



P-ISSN: 2349-8528

E-ISSN: 2321-4902

[www.chemijournal.com](http://www.chemijournal.com)

IJCS 2020; 8(2): 1765-1771

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Received: 19-01-2020

Accepted: 21-02-2020

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## Evaluation of potential fungicides, botanicals, biocontrol agents and their combination against *M. phaseolina* inciting root rot of sesame

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i2aa.9016>

### Abstract

The most effective fungicide (vitavax power), potential antagonist/ biocontrol agent (*T. viride*) and botanical (Neem oil) which were obtained during poisoned food technique and dual culture technique under *in vitro* conditions were further tested to know their individual as well as combined effect on seedling emergence, plant growth parameters and root rot incidence in potculture with susceptible variety (VRI-1) in *M. phaseolina* inoculated soil. The results indicated that combined seed treatment with *P. fluorescens*, neem oil and vitavax power recorded maximum germination percentage (93.33%), root (5.70 cm) and shoot length (11.9 cm), dry weight (0.28 g plant<sup>-1</sup>) and maximum percentage reduction of dry root rot (71.38%) followed by seed treatment with *P. fluorescens* and vitavax power (64.11%) and least reduction in root rot incidence (16.07%) observed in sesame seeds treated with only neem oil.

**Keywords:** *Pseudomonas fluorescens* and vitavax power, root rot, *Macrophomina phaseolina*

### Introduction

Sesame (*Sesamum indicum* L.) is an important oil seed crop preferred due to rich its edible oil content (about 50%) and nutritious protein (about 23%) and having sufficient carbohydrate (15%) (Ranganatha *et al.*, 2012) [25]. The medicinal value of sesame seeds are accepted worldwide due to the rich source of linolic acid, Vitamin E, A, B1 and B2 (Brar and Ahuja, 1979) [4]. In India, sesame crop occupies an area of 1.77 Mha with production of 8.27 lakh tonnes and productivity of 426 kg ha<sup>-1</sup>. In united Andhra Pradesh it is grown over 0.97 lakh ha with a production of 0.27 lakh tonnes and productivity of 341 kg ha<sup>-1</sup>, (Indiastat, 2014-15).

Sesame (*Sesamum indicum* L.) is under constant threat to many diseases. Among these root rot/stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid (= *Rhizcotonia bataticola*) is the most important disease of sesame in India (Chattopadhyay and Sastry, 1998) [6]. It has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations (Saharan *et al.*, 2005) [27]. The pathogen is responsible for seed rot, seedling decay, stem and root rot problem in sesame (Kolte, 1985; Verma *et al.*, 2005) [16, 32] and also resulted in poor seedling development and 5-100% yield loss of the crop (Aly *et al.*, 2006) [30]. The most common symptom of the disease is the sudden wilting of plants throughout the crop growth, mainly after the flowering phase. Due to severe infection, the stem becomes black and the roots rot shredding the bark exposing large number of black, minute sclerotia on the affected portions. The present investigation was carried out under glass house conditions to assess the potential effect of fungicides, botanicals, biocontrol agents and their combination against *M. phaseolina*.

### Materials and Methods

The most effective fungicide (vitavax power), potential antagonist/biocontrol agent (*T. viride*) and botanical (Neem oil) which were obtained during poisoned food technique and dual culture technique under *in vitro* conditions were further tested to know their individual as well as combined effect on seedling emergence, plant growth parameters and root rot incidence in potculture with susceptible variety (VRI-1) in *M. phaseolina* inoculated soil.

### Mass multiplication of the pathogen

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multiplied on sorghum grains. One hundred grams of sorghum seeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with 2-3 discs of 4 day old culture of *M. phaseolina* and incubated at  $28 \pm 1$  °C for seven days in BOD incubator. After seven days, the inoculum was mixed with sterilized soil in pots at five per cent level (w/w).

The seeds of sesame were imposed with the following treatments and sown in earthen pots of size 12 inch diameter which were filled with steam sterilized soil and inoculated with the soil borne pathogen *M. phaseolina* at five per cent level (w/w) which was mass multiplied on sorghum grains earlier.

