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Studies on survivability of field pea rust caused by *Uromyces viciae-fabae* (Pers.) de Bary in Tarai region of Uttarakhand (India)

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Pea rust, which is caused by *Uromyces viciae-fabae* (Pers.) de Bary has become the important pathogen since last decade and results in significant yield losses in India. The experiment was conducted during the 2006 to 2010 crop seasons on the survivability on rust of field pea under *in vitro* condition at Pantnagar, India. The results indicated that maximum urediospores germination was recorded from 2006 to 2010 in the month of April followed by May and June. However the number of uredinia on assayed plant indicated apparent survival of urediospores increased dramatically in the viable germination test of urediospore in June, July and August in the year 2008 to 2009. The urediospore survived at 10°C for four months in infected crop debris followed by 5 and 20°C, while failed to survive at temperature (30 to 40°C). Crop debris at different depths revealed that survivability of urediospore declined sharply over period of time as well as increased in the depth of placement. Maximum survivability of fungus was recorded in samples buried at 10 cm followed by 5, 15 and 20 cm deep, respectively. However, the urediospore failed to survive at any depth beyond 4 weeks in 2007 and 2008.

Key words: *Uromyces viciae-fabae*, survivability, urediospore, aeciospores.

INTRODUCTION

The rust fungus *Uromyces viciae-fabae* (Pers.) de Bary is one of the important pathogens of field pea (*Pisum sativum* L.). It is worldwide distributed pathogen of pea and also reported from faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medic.) and sweet pea (*Lathyrus sativus* L.) (Chung et al., 2004; Emeran et al., 2005, 2008; Kushwaha et al., 2006; Shroff and Chand, 2010). In India, Field pea, is grown over an area of 0.77 million hectare with a production 0.71 million tonnes and productivity 1032 kg/ha (Anonymous, 2009). It is being cultivated in Uttar Pradesh, Madhya Pradesh, Orissa, Bihar, Rajasthan, Punjab, Himachal Pradesh, Haryana and Uttarakhand states of India. Pea rust is an important disease in Uttar Pradesh, Uttarakhand and its surrounding areas, resulting adverse effect on grain yield (Singh, 2005). Initial infection is commonly found in areas of fields bordering to faba bean and lentil crops that were infected in previous year or nearly sheltered areas of

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fields where moisture favour prolonged plant wetness. In general, rust appears late in season and causes an estimated 20% yield loss in faba bean crop (Mohamed, 1982). However, these losses could go up to 45 to 50%, if severe infection occurs early in the season (Rashid and Bernier, 1991). In the last few years, disease has been observed in almost epiphytotic form in India when rust infection recorded early in season in first fortnight of November (Sharma, 1998).

The fungus *U. viciae fabae* (Pers.) de Bary is an autoecious with aeciospores, urediospores and teliospores on the surface of host plant and completes its life cycle on the same host. However, initial inoculum has not been clearly established (Gaumann, 1998). In India under field condition, urediospores are converted to teliospores in the month of March due to the higher temperature. It is assumed to teliospores overwinter in the soil or in association with their alternate host debris (Singh, 2005). Urediospores are short-lived, but the teliospores can survive in plant debris from one season to another (Hebblethwaite, 1983). However urediospores can be preserve for two years under freezing condition has been documented by Cunningham (1973) and Davison and Vaugham (1963). Germination of teliospores takes place between 17 to 22°C temperature and at the start of next season producing basidiospores which initiated new infection cycle (Joseph and Hering, 1997; Joshi and Tripathi, 2012). The basidial stage and initiation of primary infection are not fully understood. The disease is favoured by high humidity, cloudy and rainy weather condition. Disease development in field is favoured between 20 and 22°C (Webb and Hawtin, 1981; Kushwaha et al., 2006). Limited information is available on survival of pea rust fungus *U. viciae-fabae* (Pers.) de Bary in nature. Keeping this fact in view, the present investigation was undertaken to study the effect of temperature and soil depth on the survivability of urediospore of *U. viciae-fabae* in tarai region of Uttarakhand.

