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Bio-efficacy of different antibacterial antibiotic, plant extracts and bioagents against bacterial blight of soybean caused by *Pseudomonas syringae* pv. *glycinea*

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ABSTRACT

An experiment was carried out to study efficacy of different antibacterial antibiotic, plant extracts and bioagents against bacterial blight of soybean caused by *Pseudomonas syringae* pv. *glycinea*. The results revealed that all the five antibiotics tested *in vitro* applying poisoned food technique against *Pseudomonas syringae* significantly inhibited the growth of the test pathogen over untreated control. However, antibiotic, Streptocycline + Copper oxychloride recorded minimum mean colony diameter (10.47mm) and maximum mean inhibition (83.65 mm) of growth of the test pathogen over untreated control (mean col. dia. 90.00 mm and mean inhibition, 0.00) followed by the antibiotic Streptocycline 100 ppm (mean col. dia., 15.64 mm and mean inhibition, 76.69%) and Copper oxycloride (mean col. dia., 21.42 mm and mean inhibition, 62.74%). In field, the highest mean per cent disease incidence 35.51 per cent was observed in poushamycin treatment. The lowest mean per cent disease incidence 12.74 per cent was found in treatment streptocycline 100 ppm + Copper oxychloride (@0.25%) and recorded highest seed yield (2605 kg/ha) and test weight (14.33 g). Plant leaf extract (@ 5 and 10%) of Neem recorded lowest mean colony diameter (34.72 mm) and highest mean mycelial growth inhibition (59.26%) of the test pathogen over untreated control followed by Ginger (mean col. dia, 44.42 mm and mean inhibition, 48.55%).

1. Introduction

Soybean (*Glycine max* (L.) Merril.) Is a native of Eastern Asia. It belongs to the family *Leguminoseae* and sub-family *Papilionoidae.* It is an ancient crop which is being used as food, feed and also in industry for various purposes. The crop is presently grown in most of the part of the world and is a primary source of vegetable oil and protein. It contains 44.65 per cent protein, 8.77 per cent fats, 27.12 per cent nitrogen, 5.89 per cent ash, 5.96 per cent fibre. The forty percent of world's supply of vegetable oil comes from soybean and 80 per cent which is used in margarine, salad oil, cooking oil and shortening. In India area, production and productivity of soybean during 2007-2008 were 79.720 lakh ha., 64.28 lakh metric tonnes and 802 kg/ha., respectively (Anonymous, 2007).

Bacterial blight is caused by *Pseudomonas syringae* pv. *glycinea*, young leaves are most susceptible to the bacterial infection, therefore the disease lesions are small yellow to brown spots on leave. The lesions dry out, turn reddish brown to black and become surrounded by a yellowish green halo. The small lesions may enlarge and merge to produce large, irregular, dead areas. The old lesions sometimes drop out or tear away, resulting in ragged appearance of infected leaves. The bacteria can also infect stem petioles and pods. If pod infection occurs, bacterial blight can become seed borne. Studies have reported bacterial pustule disease is wide spread in the Kirovograd region causing seed losses upto 28 per cent (Teresbchenko, 1977).

Soybean crop is know to be affected by more than 100 plant pathogens out of 35 are of economic importance. Among the major bacterial, bacterial blight caused by *Pseudomonas syringae* has been reported yield loss potential for this disease to range from 4 per cent to as high as 40 per cent under extreme condition.(Rampandu *et al.,* 1979: Meshram, 1988 and 1992: Lim, 1992, Mishra and krishana, 2001 and Patil *et al.,* 2003).

Keeping in view the economic importance of the crop and losses caused by bacterial blight disease, present investigations on the aspects *viz*., evaluation of antibiotic, botanicals and bioagents, against *Pseudomonas. syringae* were undertaken during the *Kharif*, 2009 on the research farm of the Department of Plant Pathology, College of Agriculture, Parbhani.

