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Pathological and physiological studies of Fusarium wilt pathogen of carnation

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ABSTRACT

Carnation wilt incited by *Fusarium oxysporum* f.sp. *dianthi* is one of the most serious diseases in Himachal Pradesh. The disease prevailed during the cropping seasons (2004-05) with great variation in incidence, 6.70 to 22.54%, being maximum (22.54) and minimum (6.70) in Mandi and Sirmour districts, respectively. Root dip inoculation gave highest incidence with appearance of wilt symptoms within 20 days compared to soil level and root zone inoculations under artificial conditions. The pathogen showed maximum mycelial growth and spore formation on Potato dextrose agar and Richard's solution in solid and liquid media, respectively. However, temperature and hydrogen-ion concentration (pH) were optimum at 25^{0}C and 5.5, levels.

Key words : Wilt, Carnation, *Fusarium oxysporum*

The pathogen, *Fusarium oxysporum* f.sp. *dianthi* is responsible for inducing wilt in carnation and reported to be a major disease in almost entire of Himachal Pradesh wherever carnation is grown with incidence as high as 79.0% (Katoch, 1999). The losses caused by the disease are manifolds worldwide. In Germany, the estimated disease incidence was about 46 per cent while in Italy and Poland it goes upto 60 (Jacob and Kreb, 1985; Filippi and Baganoli, 1992; Manku and Fruzysiska-Jozwick, 1992). The pathogen infects vascular system and disrupts water and nutrient supply, which causes yellowing, drooping, drying and ultimately wilting of the plants. The infected roots develop light brown colour and get completely detached from the shoots. Keeping in view the severity of the pathogen and importance of the crop, a preliminary study on pathogenicity and physiological factors such as media, temperature and hydrogen-ion concentration affecting the growth and sporulation of *F.oxysporum* f.sp *dianthi* was carried out to know the etiological behaviour and cultural requirement of the causal fungus

MATERIALS AND METHODS

Survey and pathogenicity studies :

Regular surveys for recording the incidence of carnation wilt were carried out during the crop seasons (2004-05 May-Sept.) particularly in peak period of appearance and spread. Six districts of Himachal Pradesh namely Solan, Sirmour, Shimla, Kullu, Mandi and Kangra were surveyed. The incidence was calculated as:

$$\text{Disease Incidence (\%)} = \frac{\text{No.of diseased plants}}{\text{Total no. of plants}} \times 100$$

The isolation of the pathogen was carried out as per the standard procedure from the infected carnation roots in order to conduct the pathogenicity test (McMuller and Stack, 1983). Three inoculation methods, as soil, root zone and root dip were applied for artificial inoculation of the plants. In former, the mass culture of the fungus was prepared on maize grain medium (Dohroo and Sharma, 1984). Freshly prepared fungal culture (50g) was added on upper 2 inches layer of the formalin sterilized garden soil (600g) contained in 4inches dia. plastic pots and mixed by uniform spreading. This was followed by light irrigation (Kataria and Grover, 1976). After one week, three one-month-old rooted cuttings of cultivar "Purple Chopin" were planted in each pot with five replications. Whereas in root zone inoculation, the fungus @20g grown on Maize grain cultures was added surrounding the roots of the planted cuttings after one week of establishment. However, the cuttings of the same cultivar were pre-dipped in the mycelial suspension of the test fungus for 30 minute by mixing the inoculum in sterilized distilled water (500ml). The cuttings and replications remained same as mentioned for the soil inoculation study.

Physiological studies :

The study was undertaken with regards to its cultural and physiological behaviour of the pathogen to find out the suitable medium and optimum conditions for its spread. In all the physiological studies 4mm mycelial discs of 8 days old culture obtained from periphery of the test pathogen were used for inoculating different medium poured in Petriplates and flasks. The solidification of media was achieved by 2% agar while the pH of liquid media was adjusted at 6.0 by using N/10 NaOH or HCl

solution. The sterilization of media was done in an autoclave at 15lbs p.s.i for 20 minute. Each treatment was replicated four times and pathogen inoculated media in Petriplates and flasks were kept at 25^{0}C in B.O.D incubator for recording radial mycelial growth and dry mycelial growth after 8days. The homogenized suspension of the fungus was used for recording degree of sporulation based on average number of conidia counted in ten fields of haemocytometer at 10 x 25X and indicated as absent (no conidia) and Poor (upto 10); Fair (25); Good (45), Very good (> 45).

