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EFFECT OF RHIZOBIUMAND HEAVY METALS (IRON AND COPPER) ON THE GROWTH OF V*IGNA MUNGO* (L.)

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Abstract: The application of iron and copper in combination with Rhizobium was studied with on the growth of Vignamungo(L.).The Vignamungo(L.) seeds were grown in different concentrations (0, 30, 60 and 90ppm) of iron and copper individually in natural conditions. This study has shown that copper and iron metal ions have stimulatory effects on plant growth at low concentration but it turns toxic at high concentrations. The present study concluded that copper and iron ion stress cause alteration in protein contents and nodulation which might have resulted in reduction in seed germination and growth.

Keywords: *Rhizobium, Vigna mungo(L.),* Copper, Iron.

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INTRODUCTION

Pulses, the food legumes, have been grown by farmers since millennia providing nutritionally balanced food to the people of India and many other countries in the world (R. Swaminathan, 2012). Pulses constitute an integral part of Indian diet and an important source of protein for a vast majority of Indian population and often called poorman's meal (P.K. Patra, 2009).Blackgram (*Vignamungo* L. Hepper) is an important grain legume crop grown in the tropical and subtropical regions of the Indian subcontinent. It is grown for its protein-rich edible dry seeds that, when supplemented with cereals, provide a balanced diet (Bhomkar*et al*., 2008).*Vignamungo(*L.) improves the fertility of the soil by fixing the atmospheric nitrogen in symbiotic association with the *Rhizobium* bacteria present in its root nodules.The crop is subjected to a variety of abiotic and biotic stresses which are responsible for its poor productivity. Among the abiotic stresses the crop is notably susceptible to salinity,nutrient deficiency and drought (Bhomkar*et al*., 2008).The plant needs some macronutrients and micronutrients for its normal growth (Fortescue, 1992). Fe, Mn, Mo, Ni, Zn and Cu are essential micronutrients that required for normal growth and metabolic processes in plants (Vangronsveld and Clijsters, 1994). Iron is an important plant micronutrient. It is absorbed by plants as the ferrous ion (Fe^{+2}),which is necessary for the formation of chlorophyll and functions in some of the enzymes of the plant's respiratory system(Schneider *et al.*, 1968).Copper is also an essential plant micronutrient, required for the protein components of several enzymes (Marschner,1995). However, when present in excess quantities, Cu is also highly toxic to plant growth potentially causing damage resulting in complete inhibition of growth. So, it is required to examine the effects of different concentrations of iron and copperwith *Rhizobium* and assess their best combination in terms of enhancing N fixation and productivity of *Vignaradiata* (L.).

MATERIALS AND METHODS

A Field experiment was conducted in the field of Department of Botany, C.C.S. University (Campus) Meerut, (U.P.) India, during the summer season of 2014. To determine the effect of iron and copper with *Rhizobium* on *Vignamungo*(L.), the experiments were carried out in natural conditions following standard agronomic practices.Healthy seeds of *Vignamungo* (L.) were selected and washed with distilled water.Ferric sulphate ($Fe_2(SO_4)_3.H_2O$) and Cupric sulphate ($CuSO_4{\cdot}5H_2O$)) solution of 30, 60 and 90ppm were prepared in distilled water, and tap water was used as a control. Experimental field designed in eight plots of equal size(1×1m), seven plots for treatment and one plot for control.The treatments comprised of *Rhizobium* alone in one plot and 3 levels of iron and copper (30,60 and 90ppm each) along with common application of *Rhizobium*(0.31 gm/plot in powder form with 1 ml sugar sticker)in another six plots.