2. Materials and methods

2.1. In-vitro evaluation of antibiotics

To see the efficacy of antibiotics against *Pseuodomonas syringae* pv. *glycinea* these were tested by poisoned food technique in the laboratory. A 48 hour old culture of *Pseuodomonas syringae* pv. *glycinea* was diluted in water blanks upto 10^6 to obtain approximately 150 cfc/ml of the bacterium. One ml from this was placed in the centre of the plates separately and then antibiotic mixed medium was poured over it. For this, nutrient agar medium was prepared in flasks and sterilized. After cooling (45^{o}C) to this medium requisite quantity of antibiotic *viz.,* streptocyline + copper oxychloride, streptocycline, copper oxychloride, 2 bromo 2 nitro propene 1-3 diol, poushamycin were add to obtain 100 and 200 ppm concentrations. These antibiotics were thoroughly mixed by rotary motion in palms of hands. The medium is then poured into petriplates separately @ 20 ml / (90 mm diameter plate). Suitable controls with NA medium without antibiotic were kept. There were 4 replications of each treatment. These petriplates were incubated at 25 $\pm$ 2 ^{o} C temperature for 4 days, after four days colonies of *Pseuodomonas syringae* pv. *glycinea* were counted.

2.2. In-vitro evaluation of botanicals

Plant leaf extract of 5 botanicals *viz*., Ginger, Neem, Garlic, Onion and Tulsi were evaluated against *Pseuodomonas syringae* pv. *glycinea* . Leaf extract were prepared by grinding with mixture cum grinder the 50 gm and 10 gm washed leaves of each plant speices in 100 ml distilled water and filterered through whatman NO. 1 filter paper using funnel and volumetric flask (100 ml capacity). The final clear obtained from the standard leaf extract of 100 per cent concentration extracts which were evaluated @ 5 per cent and 10 per cent) *in vitro* by using inhibition zone technique against the test pathogen. Leaf extract of plant species were tested against *Pseuodomonas syringae* pv. *glycinea in vitro* using inhibition zone technique. Nutrient agar medium was prepared in conical flask (250 ml cap) and autoclaved at 15 lbs/cm^2) pressure for 15 to 20 minutes. Adding 1-2 loopful of bacterial culture in sterile water prepared dilution of bacteria. An appropriate quantity of each leaf extract was separately mixed thoroughly with molten and cooled (40^{o}C) NA medium in conical flasks (250 ml cap) to obtain

desired concentrations (5 and 10 per cent). Nutrient agar medium autoclaved and cooled at (30-35^{o}C). Mixed the dilutions of bacterium in nutrient agar medium. The bacterial dilutions amended in nutrient agar were then poured (15-20 ml /plate) in sterilized petri-dishes (90 mm dia.) under aseptic condition. On solidification of NA, all the petriplates were seeded by placing 10 mm dia. What man No. 1 filter paper by dipping in each concentration of plan extracts in the centre of medium. Plates containing nutrient agar with bacterial suspension without any extracts were maintained as control. All these petriplates were incubated at room temperature (28 $\pm$ 2^{o}C) for 48 hours.

2.3. In-vitro evaluation of bioagents

The antagonistic potential of *Trichoderma* spp. was assessed against *Pseuodomonas syringae* pv. *glycinea* by dual culture technique on yeat glucose chalk agar media as per procedure described. For this 20 ml of sterilized and luke warm medium (chalk agar) was poured in each petriplate and allowed to solidify. With the help inoculation needle streak the bacterial culture on half side of petriplate and other side of *Trichoderma* spp. are inoculated control i.e. without inoculation of bioagent was maintained simultaneously. Observation regarding colony radius of pseudomonas and bioagent were recorded at 6, 9 days after inoculation by incubating at 26 $\pm$ 1 ^{o}C temperature.

Per cent inhibition of growth was calculated by following formula.

$$X = \frac{Y - Z}{Y} \times 100$$

Where,

X = Percent inhibition

Y = Growth of bacterium in control plot (mm)

Z = Growth of bacterium in treatment plot (mm)

3. Results and discussion

A total of five chemicals (@ 100, 200 ppm each) *viz.* Streptocycline + copper oxychloride, Streptocycline, Copper oxychloride, 2 bromo 2 nitro propene 1-3 diol, Poushamycin *in vitro* applying "Poisoned Food Technique" as described earlier against *Pseudomonas syringae*.

