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J. Mycopathol, Res, 54(3) : 355-360, 2016;
ISSN 0971-3719

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J. Mycopathol. Res. ***54(3)*** *: 355-360, 2016;* (ISSN 0971-3719)

Variability of cultural, morphological, pathogenic and impact of different fungicides on *Fusarium oxysporum* f.sp. *pisi*

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Received : 01.02.2016 Accepted : 13.05.2016 Published : 31.10.2016

Twenty isolates of *Fusarium* spp. were collected from major pea growing areas of Uttar Pradesh. They produced three kinds of spores viz., micro-conidia, macro-conidia and chlamydospores. Mycelia of the pathogen were fluffy, intermediate, and appressed to white, pale white, purple and yellowish colouration on the medium. Total twenty isolates were assigned into three groups, on the basis of colony characters, sporulation and degree of pathogenicity test. Isolates F10, F19, F17, F5, F4, and F16 showed fluffy, intermediate sporulation and white color. They showed strong virulence with 71 – 100% severity and wilting symptoms were noticed 30 days after inoculation. The physiological studies of the representative isolates of these six groups were made on six different fungicides at 0.1% concentration. The minimum mean radial growth (2.00-2.99 mm) was recorded in group C of isolates F8, F11, F12 and F13 and maximum (4.00-4.99 mm) in group-A consisting of F10, F17 and F19. The maximum micro conidia were produced by group-A (2.60–2.99 million/ml) F10, F17 and F19 while group-C of F8, F11, F12, F13 and F15 had minimum micro-conidia (1.00 – 1.99 million/ml). As regard the groups maximum macro conidia were formed by group-A (0.70 – 0.79 million/ml) of F5, F10, F16, F17 and F19. The minimum macro-conidia were produced by group-C (0.40 – 0.59 million/ml) of F8, F9, F11, F12, F13, F14 and F15.

Key words: Wilt of pea, *Fusarium oxysporum* f.sp. *pisi*, morphological, pathogenic variability, fungicides

INTRODUCTION

Pulse crops play an important role in Indian agriculture. Pulses have a special role in meeting the protein requirement of predominantly vegetarian in India. They form an integral part of diet as source of protein. Pulses are the unique crops for three reasons *i.e.* they have capability to fix atmospheric nitrogen in symbiotic association with root nodule bacterium (*Rhizobium*), pulses are versatile crops to fit in diverse cropping systems and grown during *Rabi* season. Pea belongs to the family Leguminosae, sub family Papilionaceae and genus *Pisum.* Pea is the second most important food legume in the world after pogeonpea. Uttar Pradesh (UP) ranks first, with 46.9% and Jharkhand 8.9% share in the production of the country (NHB, 2013). In India, total area under pea cultivation is about 420.9 thousand ha with the production of 4006.2 thousand mt and productivity 9.5 mt ha-1. In Uttar Pradesh, total area under pea crops is about 175.01 thousand ha with production 1877.93 thousand mt. and productivity 10.7 mt ha^{-1} (NHB, 2013). Among the fungal diseases Wilt (*Fusarium oxysporum* f. sp. *pisi)*, Downy mildew (*Peronospora pisi*), Powdery mildew (*Erysiphe polygoni*) and Rust (*Uromyces fabae*) are important. The isolates of the pea wilt pathogen obtained from various location in different districts of Uttar Pradesh, were grouped into six groups on the basis of morphological and cultural characters. The variability in the growth of twenty isolates on six different fungicides is reported in this paper. The main aim of grouping of these isolates was to get in initial understanding of variation among the isolates of *Fusarium oxysporum* f.sp. *pisi* collected from major pea growing areas.

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MATERIALS AND METHODS

Collection of diseased samples of the pea from some growing areas of Uttar Pradesh

The present investigation was carried out during 2009-10 and 2010-11. Laboratory experiments were carried out at the Department of Plant Pa-

thology, C.S.A. University of Agriculture and Technology, Kanpur, U.P. *Fusarium oxysporum* f. sp. *pisi* defected samples were collected during the year 2009-10 from different pea growing regions of U.P. The details of location and designation given for each isolates are furnished in Table 1.

