*Eco. Env. & Cons. 30 (October Suppl. Issue) : 2024; pp. (S394-S401)*
*Copyright@ EM International*
*ISSN 0971–765X*

**DOI No. http://doi.org/10.53550/EEC.2024.v30i06s.058**

# Sustainable management of Stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. by bioinoculants fortified with humic acid

**J. Evanjalin and D. John Christopher**

***Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India***

(Received 20 April, 2024; Accepted 24 June, 2024)

**ABSTRACT**

Groundnut (*Arachis hypogaea* L.) is considered as an important edible leguminous oilseed crop belongs to family Fabaceae. The cultivation of groundnut is hindered by several biotic and abiotic stresses. Stem rot disease incited by *Sclerotium rolfsii* Sacc. is one of the most destructive diseases of peanut and it deteriorates the quality and quantity of peanut. Management of the stem rot was found to be difficult, uneconomical and harmful for the environment. Keeping in view of the residual nature of fungicides on the environment, the idea of bioinoculants was undertaken. Biological control offers a sustainable management of soil borne diseases.The present study was undertaken to find out the efficacy of bioinoculants such as *Streptomyces, Rhizobium* and Bacillus alone and in combined effect of bioinoculants fortified with Humic acid against stem rot incidences. The groundnut plants were treated in combined application of *Streptomyces albofaciens*, B. *japonicum* and *Bacillus subtilis* fortified with humic acid as seed treatment @ 10g/kg of seeds plus soil application @ 2.5 kg $ha^{-1}$(T6) recorded minimum diseases incidence and significantly enhanced the yield parameters.

***Key words:*** *Groundnut, Stem rot disease, Bioinoculants, Humic acid*

## Introduction

Groundnut (*Arachis hypogeae* L.) is commonly called the poor man's nut. It is a leguminous crop plant which is widely cultivated in between 40°N and 40°S latitudes regions of the world and also considered as "king of oil seed crops". India ranks first position in terms of area with 5.75 million hectares, with production of 10.11 million tones and productivity of 1759 kg/ha. (DOES-MOAFW, 2022). In India, the major groundnut growing states are Gujarat, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh and Rajasthan. In Tamil Nadu groundnut is grown in 0.37 million hectares, with production of 0.95 million tones and productivity is 2553 kg/ha. (DOES-MOAFW, 2022). In India groundnut production is affected by various biotic and abiotic factors viz., fungi, bacteria, viruses, lack of resistance to drought, salinity and temperature (El-Sherbeny *et al.,* 2020). Among many soil borne diseases, stem rot or white mould caused by *Sclerotium rolfsii* Sacc (Saccardo, 1911) is an important disease-causing significant yield loss in several groundnut growing countries. The average yield losses due to stem rot disease range from 10 to 40% and it goes up to 80% in severe conditions (Baskey *et al*., 2020).

The undesirable changes to the environment and

([1]Ph. D Scholar, [2]Professor)

the toxic effects on non- target organism due to persistent, injudicious use of chemicals has necessitated to find out new innovative and indigenous technologies to manage the stem rot diseases of groundnut. One of the emerging strategies for managing plant disease is the use of microbial antagonists combined with bio stimulants. Therefore, with the knowledge of the antagonistic potential of bio inoculants Viz., Actinobacteria, Rhizobia, *Bacillus sp* the present study was undertaken to investigate the synergistic effect of antagonistic bio inoculants fortified withHumic acid - as a novel approach for the management of the stem rot disease of groundnut.

## Materials and Methods

### Identification, Isolation and morphological characterization of *Sclerotium rolfsii*

The fungus *Sclerotium rolfsii*, was isolated from stem rot infected plants collected from different groundnut growing districts of Tamil Nadu. The pathogenic isolates of *S. rolfsii* were designated as (Sr1 to Sr20) respectively and maintained on potato dextrose agar (PDA) plates incubated at 25 $^0$C in a BOD incubated for five days. Each isolate colony's features, morphology, sclerotia development, size, shape colour was examined. The size of sclerotial bodies, were measured, and colour were assigned using the Mycological colour chart (Rayner 1970).