#### I. Seed treatment with Vitavax Power

The seeds of sesame cv VRI-1 were surface sterilized in 5% sodium hypochlorite for 2 min then thoroughly washed in sterilized distilled water and air dried under aseptic conditions. These seeds were treated with Vitavax Power @ 2 g kg<sup>-1</sup> seed using gum (5 ml kg<sup>-1</sup>) as sticker and the treated seeds were used for sowing.

#### II. Seed treatment with *T. asperellum* / *P. fluorescens*

The talc based bio-control agents *T. asperellum* @ 4 g kg<sup>-1</sup> seed and *P. fluorescens* 10 g kg<sup>-1</sup> seed were used for treating the surface sterilized seeds by using gum (5ml kg<sup>-1</sup>) as sticker. The treated seeds were spread over a clean paper and dried in a cool shady place. The seeds were sown immediately after drying.

### III. Combined seed treatment with fungicides, botanical and biocontrol agent

For treatments involving combinations of fungicide, biocontrol agent and botanical, the surface sterilized seeds were first treated with Vitavax Power, followed by neem oil, and then mixed with *Trichoderma asperellum* and *Pseudomonas fluorescens* at recommended doses using gum as sticker. The treated seeds were shade dried over a clean paper and used for sowing.

The experiment was conducted in completely randomized block design with twelve treatments and replicated three times. Data on seedling emergence was taken on 10 days after sowing. Per cent disease incidence and plant growth characters viz., shoot length, root length, shoot and root dry weights were recorded on 40 days after sowing and vigour index was also calculated. The details of treatments are mentioned in Table 1.

Germination Percentage (GP) was calculated using the formula

$$GP = \frac{D}{S} \times 100\%$$

Where,

D = Number of germinated seeds on the tenth day

S = Number of total seeds sown

Vigour Index was calculated

Vigour index-I = % Germination x Seedling growth (shoot length + root length)

Vigour index-II = % Germination x Seedling dry matter

Per cent disease incidence (PDI) was calculated following the formula given below

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

**Table 1:** Details of the treatments imposed for Sesame root rot management under green house conditions

Treatment Number	Treatments
T <sub>1</sub>	Potential Fungal Antagonist ( <i>Trichoderma viridae</i> @ 4 g kg <sup>-1</sup> )
T <sub>2</sub>	Potential Bacterial Antagonist ( <i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> )
T <sub>3</sub>	Effective Botanical (Neem oil @ 10ml kg <sup>-1</sup> )
T <sub>4</sub>	Effective Fungicide (Vitavax Power @ 2 g kg <sup>-1</sup> )
T <sub>5</sub>	<i>Trichoderma viridae</i> @ 4 g kg <sup>-1</sup> (T <sub>1</sub> ) + <i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> (T <sub>2</sub> )
T <sub>6</sub>	<i>Trichoderma asperellum</i> @ 4g kg <sup>-1</sup> (T <sub>1</sub> ) + Neem oil @ 10 ml kg <sup>-1</sup> (T <sub>3</sub> )
T <sub>7</sub>	<i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> (T <sub>2</sub> ) + Neem oil @ 10 ml kg <sup>-1</sup> (T <sub>3</sub> )
T <sub>8</sub>	<i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> (T <sub>2</sub> ) + Vitavax Power @ 2 g kg <sup>-1</sup> (T <sub>4</sub> )
T <sub>9</sub>	Neem oil @ 10 ml g kg <sup>-1</sup> (T <sub>3</sub> ) + Vitavax Power @ 2 g kg <sup>-1</sup> (T <sub>4</sub> )
T <sub>10</sub>	<i>Trichoderma viridae</i> @ 4 g kg <sup>-1</sup> (T <sub>1</sub> ) + <i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> (T <sub>2</sub> ) + Neem oil @ 10 ml kg <sup>-1</sup> (T <sub>3</sub> )
T <sub>11</sub>	<i>Pseudomonas fluorescens</i> @ 10 g kg <sup>-1</sup> (T <sub>2</sub> ) + Neem oil @ 10 ml kg <sup>-1</sup> (T <sub>3</sub> ) + Vitavax Power @ 2 g kg <sup>-1</sup> (T <sub>4</sub> )
T <sub>12</sub>	Uncoated seed (Control) + pathogen

#### Statistical Analysis

The data obtained in different experiments were statistically analyzed following completely randomized block design (CRD) as per the procedures suggested by Snedecor and Cochran (1967) [31] and Panse and Sukhatme (1978) [23]. The data pertaining to percentage were subjected to angular transformation wherever necessary.

