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Response of Seed-Borne Pathogens of Cereal Crops to *Azotobacter chroococcum* Strains

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ABSTRACT

Studies on cereal seed inoculation with *Azotobacter chroococcum* (Beijerinck) isolates conclusively showed that selection of suitable isolates of *A. chroococcum* is essential to derive maximum benefits from seed bacterization. Comparatively, isolates A_5, M_4 and M_8 proved to be more effective than others in improving the seed germination and suppressing seed - borne fungi of cereal crops. Combined application of pesticides with *Azotobacter* inoculation increased the grain yield of sorghum with a saving of 40 kg N ha^{-1}.

KEY WORDS : *Azotobacter chroococcum*, antagonism, seed pathogens, cereal crops

Control of seed-borne diseases of cereal crops by natural means is essential to improve the seed germination as, these seed - borne pathogens are capable of producing most devastating diseases destroying 90% or more of cereal crops. Screening of the isolates of *Azotobacter chroococcum* (Beijerinck) for their antagonism on agar plates against pathogens isolated from the infested seeds and plants of cereals has already been reported (Meshram *et al.*, 1990). The beneficial effects of *A. chroococcum* on cereal crops following inoculation of seeds or seedlings has been attributed to multiple action of the antagonist in soil *viz.*, 'N' fixation, suppression of plant pathogens, production of growth promoting substances, effect on other beneficial microorganisms, and mobilization of soil phosphate (Brown, 1974; Mishustin and Naumova, 1962; Shende *et al.*, 1975). The aim of the present study was to test the antagonistic activities of isolates of *A. chroococcum* against seed-borne fungi of cereals *in vitro* and subsequently its influence particularly on sorghum in relation to the effect on germination and yield of grains under field condition.

MATERIALS AND METHODS

A total of 124 *A. chroococcum* isolates were obtained from the rhizospheres of wheat, maize, sorghum and rice grown in Vidharbha region. These isolates were identified as per the methods adopted while screening of *Azotobacter* spp. by Apte (1978). In the present study, the isolates of *A. chroococcum* were selected on the basis of the maximum inhibition zone formed on agar plates against the growth of various pathogens of cereal crops.

The experiment was carried out in Petri-plates using maize, wheat, rice and sorghum seeds. Seven-day-old cultures of *A. Chroococcum* isolates ranging from 30-35 X 10^8 cells ml^{-1} were prepared in Ashby's liquid medium. Seeds of maize, wheat, rice and sorghum were kept in sterilized Petri-plates containing water-soaked cotton and blotting paper and inoculated with 0.1 ml culture of *A. chroococcum*. In the control, 0.1 ml sterilized uninoculated broth medium was used as an inoculum. These Petri-plates were kept at 28°c and percentage of germination was recorded after 24 h.

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The second set of experiments was carried out with seed treatment of 0.1% $HgCl_2$ solution followed with 3 distilled water washes and an inoculation of 0.1 ml broth culture of *Azotobacter*. In the control, seeds were treated with $HgCl_2$, washed and inoculated with 0.1 ml sterilized Ashby's broth.

The effect of various treatments on the germination and plumule formation of wheat, sorghum, maize and rice along with the percentage of pathogen infection was recorded after 48 h with a final observation after 120 h. The pathogens that infected the seeds were identified by Plant Pathology Department, College of Agriculture, Nagpur.

A field plot experiment with sorghum (CvCSH-1) was conducted during the *kharif* season of 1990 with ten treatments, each in triplicate in randomised block design. An individual net plot size of 0.92 x 0.92 m^2 was maintained. Soil of the experimental field was sandy loam with a pH of 7.9 and contained 0.021% total N, 0.179% organic carbon and 0.001% available phosphorus. Basal doses of NPK were applied @ 80 Kg N ha^{-1} in split form, 50 kg P ha^{-1} and 40 Kg K ha^{-1}. However, plots inoculated with *Azotobacter* received only 40 kg N ha^{-1} as urea in a split application, one half applied at sowing time and the other half as a top dressing 40 days after sowing.

