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Characterization of Pod Rot Fungus of Blackgram and its Management using Fungicides and Bioagents for Enhancing Seed Germination and Vigour

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ABSTRACT: Pod rot is one of the major causes for deterioration of blackgram seed during untimely rains at harvest. Studies on blackgram pod rot at RARS, Lam, Guntur during *Kharif* 2022 revealed that *Fusarium* sp. is the major culprit for pod deterioration that results in low seed germination. The major pod rot fungus was characterized as *Fusarium chlamydosporum* using ITS primers. An attempt has been made in this study to evaluate various fungicides and biocontrol agents as seed treatment to enhance seed germination and vigour of pod rot affected seed. Eight fungicides and biocontrol agents were tested *in vitro* for their *Fusarium* suppressing and seed growth promoting ability using poisoned food, dual culture and roll paper towel methods. Among different fungicides tested at 500 ppm and 1000 ppm concentrations, tebuconazole, hexaconazole, carbendazim, carbendazim + mancozeb, propiconazole showed 100 per cent inhibition of mycelial growth of *Fusarium* sp. In case of bioagents, *Pseudomonas aeruginosa* P20 showed highest mycelial growth inhibition (88.24%) followed by *Pseudomonas chlororaphis* P19 (87.65%), *Bacillus vallismortis* B20 (77.65%) and *Bacillus inaquosorum*B2 (74.12%) in dual culture assay. Seed treatment with fungicides and bioagents followed by roll towel paper method of seed germination revealed improvement of seed germination and other growth parameters besides reducing seed borne fungal infection when compared to control. Highest germination and seedling vigour were obtained in *Bacillus inaquosorum* treated blackgram seeds when compared to all other treatments. Though, germination and seed infestation were found to be enhanced in most of the chemical treatments, some of the fungicide formulations used were found inhibitory to radicle and plumule growth. Overall, the study indicated that the germination of pod rot affected blackgram seeds could be improved by seed treatment with fungicides and biocontrol agents.

Keywords: Urdbean, *Vigna mungo*, pod rot, seed germination, seedling vigour, fungicides, bioagents.

INTRODUCTION

Blackgram (*Vigna mungo* L.), popularly known as Urd bean, urid or mash is an important pulse crop grown in India. It is a rich source of protein and contains 26.2% crude protein, 1.2% fat and 56.6% carbohydrates (Raju, 2019). India is the largest producer and consumer of blackgram in the world. Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu and Uttar Pradesh accounts for about 75% of total production in India (Annual Report, 2020). In Andhra Pradesh, blackgram occupies an area of 0.31 million hectares with a production of 0.32 million tonnes and productivity of 977 kg/ha (Department of Agriculture and Cooperation, GOI, 2018-19). Blackgram suffers from various biotic and abiotic stresses influencing production as well as quality of seeds. Among biotic

stresses, pod rot fungi lead to quantitative and qualitative losses of seed, especially during untimely rains at the time of harvest. Seeds carry several fungi belonging to the fungal genera, viz., *Alternaria, Chaetomium, Cladosporium, Fusarium, Aspergillus, Curvularia*and *Penicillium*, on their surface that deteriorate seed germination and vigour besides production of mycotoxins based on storage conditions (Ahmad, 1993). These fungi have negative effects on developing seeds, including seed discolouration, seed surface deformation, endosperm deterioration, and reduced seed filling, which leads to seed deformities such as shriveling of seeds and the development of small distorted seeds. Fungi that infect seeds ultimately results in poor germination and reduction in seedling vigour (Bilgrami *et al*., 1978). In order to enhance the germination and vigour of the seeds the harmful seed mycoflora must be eliminated using fungicides or biocontrol agents which could enhance seed germination and seedling vigour. Keeping these points in view, an experiment was conducted to characterize major pod rot fungus associated with pod rot in blackgram and its management using fungicides and biocontrol agents to improve seed germination and seedling vigour.

