



# Microbial Interactions in the Management of Groundnut Stem Rot

K. Yamunarani <sup>a</sup>, S. Sangeetha <sup>a\*</sup>, K. Chitra <sup>b</sup>,  
K. Dhanalakshmi <sup>a</sup> and V. Dhanushkodi <sup>c</sup>

<sup>a</sup> Horticultural College and Research Institute for Women, Tiruchirappalli, Tamil Nadu, India.

<sup>b</sup> ICAR-Krishi Vigyan Kendra, Vellore, Tamil Nadu, India.

<sup>c</sup> ICAR-Krishi Vigyan Kendra, Thiruvavarur, Tamil Nadu, India.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop in the world belonging to the Leguminosae family. It is one of the essential food and cash crops of our country. In India, a large number of diseases attack groundnut [1]. Among soil borne diseases, stem rot or white mold caused by *Sclerotium rolfsii* Sacc. [2] is an important disease causing significant yield losses in several groundnut growing countries (Mehan et al., 1994). *Sclerotium rolfsii* Sacc. is a destructive soil borne fungal pathogen of oil seed crop in India. Different practices are recommended for management of groundnut stem rot such as deep summer ploughing, destruction of plant debris, crop rotation with jowar and bajra, seed treatment with carbendazim or captan or mancozeb or tebuconazole, soil drenching with hexaconazole, application of ammonium sulphate or calcium ammonium nitrate instead of urea and application of gypsum at flowering stage. Further, no single treatment is full proof and disease continues to cause losses in farmers' fields. Biological control offers an interesting alternative to fungicides for sustainable management of soil borne diseases. Biocontrol is a non-chemical measure, could be effective as chemical control by various

\*Corresponding author: E-mail: sangeetha.s@tnau.ac.in;

techniques. Among the techniques, the mixture of antagonists was studied to enhance the control efficacy. In most studies, the involvement of only mechanism of biological control is demonstrated. Involvement of more than one mechanism has been reported in only a few systems. Use of several biocontrol agents with several mechanisms of control fits in well with the concepts of integrated disease management, in which several means of disease suppression are applied concurrently. When single or more means of mechanisms are not effective, the others may compensate for the former absence. The present study, involved three major bacterial antagonists viz., *Streptomyces violaceusniger*, *Streptomyces exfoliatus* and *Pseudomonas fluorescens* to find out the effective dose of mixtures. The different doses from  $10^{-1}$  to  $10^{-5}$  were studied, the dose  $10^{-1}$  of *Streptomyces violaceusniger* have more efficiency of 86.70, than *S. exfoliatus*. The efficacy of *Streptomyces violaceusniger* with combination of other antagonists were tested and found that, the mixture of *Streptomyces violaceusniger* and *P. fluorescens* have synergistic activity than any other combinations which have synergistic factor greater than one.

**Keywords:** Groundnut; stem rot; *Streptomyces violaceusniger*; *Streptomyces exfoliatus*; *Pseudomonas fluorescens*.

## 1. INTRODUCTION

“Groundnut is cultivated worldwide in an area of 28 M ha with a total production of 47 Mt averaging a productivity of  $1.6 \text{ t ha}^{-1}$ ” [3]. “The leading producers of the groundnut crop include China (54%), India (22%), and USA (9.03%). In India, the crop is mostly grown in the states of Gujarat, Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Rajasthan and Maharashtra constituting about 80 percent of the total area and production of groundnut. In India, a large number of diseases attack groundnut” [1]. “Among many soil borne diseases, stem rot or white mold caused by *Sclerotium rolfsii* Sacc. is an important disease, causing significant yield losses in several groundnut growing countries” [4]. The pathogen attacks host plant during all stages when conditions are favourable [5] and yield losses over 25% have been reported [1]. “In most studies, the involvement of only one mechanism of biological control is demonstrated. Involvement of more than one mechanism has been reported in only a few systems. Use of several biocontrol agents with several mechanisms of control fits in well with the concepts of integrated disease management, in which several means of disease suppression are applied concurrently. When one or more means of mechanisms are not effective, the others may compensate for the former absence first to report the additive contribution of several biocontrol mechanisms to total disease suppression” [6]. “This work provides a theoretical explanation of reduced disease control variability by mixing *Pichia guillemontii* and *Bacillus mycoides* against *Botrytis cineria*. This is a novel approach to biological control that