Six treatment of Ferric Sulphate ($Fe_2(SO_4)_3.H_2O$) and Cupric sulphate ($CuSO_4 \cdot 5H_2O$) solution of 30, 60 and 90ppm and *Rhizobium* were applied in the plots before one day of sowing to moist soil. 50 healthy seeds of *Vignamungo* (L.) were sown in each plot. The seed germination percentage was calculated after counting the difference between germinated (coming out of the soil) and non-germinated seeds (remaining inside, non-emergent).After 30 days of seed sowing five plants were selected randomly from each plot and tagged for recording plant height, fresh weight/ plant, dry weight/ plant and number of nodules/ plant. Nodules were detached from the plant roots with the help of forceps. Fresh weight of nodules was measured immediately and followed by the measurement of their dry weight after drying them at 60ºC for 24 hours to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. The proline content of fresh leaves was estimated as devised by Sadasivam and Munikam (1996).IBM SPSS statistics 20 was used for Statistical analysis.

RESULT AND DISCUSSION

Seed germination

Germination in the *Rhizobium*+30ppm Copper and *Rhizobium*+30ppm Iron treated seeds were found higher than other treated seeds.*Rhizobium*improves the germination percentage of seeds as compared to control. Statistical analysis shows that the mean difference is significantat the 0.05 levelof significance. *Rhizobium*+30ppmCopper and *Rhizobium*+30ppm Iron treated seedshave high significant value in the comparison to others treatment as shows in figure1. Lowest difference is observed between *Rhizobium*+Copper30 and *Rhizobium+*90ppm Copper. Higher concentrationsof Copper and Iron have shown inhibitory effect on seed germination. Ionic toxicity may be the cause of drastic effects of heavy metal salts on germination, or it could be an account of osmotic effect (Shaukat*et al.,* 1999).

The heavy metals have induced delayed response in case of germination because the number of seeds germinated after 24 hr was less in comparison to those which were noticed after 48 and 72 hr (Dhankhar*et al*., 2011). Reduction in seed germination rate can also be attributed to the alterations in the selection permeability properties of the cell membrane (Muhammad *et al.,* 2008). The delay in seed germination in the presence of heavy metal is probably connected with the process of cell-stretching, break and fission (Kumar *et al*1995).

Shoot and root length

The effect of *Rhizobium* and two micronutrients on *Vignamungo* L. growth are given in table 2 and figure 2. Plants treated with concentrations of copper 60 and 30ppm and iron 60 and 30ppm showed an increase in root length and shoot length when compared to control and other treatments. In general root and shoot length of plant were found significantly higher with

Rhizobium+Copper60ppm and *Rhizobium*+Iron60ppm. Statistical analysis shows that the difference between treatments is significant at 0.01 level of significance. Significant increase in the root and shoot growth was possibly due to their nutrient value required by plants in trace amount (Reichman, 2002). Higher concentrations showed a decrease in the root and shoot length. The reduction in plant growth could also be due to the decline in photosynthetic pigments (Bibi and Hussain, 2005) and Rubisco activity (Sheoran*et. al*., 1990).Control shows the minimum shoot and root length in the comparison of other treatments. The inhibition was stronger in roots than in the shoot because the plant roots are the first point of contact with these heavy metals (Dhankaret. al, 2011). Copper in excess has been reported to have a negative effect on mineral nutrition and enzyme activities related to metabolism of plants and may be toxic to plants at higher concentrations (Jain *et al*., 2010). Excess copper has also been found to lead to the inhibition of young seedling growth and root elongation and to damage root epidermal cells and root cell membranes(Lequeux*etal*., 2010).The inhibitory action of excess copper and iron in root and shoot length may be due to reduction in cell division, toxic effect of heavy metal on photosynthesis, respiration and protein synthesis. These obviously contribute to the retardation of normal plant growth (Sonmez*etal*., 2006).

