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# Influence of bioagents on seed yield and quality of soybean (*Glycine max* (L.) Merrill)

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Field and Laboratory experiments were conducted to know the influence of bioagents *viz.,* soybean *Rhizobium, Pseudomonas fluorescence*, *Bacillus subtilis*, *Trichoderma viridae*, VAM and co-inoculation on seed yield and quality of soybean. Seeds treated with soybean *Rhizobium* @ 20 g/kg seed + *Bacillus subtilis* @ 20 g/kg seed + VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed recorded significantly higher number of*nodules in plant* (39.3 and 54.1 at 30 and 60 days after sowing respectively), number of pods (54.7 and 77.0 at 60 days after sowing harvest, respectively), seed yield (21.61 g/plant and 29.95 q/ha), net returns (Rs 58,244), benefit**:**cost (4.5), 100 seeds weight (14.85 g), total dehydrogenase activity (0.950), protein content (41.73 %), germination (97%), seedling length (34.3 cm), seedling dry weight (58.8 mg), vigour index-I (3323) and II (6676), and number of bacteria and fungal colonies (42 x $10^6$ cfu/g soil and 16 x $10^3$ cfu/g soil).

Key words : Antagonistics, Bacillus, Bioagents, *Glycine max* (L.) Merrill, *Rhizobium,* Seed quality, Seed vigour

## INTRODUCTION

The continuous use of chemical inputs in agriculture is one of the main causes of imbalance in the soil microbial activity, which leads to the outbreak of many diseases in crop plants. The crop is affected by several species of fungi that cause severe yield losses and majority of them are pathogenic and are seed-transmitted, thus demanding chemical seed treatment. Brazil is the world's second largest producer of soybean, has more than 90 per cent of the farmers treating seeds with fungicides. However, besides the negative effects on the environment and human health, one main problem reported in the country is that fungicides often drastically reduce the viability of *Bradyrhizobium* cells, decreasing nodulation and nitrogen fixation rates (Hungria *et al.,* 2005). In this context, instead of using chemicals use of micro-organisms as biological agents may represent an alternative method to control pathogenic fungi (Cubeta *et al*., 1985). Introduction of bacterized seeds having growth-promotion capabilities and antagonistic characteristics offer a valid alternative to chemical protectants. In view of the above facts, the present study is to investigate the Influence of bioagents on seed yield and quality of soybean (*Glycine max* (L.) Merrill)*"* was undertaken.

## MATERIALS AND METHODS

The cultures of soybean *Rhizobium, Pseudomonas fluorescence, Bacillus subtilis, Trichoderma viridae* and VAM were obtained from Bio-Fertilizer Scheme, UAS, GKVK, Bangalore. Soybean variety JS-335 seeds were treated with bioagents along with jaggery solution as an adhesive and Dithane M-45 and Carbendizem were treated as dry seed treatment. Treated seeds were dried under shade for half an hour and then used for field performance study.

Treated seeds of soybean variety JS-335 were sown in plot of 3.6m x 3 m with a spacing of (45cm x 15cm) by following RCBD design. At the time of sowing entire dose of recommended fertilizers *i.e.*, 30:80:37.5 kg NPK per hectare was applied in the form of urea, single super phosphate and muriate of potash, respectively. Crop was raised following all recommended practices. Field

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emergence was recorded after 16 days after sowing and days to 50 per cent flowering during crop growth. While, five plants from each plot were randomly selected for recording number of pods and nodules per plant, seed yield per plant and hectare was recorded. The harvested plants of each treatment were sun dried sufficiently and threshed manually by beating with stick. Post harvest seed quality observations *viz.*, germination, seedling length, seedling dry weight, vigour index, protein content, electrical conductivity, 100 seed weight and accelerated ageing test response were recorded.