### Pathogenicity proving and molecular characterization of the fungal pathogen

The efficacy of twenty isolates of pathogenic fungi *S. rolfsii*were tested bypathogenicity proving study and the isolate $Sr_4$ were found to be effective in causing the disease symptom inpot cultural condition. Hence *Sclerotium rolfsii* were used for further studies. From theresult of the pathogenicity test the virulent isolate of pathogen Sr4 were subjected to molecular confirmation through ITS region sequencing, the sequence thus obtained was analysed by using the BLAST analysistool of the NCBI database. Based on the BLAST search, isolate $Sr_4$ was confirmed as *Athelia rolfsii*. The sequences analysed were deposited in the GenBank database and obtained the accession number bearing OR743927.

### Isolation of bioinoculants

### Actinomycetes

Soil sample were collected from rhizosphere soils of healthy groundnut plants collected from different groundnut growing areas of Tamil Nadu. Soil samples were suspended in sterile water (10 g in 100 ml) and agitated for 45 min at 160 rpm. The supernatants were serially diluted upto $10^{-6}$. One ml of suspension drawn from each $10^{-3}$ to $10^{-6}$ dilutions and transferred to sterile petri plate containing Actinomycetes isolation agar. The plates had been incubated at 30 $^0$C for 7 days. Colonies of actinomycetes on agar plates were picked up on the basis of their morphological characteristic and purified by single hyphal tip method (Adhilakshmi *et al.,* 2013). The isolates were identified as *Streptomyces sp*.by referring to the Bergey's Manual of Systematic Bacteriology.

### Isolation of Rhizobium from root nodules of groundnut

Groundnut plants were uprooted carefully so that intact roots could be obtained. Healthy groundnut nodules were detached from the root. The detached root nodules were washed in tap water to remove the adhering soil particles from nodule surface. Fresh root nodules from groundnut were collected and surface sterilized with 70% ethanol and 0.1% mercuric chloride and washed thrice with sterile distilled water. Root nodules were crushed in saline solution. Rhizobium was isolated by means of spreading 0.1 ml overwhelmed root nodule suspension on YEM (Yeast extract mannitol, pH.7.0) agar plate and incubated at 36 °C. Rhizobium colonies observed in 2-3 days (Vrutuja Naik and Praveen Rahi, 2022).

### Isolation of Bacillus

Ten Bacillus isolates were isolated from different groundnut growing areas by serial dilution technique. 1 g of soil was taken in a test tube containing 9 ml of distilled water. The sample was mixed thoroughly by shaking the flask on a rotatory shaker for 5 min and serial dilutions of soil suspensions were prepared. Then one ml of respective dilutions was spread on sterilized petri plates containing Nutrient agar. The inoculated plates were incubated at 30 ± 2 $^o$C for 2 days. Rough and abundant colonies with waxy growth and irregular spreading edge were obtained on the Petri dishes. After the incubation, dominant bacterial colony was purified on same nutrient agar and kept at 4$^o$C for further studies

**Molecular characterization of bioinoculants**

The isolates of bioinoculants (*Streptomyces* spp, *Rhizobium*, spp, *Bacillus* spp) were tested for their efficacy to inhibit mycelial growth of *S. rolfsii*in vitro by dual culture technique. The strain which showed the maximum percentage of inhibition of mycelial growth of pathogen was considered as virulent strain. The bioinoculants subjected to molecular confirmation through ITS region sequencing. The sequence thus obtained was analysed by using the BLAST analysis tool of the NCBI database. Based on the BLAST search, bioinoculants were confirmed. The sequences analysed were deposited in the Gen Bank database and the accession number is obtained which are as follows.

| Isolate | Organism | Accession number |
|---|---|---|
| So 2 | S*treptomyces albofaciens* | OR740535 |
| Rz 5 | *Bradyrhizobium japonicum* | OR740541 |
| Bs 3 | *Bacillus subtilis* | PP493926 |