Fresh and dry matter production

Fresh and dry weights of plants are given in the table 3 . The maximum root and shoot fresh and dry weight were recorded in *Rhizobium*+Iron 60ppm which were higher than other treatments. The minimum fresh and dry weight of root and shoot wererecorded in control. The experimental results suggest that a normal level of iron produced maximum shoot and root biomass whereas above and below this level the shoot and root biomass significantly got reduced. The difference of shoot and root biomass is significant at 0.05level of significance (figure3). Samelevels of results were obtained by Shrivastava and Ahlavat (1995). Plants treated with Copper at low concentration showed a significant increase in dry matter production of root and shoot but at higher concentration, it showed a gradual decline in dry matter production. Application of copper slightly increased the dry weight at lower concentration, while its excess reduced the biomass (Xiong*et al*., 2006). Similar reduction in dry matter yield of greengram at higher concentration of heavy metals was also observed by Madhavi and Rao (1999) due to cadmium. The decrease in biomass in excess copper treated greengram might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Wani*et al*., 2007).

Nodulation

The numbers of the root nodules in *Vignamungo* L. were higher in *Rhizobium*+copper 30ppm and *Rhizobium*+iron30ppm treatment as compared to other treatment. The maximum number of nodules were found in Copper 30ppm and Iron 30ppm applied with *Rhizobium* while minimum number found with90ppm (table no. 4).Observed statistical analysis also shows that the difference between treatments and control is significant at 0.05 level of significance (figure 4). Similar reduction in nodule number under copper treatment was reported in*Trifolium pretense* L. by McIlveen and Coole(1974) and in *Vignaunguiculata* by Jain *et al.*(1994). A decrease in the number of nodules in greengram plants, due to elevated level of copper, would be attributed to the reduction in the development of root system as well as the direct toxicity of copper on soil microbes. The toxicity of metals to nodule bacteria in vitro or legume-plant varies widely. Heavy metals, therefore, affect the viability of *Rhizobium* (Broos*et al*., 2005), and consequently the legume-*Rhizobium* symbiosis (Broos*et al*., 2004).The maximum fresh weight, dry weight and nodule volume were obtained in Copper 30ppm and Iron 60ppm and minimum in Copper 90ppm have also been reported by Khan *et al.*(2014). The application of Fe 2 kg ha^{-1} through ferrous sulphate significantly increases the number and weight of nodules and biomass production of black gram over control (Balachandra*et al*., 2003).Deficiencies of iron can affect initiation and development of the nodule. The requirement for iron by legumes with an active symbiosis is large because many symbiotic proteins incorporate iron. Iron is required by numerous bacteroids for the synthesis of the nitrogen-fixing enzyme, nitrogenase, as well as cytochromes, ferredoxin, and hydrogenase (Peters and Szilagyi, 2006).

Protein

The observation with present experiments indicate that of protein content decreases with increasing Copper and Iron concentrations (table 5).The maximum accumulation of protein was obtained in Copper (30ppm) and Iron (30ppm) as compare to other treatments.Protein was appreciably reduced in wheat at the higher concentration of copper exposure. It has been further showed that excessive Cu reduced protein amount of many plant species (Singh *et al.*, 2007). It is apparent from Table 6 that at 30 and 60ppm concentration there was showed an enhancement of protein content but at 90ppm there was a decrease was recorded. The reduction in protein content might be due to enhanced rate of denaturation of protein (Tripathi and Gautam, 2007). The enhance protein denaturation and breakdown of existing protein to amino acid is the main cause of declinein protein content (Slatni*et al*., 2011). Marschner (1995) indicated that iron is involved in the formation of chlorophyll, N assimilation, nitrate reduction, and protein synthesis,thus reflecting on grain protein recovery. However the statistical analysis shows that the difference is not significant and it can be by chance.

Proline content

Proline accumulation accepted as an indicator of environmental stress, is also considered to have important protective roles. The results indicate that accumulation of proline increases with increasing Cu and Fe concentration (Table 5 and figure 5). The maximum accumulation of proline was found in 90ppm Cu and 90ppm Fe than other treated plants and as well ascontrol plants.However Statistical analysis shows that the difference within treatments and control is not significant. The results ofincreased proline content also corroborated with the findings of Bassi and Sharma (1993) who also found that the Copper proved to be a stronger inducer of proline accumulation in wheat seedlings. It has been determined that, as a response to heavy metals generated stress, plants increase their proline and abscisic acid (Zengin and Kirbag, 2007).

