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EVALUATION OF ECOFRIENDLY COMPONENTS *VIZ*., FORTIFIED LIGNITE FLY ASH, ANNAMALAI MIXTURE AND BIO-INOCULANTS AGAINST GROUNDNUT STEM ROT DISEASES CAUSED BY *SCLEROTIUM ROLFSII* SACC.,

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Abstract

Efficacy of various ecofriendly components *viz*., lignite fly ash, Annamalai mixture and bioinoculants were tested for the management of stem rot disease of groundnut incited by *S. rolfsii.* In green house condition and field condition combined application of talc based formulation *T. viride*as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus *P. fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of Annamalai mixture as seed treatment @ 10g/kg of seed, foliar spray @ 20 lit/ha at 25 and 45 DAS, (T_7) significantly reduced stem rot disease incidence of 10.11, 11.62, 13.77, and 15.25 per cent in pot culture and field condition 7.46, 8.97, 9.49 and 12.24 per cent at 45, 60, 75 and 105 DAS respectively, and it is superior than the standard chemical check Carbendazim. The same treatment significantly increase the pod yield in pot culture (67 g/plant) and field condition (2060 kg/plant) than the standard chemical check and other treatment.

Key words: Groundnut, Ecofriendly components, Stem rot.

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop belongs to *Fabaceae* family and it is grown in all over the world. It is called as king of oil seed. The crop was highly affected by many of the fungal, bacterial and viral plant pathogens. Among the various plant pathogens the soil inhabiting pathogen *Sclerotium rolfsii* Sacc., causing stem rot disease is a more destructive, which has a wide host range of over 500 species including almost all the Agricultural and Horticultural crops (Liamngee Kator *et al.,* 2015). The pathogen *S. rolfsii* has produce abundant white mycelium and mustered like sclerotia in basal portion of plant. Initially white in color, later it becomes light brown to dark brown at maturity and cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debris (Mehrotra and Aneja, 1990).

Chemical fungicides are being used to control stem rot of groundnut. Unfortunately, these chemical fungicides are not readily biodegradable and indiscriminate use of synthetic agrochemicals also causes environmental pollution and ill effects on health (Oruc, 2010). Therefore, some alternative ecofriendly compounds are needed to combat the menace. In recent years, scientists have explored large number of natural resources against plant pathogens and very encouraging results have been obtained (Javaid and Shoaib, 2012). Keeping these points in view locally available eco friendly components *viz.,* lignite fly ash, Annamalai mixture and bioinoculants were evaluated in the management of stem rot pathogen under pot culture and field condition.

Materials and methods

Isolation and mass multiplication of *S. rolfsii*

The pathogen *S. rolfsii* was isolated from the typical symptom of stem rot infected groundnut crop by tissue segmentation method (Rangaswami, 1972). Pure culture of *S. rolfsii* was obtained by single hyphal tip method (Rangaswami, 1972). The auxenic culture of pathogen was maintained in slants contain PDA media at room temperature (27±2°C) for further studies. Mass

multiplication of pathogen was carried on glucose bottle contain autoclaved 50% moistened sand maize medium (sand: maize at 19:1 w/w). The pathogen was inoculated into the sand maize medium from five days old auxenic culture by using 9mm cork borer and incubates at room temperature for 10 days.

Preparation of Ecofriendly components

Preparation of bio inoculants

Trichoderma viride and *Pseudomonas fluorescens* were isolated from the rhizosphere soil of healthy groundnut cultivating fields by serial dilution technique and prepare as talc based formulation (Vidhyasekaran and Muthamilan 1995)

Preparation of Annamalai mixture

The extracts of cow urine, cow dung, sheep dung, poultry litter and neem cake at 100% concentration were taken and mixed thoroughly at the ratio of 1:1:1:1:1 (Kurucheve *et al.*, 1999).

