



Morphological Studies and Its Biological Management Practices of *Fusarium* Wilt (*Fusarium oxysporum* F.sp. Lini) of Linseed

**Himendra Raj Raghuvanshi ^{a*}, Narendra Singh ^a,
Puskar Shukla ^a, Vivek Singh ^a and Utkarsh Upadhyay ^a**

^a Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology & Kanpur, PO- CSAUAT Campus, 208002, Uttar Pradesh, India.

Authors' contributions

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

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ABSTRACT

The experiment was carried out in a warehouse at the Department of Plant Pathology at C.S. Azad University of Agriculture and Technology in Kanpur with the aim of Isolation, purification, identification & pathogenicity of linseed wilt pathogen, integrated disease management practice against *Fusarium* wilt, soil colonizing ability of *Trichoderma viride* in sick field plots, Effect of different pH levels and different temperature levels on the growth and sporulation of the pathogen (*in-vitro*). The experiment used an RBD design On eleven cultivars out of eleven cultivars (Jeevan, Shweta, Parvati, Surbhi, Shekhar, NDL-2004-05, Kiran, RLC-92, Indira Alsi, Dipika, and Chambal) tested, the minimum percentage of wilting was recorded in cultivar RLC-92 (6.64 percent) followed

*Corresponding author: E-mail: raghuvanshihimendra@gmail.com;

by Jeevan (8.85 percent) and Indira Alsi (9.70 percent), respectively but all were at par statistically, with 13 treatments, by reducing plant wilting, all treatments were found to be noticeably better than the control (untreated) at reducing disease severity, among all treatments minimum percent wilting with maximum disease control was recorded with treatment T₄ [seed treatment (5g/kg seed) + soil treatment with *Trichoderma harzianum* (10g/kg soil)] followed by T₂ (5 g/kg seed) + soil treatments with *Trichoderma viride* (10 g/soil)] (19.46 percent) and T₁₂ (seed and soil treatment with carbendazin (0.2 percent) (23.50 percent), respectively under pot culture condition.

Keywords: Bio agents; extract; treatment; *Trichoderma*; wirehouse.

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.) is a member of the genus *Linum* in the family *Linaceae*. It is known as Tisi in Hindi, and Avishallu in Telugu. Linseed is a winter season crop usually grown for extracting oil and fibres, and used to make cloth (commonly called linen cloth). Linen cloth is much older than cotton and wool. Linseed is one of the oldest grown crops, its cultivation originated in Europe, about centuries ago. In India, linseed is commonly known as "Alsi".

The spread of the mechanical cotton gin in the early 1800s, in addition to being a fibre source, linseed was also an important oilseed crop. Linseed oil, squeezed out of linseed seed, which is used as a preservative finish on wood. Linseed oil is a "drying oil", as it can polymerize into a solid form, it is an edible oil but, because of its strong flavour and odour, is only a minor constituent of human nutrition. Linseed fibre is obtained from the stem of plants, from a blue flowered plant and woven into a fabric generally known as "Linen Linseed".

"Linseed is currently grown on about 12 million acres worldwide, with the majority of the production in northern Europe and Russia. In India linseed is mostly grown as oilseed crop on approximate area of 3.2 lakh ha with production of 1.74 lakh metric tons" (FAO STAT, 2019). It is cultivated in the temperate and sub-tropical environments as rainfed crop in the states of Madhya Pradesh, Chhattisgarh, Uttar Pradesh, Maharashtra, Rajasthan, Bihar, Odisha, Jharkhand, Karnataka and Assam that account for more than 97% of the total linseed area.