**Preparation of talc-based formulation of bioinoculants and fortification with humic acid**

Virulent bacterial bioinoculants (*Streptomyces* spp, *Rhizobium*, spp, *Bacillus* spp) were grown in Ken knight & Munaier's broth, YEMA broth and NA broth respectively @ 28 ± 2 ºC for 48 hours.One hundred grams of carrier material (Talcpowder) was taken and the pH was adjusted to 7 by adding CaCO3 at the rate of 15g/kg.Carboxy methyl cellulose (CMC) was thenadded at the rate of 10g/kg and mixed well.The mixtures were then autoclaved for 30 minat 121 ºC (15lb/inch$^2$). After autoclaving, 400 ml of bioinoculants suspension ($1\times10^8$ cfu/ml) was added to the sterilized carrier material (1kg) and thoroughly mixed followed by drying aseptically and then grounded to powder. Humic acid powder was obtained from ARVEEBIOTECH, Chidambaram. The talc-based formulation of bioinoculants were mixed with Humic acid powder @ 100g/ kg then packed in sterile polythenebags and stored at 4°C (Vidhyasekaran and Muthamilan, 1995).

The concentration ofcolony-forming units were obtained using theformula:

$$\text{Number of cfu/g} = \frac{\text{Number of colonies}}{\text{Amount of sample plated x Dilution}} \times 100$$

**Pot culture experiment**

A pot culture study was conducted to test the antagonists of bioinoculants fortified with humic acid for their efficacy in vivo with sterilized garden land soil in completely randomized block design with nine treatments and three replications each at Department of Plant Pathology, Annamalai University, Annamalai Nagar fromOctober 2022 to March 2023. Plastic pots of size 25 x 22x 15 cm filled with 25 kg of red loamy soil and TMV-7 seeds were used for this study. The plants in the pots have been maintained with uniform, regular and judicious watering. The combined application of Humic acid (HA) fortified bioinoculants (*Streptomyces albofaciens, Bradyrhizobium japonium, Bacillus sublitis*,) and chemical Nativo 75 WP were tested against *Sclerotium rolfsii*.The chemical Nativo75 WP @ 1g/Kg of seeds as seed treatment on TOS is standard chemical check. Mass multiplication of *Sclerotium rolfsii* is grown in sand maize medium and 20 days old culture were inoculated in 30 days old plants. The inoculated plants were incubated in a humid chamber for 48 h and subsequently moved to the greenhouse and it is maintained at 22-28 $^0$C, 70-90% relative humidity. Under a light intensity of 85 µmol $m^{-1}$ $S^{-1}$, 12 h photoperiod and subsequently transfer to pot culture yard. The treatment schedules were designed on the basis of the above phenomena.

**Treatment schedule**

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 50g/ pot on TOS, T2- Seed treatment with *B. japonicum* at @ 10 g/ kg of seed + Soil application of *B. japonicum* @ 50 g /pot on TOS, T3- Seed treatment of *Bacillus subtilis* @ 10g/kg of seeds + Soil application of *Bacillus subtilis* @ 50g/ pot on TOS+, T4 - T1+ T2, T5 - T3 + T4, T6 - T5 + Fortification with humic acid @ 100g/ kg of bio inoculant as soil application on TOS , T7 - Seed treatment with Nativo 75 WP @ 1g/Kg of seeds, T8- Healthy control, T9- Inoculated control .

**Assessment of the disease severity in the field**

Twelve plants from each plot were randomly selected and tagged for grading the severity of diseases. The severity of *S. rolfsii* was measured as per the standard evaluation system (SES) for groundnut. The disease severity was recorded at 30, 60, 90, 105 DAS and per cent diseases index was determined us usual.

### Field trial

The field trials were conducted at Kille, Cuddalore district of Tamil Nadu during May 2023 to August 2023, in field with a history of Stem rotincidence. The trial was laid out in plots (5x4 m) arranged in randomized block design. Groundnut seeds of cv. TMV-7 were sown in row/ plant spacing of 30 x 10 cm. Three replicate plots were maintained for each treatment. Regular cultivation practices have been accompanied asaccording to the recommendation. Treatment application details and experimental observation were the same as in greenhouse experiment.

