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IN-VITRO INTERACTION BETWEEN FUNGICIDES AND RHIZOBACTERIA ISOLATED FROM FENUGREEK ROOT NODULES

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ABSTRACT

Fenugreek is an important seed spice also has medicinal value. Chemical control of soil born insect-pest and disease in fenugreek has been achieved through use of insecticides and fungicides. However, these chemicals are also hazardous for beneficial organisms like Rhizobia. Therefore, an experiment was conducted to study the effects of different concentrations (50, 100, 200, 300, 500 ppm) of seed dressing fungicides viz. Carbendazim, Captan, Thiram and Metalxyl on growth of fifteen Rhizobium isolates of fenugreek. Colony size of isolates ATFG-1, ATFG-2, ATFG-5, ATFG-12 and BTFG-17 were reduced significantly with increasing concentration of carbendazim, the r^2 values of their relationships were -0.933, -0.935, -0.920, -0.940 and -0.964, respectively. Reduction in size of ATFG-1, BTFG-13 and BTFG-18 was negatively correlated with captan concentration i.e r^2 values being-0.988, -0.855 and -0.988, respectively. All these isolates were found tolerant to carbendazim and captan with their lower concentrations i.e. ≤50 ppm. However, these isolates were found sensitive to Carbendazim beyond its concentration of 500 ppm. Moreover, Captan had harmful effect on all the isolates beyond 300ppm. Per cent reduction of colony sizes of both the series of isolates ranged from 9.1-75% with carbendazim. However, the per cent range of reduction was 10 to 80% with captan. Increasing concentrations of Carbendazim and Captan reduced the growth of Rhziobium isolates. Moreover, none of the isolate was found tolerant to Metalxyl and Thiram and any of their concentrations.

Key words: *Trigonella foenum-graecum, Rhizobium,* Carbendazim, Captan, Metalxyl and Thiram

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important seed spice and also used as vegetable as well as medicinal plant. It is extensively grown in the tropical and sub-tropical regions of Indian sub-continent during winter season. Being a leguminous plant, it utilizes atmospheric nitrogen through symbiosis of *Rhizobia* (FAO, 1984). Rhizobia are soil bacteria those are able to invade the root hairs of compatible host legumes and initiate the formation of nodules. Rhizobum exists as an intracellular symbiont within the developed nodules, converting atmospheric nitrogen into ammonia and that is utilized by the plant in exchange of plant-derived organic acids. Signal exchange takes place between the *Rhizobia* and the plant, occurs through all stages of nodule development (Simon *et al.,* 2007). *Rhizobium* faces various stresses which affects its growth and nitrogen fixing capacity (Panwar *et al.,* 2012). Chemical control of soil born disease of fenugreek could be achieved through use of fungicides (Dodan *et al.*, 1994). These chemicals are very hazardous to human, animals and environment. Most of the times fungicides affect the growth of root nodulating Rhizobacteria. Using fungicide for disease control in legumes has contributed to increase their yield and quality (Fox *et al.*, 2007). Application of fungicides as seed dressing in leguminous plants may affect the symbiotic relationship of *Rhizobium* and their nodulation status. These also affect enzymatic activities in root and soil rhizosphere like nitrogenase and hydrogenase enzymes (Bikrol *et al.,* 2005). Nodulating ability of the surviving bacteria is an important factor for determining compatibility with seed dressing fungicides. Some fungicides affect the growth of root nodulating bacteria. Mode of action of fungicide have never been studied and classified well. The side effects of these important chemicals are not fully understood also. Therefore, fungicides use may have negative impacts and that are difficult to predict (Lo, 2010). However, some studies available with the effect of different fungicides on rhizobial growth and its nitrogen fixation capacity (Rennie and Dubetz, 1984). Cevheri *et al.*, (2011) reported that *Rhizobium* isolates have different capacity to resist against various chemical stresses like fungicides, antibiotics and salinity. Moreover, the work done on the aspect is very limited and also not specific, needs extensive study on specific rhizobecteria. Keeping in view the above facts, present investigation was undertaken to examine the effects of some commonly used fungicides.

