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ORIGINAL ARTICLE

"STUDY OF INTERACTIVE EFFECT OF RHIZOBIUM, AZOTOBACTER AND TRICHODERMA ALONE AND IN COMBINATION ON TRIGONELLA FOENUM-GRACEUM L."

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Abstract:

Trigonella foenum-graceum L. has unique ability to maintain a high protein level throughout the growing season therefore it is cultivated as a commercial crop. The use of biofertilizers on Trigonella plant showed the significant effect on growth and leaf, seed protein content. Compared to control shoot length and root length was more in the pot containing different biofertilizers. The total Chlorophyll content was maximum in the leaves of plants grown in the pot containing 50 gm of Rhizobium and 50 gm Azotobacter. The maximum leaf protein content was noticed in the plant grown in the pot containing 50 gm of Rhizobium and 50 gram of Azotobacter. The maximum seed protein was noticed in the plants grown in soil containing 50 gm of Rhizobium and 50 gm of Azotobacter.

KEYWORDS:

Interactive , Combination , Azotobacter , Rhizobium.

INTRODUCTION

Trigonella foenum-graceum L. belongs to family Leguminoceae sub family Fabaceae. It is widely cultivated in warm temperature and tropical regions in the Europe and Asia. Because of its unique ability to maintain a high protein level throughout the growing season Trigonella cultivated as a commercial crop.

Keeping in a view of the importance of Trigonella foenum-graceum, Rhizobium, Azotobactor & Trichoderma were planned to supply as an effective biofertilizer and to examine the effect of these biofertilizer on growth and development of Trigonella, also on protein contents in leaves & fruits as well as on other physiological parameters.

The main objectives of the present study was to study the comparative effect of interaction of microorganisms of biofertilizers with Trigonella, to study the response of Trigonella plant to artificial inoculation of biofertilizer, to study the effect of different biofertilizers alone and in combination on nodulation and nitrogen fixation.

MATERIALS & METHODS:

To study the response of Trigonella foenum-graceum L. to artificial inoculation of different biofertilizers pot culture experiments were conducted. The efficiency of biofertilizers was judged by nodule number, plant growth & development, protein content in leaf and fruit & other physiological parameters. For the present study the garden soil was used. The soil was sterilized in the autoclave at 15 lbs

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pressure for 20 minutes to make free from micro-organisms and with the help of pH meter soil pH was checked.

The sterilized soil was cooled, dried in shade conditions and were filled in the pots with same quantity. The different biofertilizers such as Rhizobium, Azotobacter & Trichoderma were obtained from Department of Biofertilizer M.K.V. Agriculture College, Shivajinagar, Pune-5. The biofertilizer packets were checked for expiry date and the biofertilizer powder was added in the earthen pots-alone and in combinations. One earthen pot was used as control which was filled with only sterilized soil without any biofertilizer powder. Next three pots were used for Rhizobium biofertilizer. As the Trigonella foenum-graceum belongs to family Leguminoceae the Rhizobium were used from 50 gm per pot to 100 & 200 gm per pot. For the next pot the non symbiotic Azotobacter was inoculated along with Rhizobium 50 gm each. In the last pot the three different biofertilizers like Rhizobium, Azotobacter & Trichoderma (50gm each) were used.

The biofertilizer mixture was thoroughly mixed with sterilized dried soil in the pot. The seeds of Trigonella foemum-graceum were obtained from Damaniya seed providing shop of Riccia Seed Company. The seeds were surface sterilized with 0.1% HgCl2 solution for 5-6 minutes. The surface sterilized seeds were washed thoroughly 3-4 times with sterile distilled water to remove the traces of HgCl2.

The surface sterilized seeds were sown in 1-6 pots containing sterilized soil and different biofertilizers alone and in combination. All the pots (1-6) were maintained under same environmental conditions and watered after one to two to three days of interval. After seedling growth stage the observations were recorded after every 5 days of interval. The morphological characters & root nodule development was recorded in tabulated form.

BIOCHEMICALANALYSIS

Chlorophyll estimation: Chlorophyll content was estimated by method described by Arnon's (1949). Chlorophylls were extracted in 80% acetone and the absorbance was taken at 663 nm on spectrophotometer using the absorption co-efficient. The chlorophyll content was calculated using formula.

Protein Estimation: Protein content was estimated by Lowery et al (1951) method. Protein contains different amount of aromatic amino acid like tryptophan & tyrosine .These amino acids reduce the foline phenol reagent giving blue colour. The intensity of colour was measured on calorimeter or spectrophotometer at 750nm. With the help of standard graph the amount of protein in the samples was calculated.