Cultural and Morphological studies of the pathogen

Twenty isolates of *Fusarium* spp. obtained upon isolation from wilted pea plants were compared for variation in morphological and cultural characters on PDA medium. Five ml medium was poured into each sterilized Petri plate and 5 mm mycelia disc from actively growing seven days old culture of each isolates of *Fusarium* spp. was inoculated at the centre of PDA and Petri plate were incubated at 25±1^{0}C for seven days. Observations on colony colour and linear growth measurements were recorded up to seven days. Spore measurements were taken with the help of filer micrometer.

Pathogenic variability of the pathogen

The fungus was multiplied on PDA aseptic conditions in Petri plates. When fully covered with fungal growth (seven days old) it was harvested with the help of a sterile scalpel and washed with 100 ml of sterile water. The contents of each plate were filtered through a muslin cloth to get a spore suspension. The suspension was adjusted to have a concentration of 5×10^6 spores/ ml of sterile water. Four holes of 4-5 cm deep were made with the help of small sticks in the soil around collar region of each seedling of four weeks grown in pots containing sterilized soil. 10 ml of the spore suspension was poured into the holes and covered with the soil. Control plants were applied with sterilized tap water.

Variability of different fungicides of the pathogen

All the pathogenic isolates were grown on six fungicides, namely Bavistin (Carbendazim), Copper oxychloride, Propiconazole, Tebuconazole, Mancozeb and SAAF at 0.1% concentration *in vitro*. Fungicides were tested by Poisoned food technique. The desired quantity of the fungicide was incorporated in the molten Potato Dextrose Agar Media. Three replications were maintained for each fungicides and isolates. The medium without fungicide served as control. Five mm disc from the margin of seven days old pure culture was transferred to each petriplate containing fungicide amended medium. The plates were then incubated at 25±1^{0}C for seven days. The diameter of the colony was measured in mm after seven days of incubation. The growth pattern along with the pigmentation of medium both on upper and lower surfaces was noted. On 10th days of incubation one disc (5 mm dia.) of the culture was cut randomly from each of the Petri plates, suspended in 2.0 ml of sterilized water shaken well and was examined under the low power microscope on haemocytometer. The number of macro and micro conidia/ml of suspension were calculated using the following formula:

$$\text{Conidia/ml} = X \times 250000$$

where, X = number of conidia/square of haemocytometer of 1/25 mn^2

RESULTS AND DISCUSSION

Cultural and Morphological characters of the Fusarium oxysporum f.sp. *pisi*

The morphological characters of *Fusarium oxysporum* f.sp. *pisi* isolates indicated that mycelia of pathogen were cottony white to yellowish. Micro conidia were abundant, oval, ellipsoid, straight to curved, macro-conidia, sparse to abundant, three to five septate, fusoid- subulate and pointed at both ends and had pedicellate base. Three septate spores were predominant. Chlamydospores were both smooth and rough walled (Table 1). The present studies are in agreement with Gerlach and Nirenberg (1982) who found that *Fusarium oxysporum* f.sp. *lycopersici* was identified based on its morphological characters. Twenty isolates of *Fusarium oxysporum* f.sp. *pisi* were classified into three groups (Table 2). The first group isolates showed fluffy, intermediate, pale white, white, scanty white, chlamydospores round to slightly elliptical, single or two called with abundant sporulation. Group second isolates showed fluffy, intermediate, appressed, purple, yellowish, pale white with good sporulation, chlamydospores terminal or intercalary, one or two called, circular to oval. Third group isolates exhibited fluffy, pale white apprised, white, sporulation moderate to poor, one or two called, terminal or intercalary chlamydospores.