MATERIALS AND METHODS

Four fungicides selected for the experiment were carbendazim, captan, thiram and metalxyl. Fifteen elite *Rhizobia* selected for the study were

isolated from fenugreek root nodules and used in this study namely ATFG-1, ATFG-2, BTFG-3, ATFG-4, ATFG-5, ATFG-6, ATFG-8, ATFG-9, ATFG-12, BTFG-13, BTFG-14, BTFG-15, BTFG-16, BTFG-17 and BTFG-18. All isolates were morphologically and biochemically characterized according to Pryor and Lowther (2002); Panwar *et al.*, (2012).

Five concentrations (50, 100, 200, 300, 500 ppm,) of four fungicides i.e. carbendazim, captan, thiram and metalxyl were tested on the growth of *Rhizobium* using yeast extract mannitol agar media with Congo red dye (CRYEMA). The media was prepared by adding different concentrations of seed dressing fungicides and adjusted the pH 6.8. Media was autoclaved and poured into sterilized petri plates. After solidify the media, 48 hrs old *Rhizobia* colonies streaked on media containing fungicides. Petri plates were incubated in invert position for three days at 27 ± 1^{o}C. CRYEMA media without seed dressing fungicides served as control. The mean of three replicated data were taken for interpretation of results. Growths of colonies were measured after the incubation period of 72 hrs.

RESULTS AND DISCUSSION

Several fungicides are available in the market but all were not tested for compatibility with rhizobacteria. In the present investigation fifteen elite *Rhizobial* isolates were evaluated against four different fungicides i.e. carbendazim, captan, thiram and metalxyl. All *Rhizobium* isolates showed differed response to fungicides.

Carbendazim: At 50 ppm concentration, colony size of ATFG-1 and ATFG-4 was the biggest followed by AFTG-2 and BFTG-14 (Table 1). However, at 100 ppm BFTG-17 performed better than ATFG-1 and ATFG-4 whereas BTFG-3, ATFG-6 and ATFG-8 were unable to grow beyond the 100 ppm (Fig: 1a). At 200 ppm BTFG-14 and BTFG-12 performed better than BTFG-17. The colony size of BTFG-13 was the biggest at 50 and 100 ppm than BTFG-18 at same concentration but at 200 ppm BTFG-18 performed better than BTFG-13 (Fig: 1b). Colony size of isolates ATFG-1, ATFG-2, ATFG-5, ATFG-12 and BTFG-17 reduced significantly with increasing concentration of carbendazim (Fig: 2). Growth of following isolates ATFG-1, ATFG-2, ATFG-5, ATFG-12 and BTFG-17 were negatively correlated with higher concentrations of carbendazim, the r^2 values of their relationship were -0.933, -0.935, -0.920, -0.940 and -0.964, respectively (Table 2). Colony sizes of *Rhizobial* isolates ATFG-1, ATFG-2, ATFG-4, ATFG-9, BTFG-14 and BTFG-17 decreased with increasing concentration of carbendazim (Fig: 2). Adaptability of isolates ATFG-1 and ATFG-9 was highest with various concentration of carbendazim and was least of BTFG-18. Rest of the isolates was found in the middle range for their adaptability with carbendazim (Table 3). Increasing the concentration of captan, thirum, Luxan, Femasan-D and Milcurb reduced the growth of *Rhziobium* and *Bradyrhizobium* strains as reported by Ahmed *et al.* (2007). Fawole *et al.* (2008) found that cellulase and pectinase activity was significantly lowered with high concentration of fungicides. Ahemad and Khan (2013) reported that carbendazim had some adverse effect on soil microflora and inhibited the growth of *Rhizobium japonicum*. It produces more toxins to the nodulating bacteria and affects plant vigor, number of nodules, nitrogenase activity, chlorophyll contents, nitrogen uptake and ultimately seed yield. Muthuviveganandavel *et al.* (2011) reported that use of individual or in combination with different concentrations of fungicides those generally leaves a toxic effect on animals and also occurs on early enzymatic and histological changes.