Table 1: Seed germination percentage of *Vignamungo* (L.) with *Rhizobium* and different concentrations of iron and copper.

Treatment	5 DAS	10 DAS	15 DAS
Control	66	72	82
Rhizobium	76	82	90
Rhizobium+30ppm Fe	78	88	90
Rhizobium+60ppm Fe	70	82	88
Rhizobium+90ppm Fe	66	68	75
Rhizobium+30ppm Cu	75	90	92
Rhizobizum+60ppm Cu	64	82	86
Rhizobium+90ppm Cu	58	62	68

*All values are mean of three replicates.

Table 2: Root length and shoot length of *Vignamungo*(L.) with *Rhizobium* and different concentrations of iron and copper after 30 days of sowing.

Treatment	Root length(cm.)	Shoot length (cm.)
Control	13.1	26.2
Rhizobium	11.2	30
Rhizobium+30ppm Fe	10.3	30.2
Rhizobium+60ppm Fe	12.2	33
Rhizobium+90ppm Fe	9.6	29.8
Rhizobium+30ppm Cu	11.8	31.6
Rhizobium+60ppm Cu	13.2	36
Rhizobium+90ppm Cu	12	32

Table 3: Root and shoot fresh and dry weight of *Vignamungo*(L.) with *Rhizobium* and different concentrations of iron and copper after 30 days of sowing.

Treatment	Root		Shoot	
	Fresh wt. (gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt. (gm)
Control	0.405	0.165	7.03	1.467
Rhizobium	0.499	0.223	9.713	2.131
Rhizobium+30ppm Fe	0.571	0.244	11.167	2.833
Rhizobium+60ppm Fe	0.629	0.266	14.460	2.844
Rhizobium+90ppm Fe	0.518	0.233	13.445	2.436
Rhizobium+30ppm Cu	0.499	0.231	10.358	1.684
Rhizobium+60ppm Cu	0.603	0.243	13.907	2.001
Rhizobium+90ppm Cu	0.488	0.188	9.020	1.501

Table 4: Nodule number, volume, fresh and dry weight of *Vignamungo* (L.) with *Rhizobium* and different concentrations of iron and copper after 30 days of sowing.

Treatment	Nodule number	Volume	Fresh wt. (gm)	Dry wt. (gm)
Control	19	0.6	0.014	0.012
Rhizobium	29.8	1	0.037	0.029
Rhizobium+30ppm Fe	36.8	2.8	0.056	0.032
Rhizobium+60ppm Fe	30.8	1.2	0.404	0.023
Rhizobium+90ppm Fe	18.4	0.8	0.104	0.001
Rhizobium+30ppm Cu	40	3.5	0.164	0.040
Rhizobium+60ppm Cu	13.2	1.8	0.071	0.019
Rhizobium+90ppm Cu	7.2	0.3	0.018	0.003

Table 5: Protein and proline content of *Vignamungo*(L.) with *Rhizobium*and different concentrations of iron and copper after 30 days of sowing.

Treatment	Protein mg/g fresh wt.	ProlineµM/g tissue
Control	0.00494	2.0582
Rhizobium	0.01114	0.5856
Rhizobium+30ppm Fe	0.03379	0.7864

***Rhizobium*+60ppm Fe**	0.028421	1.43906
***Rhizobium*+90ppm Fe**	0.011285	2.5769
***Rhizobium*+30ppm Cu**	0.021345	1.1462
***sRhizobium*+60ppm Cu**	0.014685	1.1964
***Rhizobium*+90ppm Cu**	0.011822	4.9949

MEAN PLOTS

Figure 1: Seed germination percentage of *Vignamungo*(L.) with *Rhizobium* and different concentrations of iron and copper.

