



Evaluation of Efficient Isolates of Fluorescent Pseudomonads for their Antagonistic Activity Against *Colletotrichum truncatum* of Soybean

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ABSTRACT

The fungal pathogen *Colletotrichum truncatum* causes anthracnose on soybean crop which results in yield reduction upto 30 per cent. The present investigation was undertaken on effect of fluorescent pseudomonads against *C. truncatum*. In soybean challenge inoculated with *C. truncatum*, the treatment T₃ (DFP54 + *C. truncatum*) recorded highest plant height, number of branches, nodule number, chlorophyll content, root, shoot and total dry weight, number of pods per plant, seeds per plant and highest seed weight per plant. Also recorded least anthracnose incidence (*C. truncatum*) of 11.90 and 13.87 PDI at 30 and 60 DAS respectively and maximum peroxidase activity (ISR activity) at 12 hr, 24 hr, 48 hr and at 72 hr after spray inoculation of pathogen (*C. truncatum*).

Keywords: Soybean, Anthracnose, *Colletotrichum truncatum* fluorescent pseudomonads, biocontrol

Received 19.06.2019

Revised 15.07.2019

Accepted 28.08.2019

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the important world's leading oilseed legume stands third among oilseed crops next only to groundnut and rapeseed. Due to its high nutritional properties it is also called as "Golden bean" and "poor man's meat". It is widely adapted to diverse environmental conditions hence this crop is also called as "miracle crop". Diseases play a major role in yield reduction in soybean. More than 100 pathogens are known to affect soybean of which 66 fungi, 6 bacteria, 8 viruses and 7 nematodes are involved [13]. The world loss of more than seven million tonnes per annum was reported only due to diseases alone [14]. Among them fungal pathogens are major production constraints in all soybean growing areas of India. This includes anthracnose (*Colletotrichum truncatum*) results in yield reduction upto 30 per cent.

Chemical fungicides which are used on crop plants are posing threat in the form of environment and health hazards and also fails to provide adequate control of the disease [4]. In modern era of organic farming the use of chemicals is being discouraged. Also the development of resistant pathogen populations against the chemicals has stimulated interest in the formulation of integrated disease control system. It is therefore, necessary to search for novel antifungal agents that are cost effective, nontoxic and that eliminate or reduce the incidence of soil-borne diseases of soybean.

Biological control of plant diseases opened an era of new technology to manage crop diseases and received the attention of researchers throughout the world, which will enhance the sustainability of agricultural production systems and to reduce the use of chemical pesticides. A few of the available biocontrol agents mostly belonging to *Pseudomonas* sp. show broad-spectrum antifungal activity by virtue of volatile and diffusible antibiotics [5].

MATERIAL AND METHODS

Five efficient fluorescent pseudomonads were selected based on their maximum per cent inhibition under *in vitro* condition. Pot experiments was conducted with challenge inoculation of *C. truncatum* along with appropriate control taking soybean as test crop.

Preparation and inoculation of selected fluorescent pseudomonads

The selected 5 fluorescent pseudomonad isolates were multiplied in King's B broth for 96 h at 30°C under shaking conditions (175 rpm). These broth cultures were diluted to maintain the population of 10^8 - 10^9 CFU/ml and applied @ 10 ml per pot just one day after sowing. Soybean seeds were treated with respective isolates at the rate of 10 gm per kg of seed, 10-15 min prior to sowing and for the chemical control treatment; the seeds were treated with Carboxin 37.5 % + Thiram 37.5 % at the rate of 4 g/kg of seeds.

Preparation and spray inoculation of foliar pathogen (*C. truncatum*)

Fifteen days old *C. truncatum* culture was multiplied in potato dextrose broth for 15 days at 30 °C under shaking conditions (175 rpm). This broth culture was diluted to maintain 10^5 spores per ml of broth and sprayed with pressure on foliage of 20 days old seedling of soybean by using a hand sprayer. Whole plant was covered with polythene covers for a day to create humidity for easy disease development. The spray was repeated for next two days to ensure maximum fungal infection.

