium* mediated zinc oxide nanoparticle (T_4), 400 ppm of *Parthenium* mediated zinc oxide nanoparticle (T_5) were sprayed with 0.03% adjuvant. T_6 is a negative control. A two set of experiments were conducted in pot culture, one is to study the effect of seed treatments on seed germination (10 seeds per pot) and second one is to analyse the growth and yield parametric studies (one plant per pot was maintained for growth and yield parameter analysis). Three replicates were maintained for this investigation.

2.5 Analysis of growth and yield parameters

The germination percentage of *Arachis hypogaea* was calculated on day 7 and 14 after sowing. Different growth [30th, 60th and 90th Days after sowing (DAS)] parameters like fresh weight, dry weight, shoot and root length and number of root nodules were noted. Fresh leaves were collected on 30th, 60th and 90th DAS and used for estimation of chlorophyll, protein, total free phenols, reducing sugars and total soluble sugars. Chlorophyll a, b and total chlorophyll were determined as described in Arnon (1949). The total protein content was assessed by the method of Lowry et al. (1951). The total free phenolic content was determined by the method of Malick and Singh (1980) method using Folin– Ciocalteu reagent and concentration of total phenols were determined by using standard calibration curve of catechol. The reducing sugar was analysed by dinitrosalicyclic acid method of Miller (1972). The total soluble sugar was determined by anthrone method (Mahadevan and Sridhar, 1986) and the concentration of total sugar was calculated using a standard curve prepared from glucose. After harvesting on 120 DAS, number of pods per plant and weight of grains per pod were recorded. Oil from groundnut seed was extracted using petroleum ether (boiling point 40–60 ºC) by soxhlet apparatus for 7 h. Finally, groundnut oil was calculated on weight basis (Surwase et al. 2011).

2.6 Statistical analysis

The experiment was conducted twice and One-way ANOVA was used to analyse the significant differences among different treatments for studied parameters. Tukey's test was performed to detect the homogeneous type of the treatments for their different properties. All the data were analysed using SPSS 16.0 software. Probability levels used for statistical significance were $P < 0.05$ for all tests.

3. Results and Discussion

3.1 Analysis of physical properties and nutritional levels

Table 1 shows the physical properties and nutritional levels of *Parthenium* based vermicompost. Vermicompost pH was strongly acidic in nature. Hogg et al. (2002) reported the pH range 6.0–8.5 for vermicompost application to the soils which is suitable for plant growth. The macro nutrients (N, P and K) and micro nutrients (Zn, Fe, Mn and Cu) level was significantly high in vermicompost, which is very similar to previous studies (Yadav and Garg, 2011).

3.2 Characterization of the zinc oxide nanoparticles

Figure 1a shows the X-ray diffraction of zinc oxide phase of nanoparticles. The diffraction XRD peaks were identified as (100), (101), (102), (110), (112) and (202) reflections, respectively. The crystallite size of nanoparticles was calculated by Scherrer's formula using the diffraction peaks observed in XRD spectra. The strong and narrow diffraction peaks indicate the zinc oxide nanoparticles are spherical and in crystalline nature. Figure 1b shows the TEM images of the zinc oxide nanoparticles and clearly reveals that the particles are spherical in shape without agglomeration and the particles average size is 28 nm, which is in good agreement with the previous works (Ni et al. 2005; Nawaz et al. 2011; Jayaseelan et al. 2012).

3.3 Analysis of zinc level on nanoparticles treated seeds

Zinc level was significantly high in zinc oxide nanoparticle treated seeds (i.e. 400 ppm > 300 ppm > 200 ppm > 100 ppm treated seeds) (Figure 2) when compared to control seeds (i.e. distilled water treated seeds), results are very similar to Prasad et al. (2012).

3.4 Effect of zinc oxide nanoparticle treatment on growth and seed yield

A germination study of *A. hypogaea* grown under various doses of zinc oxide nanoparticles treatment is shown in Fig. 3a. Highest germination percentages (80 % on 7 DAS and 96.66% on 14 DAS) were recorded in *A. hypogaea* seedlings grown in T_4 treatment. The lowest germination percentages (60% on 7 DAS and 66.66% on 14 DAS) were registered in T_1 treatment (control). At highest concentration of zinc oxide nanoparticles (400 ppm), 63.33% on 7 DAS and 66.6 % on 14 DAS seeds germinated in T_5 treatment. Prasad et al. (2012) reported that zinc oxide nanoparticles treated groundnut seeds germinated at 99% in 1000 ppm concentration where as 88% of groundnut seeds germinated at high concentration (2000 ppm). Carbon nanotubes (CNT) were discovered to enhance germination of tomato seeds (Khodakovskaya et al. 2009). TiO_2 and SiO_2 nanoparticles have been found to promote the germination and growth of *Glycine max* seeds (Lu et al. 2002).

Growth (root and shoot), fresh and dry weight of *A. hypogaea* seedlings as influenced by the application of different concentration of zinc oxide nanoparticles and vermicompost, are presented in Fig. 3 (b-e). Application of zinc oxide nanoparticle caused an improvement in average root length, shoot length and number of nodules, as compared to the untreated seedlings on zinc deficient soil. The root and shoot length were maximum in T_4 treatment when compared to other treatments. On 30, 60 and 90 DAS, the root length was highly recorded in T_4 (16.56, 17.35 and 19.69 cm). Among all the treatments, T_1 treatment has very short root formation (6.9, 7.5 and 7.9 cm) on 30, 60 and 90 DAS respectively. The highest shoot formation (15.36, 18.63 and 24.48 cm) was seen in T_4 and the lowest (8.16, 10.26 and 11.8 cm) was obtained in T_1 on 30, 60 and 90 DAS. Seif et al. (2011) reported that silver nanoparticle treatment had enhanced growth of plant (*Borago)* by different parameters such as height. Arora et al. (2012) concluded that gold-nanoparticle treatment interferes with the action of endogenous plant hormones, and induces changes in the growth profile of the treated seedlings. Carbon nanotubes (CNT) were discovered to increase germination and root elongation of tomato seeds (Khodakovskaya et al. 2009), Nano-Al was shown to enhance root elongation of radish and rape seedling (Lin and Xing, 2007).

Maximum fresh and dry weight of whole plant has been increased in T_4 treatment (300 ppm zinc oxide nanoparticles), as compared to the control seedlings. However, 400 ppm zinc oxide nanoparticle treatment has not increased the fresh and dry weight per plant, which was significant to control (Fig. 3d and Fig. 3e). Increase in fresh weight and dry weight varied from 34 to 75.6% and 57 to 78% respectively, under different concentration of zinc oxide nanoparticles treatments. Maximum amount of root nodule formation was reported in T_4 and minimum was found in T_1 (Fig. 3f).