### Treatment schedule

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 2.5 kg $ha^{-1}$ on TOS , T2 - Seed treatment with *B .japonicum* at @ 10 g/ kg of seed + Soil application of *B .japonicum* @ 2.5 kg $ha^{-1}$ on TOS , T3- Seed treatment with *Bacillus subtilis* @ 10g/kg of seeds + Soil application of *Bacillus subtilis* @ 2.5 kg $ha^{-1}$ on TOS , T4 - T1+ T2, T5 -T3 + T4, T6 - T5 + Fortification with humic acid @ 100g/ kg of bio inoculant as soil application on TOS, T7 - Seed treatment with Nativo 75 WP @ 1g/kg of seeds , T8- Untreated control.

### Data analysis

The data obtained from the studies conducted under laboratory and field conditions were subjected to the analysis of variance techniques (ANOVA) and were applied to completely randomized design (CRD) and randomized block design (RBD). The data obtained on per cent inhibition were transformed using angular (arc sine) transformation.

## Results

### Efficacy of combined applications of *Streptomyces albofaciens*, *B. japonicum* and *Bacillus subtilis* plus fortification with humic acid against stem rot incidence under pot culture and field trial

**Pot culture condition:** The pot culture experiment was carried out to evaluate the efficacy of combined application of bioinoculants fortified with Humic acid (HA) against root rot incidence of groundnut. The result revealed that all the bioinoculants were found to be effective in inhibiting the progress of disease development than the untreated control (Table 2). Among the various treatments, combined application of *Streptomyces albofaciens*, *B. japonicum* and *Bacillus subtilis* plus fortification with humic acid @ 100 g/ kg of bioinoculantsas seed treatment @ 10 g/kg of seed and soil application @ 100g/ pot (T6) recorded minimum stem rot disease incidence which recorded 5.86 %, 6.12%, 7.15%, 7.92% percent disease incidence on 30, 60, 90 and 105 DAS respectively, it is superior than the standard chemical check Nativo and it was followed bythe combined application of *Streptomyces albofaciens*, *B. japonicum* and *Bacillus subtilis* as seed treatment @ 10 g/kg of seed and soil application @ 50g/ pot(T5)recording theroot rot incidence of 6.38%, 7.02%, 8.53% and

**Table 1.** Evaluation of combined applications of bioinoculants fortification with humic acid on growth parameters and yield of groundnut under pot culture condition

| Name of the treatments | Germination (%) | Shoot length (cm) | Root length (cm) | Biomass (g/plant ) | Pod Yield (g/plant) |
|---|---|---|---|---|---|
| T1 | 90.45( 71.99) | 25.18 | 18.34 | 38.82 | 52.72 |
| T2 | 89.08( 70.70) | 23.69 | 17.65 | 35.47 | 48.86 |
| T3 | 87.72(69.48) | 22.56 | 16.36 | 30.05 | 45.25 |
| T4 | 91.48( 73.02) | 27.67 | 18.34 | 43.15 | 56.35 |
| T5 | 94.37(76.93) | 29.74 | 20.28 | 45.29 | 59.21 |
| T6 | 97.93( 81.72) | 34.89 | 22.71 | 48.07 | 63.79 |
| T7 | 92.89(74.53) | 28.43 | 19.42 | 44.56 | 57.35 |
| T8 | 82.06( 64.94) | 21.78 | 11.67 | 29.02 | 37.86 |
| T9 | 68.96( 56.14) | 17.60 | 9.42 | 25.46 | 20.74 |

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 50g/ pot, T2- Seed treatment with *B.japonicum* at @ 10 g/ kg of seed + Soil application of *B.japonicum* @ 50 g /pot, T3- Seed treatment of *Bacillus subtilis* @ 10g/kg of seeds + soil application of *Bacillus subtilis* @ 50g/ pot, T4 - T1+ T2, T5 - T3 + T4, T6 - T5 + Fortification with humic acid @ 100g/ kg as soil application @ 100g/pot, T7 - Seed treatment with Nativo 75 WP @ 1g/Kg of seeds, T8-Inoculated control, T9-.Healthy control.