Result (Table 1 and Fig. 1) indicated that all the chemicals tested significantly inhibited the growth of *Pseudomonas syringae* over untreated control at all the concentration tested (Plate 1). Among the chemicals, tested Streptocycline + copper oxychloride recorded least mean colony diameter (10.47 mm) and highest inhibition (83.65%) of growth of the test pathogen over untreated control. This was followed by the chemicals, Streptocycline which recorded mean colony diameter of 15.64 mm and mean growth inhibition of 76.69 per cent, followed by copper oxychloride, 2 bromo 2 nitro propene 1-3 diol, which recorded mean colony diameter of 21.42 mm, 25.79 mm, 31.99 mm, mean inhibition of 62.74, 53.09, per cent, respectively. Chemical poushamycin was found comparatively least effective and recorded 31.99 mm and 46.68 per cent mean colony diameter and mean growth inhibition, respectively.

All the concentrations of the chemicals tested significantly inhibited growth of the test pathogen. However, higher concentration (@ 200 ppm) recorded maximum inhibition (Range, 49.30 to 86.48 %) followed by 100 ppm (Range, 44.06 to 80.82 % inhibition).

Thus, all the chemicals tested at various concentrations significantly inhibited the growth *Pseudomonas syringae.* However, streptocycline + copper oxychloride was found most effective followed by *Streptocycline*, copper oxychloride, 2 bromo 2 nitro propene 1-3 diol. Poushamycin was found comparatively least effective against the test pathogen. This study was similar to Taylor and Dye, (1976), Taylor and Dudley; (1977), Meshram *et al.,* (1985), Jeyachandran and Shanmugam (1979), Thind and Mehara (1992) and Govindappa *et al.,* (2008).

3.1. In vitro effect of plant extract

Five chemicals / plant leaf extracts (Garlic, Onion, Ginger, Neem and Tulsi) were evaluated (@ 5 and 10%) *in vitro* against *Pseudomonas syringae* applying poisoned food technique.

Results (Table 2) indicated that all the botanicals tested were found inhibitory and caused significant inhibition of the test pathogen over untreated control. Among five aqueous leaf extracts tested (@ 5 and 10%),

Neem recorded least mean colony diameter (34.72 mm) and highest mean growth inhibition (59.26%). This was followed by Ginger, Garlic, and Onion which recorded mean colony diameter of 44.42 mm, 46.58 mm, 51.89 mm and mean growth inhibition of 48.95, 43.91, 39.67 and 43.72 per cent, respectively. Tulsi was found least effective and caused minimum inhibition (21.52 %) of the test pathogen (Fig. 2).

Table 1

In vitro effect of chemicals at different concentrations on radial growth of *Pseudomonas syringae*.

Tr. No.	Chemicals	Colony Diameter* (mm)		Mean (mm)	Inhibition %		Mean %
		100 ppm	200 ppm		100 ppm	200 ppm	
T_1	Streptocycline + copper oxy-chloride	12.80 (7.35)	8.14 (4.66)	10.47	80.82	86.48	83.65
T_2	Streptocycline	17.90 (10.31)	13.38 (7.68)	15.64	71.16	77.22	76.69
T_3	Copper oxychloride	22.98 (13.28)	19.86 (11.45)	21.42	60.36	65.12	62.74
T_4	2 bromo 2 nitro propene 1-3 diol	27.48 (15.94)	24.10 (13.94)	25.79	51.98	55.82	53.09
T_5	Poushamycin	33.90 (19.81)	30.08 (17.50)	31.99	44.06	49.30	46.68
T_6	Control	90 (64.15)	90 (64.15)	90	0.00	0.00	0.00
	SE ±	0.24	0.24				
	CD at 1%	0.78	0.78				

*Average of four replications.

Figures in parenthesis are angular transformed values.