The effect of *Parthenium* mediated zinc oxide nanoparticles on *A. hypogaea*'s biochemical parameters are presented in Fig. 4 (a-e). The highest leaf protein (4.46, 7.16 and 8.32 mg/g^{-1}), total free phenols (4.02, 4.97 and 5.19 mg/g^{-1}), reducing sugars (9.6, 11.0 and 11.86 mg/g^{-1}) and total soluble sugar (22.73, 24.13 and 25.06 mg/g^{-1}) content on 30, 45 and 90 DAS was found to be in T_4 treatment among all the treatments and the lowest protein (1.16, 2.4 and 2.8 mg/g^{-1}), total free phenols (1.24, 1.85 and 2.25 mg/g^{-1}), reducing sugars (8.3, 9.0 and 9.8

mg/g^{-1}) and total soluble sugar (18.86, 20.06 and 21.33 mg/g^{-1}) level was obtained in T_1. Chlorophyll a, b and total chlorophyll contents were increased in the seedlings treated with 300 ppm zinc oxide nanoparticles. Increasing level of chlorophyll a, b and total chlorophyll contents leads to an increase in the total photosynthate produced. The effect of different doses of zinc oxide nanoparticles in various treatments on photosynthetic pigment contents of *A. hypogaea* seedlings are reported in Fig. 5a-c. On 30, 45 and 60 DAS, the chlorophyll 'a' (0.23-0.29 mg g^{-1}), chlorophyll 'b'(0.15-0.23 mg g^{-1}), total chlorophyll content (0.40-0.49 mg g^{-1}) level was highest in T_4 treatment. The lowest chlorophyll 'a' (0.03-0.17 mg g^{-1}), chlorophyll 'b' (0.06-0.10 mg g^{-1}), total chlorophyll (0.12-0.26 mg g^{-1}) levels were recorded in *A. hypogaea* seedlings grown T_1 treatment. An et al. (2008) and Namasivayam and Chitrakala (2011) have been reported that silver nanoparticle treatment could induce higher chlorophyll contents in *Asparagus* and sorghum. Some researchers have reported that nano TiO_2 can improve photosynthesis and nitrogen metabolism and greatly improve the growth of spinach in an appropriate concentration (Zheng et al. 2005; Hong et al. 2005; Yang et al. 2007).

3.5 Yield Components for *A. hypogaea*

Table 3 revealed that zinc oxide nanoparticle significantly ($P < 0.05$) improved yield parameters such as number of pods per plant and weight of grains per pod of *A. hypogaea*. Among all the treatments, zinc oxide nanoparticle treated seedling *A. hypogaea* showed significantly increased yield parameters compared with control plants. T_4 treatment showed maximum (28.3) number of pods per plant among all other treatments. Control plants showed minimum (5.6) level of number of pods per plant. Weight of grains per pod (1.9 g) was high in T_4 treatment and low level (0.4 g) was obtained in T_1. Owolade et al. (2008) reported that seed yield of cowpea (*Vigna unguiculata* Walp) increased when treated (as foliar application) with nano sized TiO_2. Nano iron chelate fertilizer treatments showed highest fruit yield, number of fruits per plant, fruit length and fruit width from 2 g/L nano iron chelate foliar spraying (Bozorgi, 2012). The highest level (46.60%) of oil was obtained in T_4 and low level (23.20%) was found in T_1. Gold nanoparticles induced increase in the per cent oil content by 3–4%, over that of control plants (*Brassica juncea*) (Arora et al. 2012).

4. Conclusion

Micronutrients such as zinc can be supplied into *A. hypogaea* seeds through zinc oxide nanoparticles. A higher level of zinc was noticed in the nanoparticles treated seeds and it enhanced the growth of seeds which would increase crop yield in zinc deficient soil. According to above study, it could be concluded that the application of 25 g of *Parthenium* based vermicompost and zinc oxide nanoparticles (300 ppm) might increase the growth attributes, quality parameters and yield components of *A. hypogaea*.

5. Acknowledgements

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6. Conflict of interest

The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 Characterization of zinc oxide nanoparticles (a) XRD analysis of zinc oxide nanoparticles (b) TEM analysis of zinc oxide nanoparticles

Fig. 2 Zinc level in *A. hypogaea* seeds treated with different concentration of zinc oxide nanoparticles. Data represent Mean ± SE.

Fig. 3 (a) Percent germination, (b) root length, (c) shoot length, (d) fresh weight of whole plant (e) dry weight of whole plant, (f) root nodules in *A. hypogaea* treated with different concentrations of zinc oxide nanoparticles. Data represent Mean ± SE.

Fig. 4 (a) Protein level, (b) Total carbohydrate, (c) phenol level, (d) reducing sugar (e) total soluble sugar in *A. hypogaea* treated with different concentrations of zinc oxide nanoparticles. Data represent Mean ± SE.

Fig. 5 (a) Chlorophyll a, (b) chlorophyll b, (c) total chlorophyll content in *A. hypogaea* treated with different concentrations of zinc oxide nanoparticles. Data represent Mean ± SE.

Table 1. Analysis of zinc deficient soil and *Parthenium* mediated vermicompost

Parameters	**Zinc deficient soil**	***Parthenium* mediated vermicompost**
Soil texture	Sandy loam	-
Soil type	Reddish Brown	Brown
Lime status	Non-Calcareous	-
pH	7.90	6.60
EC (dS m^{-1})	0.03	1.40
Nitrogen (%)	0.68	1.54
Phosphorous (%)	0.60	0.77
Potassium (%)	1.05	1.58
Copper (ppm)	0.54	95.00
Zinc (ppm)	0.27	248.00
Iron (ppm)	8.99	96.00
Manganese (ppm)	2.92	150.00

Table 2. Treatment details

Treatments	**Details**
T_1	Control+ seeds soaked in distilled water (Without NPK)
T_2	25 g of *Parthenium* mediated vermicompost+ NPK (17:35:50)+ seeds soaked in 100 ppm of *Parthenium* mediated zinc oxide nanoparticles
T_3	25 g of *Parthenium* mediated vermicompost+ NPK (17:35:50)+ seeds soaked in 200 ppm of *Parthenium* mediated zinc oxide nanoparticles
T_4	25 g of vermicompost+ NPK (17:35:50)+ seeds soaked in 300 ppm of

	Parthenium mediated zinc oxide nanoparticles
T_5	25 g *Parthenium* mediated vermicompost+ NPK (17:35:50)+ seeds soaked in 400 ppm of zinc oxide
T_6	25 g *Parthenium* mediated vermicompost+ NPK (17:35:50)+ seeds soaked in (400 ppm) zinc sulphate (2 g per litre)

Table 3. Number of pods per plant, weight of grains per pod and oil content in *A. hypogaea* treated with different concentrations of zinc oxide nanoparticles.

Treatments	Number of pods per plant	Weight of grains per pod (g)	Oil yield (%)
T_1	5.6 ± 1.02^{d}	0.4 ± 0.04^{e}	23.20^{e}
T_2	20.3 ± 0.5^{b}	1.1 ± 0.04^{c}	30.15^{c}
T_3	24.6 ± 1.05^{a}	1.2 ± 0.05^{c}	32.24^{b}
T_4	28.3 ± 1.5^{a}	1.9 ± 0.05^{a}	46.60^{a}
T_5	14 ± 1^{c}	0.8 ± 0.01^{d}	28.00^{d}
T_6	20.6 ± 1.2^{b}	1.5 ± 0.02^{b}	31.10^{b}

Mean followed by different lower case alphabets differ significantly ($P < 0.05$) as established by ANOVA (Tukey’s test).