Preparation of Fortified Lignite Fly Ash

Strain of *T. viride* and *P. fluorescens* were grown in nutrient broth for 48 hrs as a shake culture in rotary shaker at 150 rpm. At room temperature (25±2°C). Lignite fly ash (class F) was collected from Neyveli lignite corporation, Neyveli. One kg of lignite fly ash was added with 10g of carboxymethyl cellulose and mix well. These carriers were auto claved for 30 min of two consecutive days. One kg of carrier material was added with Four hundred ml of the each antagonistic suspension, containing 9×10^8 colony forming units (CFU) plus 20 ml of molasses and mixed well and shade dry for 2 hrs under sterile conditions. The above mentioned product is named as Fortified lignite fly ash. Then packed in polythene bags, sealed and stored at room temperature (25±2°C). The population of antagonistic was estimated at monthly interval for 3 month by using serial dilution technique (John Christopher and Kavi Newton, 2017).

Pot culture

The pot culture study was conducted with 10 treatments and three replications each at Department of Plant Pathology, Annamalai University, Annamalainagar from April to July 2017. Fifteen kg of top soil, collected from a ground nut growing field, was steam pasteurized and filled in 45 × 30 cm size cement pots. Ground nut seeds (variety VRI-2) were sown in pot. The Ecofriendly components *viz*., *T. viride, P. fluorescens*, Annamalai mixture, and Fortified lignite fly ash were tested against stem rot (*S. rolfsii)* of groundnut.

The talc based formulation of *T. viride* and*P. fluorescens* were used @ 2×10^{-8} CFU g^{-1}. The seeds were treated @ 10 g / kg of seed and dried in shade condition for four hours before sowing, talc based formulation *T. viride* and *P. fluorescens* were applied to the soil @ 10kg/ha. Fortified lignite fly ash was applied to the seed @ 10 g / kg and soil application @ 40kg/ha. Twenty per cent conc. of Annamalai Mixture was used for seed treatment and also used as foliar spray @ 20 lit/ ha. The chemical Carbendazim was used as seed treatment @ 4g/kg of seed and foliar spray @ 0.1 g/lit as standard chemical check. The challenge inoculation of test pathogen *S. rolfsii* was multiplied and applies to the pot @ 5g/pot at 10 days after sowing. The inoculated plant were kept in the laboratory for 24 hours to maintain a high relative humidity and subsequently moved to a green house maintained at 28±2°C, 70 to 90% relative humidity, under a light intensity of 85 µmol m^{-1} S^{-1}, 12 hour photo period and subsequently transferred to pot culture yard. The treatments in the table were designed on the basic of the above phenomena and depicted in Table. The disease incidence was assessed at 45, 60, 75 and 105 DAS.

Assessment of the disease severity was worked out using the 0 to 9 scale according to Phytopathometry by Mayee and Datar (1986) Scale 0-No symptoms on any plant,1- 1% or less plants killed, 3 - 1-10% plants killed, 5 - 11-20% plants killed, 7- 21-50% plants killed, 9- 51% or more plants killed and the PDI = Number of plants affected/ Total number of plants observed × 100. The pod yield was also calculated @g/plant.

The treatments mentioned in the tables were designed on the basic of the above phenomena and depicted in Table. The disease incidence was assessed at 45, 60, 75 and 105 DAS.

Field trial

The field trial was conducted during *Kharif* season at Poovanur, Vridachalam, Cuddalore district, Tamil Nadu during May to August 2017. in a field with a history of stem rot disease incidence of groundnut. The trial were laid out in plots (4m × 4m) arranged in a randomized block design. Seeds were sown in field plots in rows with row / plants spacing of 30 × 10 cm. Three replicated plots were maintained for each treatment. Treatment application details and experimental observation were the same as in green house experiment. Regular cultivation practices were followed as per the recommendation.

Experimental design and data analysis:

Data were analyzed using GENSTAT computer statistical package for ANOVA to determine significant differences between treatments. Comparison between means was done using Duncan's Multiple Range Test

(DMRT). A regression analysis was done to find out the correlation between the disease levels and percent loss in yield.

