# EFFECT OF ORGANIC AMENDMENTS AGAINST SCLEROTIUM ROLFSII SACC. CAUSING DAMPING OFF AND STEM ROT OF COWPEA

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**Abstract.** A study was conducted to check the efficacy of various organic amendments viz. Vermicompost, Coirpith compost, Neem cake, Vermiwash, Panchagavya and Fish amino acid at different concentrations under in vitro as well as pot conditions against *Sclerotium rolfsii* in cowpea. In the laboratory test, significant control over *S. rolfsii* was observed for the treatments Vermicompost (5% conc.), Coirpith compost (15-25% conc.), Neem cake (25% conc.), Vermiwash (25% conc.), Panchagavya (15% conc.) and Fish amino acid (5% conc.). According to the data obtained under pot conditions, disease incidence was the least in the case of vermicompost application compared to the control with inoculum. Moreover, Vermicompost at 15% concentration showed maximum inhibition with only 8.33% disease incidence followed by 10% Vermiwash and 1% Panchagavya with 42% disease incidence. Nonetheless, all other treatments of organic amendments in varied concentrations showed more than 50% disease incidence. Although fungicides are reported to control *S. rolfsii*, the adoption of organic management over chemical control can evidently reduce the negative impact of chemicals, promoting a sustainable environment.

Keywords: vermicompost, coirpith compost, neem cake, vermiwash, panchagavya, fish amino acid

#### Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important legumes of the tropics belonging to Phaseoleae tribe of Leguminosae family (Timko et al., 2007). It is the key source of dietary protein that complements low protein cereals and tuber crops nutritionally and a major source of income for farmers and traders, thus becoming the most significant and dependable produce in the lives of many Africans (Langyintuo et al., 2003). It is also known as vegetable meat, poor man's meat, rich man's vegetable, and also the "hungry-season crop" given that it is the first crop to be harvested before the cereal crops are ready. Its nutritional components include 23.4% protein, 1.8% fat, 60.3% carbohydrate and high amount of calcium (0.08%–0.11%) and iron (0.005%) along with few essential amino acids (lysine, leucine and phenylalanine) in its seeds (Atachi, 1984). In the world, dried cowpea production has crossed 7.4 million tons in 2017 (http://faostat.fao.org, 2018), with Africa as the highest producer (7.1 million t). The largest producer and consumer is Nigeria, accounting 48% of production in Africa and 46% worldwide (IITA, FAO). In India cowpea production covers an area of 1.5 million ha with 0.5 million tons annually.

Cowpea is found to be affected by many fungal, bacterial, viral and nematode pathogens. More than 70 diseases have been reported in cowpea out of which India faces more than 50% crop loss from ashy stem blight or charcoal rot, anthracnose, mosaic and bacterial blight (Upadhyay et al., 1998). The major fungal disease observed were Stem rot, Southern wilt or blight by *Sclerotium rolfsii* Sacc. (Adandonon et al., 2006); Dry root rot, Charcoal rot, Ashy stem blight, Stem canker, Collar rot, Web blight or Pod blight by *Macrophomina phaseolina* (Tassi.) Goid. or *Rhizoctonia bataticola* (Taub.) Butler (Lakshmanan et al., 1979; Vavilapalli et al., 2014); Root rot or Dry root rot by *F. equiseti* (Li et al., 2018), *F. oxysporum*, *F. proliferatum* (Shrestha et al., 2016); Target leaf spot by *Corynespora cassiicola* from China (Li et al., 2014); Leaf spot by *Pestalotiopsis* species and *Dactuliophora* species (Mahadevakumar and Janardhana, 2012); Rust by *Uromyces appendiculatus* (Pers.) Lev. and Powdery mildew by *Erysiphe polygoni* DC (Upadhyay et al., 1998).

Stem rot in cowpea is caused by *Sclerotium rolfsii* Sacc., a soil borne, ubiquitous phytopathogen having wide host range. The fungus is a facultative parasite and causes root rot in over 500 plant species (Punja, 1985). The ability of the pathogen to infect a number of plant species has increased the challenge to manage it. Seed treatment and soil application of fungicides is a common practice. But the adverse effect of such synthetic chemicals in the environment has become a fact of concern. Alternatives to this through integrated disease management, giving more effort on sustainable and ecofriendly management has been a recent limelight. The involvement of botanicals, bio-control agents and organic amendements in crop production not only provides management to several plant diseases but can also ensure adequate nutritional availability and maintenance of soil microbiota. Hence, to investigate the efficiency of various organic amendments against damping off in cowpea caused by *Sclerotium rolfsii*, organic amendments viz. vermicompost, coipith compost, vermiwash and panchagavya and two commercial products viz. neemcake and fish amino acid were utilized in this experiment.