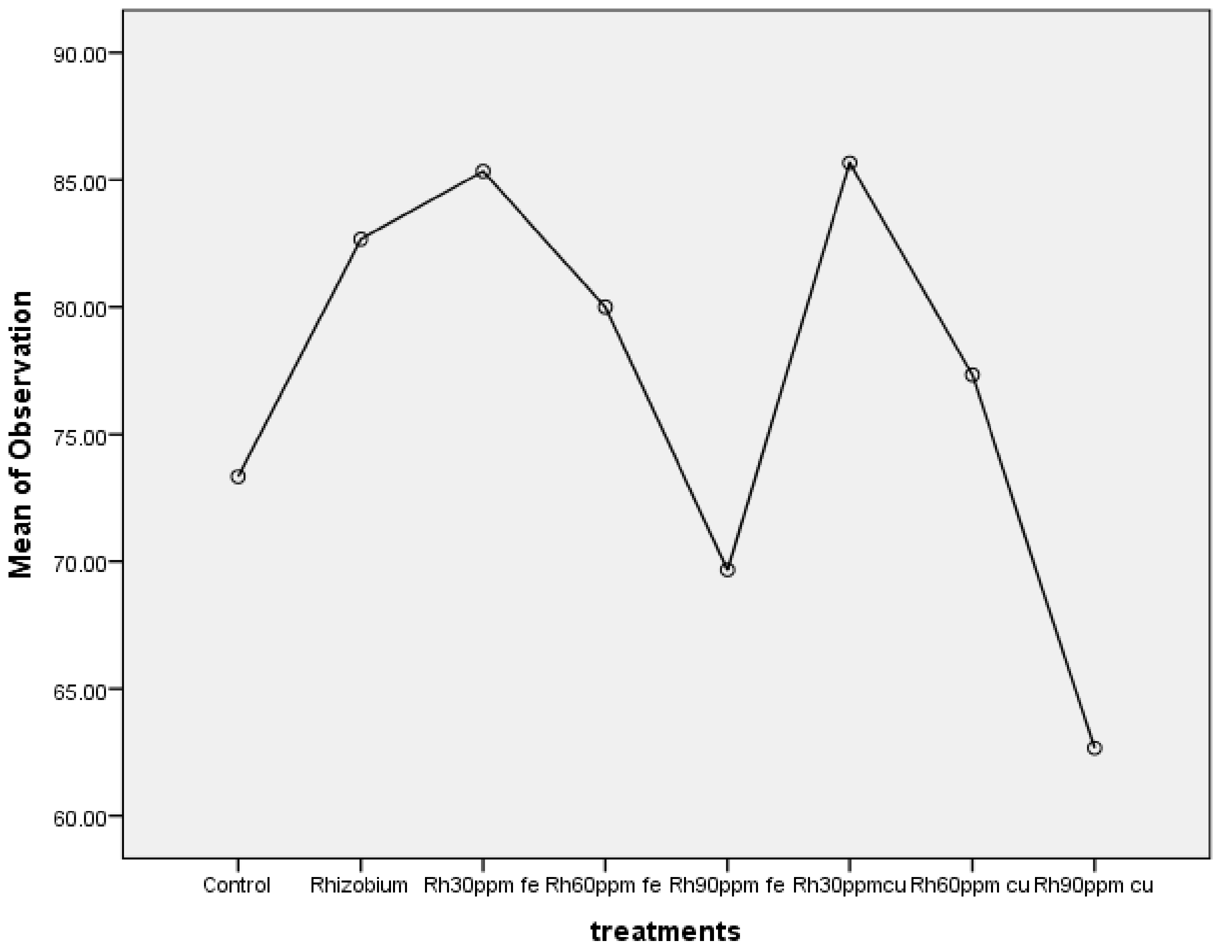


Figure 2: Root length and shoot length of *Vignamungo*(L.) with *Rhizobium* and different concentrations of iron and copper after 30 days of sowing