MATERIALS AND METHODS

Present investigation was commenced to study the effect of temperature and soil depth on the survivability of urediospore of *U. viciae-fabae* in Tarai region of Uttarakhand (India). The following methods were adopted to study the specific objective of experiment.

Production of aeciospores /urediospores in glasshouse condition

The experiment was conducted during 2006 to 2007 and 2009 to 2010 in glasshouse, Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The susceptible field pea variety “Aparna” was inoculated with rust urediospores and aeciospores collected from the naturally infected leaves of field pea plants. Spores from field infected plants were collected and dried over calcium sulphate (8 mesh) at 4°C for 20 h, then maintained in 1.0 ml cryogenic vials at 15°C. Single plant was grown in 6.5 cm square plastic pots filled with potting medium (sterilized sand, soil and farm yard manure mixture, 1:2:1). One month after planting in late October plants were inoculated by spraying a suspension of 10 ml Soltrol 170 oil carrying 0.03 g rust spores through an atomizer (Browder, 1971). Thereafter evaporation of oil, plants were wetted with a solution of 0.1% Tween 20 and transferred to a humidity chamber (+ 90% relative humidity at 18°C) for 16 h. Field pea plants were incubated in glasshouse at 22°C for 14 days for the development of rust pustules and production of aeciospores/ urediospores.

Exposure of infected leaves

Uniformly infected leaves, each with approximately more than 100 pustules, were removed from arbitrarily randomly selected plants and placed six leaves per bag into each of 16 nylon mesh bags, which were stapled. Bags were randomly divided in two groups and each group contained a wire mesh basket to provide protection from animals. The wire mesh baskets were attached to a small tree in shelterbelt at Crop Research Centre of the G.B. Pant University of Agriculture and Technology Pantnagar, in the third week of March. At each site, one basket was fixed 0.3 m above the ground level and one was placed on the ground. Within the first 10 days of each month a single nylon bags was removed from each wire enclosed and plant tissues were assayed for viable urediospores. Standard germination test method was opted to test the viability of aeciospores/urediospores (Shroff and Chand, 2010). Identical tests were made each year over a four year period.

The spore suspension (aeciospores/urediospores) of *U. viciae-fabae* was prepared. A drop of 20 µl of spore suspension prepared in distilled water was placed on glass cavity slides on moist Petriplates with the help of a Gilson micropipette. The aeciospores/urediospores of *U. viciae-fabae* were incubated at 15 ± 2°C. Three replications were maintained for each treatment. After 12 h incubation period, the slides were taken out and 10 µl lactophonal was added to each slide immediately to check further germination of spores. The observations for spore germination were recorded and the percent spore germination was calculated by using following formula:

$$\text{Spore germination (\%)} = \frac{\text{Number of spores germinated}}{\text{Total number of spores observed}} \times 100$$

Assay of plant tissue

The experimental leaf debris was weighted, crushed, suspended in 0.1% Tween 20 solution within 4 h of recovery from the exposed mesh bags and sprayed onto 20 leaves of susceptible field pea cultivar “Aparna” in the glasshouse. Inoculated plants were gently sprayed with Tween 20 solution and incubated in moist condition in the greenhouse. Rust pustules on each leaf were counted 14 days after incubation in the glasshouse. Infected recovered material were leaves and plant part segregated on separate aluminum foil sheets and conditioned at 4°C temperature and 90% relative humidity for 7 days in a refrigerator. Conditioned material from each exposure was weighted and grinded with mortar and pestle. The crushed material was loaded into a 3 cc syringe and suspended in 1.5 ml Soltrol 170 oil and thoroughly mixed. The syringe was attached to a Swinney 13 mm filter holder fitted with a 300 mesh screen. The suspension was passed through the screen and filtrate was collected in empty gelatin capsules. Each capsule was fitted into an atomizer and the contents were dispersed onto a group of 20 to 25 days old susceptible cultivar of field pea (Aparna) grown in a rust free glasshouse. Each group of plants were placed in a separate humidity chamber and later kept in glass house with growing

Table 1. Mean number of viable urediospore obtained in germination test.