REDUCING SUGARS

Reducing sugars were estimated by Dinitrosalicyclic acid reagent (DNSA reagent) method. Reducing sugars were extracted in 80% ethanol and absorbance was read at 510 nm using spectrophotometer. With help of standard graph the amount of reducing sugar in the samples was calculated.

CARBOHYDRATE

The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide's and the absorbance was read at 630 nm. With help of standard graph the amount of carbohydrate in the samples was calculated.

Table No-1 Effect of Rhizobium, Azotobacter and Trichoderma on different bimolecule content in leaf and seed of Trigonella foenum-gracum L.

No	Chemical Parameters	Control	50 gm Rhizobium	100 gm Rhizobium	20 gm Rhizobium	50 gm Rhizobium + 50 gm Azotobacter	50 gm Rhizobium + 50 gm Azotobacter + 50 gm Trichoderma
1	Total Chlorophyll (mg/gm)	0.010±2	0.012±1	0.030±3	0.057±2	0.093±1	0.061±1
2	Protein content in Leaf (ug/gm)	40.0±4	42.8±1	62.2±2	68.4±2	87.2±3	66.8±1
3	Protein content in Seed (ug/gm)	40.0±1	50.0±3	62.0±4	65.0±1	68.0±1	66.0±2
4	Carbohydrate (ug/gm)	390.0±2	392.0±1	394.0±3	430.0±3	450.0±1	420.0±1
5	Total Reducing Sugar (ug/gm)	218.0±4	66.0±1	164.0±2	108.0±3	310.0±1	120.0±2
6	Starch (ug/gm)	72.0±1	52.0±3	64.0±1	61.0±2	60.0±2	112.0±4

The value represents the mean ± SE calculated on three independent experiments.

Fig no. 1 Effect of Rhizobium, Azotobacter and Trichoderma on growth and development of Trigonella foenum-gracum L.

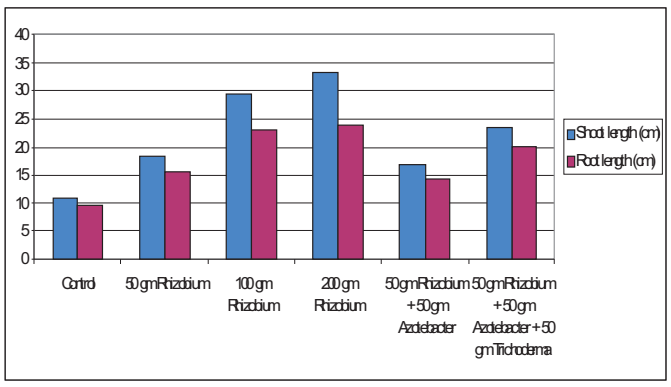
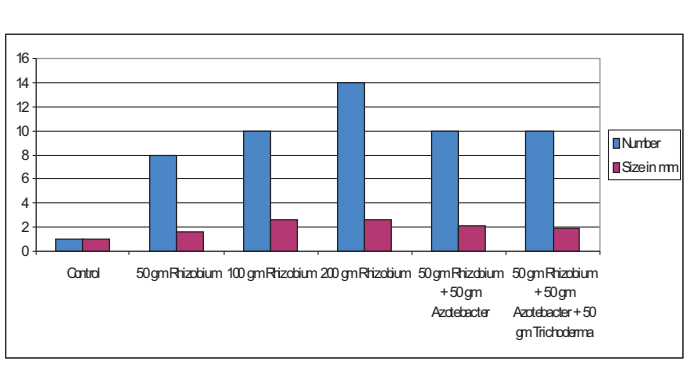


Fig no. 2 Effect of Rhizobium, Azotobacter and Trichoderma alone and in combination on nodule formation in roots of Trigonella foenum-gracum L.



RESULT

The Morphological observations are shown (Fig. 2) at different conc. of Rhizobium (20,100,200 gm/pot) maximum shoot length (33.8 cm) was recorded in the pot containing 200 gm of Rhizobium compared to 50 & 100 gm of Rhizobium per pot. Compared to control supplementation of the 50 & 100 gm Rhizobium bio-fertilizer showed more shoot length. Thus it was noticed that supplementation of Rhizobium in the soil to the Trigonella foenum enhance shoot growth compared to the control. Data recorded (Fig. No. 1) on root length showed maximum root length in the pot containing 200gm of Rhizobium. It was maximum (23.9 cm) in pot containing 200 gm Rhizobium biofertilizer compared to 50 & 100 gm Rhizobium bio-fertilizer supplementation. It was found that the first leaf primordial emerged in the pot containing 200 gm of Rhizobium at the 13th day after sowing the seeds in pot. While in the pot containing 50 & 100 gm Rhizobium 1st leaf primordial emerged on 16th day after sowing the seeds. Compared to control the emergence of leaf primordial was early in the pot containing 200 gm of Rhizobium.