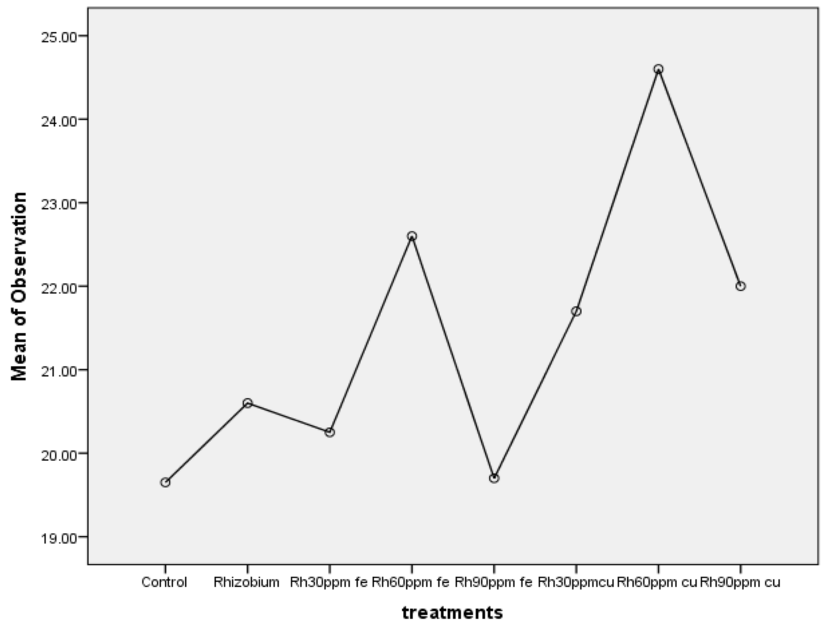
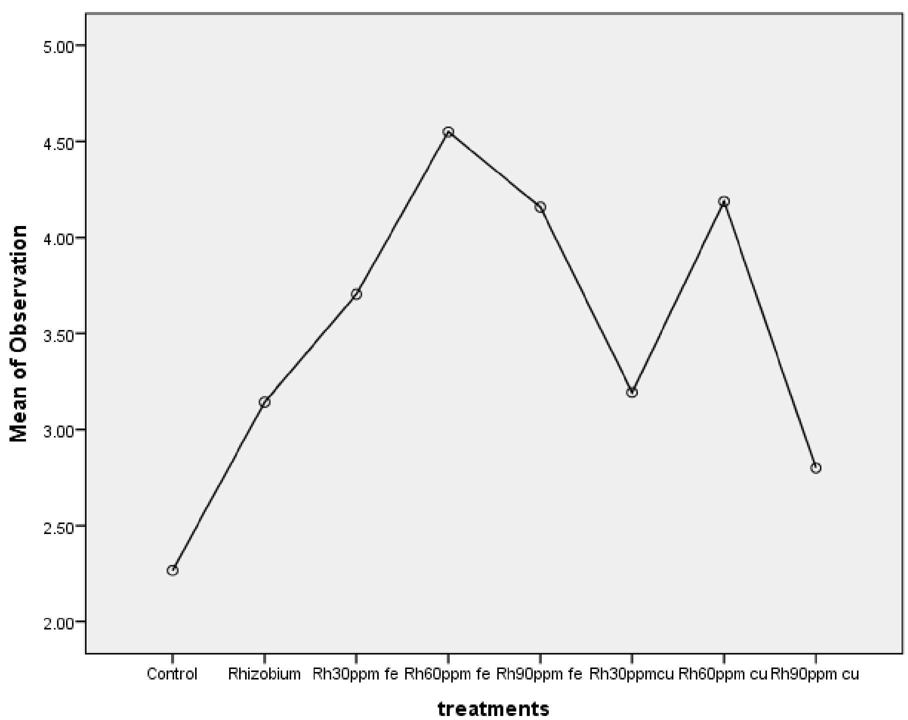


Figure 3: Root and shoot fresh and dry weight of *Vignamungo*(L.) with *Rhizobium* and different concentrations of iron and copper after 30 days of sowing.