"The average yield of 544 kg/ha was found in 2018, very low compared to world average yield of 927 kg/ha and highest average yield of 1497

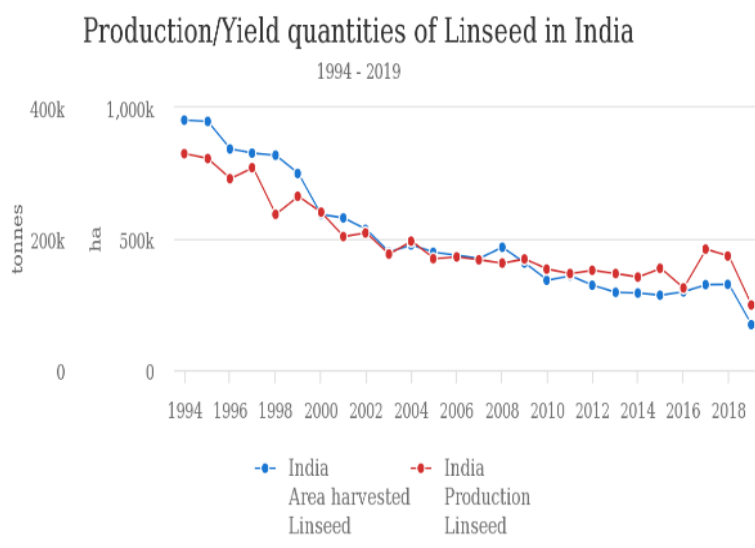
kg/ha in Canada" (FAOSTAT, 2019 <http://www.fao.org/statistics/en/>)

"At the end of year 2019, India produced nearly 100 thousand metric tons of linseed. This was a decrease from the previous financial year, which was 174 thousand metric tons. During financial year 2020, the south Asian nation produced over 33 million metric tons of oilseeds, among them India's production was nearly 110 thousand metric tons. The contribution of U.P in linseed production during FY 2019-20 was approx. 688 kg/ha" (Source: Directorate of Economics and Statistics, DAC & FW).

The important diseases damaging linseed crops in India are mainly fungal, causing crop losses up to 80-100 per cent under epidemic condition. Rust (*Melapsora lini* Erenb), Wilt (*Fusarium oxysporum* f.sp. *lini* (Bolley) Snyder & Hansen), Alternaria blight (*Alternaria lini* Dey, *A. linicola* Groves and Skolko), Powdery mildew (*Oidium lini* Skoric, *Leveillula taurica* Arnaud) are the major ones.

Wilt is one of the most serious diseases of linseed and has been reported from almost all the linseed growing countries of the world. The first report of this disease was by Luggar [1] from Minnesota, USA. He observed that the disease was transmitted through straw of linseed or water from infested fields flowing through non-infested field. Wilt of linseed is a soil borne disease.

"In India, the disease was first reported from Madhya Pradesh in 1923. Since then, it has been found in other linseed growing areas of the country" [2]. Sattar and Hafiz (1952) and Kumar et al. [3] reported losses up to 80 per cent under conditions favourable for wilt development. In the year 1952, it appeared in epidemic form at the Govt. research farm Kanpur'.



Production share of Linseed by region
Average 1994 - 2019

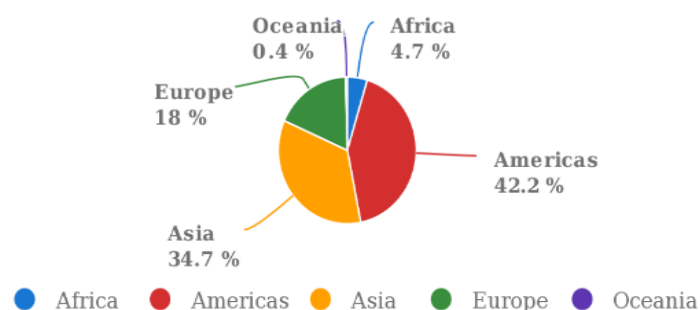


Image 1. Area wise distribution of Production share of Linseed
Image Source: (FAOSTAT, 2019<http://www.fao.org/statistics/en/>)

2. MATERIALS AND METHODS

2.1 Diseased Sample Collection

During the Rabi 2020-2021 Crop Research Farm Nawabganj, C.S. Azad University of Agriculture and Technology, Kanpur, naturally infected linseed plants with symptoms of wilt disease were collected. The infected plants were taken to the lab and thoroughly tested for the existence of the causative organism.