The electrical conductivity (EC) of seed leachate was determined as per the procedure outlined by (Perl *et al.*, 1978). The total dehydrogenase activity was determined by method described by (ISTA, 1996) with slight modifications. Three seeds were selected randomly from the EC observation. After removing the seed coat, the cotyledons were soaked in 0.5 per cent tetrazolium solution at $25^0 \pm 1^0$C for a period of 24 hours. Then they were washed thoroughly with distilled water. The red colour (formazan) was eluted from the stained cotyledons by soaking in 5 ml of 2-methoxy ethanol for 6 to 8 hours in airtight vials. The extract was decanted and the colour intensity was measured at 480 nm using spectro UV-VIS double beam pc scanning spectrometer UVD-2950. The nitrogen percentage in soybean seeds was estimated by modified kjeldhal's method then protein content was calculated by multiplying nitrogen percentage with 6.25.

The germination test was conducted in the laboratory using between paper method as per ISTA (1996). Germination percentage was recorded on $8^{th}$ day. Ten seedlings from each replication were selected randomly for the germination test and seedling length was measured and means seedling dry weight was recorded and expressed in milligrams.

Seeds from each treatment were subjected to accelerated ageing at $42^0 \pm 1^0$C and 90 per cent relative humidity for 72 h in an accelerated ageing chamber. Seeds were removed after 24 h, and then aged seeds were subjected to germination test in three replications of hundred seeds. Normal seedlings were counted on the eighth day and expressed in percentage.

Soil microflora was analysed by serial dilution plate count technique (Bunt and Rovira, 1955; Kuster and Williams, 1964; Martin, 1950). The economics of seed production was computed by taking into consideration the cost of bioagents and chemicals apart from the other costs that are common to all treatments as per package of practice. The total value of the actual produce was calculated and the net income was worked out by deducting the cost of seed production from the value of actual produce. The benefit cost ratio was worked out using net income and total cost of seed production.

## Results and Discussion

Seeds treated with soybean *Rhizobium* @ 20g/kg seed and *Bacillus subtilis* @ 20g/kg seed have taken less number of days (32 days) to 50 per cent flowering. While Dithane M-45 @ 2g/kg seed and Carbendizem @ 2g/kg seed treated seeds took more number of days (34 days) to 50 per cent flowering (Table 1 and Fig. 1). The delay in flowering may be due to non-availability of plant growth promoting substances, less nutrient mobilization and low nutrient uptake. Higher number of nodules per plant at 30 and 60 DAS (39.3 and 54.1) was recorded in soybean *Rhizobium* @ 20 g/kg seed + *Bacillus subtilis* @ 20 g/kg seed + VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed ($T_9$), while it was lowest (11.6 and 17.6) in untreated (Table 1 and Fig. 2). The increased nodule number was due to increased nitrogen and phosphorus availability. Continuous supply of phosphorus