may facilitate the more efficient use of this type of control on a larger commercial scale. Combinations of fungi and bacteria have also been shown to provide enhanced biocontrol. For instance, *Trichoderma koningii* combined with either *Pseudomonas chlororaphis* or *Pseudomonas fluorescens* provided greater suppression of take-all of wheat than *T. koningii* alone, *Trichoderma virens* GL-3 combined with *Burkholderia cepacia* provided greater protection than either antagonist used alone in the presence of a mixture of up to four soil-borne pathogens” [7]. “The non-pathogenic *Fusarium oxysporum* Fo47 combined with *Pseudomonas putida* WCS358 provided better suppression of *Fusarium* wilt of flax caused by *F. oxysporum* f. sp. *lini* than either alone” [8]. “Enhanced plant growth promotion has also been recorded in the absence of pathogens by applications of combinations of bacterial or fungal plant growth promoting microorganisms. For example, *Trichoderma aureoviride* Rifai inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* enhanced growth of *Citrus reshni* more than the *G. intraradices* used alone” [9]. “A commercial product consisting of a mixture of three *Bacillus subtilis* strains was used to control fungal soil pathogens after disinfection by seed-treating fungicides. These strains exhibit several biocontrol mechanisms, including production of antifungal compounds (including antibiotics and hydrogen cyanide), competition for ferric iron, competition for infection sites, and production of lytic enzymes” [10]. “A seed application of a combination of three PGPR, *Bacillus pumilus*, *Bacillus subtilis* and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on

cucumber than when any were inoculated singly” [11]. Combinations of *Paenibacillus* sp. and a *Streptomyces* sp. suppressed *Fusarium* wilt of cucumber better than when either was used alone [12] and a combination of *Pseudomonas fluorescens* and *Stenotrophomonas maltophilia* improved protection of sugar beet against *Pythium* damping off in comparison with either applied individually [13]. Combinations of fungi and bacteria have also been shown to provide enhanced biocontrol. Mixture of *Pichia guillemontii* and *Bacillus mycoides* resulted in additive activity compared with their separate application against *Botrytis cineria*. The combined activity was due to the summation of biocontrol mechanisms of both agents [6]. The present study, involved three major bacterial antagonists viz., *Streptomyces violaceusniger*, *Streptomyces exfoliatus* and *Pseudomonas fluorescens* to find out the effective dose of mixtures.

## 2. METHODOLOGY

In order to study the dose effect of individual antagonistic organism and also to find out the ED<sub>50</sub> and ED<sub>90</sub> value for individual antagonistic organism, different dilutions such as 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> were prepared and used for dual culture technique to find out the individual antagonistic effect at different doses. Then the antagonistic organisms viz., *Pseudomonas fluorescens* + *Streptomyces violaceusniger*, *Pseudomonas fluorescens* + *Streptomyces exfoliatus* and *Streptomyces violaceusniger* + *Streptomyces exfoliatus* were mixed together at different dilutions in order to study the combination dose response effect. Efficacy of mixture of *Streptomyces* spp. and *Pseudomonas fluorescens* against *S. rolfsii* was studied as follows.

### 2.1 Efficacy of Mixture of *Streptomyces* spp. and *Pseudomonas fluorescens* against *S. rolfsii*

The benefits of using a mixture of *Streptomyces* spp. and *Pseudomonas fluorescens* compared with separate application of each biocontrol agent were estimated in all experiments. Expected disease control and synergism of *Streptomyces* spp. control were calculated according to Abbott's formulas [14].

$$E(\text{exp}) = a + b - a \times b/100 \text{ and } SF = E(\text{obs})/E(\text{exp}),$$

where

a = control efficacy by *Streptomyces* spp. when applied alone

b = control efficacy by *Pseudomonas fluorescens* when applied alone

E(exp) = expected control efficacy by the mixture

E(obs) = observed control efficacy by the mixture

SF = the synergy factor achieved by the mixture.

When SF = 1, the interaction between the biocontrol agents is additive; when SF > 1, the interaction is synergistic and when SF < 1, the interaction is antagonistic [14].

## 2.2 Control Efficacy

Per cent control efficiency was determined for each treatment by measuring the diameter of mycelial growth in treated and untreated control (*D<sub>t</sub>* and *D<sub>c</sub>*, respectively), in the following formula: CE = 100 – (*D<sub>t</sub>*/*D<sub>c</sub>*) × 100.