Pathogenic variability in isolates of Fusarium oxysporum f.sp. *pisi*

Twenty isolates collected from different locations

showed varying degrees of aggressiveness in inoculated plants, six isolates (F10, F19, F17, F5, F4 and F16) showed strong virulence with 71-100% severity and wilting symptoms were noticed 30 days after inoculation. Group second eleven isolates (F2, F18, F6, F7, F20, F1, F9, F15, F3, F14 and F13) were the next strong virulent isolates producing wilting symptoms 30 days after inoculation and showed 51-70% severity. Third group three isolates (F18, F11 and F12) were less aggressive and took more time to cause symptoms 60 days after inoculation and severity varied from 0-50%. The main aim of the groupings of these isolates was to understand pathogenic variation among the isolates of *Fusarium oxysporum* f.sp. *pisi*.

Variability of different fungicides in isolates of Fusarium oxysporum f.sp. *pisi*

The observations were recorded on the radial growth (mm), and the number of micro and macro conidia/ml. All the groups of isolates of *Fusarium oxysporum* f.sp. *pisi* differed from each other. The radial growth (mm) on six fungicides at a 0.1% concentration *in vitro* is given in Table 3 and 4. The data revealed that all the fungicides reduced the radial growth significantly over control. The minimum mean radial growth (2.00-2.99 cm) was obtained in case of group C. Consisting of F8, F11, F12 and F13 while maximum mean growth (4.00 – 2.99 cm) in group A of F10 , F17 and F19. Radial growth of the pathogen was different on the most of the fungicides. The maximum mean radial growth (5.68 cm) was observed on SAAF and minimum in Bavistin (00.00 cm) as compared to 7.49 cm in untreated (control). The interaction effect between isolates and fungicides was also found statistically significant. Nikam *et al*, (2007) and Gupta *et al,* (1997) screened 6 fungicides against *F. oxysporum* f. sp. *ciceri* and reported Carbendazim @100 mg/ml as most effective in inhibiting the growth of fungus *in vitro*.

Microconidia

The number of micro-conidia of *Fusarium oxysporum* f.sp. *pisi* on six different fungicides are recorded in Table 5 and 6. It is clear from the data that, all the fungicides reduced the sporulation over control. The maximum sporulation (3.10 million/ml) was recorded on SAAF. The sporulation was reduced significantly in all the other fungicides. It was minimum in Bavistin (0.00 million/ml), Tebuconazole (1.94 million/ml), Propiconazole (1.33 million/ml) and Copper oxychloride (0.69 million/ml) as compared to 6.08 million/ml in control. The maximum micro conidia were produced by group-A (2.60–2.99 million/ml) F10, F17 and F19 while group C of F8, F11, F12, F13 and F15 had minimum micro-conidia (1.00 – 1.99 million/ml). The interaction effect between isolates and fungicides with regard to micro conidia production

Table: 1 : Morphological, cultural and pathogenic variability in 20 isolates of the pathogen from different growing pea areas of Uttar Pradesh

Districts of U.P.	Isolates	Radial growth (cm)	Sporualation	Colony Characters	Conidia		Mycelial growth rate	Disease incidence (%)
					Micro-conidia	Macro-conidia		
Allahabad	F1	3.4	++	Fluffy, Yellowish	Fusifrom without septum	Fusiform with 5 septum	S	60.50
Fatehpur	F2	4.1	+++	Fluffy, Purple	Oval round	Fusiform with 3 septum	S	66.20
Varanasi	F3	3.1	++	Fluffy, white	Fusifrom without septum	Fusiform with 4 septum	S	55.50
IIPR, Kanpur	F4	4.8	+++	Fluffy, Scanty white	Oval round	Fusiform with 4 septum	S	72.40
Basti	F5	5.1	++++	Intermediate, white	Oval round	Fusiform with 3 septum	S	75.20
Jaunpur	F6	3.4	++	Intermediate, pale white	Oval round	Fusiform with 5 septum	S	65.30
Etawah	F7	4.3	+++	Fluffy, white	Fusifrom without septum	Fusiform with 5 septum	S	64.20
Kannauj	F8	3.8	++	Fluffy, pale white	Fusifrom without septum	Fusiform with 4 septum	S	49.50
Banda	F9	4.2	+++	Fluffy, pale white	Fusifrom without septum	Fusiform with 5 septum	S	58.30
Lucknow	F10	6.7	++++	Fluffy, pale white	Fusifrom without septum	Fusiform with 5 septum	M	83.76
CSA, Kanpur	F11	3.8	+++	Apprised, Pale white	Oval round	Fusiform with 3 septum	S	48.30
Faizabad	F12	3.5	++	Fluffy, white	Fusifrom without septum	Fusiform with 4 septum	S	37.76
Aligarh	F13	3.7	++	Intermediate, pale white	Fusifrom without septum	Fusiform with 5 septum	S	52.36
Kasganj	F14	4.2	+++	Apprised, Pale white	Oval round	Fusiform with 3 septum	S	55.00
Mirzapur	F15	3.8	+++	Fluffy, Yellowish	Oval round	Fusiform with 4 septum	S	56.73
Bareilly	F16	5.4	++++	Fluffy, pale white	Oval round	Fusiform with 3 septum	M	71.20
Jhansi	F17	6.5	++++	Fluffy, white	Fusifrom without septum	Fusiform with 5 septum	M	75.80
Hardoi	F18	5.7	++++	Fluffy, white	Fusifrom without septum	Fusiform with 4 septum	M	65.32
Meerut	F19	6.3	++++	Fluffy, white	Oval round	Fusiform with 3 septum	M	80.52
Gonda	F20	3.6	++	Fluffy, white	Oval round	Fusiform with 3 septum	S	60.78