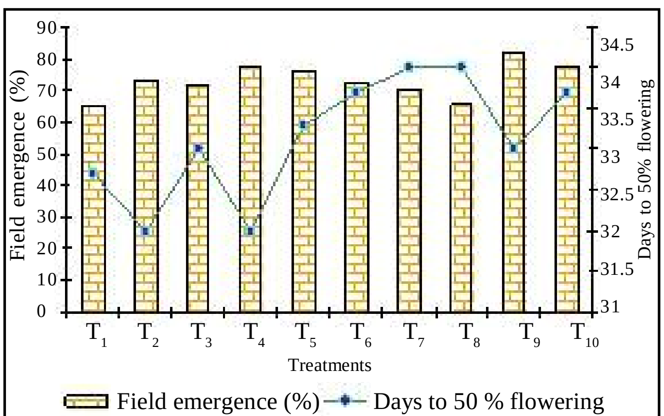


**Fig. 1: Effect of bioagents on field emergence and days to 50 per cent flowering in soybean var. JS-335**

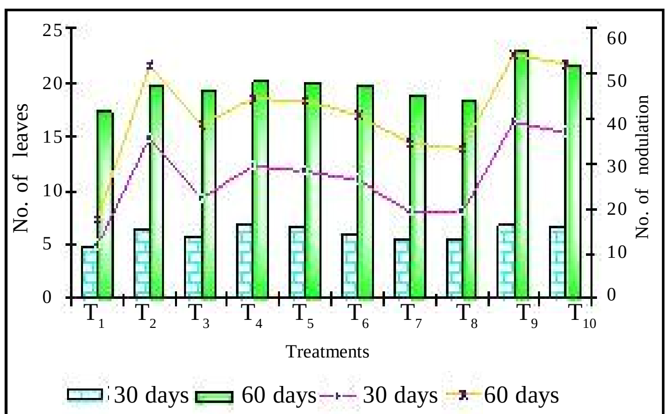


**Fig. 2: Effect of bioagents on number of leaves and number nodules in soybean var. JS-335**

**Table 1 : Effect of bioagents on field performance and seed yield in soybean var. JS-335**

| Treatment | Days to 50 % flowering | No. of nodules DAS | | No. of pods at | | Seed yield (g/plant ) | Seed yield (q/ha) |
|---|---|---|---|---|---|---|---|
| | | 30 | 60 | 60 DAS | harvest | | |
| $T_1$ | 32.7 | 11.6 | 17.6 | 44.7 | 56.6 | 12.95 | 20.21 |
| $T_2$ | 32.0 | 35.8 | 51.7 | 50.2 | 63.4 | 17.91 | 25.11 |
| $T_3$ | 33.0 | 22.3 | 38.8 | 47.2 | 61.6 | 15.35 | 22.75 |
| $T_4$ | 32.0 | 29.4 | 44.2 | 52.1 | 68.9 | 18.65 | 26.98 |
| $T_5$ | 33.3 | 28.2 | 44.0 | 51.3 | 64.6 | 17.98 | 25.34 |
| $T_6$ | 33.7 | 26.2 | 40.8 | 49.1 | 62.7 | 17.20 | 24.63 |
| $T_7$ | 34.0 | 19.4 | 34.5 | 47.1 | 60.9 | 15.25 | 22.64 |
| $T_8$ | 34.0 | 19.1 | 33.4 | 46.1 | 59.8 | 13.44 | 20.44 |
| $T_9$ | 33.0 | 39.3 | 54.1 | 54.7 | 77.0 | 21.61 | 29.95 |
| $T_{10}$ | 33.7 | 36.7 | 52.3 | 52.2 | 71.4 | 19.36 | 28.68 |
| S.Em. ± | 0.34 | 0.62 | 0.57 | 1.86 | 3.77 | 0.78 | 0.78 |
| CD (P=0.05) | 1.01 | 1.84 | 1.68 | 5.54 | 11.19 | 2.31 | 2.32 |
| CV (%) | 1.77 | 4.01 | 2.38 | 6.53 | 10.06 | 7.93 | 5.48 |

Treatment details:
$T_1$: Untreated $T_2$: Soybean *Rhizobium* @ 20 g/kg seed, $T_3$: *Pseudomonas fluorescence* @ 20 g/kg seed,
$T_4$: *Bacillus subtilis* @ 20 g/kg seed $T_5$: *Trichoderma viridae* @ 5 g/kg seed, $T_6$: VAM @ 10 kg/ha $T_7$: Dithane M-45 @ 2 g/kg seed,
$T_8$: Carbendizem @ 2 g/kg seed,
$T_9$: Soybean *Rhizobium* @ 20 g/kg seed + *Bacillus subtilis* @ 20 g/kg seed + VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed
$T_{10}$: Soybean *Rhizobium* @ 20 g/kg seed + *Pseudomonas fluorescence* @ 20 g/kg seed +VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed

in soil stimulates multiplication of *Rhizobia* and development of their motile forms which are essentially required to migrate through soil towards the root system and also due to synergistic activity among bioagents, which may led to higher number of nodules. Higher number of pods at 60 DAS and harvest was recorded in $T_9$ (54.7 and 77.0, respectively) and lowest (44.7 and 56.6 respectively) in untreated (Table 1). The increase in number of pods may be due to the more number of branches per plant, decreased flower drop and increased pod setting, nutrient mobilization, nutrient uptake, release of plant growth promoting substances by microbial inoculants and antagonistic activity against pathogens.

