Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2017 Vol.6 (4) October-December, pp.50-57/Vadeo et al.
Research Article

STUDIES ON DIVERSITY AMONG ISOLATES OF *FUSARIUM SOLANI* CAUSING STEM CANKER DISEASE OF POPLAR (*POPULOUS DELTOIDES)*

Aruku Dazo Vadeo [1] Yash Pal Singh [2] Archana Bagwari [2]

[1]ICAR Umiam, Barapani Shillong, [2]Forest Pathology Division, Forest Research Institute, Dehradun

**Author for Correspondence: arukudazo@gmail.com*

ABSTRACT

In the present study, Diversity among isolates of *Fusarium solani* causing stem canker disease of poplar (*Populous deltoids*) was evaluated. Fifteen isolates of *Fusarium sp.* were collected from the diseased clones of *Populous deltoides* from Rudrapur, Udham Singh Nagar, Uttarakhand. Poplar suffers from a number of diseases as they are being raised as single clone monocultures. *F. roseum* and *F. solani* were considered as important canker inducing agents of eastern cotton wood and hybrid poplars trees. The isolates of fungus were grown on different growth media viz. Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) and Malt Extract Agar (MEA). Morphological parameters like Colony types, colour, spore size and pigmentation were observed, cottony and its variants like flat and woolly-cottony were the dominant colony types. Pathogenicity test was conducted on three month old poplar nursery plants for *Fusarium solani* causing stem canker disease in poplar. Fungicidal sensitivity of *F. solani* was tested against Propiconazole (a systemic fungicide). The systemic fungicide Propicanazole was effective in inhibiting in vitro growth of F.solani isolates at the highest concentration of 200ppm .Bio-agent sensitivity test, *in vitro* with *Trichoderma harzianum* as antagonist showed differential inhibition (9.5-29.6 %) against the pathogen isolates.

Key Words*: Fusarium solani, monoculture, fungicidal sensitivity, Trichoderma harzianum, Bio-agent Sensitivity*

INTRODUCTION

Populous is a genus of 35 species of deciduous flowering plants in the family *salicacaea,* native to most of the Northern Hemisphere. There are six poplars indigenous to India viz. *P.ciliata, P.gamblei, P.Jacquemontii var glauca, P.rotundifolia, P.euphratica, P.alba* (Mathur and Sharma, 1983). *Populous* is widely planted above 28^0 N latitude in India in Arunachal Pradesh, Haryana, Jammu and Kashmir, North Bengal, Punjab, and Uttar Pradesh (Tiwari, 1993). The genus has a large genetic diversity and can grow from anywhere between 15-50m tall, with trunks up to 2.5m diameter. The tree develops a tall straight bole. The shoots are smooth, white to greenish or dark grey, leaves are spirally arranged and vary in shaped from triangular to circular or lobed with long petiole (ICFRE, 1994). Poplar is an important agroforestry plantation. About 90 percent of the poplar plantation in India is based on clones G-48, D-121, S7C15 and G-3 (Newman, 1997).Most of the poplar cankers are caused by fungi. Cankers may appear as circular to elongated sunken areas with a raised greyish to black margin. Cankers caused shrinking and drying of tissue at a localized area of the bark, on branches and trunks of the host tree. Fungi of the genus *Fusarium* are worldwide in distribution occurring as plant pathogens which cause severe damage to numerous cultivable plants (Wieland and Sundbak, 2000; Li *et al*, 2008) with the highest economical loss upon infection of maize, wheat and barley (Windels, 2000; Ngange *et al*, 2004). Various species of the form-genus Fusarium Link cause characteristic cankers, sometimes called fusarioses, on poplar trees. They all exhibit polyphagous, both on poplars and on other broad-leaved trees and herbaceous plants in the temperate zones. *Fusarium solani* as a canker causing agent, in order to find scientific validation different parameters were taken into consideration for the experiment.

MATERIALS AND METHODS

Fusarium sp. isolates were collected from the diseased clones of *P. deltoides* from Rudrapur, Udham Singh Nagar, Uttarakhand. The individual cankers were cut by sterilized sharp knife into small pieces of about 1cm, with small healthy area. Surface sterilization of stem section was done with disinfectant (1.0% NaOCl) for fixed time duration (15-20 sec.). Washing of stem section with sterilized distilled water (4-5 times) was performed. This was followed by Blotting stem tissue section with sterilized paper towel to remove excess distilled water. Stem tissue section was placed on to Potato Dextrose Agar (PDA) medium in a Petri plate (Dhingra and Sinclair,1985).The apparently pure growth of the fungus was, then, transferred from the Petri plate to slants after a week or so. The isolates were maintained by sub culturing after every two months. These tubes were used as starter cultures.

