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Efficacy of Different Bioagents and Botanicals on Seed Borne Mycoflora of Onion (*Allium Cepa* L.)

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Abstract

During present investigation it is observed that Biocontrol agents are highly effective for the growth of seedlings of onion as well as for controlling many seed borne pathogens. Whereas Botanical extracts (*Azardirachta indica, Ocimum sanctum, Zingiber officinale, Allium sativum, Allium cepa, Eucalyptus globulus*) can be considered as an ecological approach in which selected fungal antagonist extracts were used against seed borne pathogens. Five seed borne fungi were identified and isolated from the seeds of onion internally and externally (*Alternaria alternata, Fusarium oxysporum, Penicillium* spp., *Aspergillus niger, Aspergillus flavus*) Biological seed treatments provide an alternative to chemical control with additional benefits of induced diseases resistance, ecofriendly nature and sustainable disease management. Onion (*Allium cepa* L.) seeds are considered susceptible to seed-borne pathogens, causing significant yield losses. The investigation depicts that biocontrol agents and botanical extracts are effective for fungal control, effective seed germination and increased seedling vigour index. Biocontrol agents such as MPKV fungal consortia, Trichoderma viride, *Trichoderma harzianum, Pseudomonas fluorescens + Trichoderma viride* and *Pseudomonas fluorescens + Trichoderma harzianum* were investigated in which MPKV fungal consortia @ 1% conc. was observed as most effective followed by MPKV fungal consortia @0.5% conc. and *Pseudomonas fluorescens + Trichoderma harzianum*. Whereas in case of botanical extracts, the *Allium sativum* clove extract was recorded as more effective against seed borne mycoflora followed by *Azardirachta indica* leaf extracts with remarkable decrease in incidence of fungi, increased seed germination percentage and increased seedling vigour index.

Key words : Biocontrol agents, Onion, Seed-borne pathogens, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Fungal consortia*.

Onion (*Allium cepa* L.) is a biennial, bulbous, vegetable crop (2n = 16). It belongs to the family Alliaceae. The seed is monocotyledonous and usually cross pollinated. The primary centre of origin of onion is Central Asia while the near East and Mediterranean are the secondary centre of origin (Vavilov, 1951). It was introduced in India from Palestine. Onion is a popular vegetable grown for its pungent bulbs and flavourful leaves. It is important pulse crop native of India. Optimum temperatures for plant development are between 13 and 24°C, although the range for seedling growth is narrow, 20- 25°C. Onion is used in almost every kitchen because of its highly valued pungency and nutrition and is also considered as "Queen of the Kitchen" (Selvaraj, 1976; Griffiths *et al*., 2002). Onion contains a volatile compound "Allyl propyl disulfide" which causes the pungency in onion. The bulb is composed of certain macronutrients such as carbohydrates (9.3 g), protein (1.1 g), Fiber (1.7 g) and about 40 calories per 100 g of raw onion. It also contains various vitamins and minerals such as vitamin C (7.4 mg), Vitamin B6 (0.1 mg), Folate (19 µg), small amounts of vitamin A, E and K, potassium (146 mg), calcium (23 mg), magnesium (10 mg), phosphorus, sodium, zinc and manganese in smaller quantities per 100 g of onion. Various species of fungi have been determined in onion seeds produced in different climatic regions. They include Botrytis allii, *B.*

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cinerea, *Fusarium oxysporum, F. solani, F. moniliforme, F. avenaceum, F. moniliforme* var. subglutinans, *Alternaria alternata, A. porri, Aspergillus niger, A. flavus, A. fumigatus, Stemphylium botryosum, S. vesicarium, Penicillium* sp. and *Pythium* sp. (Köycü and Ozer, 1997). To treat different seed borne fungi different types of fungicides, biocontrol agents and botanicals are used. As seed treatment for fungi is one of the oldest methods of plant protection, this seed treatment with different bioagents and fungicides are also used to control the seed borne mycoflora. Seed treatment with different fungicides prevent the formation of mycoflora around the seed and also protects the plant from different seed borne and soil borne diseases of fungi.