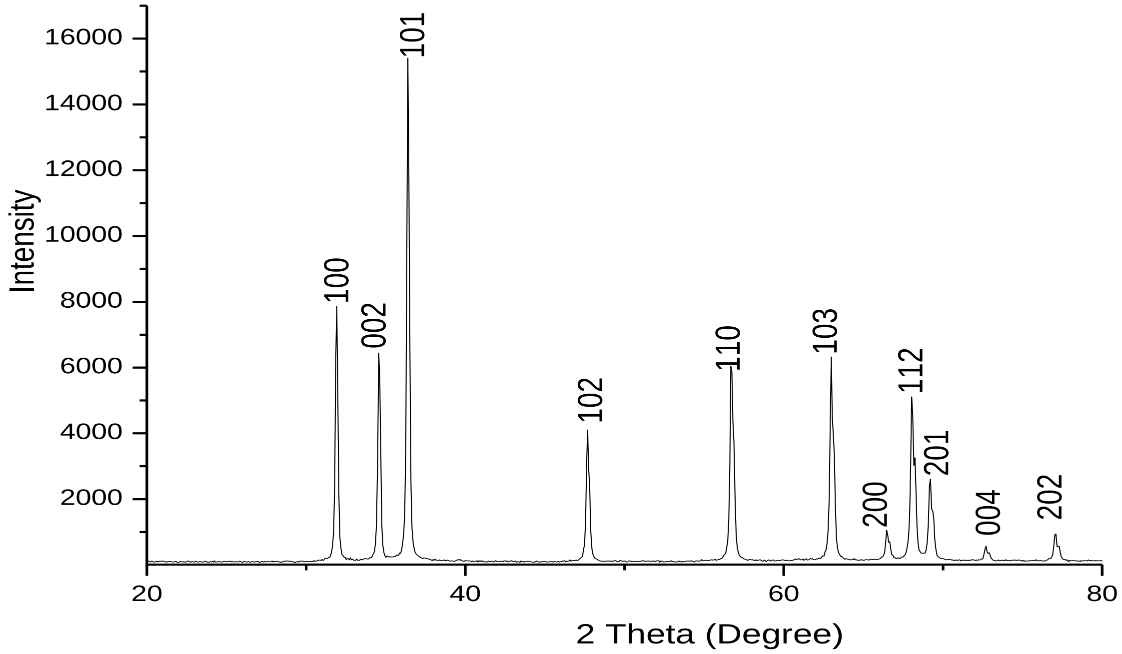
Intensity
16000
14000
12000
10000
8000
6000
4000
2000
20
40
60
80
2 Theta (Degree)
100
002
101
102
110
103
200
112
201
004
202

Fig. 1a

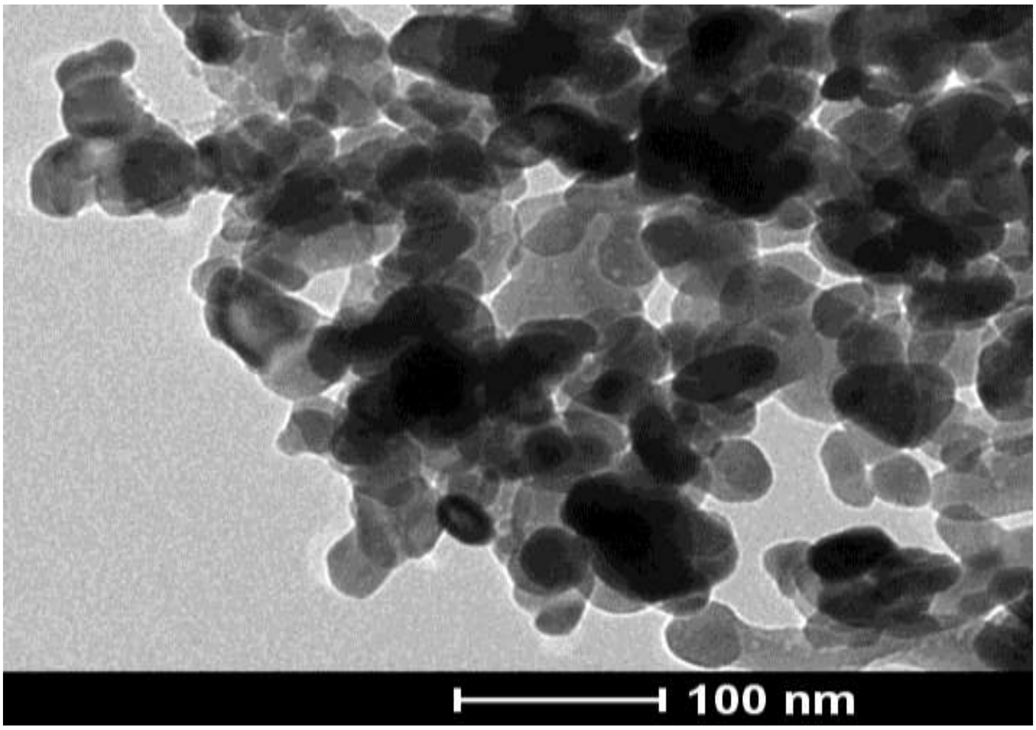
100 nm

Fig. 1b

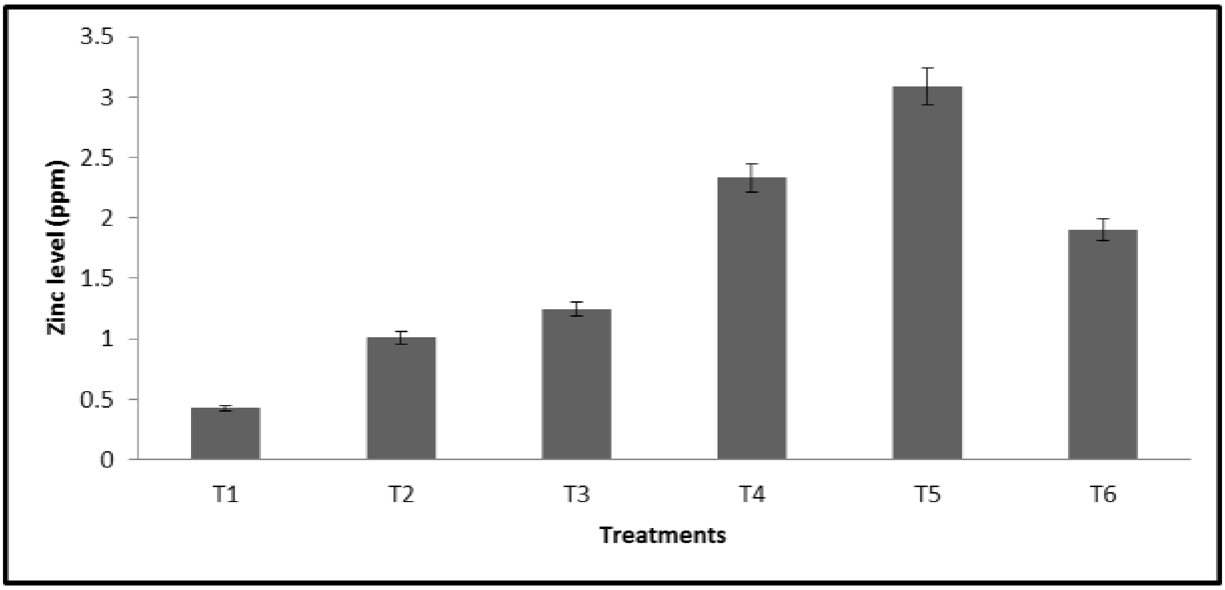
Zinc level (ppm)
3.5
3
2.5
2
1.5
1
0.5
0
T1
T2
T3
T4
T5
T6
Treatments

Fig 2.