#### Results and Discussions

##### a. Effect on Germination percentage

The data on germination was recorded ten days after sowing. It is evident from the results presented in Table 2 and figure 1 that germination percentage was significantly higher in all the treatments as compared to control (56%). Maximum germination (93.33%) was observed in combined treatment of *P. fluorescens* + neem oil + vitavax power followed by seed treatment with *P. fluorescens* + vitavax power (87.2%) and

the minimum germination was recorded in treatment with only neem oil (62.8%).

#### b. Effect on Growth Parameters Dry weight

The results (Table 3) recorded that there was a significant increase in dry weight of plants by all the treatments when compared to control (0.15 g plant<sup>-1</sup>) and the increase in dry weight ranged from (0.179 to 0.28 g plant<sup>-1</sup>). Combined seed treatment with *P. fluorescens*, neem oil and Vitavax power recorded maximum percentage increase in dry weight (86.66%) followed by combined seed treatment with *P. fluorescens* and Vitavax power (73.33%) and the minimum increase was observed in seed treatment with neem oil (19.33%) compared to control plants.

#### c. Effect on Root Length

It is evident from the Table 3 that root length was significantly increased in all the treatments when compared to control (3.10 cm). Increase in root length ranged from 3.45 to 5.70 cm. Combined seed treatment with *P. fluorescens*, neem oil and vitavax power recorded maximum percentage increase in root length (83.87%) followed by seed treatment with *P. fluorescens* and vitavax power (77.41%) compared to control and the least per cent increase was observed in neem oil treated seeds (11.29%).

Increased growth response induced by micro flora is might be either due to antagonistic activity and biological control of plant pathogens in the soil or due to growth regulatory metabolites produced microbial agents in the soil (Kleopfer and Schroth, 1981) [15]. Thus the rate of germination and dry weight of root and shoot were increased (Windham *et al.* 1986) [34]. Similar reports of increase in vegetative growth using fungicide and *Trichoderma* spp. was also recorded in greengram (Rajeswari *et al.*, 1999) [24], in chilli (Mahmohndas and Sivaprakasam, 1994) [19], and in tomato (Manoranjitham *et al.*, 2001) [21].

#### d. Effect on Dry Root Rot Incidence

Data on dry root rot disease incidence was recorded at 40 DAS and the results are presented in the Table 4 and figure 2. The results indicate that individual as well as combined seed treatment with *T. viride*, neem oil and vitavax power significantly reduced dry root rot incidence ranging from 16.03 to 47 per cent compared to control (56%). Combined seed treatment with *P. fluorescens*, neem oil and vitavax power recorded maximum percentage reduction of dry root rot (71.38%) followed by seed treatment with *P. fluorescens* and vitavax power (64.11%) and least reduction in root rot incidence (16.07%) observed in sesame seeds treated with only neem oil.

In the present study the germination percentage, plant growth parameters significantly increased and percentage of dry root rot incidence was significantly reduced when fungicide, potential biocontrol agent and botanical were used in combination. These results are in agreement with the results obtained by previous workers. Mallaiah and Krishna Rao (2016) [20] reported that in pot culture studies combined sesame seed treatment with *T. viride*, *P. fluorescens*, Neem oil and thiram seed was found to be highly effective in increasing seedling emergence (91.3%), plant dry weight (98.6%), shoot length (73.9%) and root length (70.8%). Seed treatment with all four combinations was found to be superior in reducing dry root rot incidence (87.8%).

Vyas (1994) [33] reported that root rot of soybean caused by *Macrophomina phaseolina* can be effectively controlled by integration of carbendazim with *T. harzianum* (or) *T. viride*. Jayasree *et al.* (2000) [13] reported that maximum root length and shoot length of sesame and black gram were recorded in combined seed treatment and soil application of *T. harzianum* and *P. fluorescens*.