Inoculation of selected fluorescent pseudomonads

Soybean seeds treated with respective isolates at the rate of 10 gm per kg of seed were used for sowing. Foliar spraying of isolate with a population of 10^8 - 10^9 CFU per ml of broth was done at 10 days after spray inoculation of foliar pathogen *i.e.* 30 DAS and second spray was done at 60 DAS and in chemical control, Hexaconazole @ 2 % was sprayed both at 30 and 60 DAS.

Observations were taken plant height (cm), Nodule number per plant, Chlorophyll content (SPAD), Number of branches, Shoot, root dry weight and total biomass accumulation (g/plant), Number of pods per plant. Number of seeds and Seed weight (g/plant).

Disease scoring

Observation on anthracnose disease caused by *Colletotricum truncatum* was recorded at 30 and 60 DAS. For recording per cent disease index, disease index 0 to 9 scale was used [2].

The observations were recorded from tagged plants and the percent disease index (%) was calculated as per the procedure given by Mayee and Datar [7].

$$\text{Per cent disease index (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total leaves observed in a set} \times \text{Max. grade}} \times 100$$

Assay of defense related enzymes

Assay of peroxidase (PO) activity

The peroxidase activity in fresh leaf samples was determined by following the method of Mahadevan and Sridhar [6]. Using pestle and mortar, acetone powder was extracted with 10 ml of 0.1 M phosphate buffer (pH 7.0).

Three ml buffer solution, 0.05 ml guaicol solution (0.04 %), 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide (H_2O_2) solution were taken into a cuvette. The initial absorbance was adjusted to zero at 436 nm in a UV-vis spectrophotometer. Later, the change in absorbance was recorded every 20 seconds after adding 0.5 ml of 2 per cent H_2O_2 and inverting the cuvette. The enzyme activity was expressed as change in optical density (ΔOD) per gram protein per minute.

Statistical analysis

The statistical analyzes of the data were carried out by employing completely randomized design (CRD). The critical differences were calculated at $P = 0.01$ for the *in-vitro* and pot culture experiments wherever F tests were significant and interpretation of the results was carried out in accordance with Pansey and Sukhatme [8].

RESULTS AND DISCUSSION

Plant height

All the treatments receiving foliar inoculation of different FP isolates showed significantly highest plant height over the pathogen alone at 30, 60 and 90 DAS (Table 01).

At 30 DAS, treatment T_3 (DFP54 + *C. truncatum*) recorded significantly highest plant height of 22.67 cm over all other treatments, which was followed by the treatment T_5 (DFP62 + *C. truncatum*) (19.33 cm). The treatments T_6 (Hexaconazole+*C. truncatum*), Lowest plant height was recorded in the T_7 (*C. truncatum* alone) (14.73 cm) and at 60 and 90 DAS significantly increased plant height (33.13 cm and 33.97 cm) was recorded in treatment T_3 (DFP54 + *C. truncatum*) and Lowest plant height of 25.62 cm and 28.32 cm was recorded in the T_7 (*C. truncatum* alone) at 60 and 90 DAS respectively.

Number of branches

At 60 and 90 DAS significantly more number of branches (7.50/plant and 10.40/plant) respectively were recorded in treatment T₃ (DFP54 + *C. truncatum*) over all other treatments, Pathogen sprayed treatment recorded lowest number of branches of 5.03/plant and 9.07/plant

Nodule number

With respect to nodule numbers, all the treatments recorded significantly more number of nodules /plant at 30, 60 and 90 DAS (Table 02).

At 30 and 60 DAS, significantly more number of nodules was recorded by the treatment T₁ (BFP22 + *C. truncatum*) (15/plant and 22.96/plant) respectively, Lowest nodules per plant (4.60/plant and 9.81/plant) were recorded in the treatment with pathogen (T₇).