## 3. RESULTS AND DISCUSSION

In present study, the dose effect of individual antagonistic organism studied by preparing individual antagonistic effect at different doses. Then the antagonistic organisms viz., *Pseudomonas fluorescens* + *S. violaceusniger*, *Pseudomonas fluorescens* + *Streptomyces exfoliatus* and *Streptomyces violaceusniger* + *Streptomyces exfoliatus* were mixed together at different dilutions in order to study the combination dose response effect. Effectiveness of antagonists against *S. rolfsii* was studied under *in vitro* condition and ED<sub>50</sub> and ED<sub>90</sub> were calculated. The ED<sub>50</sub> and ED<sub>90</sub> of *Streptomyces violaceusniger* values were 5.57 and 0.50 respectively. The *Streptomyces exfoliatus* ED<sub>50</sub> and ED<sub>90</sub> values were 4.56 and 1.16 respectively. The *Pseudomonas fluorescens* showed ED<sub>50</sub> of 4.85 and ED<sub>90</sub> of 0.45 (Table 1,2,3).

### 3.1 Efficacy of Mixture of *Streptomyces violaceusniger* and *Streptomyces exfoliatus* against *S. rolfsii*

The different dilutions of *Streptomyces violaceusniger* and *Streptomyces exfoliatus* were studied to find out the suitable combination to get higher efficacy. Among the 25 different dilution combinations, the most effective combination

was  $10^{-1}$  of each antagonist with synergistic factor of 0.88 (Table 4). Since the synergistic factor is less than one, the combination shows antagonistic effect.

**Table 1. Efficacy of *Streptomyces violaceusniger* at different dilutions against *Sclerotium rolfsii***

S. No	Dilution	Control efficiency (%)
1	$10^{-1}$	86.70
2	$10^{-2}$	70.00
3	$10^{-3}$	67.80
4	$10^{-4}$	65.60
5	$10^{-5}$	62.20
		ED <sub>50</sub> = 5.57
		ED <sub>90</sub> = 0.50

**Table 2. Efficacy of *Streptomyces exfoliatus* at different dilutions against *Sclerotium rolfsii***

S. No.	Dilution	Control efficiency
1	$10^{-1}$	75.5
2	$10^{-2}$	68.8
3	$10^{-3}$	66.6
4	$10^{-4}$	61.6
5	$10^{-5}$	57.7
		ED <sub>50</sub> = 4.56
		ED <sub>90</sub> = 1.16

**Table 3. Efficacy of *Pseudomonas fluorescens* at different dilutions against *Sclerotium rolfsii***

S. No.	Dilution	Control efficiency
1	$10^{-1}$	32.20
2	$10^{-2}$	27.40
3	$10^{-3}$	25.20
4	$10^{-4}$	15.80
5	$10^{-5}$	10.00
		ED <sub>50</sub> = 4.85

### 3.2 Efficacy of Mixture of *Streptomyces violaceusniger* and *Pseudomonas fluorescens* against *S. rolfsii*

Among the combinations of different dilutions ( $10^{-1}$  to  $10^{-5}$ ) of *Streptomyces violaceusniger* and  $10^{-1}$  to  $10^{-5}$  dilutions of, invariably all combinations were found to have synergistic activity. The most effective combination was  $10^{-2}$  of *Streptomyces violaceusniger*+ $10^{-5}$  of *P.*

*fluorescens*. (SF=1.12) (Table 5). Since the synergistic factor greater than one the combination shows synergistic effect.

### 3.3 Efficacy of Mixture of *Streptomyces exfoliatus* and *Pseudomonas fluorescens* against *S. rolfsii*

The combination of *Streptomyces exfoliatus* and *Pseudomonas fluorescens* was also found effective having synergistic effect in all dilution combinations with synergistic factor (SF=1.087) greater than one. The most effective combination was  $10^{-1}$  of *S. exfoliatus*+ $10^{-4}$  of *P. fluorescens* (Table 6).