MATERIAL AND METHODS

Collection of samples and characterization of major pod rot fungus. Blackgram pods of cultivar, LBG 623 with visible growth of mycoflora were collected during *kharif* 2022 and the pathogen was isolated as per standard protocols. Seeds from infected pods were surface disinfected with sodium hypochlorite (NaOCl, 3%) for three minutes, rinsed thrice with sterilize distilled water and blotted dry on sterilize filter paper before platting on potato dextrose agar (PDA) medium. Petri dishes were incubated at 25°C for four days. Mycoflora growing from seeds were identified by morphological and molecular methods. For morphological identification, colony characters, type of conidia produced, conidial length and width were recorded. For molecular identification, DNA was isolated from 5 days old mycelial growth of fungus on potato dextrose broth and genomic DNA was isolated using HiPurA Fungal DNA Purification kit (Himedia Laboratories, Maharashtra, India) and amplified by ITS1 and ITS4 primers (White *et al*., 1990). The thermocyclic conditions included denaturation at 94°C for 4 min followed by 38 amplification cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 90 sec followed by final extension for 30 min. The amplified product was separated on 1% agarose gel and outsourced for sequencing. The sequences thus obtained were subjected to NCBI-BLAST and the fungus was identified based on per cent similarity of the sequence with other available sequences in NCBI. Phylogenetic analysis was done using MEGA 11.0 software.

Evaluation of bioagents and fungicides against pod rot fungus

In vitro* evaluation of fungicides against *F. chlamydosporum. Eight fungicides belonging to triazoles, strobilurins, benzimidazoles and dithiocarbamate groups (Table 1) were evaluated at two concentrations (500 and 1000 ppm) for their efficacy against *F. chlamydosporum* by poisoned food technique on PDA as basal medium (Nene and Thapliyal, 1993).The radial growth of mycelium in each plate was recorded at 7 days interval, till the control plate was fully covered with the fungal mycelium and the per cent inhibition of fungal growth was estimated by using the formula of Vincent (1947).

In vitro* evaluation of biocontrol agents against *Fusarium chlamydosporum. The effectiveness of antagonists against *F.chlamydosporum* was assessed by means of dual culture technique (Dennis and Webster 1971) on PDA medium. A total of eight biocontrol agents (4 *Trichoderma* sp., 2 *Pseudomonas* sp. and 2 *Bacillus* sp.) were tested for their antagonism against *F. chlamydosporum* by dual culture method. The details of the biocontrol used, their source and identity were mentioned in Table 2. The radial growth of the pathogen was measured after 7 days of incubation and per cent inhibition over control was found out as per equation suggested by Vincent (1947).

Evaluation of seed treatment with fungicides and bioagents on seed germination and seedling vigour of *F.chlamydosporum* affected blackgram seed by paper towel method. The blackgram seed collected from infected pods were treated with eight fungicides and biocontrol agents and assessed for their germination potential using paper towel method. Fungicides were tested at two concentrations, i.e., 500 ppm and 1000 ppm. Fungal and bacterial bioagents were tested at concentration of $2x10^6$ and $2x10^8$CFU/ml, respectively. Seeds were dipped in a solution of fungicides or biocontrol agents for 15 minutes and blotted dry on blotter paper and placed on wetted paper towels for vigour test. Hundred seeds were placed for each treatment and replicated thrice. The seeds treated with sterile distilled water served as control. The rolled paper towels were kept in wax coated paper bags and incubated in a seed germinator at 25±1 ºC for one week. After incubation, rolled paper towels were carefully opened and seed germination percentage [(Number of seeds germinated/Total number of seeds incubated) × 100], radicle and plumule length were recorded and seedling vigourindex (I) was computed as germination percentage × (radicle length + plumule length) (Thakare *et al*., 2013). Further, per cent frequency of seed infected with *Fusarium* was also recorded using the formula: [Number of seeds on which Fusarium appear/Total number of seeds incubated)] × 100