At 90 DAS, significant differences were observed among the treatments. Significantly maximum number of nodules per plant was recorded by the treatment T₃ (DFP54 + *C. truncatum*) (29.03/plant), which was followed by the treatment T₁ (BFP22 + *C. truncatum*) (26.03/plant). Lowest number of nodules per plant (10.21/plant) was recorded in the treatment with pathogen (T₇).

Chlorophyll content (SPAD reading)

Treatment with foliar inoculation of different FP isolates had significant influence on chlorophyll content of leaves at both 45 and 60 DAS (Table 02).

At 45 and 60 DAS, T₃ (DFP54 + *C. truncatum*) recorded significantly higher chlorophyll content in leaves i.e. 48.60 and 40.05 SPAD value respectively (SPAD values), which is on par with T₅ (DFP62 + *C. truncatum*) (48.43 and 39.73 SPAD values respectively), Lowest chlorophyll content of 44.25 and 34.45 SPAD value respectively was recorded in T₇ (*C. truncatum* alone).

Number of pods/plant

Treatments with foliar inoculation of FP isolates also had a significant influence on number of pods per plant at both 60 DAS and at harvest over the pathogen alone inoculated treatment (Table 02).

At 60 DAS, among the treatments T₃ (DFP54 + *C. truncatum*) recorded more number of pods (17.87/plant) and it was significantly superior over all other treatments. It was followed by the treatment T₂ (BFP42 + *C. truncatum*) (14.90/plant) which is on par with T₅ (DFP62 + *C. truncatum*) (14.87/plant). Lowest number of pods (10.90/plant) was recorded in T₇ (*C. truncatum* alone). At 90 DAS, same trend was followed.

Table 01: Effect of efficient fluorescent pseudomonad isolates on plant height and number of branches of soybean spray inoculated with *C. truncatum*

| Treatments | Plant height (cm) | | | No. of branches/plant | |
|--|-------------------|-------------|-------------|-----------------------|-------------|
| | 30 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| T ₁ : BFP22 + <i>C. truncatum</i> | 15.27 | 25.98 | 29.50 | 6.10 | 9.80 |
| T ₂ : BFP42 + <i>C. truncatum</i> | 16.35 | 28.47 | 28.67 | 6.87 | 9.43 |
| T ₃ : DFP54 + <i>C. truncatum</i> | 22.67 | 33.13 | 33.97 | 7.50 | 10.40 |
| T ₄ : DFP56 + <i>C. truncatum</i> | 16.43 | 28.52 | 32.07 | 6.20 | 9.87 |
| T ₅ : DFP62 + <i>C. truncatum</i> | 19.33 | 30.48 | 33.70 | 5.90 | 10.07 |
| T ₆ : Hexaconazole+ <i>C. truncatum</i> | 17.27 | 29.45 | 29.88 | 5.50 | 9.93 |
| T ₇ : <i>C. truncatum</i> alone | 14.73 | 25.62 | 28.32 | 5.03 | 9.07 |
| T ₈ : Absolute control | 16.37 | 27.23 | 28.55 | 5.43 | 10.03 |
| S.Em. ± | 0.49 | 0.49 | 0.50 | 0.08 | 0.08 |
| C.D. @ 1 % | 1.92 | 1.88 | 1.95 | 0.33 | 0.34 |

Table 02: Effect of efficient fluorescent pseudomonad isolates on nodule number, chlorophyll content and number of pods of soybean spray inoculated with *C. truncatum*