Materials and Methods

Present investigation was carried out to study the effect of biocontrol agents and botanical extracts on different seed borne fungi of onion in the Seed Pathology Laboratory, Department of Plant Pathology and Microbiology, MPKV, Rahuri. The experimental seed crop was raised during kharif 2022-2023 in glasshouse conditions. Seeds of onion variety Phule Samarth was selected for the investigation. Detection of externally seed borne mycoflora in onion were done by ISTA's standard blotter test described by Neergard (1979), whereas the internally seed borne mycoflora associated with seeds of onion were detected by ISTA's standard agar plate test. As soon as fungal colonies found on seeds of onion on plates were transferred aseptically on potato dextrose agar (PDA) slants with the help of sterilized inoculating needle under aseptic condition. The isolates were maintained in pure form on potato dextrose agar slants, labelled carefully and preserved at low temperature in refrigerator. The seeds were inoculated with fungi by dipping the seed in concentrated suspension of spores/active hyphae of important seed mycoflora of onion sees for 12 hours and then seeds were dried under shade for 12 hours. The fungi found pathogenic were preserved in pure form on PDA slants and identified up to species level with the help of published literature (Booth, 1971, Barnet and Barry, 1972). The effect of pathogenic seed borne mycoflora isolated from seeds of onion on seed germination and seedling vigour index was studied by adopting the procedure of 'Between Paper' (rolled towel paper) method as described by ISTA's rules (Anonymous, 1999).

SVI = [Mean root length + Mean shoot length] (no unit) x Seed germination (%)

The data was subjected to statistical analysis (Panse and Sukhatme, 1985). Talc based formulations of the bioagents were weighted as per the required dose and mixed with seeds of onion. The material was slightly moistened with sterilized water, shakes slightly so as to cover the entire seed surface by bioagent and was used for germination and blotter test and the crude extracts of desired concentration were taken with the help of sterilized pipette and treated by smearing on surface of the seeds, then these were used for seed germination and blotter test.

The observations such as Incidence of seed mycoflora on artificially inoculated seeds, their germination percentage and Seedling vigour index are taken.

Mentioned in the tables below:

Bioagents :

Treatments	**Conc. (%)**
MPKV Fungal Consortia	0.5
MPKV Fungal Consortia	1.0
Trichoderma viride	0.5
Trichoderma harzianum	0.5
Pseudomonas fluorescens + T. viride	0.5
Pseudomonas fluorescens + T. harzanium	0.5
Control	-

Botanical Extracts and their Parts used

Treatments	Parts used	Conc. (%)
Azardirachta indica	Leaf	10
Ocimum sanctum	Leaf	10
Zingiber officinale	Rhizome	10
Allium sativum	Clove	10
Allium cepa	Bulb	10
Eucalyptus globulus	Leaf	10
Control	-	-

Results and Discussion

Five seed borne mycoflora namely, *Fusarium oxysporum, Alternaria alternata, Penicillium* spp. *Aspergillus niger* and Aspergillus flavus were identified and isolated from the incubated inion seeds. The results of internally seedborne mycoflora associated with onion seeds showed that three seedborne mycoflora viz., Fusarium oxysporum and Alternaria alternata were found internally associated with onion seeds. All of the pathogens detected were found to be pathogenic to onion seeds. Seed rotting, post-emergence damping-off of seedlings and black, brown-grey, greenish-blue, or greenish fungal growth on seed and seedlings were observed symptoms of pathogenic organisms. All the seed borne mycoflora of onion seeds were pathogenic and produced the different symptoms on seeds. The seeds infected with Fusarium oxysporum showed the whitish fungal growth on seeds and caused rotting of seeds. The Alternaria alternata grey to black mycelial growth on seeds followed by rotting of seedlings and seeds. The *Penicillium* spp. showed the seeds covered with blue to green mycelium and spores, followed by rotting of seeds. The *Aspergillus niger* produced the symptoms like black fungal growth on seeds and browning of the seedling shoots. *Aspergillus flavus* showed the greenish fungal mycelial growth on seed along with spores and seed rotting. All the

Table 1. Efficacy of bioagents on *Alternaria alternata and their effect on seed germination and seedling vigour index of onion* seeds

Treatments	Incidence of *A. alternata* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
MPKV Fungal Consortia (0.5%)	16.63 (24.06)	67.56	78.75 (62.54)	19.77	687.10	33.98
MPKV Fungal Consortia (1.0%)	13.25 (21.34)	74.15	82.25 (65.08)	25.10	715.58	39.53
Trichoderma viride (0.5%)	24.63 (29.75)	51.95	72.00 (58.05)	9.51	570.60	11.26
Trichoderma harzianum (0.5%)	20.50 (26.92)	60.00	74.75 (59.83)	13.69	604.30	17.83
Pseudomonas fluorescens + *Trichoderma viride* (0.5%)	18.50 (25.47)	63.90	76.25 (60.83)	15.97	650.33	26.81
Pseudomonas fluorescens + *Trichoderma harzianum* (0.5%)	17.63 (24.82)	65.61	77.50 (61.68)	17.87	674.25	31.47
Control	51.25 (45.71)	-	65.75 (54.18)	-	512.85	-
S. Em ±	0.30		0.52		4.64	
CD @ 1%	0.88		1.53		13.66	
C. V. (%)	2.58		1.38		1.47	