Prior to inoculation and sowing of seeds of sorghum, surface sterilization with 0.1% $HgCl_2$ (2 min.) followed by washing with water was carried out. As per treatments, seeds were treated with heavy (15-day-old) broth cultures of *Fusarium equiseti* (Corda Saccardo) + *Cladosporium oxysporum* (Berkeley & Curtis) (10^7 to 10^8 propagules ml^{-1}). The pathogenicity test was done with reference to seed-borne pathogens of sorghum. According to the treatment, *Azotobacter* isolates and pesticide application were done after the pathogen inoculation. Seeds were treated with 10-day-old *Azotobacter* culture prepared in Ashby's liquid medium containing fine mesh powder of charcoal. The density of *A. chroococcum* isolates ranged from 16-24 x 10^8 cells ml^{-1}. The *Azotobacter* M_4 & A_5 were selected on the basis of constant antagonistic phenomenon shown under laboratory condition. Within 10 days of incubation, isolates M_4 and A_5 fixed N_2 7.90 to 8.70 mgN/gm of sucrose consumed respectively. In case of pesticide treatments, dry seed dressing of Thiram @ 0.25% + Vitavax @ 0.1% was done. Other agronomic practices were followed commonly during the vegetative period of the crop.

Seed germination per cent was recorded after 20 days of sowing and no gap filling was done. The infestation of inoculated pathogens and other microflora of non-germinated seeds was investigated. At harvest data on grain yield were collected. The statistical differences in results were compared at 5% level by adopting the technique of 'analysis of variance' (Fisher, 1958).

RESULTS AND DISCUSSION

Inoculation of *Azotobacter* isolates suppressed the growth of pathogens, and enhanced the seed germination in wheat depending upon the type of isolates used (Table 1). Isolates A_5, M_4 and M_7 proved to be much better than the other recording 10[illegible] per cent germination of the seeds. *Azotobacter* inoculation combined with $HgCl_2$ treatment improved the seed germination of rice (Table 1). But seed germination in rice was lower than in wheat. The per cent of seed germination along with their plumule formation of maize was comparatively higher with *A. chroococcum* A_5, M_4 and M_8 isolates. The *Azotobacter* isolate R_2 in combination of $HgCl_2$ treatment resulted in 100% seed germination as well as plumule formation in sorghum. *Azotobacter* inoculation alone and in combination of $HgCl_2$ treatment could control the infestation of various seed-borne microflora of rice crop as compared to the control (Table 2). However, infection b[illegible]

Table 1. Effect of *Azotobacter* and other treatments on the percentage germination (G) and plumule formation (P) of cereals

Treatment	Wheat		Rice		Maize		Sorghum	
	G	P	G	P	G	P	G	P
Control (Sterilized medium)	70	70	55	55	76.6	73.3	75	50
Azotobacter								
A_2	85	80	65	55	70.0	56.6	80	70
A_5	100	100	65	60	86.6	83.3	95	90
A_6	70	70	40	40	66.6	63.3	90	70
M_4	100	100	65	60	93.3	86.6	90	90
M_7	100	95	35	35	76.6	73.3	70	50
M_8	95	90	65	65	73.3	70.0	95	85
R_2	90	80	60	60	76.6	63.3	80	45
Control (Sterilized medium + $HgCl_2$)	80	80	70	70	70.0	70.0	75	75
Azotobacter + $HgCl_2$								
A_2	85	75	75	75	70.0	66.6	80	70
A_5	100	100	80	75	93.3	90.0	80	85
A_6	70	70	65	60	83.3	76.6	80	65
M_4	100	100	70	70	90.0	90.0	85	70
M_7	100	95	70	65	83.3	83.3	90	90
M_8	95	90	80	80	90.0	86.6	90	90
R_2	90	90	55	50	83.3	80.0	100	100