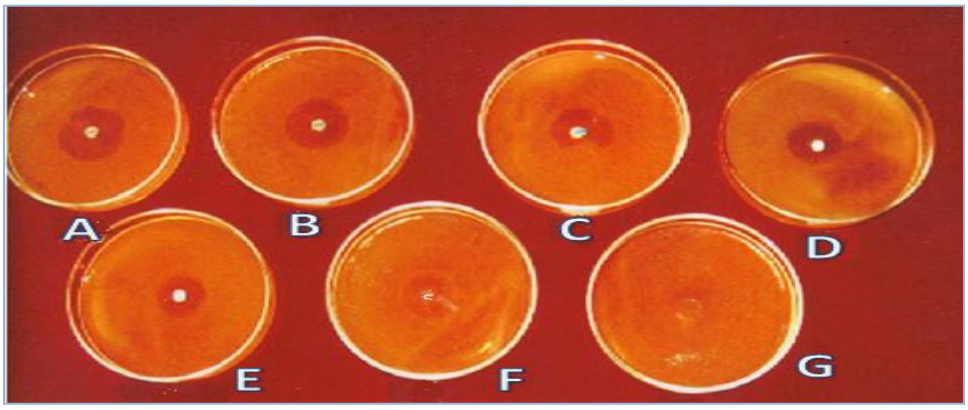

Plate 1. *In vitro* antibiotics against *Pseudomonas syringae* pv. *glycinea.*

A=2 bromo 2 nitro propene 1-3 diol (500 ppm)
B=*Poushamycin (*200 ppm)
C= Copper oxychloride (0.25%)
D=Streptocycline + copper oxychloride (100 ppm +0.25%)
E=Streptocycline (100 ppm)
F=*Pseudomonas fluorescens (0.2%)*
G=Control (Water spray)

Both concentrations (@ 5 and 10%) of the plant extracts tested were found effective in the inhibition of the test pathogen. The plant leaf extract of Tulsi was found least effective and reported maximum mean colony diameter (52.58 mm) and lowest inhibition (21.52%) of the test pathogen. This experiment was showed same result to Mangamma and Screeramulu (1991), Chaturbhuj *et al.,* (2004).

Thus, all the botanicals tested *in vitro* against *Pseudomonas syringae* were found effective in inhibiting the growth of the test pathogen over untreated control.

Table 2

In vitro effect of different plant extracts on radial growth of Pseudomonas *syringae.*

Sr.no.	Treatment	Colony diameter (mm) at conc.		Mean (mm)	% inhibition at conc.		Mean (mm)
		5%	10%		5%	10%	
1	Ginger	46.14 (27.47)	42.71 (25.28)	44.42	47.77 (28.53)	50.13 (30.08)	48.95
2	Neem	35.37 (20.71)	34.08 (19.92)	34.72	58.57 (35.85)	59.96 (36.83)	59.26
3	Garlic	50.23 (30.14)	42.91 (25.42)	46.58	42.75 (25.31)	45.08 (26.79)	43.91
4	Onion	53.98 (32.66)	49.8 (29.87)	51.89	37.14 (21.80)	42.21 (24.96)	39.67
5	Tulsi	54.26 (32.85)	51.1 (30.72)	52.68	20.71 (11.95)	22.33 (12.90)	21.52
6	Contol	90 (64.15)	90 (64.15)	90	0 (0.00)	0 (0.00)	0
	SE ±	0.4	0.33		0.34	0.28	
	CD at 1 %	1.27	1.03		1.07	0.9	

*Average of four replications.

Figures in parenthesis are angular transformed values

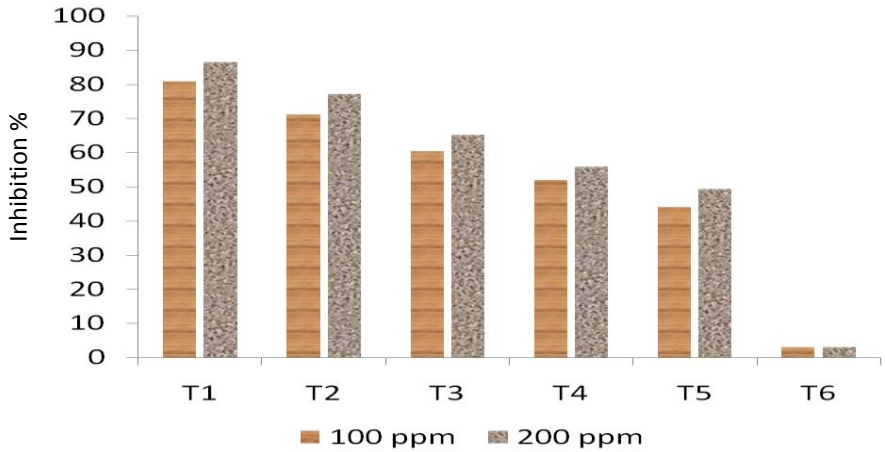

Fig. 1. *In vitro* effect of chemicals at different concentrations on radial growth of *Pseudomonas syringae.*