RESULTS AND DISCUSSION

Characterization of the fungus associated with blackgram pod rot. The pod rot fungus produced white fluffy mycelium on Potato Dextrose Agar (PDA) and turned pinkish in older portions of the colony. The reverse side of culture appears orange in colour. The fungus produced micro- and macroconidia on short conidiophores. Microconidia are hyaline with 0-2 septa and measured 5-20µm × 2-4 µm in size. Macroconidia are produced on mono- or polyphialides, hyaline,

straight to slightly falcate with 3 to 5 septa measuring 32-35µm × 2.5 – 4.0µm in size. Chlamydospores were abundantly produced in chains or clusters. Based on the morphological characters the fungus was putatively identified as *F. chlamydosporum*. Similar descriptions of the *F. chlamydosporum* were reported earlier in various crops (Siddiquee *et al*., 2010; Parihar *et al*., 2022). The identity of the fungus was further confirmed by amplification of ITS region using PCR. The NCBI-BLAST analysis of the partial genome sequence amplified by ITS1 and ITS 4 primers has shown 99.41% identity with the accession number, ON242107. The sequence was submitted to Gene bank of NCBI and obtained accession number (OR229711). Further, phylogenetic tree was constructed to ascertain the grouping of the characterized *F.chlamydosporum* isolate. Relevant sequences from NCBI database were used for phylogenetic tree construction. Phylogenetic tree was constructed using Maximum parsimony method after aligning the sequences using Clustal W software. MEGA software version 11.0 was used for phylogenetic tree construction. The results clearly revealed that there is separate clustering for the different species of Fusarium (Fig. 1). The test sequence clustered with *F. chlamydopsorum* confirming the identity of pod rot fungus of blackgram. DNA barcoding using the internal transcribed spacer region (ITS) was used for the identification of *F. chlamydoporum*, the wilt causing pathogen of chilli and brinjal in Kashmir Himalayas (Parihar *et al*., 2022).

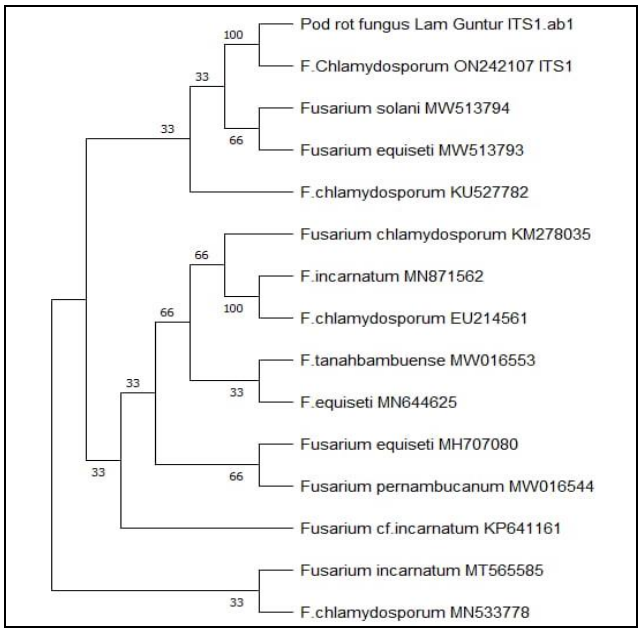

Fig. 1. Dendrogram of ITS sequences of Fusarium using Maximum parsimony method.