| Treatments | Nodule number /plant | | | Chlorophyll content (SPAD reading) | | Number of pods/plant | |
|--|----------------------|-------------|-------------|------------------------------------|-------------|----------------------|-------------|
| | 30 DAS | 60 DAS | 90 DAS | 45 DAS | 60 DAS | 60 DAS | At harvest |
| T ₁ : BFP22 + <i>C. truncatum</i> | 15.00 | 22.96 | 26.03 | 48.02 | 39.47 | 11.83 | 12.90 |
| T ₂ : BFP42 + <i>C. truncatum</i> | 9.99 | 16.99 | 21.27 | 48.07 | 37.93 | 14.90 | 16.17 |
| T ₃ : DFP54 + <i>C. truncatum</i> | 11.20 | 18.09 | 29.03 | 48.60 | 40.05 | 17.87 | 19.50 |
| T ₄ : DFP56 + <i>C. truncatum</i> | 13.03 | 15.88 | 19.96 | 47.80 | 38.97 | 12.67 | 13.43 |
| T ₅ : DFP62 + <i>C. truncatum</i> | 10.18 | 16.92 | 19.08 | 48.43 | 39.73 | 14.87 | 16.37 |
| T ₆ : Hexaconazole+ <i>C. truncatum</i> | 9.91 | 11.02 | 15.50 | 47.22 | 39.25 | 12.47 | 13.20 |
| T ₇ : <i>C. truncatum</i> alone | 4.60 | 9.81 | 10.21 | 44.25 | 34.45 | 10.90 | 11.87 |
| T ₈ : Absolute control | 11.00 | 15.11 | 19.11 | 45.73 | 38.95 | 11.17 | 12.80 |
| S.Em. ± | 0.06 | 0.07 | 0.08 | 0.49 | 0.68 | 0.08 | 0.10 |
| C.D. @ 1 % | 0.25 | 0.28 | 0.33 | 1.91 | 2.60 | 0.32 | 0.42 |

Table 03: Peroxidase activity of selected fluorescent pseudomonad isolate and their effect on per cent disease incidence of *C. truncatum*

| Treatments | Per cent disease incidence | | Peroxidase activity(Δ OD/g protein/min)(Hours after spray inoculation with <i>C. truncatum</i>) | | | |
|---|----------------------------|----------------|--|-------------|-------------|-------------|
| | 15 DAS | 45 DAS | 12 | 24 | 48 | 72 |
| T ₁ : BFP22 + <i>C. truncatum</i> | 18.27 (25.29)* | 24.17 (29.43)* | 6.99 | 8.06 | 10.18 | 9.99 |
| T ₂ : BFP42 + <i>C. truncatum</i> | 15.29 (23.00) | 19.67 (26.31) | 7.23 | 8.42 | 12.29 | 11.97 |
| T ₃ : DFP54 + <i>C. truncatum</i> | 11.90 (20.17) | 13.87 (21.85) | 8.33 | 9.63 | 13.60 | 13.02 |
| T ₄ : DFP56 + <i>C. truncatum</i> | 15.80 (23.41) | 17.93 (25.04) | 7.07 | 8.55 | 11.33 | 10.96 |
| T ₅ : DFP62 + <i>C. truncatum</i> | 18.67 (25.59) | 23.10 (28.71) | 8.18 | 9.36 | 12.68 | 12.06 |
| T ₆ : Hexaconazole + <i>C. truncatum</i> | 02.80 (9.58) | 09.80 (18.29) | 6.66 | 8.26 | 12.04 | 11.29 |
| T ₇ : <i>C. truncatum</i> alone | 29.79 (33.06) | 45.10 (42.15) | 6.62 | 7.18 | 9.00 | 8.90 |
| T ₈ : Absolute control | 1.47 (6.95) | 2.30 (8.66) | 6.96 | 7.25 | 9.15 | 8.99 |
| S.Em. \pm | 0.39 | 0.44 | 0.14 | 0.11 | 0.05 | 0.13 |
| C.D. @ 1 % | 1.64 | 1.84 | 0.59 | 0.46 | 0.23 | 0.54 |