Many management practices have been used to remove or reduce the inoculum load of soil borne pathogens, which includes soil solarisation, bio-agent application in the soil, amendment of organic substrates, soil drenching, use of chemical fungicides etc. Few of the triazole group fungicides have been reported to give more than 90%

efficiency in eliminating root rot fungi. However, keeping in view of the repercussions of chemical residues, this study has focused on various organic amendments that can be administered to soil to lessen the fungal inoculum.

#### Materials and methods

# Location of experimental site

The experiments were conducted in the fields of CPCRI, Kasaragod, Kerala located geographically at 12.5279° N latitude, 74.9685° E longitude with an altitude of 3.05 m above MSL, with a mean maximum temperature of 32°C and mean annual rainfall of 2564 mm. The soil texture is sandy to loamy sand with pH ranging from 5-6.

# Development of the experiment

Laboratory experiments were conducted in the Department of Plant Pathology, CPCRI, Kasaragod, Kerala and Field experiments were carried out in pots of 6.5 cm radius in the Rabi season, 2020-2021 at CPCRI field, Kasaragod, Kerala.

# Sample collection and isolation of the pathogen

The pathogen was collected from the rhizosphere and rhizoplane of cowpea (Mian, 1995). Soil samples were taken from the rhizosphere of cowpea crop in the field and subjected to serial dilution. Also infected plant parts such as root and stem portion were taken during seedling and vegetative stage of the crop.

Soil samples were subjected to serial dilution for isolation of the pathogen. Isolation was also carried out from the diseased crop parts. The roots of diseased plant showing symptoms along with the stem, were washed with water thoroughly and small pieces of infected roots and the collar portion along with some healthy tissues were cut out using a sterilized blade. These pieces were surface sterilized with  $HgCl_2$  (0.1%) for 1 min followed by washing three times with sterilized distilled water to remove trace elements of mercuric chloride. The pieces were then transferred to petri plates containing PDA media with streptomycin aseptically (Pitt and Hocking, 1997). Inoculated petri plates will be incubated (according to the International Commission on Food Mycology-ICFM) at  $25 \pm 2$ °C for 5 days and the growth of the fungus was examined at 3 days intervals.

# Mass multiplication of the fungus

The required amount of sorghum grains were washed thoroughly to remove impurities and soaked in water overnight. The grains were then dried partially on blotting paper and filled into conical flasks @ 250 g per 500 ml flask. The flasks were then subjected to double autoclave for 2 h in two successive days. The sterilized flasks with sorghum grains were inoculated aseptically with 10 mycelial discs (9 mm diameter) per flask taken from the 7-day old *S. rolfsii* on PDA. The inoculated flasks were then incubated at  $25 \pm 2^{\circ}$ C for 20 to 22 days and further used for pot experiments (Bekriwala et al., 2016; Shokes et al., 1996).

## Organic amendments used in the experiment

Both in vitro and in vivo experiments included the use of Vermicompost, Coirpith compost, Neem cake, Vermiwash, Panchagavya and Fish amino acid formulation. Vermicompost, Coirpith compost and Vermiwash were obtained from CPCRI,

Kasaragod. Neem cake and Fish amino acid formulation were commercial products from Neemex, Agro Extracts Limited and Haritha, Krishna Agro Chemicals respectively while Panchagavya was prepared using the ingredients and protocol presented in *Table 1*.