2.2 Cleaning and Sterilization of Glassware

All of the glassware used in this study was first cleaned with Sterilizing glassware by heat using

an autoclave, then with a 0.1 percent mercuric chloride solution, and finally with tap water.

2.3 Preparation of Cultural Media

The Singh and Chaubey [4] method was used to isolate the *Fusarium* wilt pathogen on potato-dextrose-agar medium. For preserving a pure culture of the wilt, a potato-dextrose-agar medium was used.

2.3.1 Potato-Dextrose-Agar medium (PDA medium)

For this analysis, a potato-dextrose-agar medium with the following composition was prepared using the method defined by Johnston and Booth [5] and Singh [6].

Table 1. Composition of potato-dextrose-agar medium

Ingredients	Gm/ml
Peeled potato	200.0 g
Dextrose	20.0 g
Agar agar	20.0 g
Distilled water to make volume	1000.0 ml

The potatoes were peeled and cut into 12 mm cubes. 200.00 g of potato cubes were rinsed and boiled in 500 mL water for 20 minutes. Cheese cloth was used to filter the potato broth. Agar was also melted in 500 mL of water by heating at the same time. The potato broth was then poured into the molten agar, along with dextrose.

By adding distilled water, the final volume was increased to 1000 ml. The pH level was raised to 7.0. While tubing, the stock solution was agitated to ensure that each sample had a proportion of solid matter. After that, it was sterilized in an autoclave at 15 psi for 20 minutes.

2.4 Sterilization of Metallic Objects

Before usage, metallic objects such as blades, forceps, and inoculation needles were sterilized by dipping them in spirit and heating them over a flame until

3. RESULTS AND DISCUSSION

3.1 Evaluation of Suitable Sowing Date to Manage the Wilt under Wilt-sick Field Condition

Management of disease through manipulation of date of sowing is a cheapest and best method of disease control. To find out the suitable date for the management of *Fusarium* wilt in linseed, linseed cultivar Chambal was sown in 7 different dates starting from 8 October, 2020 to 15 December, 2020 of 7 days intervals. The incidence of disease and seed yield was recorded and data were presented in Table 1.

Maximum wilting of plants (78.46 Per cent) was noted in 1st date (08-10-2020) of sowing which decreased with delayed sowing and minimum (16.10 Per cent) was recorded in December sown (19- 11-2020) crop. Significant difference in wilting percentage was noted in crops sown on different dates. Maximum yield of 930.45kg/ha was noted in crop sown on 12-11-2020 followed by crop sown on 29-10-2020 (778.41kg/ha) and 30-10-2020 (210.14kg/ha), respectively. The yield obtained from the crop sown on 12-11-2020

was significantly higher over other date of sowing while the yield obtained in the crops sown on 19th November, and 29th October, 2020 was at par. Sown in (Table-2).

Crop sown under wilt free conditions (normal condition) has been noted to yield highest under 5 November sowing, followed by 15 November [7]. Thus, 19 November seen to be an ideal date for sowing under wilt infested conditions. However, to spread the field operations over a reasonably longer period of time, period from 09 November to 19 November seems to be reasonably good as yields obtained these two dates are higher concurrent with present findings Singh and Singh [8] have also reported that sowing of linseed between 5 and 15 November in wilt sick field results in lower disease severity and higher seed yield. Maximum incidence of wilt was also reported in 20 October sown crop and minimum in 09 December sown by Kishore et al. [9] from Kanpur. They again recorded maximum yield in 19 November sown crops. This supports the present findings, Ved Ratan and Biswas [10] have also reported the higher level of wilt incidence in early sown (October sown) chickpea as comparison to late sowing.