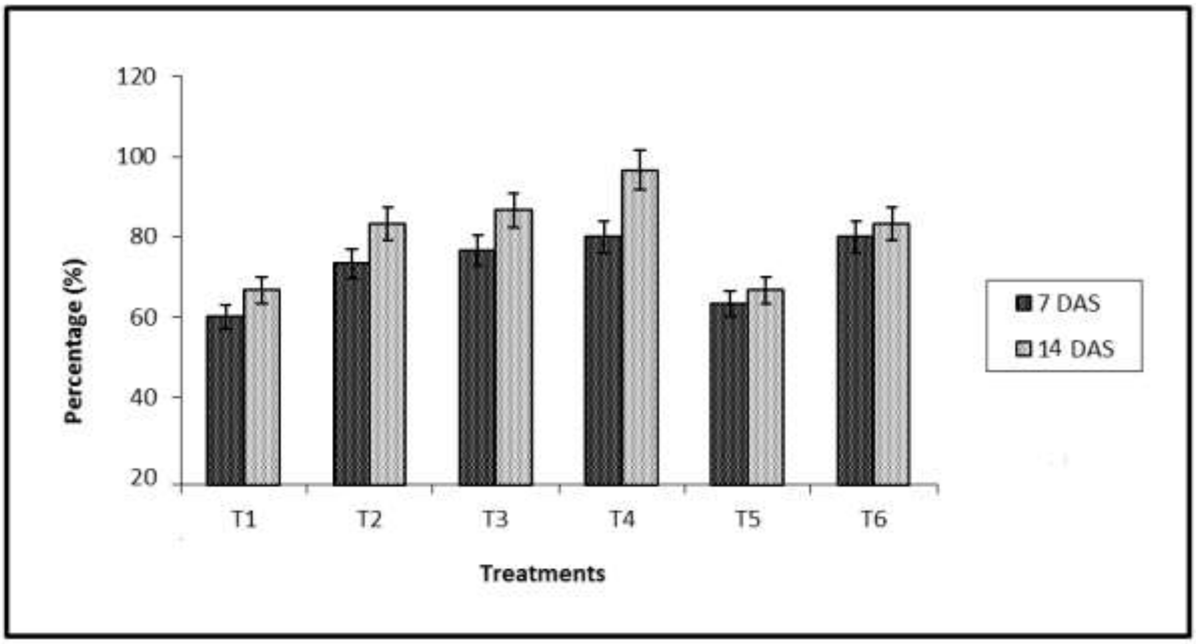
Percentage (%)
120
100
80
60
40
20
T1
T2
T3
T4
T5
T6
Treatments
7 DAS
14 DAS

Fig. 3a

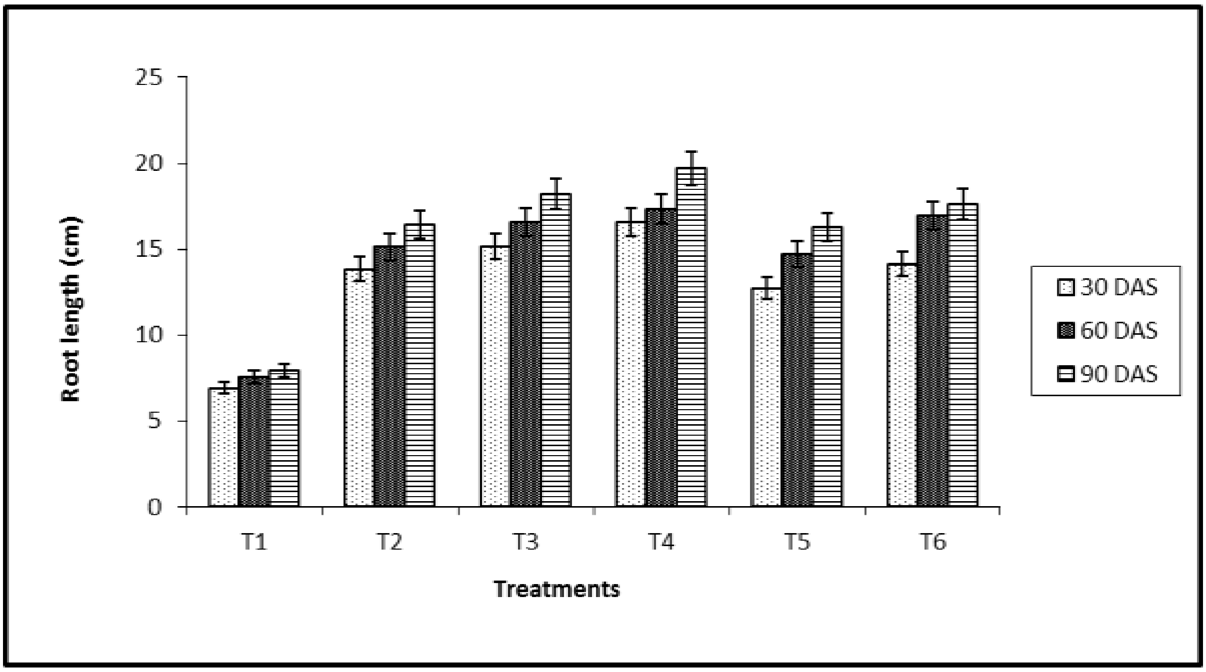
25
20
15
10
5
0
Root length (cm)
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 3b

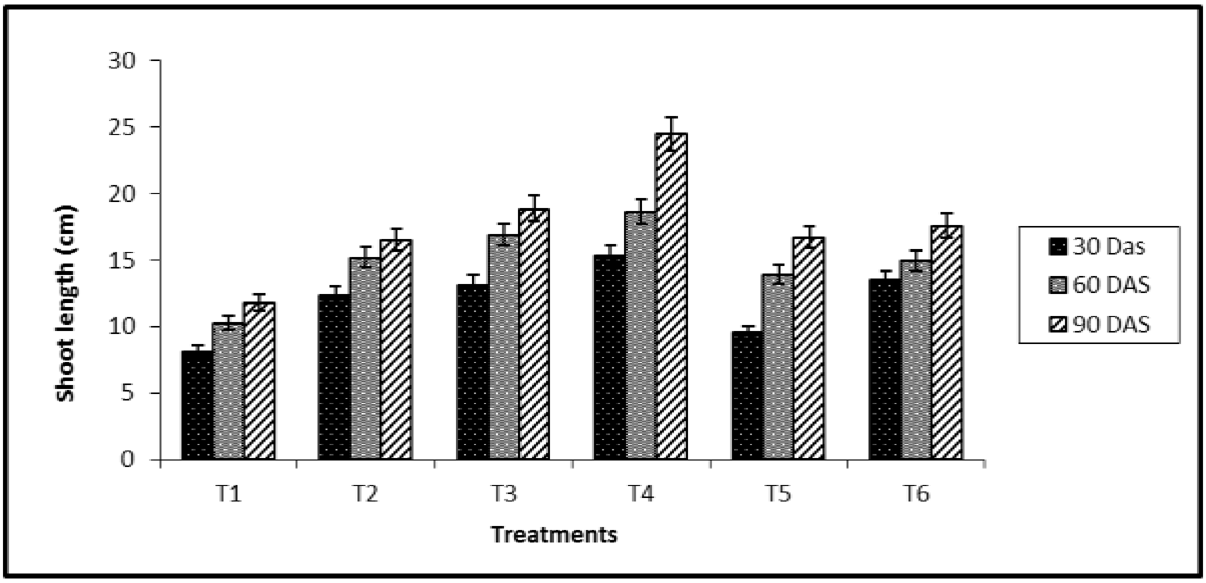
30
25
20
15
10
5
0
Shoot length (cm)
T1
T2
T3
T4
T5
T6
Treatments
30 Das
60 DAS
90 DAS