9.13% percent disease incidence on 30,60, 90,105 DAS respectively and followed by standard chemical check Nativo 75 WP @ 1g/kg of seeds (T7) registered the root rot disease incidence of 6.92%, 7.22%, 9.10% and 10.11% percent disease incidence on 30, 60, 90, 105DAS respectively. All the bioinoculants treated plants significantly reduced the disease incidence as compared to control. Combined application of bioinoculants fortified with humic acid has reduced the disease incidence than the individual application of bioinoculants and standard chemical check.

**Field trial:** The same trend as pot culture was observed in field trial also. The minimum disease incidence was registered with combined application of *Streptomyces albofaciens, B.japonicum* and *Bacillus subtilis* plus fortification with humic acid @ 100g/ kgas seed treatment @ 10 g/kg of seed and soil application @ 2.5 Kg $ha^{-1}$ of each (T6) recorded minimum stem rot disease incidence which recorded 4.52%, 6.36%, 7.41% and 9.53% percent disease incidence on 30, 60, 90, 105 DAS respectively and followed bythe combined application of *Streptomyces albofaciens, B.japonicum* and *Bacillus subtilis* as seed treatment @ 10 g/kg of seed and soil application @ 2.5 kg/ha (T5) recording theroot rot incidence of 5.95%, 7.41%, 8.77% and 9.53% percent disease incidence on 30,60, 90, 105 DAS respectively and followed by plants treated with standard chemical check Nativo 75 WP as @ 1g/kg of seeds (T7) registered the root rot disease incidence of 6.44%, 8.23%, 9.93% and 11.87% percent disease incidence on 30, 60, 90, 105 DAS respectively. All the bioinoculants treated plants significantly reduced the disease incidence as compared to control. Combined application of bioinoculants fortified with humic acid has reduced the disease incidence than the individual application of bioinoculants and standard chemical check (Table 4).

**Evaluation of combined applications of *Streptomyces albofaciens, B. japonicum* and *Bacillus subtilis* plus fortification with humic acid ongrowth parameters and yield of groundnut under pot culture condition and field trial**

**Pot culture condition:** The results pot culture experiment revealed that among the nine treatments, the plants that are treated with combined application of *Streptomyces albofaciens, B. japonicum* and *Bacillus subtilis* plus fortification with humic acid @ 100g/ kg of bioinoculantsas seed treatment @ 10 g/ kg of seed and soil application @ 100g/ pot (T6) recorded maximum shoot length (34.89cm), root length (22.71cm), (48.07 g/plant) and pod yield (63.79 g/plant). It was followed by combined application of combined application of *Streptomyces*

**Table 2.** Evaluation of combined effect of bioinoculants fortified with Humic acid against stem rot of groundnut incited by *Sclerotium rolfsii* in pot culture condition

| Name of the treatments | Disease incidence (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 DAS | Percent disease reduction over control | 60 DAS | Percent disease reduction over control | 90 DAS | Percent disease reduction over control | 105 DAS | Percent disease reduction over control |
| T1 | 9.54(17.99) | 70.40 | 10.95(19.32) | 69.53 | 11.33(19.66) | 75.21 | 13.67(21.69) | 74.17 |
| T2 | 10.87(19.25) | 66.28 | 12.57( 20.76) | 65.02 | 13.69(21.71) | 70.05 | 14.96(22.75) | 71.74 |
| T3 | 12.01(20.27) | 62.74 | 13.68(21.64) | 61.93 | 15.75(23.38) | 65.55 | 16.51(23.97) | 68.81 |
| T4 | 7.98(16.40) | 75.24 | 9.21(17.66) | 74.37 | 10.41(18.82) | 77.23 | 11.87(20.15) | 77.57 |
| T5 | 6.38(14.63) | 80.21 | 7.02(15.36) | 80.30 | 8.53(16.98) | 81.12 | 9.13(17.58) | 82.86 |
| T6 | 5.86(14.09) | 81.82 | 6.12(14.32) | 82.97 | 7.15(15.50) | 84.36 | 7.92(16.65) | 85.03 |
| T7 | 6.92(15.25) | 78.53 | 7.92(16.34) | 77.96 | 9.10(17.55) | 80.97 | 10.11(18.53) | 81.56 |
| T8 | 32.24(34.59) | | 35.94(36.83) | | 45.72(42.54) | | 52.94(46.68) | |
| T9 | 21.65(27.723) | | 29.46(32.87) | | 32.13(34.52) | | 39.27(38.52 ) | |
| C.D. at 5 % | 0.80 | | 0.97 | | 1.15 | | 1.35 | |
| S. Em. ± | 0.26 | | 0.32 | | 0.38 | | 0.44 | |