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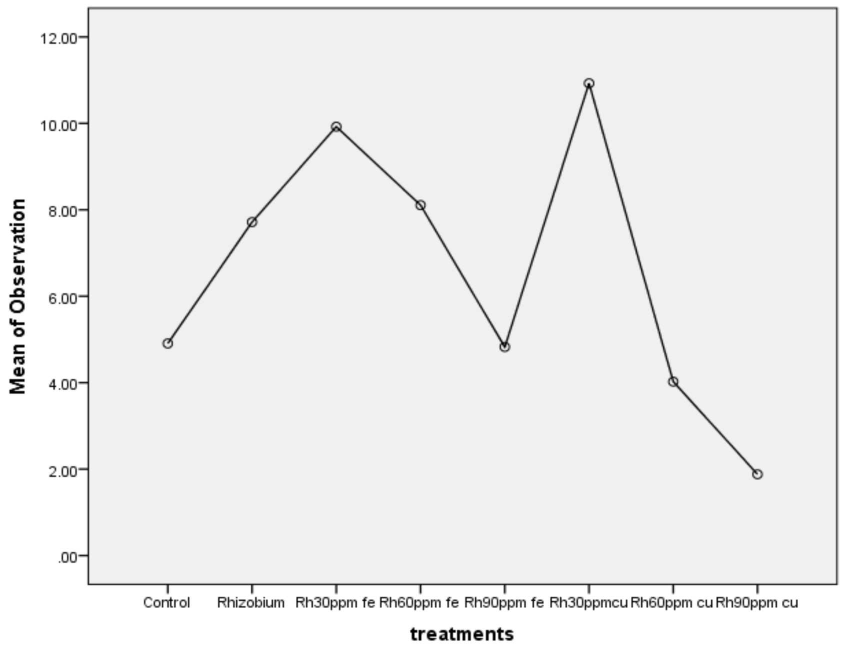
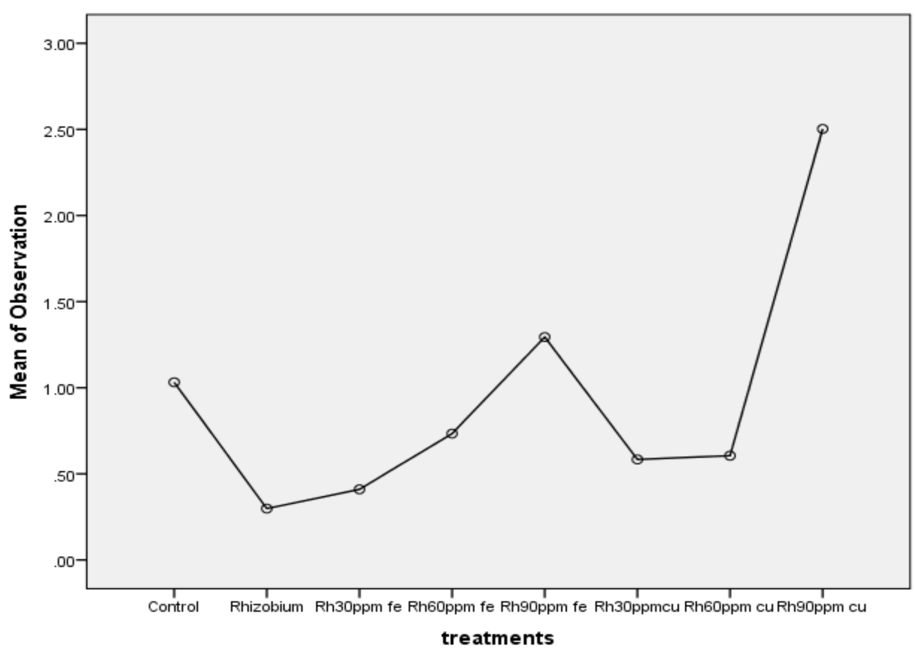


Figure 5: Protein and proline content of *Vignamungo*(L.) with *Rhizobium*and different concentrations of iron and copper after 30 days of sowing.



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