Fig. 3c

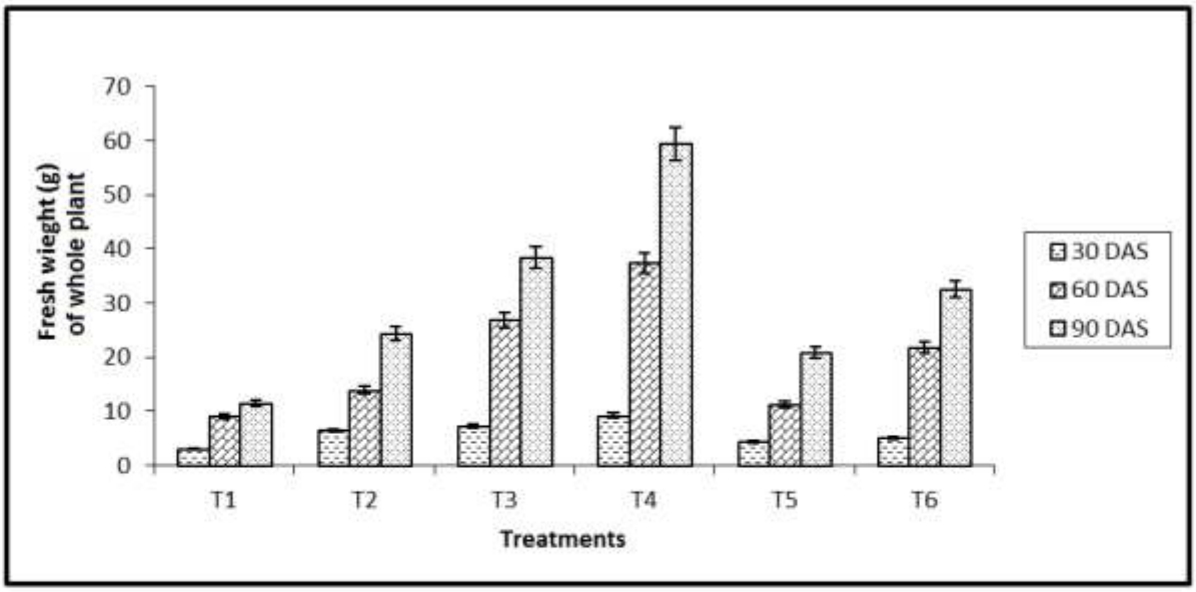
Fresh wieght (g) of whole plant
70
60
50
40
30
20
10
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 3d

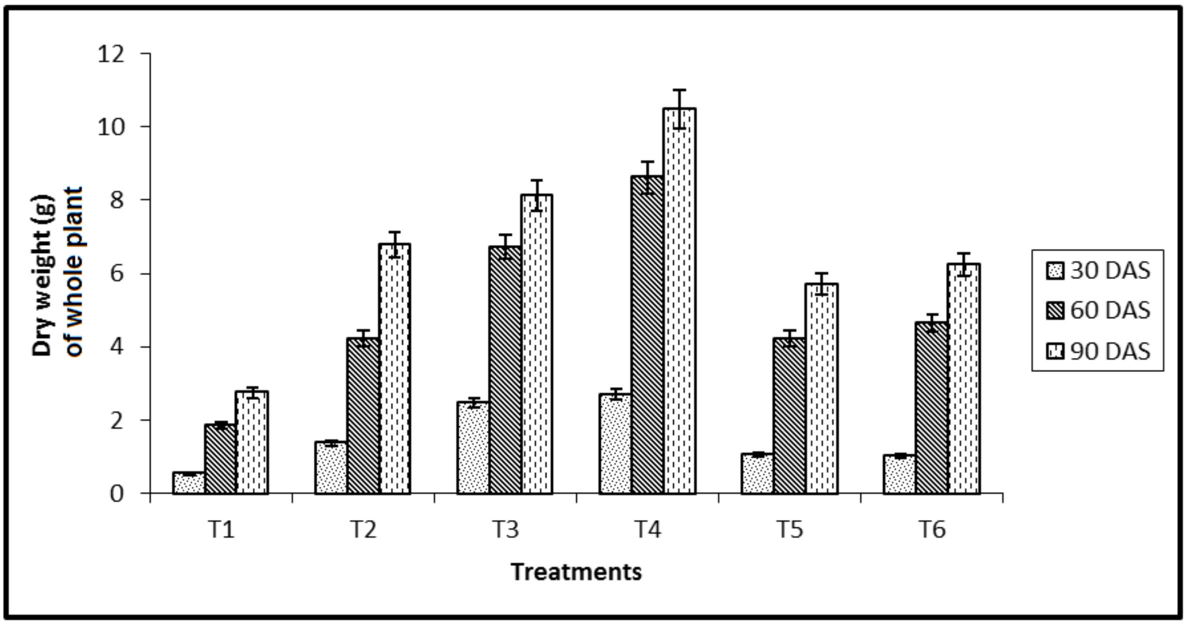
Dry weight (g) of whole plant
12
10
8
6
4
2
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 3e

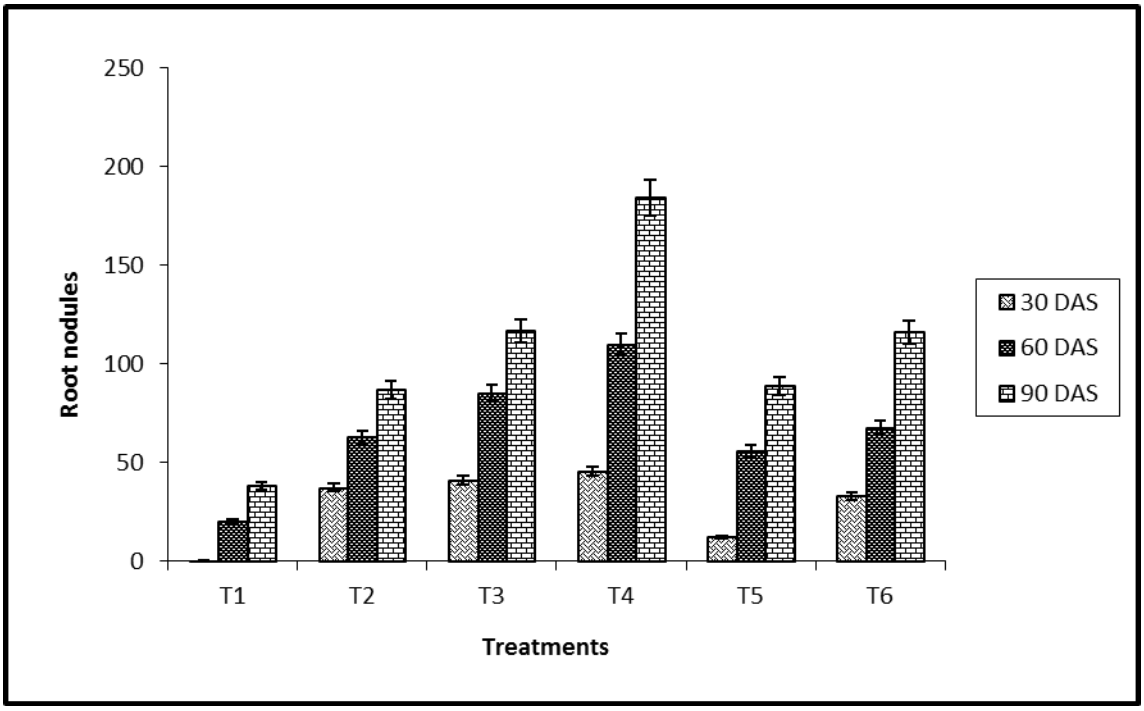
Root nodules
250
200
150
100
50
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 3f