Four solid media namely, Potato dextrose agar (PDA), Corn meal agar, Carnation leaf agar, Kamada's and four liquid media *viz*., Richard's solution, Czapeck's Dox, Glucose-asparagine and Coon's solution were prepared (Tuite,1969). Carnation leaf agar medium was prepared by mixing extract of carnation leaves (40g) with 300ml of distilled water, which was mixed with 20g of agar to make volume to one liter.

However, mycelium mats obtained from liquid media after 20days of incubation were filtered on circular discs weighed Whatman's No. 42 filter papers. These were washed thoroughly by distilled water, dried to a constant weight at 60-80^{0}C for 24 h and further weighed. Dry weights recorded were used for comparing the different treatments. The data were analyzed and results have been discussed at 5% level of probability.

In order to determine the effect of temperature on mycelial growth and spore formation, six temperatures *i.e.* 10, 15, 20, 25, 30 and 35^{0}C were adjusted in B.O.D incubators. Best solid medium PDA was used. The pathogen inoculation method remains same as discussed earlier and treatments under considerations were replicated five times. The data related to average radial growth (mm) after 8days and spore count after 12 days were recorded and analyzed statistically following analysis of variance. Similarly ten pH values *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 10.0 and 12.0 were maintained on liquid medium (Potato dextrose) prior to fungal growth with help of N/10 NaOH or HCl solution. The mycelium discs of 4mm size were transferred in 100 ml of PDA broth contained in 150ml Erlenmeyer flasks. The average dry mycelial weight (mg) and sporulation of fungus was recorded after 20 days of incubation.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Survey and pathogenicity tests :

The explanation of the present findings revealed that during the periodical surveys in the cropping seasons, the incidence of Fusarium wilt was reported from all the districts of Himachal Pradesh (Table 1). The wilt incidence varied from 6.70 to 22.54%, the maximum (22.54) being recorded in Mandi district showing more congenial environment for the disease spread compared to Sirmour, Kullu, Solan, Kangra and Shimla districts. The Kullu, however, recorded lowest wilt infection. The occurrence of wilt pathogen in this crop was observed and reported much earlier from United States and European countries (Sturgis, 1897; Wickens, 1935; Hood and Stewart, 1957; Baayen and Maat, 1987; Baayen and Elgersma, 1985). Henceforth, many reports came from different parts of the world particularly from Nepal, Israel and Iran (Villiam *et al*., 1972; Ben-Yephet *et al.,*1993 ; Etabarian, 1996).

Table1 : Incidence of carnation wilt in different districts of Himachal Pradesh

District	(%)
Kangra	9.13
Kulhi	6.70
Mandi	22.54
Shimla	10.20
Sirmour	19.10
Solan	8.71
C.D. (P=0.05)	1.33

Out of three methods tested under pathogenicity test, root dip inoculation gave highest (45.57%) disease incidence within 20days in comparison to others. Soil inoculation was next best procedure for developing symptoms on carnation as mortality rate gone as high as 38.75% whereas root zone inoculation showed less infection upto 28.88% between 26-40 days in artificially inoculated conditions. The procedure of plant inoculation will be helpful in screening out large number of varieties/ cultivars of this crop. Hood and Stewart (1957) also reported similar results with root dip inoculation while proving the pathogenicity in carnations.

Physiological parameters on pathogen vegetative growth and sporulation:

The different media behaved differently in supporting the vegetative growth of the fungus and sporulation. The data on average vegetative growth in various solid and liquid media after 8 days of incubation period are presented in Table 2. The fungus could grow on all media tested with maximum (77.52) on Potato dextrose agar followed by Carnation leaf agar (69.82). However, the least growth was observed on Corn meal agar (41.40). As regards to

Table 2 : Growth and sporulation of *Fusarium oxysporum* f sp.*dianthi* on different solid and liquid media

Growth media	Av. Radial mycelial growth dry mycelial wt(mg)	Sporulation
Solid		
Potato Dextrose Agar	77.52(1.89)	Very good
Carnation Leaf Agar	69.82(1.85)	Good
Kamada's medium	53.5(1.74)	Good
Corn Meal Agar	41.40(1.63)	Poor
Liquid		
Richard's solution	621.60(2.79)	Very good
Czapeck's	321.40(2.51)	Fair
Glucose Asparagine	106.40(2.03)	Poor
Coon's solution	76.50(1.80)	Absent

C.D. (P=0.05) For sold medium : 1.99 and for liquid medium : 1.53
Figures in parentheses are log transformed (X+1) values

sporulation, same medium proved most effective in recording the spore count. Carnation leaf agar and Kamada's media were found equally good for spore production. However, a poor spore count was reported from Corn meal agar. The results are in conformity with the earlier records (Nightingale and Ramsey, 1937; Dohroo, 1982).