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 50g/ pot, T2- Seed treatment with *B.japonicum* at @ 10 g/ kg of seed + Soil application of *B.japonicum* @ 50 g /pot, T3- Seed treatment of *Bacillus subtilis* @ 10g/kg of seeds + soil application of *Bacillus subtilis* @ 50g/ pot, T4 - T1+ T2, T5 - T3 + T4, T6 - T5 + Fortification with humic acid @ 100g/ kg as soil application @ 100g/pot, T7 - Seed treatment with Nativo 75 WP @ 1g/Kg of seeds, T8-Inoculated control, T9-.Healthy control.

*albofaciens, B. japonicum* and *Bacillus subtilis* as seed treatment @ 10 g/kg of seed and soil application @ 50g/ pot (T5) which registered shoot length ( 29.74 cm), root length ( 20.28 cm), biomass ( 45.29g/plant) pod yield ( 59.21g/ plant) and followed by standard chemical check Nativo 75 WP @ 1g/Kg of seeds ( T7) recording shoot length (28.43cm), root length (19.42cm), biomass ( 44.56g/plant), pod yield (57.35g/plant) (Table 1).

**Field trial:** The same trend as pot culture was observed in field trial also. The maximum growth and yield was observed withcombined application of *Streptomyces albofaciens, B.japonicum* and *Bacillus subtilis*plus fortification with humic acid @ 100g/ kg of bioinoculantsas seed treatment @ 10 g/kg of seed and soil application @ 2.5 kg $ha^{-1}$ (T6) which recorded maximum plant height ( 68.28 cm), biomass (45.09 g/plant), Number of pods ( 46.00 /plant), pod yield ( 1755 kg/ha) and followed by the combined application of *Streptomyces albofaciens, B. japonicum* and *Bacillus subtilis* as seed treatment @ 10 g/kg of seed and soil application @ 2.5 kg/ ha (T5)

**Table 3.** Evaluation of combined application of bioinoculants fortification with humic acid on growth parameters and yield of groundnut in fieldcondition

| Name of the treatments | Germination (%) | Plant height(cm) | Biomass (g/plant ) | No of pods/plant | Pod Yield (kg/ha) |
|---|---|---|---|---|---|
| T1 | 87.52(69.31) | 57.43 | 36.49 | 33.00 | 1524 |
| T2 | 85.62(67.71) | 52.36 | 30.42 | 32.00 | 1439 |
| T3 | 83.15(65.76) | 48.68 | 29.83 | 29.00 | 1298 |
| T4 | 89.31(70.91) | 61.89 | 41.20 | 34.00 | 1576 |
| T5 | 93.75(75.52) | 65.17 | 43.67 | 42.00 | 1703 |
| T6 | 95.21(77.35) | 68.28 | 45.09 | 46.00 | 1755 |
| T7 | 90.48(72.02) | 62.53 | 39.24 | 37.00 | 1688 |
| T8 | 78.03(62.04) | 34.88 | 28.81 | 23.00 | 942 |

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 2.5 kg $ha^{-1}$ on TOS , T2 - Seed treatment with *B .japonicum* at @ 10 g/ kg of seed + Soil application of *B .japonicum* @ 3 $ha^{-1}$ on TOS , T3- Seed treatment with *Bacillus subtilis* @ 10g/kg of seeds + Soil application of *Bacillus subtilis* @ 2.5 kg $ha^{-1}$ on TOS , T4 - T1+ T2, T5 -T3 + T4, T6 - T5 + Fortification humic acid @ 100g/ kg as soil application @ 20kg $ha^{-1}$ on TOS, T7 - Seed treatment with Nativo 75 WP @ 1g/kg of seeds , T8- Untreated control.