Figures in parantheses indicates arc sin transformed values

Table 2. Efficacy of botanicals on *Alternaria alternata* and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *A. alternata* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
Azardirachta indica leaf extract (10%)	22.50 (28.31)	51.61	80.00 (63.43)	24.03	621.93	26.87
Ocimum sanctum leaf extract (10%)	24.50 (29.66)	47.31	77.50 (61.68)	20.16	602.68	22.94
Zingiber officinale rhizome extract (10%)	25.75 (30.49)	44.62	75.50 (60.33)	17.05	586.98	19.74
Allium sativum clove extract (10%)	19.00 (25.84)	59.14	84.00 (66.42)	30.23	648.90	32.37
Allium cepa bulb extract (10%)	28.50 (32.26)	38.71	72.25 (58.21)	12.02	561.80	14.61
Eucalyptus globulus leaf extract (10%)	29.50 (32.89)	36.56	70.00 (56.78)	8.53	544.25	11.03
Control	46.50 (42.99)	-	64.50 (53.42)	-	490.20	-
S. Em ±	0.37		0.48		6.35	
CD @ 1%	1.09		1.42		18.68	
C. V. (%)	2.66		1.29		2.19	

Figures in parantheses indicates arc sin transformed values

Table 3. Efficacy of bioagents on Fusarium oxysporum and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *F. oxysporum* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
MPKV Fungal Consortia (0.5%)	12.00 (20.26)	67.57	83.25 (65.84)	38.17	684.75	43.86
MPKV Fungal Consortia (1.0%)	6.25 (14.47)	83.11	88.00 (69.73)	46.06	721.60	51.50
Trichoderma viride (0.5%)	27.63 (31.70)	25.34	68.50 (55.85)	13.69	554.85	16.57
Trichoderma harzianum (0.5%)	25.25 (30.16)	31.76	75.50 (60.33)	25.31	611.55	28.48
Pseudomonas fluorescens + Trichoderma viride (0.5%)	20.88 (27.18)	43.58	76.25 (60.83)	26.56	625.25	31.36
Pseudomonas fluorescens + Trichoderma harzianum (0.5%)	12.75 (20.92)	65.54	83.25 (65.84)	38.17	682.65	43.42
Control	37.00 (37.46)	-	60.25 (50.91)	-	475.98	-
S. Em ±	0.40		0.56		4.79	
CD @ 1%	1.18		1.66		14.10	
C. V. (%)	3.97		1.48		1.54	

Figures in parantheses indicates arc sin transformed values

Table 4. Efficacy of botanicals on *Fusarium oxysporum* and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *F. oxysporum* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
Azardirachta indica leaf extract (10%)	15.00 (22.78)	60.00	74.50 (59.67)	19.68	603.45	22.71
Ocimum sanctum leaf extract (10%)	21.63 (27.71)	42.33	74.00 (59.34)	18.88	599.40	21.88
Zingiber officinale rhizome extract (10%)	22.50 (28.31)	40.00	72.50 (58.37)	16.47	587.25	19.41
Allium sativum clove extract (10%)	11.25 (19.59)	70.00	78.25 (62.20)	25.70	633.83	28.88
Allium cepa bulb extract (10%)	24.63 (29.75)	34.33	69.50 (56.47)	11.65	562.95	14.47
Eucalyptus globulus leaf extract (10%)	25.50 (30.32)	32.00	67.00 (54.93)	7.63	542.70	10.35
Control	37.50 (37.76)	-	62.25 (52.09)	-	491.78	-
S. Em ±	0.41		0.60		4.87	
CD @ 1%	1.20		1.77		14.32	
C. V. (%)	3.63		1.69		1.69	