Year	No. of viable urediospore (mean spore germination of 03 sample)				
	April	May	June	July	August
2006-2007	44	8	5	0	NT
2007-2008	56	10	5	1	NT
2008- 2009	67	12	35	17	5
2009-2010	60	13	8	0	NT

NT = Not tested because in month of August due to no viable spore was observed in July.

conditions as previously described.

Survival of urediospores at different temperature

Infected crop debris having urediospores /teliospores were collected from the field during the month of April 2007 and 2008, respectively and they were cut into small pieces and wrapped in butter paper. These pieces were stored in incubator at different temperature viz., 10, 20, 30, 40 and at 5°C in refrigerator. Germination of urediospores /teliospores from crop debris was tested at monthly interval by serial dilution technique to ascertain the survivability of spores.

Survival of aeciospores/urediospores at different soil depth

The experiment was conducted for two consecutive years (2007 and 2008) from April to May under glasshouse condition to observe the survivability of spores. The small pieces of infected plant debris were placed in nylon mesh bags (50 g/bag) and buried at different depths viz., 5, 10, 15 and 20 cm, respectively in plastic pots filled with natural soil. These inoculated pots were replicated 4 times and randomly kept in glasshouse. At weekly interval, one gram of debris was taken out from each sample and germination of spore was tested by serial dilution technique as above to ascertain the survival of urediospore/ teliospores at different soil depths, if any.

RESULTS and DISCUSSION

Production of aeciospores/urediospores in glasshouse

Field pea plants were inoculated with rust urediospores and aeciospores collected from the naturally infected leaves and incubated in glasshouse at 22°C for 14 days for the development of rust pustules and production of aeciospores/urediospores. The survival of viable aeciospores/urediospore from detached debris and standing plant residue was apparent. Therefore data were combined and mean spore germination was calculated for each month. Within the first 10 days of each month plant tissues were sampled three times and assayed for viable urediospores. The table indicated that maximum urediospore germination was recorded during 2006 to 2010 in the month of April followed by May and June (Table 1). For the first 2 years (2006 to 2007 and 2007 to 2008) of the study, the limited number of uredinia developed on the assayed plant indicated that the urediospore survival was poor or lasted up to the month of July.

In June 2008 to 2009, urediospores were left in the refrigerator for 1 week because susceptible test plant had poor/no germination and had to be replanted. The number of uredinia on assayed plant indicated apparent survival increased dramatically in June, July and August in the year 2008 to 2009 (Table 1). The difference was interpreted as the result of spore conditioning. The work has been duly supported by earlier investigation on bean with rust pathogens as conditioning increases urediospores viability (Beard and Hamlin, 1995). In the year 2008 to 2009, conditioned spores were assayed and urediospores found survived up to August. In general rust survival declined over time from April onwards. Temperature was warmer than normal in 2006 to 2007 and cooler than normal for 2008 to 2009. Although most uredinia are converted to telia on mature and senescent plant tissues, uredinia are also common on field pea at or after harvest. Overwintered urediniospores could contribute to early initiation of disease and also to build-up of new races known to attack on field pea in Pantnagar.

Effect of different temperatures on survival of urediospore

The results revealed in year 2007 that initial survival of urediospore was 65.0%, declined up to 8.67% after lapse of 4 month period when stored at 10°C, while at 5°C temperature only 5% survivability of spore was observed in the month of July. Urediospore incubated at 40°C and 30°C in the month of April and onward, only 4.0% survival was found in the month of April at 30°C whereas no germination was recorded at 40°C temperature. At 20°C by the end of June 14.0% survivability was recorded and thereafter no germination of spores at all (Table 2). Similar trend was observed in respect to effect of different temperature on survival of urediospore during the year 2008. Maximum survivability (67.0%) was recorded at low temperature when urediospore were incubated at 10°C followed by 5°C (65.0%), 20°C (60.33%) and 30°C (8.33%) in the month of April. In the month of July

Table 2. Effect of different temperatures on survival of urediospores of *U. viciae-fabae*.