Result and discussion

Effects of eco friendly components against stem rot disease of groundnut

Pot culture condition:- The pot culture experiment was carried out to evaluate the effect of eco friendly components *viz*., *T. viride*, *P. fluorescens,* Annamalai mixture and lignite fly ash against stem rot (*S. rolfsii)* disease incidence of groundnut. All the ecofriendly components significantly reduced the stem rot disease incidence than the control (table1). Among the treatments, combined application of talc based formulation *T. viride*as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus *P. fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of Annamalai mixture as seed treatment @ 10g/kg of seed, foliar spray @ 20 lit/ha at 25 and 45 DAS, (T_7) significantly reduced stem rot disease incidence of 10.11, 11.62, 13.77, and 15.25per cent at 45, 60, 75 and 105 DAS respectively, it is superior than the standard chemical check Carbendazim, and followed by combined application of *T. viride*, as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS plus *P. Fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS plus application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS (T_6) which recorded stem rot disease incidence of 12.24, 13.87, 15.26 and 18.48 per cent at 45, 60, 75 and 105 DAS respectively, and followed by Seed treatment @ 2g/kg, foliar application @ 0.1 g/lit of Carbendazim @ 45 DAS (T_8) which recorded stem rot disease incidence of 12.66, 14.21, 16.82 and 19.11 per cent at 45, 60, 75 and 105 DAS respectively. The treatments T_6 and T_8 were statistically on par with each other. All the ecofriendly components treated plants significantly increased pod yield as compare to control. Among the various treatments the treatment T_7 recorded maximum pod yield (67 g/plant)

Table 1: Effects of ecofriendly components against stem rot disease of groundnut under pot culture condition

Treatment	Stem rot								Pod Yield (g/plant)
	Diseases incidence (%)				Decrease over control (%)				
	45 DAS	60 DAS	75 DAS	105 DAS	30 DAS	45 DAS	60 DAS	105 DAS	
T_1	15.73 (23.36)^{fg}	16.97 (24.32)^{fg}	18.98 (25.82)^{fg}	23.04 (28.68)^{fg}	27.84 (31.84)^{fg}	26.79 (31.17)^{fg}	29.49 (32.89)^{fg}	24.90 (31.84)^{fg}	49^{g}
T_2	15.93 (23.52)^{fh}	17.03 (24.37)^{fh}	19.21 (25.99)^{fh}	23.72 (29.14)^{fh}	26.92 (31.25)^{fh}	26.53 (31.00)^{fh}	28.64 (32.35)^{fh}	22.68 (28.43)^{fh}	47^{h}
T_3	13.43 (21.49)^{cd}	15.35 (23.06)^{cd}	17.16 (24.47)^{cd}	20.36 (26.82)^{cd}	38.39 (38.28)^{cd}	33.77 (35.52)^{cd}	36.25 (37.01)^{cd}	33.63 (35.44)^{cd}	56^{d}
T_4	14.89 (22.69)^{e}	16.54 (23.99)^{e}	18.08 (25.16)^{e}	22.04 (27.99)^{e}	31.69 (34.25)^{e}	28.64 (32.35)^{e}	32.83 (34.95)^{e}	28.16 (32.05)^{e}	53^{e}
T_5	15.12 (22.88)^{ef}	16.87 (24.25)^{ef}	18.45 (25.43)^{ef}	22.65 (28.41)^{ef}	30.64 (33.60)^{ef}	27.22 (31.44)^{ef}	31.46 (34.11)^{ef}	26.17 (30.76)^{ef}	52^{ef}
T_6	12.24 (20.47)^{b}	13.87 (21.86)^{b}	15.26 (22.99)^{b}	18.48 (25.46)^{b}	43.85 (41.46)^{b}	40.16 (39.32)^{b}	43.31 (41.15)^{b}	39.76 (39.09)^{b}	59^{b}
T_7	10.11 (18.53)^{a}	11.62 (19.93)^{a}	13.77 (21.78)^{a}	15.25 (22.98)^{a}	53.62 (47.07)^{a}	49.87 (44.92)^{a}	48.84 (44.33)^{a}	50.29 (45.16)^{a}	67^{a}
T_8	12.66 (20.84)^{bc}	14.21 (22.14)^{bc}	16.82 (24.21)^{bc}	19.11 (25.92)^{bc}	41.92 (40.35)^{bc}	38.69 (38.46)^{bc}	37.51 (37.76)^{bc}	37.71 (37.88)^{bc}	58^{bc}
T_9	13.76 (21.77)^{de}	15.49 (23.17)^{de}	17.67 (24.85)^{de}	20.76 (27.10)^{de}	36.88 (37.39)^{de}	33.17 (35.16)^{de}	34.36 (35.88)^{de}	32.33 (34.65)^{de}	56^{d}
T_{10}	21.80 (27.83)^{i}	23.18 (28.78)^{i}	26.92 (31.25)^{i}	30.68 (33.63)^{i}	—	—	—	—	45^{i}