Table 1. Ingredients used for the preparation of panchagavya

Component	Quantity
Fresh cow dung	3 ½ kg
Cow urine	1 ½ litre
Cow milk	1 litre
Cow curd	1 litre
Cow ghee	½ kg

# Method of preparation (Raghavendra et al., 2014; Kumar et al., 2010)

The components mentioned in *Table 1* were obtained from desi cow. Firstly, 3 ½ kg of cow dung and ½ kg of cow ghee were mixed in a 10 L plastic container or bucket. After 2 days of incubation of the above mixture, 1 ½ L of cow urine and 5 L of water were added. This mixture was incubated further for 1 week giving it stir both morning and evening only in one direction. After 1 week, cow milk and curd were added, 1 L each. The lid of the container was kept closed after adding and mixing each ingredient. The whole mixture was incubated for another 2 weeks and finally filtered through clean double layered muslin cloth. This was stored in bottles or closed containers for further use (*Fig. 1*).

Observation was taken after 10 days of inoculation and disease incidence was calculated with the formula (Wheeler, 1969) (Eq. 1):

Disease incidence = 
$$\frac{Number\ of\ plants\ infected}{Total\ number\ of\ plants\ observed} X100 \tag{Eq.1}$$

### In-vitro evaluation of efficacy of organic amendments against Sclerotium rolfsii

Extract preparation of organic amendments

The extract preparation was necessary for solid additives while for liquid amendments, only centrifugation to remove impurities was carried out.

# Procedure for extract preparation

Known amount (50 g) of vermicompost, coirpith compost and neem cake were dissolved in 100 ml of distilled water in the ratio 1:2 (w/v) in a conical flask and covered with an aluminum foil.

The mixture was subjected to vigorous shaking in a shaker for 30 min. This was then filtered through a clean muslin cloth and the extract was separated. The extract and the liquid amendments were subjected to centrifugation @ 1000 rpm for 5 min and the supernatant was collected carefully. The obtained extracts were 100% concentration and were used for PFT and KB tests. 10% concentration (10 ml in 90 ml water) of FAA (Fish Amino Acid) was used for centrifugation as it was highly concentrated semi solid formulation.



Figure 1. Steps in preparation of Panchagavya

# Poisoned food technique

The organic amendments used were evaluated for its toxicity against *Sclerotium rolfsii* by poisoned food technique (PFT) (Grover and Moore, 1962). Organic amendments in both crude and extract form were incorporated into sterilized PDA (without streptomycin) to obtain 5, 15 and 25% concentration and poured into sterilized petri plates. After solidification of the media, the plates were inoculated with 9 mm mycelial discs of the pathogen taken from 5 days old actively growing cultures. Three replications per treatment were maintained and the control plates were not

supplemented with any of the organic amendment. The plates were then incubated at  $28 \pm 1$  °C for 3-7 days and the growth of the pathogen was measured. Percent inhibition of mycelial growth was calculated because of growth in control treatments.

The percent growth inhibition of the mycelium was calculated using the following formula (*Eq. 2*):

Percent growth inhibition = 
$$\frac{\text{Radial growth in control} - \text{Radial growth in treated plates}}{\text{Radial growth in control}} \times 100$$
 (Eq.2)

# Management under in-vivo conditions

The treatments were carried out in the cowpea crop planted in pots in CPCRI. The organic amendments were applied individually to the crop at 3 different concentrations as depicted in *Table 2*.

**Table 2.** Different concentrations of organic amendments used under pot conditions to examine the seed germination percentage and disease incidence

Treatment	Name of the organic amendment		
T1	Soil + FYM + inoculum + 15% Vermicompost		
T2	Soil + FYM + inoculum + 25% Vermicompost		
Т3	Soil + FYM + inoculum + 50% Vermicompost		
T4	Soil + FYM + inoculum + 15% Coirpith compost		
T5	Soil + FYM + inoculum + 25% Coirpith compost		
T6	Soil + FYM + inoculum + 50% Coirpith compost		
<b>T</b> 7	Soil + FYM + inoculum + 3% Neem cake		
T8	Soil + FYM + inoculum + 5% Neem cake		
Т9	Soil + FYM + inoculum + 10% Neem cake		
T10	Soil + FYM + inoculum + 3% Vermiwash		
T11	Soil + FYM + inoculum + 5% Vermiwash		
T12	Soil + FYM + inoculum + 10% Vermiwash		
T13	Soil + FYM + inoculum + 1% Panchagavya		
T14	Soil + FYM + inoculum + 3% Panchagavya		
T15	Soil + FYM + inoculum + 5% Panchagavya		
T16	Soil + FYM + inoculum + 0.5% Fish amino acid		
T17	Soil + FYM + inoculum + 1% Fish amino acid		
T18	Soil + FYM + inoculum + 2% Fish amino acid		
Control*	Soil + FYM + S. rolfsii inoculum		
Control	Soil + FYM		