Table 04: Effect of efficient fluorescent pseudomonad isolates on root and shoot dryweight and total biomass accumulation of soybean spray inoculated with *C. truncatum*

| Treatments | Total biomass accumulation (g/plant) | | | | | | | | |
|--|--------------------------------------|-------------|-------------|--------------------------------|-------------|-------------|--------------------------------|-------------|-------------|
| | Root dry weight (g/plant) DAS | | | Shoot dry weight (g/plant) DAS | | | Total dry weight (g/plant) DAS | | |
| | 30 | 60 | At harvest | 30 | 60 | At harvest | 30 | 60 | At harvest |
| T ₁ : BFP22 + <i>C. truncatum</i> | 0.08 | 0.22 | 0.38 | 0.37 | 3.03 | 3.62 | 0.45 | 3.25 | 4.00 |
| T ₂ : BFP42 + <i>C. truncatum</i> | 0.07 | 0.21 | 0.41 | 0.49 | 3.11 | 4.04 | 0.56 | 3.32 | 4.45 |
| T ₃ : DFP54 + <i>C. truncatum</i> | 0.35 | 0.40 | 0.80 | 0.41 | 6.33 | 6.63 | 0.76 | 6.73 | 7.43 |
| T ₄ : DFP56 + <i>C. truncatum</i> | 0.09 | 0.21 | 0.39 | 0.64 | 3.89 | 4.30 | 0.73 | 4.10 | 4.69 |
| T ₅ : DFP62 + <i>C. truncatum</i> | 0.10 | 0.24 | 0.68 | 0.55 | 4.07 | 4.85 | 0.65 | 4.31 | 5.53 |
| T ₆ : Hexaconazole+ <i>C. truncatum</i> | 0.07 | 0.18 | 0.33 | 0.37 | 2.38 | 4.07 | 0.44 | 2.56 | 4.40 |
| T ₇ : <i>C. truncatum</i> alone | 0.06 | 0.17 | 0.24 | 0.36 | 2.35 | 2.60 | 0.42 | 2.52 | 2.84 |
| T ₈ : Absolute control | 0.11 | 0.20 | 0.35 | 0.43 | 3.55 | 3.06 | 0.54 | 3.75 | 3.41 |
| S.Em. \pm | 0.01 | 0.01 | 0.03 | 0.03 | 0.22 | 0.13 | 0.01 | 0.01 | 0.41 |
| C.D. @ 1 % | 0.05 | 0.04 | 0.10 | 0.09 | 0.84 | 0.50 | 0.03 | 0.03 | 1.69 |

Table 05: Effect of efficient fluorescent pseudomonad isolates on yield parameters of soybean spray inoculated with *C. truncatum*

| Treatments | Number of seeds/plant | Seed weight (g/plant) |
|---|-----------------------|-----------------------|
| T ₁ : BFP22 + <i>C. truncatum</i> | 45.47 | 5.30 |
| T ₂ : BFP42 + <i>C. truncatum</i> | 33.37 | 5.80 |
| T ₃ : DFP54 + <i>C. truncatum</i> | 55.77 | 9.83 |
| T ₄ : DFP56 + <i>C. truncatum</i> | 40.07 | 7.90 |
| T ₅ : DFP62 + <i>C. truncatum</i> | 26.73 | 6.67 |
| T ₆ : Hexaconazole + <i>C. truncatum</i> | 27.90 | 4.90 |
| T ₇ : <i>C. truncatum</i> alone | 19.90 | 4.03 |
| T ₈ : Absolute control | 26.87 | 4.80 |
| S.Em. \pm | 1.18 | 0.07 |
| C.D. @ 1 % | 4.96 | 0.28 |

Biocontrol potential of fluorescent pseudomonads against *C. truncatum*

A green house experiment was conducted to study the biocontrol potential of selected five efficient isolates against anthracnose of soybean caused by *Colletotrichum truncatum*. Among the At 15 days after spray inoculation (1st spray), treatment T₈ (Absolute control) recorded significantly lowest percent disease index (1.47 PDI), which was followed by the treatment T₆ (Hexaconazole+*C. truncatum*) (2.80) and T₃ (DFP54 + *C. truncatum*) (11.90). Maximum percent disease index (29.79 PDI) was recorded in T₇ (*C. truncatum* alone). At 45 days after spray inoculation of FP isolates T₃ (DFP54 + *C. truncatum*) recorded lowest PDI of 13.87 however treatment T₆ (Hexaconazole+*C. truncatum*) has recorded (9.80) lesser PDI than T₃ Maximum percent disease index (45.10 PDI) was recorded in T₇ (*C. truncatum* alone).