Culture and morphological studies: The diversity of 15 isolates was studied on the basis of their colony type, pigmentation, rate of growth and spore size on different growth medium such as Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) and Malt Extract Agar (MEA). The fungus colony diameter was measured every 24 hours after incubating it at 22+_1^{o}C.

Pathogenicity Test (Koch's Postulates)*:* The Isolate F-12 was used to prove Koch's postulates on poplar clones viz., WSl-22, WSL-39 and G-48. Stem cutting of WSl-22, WSL- 39 and G-48 clones were transferred in poly-bags and four replicates for each treatment were maintained. Colony of *Fusarium sp*. was taken from isolate no. F-12 and were cut with borer and were inoculated to bark of poplar sampling and rub around with a cotton and tape. Treated plants were kept under control condition i.e., high humidity and temperature, plants were watered and observed periodically for appearance of symptoms. Fungus was re-isolated from the disease plants and was matched with the original culture of the pathogen for conformation.

Fungicidal Sensitivity Test: Fifteen isolates of Fusarium sp. were tested against Propiconazole *in vitro* by food poison technique. The experiment was conducted at 5 different concentrations as 100,125, 150, 175 and 200 ppm (parts per million). Preparation and sterilization of CDA was done. Requisite quantity of fungicides Weighed and added into CDA medium under sterile conditions. Control plate was prepared without fungicides. Measurement of the diameter of fungus colony was taken after every 24 hours till control plate is completely filled.

Bio-agent Sensitivity Test: Fifteen isolates of *Fusarium sp*. were tested individually for antagonism against isolate of *Trichoderma harzianum* isolated from soil by dual culture method on PDA (Dhingra and Sinclair, 1985).The cultures of antagonists were obtained from Forest Pathology Division, Forest Research Institute (FRI), Dehradun.

Data for different parameters were analysed with the help of Genstat 5 Release 3.22. One- way analysis was followed for growth data and two-way analysis was used for growth, sporulation, biogenic and fungicidal sensitivity data. Treatments means were compared at 5 per cent level of significance.

RESULTS AND DISCUSSION

Canker affected seedlings of WSL-81 clone of *P.deltoides* showed blightening symptom in the advanced stage of the disease. They stood distinct among the healthy seedlings in the nursery. The disease initiated on stem as blisters get ruptured oozing watery substance that led to a typical mark of a stream on the affected stem, these ruptured pustules turn into canker like structure with distinct callus lips. Epicormic branching was also noticed in disease affected plants especially on the lower side.

Colony Characteristics

On PDA , cottony colony type were dominant with nine isolates, followed by three isolates of woolly type, two isolates were cottony to woolly and one isolate had effuse to flat surface. Various colony color like off white, pale pinkish cinnamon, pale smoke grey, pale olive grey and light vinaceous fawn were observed. The pigmentation observed were buff pink, onion skin pink,Verona brown,shell pink,sudan brown, sayal brown, vinaceous tawny,cream buff and chamosis (Ridgway, 1912). (Table.1.1)

Table 1.1: COLONY CHARACTER OF DIFFERENT ISOLATES OF *F. SOLANI* ON PDA

Isolate no.	Medium (PDA)		
	Colony type	**colour**	**Pigmentation**
F-1	Cottony	Pale olive grey	Verona brown
F-2	Cottony	Off white	Buff pink
F-3	Cottony	Off white	Shell pink
F-4	Woolly	Off white	Onion skin pink
F-5	Woolly	Tileul buff	Sudan brown
F-6	Woolly to cottony	Off white	Sayal brown
F-7	Cottony	Pale smoke grey	Vinaceous tawny
F-8	Woolly	Pale pinkish cinnamon	Cream buff
F-9	Cottony	Pale smoke grey	Chamosis
F-10	Cottony	Pale pinkish cinnamon	Buff pink
F-11	Cottony to woolly	Pale pinkish cinnamon	Buff pink
F-12	Cottony	Off white	Buff pink
F-13	Cottony	Pale olive grey	Onion skin pink
F-14	Cottony	Pale smoke grey	Onion skin pink
F-15	Effuse to flat	Light vinaceous fawn	Buff pink

On CDA, 7 isolates showed woolly type colony, 5 isolates were cottony, 2 isolates had flat to cottony while one isolate exhibited cottony to woolly type colony. Seven isolates had pale olive grey type colonies, Tileul buff, pale pinkish cinnamon, smoke grey, and pale olive buff color were also observed. Chamosis was dominant pigmentation, verna brown, sayal brown and pink buff pigmentation were also released (Ridgway, 1912).