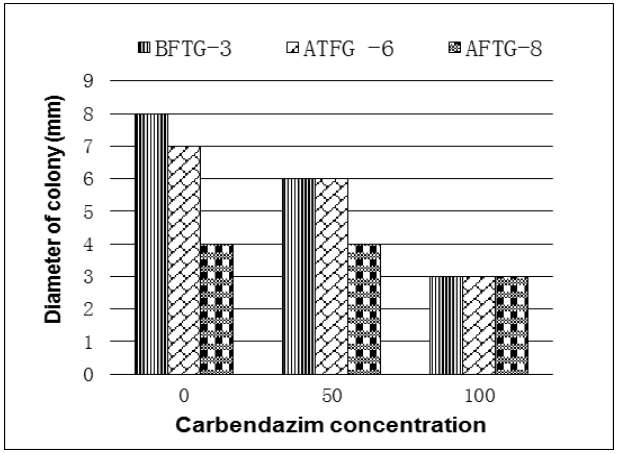
Captan: At 50 ppm concentration, all isolates were found compatible with this fungicide. At 50 ppm, the colony size of BTFG-13 was the biggest. However, at 100 ppm ATFG-1 performed better than BTFG-13 followed by BTFG-18. Moreover, BTFG-18 was unable to grow beyond the 100 ppm. Among the fifteen isolates, BTFG-13 was the most tolerant, ATFG-1 and BTFG-18 were moderately tolerant (Fig: 3) while remaining isolates were least tolerant against the various concentrations. At 100 ppm of captan, colony size of BTFG-13 was reduced by three times over the 50 ppm. Reduction in size of ATFG-1, BTFG-13 and BTFG-18 was negatively correlated with its concentration i.e r^2 values being -0.988, -0.855 and -0.988, respectively (Table 2). ATFG-1 was least adaptive against various concentrations of captan. However, BTFG-13 was more tolerant against various concentrations of captan than the other isolates (Table 3). Ahemad and Khan (2013) reported that fungicides treated seeds were inoculated with rhizobia affects the physiology and growth characteristics of both rhizobia and legume plants by blocking signaling between them and ultimately effects the symbiotic nitrogen fixation.

Thiram and Metalxyl: None of the isolate was found tolerant against thiram and metalxyl, as these isolates were very sensitive with these fungicides. These finding are corroborated by Ahmed *et al.*, (2007). Besides the individual observations, overall performances of isolates were fair at low concentration (50 ppm) of carbamdazim and captan.

Table 1: Effect of fungicides on development of colony of *Rhizobium* isolates

Rhizobium isolates	Concentrations fungicides (ppm)																			
	Carbendazim					Thiram					Metalxyl					Capton				
	50	100	200	300	500	50	100	200	300	500	50	100	200	300	500	50	100	200	300	500
ATFG-1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
ATFG -2	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -4	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -5	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -9	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATFG -12	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -13	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
BTFG -14	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -15	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -16	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -17	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
BTFG -18	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

Note: '+' = Colony developed; '-' = Colony not developed



1a

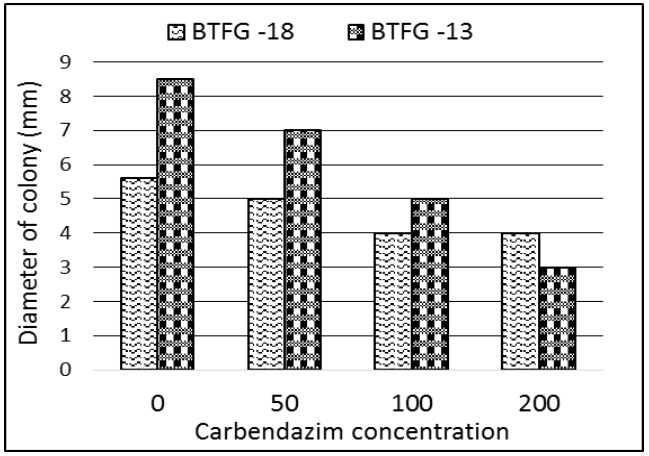


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Fig: 1a- Effect of lower concentrations of carbendazim on BTFG-3, ATFG-6 and ATFG-8 isolates
1b- Effect of higher concentrations of carbendazim on BTFG-18 and BTFG-13 isolates

Table 2: Relationship of concentrations of fungicides and growth of Rhizobia colony

Isolates	Carbendazim	CD at 5%	Captan	CD at 5%
	r^2 value		r^2 value	
ATFG-1	-0.933	S	-0.982	S
ATFG-2	-0.935	S	-	-
BTFG -3	-	-	-	-
ATFG -4	-0.818	NS	-	-
ATFG -5	-0.920	S	-	-
ATFG -6	-	-	-	-
ATFG -8	-	-	-	-
ATFG -9	-0.882	NS	-	-
ATFG -12	-0.940	S	-	-
BTFG -13	-	-	-0.855	NS
BTFG -14	-0.844	NS	-	-
BTFG -15	-0.865	NS	-	-
BTFG -16	-0.888	NS	-	-
BTFG -17	-0.964	S	-	-
BTFG -18	-0.878	NS	-0.981	S