The solid amendments were mixed with soil and farmyard manure (FYM) during potting while the liquid amendments were drenched into the pots. Soil and FYM were mixed in the ratio 2:1 and vermicompost, coirpith compost and neem cake were mixed in the required concentrations to the total volume of the pot. The mixture was filled in the pots maintaining 5 replications per treatment. For liquid amendments, the pots were filled with the soil and FYM mixture and then drenched with the amendments dissolved

in water making the volume up to 200 ml. The inoculum mixture of *S. rolfsii* (prepared with sorghum grains) was then inoculated in the pots @ 25 g/kg soil after 1 week of application of the amendments. After 5 days of inoculation, 4 cowpea seeds of Lola variety were sown in each pot. The cowpea seeds were surface sterilized using 70% alcohol followed by three washings in sterile distilled water. The pots with inoculum and devoid of amendments served as control\* and the one without inoculum and amendment served as control. The observation of seed germination was taken after 10 days of sowing (Eq. 3).

Since germination of cowpea seeds, the germination percentage was evaluated with the following formula:

Germination percentage = 
$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$
 (Eq.3)

# Data analysis

All the data were statistically analyzed with computer software SAS and subjected to analysis of variance (ANOVA). The treatment means were separated at 5% significance level using Duncan's Multiple Range Test (DMRT).

#### Results and discussion

# Efficacy of organic amendments against Sclerotium rolfsii through poisoned food technique

All the organic amendments tested were found efficient in reducing the mycelial growth of *Sclerotium rolfsii* over untreated control, wherein complete inhibition (100%) was observed in all the concentrations (5%, 15%, 25%) of Vermicompost and Fish amino acid, 15% concentration of Coirpith compost, 15% and 25% concentration of Vermicompost extract, Neem cake extract and Panchagavya. This was followed by 5% concentration of Coirpith compost (94.44%), 25% and 15% concentration of Neemcake (92.22% and 90.74% respectively). Additionally, percent inhibition by 25% Vermiwash and Coirpith Compost Extract and 5% of Vermicompost Extract ranged between 81%-84%, while inhibition of 72-79% was exhibited by 5% of Neem cake and Neemcake Extract, 25% of Coirpith compost and 5% and 15% of Vermiwash. Moreover, 60%-64% inhibition was shown by 5% and 15% concentration of Coirpith Compost Extract, whereas the least inhibition of 29.63% was manifested by 5% of Panchagavya (*Table 3*).

Among the different concentration of organic amendments used, most of them showed a gradual increase in the percent inhibition as the concentration improved (*Fig.* 2). However, the results of Coirpith compost were fluctuating. The latter was found to give complete suppression at 15% concentration, followed by 5% concentration (94.4%) and 25% concentration (75.93%). Moreover, in Panchagavya, a dramatic rise from 29.63% to 100% inhibition was observed when the concentrations were improved from 5% to 15% (*Table 4*).

# Evaluation of germination percentage of cowpea seeds under in-vivo conditions

Under *in-vivo* conditions, the untreated control gave 100% germination whereas the untreated control with the inoculum of *S. rolfsii* gave 33.33% germination. Among the

treated pots with the inoculum, only VCE gave more than 80% germination of the cowpea seeds, in which VC at 15% and 50% showed 91.66% germination whereas that at 25% gave comparatively lesser germination percentage (83.33%). Moreover, 58.33 germination percentage was observed in treatments with 10% VW and 1% PAN (*Table 5*).

Among the different concentrations of CC, the most effective was 25% CC which exhibited 41.66% germination. Additionally, in NC, both 3% NC and 10% NC showed similar results with 16.66 germination percentage, while 5% NC completely inhibited seed germination. Nevertheless, 3% VW and 5% VW also had null effect on seed germination while on contrary, higher concentration of 10% VW gave satisfactory result. Despite that, PAN at lower concentration (1%) was found to manifest higher germination percentage than its higher level. Similarly, 1% FAA showed mild efficiency in seed germination (33.33%) compared to its lower and higher concentration.