TABLE 1.2: COLONY CHARACTER OF DIFFERENT ISOLATES OF *F.SOLANI* ON CDA

Isolate no.	Medium (CDA)		
	Colony type	**colour**	**Pigmentation**
F-1	Cottony	Pale olive grey	Verona brown
F-2	Woolly	Pale olive grey	Verona brown
F-3	Woolly	Tileul buff	Deep coonial bay
F-4	Woolly	Tileul buff	Chamosis
F-5	Woolly	Pale olive buff	Chamosis
F-6	Cottony	Pale olive grey	Sayal brown
F-7	Cottony	Pale pinkish cinnamon	Chamosis
F-8	Cottony	Pale pinkish cinnamon	Pimk buff
F-9	Woolly	Pale olive grey	Chamosis
F-10	Flat to Cottony	Tileul buff	Chamosis
F-11	Woolly	Pale olive grey	Sayal brown
F-12	Cottony to woolly	Pale olive grey	Chamosis
F-13	Cottony	Pale olive grey	Pink buff
F-14	Woolly	smoke grey	Chamosis
F-15	Flat to cottony	smoke grey	Raw sienna

Woolly type colony was dominant and showed by 10 isolates on MEA, cottony and flat to cottony were exhibited. Various colors were observed on MEA. Light vinaceous brown, pale olive grey, pale vinaceous

fawn, and pale ocharaceous. Chamosis and pinkish cinnamon were dominant pigment released (Ridgway, 1912).

Table 1.3: COLONY CHARACTER OF DIFFERENT ISOLATES OF *F.SOLANI* ON CDA

Isolate no.	Medium (MEA)		
	Colony type	**colour**	**Pigmentation**
F-1	Woolly	Pale olive grey	Chamosis
F-2	Woolly	Pale vinaceous fawn	Buff pink
F-3	Cottony	Light vinaceous brown	Chamosis
F-4	Cottony	Light vinaceous brown	Chamosis
F-5	Woolly	Pale olive grey	Chamosis
F-6	Woolly	Pale ochraeous salmon	Raw sienna
F-7	Woolly	Light ochraeous salmon	Ochraeous
F-8	Cottony	Off white	Sudan brown
F-9	Woolly	Light vinaceous brown	Chamosis
F-10	Woolly	Pale vinaceous fawn	Pinkish cinnamon
F-11	Woolly	Light vinaceous brown	Chamosis
F-12	Cottony	Pale ochraeous brown	Honey brown
F-13	Woolly	Light vinaceous brown	Chamosis
F-14	Woolly	Sayal brown	Clay colour
F-15	Flat to cottony	Smoke grey	Raw sienna

Spore size:

The relative growth of *F.solani* on MEA,CDA and PDA media showed differential growth after 6 days of inoculation when the first plate was completely filled. CDA, in general, supported significantly more and maximum growth (6.3 cm) among all the media used followed by MEA (5.5cm) and PDA (4.0cm). The identification of *F.solani* species is primarily based on the morphology of asexual spores (Booth, 1985).The spore size varied from media to media. While both Macro-conidia and Micro-conidia were observed in CDA and MEA, only Micro-conidia was observed in case of PDA. The spore size of Micro-conidia and Macro-conidia differed in sizes from media to media (table.2.1).