Induction of systemic resistance against *C. truncatum*

Peroxidase activity

The data presented in Table 03 indicated all the treatments showed significantly increased peroxidase activity i.e. Δ OD/g protein/min values in the leaves at 12, 24, 48, 72 hours after challenge spray inoculation of different FP isolates. Maximum peroxidase activity was observed in treatment T₃ (DFP54 + *C. truncatum*) (8.33 Δ OD/g protein/min), at 12 hours after spraying of pathogen. And same treatment has shown gradual increase in peroxidase activity at 24, 48 and 72 hours of 9.63, 13.60 and 13.02 Δ OD/g protein/min and least peroxidase activity was recorded in T₇ (*C. truncatum* alone) at all different intervals.

Total dry matter production

T₃ (DFP54 + *C. truncatum*) has recorded highest dry matter production of 0.76 g/plant, of 6.73 g/plant and 7.43 g/plant at 30, 60 DAS and at harvesting respectively and lowest dry matter production was recorded by T₇ (*C. truncatum* alone) (table 4)

Number of seeds per plant and seed weight (g/plant)

The Treatment T₃ (DFP54 + *C. truncatum*) recorded highest number of seeds per plant (55.77/plant) and seed weight 9.83 g/plant which is significantly superior over pathogen alone treatment which has recorded lowest number of seeds and seed weight of 19.90/plant and 4.03 g/plant respectively (Table 05).

Based on antagonistic activity against *C. truncatum* under *in vitro* condition, 5 potential antagonistic (BFP22, DFP62, DFP56, DFP54 and BFP42) isolates were further tested under green house experiment in sterile soil condition with challenge spray inoculation of *Colletotricum truncatum* followed by spraying of selected FP isolates one day after inoculation of pathogen. (refer: Priyanka and Geetagoudar latest article) The treatments T₈ (Absolute control) and T₆ (Hexaconazole + *C. truncatum*) recorded lowest PDI of anthracnose disease intensity caused by *C. truncatum*. Among FP inoculated treatments, T₃ (DFP54 + *C. truncatum*), T₂ (BFP42 + *C. truncatum*) and T₄ (DFP56 + *C. truncatum*) recorded least anthracnose disease intensity caused by *C. truncatum* at 30 and at 60 DAS. The isolates also recorded highest plant height, number of branches nodules, number of seed per plant, seed weight, root, shoot and total dry weight, and highest chlorophyll content.

The plant growth promotional ability in this case is also due to production of highest amount IAA and GA also to solubilize more P. Their biocontrol ability could be linked with triggered activity of defense molecules like peroxidase. These isolates recorded highest per cent inhibition (55.56 % to 74.36 %), and recorded highest peroxidase activity which ranges from 8.33 Δ OD/g protein/min to 13.02 Δ OD/g protein/min which may be directly involved in inhibiting pathogen growth. These multifunctional properties of the selected isolates strongly substantiate the performance of these isolates in terms of *in-vivo* biocontrol activity coupled with significant growth promotion in soybean. Similar results on biocontrol activity of the fluorescent pseudomonads coupled with plant growth promotional ability have been reported by a number of authors [3, 17, 10, 1, 11, 12, 15].

In agriculture, numerous studies have been reported on the antagonistic activity against mainly soil-borne plant pathogens, but only a few studies have investigated the antagonistic activity against *Colletotricum* spp. of different crops. Fluorescent pseudomonad was found to decrease significantly the incidence of *C. truncatum* which causes anthracnose severely [16]. All the selected FP's isolates has shown adverse effect on pathogen and helps in good plant health and increased plant growth in both directly and indirectly. The use of these biocontrol agents could be an economically feasible alternative to chemical biocides and environmental friendly in suppressing the anthracnose disease in biological control programs of soybean.