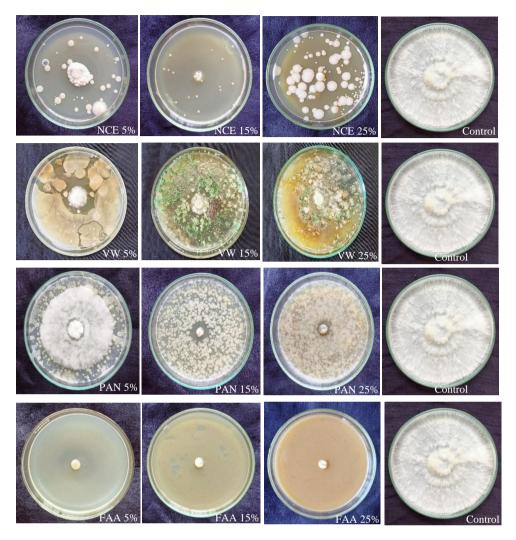


Figure 2. Efficacy of different organic amendments against S. rolfsii in PFT

**Table 3.** Radial growth of mycelium of S. rolfsii exposed to 5%, 15% and 25% concentrations of various organic amendments

Ougania amandmenta	Radial growth (cm)			
Organic amendments	5%	15%	25%	
Coirpith compost	$0.5000 \pm 0.08^{HI}$	$0.0000 \pm 0.0^{I}$	$2.1700 \pm 0.41^{EF}$	
Vermicompost	$0.0000\pm0.0^{I}$	$0.0000 \pm 0.0^{I}$	$0.0000\pm0.0^{I}$	
Neemcake	$1.8300 \pm 0.31^{EF}$	$0.8300 \pm 0.25^{G}$	$0.7000 \pm 0.26^{GH}$	
Coirpith compost extract	$3.5300 \pm 0.75^{C}$	$3.1700 \pm 0.2^{CD}$	$1.6000 \pm 0.46^F$	
Vermicompost extract	$1.6700 \pm 0.72^{\mathrm{F}}$	$0.0000 \pm 0.0^{I}$	$0.0000\pm0.0^{I}$	
Neemcake extract	$2.4700 \pm 0.67^{DE}$	$0.0000 \pm 0.0^{I}$	$0.0000\pm0.0^{I}$	
Vermiwash	$2.4000 \pm 0.72^{DE}$	$2.1000 \pm 0.36^{EF}$	$1.5000 \pm 0.36^{F}$	
Panchagavya	$6.3300 \pm 1.04^{B}$	$0.0000 \pm 0.0^{I}$	$0.0000\pm0.0^{I}$	
Fish amino acid	$0.0000\pm0.0^{I}$	$0.0000 \pm 0.0^{I}$	$0.0000\pm0.0^{I}$	
Control	$9.0000 \pm 0.0^{A}$	$9.0000 \pm 0.0^{A}$	$9.0000 \pm 0.0^{\rm A}$	

p-value -<.0001, CV% - 22.0749, CD at 5% - 0.69

Treatments with the same letter are not significantly different from each other, while treatments with different letters are significantly different

**Table 4.** Inhibition percentage of S. rolfsii exposed to 5%, 15% and 25% concentrations of various organic amendments

Owen is a sure dimenta	Percent inhibition		
Organic amendments	5%	15%	25%
Coirpith compost	94.44	100	75.93
Vermicompost	100	100	100
Neemcake	79.63	90.74	92.22
Coirpith compost extract	60.74	64.81	82.22
Vermicompost extract	81.48	100	100
Neemcake extract	72.59	100	100
Vermiwash	73.33	76.67	83.33
Panchagavya	29.63	100	100
Fish amino acid	100	100	100
Control	0	0	0

**Table 5.** Germination percentage of cowpea seeds at various concentrations of organic amendments under in vivo condition