Note:- ++++: High sporualation, +++: Medium sporualation, ++: Low sporualation H: High radial growth, M: Medium radial growth, S: Slow radial growth

Table 2 : Grouping of morphological, cultural characters and pathogenicity of the *Fusarium oxysporum* f.sp. *pisi*

Groups	Colony Characters	Micro-conidia	Macro-conidia	Disease incidence (%)
Group-1 F10, F19,F17, F5, F4 and F16	Fluffy, pale white, white, intermediate, Scanty white: Chlamydospores rounded to elliptical, single or two called sporualation	Fusiform without septum to oval rounded	Fusiform with 3-5 septum	71-100
Group-2 F2, F18, F6, F7, F20, F1, F9, F15, F3, F14 and F13	Fluffy, Purple, Fluffy white, intermediate yellowish, pale white, Appressed, good sporualation, chlamydospores terminal or intercalary, one or two called circular to oval.	Oval round to fusiform without septum	Fusiform with 3-5 septum	51-70
Group-3 F8, F11 and F12	Fluffy, palewhite, Appressed, white, sporualation moderate to poor chlamydospores terminal or intercalary one or two called.	Fusiform without septum to oval rounded	Fusiform with 3-5 septum	0-50

Table 3 : Radial growth (cm) of 20 isolates of *F.o.*f.sp. *pisi* on different fungicides at 0.1% concentration *in vitro*

Name of Isolate	Bavistin	Copper oxychloride	Propiconazole	Tubeconazole	Mancozeb	SAAF	Control	Mean
F 1	0.00	0.00	2.27	3.07	5.10	5.83	7.93	3.46
F 2	0.00	1.30	1.73	2.47	4.90	5.80	8.10	3.47
F 3	0.00	0.33	1.97	3.10	4.60	5.93	7.90	3.40
F 4	0.00	1.80	2.30	3.00	5.87	5.97	8.33	3.90
F 5	0.00	1.90	2.47	3.07	5.50	5.53	7.07	3.65
F 6	0.00	0.00	1.40	2.53	4.80	5.73	7.33	3.11
F 7	0.00	0.80	1.80	2.90	5.00	5.80	8.47	3.54
F 8	0.00	0.00	1.40	2.77	3.80	5.07	6.60	2.80
F 9	0.00	1.30	2.10	3.03	3.93	4.80	6.10	3.04
F 10	0.00	2.57	2.77	3.37	5.30	6.27	8.70	4.14
F 11	0.00	0.00	1.30	2.47	3.87	4.80	6.30	2.68
F 12	0.00	0.00	0.00	3.03	4.50	5.70	6.20	2.78
F 13	0.00	0.00	1.80	2.50	3.43	5.03	6.80	2.80
F 14	0.00	1.23	1.83	2.87	3.73	5.13	7.07	3.12
F 15	0.00	0.00	2.07	2.90	3.53	5.47	7.47	3.06
F 16	0.00	2.27	2.83	2.50	4.87	6.33	8.57	3.91
F 17	0.00	2.50	2.63	3.37	5.07	6.70	8.53	4.11
F 18	0.00	2.10	2.57	3.53	4.60	5.53	7.07	3.63
F 19	0.00	2.63	3.07	3.80	5.07	6.60	8.17	4.19
F 20	0.00	1.03	2.20	2.60	4.33	5.53	7.10	3.26
Mean	0.00	1.09	2.03	2.94	4.59	5.68	7.49	3.40