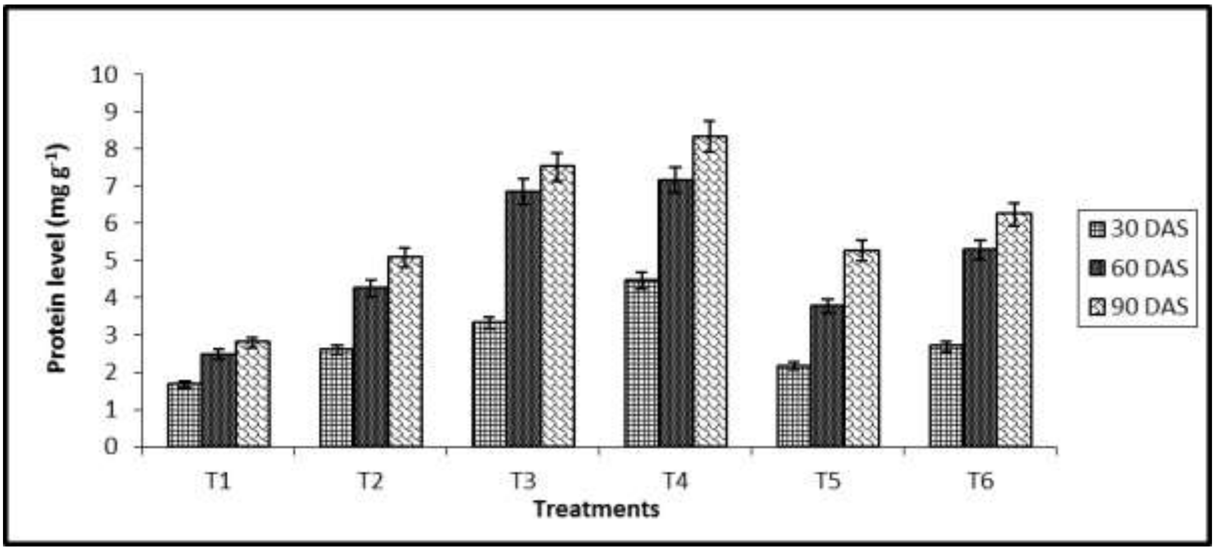
Protein level (mg g-1)
10
9
8
7
6
5
4
3
2
1
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 4a

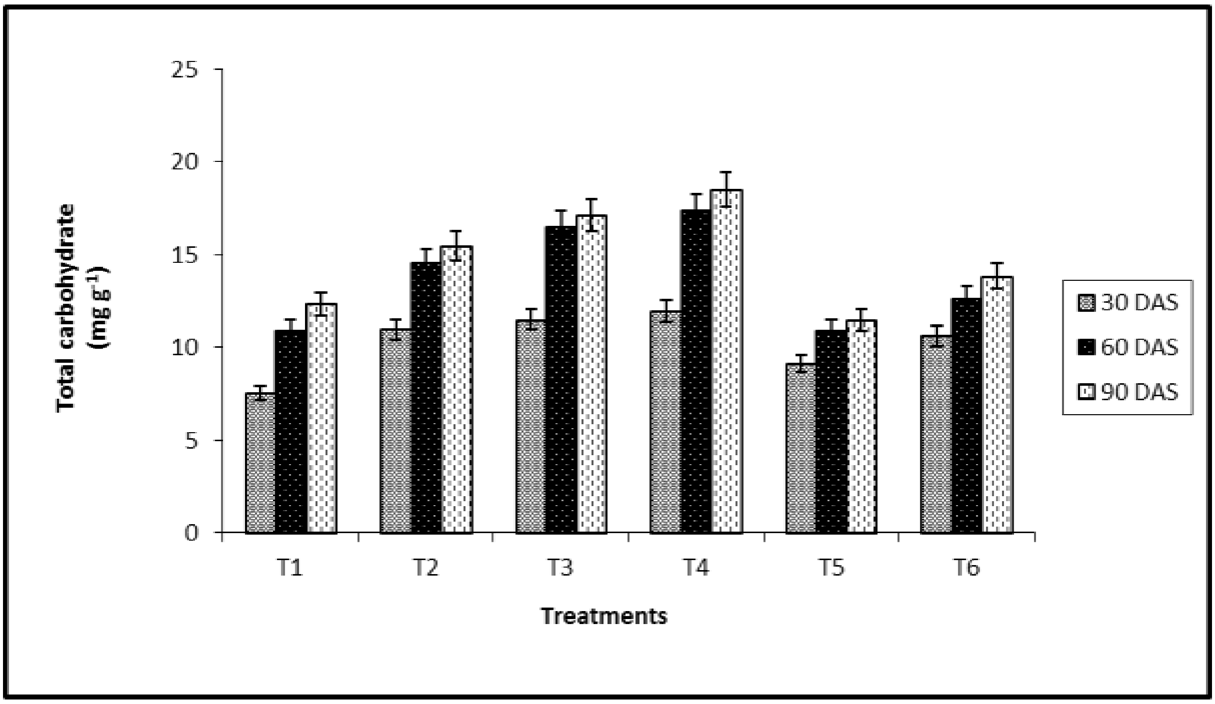
25
20
15
10
5
0
Total carbohydrate (mg g^{-1})
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 4b