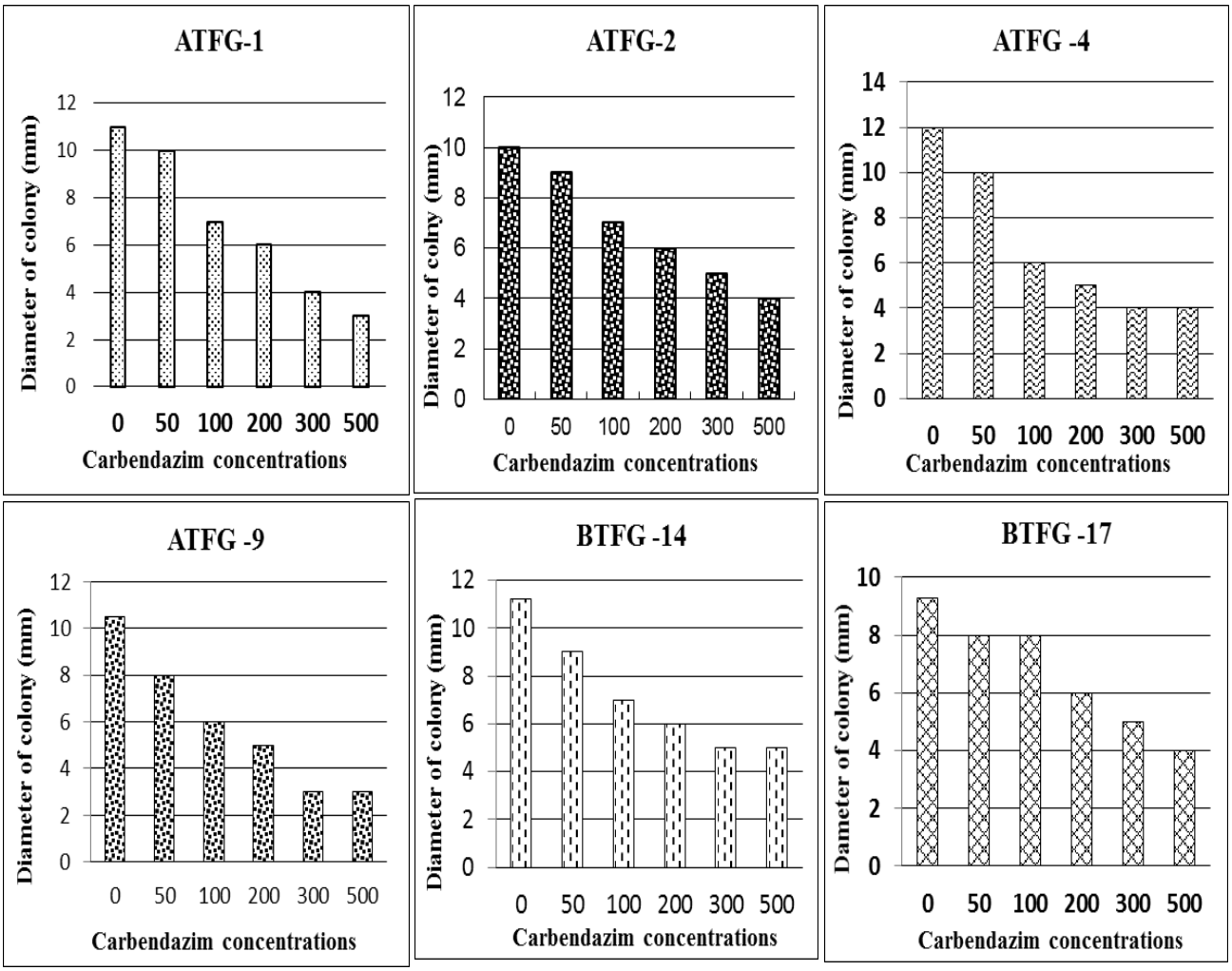

Fig: 2: Effect of carbendazim concentrations on ATFG-1, ATFG-2, ATFG-4, ATFG-9, BTFG-14 and BTFG-17 isolates