***In vitro* evaluation of fungicides against *F.chlamydosporum*.** Eight fungicides of two concentrations were assessed for their effect on radial growth of *F. chlamydosporum* using poisoned food technique (Table 3). All the fungicides tested were found to inhibit the mycelial growth of *F. chlamydosporum* when compared to control. Highest per cent inhibition (100%) was recorded in tebuconazole, hexaconazole, carbendazim, carbendazim+mancozeb and propiconazole treatments at both the concentrations, *i.e*., 500 and 1000 ppm. The least per cent inhibition was observed in mancozeb (40%) and azoxystrobin (45.59%) treatments at 500 and 1000 ppm, respectively. At 1000 ppm concentration, all fungicides tested reported 100% inhibition of mycelial growth except azoxystrobin and mancozeb. Trifloxystrobin+tebuconazole has shown better efficacy at 1000ppm concentration than at 500 ppm concentration.Similarly, Harwinder Singh *et al.* (2023) reported trifloxystrobin+tebuconazole as the most effective fungicide in the management of mungbean pod rot. In this study, triazole and benzimidazole compounds were found effective for inhibiting the mycelial growth of *F. chlamydosporum* compared to dithiocarbamates and strobilurins. Mesterhazy *et al*. (2003) reported the efficacy of fungicides with triazoles as active ingredient in management of plant diseases caused by *Fusarium* species. Conidiospore production of *F. chlamydosporum* was found to be adversely affected in the presence of the fungicide, penconazole (Al-Awlaqi, 2011).

***In vitro* evaluation of biocontrol agents against *Fusarium chlamydosporum*.** Four fungal (*Trichoderma* sp.) and four bacterial bioagents (2 *Pseudomonas* sp. and 2 *Bacillus* sp.) were tested for their growth suppressing ability against *F. chlamydosporum* by dual culture method (Table 4). Results revealed that all the bioagents tested have significantly suppressed the mycelial growth of *F. chlamydosporum in vitro* compared to control. Among the bioagents tested, *Pseudomonas aeruginosa* and *Pseudomonas chlororaphis* subsp. *aurantiaca*, were found superior to other bioagents in suppressing mycelial growth of the test fungus with an inhibition percentage of 88.24 and 87.65 per cent, respectively. Badrakia *et al*. (2021) reported the biocontrol efficacy of *Pseudomonas aeruginosa* against *F. chlamydosporum*, the incitant of root rot disease in soybean. The biocontrol potential of

P. aeruginosa was attributed to the production of glucanase and chitinase upon interaction with *F. chlamydosporum*. *Bacillus vallismortis* and *Bacillus inaquosorum* used in the present study have recorded mycelial inhibition percentage of 77.65 and 74.12 per cent, respectively. Bacillus species were reported as potential antagonists of different Fusarium species causing several plant diseases (Kishore Varma *et al*., 2017). Various types of antimicrobial peptides (AMPs) are synthesized by Bacillus species which have been implicated in the biological control of several plant pathogens. Antimicrobial peptides elaborated by the Bacillus species like bacillomycin, bacilysin, fengycin, iturin, surfactin and subtilin are known to enhance their biocontrol efficiency as well as their sustenance in the plant environment (Mora *et al*., 2011). *Bacillus amyloliquefaciens* was identified as a potential bioagent for the management of *F. chlamydosporum*, the causal agent of stem rot in *Jacaranda acutifolia* (Han-mingyue Zhu and Yuan-zhi Pan 2019). Among the *Trichoderma* isolates tested against mycelial growth of *F. chlamydosporum*, *Trichoderma asperellum* T19012, *T. erinaceum* and *Trichoderma* sp. T19015 were found at par with each other with mycelial growth inhibition of 67.65%, 66.18% and 65%, respectively. Least inhibition of mycelial growth of the test fungus was obtained with *Trichoderma asperellum* T19007 isolate (60.88%). Bacterial bioagents were found superior over fungal bioagents in suppressing the mycelial growth of *F.chlamydosporum* under dual culture assay.

Evaluation of seed treatment with fungicides and bioagents on seed germination and seedling vigourof *F.chlamydosporum* affected blackgram seed by paper towel method