Initiation of flowering & fruiting showed more or less similar results in control as well as the pot containing 50, 100 & 200 gm Rhizobium biofertilizer. The effect of Azotobacter in combination with Rhizobium and the cumulative effect of Azotobacter & Trichoderma and Rhizobium were studied in the pot culture experiment on Trigonella foenum-graceum L. The data recorded (Fig. no.1) shows that the cumulative effect of Azotobacter, Trichoderma and Rhizobium has better result compared to control but the shoot length was less than that of obtained in the pot containing 200 gm of Rhizobium supplementation alone. The emergence of first leaf primordia was more or less similar in the pot containing Rhizobium and Azotobacter (50 gm each) and Rhizobium, Azotobacter and Trichoderma (50 gm each) in combination, as well as in control. The initiation of flowering & fruiting showed more or less similar result in pot containing Azotobacter & Rhizobium (50 gm each/pot) as well as control.

The studies on nodule formation and morphology of nodule are shown in fig. no. 2. The no. of nodule per plants was 0 to 1 in control pot. The no. of nodules per plant showed significant increase in number with the concentration of Rhizobium biofertilizer and use of combination of biofertilizer used. The no. of nodule per plant was 8 in the pot containing 50 gm Rhizobium. The no. of nodules per plant was increased from 10 to 14 in the pot containing 100 and 200 gm Rhizobium biofertilizer. The no of nodules per plant was more or less similar in the pot containing 50 gm of Azotobacter and 50 gm of Rhizobium as well as Azotobacter, Rhizobium and Trichoderma (50:50:50 gm/pot). The size of nodules per plant was also shown the variation in pot containing different biofertilizer. The size of nodule was minimum (0.5 to 1 mm) in the pot containing sterile soil. The pot containing Rhizobium biofertilizer 50 gm, 100 gm and 200 gm per pot showed maximum (2.6 mm) size of nodule.

The colour of nodule showed variation from white to whitish pink colour. The color of nodule was white in the pot containing sterile soil. The colour of nodule was white to whitish pink in all pots containing Rhizobium biofertilizer alone and Rhizobium in combination with Azotobacter and Trichoderma. The appearance of nodule was smooth in all the plants containing Rhizobium biofertilizer alone and in combination with Azotobacter and Trichoderma. The position of nodules on plant root was more or less similar in all the plants. The position of nodule was on main root and on lateral branches of root in all the plants grown in the pots containing Rhizobium and combination of Rhizobium with Azotobacter and Trichoderma.

BIOCHEMICALANALYSIS

The studies on biochemical analysis are shown in the table no.1. The total chlorophyll content was minimum (0.010 mg/gm) in the leaves of plants grown in the sterile soil compared to it total chlorophyll content was maximum (0.093 mg/gm) in the leaves of plants grown in the pot containing of Azotobacter and Rhizobium 50 gm each. Less to it was (0.061mg/gm) in leaves of plants grown in pot containing Azotobacter, Rhizobium and Trichoderma 50 gm each.

The leaf protein content was maximum (87.2 ug/gm) in the leaves of plants grown in the pot containing 50 gm of Rhizobium plus 50 gm of Azotobacter, which was twice compared to control plant leaves. The protein content was more or less similar in the leaves of plants grown in the pots containing 50 gm of Rhizobium and the pots containing combination of Azotobacter, Rhizobium and Trichoderma 50 gm each. But it was less compared to pot containing 50 gm of Azotobacter plus 50 gm of Rhizobium and more compared to control plants.

The carbohydrate content was more or less similar in the leaves of plants grown in the pot containing Rhizobium alone and Azotobacter plus Rhizobium and the pots containing combination of Azotobacter, Rhizobium and Trichoderma. Starch content was maximum (112 ug/gm) in the leaves of plants growing in

the soil containing combination of Azotobacter, Rhizobium and Trichoderma 50 gm each biofertilizer and minimum starch (52 ug/gm) noticed in the leaves of plants growing in the pot containing 50 gm of Rhizobium biofertilizer. Total reducing sugar content was maximum in the plants grown in the pot containing 50 gm of Rhizobium plus 50 gm Azotobacter biofertilizer which was more than control plants. The data recorded on seed protein content showed variations. The maximum (68.0 ug/gm) was recorded in the seeds of plants grown in the soil containing 50 gm of Azotobacter plus 50 gm Rhizobium. The minimum (48.0 ug/gm) protein content was recorded in the seeds of plants grown in the pot containing sterile soil. Increase in level of protein content was recorded in the seed of plants grown in the soil containing 50 gm, 100 gm and 200 gm of Rhizobium biofertilizer.