Figures in parantheses indicates arc sin transformed values

Table 5. Efficacy of bioagents on *Penicillium* spp. and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *Penicillium* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
MPKV Fungal Consortia (0.5%)	8.63 (17.07)	78.70	81.25 (64.34)	35.98	706.88	49.75
MPKV Fungal Consortia (1.0%)	7.50 (15.89)	81.48	86.75 (68.65)	45.19	754.73	59.89
Trichoderma viride (0.5%)	21.63 (27.71)	46.60	69.75 (56.63)	16.74	563.25	19.33
Trichoderma harzianum (0.5%)	15.75 (23.38)	61.11	76.75 (61.17)	28.45	619.80	31.31
Pseudomonas fluorescens + *Trichoderma viride* (0.5%)	12.00 (20.26)	70.37	75.50 (60.33)	26.36	656.85	39.15
Pseudomonas fluorescens + *Trichoderma harzianum* (0.5%)	9.88 (18.31)	75.62	78.00 (62.02)	30.54	678.60	43.76
Control	40.50 (39.52)	-	59.75 (50.62)	-	472.03	-
S. Em ±	0.35		0.56		5.17	
CD @ 1%	1.05		1.65		15.20	
C. V. (%)	4.33		1.49		1.62	

Figures in parantheses indicates arc sin transformed values

Table 6. Efficacy of botanicals on *Penicillium* spp. and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *Penicillium* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
Azardirachta indica leaf extract (10%)	15.75 (23.38)	60.87	79.25 (62.90)	25.79	639.93	28.58
Ocimum sanctum leaf extract (10%)	17.63 (24.82)	56.21	75.00 (60.00)	19.05	605.65	21.69
Zingiber officinale rhizome extract (10%)	20.13 (26.65)	50.00	72.00 (58.05)	14.29	581.40	16.82
Allium sativum clove extract (10%)	11.00 (19.36)	72.67	79.50 (63.07)	26.19	641.98	28.99
Allium cepa bulb extract (10%)	22.63 (28.40)	43.79	69.75 (56.63)	10.71	563.23	13.17
Eucalyptus globulus leaf extract (10%)	23.63 (29.08)	41.30	69.25 (56.32)	9.92	559.20	12.36
Control	40.25 (39.37)	-	63.00 (52.53)	-	497.70	-
S. Em ±	0.34		0.47		4.35	
CD @ 1%	1.02		1.40		12.79	
C. V. (%)	3.23		1.31		1.48	

Figures in parantheses indicates arc sin transformed values

Table 7. Efficacy of bioagents on *Aspergillus niger* and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *A. niger* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
MPKV Fungal Consortia (0.5%)	14.00 (21.97)	73.21	82.50 (65.27)	28.91	692.85	37.04
MPKV Fungal Consortia (1.0%)	8.63 (17.07)	83.49	87.50 (69.29)	36.72	743.75	47.10
Trichoderma viride (0.5%)	22.13 (28.05)	57.66	68.75 (56.01)	7.42	584.38	15.58
Trichoderma harzianum (0.5%)	20.63 (27.01)	60.53	73.25 (58.85)	14.45	622.63	23.15
Pseudomonas fluorescens + *Trichoderma viride* (0.5%)	19.50 (26.20)	62.68	79.00 (62.72)	23.44	671.50	32.81
Pseudomonas fluorescens + *Trichoderma harzianum* (0.5%)	15.88 (23.48)	69.62	81.25 (64.34)	26.95	690.63	36.60
Control	52.25 (46.28)	-	64.00 (53.13)	-	505.60	-
S. Em ±	0.44		0.53		4.52	
CD @ 1%	1.30		1.58		13.31	
C. V. (%)	4.05		1.40		1.40	

Figures in parantheses indicates arc sin transformed values

Table 8. Efficacy of botanicals on *Aspergillus niger* and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *A. niger* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
Azardirachta indica leaf extract (10%)	21.50 (27.62)	57.84	76.25 (60.83)	17.76	610.00	19.25
Ocimum sanctum leaf extract (10%)	26.00 (30.65)	49.02	71.25 (57.57)	10.04	570.00	11.43
Zingiber officinale rhizome extract (10%)	26.50 (30.98)	48.04	73.00 (58.69)	12.74	584.00	14.17
Allium sativum clove extract (10%)	16.00 (23.57)	68.63	81.25 (64.34)	25.48	651.98	27.46
Allium cepa bulb extract (10%)	30.25 (33.36)	40.69	69.25 (56.32)	6.95	554.00	8.30
Eucalyptus globulus leaf extract (10%)	35.00 (36.27)	31.37	67.25 (55.09)	3.86	538.00	5.17
Control	51.00 (45.57)	-	64.75 (53.57)	-	511.53	-
S. Em ±	0.49		0.54		3.97	
CD @ 1%	1.46		1.59		11.68	
C. V. (%)	3.37		1.50		1.38	

Figures in parantheses indicates arc sin transformed values

Table 9. Efficacy of botanicals on *Aspergillus flavus* and their effect on seed germination and seedling vigour index of onion seeds