3.2 Effect of Different pH Levels on the Growth and Sporulation of the Pathogen

The hydrogen ions concentration is also known to have considerable effect on the growth of the fungi. To find the optimum pH level the pathogen was grown on Potato dextrose medium (liquid) adjusted at different pH levels ranging from 4.5. to 8.5 After 10 days of incubation at 28±1°C, the mycelial weight was recorded in each treatment as per procedure. The data are presented in Table 3.

In the present study it was found that the pathogen grew over a wide range of pH i.e. from 4.5 to 8.5. Maximum mycelial growth was recorded at 6.5 pH level and minimum at pH 4.5. The results are in agreement with finding of Nair (1957) he reported that the incidence of wilt appeared to be more at soil pH 5.5 to 7.5 than at

higher and lower level. Similar result was also observed by Souramma and Singh [11] found that 6.5 to be the most suitable pH level for growth and sporulation of *Fusarium oxysporum* f. sp. *lini*.

It was evident from the data that, although the pathogen grew over a wide range of pH i.e. from 4.5 to 8.5, but the most suitable pH for its growth was observed to be 6.5 at which the maximum

growth of the fungus was recorded which was closely followed by 5.5. These two pH levels supported statistically similarly growth of the pathogen. However, with the increase of acidity or alkalinity. The growth of the fungus was hampered and lowest growth was recorded at pH 4.5. The excellent conidia were counted at 6.5 and 7.0 pH, good at 5.5 and 7.5 pH, whereas fair at 5.0 pH level and poor at 4.5 and 8.5 pH levels.

Table 2. Effect of date of sowing on disease severity and seed yield

Date of sowing	Date of disease appearance	Initial plant population	No. of wilted plant	Final plant population	% plant wilted	Seed yield/plot (Kg)	Seed yield (Kg/ha.)
08/10/2020	26/10/2020	495.62	352.32	482.23	78.46	118.02	200.14
15/10/2020	03/11/2020	523.89	291.93	514.07	62.33	131.53	289.17
22/10/2020	11/11/2020	517.73	223.14	509.26	47.92	152.86	336.79
29/10/2020	17/11/2020	503.07	77.90	494.08	28.30	353.49	778.41
05/11/2020	25/11/2020	441.35	313.52	436.15	77.89	93.25	259.13
12/11/2020	1/12/2020	522.52	64.47	512.71	34.53	419.17	930.45
15/12/2020	28/12/2020	513.75	167.19	504.34	16.10	215.03	474.08
SE(m)±		0.235	0.198	0.885	0.427	319.25	458.68
CD 5%		20.08	17.75	5.87	19.47	22.56	44.97

Table 3. Effect of different pH levels on growth and sporulation of *Fusarium oxysporum* f.sp. *lini* after 10 days of incubation

S. No.	pH	Dry weight of mycelium (mg)	Sporulation
1	4.5	106.49	Poor
2	5.0	108.71	Poor
3	5.5	129.86	Poor
4	6.0	173.43	Fair
5	6.5	248.13	Excellence
6	7.0	222.27	Excellence
7	7.5	205.20	Good
8	8.0	184.37	Good
9	8.5	122.44	Poor
SEm±	2.23		
CD at 5 %	5.74		