a. ***Effect of seed treatment with fungicides on seed germination and vigour.*** The efficacy of eight fungicides on improving seed germination and vigour of *F. chlamydosporum* affected blackgram seed was tested by roll paper towel method (Table 5, 6). It is evident from the results that all the fungicides have drastically reduced the Fusarium infection of seed compared to control. Fusarium growth was not observed on the seed treated with carbendazim and incubated in a roll paper towel for germination. The seed germination was found to be higher in Tebuconazole treated seeds followed by mancozeb, carbendazim+mancozeb, hexaconazole, trifloxystrobin+tebuconazole, azoxystrobin, propiconazole and carbendazim at 500 ppm concentration. However, at 1000ppm, highest germination was obtained with mancozeb followed by carbendazim+mancozeb, azoxystrobin/ carbendazim, tebuconazole/ trifloxystrobin+tebuconazole/ propiconazole and hexaconazole. This shows that certain fungicides have inhibitory effects on seed germination when used at higher concentrations. For example, seed treated with an Emulsifiable Concentrate (EC) formulation of tebuconazole has shown 76 % seed germination at 500 ppm, but germination percentage was reduced to 58% at a concentration of 1000 ppm. At both the concentrations tested for pathogen suppression and seedling growth promotion, canbendazim+mancozeb was found effective compared to all other treatments with a seedling vigour index of 6292 and 5353, respectively at 500 and 1000 ppm concentrations. Carbendazim at 0.2% was found effective for management of seed mycoflora and enhancing seed germination and seedling vigour in mungbean (Dolas *et al*., 2018). Though seed germination percentage is high in the treatments, tebuconazole and mancozeb, at 500 ppm concentration, further growth of radicle and plumule were restricted when compared to carbendazim+mancozeb. Devamani *et al*. (2017) evaluated various fungicides as seed treatment to reduce seed borne mycoflora of mungbean and reported that seed treatment with captan at the rate 4 g/kg has reduced seed mycoflora effectively.

b. Effect of seed treatment with bioagnets on seed germination and vigour. Among the bioagents tested to enhance the seed germination and seedling vigour of *F. chlamydosporum* infected seed, *Bacillus inaquosorum* treated seed has recorded highest germination percentage (90%), low percentage of seed infected with *Fusarium* (10%) and high seedling vigour index (11191) when compared to all other treatments (Table 7). Only in few treatments, viz., seed treatment with *Trichoderma* sp. T19015, *Bacillus vallismortis* and *B. inaquosorum*, the seed germination percentage is more than 50 percent with less than 25% of the seed with aerial growth of *Fusarium*. Seedling vigour index (SVI) is high in *B. inaquosorum* followed by *Pseudomonas chlororaphis* subsp. *aurantiaca*, *T. asperellum* T19012, *Pseudomonas aeruginosa* and *T. asperellum* T19007. Seedling vigour index of 960 was observed in untreated control. However, SVI is least in the seed treated with *T. erinaceum*and *Bacillus vallismortis* in roll paper towel method. Seed treatment with *Trichoderma viride* was found effective in reducing seed mycoflora and enhancing seed germination and seedling vigour index in mungbean when treated at a concentration of 0.6 percent (Dolas *et al*., 2018). Similarly, treating mungbean seed with *Trichoderma harzianum* at the rate of 8g/kg reduced the seed mycoflora significantly when compared to untreated seed (Devamani *et al*., 2017).

CONCLUSIONS

Overall, the results of the present study revealed the association of *Fusarium chlamydosporum* with the pod rot of blackgram in Guntur district of Andhra Pradesh during Kharif, 2022. Bacterial bioagents and triazole compounds were found inhibitory to the mycelial growth of *F. chlamydosporum* under laboratory conditions compared to other bioagents and other group of fungicides studied. Seed treatment with *Bacillus inaquosorum* could be advocated to reduce seed infection of pod rot fungus of blackgram thereby improving seed germination and seedling vigour. In future, the effective treatments have to be assessed as pre-harvest sprays *in vivo* to reduce the incidence of pod mycoflora that affect the blackgram seed germination.

Table 1: List of fungicides used in the study.

Sr. No.	Trade name	Chemical name	Active ingredient
1.	Folicur	Tebuconazole	25.9% EC
2.	Nativo	Tebuconazole + Trifloxystrobin	50%+25%WG
3.	Amistar	Azoxystrobin	23% SC
4.	Contaf	Hexaconazole	5% EC
5.	Zoom	Carbendazim	50% WP
6.	M-45	Mancozeb	75%WP
7.	SAAF	Carbendazim + Mancozeb	12%+63%WP
8.	Tilt	Propiconazole	25% EC

Table 2: Details of the biocontrol agents used for dual culture assay against *F. chlamydosporum*.