Table 2.1: SIZE OF MICRO-CONIDIA AND MACRO-CONIDIA IN DIFFERENT MEDIA

Isolate no.	Medium				
	PDA	**CDA**		**MEA**	
	Micro-conidia	**Macro-conidia**	**Micro-conidia**	**Macro-conidia**	**Micro-conidia**
F-1	17.5X14.3-10.3X7.3	154X29.4-80.9X21	54.8X21-43.2X10.3	168.1X32.1-122X22.8	58.6X24.3-3.2X15.2
F-2	24.3X14.5-15.2X8.9	254X34-200.3X23	58.6X24.3-42.2X12.3	89.6X15-73.5X10.3	26.1X15.2-20.5X10.2
F-3	20.1X24.5-12.65X10.2	112X25-97X18	89.6X15.2-76.4X10.2	89.6X15.2-76.4X10.2	27.1X15.1-21.1X11.2
F-4	20.6X23.6-14.2X10.2	98X15-83.5X12	27.6X15.3-20.5X10.2	89.5X14.3-75.6X10.2	27.12X15.2-22.1X10.2
F-5	22.1X16.5-15.2X8.9	234.2X32.6-200.2X27.4	25.3X15.2-22.1X10.2	68.2X15.2-56.3X10.3	27.2X15.2-22.1X10.2
F-6	23.1X12.3-22.1x12	98.6X15.1-82.4X10.2	25.3X15-21.3X10.2	65.2X12.4-43.2X6.7	25.2X15.1-22.1X10.2
F-7	27.2x15.2-	58.6X10-	25.2X15.1-	69.4X15.2-	27.6X15.2-

	24.1x11.4	43.2X6.7	22.1X10.2	59.11X11	24.1X11.4
F-8	27.1x15.2-21.1x11.2	92.1X16.44-80.4X10.5	27.1X15.2-22.1X10.1	89.8X15.4-76.4X10.5	27.1X15.2-21.12X12
F-9	27.6x12.3-23.1x10.2	98.6X14.8-82.4X10.2	25.3X14.2-22.1X10.2	112X14.2-97.1X10.1	27.6X14.2-23.1X10.2
F-10	25.4x15.2-22.1x10.2	98.4X14-84.0X11.3	27.1X15.2-22.1X10.1	154.2X25-112.1X21	24.5X15.2-22.1X12
F-11	25.3x13.2-21.1x10.3	57.3X10-43.2X6.4	27.6X15.2-22.1X11	77.2X12.5-59.4X11	26.1X13.2-21.2X10.2
F-12	25.3x12.3-22.1x10.2	6.1X13.2-55.2X10.2	25.6X14.2-22.1X10.2	89.6X12.8-73.5X10.3	26.5X14.2-22X11.5
F-13	24.3x13.2-22.1x10.2	142.5X2.56-80.9X21	25.3X15.2-22.1X10.2	69.4X12.8-59.11X11.2	27.5X12-22.1X10.2
F-14	27.2x15.2-21.1x10.3	93.2X12.5-83.4X10.2	27.6X15.2-22.1X11.5	107.5X14.2-97X14	24.5X13.2-21.0X10.3
F-15	24.5x12.4-22.1x10.2	56.2X9.4-43.2X6.7	24.6X15.2-23.1X10.2	87.2X12.9-75.6X10.3	24.5X12.1-22.1X10.2

Pathogenicity: The inoculated plants of G-48, WSL-22 and WSL-39 clones showed typical canker at the inoculation point and *F.solani* was isolated from the cankered portions. In case of WSL-22, a canker was observed away from the point of inoculation revealing the development of disease in the inoculated seedlings. The disease symptom appeared within fifteen days of inoculation. The presence of inoculated fungus was found inside stem cambium tissues and from it the fungus has been re-isolated, fulfilling Koch's postulation.

Figure 1: symptoms of pathogenicity experiments on different clones. A. G-48, B. WSL 22, C. WSL-39 and D. canker developed on the stem away from the point of inoculation.

Sensitivity to Fungicides *in vitro*

To evaluate its agrochemical sensitivity we grew the *F.solani* with Propicanazole on PDA plate containing different concentration of 100,125.150,175 and 200 ppm of the fungicide. Propiconazole was found effective against *R. solani* at all the concentrations exhibiting cent per cent inhibition (Muneeb

Andrabi *et al* 2011). Irrespective of the isolates there was a linear suppression of fungal growth with increasing concentration of fungicide (Table.2.1).