Kishan *et al.* (1999) [14] observed that combination of fungicide (soil application) and conidia (seed coating) reduced the disease to 5% in his studies on management of sesame dry root-rot *Rhizoctonia bataticola* (Taub.) Butl. through biological and chemical means.

Animisha *et al.* (2012) [2] observed that chickpea wilt incited by *Fusarium oxysporum* can be effectively controlled by integration of *T. viride*, carbendazim and neem cakes. Combined application of carbendazim, *T. viride* and *P. fluorescens* were superior in management of Pigeon pea wilt disease caused by *Fusarium udum* var *cajani* (Mahesh *et al.* 2010) [18]. Combination of PBP 4G (*T. viride*) for soil application and Pusa 5SD (*T. harzianum*) for seed treatment together with fungicide carboxin, provided the highest seed germination, shoot and root lengths and grain yield with the lowest incidence of wilt in chickpea under field conditions (Dubey *et al.* 2013) [9].

Sindhan *et al.* (2002) [29] reported satisfactory disease control when *Pseudomonas fluorescens* was used as seed treatment along with carbendazim against *Macrophomina phaseolina* in chickpea. It is attributed that carbendazim could arrest the pathogen and antagonists could parasitize the pathogen and promote growth by secreting growth promoting metabolites. Different workers have also been reported that *P. fluorescens* was effective biocontrol agent for different fungal pathogens (Meyer *et al.*, 1992; Buysens *et al.*, 1996; Reimmann *et al.*, 1988; Singh *et al.*, 2006) [22, 5, 26, 30]. *P. fluorescens* could act as strong elicitors of plant defense reactions (M' Piga *et al.*, 1997) [17]. Recent studies imply that prior application of fluorescent *pseudomonads* strengthens host cell wall structure that results in restriction of pathogen invasion in plant tissue (Benhamou *et al.*, 2000; Chen *et al.*, 2000; Conrath *et al.*, 2002; Dwivedi and Johri, 2003) [3, 7, 8, 10].

The present findings are supported by earlier workers who also reported that integration of biocontrol agent with compatible fungicide gave significantly higher disease control in several crops than obtained by either biocontrol agent (or) fungicide. (Henis *et al.* 1978, Sawant and Mukhopadhyay, 1990) [11, 28].

This is particularly relevant for soil borne pathogens such as *M. phaseolina* where use of only fungicides for drenching the soil is not only deleterious to the environment but is also practically not feasible due to high cost as sesame is generally cultivated without much investment for crop production as well as protection.

#### Conclusions

The results indicated that germination percentage was significantly higher in all the treatments as compared to control (56%). Maximum germination (93.33%) was observed in combined treatment of *P. fluorescens* + neem oil + vitavax power followed by seed treatment with *P. fluorescens* + vitavax power (87.2%). Significant increase in dry weight of plants by all the treatments when compared to control (0.15 g plant<sup>-1</sup>) and the increase in dry weight ranged from (0.179 to 0.28 g plant<sup>-1</sup>). Combined seed treatment with *P. fluorescens*, neem oil and Vitavax power recorded maximum percentage increase in dry weight (86.66%). Increase in root length

ranged from 3.45 to 5.70 cm. Combined seed treatment with *P. fluorescens*, neem oil and vitavax power recorded maximum percentage increase in root length (83.87%), individual as well as combined seed treatment with *T. viride*, neem oil and vitavax power significantly reduced dry root rot incidence ranging from 16.03 to 47 per cent compared to control (56%). Combined seed treatment with *P. fluorescens*, neem oil and vitavax power recorded maximum percentage reduction of dry root rot (71.38%) followed by seed treatment with *P. fluorescens* and vitavax power (64.11%) and least

reduction in root rot incidence (16.07%) observed in sesame seeds treated with only neem oil.