Table 4 : Grouping of radial growth of 20 isolates on the *Fusarium oxysporum* f.sp. *pisi*

Group	Average mean	Isolates
Group A	4.00 – 4.99	F10, F17 and F19
Group B	3.00 – 3.99	F1, F2, F3, F5, F6, F7, F9, F14, F15, F16, F18 and F20
Group C	2.00 – 2.99	F8, F11, F12 and F13

was significant statistically indicating the difference among different groups.

Macroconidia

The number of macro-conidia of *Fusarium oxysporum* f.sp. *pisi* on six fungicides are recorded in Table 7. It is evident that all the fungicides reduced macro conidia formation greatly. The maximum reduction was by Bavistin (0.00 million/ml), Copper oxychloride (0.15 million/ml), Propiconazole (0.25 million/ml), Tubeconazole (0.25 million/ml), Mancozeb (0.24 million/ml) and SAAF (0.37 million /ml) as compared to 3.13 million/ml in control. As regard the groups maximum macro-conidia (Table 8) were formed by group A (0.70 – 0.79 million/ml) of F5, F10, F16, F17 and F19. The minimum macro-conidia were produced by group C (0.40 – 0.59 million/ml) of F8, F9, F11, F12, F13, F14 and F15. The fungicides with regard to formation of macro-conidia indicated that the isolates different from each other. The radial growth of isolates in general reduced on all six fungicides to a variable extent when compared with untreated (control) treatment. On the other hand the mean radial growth did not differ much from each other.

Table 5 : Micro-conidia (million/ml) of 20 isolates of *F.o.*f.sp. *pisi* on different fungicides at 0.1% concentration *in vitro*

Name of Isolate	Bavistin	Copper oxychloride	Propiconazole	Tebuconazole	Mancozeb	SAAF	Control	Mean
F 1	0.00	0.00	1.17	1.80	3.20	3.50	6.43	2.30
F 2	0.00	0.70	1.07	1.40	2.87	3.17	6.60	2.26
F 3	0.00	0.33	1.17	2.10	2.80	3.20	6.43	2.29
F 4	0.00	1.20	1.63	2.07	3.00	3.40	6.50	2.54
F 5	0.00	1.13	1.60	1.97	3.10	3.43	6.40	2.52
F 6	0.00	0.00	1.00	2.17	2.23	3.17	6.40	2.14
F 7	0.00	0.67	1.17	2.30	2.33	3.03	6.63	2.30
F 8	0.00	0.00	1.00	1.43	1.83	2.87	5.20	1.76
F 9	0.00	1.03	1.27	2.20	2.13	2.83	5.40	2.12
F 10	0.00	1.43	1.77	2.17	3.37	3.73	6.87	2.76
F 11	0.00	0.00	1.00	1.33	2.33	2.23	5.40	1.76
F 12	0.00	0.00	0.00	2.30	2.77	2.90	5.37	1.90
F 13	0.00	0.00	1.07	1.90	2.13	2.67	5.47	1.89
F 14	0.00	1.00	1.27	1.90	2.03	2.70	5.73	2.09
F 15	0.00	0.00	1.33	1.80	2.13	2.90	5.57	1.96
F 16	0.00	1.33	1.50	1.97	2.77	3.30	6.20	2.44
F 17	0.00	1.50	2.43	2.13	2.93	3.50	6.67	2.74
F 18	0.00	1.27	1.67	2.07	2.90	3.23	6.13	2.47
F 19	0.00	1.67	2.20	2.03	3.17	3.30	6.27	2.66
F 20	0.00	0.53	1.30	1.80	3.03	2.90	5.90	2.21
Mean	0.00	0.69	1.33	1.94	2.65	3.10	6.08	2.26

