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Evaluation of Bioagents Against Seed Borne Fungi in Green Gram and Blackgram

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Abstract An experiment was conducted 2011-2012 *in vitro* the effect of different antagonists viz. *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against different seed borne fungi of green gram and black gram by dual culture technique. Among three bioagents, *Bacillus subtilis* exhibited maximum mycelial growth inhibition of *Phoma medicaginis* (73.70%), *Fusarium solani* (57.60%),*Fusarium oxysporum* (47.82%) and *Macrophomina phaseolina* (47.29%) followed by *Pseudomonas fluorescens* exhibited maximum mycelial growth inhibition of *Phoma medicaginis* (71.70%), *Macrophomina phaseolina* (62.41%) *Fusarium oxysporum* (59.97%) and *Curvularia lunata* (41.08%) respectively and lowest mycelial growth observed in *Phoma medicaginis* (68.33%), *Macrophomina phaseolina* (44.94%), *Fusarium solani* (38.95%) with *Trichoderma viride.*

Keywords Green gram, Black gram, Seed borne pathogens, Bioagents *in vitro.*

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Introduction

Green gram [*Vigna radiata* (L.) Wilczek] and Black grain [*Vigna mungo* (L.) Hepper] are the important pulse crops of India next to pigeon pea [*Cajanus cajan* (L.) Mill. Sp] and belongs to family Leguminoceae.They both are ancient pulse crops which are consumed in the form of split pulse as well as whole pulse. In addition, being an important source of human food and animal feed, both crops plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen through symbiotic action.

Most of the farmers use uncertified seed saved from the previous harvest, borrowed from neighbors or purchased from local markets, factors that encourage spread and introduction of new diseases. These seed-borne micro-organisms have adverse effects on green gram and black gram seeds. They can reduce seed germination, seedling emergence or cause blights, leaf spots and other diseases on mature plants. Several fungi have been reported by many workers in black gram viz. *Aspergillus flavus, Aspergillus niger,Curvularia lunata, Colletotrichum* sp., *Fusarium moniliforme, Fusarium semitectum, Fusarium solani, Phoma medicaginis, Macrophomina phaseolina, Penicillium* sp., some of these are seed borne in nature and seed transmissible (Shamsur Rahman et al. 1999, Raut and Ahire 1988). Seed health testing for the presence of seed borne pathogens is an important step in the management of crop diseases. Present study was undertaken to know the efficacy of some fungal and bacterial bioagents against seed borne fungi of green gram and black gram (Ali et al. 2010, Arya et al. 2004, Bagri et al. 2004,

Barua et al. 2007, Gawade et al. 2010, Jaiman and Jain 2004, Mandhare et al. 2010, Muthukumar and Bhaskaran 2007, Rajeswari and Kumari 2009, Rekha et al. 2011, Sarhan 2009).

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Materials and Methods

Sample collection

Seeds of green gram cultivars Koprergaon, AKM-8802, AKM-4, Greengold, TARM-18 and Black gram cultivars TAU-1, TAU-2, T-9, AKU-15, PPU-4 were collected from Pulses Research Unit, Akola, and also fungal and bacterial bioagents, *Trichoderma viride*, *Pseudomonas fluorescens, Bacillus subtilis* were collected from Department of Plant Pathology PGI, Akola.

Identification of fungi

Standard blotter method (ISTA 1985) is widely used for the detection of seed borne fungi. Pre-treatment is applied in seed health testing to avoid growth of fast growing saprophytic fungi and to allow the slow growing pathogenic fungi to colonize. In the present study 200 seeds of each variety were pre treated with sodium hypochlorite solution (Naocl) for two minutes and washed with three changes of sterilized distilled water. Then, the seeds were placed at equidistance on moist blotter and plates were incubated at 27 ± 2^0C, under alternate cycle of 12 h light and 12 h darkness for seven days by using two 40W white fluorescent tubes. After seven days of incubation, seeds were examined under stereoscopic microscope by using a magnification of 6X to 50X. Research microscope was also used to confirm the identification of fungi based on morphological characters given in standard mycological books and identification keys.

Isolation of fungi

Structure of seed borne fungi growing over incubated seed observed under Stereoscopic microscope. The pre dominant fungi viz. *Curvularia lunata, Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina, Phoma medicaginis* were lifted with sterilized needle and transferred aseptically on PDA media. The fungi were purified by hyphal tip method and maintained on PDA slants for further studies.