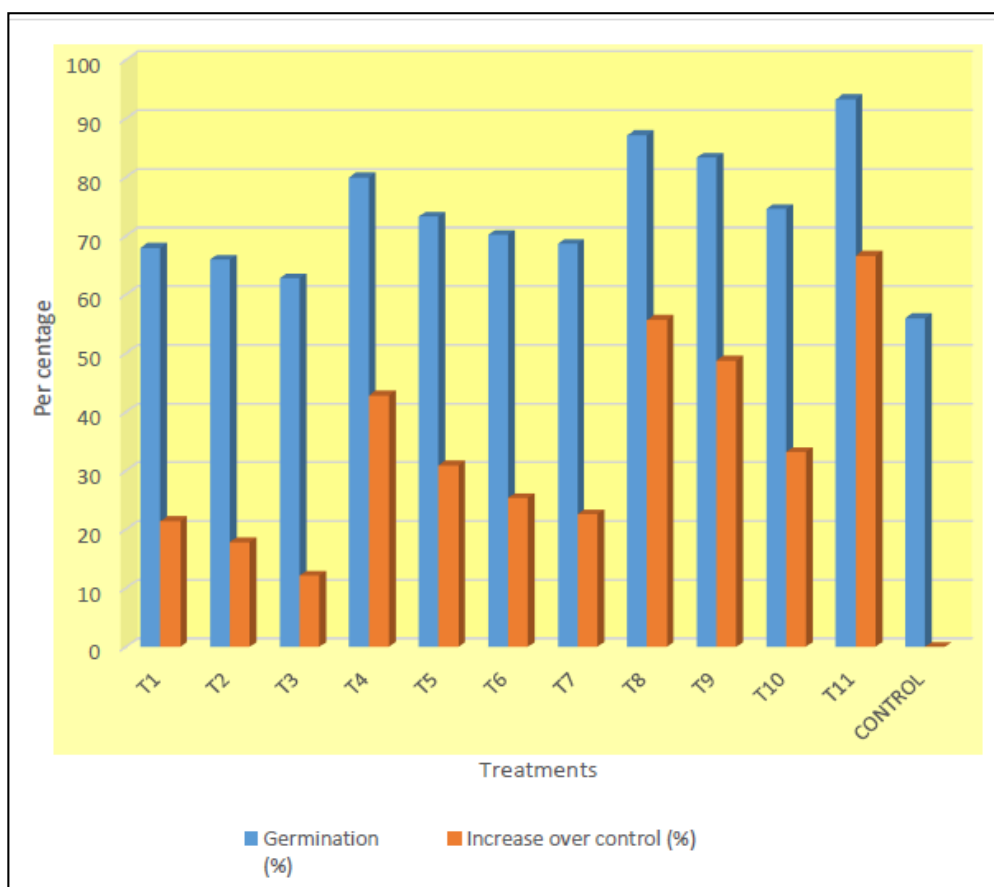
### Acknowledgements

The authors would like to acknowledge the support of Professor Jayashankar Telangana State Agricultural University and the Indian Institute of Oilseeds Research, Hyderabad, India for providing financial as well as lab and glasshouse facilities in conducting this experiment.

**Table 2:** Effect of seed treatment with effective biocontrol agents, botanical and fungicide on seedling emergence of Sesame cv. VRI-1 in *M. phaseolina* infested soil in greenhouse conditions

Treatment No.	Details of Treatment	Germination (%)	Increase over control (%)
T <sub>1</sub>	<i>Trichoderma viridae</i> (Tv1 @ 4 g kg <sup>-1</sup> ) + Pathogen	68 (55.54) (55.543)	21.42
T <sub>2</sub>	<i>Pseudomonas fluorescens</i> (pf1 @ 10 g kg <sup>-1</sup> ) + Pathogen	66 (54.32) (54.323)	17.85
T <sub>3</sub>	Neem oil (@ 10ml kg <sup>-1</sup> ) + Pathogen	62.8 (52.40)	12.14
T <sub>4</sub>	Vitavax power (@ 2 g kg <sup>-1</sup> ) + Pathogen	80 (63.45)	42.85
T <sub>5</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Pathogen	73.33 (58.91)	30.94
T <sub>6</sub>	<i>Trichoderma viridae</i> + Neem oil + Pathogen	70.2 (56.91)	25.35
T <sub>7</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	68.66 (55.95)	22.6
T <sub>8</sub>	<i>Pseudomonas fluorescens</i> + Vitavax power + Pathogen	87.2 (69.10)	55.71
T <sub>9</sub>	Neem oil + Vitavax power + Pathogen	83.33 (65.93)	48.8
T <sub>10</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	74.6 (59.74)	33.21
T <sub>11</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Vitavax power + Pathogen	93.33 (75.26)	66.66
T <sub>12</sub>	Inoculated control	56 (48.43)	
CD (p = 0.05)		3.69	
SE(m) ±		1.25	
CV (%)		3.64	

\* Figures in the parentheses are angular transformed values and are the means of three replication.