T1 Streptocycline + copper oxy-chloride T2 Streptocycline

T3 Copper oxychloride T4 2 bromo 2 nitro propene 1-3 diol

T5 Poushamycin T6 Control (water spray)

3.2. In-vitro evaluation of bioagents

Five different species of *Trichoderma viz., T viride, T. hamatum, T. harzianum, T. lignorum, T. koningiii* and one bacterial origin bioagent i.e. *P. fluorescnes* were tested as biological agents for the control of *Pseudomonas syringae* pv.*glycinea.* The effect of bioagents tested against *Pseudomonas syringae* by dual culture technique are

given in Table 3 and 4. The growth of antagonist was recorded at 6 and 9 days of incubation and per cent reduction in colony diameter (mm) of pathogen over control was calculated.

At 6 days of incubation all the species of *Trichoderma* except *T. lignorum* reduced the growth of pathogen over control. The maximum reduction of the pathogen was observed with *T. viride* (48.03 per cent) Amongest antagonists maximum growth was observed with *T. viride* (55.85 mm) followed by *T. harzianum* (50.60 mm) and *T. hamatum* (49.70 mm), (Plate 2 and Fig 3). At 9 days of incubation all the speices of *Trichoderma* reduced the growth of pathogen over control. The maximum reduction of the pathogen was observed with *T. viride* (44.12 per cent) followed by *T. hamatum* (41.70 per cent) and *T harzianum* (41.02 per cent). Amongst the antagonists maximum growth was observed with *T. viride* (60.73 mm) followed by *T harzianum (*58.85 mm) and *T. hamatum* (56.91 mm), (Plate 3 and Fig 3).

Considering the per cent reduction of colony diameter (mm) of the pathogen over control after 6 and 9 days incubation period (Table 5) the treatment. *T. viride* was found best followed by *T. hamatum* and *T harzianum.* Results obtained in respect of efficacy of bioagent in effectively inhibiting the *Xanthomonas* are in conformity with those reported earlier by Chattannovar *et al.,* (1988), Sujoy-Saha *et al.,*(2000), Sanjay-Arya *et al.,*(2002), Saha *et al.,* (2000).

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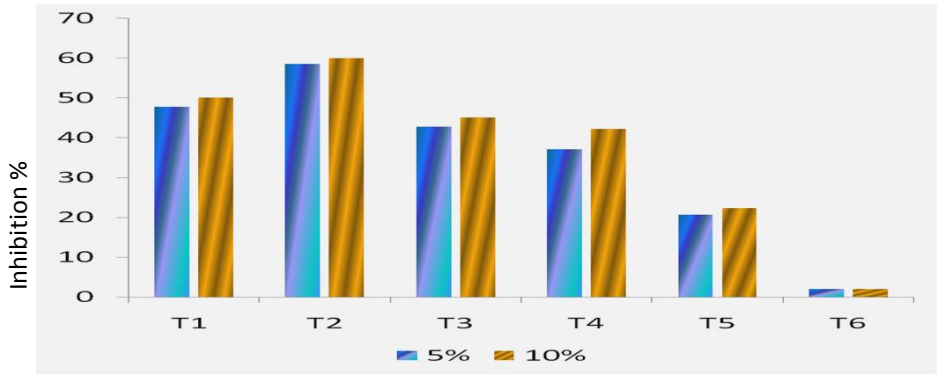

Fig. 2. *In vitro* effect of different plant extracts on radial growth of Pseudomonas *syringa.*
T1 Ginger T2 Neem T3 Garlic
T4 Onion T5 Tulsi T6 Contol