T_1 – Talk based formulation of *T. viride*, as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS,T_2 – Talk based formulation of*P. Fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS,T_3 – T_1 +T_2, T_4 – Application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS, T_5 – Application of Annamalai mixture as seed @ 10g/kg of seed, foliar spray @ 20 lit/ha at 25 and 45 DAS,T_6 – T_3 + T_4, T_7–T_6+ T_5, T_8 – Carbendazim seed treatment @ 2g/kg, foliar application @ 0.1 g/lit at 45 DAS, T_9– Inoculated control and T_{10}– Healthy control.

Table 2: Effects of eco friendly components against stem rot diseases of Groundnut under field condition

Treatment	Stem rot								Yield (g/plant)
	Diseases incidence (%)				Decrease over control (%)				
	45 DAS	60 DAS	75 DAS	105 DAS	30 DAS	45 DAS	60 DAS	105 DAS	
T_1	17.64 (24.83)^{fg}	19.20 (25.98)^{fg}	21.04 (27.30)^{fg}	25.19 (30.12)^{fg}	13.48 (21.54)^{fg}	23.07 (28.70)^{fg}	22.59 (28.37)^{fg}	13.93 (21.91)^{fg}	1797^{fg}
T_2	17.89 (25.02)^{gh}	19.74 (26.37)^{gh}	21.49 (27.61)^{gh}	25.67 (30.44)^{gh}	12.26 (20.49)^{gh}	20.91 (27.21)^{gh}	20.93 (27.22)^{gh}	12.29 (20.52)^{gh}	1778^{gh}
T_3	15.12 (22.88)^{d}	16.19 (23.72)^{d}	18.69 (25.61)^{d}	20.34 (26.80)^{d}	25.84 (30.55)^{d}	35.13 (36.34)^{d}	31.23 (33.97)^{d}	30.50 (33.52)^{d}	1893^{d}
T_4	16.88 (24.25)^{e}	18.31 (25.33)^{e}	20.28 (26.76)^{e}	24.56 (29.70)^{e}	17.21 (24.50)^{e}	26.64 (31.07)^{e}	25.38 (30.25)^{e}	16.09 (23.64)^{e}	1868^{e}
T_5	17.16 (24.47)^{ef}	18.82 (25.71)^{ef}	20.73 (27.08)^{ef}	24.73 (29.82)^{ef}	15.84 (23.45)^{ef}	24.54 (29.69)^{ef}	23.73 (29.15)^{ef}	15.51 (23.19)^{ef}	1843^{ef}
T_6	9.32 (17.77)^{b}	10.17 (18.59)^{b}	12.18 (20.42)^{b}	14.95 (22.74)^{b}	54.29 (47.46)^{b}	59.25 (50.33)^{b}	55.18 (47.97)^{b}	48.92 (44.38)^{b}	1984^{b}
T_7	7.46 (15.86)^{a}	8.97 (17.42)^{a}	9.49 (17.94)^{a}	12.24 (20.47)^{a}	63.41 (52.77)^{a}	64.06 (53.16)^{a}	65.08 (53.77)^{a}	58.18 (49.70)^{a}	2060^{a}
T_8	11.12 (19.47)^{c}	12.45 (20.66)^{c}	14.63 (22.48)^{c}	17.41 (24.66)^{c}	45.46 (42.39)^{c}	50.12 (45.06)^{c}	46.17 (42.80)^{c}	40.51 (39.52)^{c}	1938^{c}
T_9	20.39 (26.84)^{i}	24.96 (29.97)^{i}	27.18 (31.42)^{i}	29.27 (32.75)^{i}	—	—	—	—	1639^{i}

