

Effect of temperature on germination and survival of *Uromyces viciae fabae* (Pers.) de Bary

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ABSTRACT

The effect of temperature on spore germination of *Uromyces viciae fabae* (Pers.) de Bary on pea plant was studied using cavity slide germination method at different temperatures like 10, 15, 20, 25 and 30°C and spore viability was studied by germination test employing cavity slide technique at 10, 15, 30, 60, 90, 120, 150, 180 and 240 days after storage at different temperatures i.e. 15, 20, 25, 30°C in incubator and at 5 to 10°C in refrigerator. The result indicated that maximum germination of aeciospore, urediospore and teliospore was observed at 20 °C temperature. At 5 °C storage temperature, the survival of aeciospore, urediospore and teliospore was found maximum for 60, 120 and 240 days, respectively.

Keywords: Temperature, germination and survival, *Uromyces viciae fabae*

Uromyces viciae fabae (Pers.) de Bary is autoecious, macrocyclic, heterothallic rust fungi having wide range of collateral hosts including pea crops causes significant yield losses up to 20 to 100 per cent by affecting all the green plant parts including the pods (Sharma, 1998). It forms all the four spores viz., pycniospores/spermatiospores, aeciospores, urediospores and teliospores on pea plant. The pycnial stage was inconspicuous in nature occurring for a very short duration quickly followed by formation of aecia, the disease spread rapidly and aecia developed on all the above ground parts of the pea plants including pods. In India, it has been reported that aeciospores act as repeating spores and play an important role in the outbreak of the disease (Kushwaha *et al.*, 2006). In nature, the uredia were produced for very short duration quickly followed by telia and the teliospores are generally formed when the plant reaches towards maturity and environmental conditions are unfavourable for the propagation of the rust in the uredial stage. Hence, it is also known as resting spores.

This disease normally not serious may assume epidemic proportions in certain years because of favorable climatic condition for their development and spread. Infected inert plant debris mixed with seed can act as basic inoculum for the recurrence of the disease in most years due to germination and survival of *Uromyces viciae fabae* (Khare, 1981). Pathogen survives in different forms during unfavourable environmental condition and the appearance and progress of disease is region specific. The predominant form of survival, therefore, varies with the environment and

location. In Maharashtra, research works on important aspect of this pathogen previously have not been done. Limited information is available on germination and survival of pea rust fungus *Uromyces viciae fabae* (Pers.) de Bary in nature. Keeping this fact in view, it is important to study the effect of temperature on germination and survivability of pathogen.

MATERIAL AND METHODS

This experiment was conducted during 2015 in the laboratory of Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri.

Spore germination of pathogen

Effect of temperature on spore germination was studied using cavity slide germination method. The aecial and uredial stages of the pathogen were collected from heavily infected pea plants. Fresh crops of spores from recently captured aecia and uredia were taken for all treatments. Spores from samples were separated from the diseased tissue by shaking the exposed end of the sori with a needle. A water agar method was used for aeciospores germination as these spores were germinated poorly in distilled water. A water agar (1.5 per cent) was layered on clear slides after warming for 30-60 seconds. The aeciospores were brushed on the slides and incubated in petriplates lined with moistened filter paper at 10, 15, 20, 25 and 30°C. After 48 hrs of incubation, slides were taken out and 10 µl of lacto phenol was added immediately to check further germination of spores. Per cent germination was calculated by using following formula.

Table 1: Effect of temperature on *Uromyces viciae fabae* spore germination

Temperature(°C)	Germination(%)		
	Aeciospores	Urediospores	Teliospores
10	41.2(39.9)	46.29(42.87)	31.4(34.0)
15	56.9(49.0)	58.60(49.95)	37.4(37.7)
20	72.5(58.4)	69.55(56.52)	71.3(57.6)
25	21.7(27.7)	15.35(23.06)	68.2(55.7)
30	0.0(0.0)	0.0(0.0)	0.0(0.0)
CV %	3.7	3.72	4.14
SE±	0.6	0.66	0.78
CD at 5%	2.04	1.98	2.36

Figures in parenthesis are arc sin transformed values

$$\text{Per cent germination} = \frac{\text{No. of spore germinated}}{\text{Total no. of spores}} \times 100$$

For urediospore germination, spore suspension was prepared in distilled water (500 spores / ml) and 0.1 ml of spore suspension was placed on a clean cavity microslide and incubated in petriplates lined with moistened filter paper at 10, 15, 20, 25 and 30°C. Per cent germination of urediospore was recorded after 48 h of incubation.