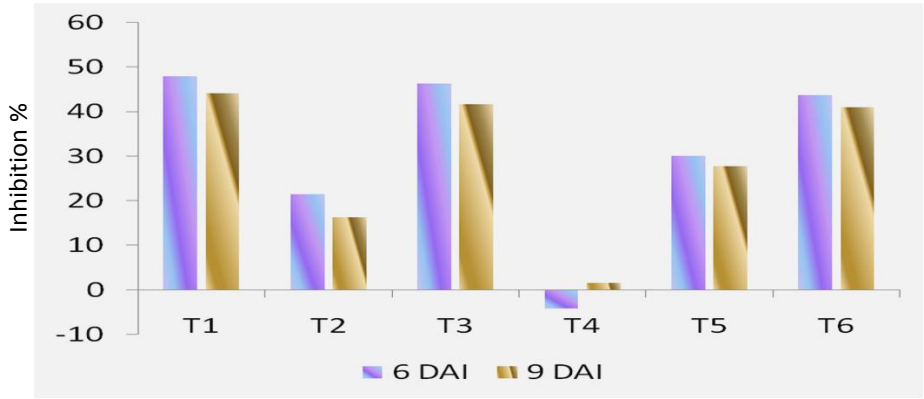

Fig. 3. Percent reduction in colony diameter of the pathogen over control.
T1 *T. viride* T2 *P. fluorescents*
T3 *T. hamatum* T4 *T. lignorum*
T5 *T. koningii* T6 *T. harzianum*

Table 3
Mean colony diameter (mm) at 6 days.

Sr. No.	Antagonists	Mean colony diameter (mm) at 6 days		
		Pathogen	Antagonist	Per cent reduction of colony diameter over control
T_1	*T. viride*	20.23	55.85	48.03
T_2	*P. fluorescents*	30.56	18.01	21.50
T_3	*T. hamatum*	20.87	49.70	46.39
T_4	*T. lignorum*	40.56	34.21	-4.19
T_5	*T. koningii*	27.20	31.61	30.13
T_6	*T. harzianum*	21.91	50.60	43.72
T_7	*Control*	38.93	--	-
	SE ±	1.05	0.78	-
	CD at 5%	3.13	2.34	-

Table 4
Mean colony diameter (mm) at 9 days.

Sr. No.	Antagonists	Mean colony diameter (mm) at 9 days		
		Pathogen	Antagonist	Per cent reduction of colony diameter over control
T_1	*T. viride*	23.81	60.73	44.12
T_2	*P. fluorescents*	35.67	50.93	16.28
T_3	*T. hamatum*	24.84	56.91	41.70
T_4	*T. lignorum*	41.95	43.67	1.55
T_5	*T. koningii*	30.78	54.82	27.76
T_6	*T. harzianum*	25.13	58.85	41.02
T_7	*Control*	42.61	--	-
	SE ±	0.53	0.78	-
	CD at 1%	1.59	0.33	-

Table 5
Percent reduction in colony diameter of the pathogen over control.

Sr. No.	Antagonists	Days after incubation	
		6	9
T_1	*T. viride*	48.03	44.12
T_2	*P. fluorescens*	21.50	16.28
T_3	*T. hamatum*	46.39	41.70
T_4	*T. lignorum*	-4.19	1.55
T_5	*T. koningii*	30.13	27.76
T_6	*T. harzianum*	43.72	41.02

4. Conclusion

All the five chemical tested *in vitro* at various concentrations significantly inhibited the growth of *Pseudomonas syringae* Howeever, streptocycline (100 ppm) + copper oxychloride (0.25%) was found most effective followed by teptocycline (100 ppm), Copper oxychloride (0.25%), 2 bromo 2 nitro propene 1-3 diol (500 ppm), while chemical poushamycin (200 ppm) was found comparatively least effective against the test pathogen. All the five botanicals / plant extracts tested *in vitro* at various concentrations significantly inhibited the growth of *P. syringae.* However, Neem found most effective followed by Ginger, Garlic, Onion, Tulsi. While plant extract of Tulsi was found least effective against test pathogen. All the five botanicals / plant extracts tested *in vivo* at various

concentrations significantly inhibited the growth of *P. syringae.* However, Neem found most effective followed by Ginger, Garlic, Onion, while plant extract of Tulsi was found least effective against test pathogen. All the six bioagents tested at various concentrations significantly inhibited the growth of *P. syringae.* However, *T. hamatum* was found most effective followed by *T. harzianum,* and *P. fluorescence.* Amongst the antagonist tested against *P. syringae* pv. *glycinea, T. viride* was significantly superior in per cent reduction of pathogen at all the incubation periods tested. The next best antagonist noticed was *T. hamatum.*