In order to study the interactions among the host, pathogen and biocontrol agents, the dose response studies were carried out and results revealed that the antagonists *Streptomyces violaceusniger* recorded ED<sub>50</sub> and ED<sub>90</sub> values of 5.57 and 0.50 respectively followed by *Streptomyces exfoliatus* having ED<sub>50</sub> and ED<sub>90</sub> values of 1.16 and 4.56 respectively. In connection with this study, [15-17] were calculated the effective foliar spray containing  $10^7$  to  $10^8$  *Trichoderma* conidia/ml necessary to suppress *Botrytis cinerea*. The mixture of *Streptomyces violaceusniger* and *Streptomyces exfoliatus* was evaluated for its efficacy, the results shows that it was not effective against *S. rolfsii* probably because of low synergistic factor (0.077 to 0.857). Similar result was also obtained by [18] reported combinations of *Paenibacillus* sp. and a *Streptomyces* sp. suppressed *Fusarium* wilt of cucumber better than when either was used alone. Hervas et al. [19] found that combination of *Bacillus subtilis* and non-pathogenic *Fusarium oxysporum* did not provide control of *Fusarium* wilt whereas either applied alone did. The efficacy of mixture *Streptomyces violaceusniger* and *Pseudomonas fluorescens* was studied with different concentration ( $10^{-1}$  to  $10^{-5}$ ). The positive synergistic factor of 1.08 was observed in this study. Since the combination of *Streptomyces* spp. and *Trichoderma viride* were shown antagonistic effect, the *Trichoderma viride* was not taken for combination studies [Fig. 1]. Leona indicated that seed treatment or soil application of powder formulations of all the three strains of *Streptomyces* sp. effectively reduced the incidence of stem rot under greenhouse [20,21].

Table 4. Efficacy of mixture of *Streptomyces violaceusniger* and *Streptomyces exfoliatus* against *S.rolfsii*

S. No.	<i>Streptomyces violaceusniger</i> dilution	<i>Streptomyces exfoliatus</i> dilution	CE of <i>Streptomyces violaceusniger</i>	CE of <i>Streptomyces exfoliatus</i>	Efficacy (EXP)	Efficacy(OBS)	SF
1	-1	-1	82.2	75.5	95.63	84.40	0.882485
2	-1	-2	82.2	68.8	94.44	77.70	0.822689
3	-1	-3	82.2	66.6	94.05	76.60	0.814419
4	-1	-4	82.2	61.1	93.07	76.60	0.822985
5	-1	-5	82.2	57.7	92.47	72.20	0.780789
6	-2	-1	70.0	75.5	92.65	77.70	0.83864
7	-2	-2	70.0	68.8	90.64	77.70	0.857237
8	-2	-3	70.0	66.6	89.98	74.40	0.82685
9	-2	-4	70.0	61.1	88.33	71.10	0.804936
10	-2	-5	70.0	57.7	87.31	66.60	0.762799
11	-3	-1	67.8	75.5	92.11	75.50	0.819663
12	-3	-2	67.8	68.8	89.95	75.50	0.839322
13	-3	-3	67.8	66.6	89.24	72.20	0.809007
14	-3	-4	67.8	61.1	87.47	71.10	0.812811
15	-3	-5	67.8	57.7	86.37	70.60	0.817325
16	-4	-1	65.6	75.5	91.57	73.30	0.800463
17	-4	-2	65.6	68.8	89.26	73.30	0.82113
18	-4	-3	65.6	66.6	88.51	68.80	0.77731
19	-4	-4	65.6	61.1	86.61	67.70	0.781589
20	-4	-5	65.6	57.7	85.44	6.66	0.077941
21	-5	-1	62.2	75.5	90.73	73.30	0.807811
22	-5	-2	62.2	68.8	88.20	72.30	0.819668
23	-5	-3	62.2	66.6	87.37	65.50	0.749644
24	-5	-4	62.2	61.1	85.29	62.20	0.729227
25	-5	-5	62.2	57.7	84.01	61.10	0.727289

Table 5. Efficacy of mixture of *S.violaceusniger* and *Pseudomonas fluorescens* against *S. rolfsii*