DISCUSSION

The data recorded in table no:1 showed that the use of Rhizobium 200 gm per pot and 50 gm of Rhizobium plus 50 gm Azotobacter and combination of Azotobacter, Rhizobium and Trichoderma 50 gm each per pot showed maximum shoot length, root length and early flowering as well as fruiting compaired to control. This might be due to increase in the nitrogen fixation of plants along with these microorganisms in the soil. The maximum number of nodules per plant was recorded on the roots of plants grown in the soil containing 200 gm of Rhizobium which might be due to increase in the number of Rhizobia cell in the soil. Similar results were obtained by Aboul Nasr et al (2006). They also noticed the increase in nodule number per plant when plants were supplemented with Rhizobium melliloti and Bacillus circulans on Trigonella foenum graceum L. The result of present investigation showed simillarities with the earlier result recorded by B.P.Jinturkar and S.B.Kale (2005) on groundnut plant, they noticed highest number of nodules per plant inoculated with strains of Rhizobium. Jat.B.L and Shekhwat M.S (2003) studied on earl millet (Pennisterum glaucum L.)and fenugreek for production of economic and nutrient content by studying effect of residual sulphur, phosphorus and biofertilizer, they noticed increase in the seed yield of Trigonella foenum-graceum L.3.4% when Trigonella plant were supplied with Rhizobium, phosphate solubilizing bacteria significantly increases the nitrogen content after harvest of Trigonella. The result of these authors shows co-relation with the present study on Trigonella plant. We have also noticed inoculation of Rhizobium in soil increased protein content in leaves and seeds of Trigonella foenum-graceum L. plant. Sharma et al. (2005) studied effect of bioinoculant on biomass productivity under the agro forestry system, they have noticed combined inoculation of AM fungi and Azotobacter gave the best result on Trigonella foenum- graceum L. and other plant .Simillar results were obtained in our present investigation on Trigonella foenum- graceum L, an increase in protein content in leaf and seed when the plants were supplied with combination of biofertilizers to Trigonella plant.

SUMMARY AND CONCLUSION

The studies on interaction between Azotobacter, Rhizobium and Trichoderma alone and in combination on Trigonella foenum graceum L. shows the following important finding.
The use of biofertilizer on Trigonella foenum graceum L plant shows the significant effect on growth and leaf, seed protein content.
Compared to control shoot length and root length was more in the pot containing different biofertilizers.
Maximum shoot length and root length was noticed in the pot containing 200 gm of Rhizobium.
The size of nodule was large on roots of plants grown in pots containing 100 gm and 200 gm Rhizobium.
The total Chlorophyll content was maximum in the leaves of plants grown in the pot containing 50 gm of Rhizobium and 50 gm Azotobacter.
The maximum leaf protein content was noticed in the plant grown in the pot containing 50 gm of Rhizobium and 50 gram of Azotobacter.
The maximum starch content was observed in leaves of plants grown in the soil containing Azotobacter, Rhizobium and Trichoderma 50 gm each.
The total reducing sugar content was maximum in leaves of plants grown in the soil containing 50 gm of Rhizobium and 50 gm of Azotobacter
The maximum seed protein noticed in the plants grown in soil containing 50 gm of Rhizobium and 50 gm of Azotobacter.
Thus use of Azotobacter and Rhizobium biofertilizer in combination showed significant effect on enhancement of protein content in Trigonella foenum graceum L, Compared to use of Rhizobium alone and Azotobacter, Rhizobium and Trichoderma in combination.

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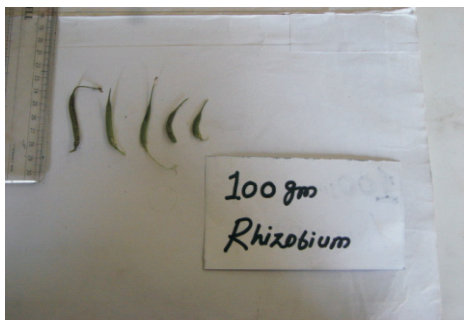

Plate no: 1. Pod of *Trigonella foenum graceum* L grown in the soil containing 100 gm of *Rhizobium*

Plate no: 2. Pod of *Trigonella foenum graceum* L grown in the soil containing 50 gm of *Rhizobium* and 50 gm of Azotobacter