T_1 – Talk based formulation of *T. viride*, as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS, T_2 – Talk based formulation of *P. fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS, T_3 – T_1 +T_2, T_4 – Application of Fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS, T_5 – Application of Annamalai mixture as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS,T_6 – T_3 + T_4, T_7– T_6 + T_5, T_8 – Carbendazim as seed treatment @ 2g/kg, foliar application @ 0.1 g/lit at 45 DAS and T_9 – Healthy control.

than other treatments.

Field trial:- (season *Kharif)* The field experiment of *kharif* season 2017 revealed that, combined application of combined application of *T. viride* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus *P. fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS plus application of Annamalai mixture as seed treatment @ 10g/kg of seed, foliar spray @ 20 lit/ha at 25 and 45 DAS, (T_7) significantly reduced stem rot disease incidence of 7.46, 8.97, 9.49, and 12.24 per cent at 45, 60, 75 and 105 DAS respectively, it is superior than the standard chemical check Carbendazim, and followed by combined application of *T. viride*, as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS, *P. fluorescens* as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 and 45 DAS, Application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS (T_6) which recorded stem rot disease incidence of 9.32, 10.17, 12.18 and 14.95 per cent at 45, 60, 75 and 105 DAS respectively, and followed by Seed treatment @ 2g/kg, foliar application @ 0.1 g/lit of Carbendazim @ 45 DAS (T_8) which recorded stem rot disease incidence of 11.12, 12.45, 14.63 and 17.41 per cent at 45, 60, 75 and 105 DAS respectively. The treatments T_6 and T_8 were statistically on par with each other. Among the various treatments the treatment T_7 record maximum pod yield (2060 kg/ha) than others.

Lignite Fly Ash (LFA)- contains high amounts of **Si, Al, Fe, Ca**, intermediate amounts of Mg, K, and Na. Silicon-fed plants naturally translocate silicic acid throughout all tissues (Karpagavalli *et al.,* 1997).

Biocontrol agent - (*Trichoderma* sp., *Pseudomonas* sp.,) are excellent colonizers and widely prevalent in Groundnut rhizosphere have been found to be most effective antagonists against *S. rolfsii*, *Pythium* sp., *Fusarium* sp., and also enhance the growth of the plant due to induction of growth promoting substances like auxins and gibberellins (Rosa Hermosa *et al.,* 2012). Lignite Fly Ash fortified with bio inoculants (*Trichoderma* sp., *Pseudomonas* sp.,) with the addition of molasses retained maximum viability of the propagules with very little reduction in the population over a prolonged period and showed improved consistency of colonization as well as the spectrum of pest and disease suppression.

An organic mixture of plant products and animal excrements (Annamalai mixture) contains volatile ammonia and silica which are fungitoxic against various rice pathogens, acts as repellent against aphids, white flies, leaf hoppers, BPH, GLH and thrips and also enhances the yield which might be due to various macro & micro elements and humus present in them (Kurucheve *et. al.,* 1999). Combined formulation of Fortified Lignite Fly Ash and Annamalai mixture have different mode of action to control the disease of ground nut and also enhance the growth and yield.

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