Biological control is an effective, ecofriendly and alternative approach for any disease management practice. The results on *P. syringae,* revealed that, all the plant extracts and antagonists significantly reduced the growth of *P. syringae,* either by over growing or by exhibiting inhibition zones. Most of antagonists inhibited colony growth of *P. syringae,* by their fast and over growing nature as observed in antagonists. This can be attributed to higher competitive ability of this *Trichoderma* spp. The antagonism of *Trichoderma* spp. against many fungi is mainly due to production at acetaldehyde compound (Robinson and Park, 1966 and Dennis and Webster, 1971). This may also be the reason for its antagonistic effect on *P. syringae*. Godtfredsen and Vagedal (1965) reported trichodermin, Pyke and Dictz (1960) found dermadin as major volatile antibiotic produced by *Trichoderma* spp., which suppress several plant pathogens.

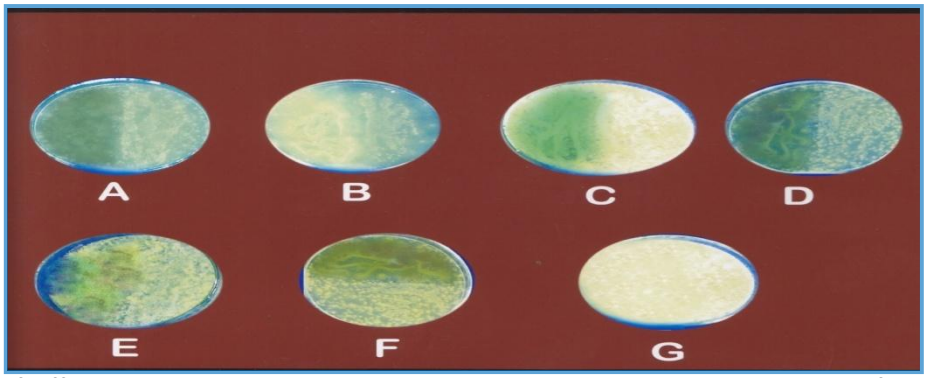

Plate 2. Effect of different *Trichoderma* spp. on *Pseudomonas syringae* pv. *glycinea* at 6 days after inoculation.
A = T. koningii B = T. lignorum C = T. viridae
D = T. hamatum E = T. harzianum F = P. fluorescens
G = Control

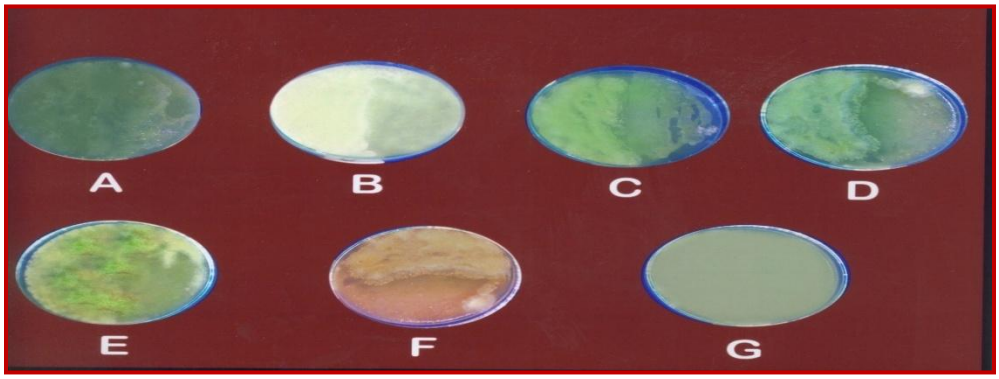

Plate 3. Effect of different Trichoderma spp. on Pseudomonas syringae pv. glycinea at 9 days after inoculation.
A = T. koningii B = T. lignorum C = T. viridae
D = T. hamatum E = T. harzianum F = P. fluorescens
G = Control

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