Aspergillus sp. was higher when compared to other pathogens. Treatment of *Azotobacter* (A_5, A_6) alone and in combination of $HgCl_2$ and isolates A_2,A_5, M_4,M_7,M_8 and R_2 proved to be highly effective. Infestation was totally absent. Comparatively, the combination of treatments proved much better controlling infestation of various microflora of wheat seeds. Whereas *Azotobacter* inoculation alone proved less effective as compared to *Azotobacter* inoculation in combination of $HgCl_2$ treatment. Infection by *Aspergillus* sp. was noticed more on wheat seeds. The combined effect of *Azotobacter* inoculation and $HgCl_2$ treatment on maize seeds proved much better in respect of all other treatments. *Azotobacter* inoculation alone, particularly isolate A_5 protected the seeds much better from the infestation of various microflora. *Azotobacter* seed inoculation alone could not protect sorghum from the infestation by various seed borne pathogens. Whereas the treatment consisting of *Azotobacter* inoculation + $HgCl_2$ could suppress the infection effectively. In this combination treatment, the isolates M_4 and M_8 were found to be most effective.

Antifungal action of *A. chroococcum* against *Aspergillus* sp., *Penicillium* spp., *Fusarium* spp. and *Alternaria* spp. have been reported by Mishustin (1966) and Lakshmi Kumari *et al.* (1972). According to Linchevskaya and Kaliberad (1958), late blight of potato incidence could be minimized or reduced by applying *Azotobacter*.

A number of workers reported that seed inoculation with *Azotobacter* inhibited or prevented the occurrence of viral, fungal and bacterial diseases of some agricultural crops (Dorosinskii, 1962; Khudyakov and Marschunova, 1966). The success of the inoculation varies with temperature, and also

Table 2. Effect of *Azotobacter* isolates and other treatments on various micro-flora of cereals seeds

Treatment	WHEAT			% Total infest-ation	RICE				% Total infest-ation	MAIZE				% Total infest-ation	SORGHUM			% Total infest-ation
	Asp.	*Pyt.*	*		*Asp.*	*Peni.*	*Fus.*	*Hel.*		*Asp.*	*Peni.*	*Fus.*	*Al.*		*Cul.*	*Pho.*	*Cla.*	
Control (Sterilized medium)	15	10	10	35	25	5	10	5	45	33.3	10.0	6.6	3.3	53.3	45	10	45	100
Azotobacter																		
A2	25	0	0	25	10	0	0	0	10	30.0	10.0	0.0	6.6	46.6	70	0	30	100
A5	25	0	0	25	0	0	0	0	0	13.3	10.0	0.0	0.0	23.3	50	25	25	100
A6	15	0	0	15	0	0	0	0	0	20.0	10.0	6.6	6.6	43.3	50	20	30	100
M4	15	0	5	20	5	0	0	0	5	10.0	13.3	3.3	0.0	26.6	45	20	35	100
M7	15	10	5	30	25	0	0	5	30	26.6	6.6	0.0	3.3	36.6	50	45	5	100
M8	10	0	0	10	0	5	0	0	5	30.0	3.3	3.3	0.0	36.6	55	20	25	100
R2	15	10	10	35	0	10	0	0	10	30.0	10.0	0.0	0.0	40.0	60	0	40	100
Control (Sterilized medium + $HgCl_2$)	20	10	5	35	15	0	0	0	15	16.6	6.6	6.6	3.3	33.3	40	5	25	70
Azotobacter $HgCl_2$																		
A2	0	0	0	0	0	0	0	0	0	10.0	0.0	0.0	0.0	10.0	40	0	20	60
A5	10	0	0	10	0	0	0	0	0	3.3	0.0	0.0	0.0	3.0	15	0	0	15
A6	0	0	0	0	5	0	0	0	5	13.3	0.0	0.0	0.0	13.3	30	0	20	50
M4	0	0	0	0	0	0	0	0	0	3.3	0.0	6.6	0.0	10.0	5	0	0	5
M7	0	0	0	0	0	0	0	0	0	3.3	0.0	0.0	0.0	3.3	35	10	20	65
M8	0	0	0	0	0	0	0	0	0	3.3	0.0	0.0	0.0	3.3	10	0	0	10
R2	0	0	0	0	0	0	0	0	0	10.0	3.3	0.0	0.0	13.3	15	10	15	40

* The fungus is yet to be identified

NOTE : *Rhizopus* was found growing vigorously in case of *Azotobactor* alone treatments.