Sr. No.	Bioagents	NCBI Accession No.	Source
1.	*Trichoderma* spp. T19015	ON969989	Dept. Plant Pathology, Bapatla
2.	*Trichoderma asperellum*T19007	ON909211	Dept. Plant Pathology, Bapatla
3.	*Trichoderma asperellum* T19012	ON810659	Dept. Plant Pathology, Bapatla
4.	*Trichoderma erinaceum*	-	RARS, Lam Farm
5.	*Bacillus vallismortis*	ON954849	RARS, Anakapalle
6.	*Pseudomonas aeruginosa*	ON964472	RARS, Anakapalle
7.	*Pseudomonas chlororaphis* subsp. *aurantiaca*	ON116377	RARS, Anakapalle
8	*Bacillus inaquosorum*	ON964473	RARS, Anakapalle

Table 3: *In vitro* evaluation of fungicides against *F. chlamydosporum.*

Tr. No.	Fungicides	500 PPM		1000 PPM	
		Colony dia. (cm)*	% Inhibition*	Colony dia. (cm)*	% Inhibition*
T1	Tebuconazole	0.00 (1.00)	100.00	0.00 (1.00)	100.00
T2	Tebuconazole+ Trifloxystrobin	1.13 (1.46)	86.76	0.00 (1.00)	100.00
T3	Azoxystrobin	4.63 (2.37)	45.59	4.60 (2.37)	45.88
T4	Hexaconazole	0.00 (1.00)	100.00	0.00 (1.00)	100.00
T5	Carbendazim	0.00 (1.00)	100.00	0.00 (1.00)	100.00
T6	Mancozeb	5.10 (2.47)	40.00	3.73 (2.17)	56.18
T7	Carbendazim + Mancozeb	0.00 (1.00)	100.00	0.00 (1.00)	100.00
T8	Propiconazole	0.00 (1.00)	100.00	0.00 (1.00)	100.00
T9	Untreated Control	8.50 (3.08)	0.00	8.50 (3.08)	0.00
CD at 1%		0.04	2.48	0.04	2.04
CV (%)		1.61	1.92	1.40	1.51

*Mean of three replications; Numbers in parenthesis indicate square root transformed values

Table 4: *In vitro* efficacy of bio-agents against mycelial growth of *F. chlamydosporum.*

Tr. No.	Treatments	Bioagents	
		Colony dia. (cm)*	% Inhibition*
T1	*Trichoderma* spp. T19015	28.75 (5.45)	66.18
T2	*Trichoderma asperellum*T19007	33.25 (5.85)	60.88
T3	*Trichoderma asperellum*T19012	27.50 (5.33)	67.65
T4	*Trichoderma erinaceum*	29.75 (5.54)	65.00
T5	*Bacillus vallismortis*	19.00 (4.46)	77.65
T6	*Pseudomonas aeruginosa*	10.00 (3.32)	88.24
T7	*Pseudomonas chlororaphis* subsp. *aurantiaca*	10.50 (3.39)	87.65
T8	*Bacillus inaquosorum*	22.00 (4.80)	74.12
T9	Untreated Control	85.00 (9.27)	0.00
CD at 1%		0.338	3.755
CV (%)		3.710	3.329

*Mean of three replications; Numbers in parenthesis indicate square root transformed values

Table 5: Effect of seed treatment with fungicides (500 ppm) on black gram seed germination, seedling vigour and seed mycoflora.