**Table 4.** Evaluation of combined effect of bioinoculants fortified with Humic acid against stem rot of groundnut incited by Sclerotium rolfsii in Field condition

| Name of the treatments | Disease incidence (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 DAS | Percent disease reduction over control | 60 DAS | Percent disease reduction over control | 90 DAS | Percent disease reduction over control | 105 DAS | Percent disease reduction over control |
| T1 | 7.52(15.91) | 72.97 | 9.63(18.07) | 71.38 | 11.73(20.02) | 67.85 | 13.34(21.42) | 66.09 |
| T2 | 8.12(16.55) | 70.82 | 10.67(19.06) | 68.29 | 12.69(20.86) | 65.22 | 14.98(21.77) | 61.92 |
| T3 | 10.97(19.34) | 60.58 | 13.84(21.84) | 58.87 | 15.57(23.24) | 57.33 | 17.69(24.87) | 55.03 |
| T4 | 6.96(15.29) | 75.70 | 8.97(17.42) | 73.34 | 10.73(18.65) | 71.96 | 12.45(20.66) | 68.35 |
| T5 | 5.95(14.10) | 78.62 | 7.44(15.82) | 77.89 | 8.77(17.22) | 75.96 | 10.85(19.23) | 72.41 |
| T6 | 4.52(12.27) | 83.75 | 6.36[a](14.60) | 81.09 | 7.41(15.79) | 79.69 | 9.53(17.98) | 75.77 |
| T7 | 6.44(14.70) | 76.85 | 8.23(16.67) | 75.54 | 9.93(18.36) | 72.75 | 11.87(20.15) | 70.25 |
| T8 | 27.83(31.83) | | 33.6(35.45) | | 36.49(37.16) | | 39.34(38.84) | |
| C.D. at 5 % | 0.77 | | 1.09 | | 1.30 | | 1.59 | |
| S. Em. ± | 0.32 | | 0.35 | | 0.42 | | 0.52 | |

T1- Seed treatment of *Streptomyces albofaciens* @ 10 g/kg of seed + Soil application of *Streptomyces albofaciens* @ 2.5 kg $ha^{-1}$ on TOS , T2 - Seed treatment with *B .japonicum* at @ 10 g/ kg of seed + Soil application of *B .japonicum* @ 3 $ha^{-1}$ on TOS , T3- Seed treatment with *Bacillus subtilis* @ 10g/kg of seeds + Soil application of *Bacillus subtilis* @ 2.5 kg $ha^{-1}$ on TOS , T4 - T1+ T2, T5 -T3 + T4, T6 - T5 + Fortification humic acid @ 100g/ kg as soil application @ 20kg $ha^{-1}$ on TOS, T7 - Seed treatment with Nativo 75 WP @ 1g/kg of seeds , T8- Untreated control.

has recorded plant height (65.17cm), biomass (43.67g/plant), Number of pods (42.00/plant), pod yield ( 1703 kg/ ha) and followed by plants treated with standard chemical check Nativo 75 WP as @ 1g/kg of seeds (T7) recording the plant height (62.53cm), biomass (39.24 g/pod), number of pods (37.00/plant), pod yield (1688 kg/ha). The treatments T4 and T7 were statistically on par with each other. All the treatments were significantly superior than the control (Table 3).