Highest seed yield per plant and yield per hectare was recorded in $T_9$ (21.61g and 29.95q, respectively) and lowest in untreated (12.95 g and 20.21 q, respectively) (Table 1). The appreciable increase in yield may be attributed to extensive root development, which resulted in uptake of N and P and their transportation towards above ground parts, plant growth promoting substance secreted by micro-organisms helped in various metabolic activities and control of plant pathogens and in the proliferation of beneficial organism in the rhizosphere. Which led to higher plant height, number of leaves, number of branches and number of pods per plant in turn led to higher seed yield per plant, net plot yield and yield per hectare.

Seed quality parameters *viz.*, 100 Seed weight (Table 2), Electrical conductivity (EC) (Table 2 and Fig. 3), Total dehydrogenase activity (Table 2 and Fig. 3), Protein

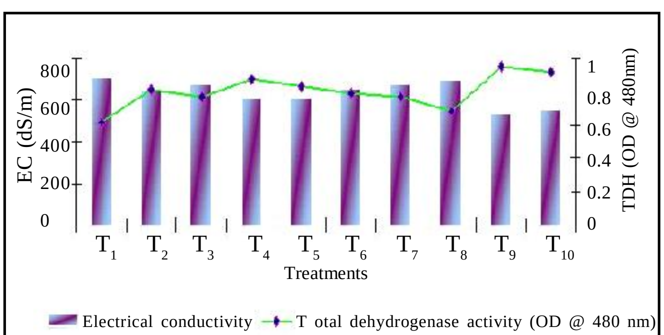


**Fig. 3: Effect of bioagents on electrical conductivity, total dehydrogenase activity in soybean var. JS-335**

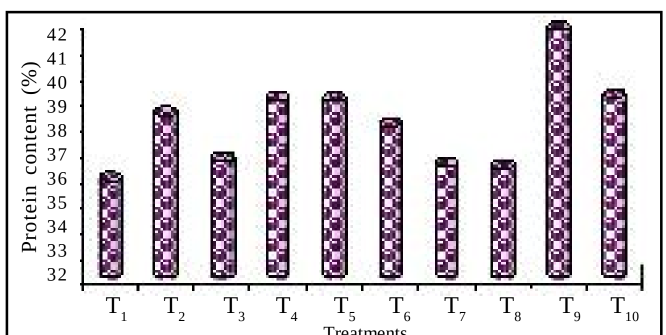


**Fig. 4: Effect of bioagents on protein content in soybean var. JS-335**

**Table 2: Effect of bioagents on post seed quality parameters in soybean var. JS-335**

| Treatments | 100 Seeds weight (g) | EC ($dSm^{-1}$) | Total dehydrogenase activity (OD @ 480ηm) | Protein content (%) | Germination (%) | Seedling length (cm) | Vigour Index-I | Seedling dry wt (mg) | Vigour index-II |
|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 12.93 | 704 | 0.622 | 35.85 | 89 | 29.0 | 2573 | 46.3 | 4997 |
| $T_2$ | 13.96 | 647 | 0.805 | 38.40 | 93 | 31.3 | 2919 | 53.3 | 5915 |
| $T_3$ | 13.56 | 670 | 0.771 | 36.58 | 92 | 30.8 | 2827 | 51.7 | 5675 |
| $T_4$ | 14.05 | 614 | 0.877 | 39.00 | 94 | 32.0 | 3016 | 54.7 | 6100 |
| $T_5$ | 13.97 | 617 | 0.830 | 38.95 | 94 | 31.9 | 3007 | 53.0 | 5946 |
| $T_6$ | 13.77 | 658 | 0.788 | 37.97 | 93 | 31.0 | 2869 | 53.3 | 5873 |
| $T_7$ | 13.51 | 671 | 0.765 | 36.40 | 89 | 30.9 | 2760 | 50.3 | 5389 |
| $T_8$ | 13.31 | 697 | 0.685 | 36.33 | 89 | 29.5 | 2616 | 50.3 | 5352 |
| $T_9$ | 14.85 | 534 | 0.950 | 41.73 | 97 | 34.3 | 3323 | 58.8 | 6676 |
| $T_{10}$ | 14.14 | 556 | 0.910 | 39.11 | 95 | 32.4 | 3068 | 54.3 | 6093 |
| S.E. ± | 0.27 | 36.47 | 0.04 | 0.56 | 1.47 | 0.81 | 79.60 | 1.40 | 187.29 |
| C.D.(P=0.05) | 0.80 | 107.59 | 0.12 | 1.66 | 4.33 | 2.39 | 234.83 | 4.13 | 552.52 |
| CV (%) | 3.39 | 9.92 | 8.98 | 2.56 | 2.75 | 4.48 | 4.76 | 4.61 | 5.59 |