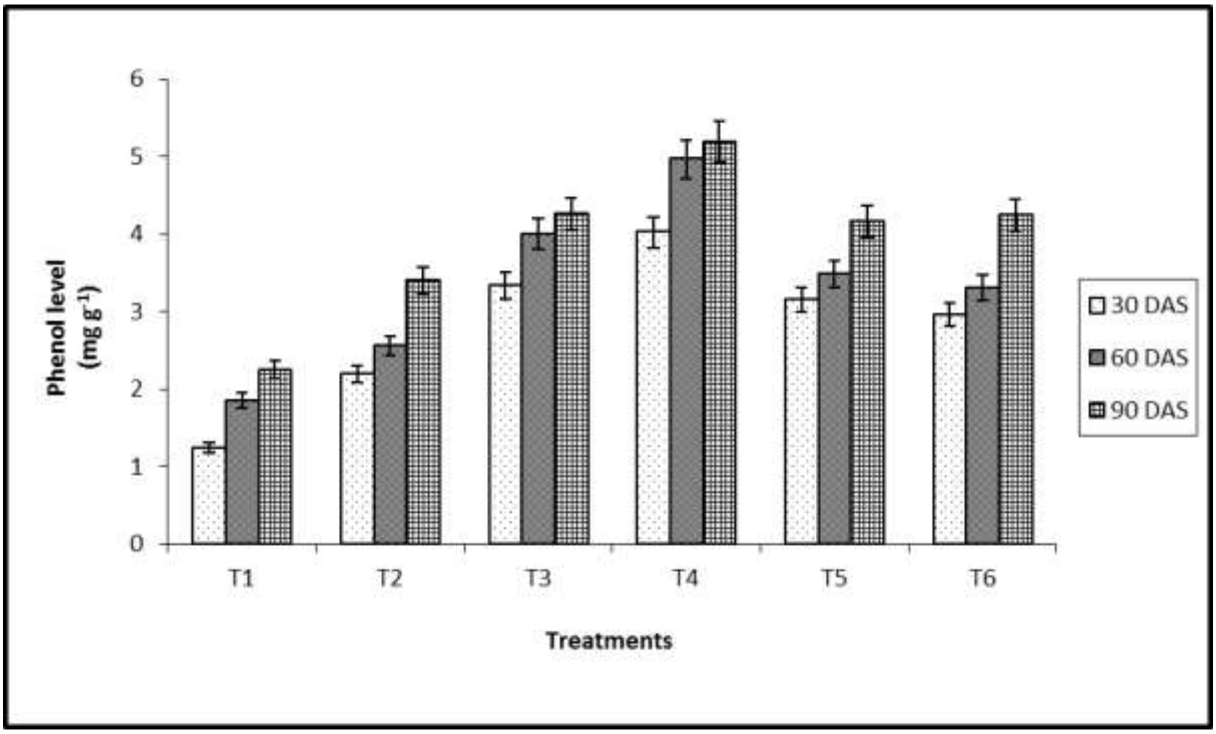
6
5
4
3
2
1
0
Phenol level (mg g^{-1})
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 4c

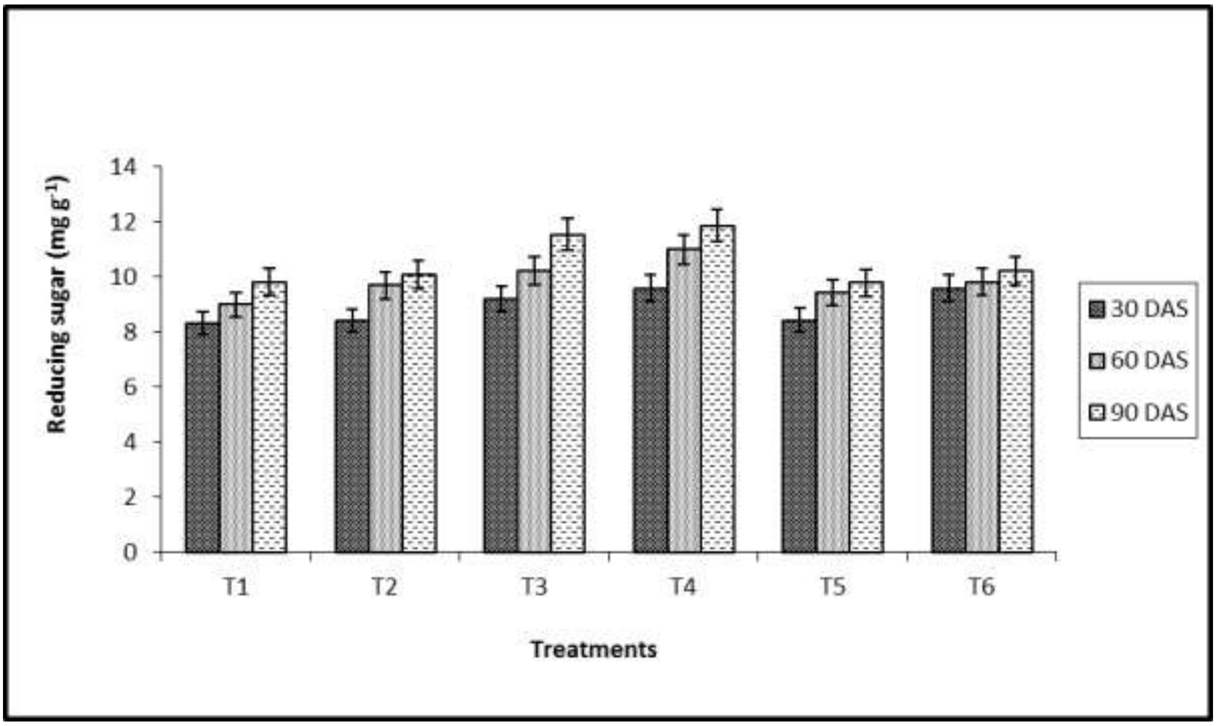
Reducing sugar (mg g^{-1})
14
12
10
8
6
4
2
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 4d

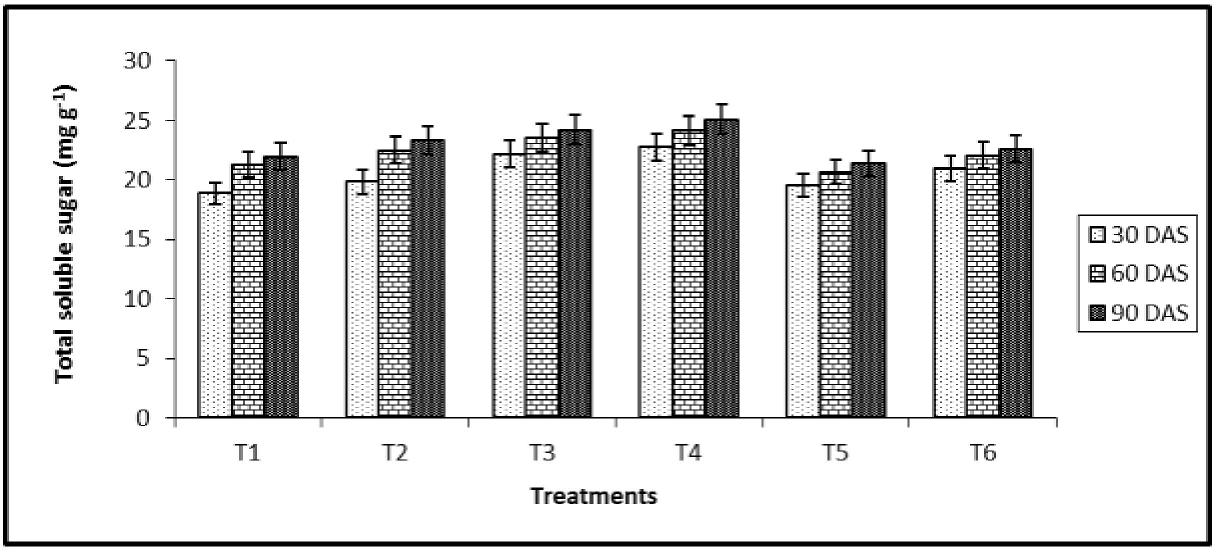
Total soluble sugar (mg g^{-1})
30
25
20
15
10
5
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 4e