Efficacy of bioagents against seed borne fungi by dual culture technique

The test of fungi and bioagents i.e. *Trichoderma viride* were prepared in petriplate. In sterilized petriplate autoclaved melted Potato Dextrose Agar was poured and allowed to solidify for obtaining leveled surface. These plates were inoculated with the culture of test fungi and bioagents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bioagents, *Pseudomonas fluorescens* and *Bacillus subtilis* were prepared by inoculating a loopful culture in sterilized conical flask containing hundred ml nutrient broth. Broth culture was incubated at room temperature for three days. Autoclaved PDA poured in each of the sterilized petriplates and allowed to solidify. Four petriplates of each bioagents were used for this study. Six mm disc of one week old test fungus and bioagent lawn culture was cut with the help of cork borer lifted and transferred in petriplates.

In each petriplate four discs of bioagents were inoculated at four peripheral points of the plates and test fungi was placed in center of petriplates. In case of *P. fluorescens* and *B. subtilis*, a three days old culture was streaked around the test fungus at two sites. Control plates were kept where, culture disc of test fungus were grown in same condition on potato dextrose agar without bioagents. All these plates were incubated at room temperature for seven days. After an expiry of incubation period the mycelial inhibition was calculated as per formula :

$$PI = \frac{C - T}{C} \times 100$$

Where,
PI = Percent inhibition, C = Growth in control plate

Table 1. Evaluation of different bioagents against seed borne fungi of green gram and black gram by dual culture technique. MCD-Mean colony diameter (mm), PGI-Percent growth inhibition (%).

Treatments	*M. phaseolina*		*F. oxysporum*		*F. solani*		*C. lunata*		*P. medicaginis*	
	MCD	PGI (%)	MCD	PGI (%)	MCD	PGI (%)	MCD	PGI (%)	MCD	PGI (%)
T. viride	43.07	44.94	48.80	37.59	48.96	38.95	39.73	31.50	28.50	68.33
P. fluorescens	29.40	62.41	31.30	59.97	60.94	24.01	34.17	41.08	25.40	71.70
B. subtilis	41.23	47.29	40.80	47.82	34.00	57.60	32.17	44.53	23.67	73.70
Control	78.23		78.20		80.20		58.00		90.00	
F test	Sig.		Sig.		Sig.		Sig.		Sig.	
SE (m)±	0.17		0.27		0.22		0.17		0.16	
CD (p=0.01)	0.86		1.37		0.93		0.88		0.82	

(mm), T = Growth in treatment plate (mm).

Results and Discussion

The data presented in Table 1 showed that highest percent growth inhibition of *Macrophomina phaseolina* observed in *P. fluorescens* (62.41%) followed by *B. subtilis* (47.29%). Lowest mycelial growth observed in *T. viride* (44.94%). Present findings are in confirmation with earlier workers Shanmugam et al. (2003) and Choudary et al. (2010) who reported effective control of the mycelial growth inhibition of *M. phaseolina* with *P. fluorescens in vitro* in Urdbean. Maximum growth inhibition of *F. oxysporum* observed in *P. fluorescens* (59.97%) followed by *B. subtilis* (47.82%) and *T. viride* (37.59%). Present result confirmed with Muthukumar and Bhaskaran (2007) who reported *Pseudomonas fluorescens* and *Bacillus substilis* against *F. oxysporum*, the cause of tuber rot in tuberose (*Polyanthus tuberose* L.) *in vitro*, and the *Pseudomonas fluorescens* reduced the growth of pathogen to an extent of 51.78%.

In case of *Fusarium solani* highest percent growth of inhibition observed in *B. subtilis* (57.60%) followed by *T. viride* (38.95%). Lowest mycelial growth observed in *P. fluorescens* (24.01%). Similar finding observed by Selverajan and Jayrajan (1996) who recorded maximum zone of inhibition due to *B. subtilis, P. fluorescens, T. viride* against *F. solani*. The data presented in Table 1 showed that highest percent growth of inhibition of *C. lunata* observed in *B. subtilis* (44.53%) followed by *P. fluorescens* (41.08%) and *T. viride* (31.50%). Similar results were reported by Sumangala et al. (2008) who observed the mycelial inhibition of *C. lunata* 97.66% due to *B. subtilis* followed by *T. viride* 96.44%. In case of *Phoma medicaginis* highest percent growth of inhibition observed in *B. subtilis* (73.70%) followed by *P. fluorescens* (71.70%). Lowest mycelial growth observed in *T. viride* (68.83).

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