*Treatment details:*

$T_1$: Untreated $T_2$: Soybean *Rhizobium* @ 20 g/kg seed,
$T_3$: *Pseudomonas fluorescence* @ 20 g/kg seed,
$T_4$: *Bacillus subtilis* @ 20 g/kg seed $T_5$: *Trichoderma viridae* @ 5 g/kg seed,
$T_6$: VAM @ 10 kg/ha $T_7$: Dithane M-45 @ 2 g/kg seed,
$T_8$: Carbendizem @ 2 g/kg seed,
$T_9$: Soybean *Rhizobium* @ 20 g/kg seed + *Bacillus subtilis* @ 20 g/kg seed + VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed
$T_{10}$: Soybean *Rhizobium* @ 20 g/kg seed + *Pseudomonas fluorescence* @ 20 g/kg seed +VAM @ 10 kg/ha + *Trichoderma viridae* @ 5 g/kg seed

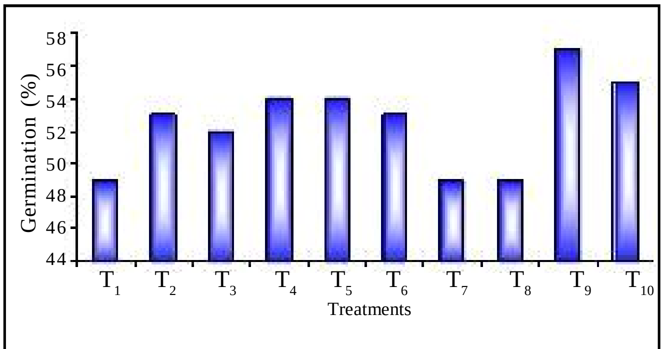


**Fig. 5: Effect of bioagents on germination, in soybean var. JS-335**

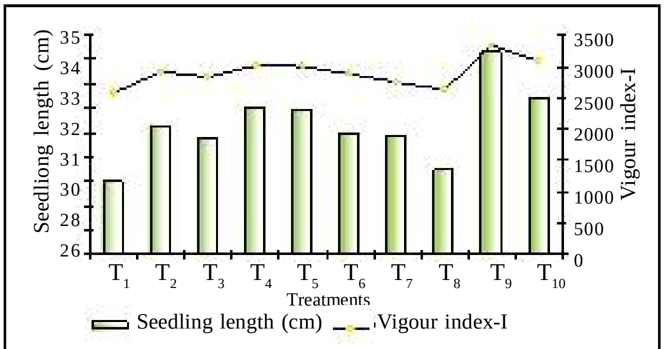


**Fig. 6: Effect of bioagents on seedling length, vigour index-I in soybean var. JS-335**

content (Table 2 and Fig. 4), germination (Table 2 and Fig. 5), seedling length (Table 2 and Fig. 6), seedling dry weight (Table 2), vigour index I (Table 2 and Fig. 6) and vigour index II (Table 2) after seed production differed significantly due to bioagents treatment. Maximum quality parameters was recorded in seeds obtained from $T_9$ (14.85g, 534 $dSm^{-1}$, 0.950, 41.73%, 97%, 34.3 cm, 58.8 mg, 3323 and 6676, respectively), while lowest was noticed in seeds of untreated plants (12.93g, 704 $dSm^{-1}$, 0.622, 35.85%, 89%, 29.0 cm, 46.3 mg, 2573 and 4997, respectively). Higher seed quality parameters in $T_9$ may be due to bioagents play important role in nitrogen and phosphorus mobilization and uptake, which were very much essential in synthesis of nucleic acid and proteins, they also reduce the infection of seed borne pathogen and they release plant growth promoters there by better diversion of photosynthates to seeds and better accumulation of food reserves in the seeds. Lower electrical conductivity may be due to high quality of seed produced by bioagents application and their involvement in the membrane integrity and there by reducing the E.C. values. The highest protein content may be due to the bioagents helped in nitrogen mobilization and in uptake, which results in synthesis of more protein than other treatments.