Samples for teliospores were collected from the field during the month of March, 2015. Soon after collection, infected leaves and bits of stem were be soaked in water overnight and teliospore suspension was used to test spore germination at different temperatures employing the method as described for urediospore germination.

Survival of pathogen

To study the length of survival of spores of the test pathogen at different temperatures, samples were collected from the field during the month of March and April, 2015 for aeciospores/urediospores and teliospores, respectively. They were wrapped in butter paper and stored in sealed glasstubes. In one set of experiment, these pieces were exposed to room temperature in laboratory and at different temperature i.e. 15, 20, 25, 30°C in incubator and at 5 to 10°C in refrigerator. Spore viability was studied by germination test employing cavity slide technique at 10, 15, 30, 60, 90, 120, 150, 180 and 240 days after storage. Further, the spore germination test was carried out at 18°C and subsequently observations were recorded 48 hrs after incubation.

RESULT AND DISCUSSION

Effect of temperature on spore germination

The data on the effect of temperature on germination

of aeciospores, urediospores and teliospores of *Uromyces viciae-fabae* are presented in Table 1.

Maximum germination of aeciospores (72.5%) was observed at 20°C, while, none of the aeciospores germinated at 30°C. The germination of aeciospores increased progressively with an increase in temperature from 10 to 20°C but decreased further significantly. Nearly 40 to 75 per cent germination of aeciospores of *Uromyces viciae fabae* was obtained between 7 to 22 °C. At 25 °C and 30°C the spore did not germinate. Negussie *et al.* (2005) reported that after 3 hrs of incubation a high percentage of spore germination was observed on 1.5 per cent water agar at 10, 15, 20 and 25°C with an optimum at 20°C (99.0 %). At sampling time the length of germ tube ranged from 66 micrometer at 10°C to a maximum 196 micrometer at 20°C. Growth of germ tube increased progressively from 10 to 20°C and then declined at 25°C. Joshi (2006) also reported that maximum germination was (73.3%) at 20°C while, no germination was observed at 30°C.

Similar trend was followed by the urediospores. Maximum germination of urediospores (69.5%) was recorded at 20°C, while there was no germination at 30°C. The germination of urediospores also increased progressively with an increase in temperature from 10 to 20°C but decreased further significantly. Joseph and Hering (1997) have reported that urediospores of *Uromyces viciae fabae* germinated well in range of 5 to 26°C, with fastest germination at 20°C while, exposure to 30 °C gave poor germination and damaged spores. Joshi (2006) also reported maximum germination of urediospores (70%) at 20°C while, no germination at 30°C.

The teliospores of the pathogen germinated at all the temperatures tried, ranging from 10 to 25°C. Maximum germination of teliospores (71.3 %) was recorded at 20°C.

Table 2: Effect of temperature on survival of aeciospores of *Uromyces viciae fabae*

Temperature(°C)	Survival of aeciospores germination (%)						Mean
	0DAS	10 DAS	15 DAS	30DAS	60 DAS	90 DAS	
5	60.9 (51.3)	60.4 (51.0)	51.5 (45.8)	29.6 (32.9)	14.0 (21.9)	0.0 (0.0)	33.8
10	60.9 (51.3)	54.6 (47.6)	45.3 (42.3)	23.4 (28.8)	0.0 (0.0)	0.0 (0.0)	28.3
15	60.9 (51.3)	59.3 (50.4)	48.4 (44.1)	25.0 (29.9)	0.0 (0.0)	0.0 (0.0)	29.3
20	60.9 (51.3)	57.8 (49.5)	50.0 (45.0)	26.5 (31.0)	0.0 (0.0)	0.0 (0.0)	29.4
25	60.9 (51.3)	32.8 (34.9)	21.8 (27.8)	17.1 (24.4)	0.0 (0.0)	0.0 (0.0)	23.1
30	60.9 (51.3)	23.4 (28.8)	12.5 (20.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	17.4
Room Temp.	60.9 (51.3)	60.9 (51.3)	50.0 (45.0)	28.1 (32.0)	0.0 (0.0)	0.0 (0.0)	29.9
Mean	51.3	44.8	38.6	26.2	3.13	0.00	27.3
CV %	4.64						
SE±	a : 0.34		b:0.40		a x b :0.90		
CD at 5%	a : 0.96		b:1.13		a x b :2.53		