Month/week	Percent survival									
	2007					2008				
	5℃	10 ℃	20℃	30℃	40℃	5℃	10 ℃	20℃	30℃	40℃
April	62.00 (51.99)	65.00 (53.76)	45.00 (42.12)	4.00 (11.35)	0.00 (0.00)	65.00 (53.76)	67.00 (55.01)	60.33 (50.98)	8.33 (16.73)	0.00 (0.00)
May	45.00 (42.12)	55.00 (47.88)	32.00 (34.44)	0.00 (0.00)	0.00 (0.00)	44.00 (41.54)	50.00 (44.99)	37.33 (37.64)	0.00 (0.00)	0.00 (0.00)
June	30.00 (33.16)	36.67 (37.25)	14.00 (20.69)	0.00 (0.00)	0.00 (0.00)	26.67 (30.39)	30.00 (33.16)	13.33 (17.21)	0.00 (0.00)	0.00 (0.00)
July	5.00 (12.92)	8.67 (17.35)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.67 (16.32)	5.33 (13.32)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
RCD (P = 0.01)	3.01	8.70	5.37	1.72	0.00	7.84	7.39	9.68	1.52	0.00
CD (P = 0.05)	1.99	13.14	8.11	2.60	0.00	11.84	11.17	14.63	2.30	0.00
GM±SEM	40.50 ± 0.57	46.33 ± 2.51	27.75 ± 1.55	1.0 ± 0.50	0.00	38.08 ± 2.26	43.08 ± 2.14	33.16 ± 2.80	2.88 ± 0.44	0.00
CV (%)	2.46	9.41	9.70	86.60	0.00	10.31	8.60	14.64	36.66	0.00

Figures given in parentheses are angular transformed values. Average of four replications.

urediospore was incubated at 5 and 10℃ temperatures. Only 7.67 and 5.33% survivability was recorded, while at higher temperature, it was also observed that at 30℃ temperature, spore survived only for one month in both the years and no germination was observed at 40℃. Similarly, optimum germination of urediospore and other spores of *U. viciae-fabae* were recorded at 20℃ temperature by Joseph and Hering (1997) and Joshi and Tripathi (2012). Experimental result indicated that constant exposure of urediospore at 30 and 40℃ temperature was lethal to the survival of urediospore as they cannot survive in hot months of May, June and July. The results of present study revealed that the fungus survived in infected crop debris in the form of urediospores at different temperature (Table 2). Similarly Singh (2005) reported that optimum temperature for germination of urediospores is 16 and 25℃. However, there was no germination observed at 28 and 29℃. Batra and Stavely (1994) reported that U. *appendiculatus* urediospore germinates best at 15 to 24℃, while the teliospore in crop debris germinates best at 10 and 15℃.

Magsi (2000) observed that germination of *U. striatus* urediospore causes the rust of Lucerne at 5, 10, 15, 20, 25, 30 and 35℃ under 24 h in dark condition. It was reported that 20℃ favoured maximum spore germination, whereas minimum germination occurred at 10 and 25℃. Singh (2005) reported in rust of chickpea caused by *U. ciceris-arietini* that the urediospore can germinate at any temperature between 5 and 30℃ but the optimum is 11 and 12℃. No germination occurs at 35℃ and above temperatures.