**Fig 1:** Effect of seed treatment with effective biocontrol agents, botanical and fungicide on seedling emergence of Sesame cv. VRI-1 in *M. phaseolina* infested soil in greenhouse conditions

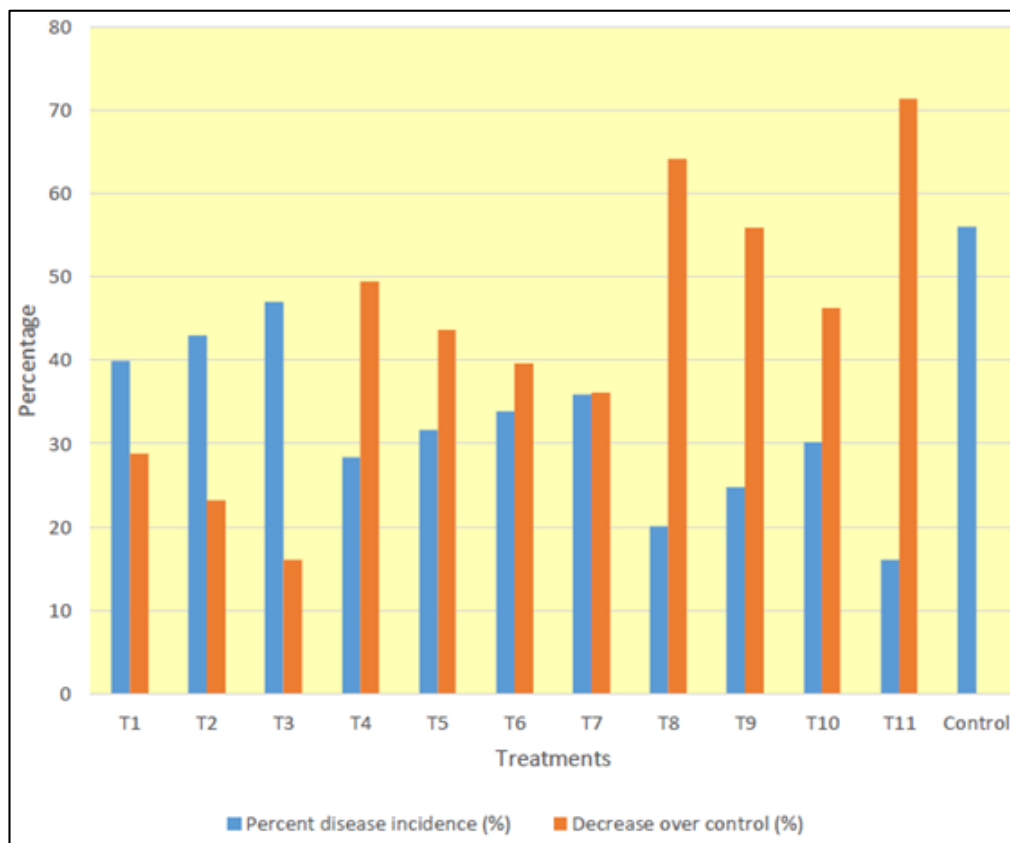
**Table 3:** Effect of seed treatment with effective biocontrol agents, botanical and fungicide on growth parameters of Sesame cv. VRI-1 in *M. phaseolina* infested soil in greenhouse conditions.