DAS : Days after storage; Figures in parenthesis are arc sin transformed values

Table 3: Effect of temperature on survival of urediospores of *Uromyces viciae fabae*

Temperature(°C)	Survival of urediospore germination (%)								Mean
	0DAS	10 DAS	15 DAS	30DAS	60 DAS	90 DAS	120 DAS	150 DAS	
5	72.7 (58.5)	63.6 (52.9)	48.4 (44.1)	42.4 (40.6)	33.3 (35.2)	24.2 (29.4)	15.1 (22.8)	0.0 (0.0)	35.4
10	72.7 (58.5)	57.5 (49.3)	51.5 (45.8)	39.3 (38.8)	27.2 (31.4)	13.6 (21.6)	0.0 (0.0)	0.0 (0.0)	30.7
15	72.7 (58.5)	66.6 (54.7)	54.5 (47.6)	40.9 (39.7)	24.2 (29.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	28.7
20	72.7 (58.5)	75.7 (60.5)	62.1 (52.0)	39.3 (38.8)	31.8 (34.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.5
25	72.7 (58.5)	48.4 (44.1)	36.3 (37.0)	28.7 (32.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	21.5
30	72.7 (58.5)	37.8 (37.9)	21.2 (27.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	15.4
Room Temp.	72.7 (58.5)	71.2 (57.5)	63.6 (52.9)	36.3 (37.0)	12.1 (20.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	28.2
Mean	58.5	51.0	43.8	32.5	21.5	7.3	3.2	0.0	27.2
CV %	4.80								
SE±	a : 0.34		b:0.34		a x b :0.89				
CD at 5%	a : 0.95		b:0.95		a x b :2.51				

DAS : Days after storage; Figures in parenthesis are arc sin transformed values

Table 4: Effect of temperature on survival of teliospore of *Uromyces viciae fabae*

Temperature(°C)	Survival of teliospore germination (%)										Mean
	0DAS	10DAS	15DAS	30DAS	60DAS	90DAS	120DAS	150DAS	180DAS	240DAS	
5	74.6 (59.8)	69.3 (56.3)	68.2 (55.7)	63.4 (52.8)	60.3 (50.9)	57.1 (49.1)	53.9 (47.2)	50.7 (45.4)	49.2 (44.5)	42.8 (40.8)	50.3
10	74.6 (59.8)	72.4 (58.3)	71.4 (57.6)	68.2 (55.7)	61.9 (51.8)	55.5 (48.1)	49.2 (44.5)	44.4 (41.8)	41.2 (39.9)	39.6 (39.0)	49.7
15	74.6 (59.8)	70.3 (57.0)	69.3 (56.3)	65.0 (53.7)	63.4 (52.8)	53.9 (47.2)	50.7 (45.4)	42.8 (40.4)	39.6 (39.0)	38.1 (38.1)	49.0
20	74.6 (59.8)	70.9 (57.3)	69.8 (56.6)	66.6 (54.7)	57.1 (49.1)	50.7 (45.4)	50.7 (45.4)	39.6 (39.0)	38.1 (38.1)	34.9 (36.2)	48.1
25	74.6 (59.8)	67.7 (55.3)	66.6 (54.7)	60.3 (50.9)	50.7 (45.4)	46.0 (42.7)	41.2 (39.9)	41.2 (39.9)	36.5 (37.1)	33.3 (35.2)	46.1
30	74.6 (59.8)	64.5 (53.4)	63.4 (52.8)	57.1 (49.1)	47.6 (43.6)	41.2 (39.9)	38.1 (38.1)	38.1 (38.1)	34.9 (36.2)	31.7 (34.2)	44.5
Room Temp.	74.6 (59.8)	72.4 (58.4)	71.9 (58.0)	69.8 (56.7)	49.2 (44.5)	47.6 (43.6)	44.4 (41.8)	42.8 (40.8)	36.5 (37.1)	34.9 (36.2)	47.7
Mean	59.8	56.6	56.0	53.4	48.3	45.1	43.2	40.8	38.8	37.1	47.9
CV%	3.19										
SE±	a : 0.33			b:0.28			a x b :0.88				
CDat5%	a : 0.93			b:0.78			a x b :2.47				