Treatments	Incidence of *A. flavus* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
MPKV Fungal Consortia (0.5%)	12.75 (20.90)	76.39	82.75 (65.45)	40.25	703.38	50.91
MPKV Fungal Consortia (1.0%)	10.50 (18.90)	80.56	88.25 (69.95)	49.58	750.13	60.94
Trichoderma viride (0.5%)	26.00 (30.65)	51.85	71.00 (57.41)	20.34	603.50	29.48
Trichoderma harzianum (0.5%)	23.25 (28.82)	56.94	76.25 (60.83)	29.24	648.13	39.05
Pseudomonas fluorescens + Trichoderma viride (0.5%)	22.25 (28.14)	58.80	78.00 (62.02)	32.20	663.00	42.24
Pseudomonas fluorescens + Trichoderma harzianum (0.5%)	14.75 (22.58)	72.69	80.50 (63.79)	36.44	684.25	46.80
Control	54.00 (47.29)	-	59.00 (50.18)	-	466.10	-
S. Em ±	0.48		0.54		4.63	
CD @ 1%	1.43		1.61		13.63	
C. V. (%)	4.17		1.43		1.43	

Figures in parantheses indicates arc sin transformed values

Table 10. Efficacy of botanicals on Aspergillus flavus and their effect on seed germination and seedling vigour index of Onion seeds

Treatments	Incidence of *A. flavus* (%)	Reduction in incidence over control (%)	Seed germination (%)	Increase in seed germination over control (%)	Seedling vigour index (SVI)	Increase in SVI over control (%)
Azardirachta indica leaf extract (10%)	27.00 (31.30)	47.32	73.25 (58.85)	28.51	586.00	30.14
Ocimum sanctum leaf extract (10%)	28.25 (32.10)	44.88	72.50 (58.37)	27.19	580.00	28.80
Zingiber officinale rhizome extract (10%)	29.50 (32.89)	42.44	68.50 (55.85)	20.18	548.00	21.70
Allium sativum clove extract (10%)	16.00 (23.57)	68.78	81.25 (64.34)	42.54	652.03	44.80
Allium cepa bulb extract (10%)	35.25 (36.42)	31.22	59.50 (50.47)	4.39	476.00	5.71
Eucalyptus globulus leaf extract (10%)	37.00 (37.46)	27.80	58.75 (50.03)	3.07	470.00	4.37
Control	51.25 (45.71)	-	57.00 (49.02)	-	450.30	-
S. Em ±	0.51		0.54		4.40	
CD @ 1%	1.51		1.61		12.95	
C. V. (%)	3.35		1.63		1.63	

Figures in parantheses indicates arc sin transformed values

isolated seedborne mycoflora reduced seed germination significantly over the control. The lowest seed germination was observed in case of seeds inoculated with *Aspergillus flavus* also these seedborne mycoflora significantly reduced the seedling vigour index of onion seeds as compared to control. The lowest seedling vigour index was observed in the seeds inoculated with *Aspergillus flavus* as against maximum in control followed by *Aspergillus niger*, *Alternaria alternata*, *Fusarium oxysporum* and *Penicillium* spp. All these results of the investigation are compatible with literature of Dumbre *et al*. (2011), Özer and Köycü (2004), Dabire *et al*. (2016), Dabire *et al*. (2021), etc.

The data and analysis regarding incidence of mycoflora, seed germination percentage and seedling vigour index is demonstrated in tables.

Conclusions

Five seed borne fungi *viz*., Fusarium oxysporum, Alternaria alternata, Penicillium spp., Aspergillus niger and Aspergillus flavus were detected and isolated from the seeds of onion variety Phule Samarth externally. Two seed borne fungi viz., Fusarium oxysporum and Alternaria alternata were found associated with the seeds of onion variety Phule Samarth internally. All isolated pathogens were found pathogenic to seeds of onion resulting into reduction in seed germination and seedling vigour index. The pathogens Fusarium oxysporum, Alternaria alternata, Aspergillus niger and Aspergillus flavus caused more damage. Among all the bioagents treatments given to artificially inoculated seeds MPKV fungal consortia with 1% conc. was found the most effective treatment in all mycoflora, with lowest incidence of seed mycoflora and increased seed germination and seedling vigour index. Among all the botanicals treatments given to artificially inoculated seeds Allium sativum clove extract with 10% conc. was found the

most effective treatment in all mycoflora, with lowest incidence of seed mycoflora and increased seed germination and seedling vigour index.

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