REFERENCES

1. Ahmadzadeh, M. and Sharifi, A., 2009. Evaluation of fluorescent pseudomonads for plant growth promotion, antifungal activity against *Rhizoctonia solanion* common bean and biocontrol potential. *Biol. Control*. 48(2) : 101-107.
2. Anonymous, 1997. Annual report, All India Co-coordinated Research Project on Soybean, UAS, Dharwad, p. 24.
3. Bhatia, S., Dubey, R. C. and Maheshwari, D. K., 2005. Enhancement of plant growth and suppression of collar rot of sunflower caused by *Sclerotium rolfsii* through fluorescent pseudomonads. *Indian Phytopathol.*, 58(1) : 17-24.
4. Hwang, S. F., Ahmed, H. U., Gossen, B., Kutche, H. R., Brandt, S. A. and Strelkov, S. E., 2009. Effect of crop rotation on soil pathogen population dynamics and canola seedling establishment. *Pl. Pathol. J.*, 8 : 106-12.
5. Krishna, K. G., Pande, S. and Podile, A. R., 2005. Biological control of collar root disease with broad-spectrum antifungal bacteria associated with groundnut. *Canadian J. Microbiol.*, 51 : 123-132.
6. Mahadevan, A. and Sridhar, R., 1986, Methods in Physiological Plant Pathology, Sivakami Publishers, Madras, pp. 103-108.

7. Mayee, C. D. and Datar, V. V., 1986. Phytopathometry, *Technical Bulletin-1 (Special Bulletin-3)*. Marathwada Agric. Uni., Parbhani, p. 95.
8. Pansey, V. S. and Sukhatme, P. V., 1985. Statistical methods for agricultural works. ICAR, New Delhi, pp. 152-155.
9. Rana, S., Sharma, R. and Kaur, M., 2015. Biochemical characterization of fluorescent *Pseudomonas* species isolated from normal and replant sites of apple orchards. *Int. J. Farm Sci.*, 5(3): 165-170.
10. Rini, C. R. and Sulochana, K. K., 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctoniasolani* and *Fusariumoxysporum* infecting tomato. *J. Trop. Agric.*, 45 : 21-28.
11. Saravanakumar, D., Harish, S., Loganathan, M., Vivekananthan, R., Rajendran, L., Raguchander, T. and Samiyappan, R., 2007. Rhizobacterial bioformulation for the effective management of *Macrophomina* root rot in mungbean. *Arch. Phytopathol. Pl. Prot.*, 40(5) : 323-337.
12. Siddiqui, Z. A., Shakeel, U. and Siddiqui, S., 2008. Biocontrol of wilt disease complex of pigeonpea by fluorescent pseudomonads and *Bacillus* spp. under pot and field conditions. *Acta Phytopathol. Entomol. Hung.*, 43 (1) : 77-92.
13. Sinclair, J. B., 1978. The seed borne nature of some soybean pathogens, the effect of *Phomopsis* Spp. and *Bacillus subtilis* on germination and their occurrence in soybean produced in Illinois. *Seed Sci. Technol.*, 6 : 957-964.
14. Sinclair, J. B., 1988. Anthracnose of soybean *In : Soybean diseases of North Central region* (Eds.) Wyllie, T. D and Suth, D. H. American phytopathological society, St. Paul, Minnesota, USA, p. 104.
15. Singh, R. and Sinha, A. P., 2009. Biological control of rice sheath blight with antagonistic bacteria. *Ann. Pl. Prot. Sci.*, 17(1) : 107-110.
16. Tripathi, M. and Johri, B. N., 2002. In vitro antagonistic potential of fluorescent pseudomonads and control of sheath blight of maize caused by *Rhizoctoniasolani*. *Indian J. Microbiol.*, 42(3) : 207-214.
17. Velusamy, P., Immanuel, J. E., Gnanamanickam, S. S. and Thomashow, L., 2006. Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. *Canadian J. Microbiol.*, 52 : 56-65.