Fig. 1. Field view of variety Chambal sown at different dates

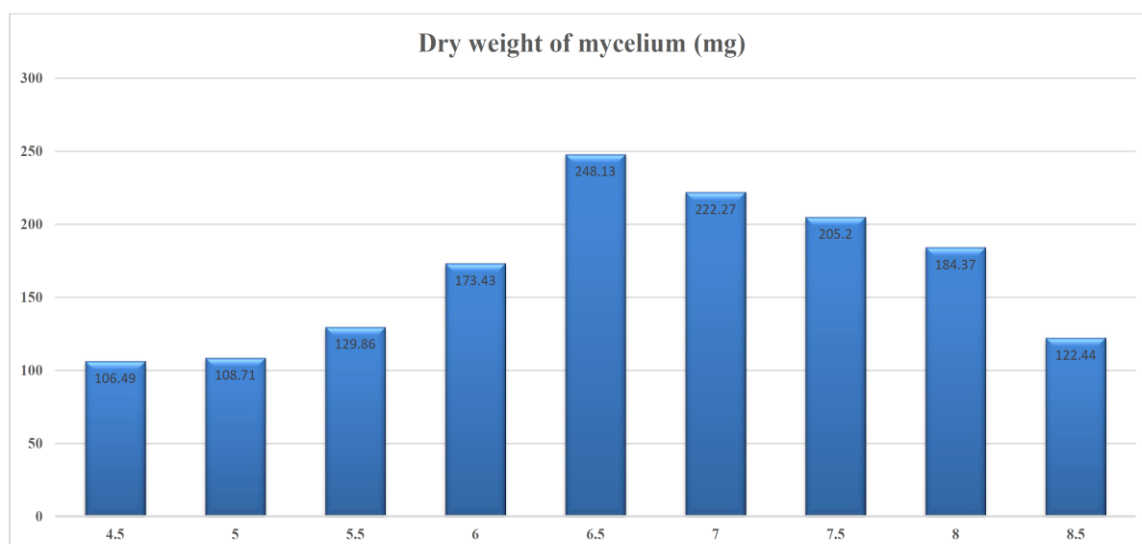


Fig. 2. Dry weight of mtcelium (mg)

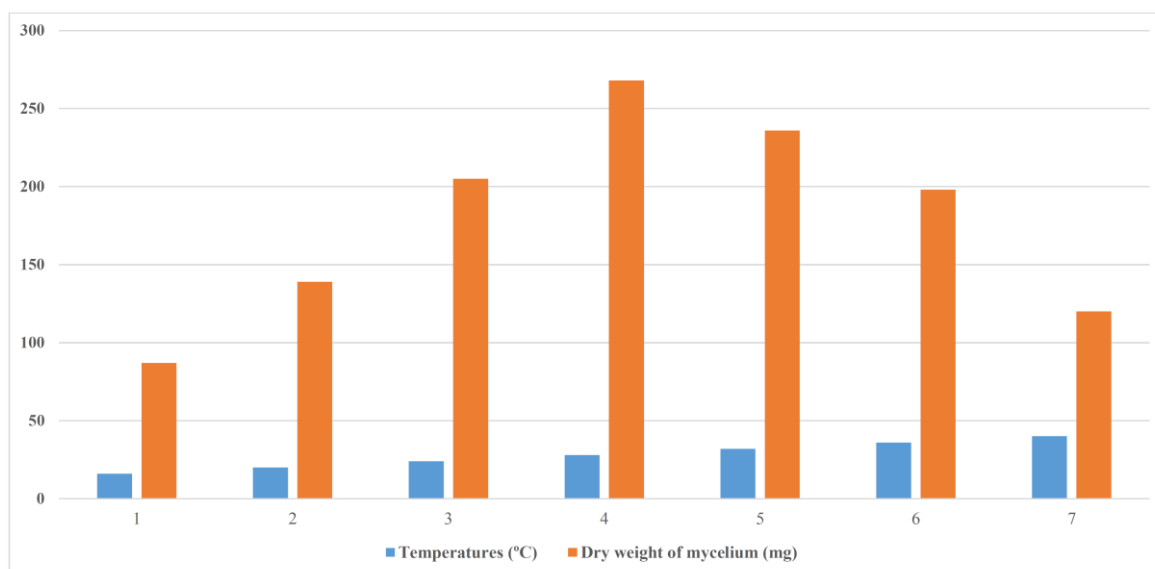


Fig. 3. Bar graph showing variation in dry weight of mycelium

Table 4. Effect of different temperatures on growth and sporulation of *Fusarium oxysporium* f.sp. lini after 10 days of incubation

S. No.	Temperatures (°C)	Dry weight of mycelium (mg)	Sporulation
1	16	87.00	Poor
2	20	139.00	Fair
3	24	205.00	Good
4	28	268.00	Excellence
5	32	236.00	Good
6	36	198.00	Fair
7	40	120.00	Poor
SEm±		1.14	
CD at 5 %		2.58	

3.3 Effect of Different Temperatures on the Growth and Sporulation of the Pathogen

It is well known that temperature influences the growth of all microorganisms. Present studies were, therefore, taken up to find out the optimum, minimum and maximum temperature requirements for the growth and sporulation of the test pathogen. In present investigation the fungus was grown on Potato dextrose medium and incubated at nine different temperatures ranging from 15°C to 40°C. The average dry mycelium weight was recorded in the Table 4.