Asp. = *Aspergillus* sp.; *Pyt.* = *Pythium* sp.; *Peni.* = *Penicillium* sp.; *Fus.* = *Fusarium* sp. *Hel.* = *Helminthosporium* sp.; *Al.* = *Alternaria* sp.; *Cul.* = *Culvularia* sp.; *Pho.* = *Phoma* sp.; *Cla.* = *Cladosporium* sp.

depends on the selection of appropriate isolates (Meshram and Jager, 1983). Inoculation with an isolate of *Verticillium biguttatum* in combination with isolates of *A. chroococcum* effectively protected sprouts, stems and stolons against the infestation with *R.solani* (Meshram, 1984); the yield also increased significantly over the control.

Results of the field trial conducted on sorghum revealed that the efficacy of *Azotobacter* isolates varied under the field condition also (Table 3). This indicates that screening of *A. chroococcum* strains in laboratory as well as field condition is essential prior to recommendation of package of treatment. The application of pesticides alone and combined with *Azotobacter* and seed-borne inoculated pathogens gave the highest germination rate. However, the seed bacterization of *Azotobacter* isolates alone proved to be statistically non- significant though higher per cent germination was observed compared to control. Inoculation of these isolates combined with seed-borne inoculated pathogens proved to be less effective. This might be due to the heavy inoculum of pathogens artificially in addition to natural presence of seed-borne microflora as recorded in Table 2 with the same variety of seeds. Further, the per cent of seed germination recorded in the treatments of inoculated seed- borne pathogens was found much lower. Most of the traceable non- germinated seeds were found totally deterioated and infected with *Fusarium, Cladosporium and Curvularia* spp.

The application of pesticides proved to be very effective when combined with *Azotobacter* inoculation, the increase of grain yield was obtained with saving of 40 kg N ha^{-1}. Of course, the significant effect of these treatments on yield is due to the high rate of germination of seeds. Besides, the gradual release of N fixed by *Azotobacter* may have resulted in higher efficiency with low level of nitrogenous fertilizers i.e. 40 kg N ha^{-1} when compared with high level 80 kg N ha^{-1}. No response to *Azotobacter* inoculation combined with pathogens was obtained. Obviously, this is due to introduction of pathogenic inoculum with seed treatment. Inoculum density is generally known to be directly proportional to disease severity (Baker, 1968). To add this, an inoculation of *Azotobacter* M_4 alone proved to be much beneficial with a grain yield of 26.20 Q/ha.

The effect of pesticides such as thiram, and vitavax on *Azotobacter* inoculant needs to be studied. The favourable effect of *Azotobacter* inoculation obtained is attributed due to multiple action. However, prior to use of

Table 3. Influence of various treatments inoculated with *Azotobacter chroococcum* isolates on seed germination and yield of sorghum (var. CSH-1)

Treatment	Seed Germination (%)	Grain yield (Q ha^{-1})
Control	53.79	21.52
Pathogens alone	43.71	18.54
Azotobacter M_4	68.66	26.20
Azotobacter A_5	61.80	22.87
Pesticides	76.50	28.17
Azotobacter M_4 + Pathogens	56.75	21.89
Azotobacter A_5 + Pathogens	52.90	21.25
Pesticides + Pathogens	71.05	26.26
Azotobacter M_4 + Pesticides + Pathogens	80.90	32.86
Azotobacter A_5 + Pesticides + Pathogens	76.46	27.91
SE (m) ±	4.04	1.17
C.D. at 5%	16.46	4.77

Azotobacter seed bacterization, screening of isolates of *A. chroococcum* is necessary under laboratory as well as field condition.

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