Tr. No.	Fungicides	500 PPM				
		Seed germination* (%)	%Frequencyof aerial growth of Fusarium *	Radicle length* (mm)	Plumule length* (mm)	Vigour index*
T1	Tebuconazole	76	6	14.00	14.33	2153.47
T2	Tebuconazole+ Trifloxystrobin	56	14	16.67	0.00	933.33
T3	Azoxystrobin	54	16	17.33	0.00	936.00
T4	Hexaconazole	60	16	19.00	0.00	1140.00
T5	Carbendazim	42	14	15.67	0.00	658.00
T6	Mancozeb	70	12	17.33	0.00	1213.37
T7	Carbendazim + Mancozeb	66	0	48.33	47.00	6292.00
T8	Propiconazole	46	12	18.00	0.00	828.00
T9	Untreated Control	46	62	20.88	0.00	960.62
CD at 1%		4.15	2.82	1.26	0.67	73.35
CV (%)		4.19	9.67	3.49	5.65	2.53

*Mean of three replications

Table 6: Effect of seed treatment with fungicides (1000 ppm) on black gram seed germination, seedling vigour and seed mycoflora.

Tr. No.	Fungicides	1000 PPM				
		Seed germination* (%)	%Frequencyof aerial growth of Fusarium *	Radicle length* (mm)	Plumule length* (mm)	Vigour index*
T1	Tebuconazole	58	12	14.67	0.00	850.67
T2	Tebuconazole+ Trifloxystrobin	58	4	17.00	0.00	986.00
T3	Azoxystrobin	60	8	18.00	0.00	1080.00
T4	Hexaconazole	52	18	16.67	0.00	866.67
T5	Carbendazim	60	16	42.00	39.33	4880.00
T6	Mancozeb	64	20	14.67	0.00	938.66
T7	Carbendazim + Mancozeb	62	0	46.33	40.00	5352.63
T8	Propiconazole	58	18	19.00	0.00	1102.00
T9	Untreated Control	46	62	20.88	0.00	960.62
CD at 1%			3.457	3.26	1.57	0.67
CV (%)			3.475	10.74	3.91	4.37

*Mean of three replications

Table 7: Effect of seed treatment with bioagents on black gram seed germination, seedling vigour and seed mycoflora.

Tr. No.	Bioagents	Roll towel paper method				
		Seed germination * (%)	Percentage frequency (PF) of occurrence of aerial growth of Fusarium *	Radicle length* (mm)	Plumule length* (mm)	Vigour index
T1	*Trichoderma* spp. T19015	58	22	20.62	0.00	1196.35
T2	*Trichoderma asperellum*T19007	44	22	52.86	32.28	3746.96
T3	*Trichoderma asperellum*T19012	44	56	73.63	50.48	5460.89
T4	*Trichoderma erinaceum*	40	58	14.38	0.00	575.30
T5	*Bacillus vallismortis*	64	16	13.15	0.00	841.34
T6	*Pseudomonas aeruginosa*	44	20	61.66	47.47	4801.89
T7	*Pseudomonas chlororaphis* subsp. *aurantiaca*	46	32	78.22	58.09	6270.76
T8	*Bacillus inaquosorum*	90	10	77.10	46.67	11191.97
T9	Untreated Control	46	62	20.88	0.00	960.62
CD at 1%		3.82	4.61	2.00	1.76	168.50
CV (%)		4.18	8.05	2.53	3.89	2.50

*Mean of three replications

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Conflict of Interest. None.

REFERENCES

Ahmad, S. K. (1993). Mycoflora changes and aflatoxin production in stored blackgram seeds. *Journal of Stored Products Research, 29*(1), 33-36

Al-Awlaqi, M. M. (2011). *In vitro* growth and sporulation of *Fusarium chlamydosporum* under combined effect of pesticides and gibberellic acid. *Research Journal of Agriculture and Biological Sciences, 7*(6), 450-455.

Annual Teport (2020). Project coordinator's report (Mungbean and Urdbean), *All India Coordinated Research Project on MULLaRP*. ICAR- Indian Institute of Pulse Research, Kanpur, 208 024.