Treatments	Organic amendments	No. of cowpea seeds germinated in pot	In- vivo germination %
T1	VC (15%) + inoculum (T1)	3.6667 <sup>A</sup>	$91.666666667 \pm 13.69^{A}$
T2	VC (25%) + inoculum (T2)	3.3333 <sup>A</sup>	$83.33 \pm 13.69^{A}$
T3	VC (50%) + inoculum (T3)	3.6667 <sup>A</sup>	$91.\ 67 \pm 13.69^{A}$
T4	CC (15%) + inoculum (T4)	0.3333 <sup>ED</sup>	$8.33 \pm 11.10^{\text{EF}}$
T5	CC (25%) + inoculum (T5)	1.6667 <sup>BC</sup>	41. $67 \pm 20.91^{CD}$
T6	CC (50%) + inoculum (T6)	$1.0000^{\mathrm{EDC}}$	$25\pm17.67^{\rm CDE}$
T7	NC (3%) + inoculum (T7)	0.6667 <sup>EDC</sup>	$16.67 \pm 13.69^{\text{CDEF}}$
T8	NC (5%) + inoculum (T8)	$0.0000^{\mathrm{E}}$	$0\pm22.36^{DEF}$
T9	NC (10%) + inoculum (T9)	0.6667 <sup>EDC</sup>	$16.67 \pm 13.69^{\text{CDEF}}$
T10	VW (3%) + inoculum (T10)	$0.0000^{\mathrm{E}}$	$0\pm0.0^{\mathrm{F}}$
T11	VW (5%) + inoculum (T11)	$0.0000^{\mathrm{E}}$	$0\pm0.0^{\mathrm{F}}$
T12	VW (10%) + inoculum (T12)	2.3333 <sup>B</sup>	$58.33 \pm 22.36^{B}$
T13	PAN (1%) + inoculum (T13)	2.3333 <sup>B</sup>	$58.33 \pm 22.36^{B}$
T14	PAN (3%) + inoculum (T14)	$1.0000^{\mathrm{EDC}}$	$25 \pm 0.0^{CDE}$
T15	PAN (5%) + inoculum (T15)	$0.0000^{\mathrm{E}}$	$0 \pm 0.0^{\mathrm{F}}$
T16	FAA (0.5%) + inoculum (T16)	$1.0000^{\mathrm{EDC}}$	$25\pm17.67^{CDE}$
T17	FAA (1%) + inoculum (T17)	1.3333 <sup>DC</sup>	$33.33 \pm 13.69^{\text{C}}$
T18	FAA (2%) + inoculum (T18)	$1.0000^{\mathrm{EDC}}$	$25 \pm 25^{\text{CDE}}$
T19	Control + inoculum	1.3333 <sup>DC</sup>	$33.33 \pm 13.69^{\circ}$
T20	Control without inoculum	4.0000 <sup>A</sup>	$100 \pm 20.91^{CD}$

p-value - <.0001, CV%- 36.2925, CD at 5%- 0.869176

# Evaluation of disease incidence under in-vivo condition

According to the data obtained, disease incidence has been the least in the case of vermicompost application compared to the control with inoculum. At 15% VC and 50% VC, only 8.33% disease incidence was observed, which is negligible (*Fig. 3*). Moreover, at 25% VC only 16.66% disease incidence was noticed. In addition, at 10% VW and 1% PAN, about 42% disease incidence was observed. Nonetheless, all other treatments of organic amendments in varied concentrations showed more than 50% disease incidence (*Table 6*).