Treatment No.	Details of Treatment	Root Length (cm)	Increase over control (%)	Shoot Length (cm)	Increase over control (%)	Vigour index I	Dry Weight (g)	Increase over control (%)	Vigour index II
T <sub>1</sub>	<i>Trichoderma viridae</i> (Tv1 @ 4 g kg <sup>-1</sup> ) + Pathogen	4.12	32.9	8.50	34.49	1,089.36	0.185	23.33	12.58
T <sub>2</sub>	<i>Pseudomonas fluorescens</i> (pf1 @ 10 g kg <sup>-1</sup> ) + Pathogen	3.83	23.54	7.80	23.41	1,017.06	0.181	20.66	11.95
T <sub>3</sub>	Neem oil (@ 10ml kg <sup>-1</sup> ) + Pathogen	3.45	11.29	6.98	10.44	898.04	0.179	19.33	11.24
T <sub>4</sub>	Vitavax power (@ 2 g kg <sup>-1</sup> ) + Pathogen	5.00	61.29	10.55	66.93	1,244.00	0.230	58.00	18.40
T <sub>5</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Pathogen	4.75	53.22	9.95	57.43	1,096.28	0.215	43.33	15.77
T <sub>6</sub>	<i>Trichoderma viridae</i> + Neem oil + Pathogen	4.56	47.09	9.50	50.31	1,018.60	0.205	36.66	14.39
T <sub>7</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	4.32	39.35	8.95	41.61	948.881	0.190	26.66	13.04
T <sub>8</sub>	<i>Pseudomonas fluorescens</i> + Vitavax power + Pathogen	5.50	77.41	11.58	83.22	1,260.04	0.260	73.33	22.67
T <sub>9</sub>	Neem oil + Vitavax power + Pathogen	5.10	64.51	10.85	71.67	1,133.29	0.250	66.66	20.83
T <sub>10</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	4.85	56.45	10.20	61.39	943.69	0.220	46.66	16.41
T <sub>11</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Vitavax power + Pathogen	5.70	83.87	11.90	88.29	1,183.42	0.280	86.66	26.13
T <sub>12</sub>	Inoculated control	3.10		6.32		527.52	0.150		8.4
	CD (p = 0.05)	0.34		0.85		42.38	0.025		0.76
	SE(m) ±	0.11		0.29		14.43	0.009		0.26
	CV (%)	4.42		5.30		2.43	7.053		2.81

**Table 4:** Effect of seed treatment with effective biocontrol agents, botanical and fungicide on root rot incidence of Sesame cv. VRI-1 in *M. phaseolina* infested soil in greenhouse conditions

Treatment No.	Details of Treatment	Per cent disease incidence	Decrease over control (%)
T <sub>1</sub>	<i>Trichoderma viridae</i> (Tv1 @ 4 g kg <sup>-1</sup> ) + Pathogen	39.9 (39.16)*	28.75
T <sub>2</sub>	<i>Pseudomonas fluorescens</i> (pf1 @ 10 g kg <sup>-1</sup> ) + Pathogen	43 (40.96)	23.21
T <sub>3</sub>	Neem oil (@ 10ml kg <sup>-1</sup> ) + Pathogen	47 (43.26)	16.07
T <sub>4</sub>	Vitavax power (@ 2 g kg <sup>-1</sup> ) + Pathogen	28.33 (32.14)	49.41
T <sub>5</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Pathogen	31.6 (34.19)	43.57
T <sub>6</sub>	<i>Trichoderma viridae</i> + Neem oil + Pathogen	33.82 (35.54)	39.61
T <sub>7</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	35.8 (36.74)	36.07
T <sub>8</sub>	<i>Pseudomonas fluorescens</i> + Vitavax power + Pathogen	20.1 (26.62)	64.11
T <sub>9</sub>	Neem oil + Vitavax power + Pathogen	24.7 (29.79)	55.86
T <sub>10</sub>	<i>Trichoderma viridae</i> + <i>Pseudomonas fluorescens</i> + Neem oil + Pathogen	30.1 (33.26)	46.25
T <sub>11</sub>	<i>Pseudomonas fluorescens</i> + Neem oil + Vitavax power + Pathogen	16.03 (23.58)	71.38
T <sub>12</sub>	Inoculated control	56 (48.43)	
	CD (p = 0.05)	0.80	
	SE(m) ±	0.27	
	CV (%)	1.34	

\* Values in the parentheses are angular transformed and are the means of three replication



**Fig 2:** Effect of seed treatment with effective biocontrol agents, botanical and fungicide on root rot incidence of Sesame cv. VRI-1 in *M. phaseolina* infested soil in greenhouse conditions

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