Badrakia, J., Patel, K. B., Dhandhukia, P. and Thakker, J. N. (2021). Mycoparasitic *Pseudomonas* spp. against infection of *Fusarium chlamydosporum* pathogen in soybean (*Glycine max*) plant. *Archives of Phytopathology and Plant Protection, 54*(19-20), 2160-2170.

Bilgrami, K. S., Sinha, R. K. and Prasad, T. (1978). Effect of fungal flora on the seed contents of moong. *Indian Phytopathology, 31*, 476-479.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society, 57*, 25-39.

Department of Agriculture and Cooperation, Government of India (2018-19). Area and production of agricultural crops in India. *www.indiaagristat.com*

Devamani, B. D., Saifulla, M. and Jayappa. (2017). Efficacy of fungicides and bioagents of seed mycoflora of Mung bean (*Vigna radiata* L.). *International Journal of Pure and Applied Bioscience, 5*(3), 231-237.

Dolas, R. M., Gawade, S. B. and Kasture, M. C. (2018). Efficacy of seed treatment of fungicides, bioagents and botanicals on seed mycoflora, seed germination and seedling vigour index of mungbean. *Journal of Pharmacognosy and Phytochemistry, 7*(5), 1074-1077.

Han-mingyue Zhu and Yuan-zhi Pan (2019). A novel antimicrobial protein of the endophytic *Bacillus amyloliquefaciens* and its control effect against *Fusarium chlamydosporum*. *Biocontrol, 64*, 737-748.

Harwinder Singh, B., Amarjit, S., Asmita, S., Anupam., Komalpreet, K., Abhishek, K., Milan Kumar, L., Rahul Kumar, T. G. And Ravinder, K. (2023). Investigating the impact of fungicides and mungbean genotypes on the management of pod rot disease caused by *Fusarium equiseti* and *Fusarium chlamydosporum*. *Frontiers in Plant Science, 14*, 10.3389/fpls.2023.1164245

Kishore Varma, P., Vijay Krishna Kumar, K., Adilakshmi, D., Suresh, M., Raja Kumar, N., Jayachandra, K. and Anitha, R. (2017). Evaluation of endophytic bacteria for plant growth promotion and pathogen suppression traits in *Saccharum officinarum*. *Journal of Agricultural Science and Technology A7*, 537-545.

Mesterhazy, A., Bartok, T. and Lamper, C. (2003). Influence of wheat cultivar, species of Fusarium and isolate aggressiveness on the efficiency of fungicides for control of *Fusarium* head blight. *Plant Disease, 87*, 1107-1115.

Mora, I., Cabrefiga, J. and Montesinos E. (2011). Antimicrobial peptide genes in Bacillus strains from plant Environments. *International Microbiology, 14*, 213-223.

Parihar, T. J., Sofi, M. Y., Rasool, R. S., Khursheed, S., Bhat, Z. A. and Hussain, K. (2022). *Fusarium chlamydosporum*, causing wilt disease of chilli (*Capsicum annum* L.) and brinjal (*Solanum melongena* L.) in Northern Himalayas: a first report. *Scientific reports, 12*, 20392.

Raju, M. (2019). Study on constraints and adoption of blackgram seed production technologies by the farmers of Cauvery delta zone of Tamil Nadu. *Journal of Pharmacognosy and Phytochemistry, 8*(4), 1031-1035.

Siddiquee, S., Yusuf, U. K. and Zainudin, N. A. I. M. (2010). Morphological and molecular detection of Fusarium chlamydosporum from root endophytes of *Dendrobium crumenatum*. *African Journal of Biotechnology, 9*(26), 4081-4090.

Thakare, D. P., Mula, M. G., Mehtre, S. P., Saxena, K. B. and Rathore, A. (2013). Seedling vigour study in pigeonpea (*Cajanus cajan* (L.) Millsp.) hybrids and varieties. *Journal of Food Legumes, 26* (1&2), 100-102.

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature, 150*, 850.

White, T. J., Burns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 IN: PCR protocols, A guide to Methods and Applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds. Academic Press, San Diego, CA.

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