Higher germination percentage, vigour index-I and vigour index-II after accelerated ageing was recorded in harvested seeds obtained from seeds treated with soybean *Rhizobium* @ 20g/kg seed + *Bacillus subtilis* @ 20g/kg seed + VAM @ 10kg/ha + *Trichoderma viridae* @ 5g/kg seed (90%, 2513 and 4165, respectively) and was lowest in seeds obtained from untreated plants (69%, 1671 and 2315, respectively). Lowest is due to absorption of more moisture, due to its lower membrane integrity and higher respiration rate which resulted in faster deterioration of seeds in AA test (Table 3).

$T_9$ plots recorded higher number of bacteria and fungal colonies (42 x $10^6$ cfu/g soil and 16 x $10^3$ cfu/g soil) and was lowest in initial soil (11.3 x $10^6$ cfu/g soil and 3.3 x $10^3$ cfu/g soil). Untreated (8 x $10^4$ cfu/g soil) plot recorded higher number of actinomycete colonies, While lowest was recorded in Dithane M-45 @ 2g/kg seed and Carbendizem @ 2g/kg seed (1 x $10^4$ cfu/g soil) treated plots (Table 3). The highest number of bacterial colonies may be due to increased availability of root exudates, availability of nitrogen and phosphorus and synergistic effect of *Rhizobium*, *Bacillus subtilis*, VAM, *Trichoderma viridae* and *Pseudomonas fluorescence* on other bacteria in the crop rhizosphere. The highest number of fungi colonies may be due to increased availability of root exudates rich in carbohydrate and amino acids and synergistic effect of introduced microbial inoculants *viz.*, *Rhizobium*, *Bacillus subtilis,* VAM, *Trichoderma viridae* and *Pseudomonas fluorescence*. The highest number of Actinomycetes colonies may be due to there was less competition from bacterial and fungi colonies and less number due to the effect of chemicals which would have inhibited the Actinomycete colonies. $T_9$ also recorded higher net returns and benefit: cost ratio (Rs. 58244 and 4.5) (Table 3). While lowest was recoded in untreated (Rs. 35574 and 3.4).

The present findings showed that co-inoculation with soybean *Rhizobium*, *Bacillus subtilis*, *Trichoderma viridae* and VAM can interact positively in improving yield

**Table 3 : Effect of bioagents on seed quality parameters after accelerated ageing, soil microflora and economics of seed production in soybean var. JS-335**