S. No.	<i>Streptomyces violaceusniger</i> dilution	<i>Pseudomonas fluorescens</i> dilution	CE of <i>Streptomyces violaceusniger</i>	CE of <i>Pseudomonas fluorescens</i>	Efficacy (EXP)	Efficacy (O)	SF
1	-1	-1	82.2	32.2	87.93	88.5	1.006464
2	-1	-2	82.2	27.4	87.07	88.2	1.012894
3	-1	-3	82.2	25.2	86.68	86.3	0.995552
4	-1	-4	82.2	15.8	85.01	86.0	1.011617
5	-1	-5	82.2	10.0	83.98	84.9	1.010955
6	-2	-1	70.0	32.2	79.66	87.8	1.102184
7	-2	-2	70.0	27.4	78.22	86.3	1.103298
8	-2	-3	70.0	25.2	77.56	84.1	1.084322
9	-2	-4	70.0	15.8	74.74	83.3	1.11453
10	-2	-5	70.0	10.0	73.00	81.9	1.121918
11	-3	-1	67.8	32.2	78.16	80.0	1.023431
12	-3	-2	67.8	27.4	76.62	79.3	1.03494
13	-3	-3	67.8	25.2	75.91	78.9	1.039329
14	-3	-4	67.8	15.8	72.88	78.6	1.078373
15	-3	-5	67.8	10.0	71.02	77.7	1.094058
16	-4	-1	65.6	32.2	76.67	72.7	0.948136
17	-4	-2	65.6	27.4	75.02	71.9	0.95834
18	-4	-3	65.6	25.2	74.26	69.3	0.933097
19	-4	-4	65.6	15.8	71.03	68.5	0.964311
20	-4	-5	65.6	10.0	69.04	68.3	0.989282
21	-5	-1	62.2	32.2	74.37	70.3	0.945253
22	-5	-2	62.2	27.4	72.55	69.6	0.959243
23	-5	-3	62.2	25.2	71.72	68.5	0.955029
24	-5	-4	62.2	15.8	68.17	66.7	0.978402
25	-5	-5	62.2	10.0	65.98	66.7	1.010912

Table 6. Efficacy of mixture of *Streptomyces exfoliatus* and *Pseudomonas fluorescens* against *S.rolfsii*

S.No	<i>Streptomyces exfoliatus</i> dilution	<i>Pseudomonas fluorescens</i> dilution	CE of <i>Streptomyces exfoliatus</i>	CE of <i>Pseudomonas fluorescens</i>	Efficacy (EXP)	Efficacy (OBS)	SF
1	-1	-1	75.5	32.2	83.38	88.2	1.057693
2	-1	-2	75.5	27.4	82.21	87.8	1.067958
3	-1	-3	75.5	25.2	81.67	87.8	1.075006
4	-1	-4	75.5	15.8	79.37	86.3	1.087299
5	-1	-5	75.5	10.1	77.97	84.1	1.078558
6	-2	-1	68.8	32.2	78.84	81.9	1.038728
7	-2	-2	68.8	27.4	77.34	81.4	1.052376
8	-2	-3	68.8	25.2	76.66	79.7	1.039623
9	-2	-4	68.8	15.8	73.72	78.5	1.064701
10	-2	-5	68.8	10.1	71.95	77.8	1.081288
11	-3	-1	66.6	32.2	77.35	77.4	1.000584
12	-3	-2	66.6	27.4	75.75	75.2	0.992718
13	-3	-3	66.6	25.2	75.01	74.9	0.998443
14	-3	-4	66.6	15.8	71.87	73.0	1.015621
15	-3	-5	66.6	10.1	69.97	71.1	1.0161
16	-4	-1	61.1	32.2	73.62	73.0	0.9915
17	-4	-2	61.1	27.4	71.75	72.2	1.006151
18	-4	-3	61.1	25.2	70.90	70.8	0.99855
19	-4	-4	61.1	15.8	67.24	69.8	1.037977
20	-4	-5	61.1	10.1	65.02	69.4	1.067218
21	-5	-1	57.7	32.2	71.32	71.3	0.999711
22	-5	-2	57.7	27.4	69.29	71.1	1.026119
23	-5	-3	57.7	25.2	68.35	69.6	1.018145
24	-5	-4	57.7	15.8	64.38	58.6	0.910172
25	-5	-5	57.7	10.1	61.97	52.8	0.851994