Jones and Tisdale [12] observed that the optimum temperature, for linseed wilt pathogen lied between 75 to 82°F (24-28°C) and infection did not occur above 96.4°F (38°C). The present results are in agreement with their findings. Similar results were reported by Mc Rae [2] from India. However, Souramma and Singh [11] and Pal [13] also found 25°C to be the best temperature for growth and sporulation of *Fusarium oxysporum* f. sp. *lini*.

The observation recorded in Table 4 indicated that, although the pathogen could grow over a wide range of temperature i.e. 16°C to 40°C, but the optimum temperature for its growth was found to be 28°C followed by 24°C and 32°C and former two did not differ statistically from each other. The optimum temperature for growth was therefore 25°C to 30°C and good growth was recorded at 24°C and 32°C, however, the minimum growth was recorded at 38°C and 15°C. No growth and sporulation were observed at 40°C temperature.

3.4 To Test the Efficacy of Bio-agents/Plant Products to Manage the Disease under Pot Culture

In this study the efforts have been made to evaluate the active bio- agents/plant products as seed dressers and as a soil application in comparison to fungicide (Carbendazin) against the *Fusarium* wilt of linseed under sick pot culture. The experiment was conducted in pots under glass house condition, during 2020-21 by using susceptible cultivar Chambal.

Wilting of the plant started in control pots just 13 to 18 days after sowing while in treated pots soil,

wilting started after 30 to 35 days of sowing during. All the treatments were found significantly superior over check (untreated control) in controlling the disease severity by checking the wilting of plants. Minimum wilting of 19.46 Per cent were recorded with treatment T₄ [Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma harzianum*] followed by T₂ [(Seed (5g/kg seed) and soil treatment (10g/kg soil) with *Trichoderma viride*], (20.76 Per cent) and T₁₂ [Seed + soil treatment with carbendazin (0.2 Per cent)] (23.50 Per cent), respectively. All these treatments were found at par in controlling the disease severity. These treatments were also found statistically at par with treatments T₆ [Seed treatment + soil treatment with *Pseudomonas fluorescens* (10g/kg soil) (27.70)] and T₈ [Seed treatment + soil treatment with seed kernel extract of *Azadirachita indica* (10 Per cent w/v) (37.79 Percent)] respectively. Seed treatments with either *Trichoderma* spp. Or *Pseudomonas* spp. or with leaf extracts were less effective as compared to treatments of seed and soil both. Only seed treatments have no long-term effect on controlling the disease incidence. Maximum percent wilting of 82.29 Per cent were recorded in untreated pot. The treatments T₁ [Seed treatment with *Trichoderma viride* (5g/kg seed)], T₅ [Seed treatment with *Pseudomonas fluorescens* (10g/kg seed)], T₇ [Seed treatment with seed kernel extract of *Azadirachita indica* (10 Per cent w/v)] and T₉ [Seed treatment with extract of *Allium sativum* (10 Per cent w/v)] were found less effective in controlling the disease in comparison to others (Table-5).

Singh and Singh [14] also evaluated commercially grown cultivars namely Jawahar-23, Jeevan, Kiran, Padmini, R-552, Surbhi, Type-397 and Chambal against wilt disease under sick field condition for evaluation of their resistance and yield concurrent with present findings They have also reported Jeevan and Surbhi as resistant and moderately resistant respectively with higher yield. However, in present finding NDL- 2004-05 gave maximum yield (955.55kg/ha) and showed resistant reaction. The cultivar jeevan and Surbhi were reported resistant by Kishor et al. [8] from Faizabad also. These cultivars may be sown in wilt prone areas of different genotypes/cultivars by different workers time to time [15,16,17,18,19].