Somewhat similar results were reported by Mishra *et al.* (2013), while they worked on *Bradirihizobium japonicum* and PSB. There are numeral studies on mode of action of fungicides on toxicology. However, very few information are there, those are also not specific and limited to mode of action of fungicides on interaction with fungal infection. Fungicides absorbed through the roots and green tissues, with translocation acropetally and act by inhibiting development of the fungal germ tubes and the growth of mycelia (Anonymous 2009). There may be the similar detrimental effect of these fungicides on rhizobacteria as explained by researcher. These chemicals cause changes in composition, diversity and functional activities like inhibiting protein synthesis, metabolic activity of enzymes Boldt *et al.* (1998) and Kapoor *et al.*, (1996) including dehydrogenase and phosphatase activity Monkiedje *et al.* (2002), Sannino and Gianfreda (2001), damaging structural proteins through biochemical alterations in membrane composition Fabra *et al.* (1998) geno-toxicity and microbial respiration Kumar *et al.* (2010) and Pham *et al.* (2004).

Table 3: Per cent reduction of colony sizes in relation to control

Isolates	Per cent reduction of colony sizes over their respective controls									
	Concentrations of carbendazim (ppm)					Concentration of captan (ppm)				
	50	100	200	300	500	50	100	200	300	500
ATFG-1	9.1	36.4	45.5	63.6	72.7	22.2	44.4	66.6		
ATFG-2	10.0	30.0	40.0	50.0	60.0	-	-	-	-	-
BTFG -3	25.0	62.5	-	-	-	-	-	-	-	-
ATFG -4	16.7	50.0	58.3	66.7	66.7	-	-	-	-	-
ATFG -5	20.0	50.0	50.0	70.0	-	-	-	-	-	-
ATFG -6	14.3	57.1	-	-	-	-	-	-	-	-
ATFG -8	-	25.0	-	-	-	-	-	-	-	-
ATFG -9	27.3	45.5	54.5	72.7	72.7	-	-	-	-	-
ATFG -12	25.0	50.0	62.5	75.0	-	-	-	-	-	-
BTFG -13	22.2	44.4	66.6	-	-	10.0	70.0	80.0	80.0	-
BTFG -14	18.2	36.4	45.5	54.5	54.5	-	-	-	-	-
BTFG -15	27.3	54.5	63.6	64.0	-	-	-	-	-	-
BTFG -16	28.6	57.1	57.0	71.4	-	-	-	-	-	-
BTFG -17	11.1	11.1	33.3	44.4	55. 6	-	-	-	-	-
BTFG -18	16.7	33.3	33.3	-	-	25.0	75.0			-

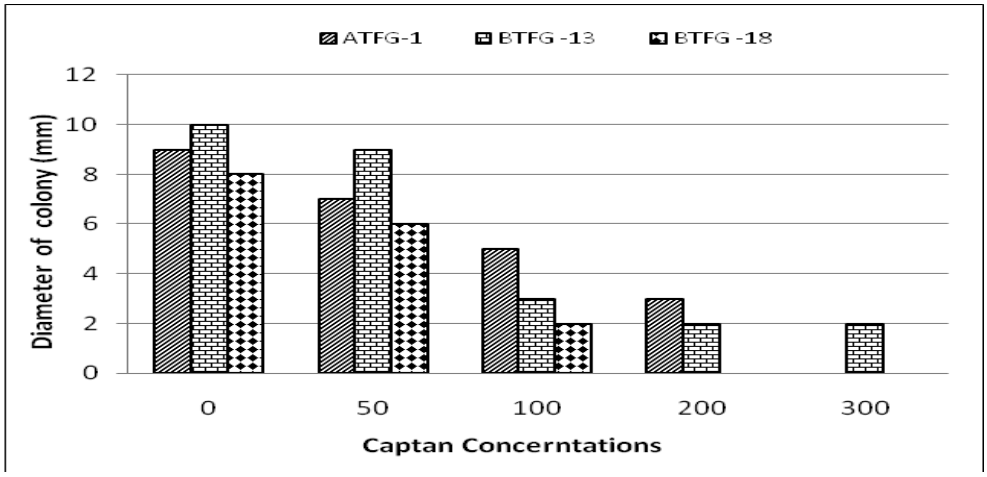

Fig 3: Effect of captan concentrations on ATFG-1, BTFG-13 and BTFG-18 isolates.

All the rhizobia isolates were found tolerant to lower concentrations of fungicides, whereas higher concentrations reduced the growth of rhizobia colonies. However, none of the isolates were found tolerant against thiram and metalxyl.

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