| Treatments | Germination (%) | Vigour Index-I | Vigour Index-II | Bacteria (No of colonies X $10^6$ cfu/g soil) | Fungi (No of colonies X $10^3$ cfu/g soil) | Actinomycets (No of colonies X $10^4$ cfu/g soil) | Net profit (Rs) | Benefit: cost ratio |
|---|---|---|---|---|---|---|---|---|
| $T_1$ | 69 | 1671 | 2315 | 12 (3.46) | 4 (1.95) | 8 (2.81) | 35574 | 3.4 |
| $T_2$ | 85 | 2223 | 3510 | 31 (5.56) | 10 (3.15) | 6 (2.44) | 47764 | 4.2 |
| $T_3$ | 83 | 2140 | 3399 | 25 (5.00) | 5 (2.23) | 5 (2.24) | 41864 | 3.8 |
| $T_4$ | 86 | 2325 | 3661 | 35 (5.90) | 13 (3.60) | 3 (1.72) | 52439 | 4.5 |
| $T_5$ | 85 | 2285 | 3564 | 33 (5.74) | 12 (3.46) | 2 (1.41) | 48339 | 4.2 |
| $T_6$ | 83 | 2163 | 3448 | 28 (5.29) | 8 (2.82) | 4 (1.99) | 45124 | 3.7 |
| $T_7$ | 77 | 1991 | 3163 | 23 (4.79) | 2 (1.38) | 1 (1.00) | 41626 | 3.8 |
| $T_8$ | 76 | 1856 | 3024 | 20 (4.47) | 1 (1.00) | 1 (1.00) | 36111 | 3.4 |
| $T_9$ | 90 | 2513 | 4165 | 42 (6.48) | 16 (4.00) | 7 (2.63) | 58244 | 4.5 |
| $T_{10}$ | 88 | 2405 | 3884 | 38 (6.16) | 14 (3.74) | 5 (2.23) | 55069 | 4.3 |
| S.E. ± | 1.70 | 75.49 | 146.47 | 0.16 | 0.16 | 0.13 | - | - |
| C.D. (P=0.05) | 5.01 | 222.70 | 432.09 | 0.46 | 0.48 | 0.38 | - | - |
| CV (%) | 3.58 | 6.0615 | 7.43 | 5.09 | 10.39 | 11.32 | - | - |
| Initial | - | - | - | 11.3 | 3 | 6 | - | - |

Details of economics of soybean seed production:
Cost of seed @ Rs 35/kg
Cost of *Rhizobium*, *Pseudomonas fluorescence, Bacillus subtilis* and *Trichoderma viridae* @ Rs 75/kg
Cost of VAM @ Rs 150/kg
Cost of Dithane M-45 @ Rs 28/100g
Cost of Carbendizem @ 47/100g
Cost of Urea (N) @ Rs10.38/kg
Cost of single super phosphate (P) @ Rs 12.80/kg
Cost of Muriate of potash (K) @ Rs 7.40/kg
Price of seed @ Rs 25/kg
Note: Cost of inputs and out put of seed production is based on present market situation

parameters and post harvest seed quality parameters in soybean.

## REFERENCES

**Bunt, J.S. and Rovira, A.D.** (1955). Microbial studies of subflantic soils, *J. Soil Sci.*, **6**: p119

**Cubeta, M.A., Hartman, G.L. and Sinclair, J.B.** (1985). Interaction between *Bacillus subtilis* and fungi associated with soybean seeds. *Plant Disease*, **69**, 506–509.

**Hungria, M., Campo, R.J., Mendes, I.C. and Graham, P.H.** (2005). Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics, the success of soybean (*Glycine max* L. Merr.) in South America, In: *Nitrogen nutrition and sustainable plant productivity*. Singh, R.P. & Shankar Jaiwal, N.P.K. (eds.), Studium Press, LLC, Houston, Texas.

**ISTA.** (1996). International Rules for Seed Testing. *Seed Sci & Technol.*, **21** supplement, 1-228.

**Kuster, E. and Williams, S.T.** (1964). Selection of media for isolation of streptomyces. *Nature*, **202**: 926-929.

**Martin, J.P.** (1950). Use of acid rose Bengal and streptomycin in plate method for estimating soil fungi. *Soil Sci.*, **69**: 225-232.

**Perl, M., Luria, I. and Gelmond, H.** (1978). Biochemical changes in sorghum seed affected by accelerated ageing. *J. Expt. Bot.*, **29**: 497-509.