Table 5. Effect of treatment on plant population and severity of *Fusarium oxysporium* f.sp. lini during 2020-21

Treatments	Initial plant population average of 3 pots	Final plant Population average of 3 pot	% plant wilted
T ₁ : Seed treatment with <i>Trichoderma viride</i> (5g/Kg seed)	9.78	4.00	52.53
T ₂ : Seed treatment + soil treatment with <i>Trichoderma viride</i> (10g/kg soil)	9.18	7.24	20.76
T ₃ : Seed treatment with <i>Trichoderma harzianum</i> (5g/Kg seed)	9.42	3.89	53.70
T ₄ : Seed treatment + soil treatment with <i>Trichoderma harzianum</i> (10g/kg soil)	9.38	7.78	19.46
T ₅ : Seed treatment with <i>Pseudomonas fluorescens</i> (10g/Kg seed)	8.58	3.52	60.90
T ₆ : Seed treatment + soil treatment with <i>Pseudomonas fluorescens</i> (20g/kg soil)	9.45	6.65	27.70
T ₇ : Seed treatment with seed kernel extract of <i>Azadirachta indica</i> (10% w/v)	9.33	3.76	68.33
T ₈ : Seed treatment + soil treatment with seed kernel extract of <i>Azadirachta indica</i> (10% w/v)	10.28	6.82	37.79
T ₉ : Seed treatment with extract of <i>Allium sativum</i> (10% w/v)	9.76	2.45	78.23
T ₁₀ : Seed treatment + soil treatment with extract of <i>Allium sativum</i> 10% w/v)	10.58	4.52	50.00
T ₁₁ : Seed treatment with carbendazin (0.2%)	10.67	5.23	49.96
T ₁₂ : Seed treatment + soil treatment with carbendazin (0.2%)	10.36	7.64	23.50
T ₁₃ : Control	9.67	1.83	82.89
SEm±	0.72	0.48	4.72
CD at 5 %	N.S.	1.42	13.58



Fig. 4. Treatments carried out in different pots

4. CONCLUSION

Based on the above findings following conclusions were drawn Out of eleven cultivars tested, the minimum percentage of wilting was recorded in cultivar RLC-92 (6.64 percent) followed by Jeevan (8.85 percent) and Indra AlsI (9.70 percent), respectively. A maximum seed yield of 1170.250 kg/ha was recorded in cultivar RLC-92 followed by Dipika (935.480 kg/ha) and NDL-2004-05 (908.670kg/ha). Maximum wilting was 63.550 percent recorded in the 08 October

sown crop which gradually decreased with delayed sowing. Minimum disease severity of 40.340 percent was recorded on 15 December, sown crop. Maximum seed yield of 572.671 kg/ha was recorded on 13 November sown crop followed by 495.000 kg/ha sown on 21 November. The maximum growth and sporulation were observed at 28°C temperature followed by 24°C and 32°C, where no growth was recorded at 40°C. The maximum growth and sporulation were observed at 6.5 pH closely followed by 5.5 pH, whereas it was minimum at pH 4.5 Minimum

percent wilting with maximum disease control was recorded with treatment T4 [seed treatment (5g/kg seed) + soil treatment with *Trichoderma harzianum* (10g/kg soil)] followed by T2 [(5 g /kg seed) + soil treatments with *Trichoderma viride* (10 g/soil)] (19.46 percent) and T12 [seed and soil treatment with carbendazin (0.2 percent)] (23.50 percent), respectively under pot culture condition. Therefore, Biological control may suggested in good management practices with low cost effective, environment friendly as well as work only on the targeted (invasive) part of the plants. It can be readily incorporated into integrated fungal management (IFM) programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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