Table 6 : Grouping of Micro-conidia of 20 isolates on the *Fusarium oxysporum* f.sp. *pisi*

Group	Average mean	Isolates
Group A	2.60 – 2.99	F10, F17 and F19
Group B	2.00 – 2.59	F1, F2, F3, F4, F5, F6, F7, F9, F14, F16, F18 and F20
Group C	1.00 – 1.99	F8, F11, F12, F13 and F15

Table 7 : Macro-conidia (million/ml) of 20 isolates of *F.o.*f.sp. *pisi* on different fungicides at 0.1% concentration *in vitro*

Name of Isolate	Bavistin	Copper oxychloride	Propiconazole	Tebuconazole	Mancozeb	SAAF	Control	Mean
F 1	0.00	0.00	0.31	0.25	0.28	0.42	3.37	0.66
F 2	0.00	0.14	0.24	0.23	0.26	0.38	3.33	0.66
F 3	0.00	0.07	0.30	0.30	0.20	0.37	3.30	0.65
F 4	0.00	0.23	0.34	0.27	0.23	0.39	3.37	0.69
F 5	0.00	0.21	0.36	0.28	0.28	0.40	3.40	0.70
F 6	0.00	0.00	0.21	0.27	0.20	0.38	3.40	0.64
F 7	0.00	0.13	0.22	0.30	0.20	0.38	3.63	0.69
F 8	0.00	0.00	0.22	0.22	0.28	0.30	3.00	0.57
F 9	0.00	0.20	0.31	0.26	0.20	0.38	2.63	0.57
F 10	0.00	0.22	0.40	0.26	0.26	0.40	3.73	0.75
F 11	0.00	0.00	0.20	0.19	0.20	0.31	2.53	0.49
F 12	0.00	0.00	0.00	0.21	0.28	0.31	2.47	0.47
F 13	0.00	0.00	0.20	0.19	0.21	0.33	2.50	0.49
F 14	0.00	0.20	0.20	0.17	0.19	0.33	2.77	0.55
F 15	0.00	0.00	0.21	0.21	0.19	0.36	2.57	0.51
F 16	0.00	0.81	0.23	0.24	0.21	0.39	3.20	0.72
F 17	0.00	0.21	0.30	0.31	0.25	0.37	3.60	0.72
F 18	0.00	0.21	0.21	0.22	0.32	0.38	3.20	0.65
F 19	0.00	0.24	0.32	0.28	0.31	0.42	3.33	0.70
F 20	0.00	0.06	0.23	0.29	0.27	0.31	3.30	0.64
Mean	0.00	0.15	0.25	0.25	0.24	0.37	3.13	0.63

Although, the data were statistically significant with regard to isolates, the significant interaction effect however, showed the variability amongst the isolates. Probably the qualitative characters can only be the best criterion for such type of grouping of isolates.

As observed, the sporulation was also influenced

by different fungicides. The higher number of micro as well as macro-conidia was recorded on untreated treatments (control). While the numbers was reduced drastically on other fungicides. The number of macro-conidia in all the cases was much less as compared to micro-conidia. The data on sporulation were statistically significant so as the isolates on the basis of these characters.

Table 8 : Grouping of macro conidia of 20 isolates on the *Fusarium oxysporum* f.sp. *pisi*

Group	Average mean	Isolates
Group A	0.70 – 0.79	F5, F10, F16, F17 and F19
Group B	0.60 – 0.69	F1, F2, F3, F4, F6, F7, F18 and F20
Group C	0.40 – 0.59	F8, F9, F11, F12, F13, F14 and F15

ACKNOWLEDGEMENT

The authors are thankfull to Head, Deptt. of Plant Pathology, C.S. Azad Univ. of Agric. and Tech. Kanpur, U.P. for providing necessary facilities. We are grateful to all lab staff for valuable support in this study.

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