An average mycelial growth in terms of dry mycelial weight was recorded as 621.60 and 321.40mg in Richard's solution and Czapeck's Dox, respectively with maximum vegetative growth and good spore count in former and poor growth and sporulation in the later. Coon's solution was however, found least effective in terms of dry mycelial weight of the fungus with no spore formation even after 20 days of incubation. Dohroo (1982) also obtain maximum growth of *Fusarium equiseti* at 25-30^{0}C in Potato dextrose agar and Richard's solution. These media were also reported superior in supporting excellent vegetative growth as well as spore production (Kloutz *et al*., 1988; Ashour and El-Kadi, 1958).

Amongst the six tested temperatures (Table 3) there was no measurable growth of the test fungus at 10^{0}C while range between 25^0-30^{0}C found to be best for the mycelial growth (83.75 and 71.80mm, respectively) and sporulation. Minimum (15.63mm) was observed at 35^{0}C. A decline in colony diameter on either side of the optimum temperature (25^{0}C) was observed and further of spore production reported below 20 and above 30^{o}C. Harling *et al*. (1988) reported considerable role of temperature on carnation wilt. He observed symptoms expression at 26^{0}C in all the commercial cultivars of carnation infected with wilt pathogen. Joffee and Nadel- Schiffman (1967) found 20-25^{0}C temperature as optimum for multiplication of Fusarium wilt. The findings of Chauhan (1963) also gave similar results showing 25^{0}C as best temperature for vegetative growth and sporulation of *Fusarium* spp. and inhibition of the fungus below 15^{0}C temperature.

Table 3 : Growth and sporulation of *Fusarium oxysporum* f. sp *dianthi* at differents temperature regimes

Temperature (^{0}C)	Av. Radial growth (mm)	Sporulation
10	0.00 (0.00)	Absent
15	28.25(1.47)	Absent
20	63.35(1.82)	Fair
25	83.75(1.93)	Very good
30	71.80(1.86)	Good
35	15.63(1.22)	Absent
C.D. (P=0.05)	0.023	

Figures in parentheses are log(X+1) transformed values

Growth and sporulation found to be variable when the fungus was cultured at 4.0 to 12.0 pH levels. The results of the data indicate that wide range of pH supported the fungus growth. The pH levels ranging between 5.0 to 6.5 favoured the growth by giving maximum mycelial dry weight at pH level 5.5, while pH value lower and higher than 5.5 resulted in gradual decline in the average dry weight mycelium indicating a poor growth in highly acidic and alkaline medium whereas no growth obtained at pH 12.0. Spore induction was rated very good and found highest at pH 5.5 levels, which were closely followed by 6.0. However, at 5.0 and 6.5 pH levels, the spore formation was fair. Whereas at 4.0, 8.0 and above 8.0pH levels, fungus failed to form spores (Table 4). Probably the induction of sporulation required slight acidic and alkaline conditions compared to heavy acidic and alkaline situations. Orozco-de-Amezquite *et al.* (1993) while working on carnation wilt found lesser incidence due to *F.oxysporum* f.sp.*dianthi* at higher pH but found acidic

Table 4 : Growth and sporulation of *Fusarium oxysporum* f sp.*dianthi* at different pH values

pH values	Av. Mycelial dry wt (mg)	Sporulation
4.0	259.00(2.24)	Absent
4.5	245.10(2.40)	Poor
5.0	495.50 (2.51)	Fair
5.5	829.10(2.92)	Very good
6.0	629.20(2.73)	Good
6.5	551.60(2.64)	Fair
7.0	336.50(2.15)	Poor
8.0	145.50(2.15)	Absent
10.0	35.87(1.41)	Absent
12.0	0.00(0.00)	Absent
C.D. (P=0.05)	0.029	

Figures in parentheses are log (X+1) transformed values

conditions to favour the growth and sporulation of the fungus. They also reported slight deviation from the observations made by the other workers. Dohroo (1979) and Chauhan (1963) reported 6.6 –7.8 and 5.6-6.0 pH most suitable for the growth of *F. oxysporum* and *F. equiseti*, respectively. In another study Burgess and Liddell (1983) and Mishra and Rath (1986) reported 6.0 to 7.0pH range as optimum for the growth of *F. equiseti* and *F. solani*.

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