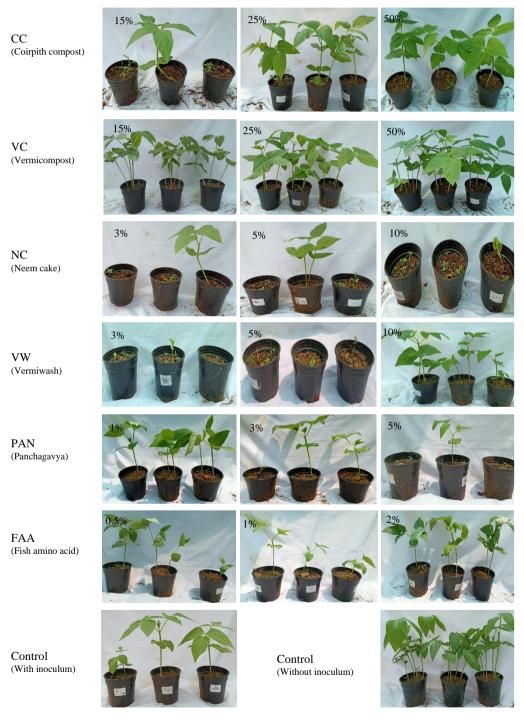


Figure 3. In vivo treatment of organic amendments in cowpea

Table 6. Percentage disease incidence in cowpea planted in treated pots

Treatments	Organic amendments	No. of infected plants in pot	% Disease incidence
T1	VC (15%) + inoculum (T1)	0.3333 <sup>E</sup>	8.333333333
T2	VC (25%) + inoculum (T2)	0.6667 <sup>E</sup>	16.66666667
T3	VC (50%) + inoculum (T3)	0.3333 <sup>E</sup>	8.333333333
T4	CC (15%) + inoculum (T4)	3.6667 AB	91.66666667
T5	CC (25%) + inoculum (T5)	2.3333 <sup>CD</sup>	58.33333333
T6	CC (50%) + inoculum (T6)	3.0000 <sup>CAB</sup>	75
T7	NC (3%) + inoculum (T7)	3.3333 <sup>CAB</sup>	83.33333333
Т8	NC (5%) + inoculum (T8)	4.0000 <sup>A</sup>	100
T9	NC (10%) + inoculum (T9)	3.3333 <sup>CAB</sup>	83.33333333
T10	VW (3%) + inoculum (T10)	4.0000 <sup>A</sup>	100
T11	VW (5%) + inoculum (T11)	4.0000 <sup>A</sup>	100
T12	VW (10%) + inoculum (T12)	1.6667 <sup>D</sup>	41.66666667
T13	PAN (1%) + inoculum (T13)	1.6667 <sup>D</sup>	41.66666667
T14	PAN (3%) + inoculum (T14)	3.0000 <sup>CAB</sup>	75
T15	PAN (5%) + inoculum (T15)	4.0000 <sup>A</sup>	100
T16	FAA (0.5%) + inoculum (T16)	3.0000 <sup>CAB</sup>	75
T17	FAA (1%) + inoculum (T17)	2.6667 <sup>CB</sup>	66.66666667
T18	FAA (2%) + inoculum (T18)	3.0000 <sup>CAB</sup>	75
T19	Control + inoculum	2.6667 <sup>CB</sup>	66.66666667
T20	Control without inoculum	0.0000 <sup>E</sup>	0

p-value - <.0001, CV% - 21.0115, CD at 5% - 0.869176

#### **Discussion**

Under in-vitro, the minimum concentration of Vermicompost that gave significant control over *S. rolfsii* was 5%, for Coirpith compost it was 15-25%, for Neem cake and Vermiwash it was 25%, for Panchagavya it was 15% and for Fish amino acid, it was 5%. Among the different concentration of organic amendments used, most of them showed a gradual increase in the percent inhibition as the concentration improved. However, the results of Coirpith compost were fluctuating. The latter was found to give complete suppression at 15% concentration, followed by 5% concentration and 25% concentration. Moreover, in Panchagavya, a dramatic rise from 29.63% to 100% inhibition was observed when the concentrations were improved from 5% to 15%.

According to the data obtained under pot conditions, disease incidence has been the least in the case of vermicompost application compared to the control with inoculum. At 15% Vermicompost and 50% Vermicompost, only 8.33% disease incidence was observed which is negligible. Moreover, at 25% Vermicompost only 16.66% disease incidence was noticed. In addition, at 10% Vermiwash and 1% Panchagavya, about 42% disease incidence was observed. Nonetheless, all other treatments of organic amendments in varied concentrations showed more than 50% disease incidence.

Vermicompost application at 20% concentration was reported to manage various soil borne pathogens whereas higher concentration of 40% increased disease incidence (Rodriguez et al., 2000). Moreover, variation in the efficacy of vermicomposts from different sources was also reported (Szczech and Smolinska, 2001).

Vermicomposts includes various beneficial microbes that can manage soil pathogens by its antagonistic effect (Gupta et al., 2010) and is also rich in micro and macro nutrients (Sutar et al., 2019). Several other composts are also reported to possess inhibitory action in sclerotial germination and viability due to the presence of the microbial consortia including *Penicillium* that was isolated from non-viable sclerotia (Hadar and Gorodecki, 1991).

Compatibility between soil application of various oil cakes including neem cake and seed treatment of *Trichoderma* sp. was reported (Lahre et al., 2012). Moreover, *Trichoderma* sp. has also been reported to actively grow in the substrate containing neem cake (Kalavathi et al., 2020). *Trichoderma* being a chitinolytic microbe can evidently cause reduction in sclerotial germination (Rodriguez-Kabana et al., 1987).