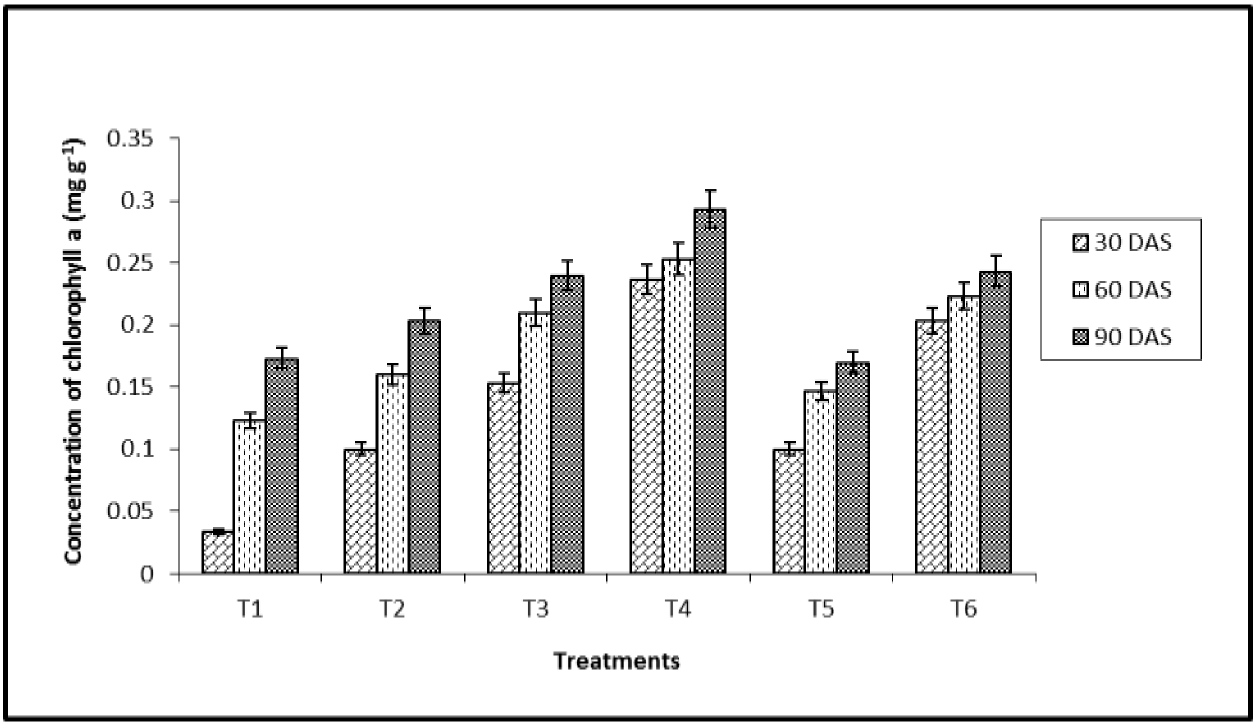
Concentration of chlorophyll a (mg g-1)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 5a

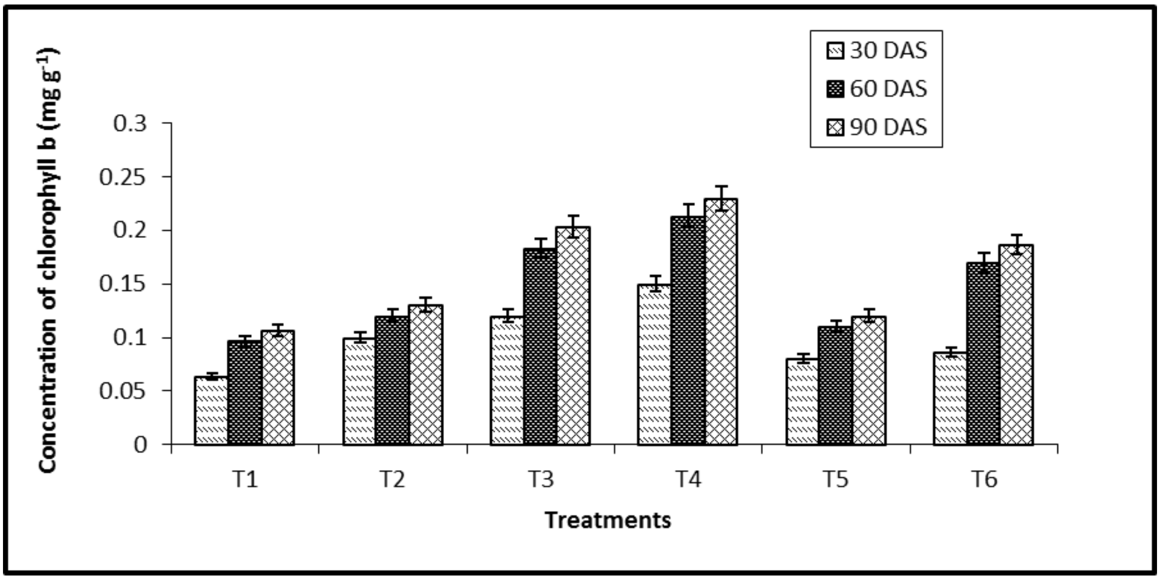
Concentration of chlorophyll b (mg g-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 5b

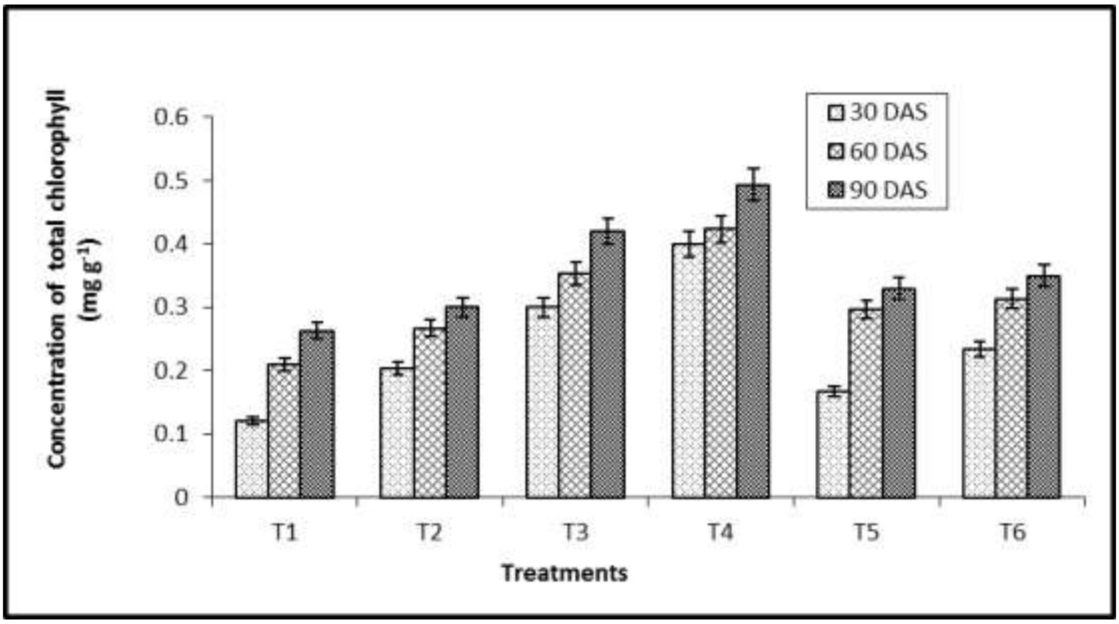
Concentration of total chlorophyll (mg g-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
T1
T2
T3
T4
T5
T6
Treatments
30 DAS
60 DAS
90 DAS

Fig. 5c