Table 2.2: EFFECT OF DIFFERENT CONCENTRATION OF PROPICANAZOLE (PPM) ON GROWTH OF *F.SOLANI* ISOLATES

Isolate no	**Concentration (ppm)/ Inhibition(%)**						**Mean**
	Check	**100**	**125**	**150**	**175**	**200**	
F-1	0.0	78.8	83.6	88.1	92.0	100.0	88.5
F-2	0.0	79.3	83.8	88.3	89.5	91.6	86.5
F-3	0.0	80.0	84.0	86.4	89.3	90.2	86.0
F-4	0.0	82.6	85.2	89.0	90.0	91.4	87.5
F-5	0.0	82.0	84.5	88.0	89.5	100.0	88.7
F-6	0.0	83.0	88.5	90.0	92.1	100.0	90.7
F-7	0.0	79.5	83.3	85.4	88.0	91.2	85.5
F-8	0.0	81.6	83.6	86.4	88.1	92.0	86.3
F-9	0.0	82.0	85.2	88.0	90.5	100.0	89.1
F-10	0.0	85.5	87.6	89.0	90.5	91.7	89.0
F-11	0.0	83.3	84.5	86.2	88.1	100.0	88.4
F-12	0.0	81.7	86.0	88.0	90.0	92.0	87.4
F-13	0.0	82.4	85.5	88.0	91.4	100.0	89.4
F-14	0.0	81.0	83.0	86.2	89.0	100.0	88.0
F-15	0.0	81.0	84.5	86.0	88.0	92.0	86.2
Mean	0.0	81.5	85.0	87.5	89.7	95.4	
		Isolate(I)		**Concentration (C)**		**Interaction (IXC)**	
SEM		0.3		0.2		0.6	
CD (5 %)		0.5		0.3		1.2	

Bio-Sensitivity Test (Dual culture method, in vitro)

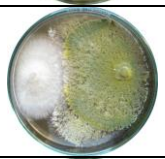
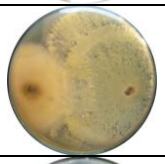
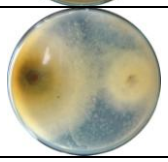
T.harzianum was used to test the isolates capacity against the antagonist. Among the fifteen isolates of *F.solani*, maximum growth of isolate no. F-13 was suppressed (29.6%). On the other hand, many isolates had at par and minimum growth suppression, for example isolate no.F-11 was reduced to minimum of 9.5% that was at par with isolate no.F-5, F-14 and F-4. Khair *et al*., 2010 tested *trichoderma spp.* against different *F.solani*. All the antagonistic reduced the in vitro mycelia growth of *F.solani* in the range of 38.9-57.5%. In the present case, the reduction was in the range of 9.5-29.6% by *T.harzianum* indicating that the antagonist had differential but low inhibition of the pathogen isolates.

Table 2.3: ANTAGONIST EFFICIENCY OF *T. HARZIANUM* AGAINST ISOLATES OF F. *SOLANI* IN DUAL CULTURE

Isolate no.	**PIMG**
F-1	18.3
F-2	25.6
F-3	19.8
F-4	11.3
F-5	9.5
F-6	21.8
F-7	20.3
F-8	12.7

F-9	19.6
F-10	23.0
F-11	12.6
F-12	20.0
F-13	29.6
F-14	13.0
F-15	22.1
SEM	2.5
CD (5%)	5.0

Figure 2: ANTAGONIST EFFICIENCY OF *T. HARZIANUM* AGAINST ISOLATES OF F. *SOLANI* IN DUAL CULTURE

Isolate no.	**Control**	**Treatment (front view)**	**Treatment (back view)**
F2			
F5			
F13			

CONCLUSION

Blisters, oozing and canker are the major symptoms of the disease under nursery condition. The inoculated plants of G-48, WSL-22 and WSL-39 clones showed typical canker at the inoculation point in pathogenicity test. In case of WSL-22, a canker was observed away from the point of inoculation revealing the development of disease in the inoculated seedling. Varied colony types, color and pigmentation were observed in isolates of *F. solani* on different growth media. Cottony and its variants like flat and woolly cottony were the dominant colony types. Ofwhite, shades of pink (pale pinkish cinnamon) grey (smoke and pale olive grey) and brown (sudan and honey brown) were dominating in terms of colony colour *of F.solani* on all the three growth media. Chamosis was a common pigment among the isolates of *F.solani*. Based on the relative growth of *F.solani* isolates, CDA was judged best growth medium followed by MEA and PDA. Both macro-conidia and microconidia were recorded on CDA, MEA and PDA. The systemic fungicide Propicanazole was effective in inhibiting *in vitro* growth of *F.solani* isolates at the highest concentration of 200ppm. Based on extent of growth inhibition of the pathogen vis-à-vis concentration the tested fungicide seems mildly effective. *T.harzianum* could reduce the growth of *F. solani* isolates from 9.5-29.6% underlying its limited efficiency.

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