Effect of different soil depth on urediospore survival

The experiment was conducted for two consecutive years (2007 and 2008) from April to May under glasshouse condition to observe the survivability of urediospore, buried at 5, 10, 15 and 20 cm depth in natural soil. The urediospore

Table 3. Survival of *U. viciae fabae* in infected crop debris buried in soil at different depth during the month of April to May in 2007and 2008 crop seasons.

Months/week	Percent survival							
	2007				2008			
	5 cm	10 cm	15 cm	20 cm	5 cm	10 cm	15 cm	20 cm
April								
1st week	44.00 (41.55)	54.00 (47.29)	16.33 (23.77)	11.33 (19.66)	51.00 (45.57)	61.67 (51.75)	21.67 (27.71)	12.67 (20.80)
2nd week	36.67 (37.20)	45.00 (42.12)	11.30 (19.21)	7.67 (16.07)	39.67 (39.03)	47.67 (43.66)	16.67 (24.08)	9.67 (18.07)
3rd week	2.400 (29.66)	35.00 (38.92)	6.67 (14.72)	5.33 (13.34)	32.67 (34.82)	36.67 (37.25)	9.67 (18.07)	6.67 (14.89)
4th week	18.00 (21.32)	20.33 (26.45)	5.00 (12.75)	0.00 (0.00)	21.67 (27.71)	23.33 (28.78)	6.33 (14.50)	0.00 (0.00)
May								
1st week	11.33 (19.56)	31.66 (34.23)	0.00 (0.00)	0.00	11.67 (19.74)	21.00 (27.70)	0.00 (0.00)	0.00
2nd week	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3rd week	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4th week	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (P = 0.01)	4.36	9.89	3.94	1.14	4.56	7.20	3.33	2.42
GM ± SEM	10.45 ± 1.98	20.16 ± 3.77	5.20 ± 0.93	3.33 ± 0.27	20.50 ± 1.93	28.95 ± 2.16	7.16 ± 0.79	3.62 ± 0.57
CV (%)	10.69	20.05	31.17	14.07	13.13	10.37	19.13	27.58

Figures given in parentheses are angular transformed values. Average of four replications.

of *U. viciae-fabae* survived up to 5 weeks in natural soil at 10 cm deep. However, the survivability was significantly declined with increase in soil depth and exposure period. The survivability of urediospore was maximum (54.0%) when it was buried at 10 cm deep in the first week of April followed by 5 cm (44.0%), 15 cm (16.33%) and 20 cm (11.33%) (Table 3). The survivability of urediospore was significantly higher (31.66%) when it was buried at 10 cm depth followed by 5 cm (11.33%) depth in the first week of May. The survivability declined from 11.33 to 0.00% by 6th week at 5 cm depth, while no survivability was recorded at 15 and 20 cm depth from 6th to 8th week burial in soil during both the seasons. The similar trend was observed during the next year also where highest survivability was recorded when urediospore were buried at 10 and 5 cm deep. There was gradual decrease in survivability with increase in soil depth as well as lapse of time. Likewise, percent survivability of *U. viciae-fabae* declined sharply with increase in depth reported by Joseph and Hering (1997) and Joshi and Tripathi (2012).

Loss of survivability of urediospores of *U. viciae-fabae* through infected crop debris at 15 and 20 cm soil depth might be due to decomposition of crop debris by saprophytic fungi like Aspergillus, Rhizopus, Mucor, Penicillium present in soil. It is presumed that maximum survivability at 10 cm due to less competition with other microorganism and comparatively lower temperature than other soil depths tried. The results and assumption was duly supported by various workers (Pandey and Sinha, 2008; Thorman et al., 2003). The role of environmental factors is more complex with air borne disease than soil borne. Apart from the direct effect of moisture and temperature on the germination, dissemination and pathogenicity of organism the complexity of soil itself is highly significant as soil environment present a physical, chemical and biological barrier for survival. Obviously all these factors interact, as has been hypothesized as an explanation for the survivability of the fungus. Therefore, this information will be apparently useful for epidemiological studies and development of integrated disease management module for pea rust disease.

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