Furthermore, it has been reported that Panchagavya and Vermiwash showed variation in their efficacy in regulating the incidence of *S. rolfsii* in different crops (Parmar et al., 2018). Despite that, both these amendments successfully controlled the pathogen both in-vitro and in-vivo in napier hybrid (Atri and Kaur, 2021). Reports revealed that Panchagavya constitutes multitude of beneficial organisms that improves plant growth and aids plant protection (Solaiappan, 2003) as well as consists of several proteins and micronutrients (Kumar et al., 2006).

Soil solarization was reported to successfully help in reducing the primary inoculum of the pathogen (Flores-Moctezuma et al., 2006) and its viability to a certain extent and organic amendments can further incorporate antimicrobial property thus suppressing the development of the pathogen (Szczech et al., 1993; Rodriguez et al., 2000; Szczech and Smolinska, 2001; Edwards and Arancon, 2004; Zaller, 2006). Individual supplement of amendments alone was reported to cause damage and increased sclerotial population. Thus, a combination of soil solarization and soil amendment along with bio-agents can explicitly bring out tremendous effect in managing soil borne pathogens.

Based on the molecular studies conducted, bacterial biocontrol agents undergo many regulatory processes at the transcriptional and post-transcriptional levels (Haas and Défago, 2005). The extracellular secondary metabolites produced by the fluorescent pseudomonads efficiently manage the expression of their own biosynthetic genes at the transcriptional level that contributes in inhibiting the growth of the pathogen. In addition, during post transcriptional level, the GacS/GacA two-component system controls the small noncoding RNAs which regulate the expression of biocontrol factors, lessening the suppression that RNA-binding proteins exert on the expression of target mRNAs. It was observed that the involvement of siderophores in biocontrol is rare contradicting to the crucial role once attributed. The recent research about biocontrol agents emphasizes on the production of antifungal antibiotics, elicitation of ISR in hosts through jasmonate- and ethylene-responsive defense pathways and interference with fungal pathogenic factors by the bacteria during root colonization.

Many toxic exo products like phenazine, pyrrolnitrin, pyoleuterin, HCN and 2,4-diacetyl phloroglucinol (Phl), lytic enzymes and exoproteases produced by *P. fluorescens* (Jousset et al., 2008; Haas and Keel, 2003; Ramamoorthy and Samiyappan 2001); iturin produced by the *Bacillus* (Gumede, 2008; Bernal et al., 2002) and antibiotics, glisoprenins, gliovirin, viridin, peptaibols, harzianic acid, heptelidic acid, alamethicins, massoilactone, tricholin and 6-penthyl- a –pyrone (Vey et al., 2001) and hydrolytic enzymes (Monte, 2001; Vizcaino et al., 2005) produced by *Trichoderma* species might be causing the inhibitory effect on fungal pathogens.

Since *S. rolfsii* exhibits variation in their sensitivity to several bioagents, application of microbial consortia could be more appropriate in managing soil borne pathogens (Palaiah et al., 2010; Thilagavathi et al., 2012).

Several cultural, biocontrol and chemical managements are practiced against *S. rolfsii*. Few of them are found to be compatible with each other, like Difenoconazole was found to decrease the growth rate of *S. rolfsii* and not that of *Trichoderma harzianum*, which indicates that they can be applied in combination (Cilliers et al., 2003). But according to this study vermicompost can manage the disease to some extent and is favorable for the growth of *Trichoderma harzianum* and *T. koningii* (LatundeDada, 1993). And also, various *Trichoderma* spp. are able to inhibit the growth of the pathogen.

# Conclusion

It was examined that all the in vitro and in vivo experiments reveal that soil application of Vermicompost can successfully manage *S. rolfsii* in cowpea causing damping off. Additionally, the minimum concentration that gave maximum efficacy was 15% Vermicompost and concentration beyond this might improve or decrease the disease incidence in pot conditions. Moreover, many of the organic amendments are harboring such antagonistic fungi and bacteria as they were successfully isolated in the study. This clearly indicates that we can substitute chemical fungicides with well decomposed organic amendments. However, the potential for phytotoxic effects with amendments such as fish amino acid necessitates careful selection.

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