## Discussion

The efficacy of bioinoculants (*Streptomyces albofaciens, Bradyrhizobium japonium, Bacillus sublitis*) and combined effect of bioinoculants fortified with humic acid were tested against root rot disease of groundnut under pot culture and field condition. Plants treated with combined application of *Streptomyces albofaciens, B. japonicum* and *Bacillus subtilis* plus fortification with humic acid @ 100g/ kg as seed treatment @ 10 g/kg of seed and soil application @ 2.5 kg $ha^{-1}$of each (T6) was found to significantly manage the stem rot disease and in influencing the growth and yield parameters.Similar findings were made by several workers using the bioinoculants of *Streptomyces, Rhizobium* and *Bacillus* sp. During the past decade actinomycetes have been given considerable importance due to their ecological role in nutrient cycling and as plant growth promoters (Franco-Correa *et al.,* 2010). The beneficial effects of actinomycetes on plants are brought about by plant growth promotion and disease suppression activities, the *Streptomyces* sp. RP1A-12 was found in effective management of groundnut stem rot disease caused by *S. rolfsii* under the greenhouse conditions (Simi *et al*., 2016). Seed treatment and soil application with powder formulation of *Streptomyces sp* strain CBE significantly increased the pod yield in field trails besides controlling stem rot incidences (Adhililakshmi *et al.*, 2013). Doumbou *et al.*, 2001 studied that the increase in pod yield due to application of *Streptomyces sp* may be associated with decrease of disease incidence and increase in plant growth because of plant- growth promoting characteristic of actinomycetes.It is highly reported that the Rhizobium strains are very good bio-control agents to control soil borne plant diseases. Rhizobial isolates from groundnut (*Arachis hypogaea* L.) inhibited up to 62.5% of the *S. rolfsii* mycelium growth diameter (Ganesan *et al*., 2007). Camila Gazolla *et al*., 2019 reported that *Rhizobium* strainsrecorded the maximum disease control over the test pathogen. Shifa *et al*. (2015) reveals the important role of *Bacillus* has a very effective antagonistic activity against *Sclerotium rolfsi* as compeer to fungicide. Gholami *et al*. (2014) reported that *Bacillus* and *Streptomyces* treatments reduced the disease severity of stem incidence up to 58.5%. Humic acid as additives has shown excellent survival, releases cell slowly at various pH, and provide uniform growth of bacteria (Meeks *et al.,* 2005). It was reported that survival and storage rate of *Bacillus subtilis* were increased for six months by adding it with humic acid (Young *et al.,* 2006).Application of HA apart from being beneficial for the growth, development, and yield of many plant species such as groundnut, sugarcane, cowpea and have also been found to reduce the incidence of several diseases by inducing systemic resistance (Hermosa *et al*., 2012).

## Conclusion

The results reported here indicated that groundnut crop treated with combined application of bioinoculants (*Streptomyces albofaciens, Bradyrhizobium japonium, Bacillus sublitis*) fortified with humic acidcan lead towards the sustainable option against chemical pesticides and fertilizers. The direct application in seed and soil decreased the viability of pathogen and increases the plant growth promoting potential. Humic acid as an additivecan also be used as polymer matrix for encapsulation.

**Conflict of Interest:** None

## References

Adhilakshmi, M., Latha, P., Paranidharan, V., Balachandar, D., Ganesamurthy, K. and Velazhahan, R. 2014. Biological control of stem rot of groundnut (*Arachis hypogaea* L.) caused by *Sclerotium rolfsii* Sacc. with actinomycetes. *Archives of Phytopathology and Plant Protection.* 47: 298-311.

Baskey, S., Khalko, S., Hembram, S., Sharma, B.R. and Ali, S. 2020. Survey for the Incidence of Stem Rot of Groundnut in North Bengal Districts of West Bengal, India. *International Journal of Current Microbiology and Applied Sciences*. 9(01): 328-333.

Doumbou, Cyr Lézin, M.K., Hamby Salove, Don, L., Crawford and Carole Beaulieu, 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. *Phytoprotection.* 82(3): 85-102.

Department of Agriculture and Farmers Welfare, MoA & FW, Government of India, India (agriwelfare.gov.in)

El-Sherbeny, T.M.S., Abeer, M., Mousa and Mostafa AZhran, 2023. Response of peanut (*Arachis hypogaea* L.) plant to bio-fertilizer and plant residues in sandy soil. *Environmental Geochemistry and Health.* 45(2): 253-265.

Rayner, R.W. 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological. Society, Kew

Saccardo, P.A. 1911. Notae Mycological. *Annales Mycologici*. 9: 249-257.

Vidhyasekaran, P. and Muthamilan, M. 1995. Development of formulations of **Pseudomonas fluorescens** for control of chickpea wilt. *Plant Disease*. 79(8):782-786.