DAS : Days after storage; Figures in parenthesis are arc sin transformed values

It was followed by 25°C (68.2 %) and 15°C (37.4 %), respectively. While, none of the teliospores germinated at 30°C. The germination of teliospores increased progressively with an increase in temperature from 10 to 20°C, but decreased further significantly. Joshi (2006) have reported that maximum germination of teliospores 71.1 per cent was observed at 20°C followed by 68.3 per cent at 25°C while, no germination at 30°C. All the results are in accordance to those reported by Prasada and Verma (1948) and Agarwal and Prasada (1997). Critical analysis of data revealed that optimum temperature for germination of aeciospores and urediospores ranges between 15 to 20°C, while the optimum temperature for germination of teliospores ranges around 20 to 25°C.

Effect of temperature on survival of aeciospores, urediospores and teliospores

Data on per cent germination of aeciospores, urediospores and teliospores stored at different temperature are presented in Table 2,3 and 4, respectively.

Aeciospores : A perusal of the data on per cent germination of aeciospores stored at different temperatures for various duration (Table 2), revealed that viability of the aeciospores

decreased with increase in time. Maximum viability of aeciospores was recorded in the samples stored at 5°C, which recorded 14.0 per cent germination after 60 days. While, at 30°C the spores remained viable only upto 15 days showing 12.5 per cent germination. At temperatures ranging from 10-25°C, the spores remained viable upto 30 days with 23.4, 25.0, 26.5 and 17.1 per cent germination at 10, 15, 20 and 25°C storage temperature, respectively. Aeciospores when stored at room temperature, the spores retained their viability upto 30 days with 28.1 per cent germination.

Urediospores : The data recorded on per cent germination of urediospores stored at various temperature for different durations (Table 3) indicate that urediospores followed the trend similar to aeciospores but somewhat longer viability was observed. Maximum viability of urediospores was recorded in samples stored at 5°C with 15.1 per cent germination after 120 days while at 30°C, the spores remained viable only up to 15 days showing 21.2 per cent germinability. The urediospores retained their viability upto 90 days showing 13.6 per cent germination when stored at 10°C, 60 days at 15°C showing 24.2 per cent germination and 20°C showing 31.8 per cent germination and 30 days when stored at 25°C showing 28.7 per cent germination. Urediospores

when exposed to room temperature, the urediospores remained viable upto 60 days with 12.1 per cent germination.

Teliospores : The data recorded on per cent germination of teliospores stored at various temperature for different durations (Table 4) indicated that teliospores retain their viability for longer durations as compared to aeciospores and urediospores. Spore germination tests made after 240 days of storage at different temperature give positive result. After 240 days of storage, maximum germination was recorded in the samples stored at 5°C giving 42.8 per cent germination and it decreased progressively with increase in temperature. At 10, 15, 20, 25 and 30°C storage temperature 39.6, 38.1, 34.9, 33.3 and 31.7 per cent germination was observed, respectively after 240 days of storage.

The teliospores exposed to room temperature also retained their viability upto 240 days giving 34.9 per cent germination. Thus, it is apparent that the aeciospores and urediospores of *Uromyces viciae fabae* have no role to play in recurrence of pea rust in India as they can not retain their viability for long durations at high temperatures and perish during the summer that follows the harvest. But the teliospores can retain their viability up to longer duration. These observations are in conformity of those reported previously by Joshi (2006). Prasada and Verma (1948) have reported similar results that the teliospores of *Uromyces viciae fabae* remain viable for nearly a year under natural conditions in the plains of India. The teliospores can germinate soon after their formation at temperature ranging from 12°C to 22°C. Since in nature, this range of temperature and high humidity are together available during the following winter (January-February), the teliospores retained their viability until then.

CONCLUSION

Maximum germination of aeciospore, urediospore and teliospore was observed at 20 °C temperature. At 5 °C storage temperature, the survival of aeciospore, urediospore and teliospore was found maximum for 60, 120 and 240 days, respectively.

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