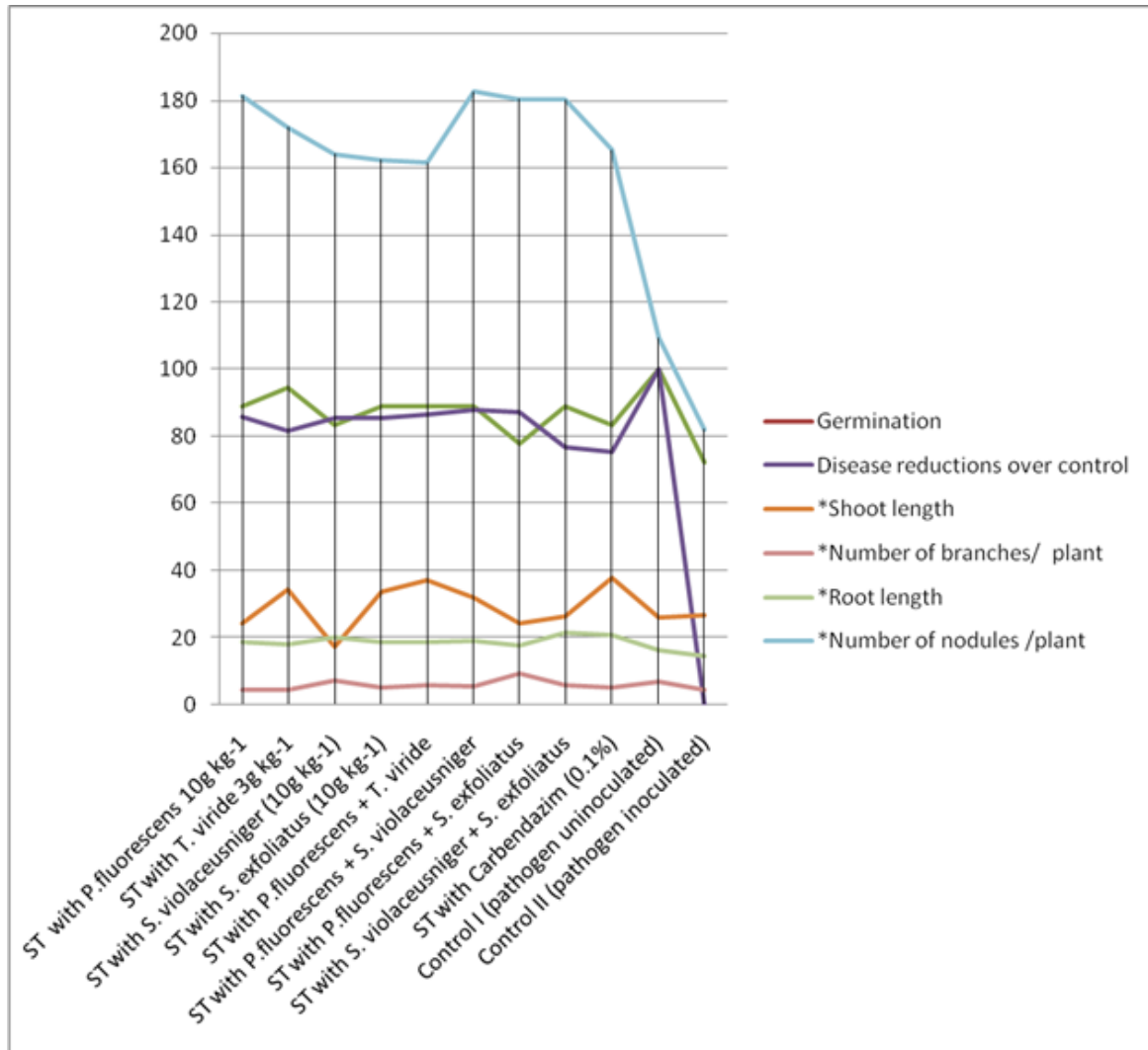


Fig.1 Effect of antagonists and their combination on stem rot incidence and growth parameter of groundnut in Pot culture

#### 4. CONCLUSION

The present study, involved three major bacterial antagonists viz., *Streptomyces violaceusniger*, *Streptomyces exfoliatus* and *Pseudomonas fluorescens* to find out the effective dose of mixtures. The different doses from  $10^{-1}$  to  $10^{-5}$  were studied, the dose  $10^{-1}$  of *Streptomyces violaceusniger* was found more efficiency of 86.70, than *S. exfoliatus*. The efficacy of *Streptomyces violaceusniger* with combination of other antagonists were tested and found that, the mixture of *Streptomyces violaceusniger* and *P. fluorescens* have synergistic activity than any other combinations which having synergistic factor greater than one. A new biocontrol combination was explored for the ecofriendly management of groundnut stem rot pathogen.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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