Effect of some abiotic factors on germination of urediospore of the coffee leaf rust fungus, *Hemileia vastatrix* (Berkeley & Broome)

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ABSTRACT

The influence of abiotic factors *viz.*, temperature and relative humidity on the germination of urediospore of *Hemileia vastatrix* Berkeley & Broome, the coffee leaf rust pathogen was assessed under *in-vitro* conditions. The laboratory study was carried out to find the percentage germination of urediospores at different temperatures of 18, 20, 22, 24 and 26°C and at various relative humidity (RH) levels of 50, 60, 70, 80 and 90%. The results indicated that maximum germination of urediospore of the coffee rust fungus was observed at 24°C (48.60%) and 70% relative humidity (40.80%). The percentage germination of urediospore was affected when the temperature was decreased to 18°C (33.00%) and also increased to 26°C (36.20%). Spore germination was reduced to 31.60% at 50% relative humidity and 26.40% at 90% relative humidity levels set for the experiment.

Key words: Abiotic factors, *Hemileia vastatrix*, coffee leaf rust, urediospore

INTRODUCTION

The economically important two species of *Coffea, Coffea arabica* L. (arabica coffee) and *Coffea canephora* Pierre ex Froehner (robusta coffee) are commercially cultivated throughout the coffee growing countries. In India, the cultivated area under arabica coffee is about 1.79 lakh ha with a production of 0.99 lakh MT. The average productivity was 657 kg ha^{-1} clean coffee in the year 2006-07 (Anonymous, 2008). Among the cultivated coffee, arabica coffee is more susceptible to diseases compared to robusta coffee (Anonymous, 2003).

The coffee leaf rust (CLR) disease incited by the fungus *Hemileia vastatrix* Berkeley & Broome is a major disease of arabica coffee causing economic loss (Bhat *et al*., 2000). This foliar disease is reported from over fifty coffee growing countries. The disease was first observed on coffee in India during 1869. The CLR fungus is an obligate parasite and is host specific. The fungus exists in different physiological forms called "races". The uredinial stage (urediosorus) is the only viable perpetuating stage of the coffee rust fungus. Urediospores are the infection propagules disseminated by wind, rain water droplets, insects etc. (Anonymous, 2003).

Urediospores are the potential source of inoculum for the coffee leaf rust disease buildup under field conditions. So, it is very important to understand the mechanism of urediospore germination and the factors that influence the spore germination. Not much information was available on the influence of temperature and relative humidity on urediospore germination and also the optimum level of these abiotic factors required for maximum spore germination of *H. vastatrix*. Hence, the present study was undertaken to assess the effect of temperature and relative humidity on the germination of the urediospore of coffee rust fungus under *in-vitro* conditions.

MATERIALS AND METHODS

Temperature on urediospore germination

The coffee leaves (arabica coffee S.795 variety) infected by *H. vastatrix* were collected freshly from the field. The infected leaves with different size of urediosorus (rust spots or pustules) which are free from contamination by the hyper-parasite (myco-parasite) *Verticillium hemileiae* Bour. (Bouriquet, 1946) was selected and the urediospores were collected in a gelatin capsule by scooping the spores from each pustules of the leaf. These urediospores from different pustules of the leaf and from different leaves formed the bulk sample (inoculum). From the bulk sample, twenty five milligram of urediospore was mixed in 25 ml of sterile distilled water present in a conical flask (Corning make) and this urediospore suspension was used for inoculation. Two per cent water agar medium was prepared and kept ready for use (Anonymous, 1953). Sterilized glass Petri-plates (Corning make) of 9 cm dia. were used for plating of agar medium. Fifteen ml of 2 per cent water agar medium was poured in to each Petri-plate and was thoroughly spread on the plate. To each Petri plate, one

ml of the urediospore suspension was added and spread uniformly on to the agar medium. Five Petri-plates of five sets each for different temperatures of 18, 20, 22, 24 and 26°C were inoculated with the urediospore suspension. The inoculated Petri-plates were incubated for 18 h (SANYO – Versatile Environmental Test Chamber, MLR-350 H, Japan) and later the Petri-plates were observed directly under a stereo microscope (Nikon – SMZ-800, Japan) for germination of urediospore (Kamanna *et al.*, 1993). Per cent urediospore germination was calculated using the formula: $PGU = \frac{TNGU}{TNU} \times 100$

Where;
PGU = Per cent germinated urediospores ,
TNGU = Total number of germinated urediospores in each microscopic field,
TNU = Total number of urediospores present in each microscopic field

Relative humidity on urediospore germination

The procedure followed for preparation of urediospore sample and water agar medium was the same as described above under the study effect of temperature on urediospore germination. Five sets of five Petri-plates each were filled with fifteen ml of 2 per cent water agar medium and spread thoroughly. One ml of the urediospore suspension was added on to the medium in each petriplate and spread uniformly. The inoculated Petri-plates were kept in a growth chamber (SANYO – Versatile Environmental Test Chamber, MLR-350 H, Japan) where RH was maintained at different humidity levels of 50, 60, 70, 80 and 90%. The inoculated Petri-plates were incubated for 18 h and later they were observed directly under a stereo microscope (Nikon – SMZ-800, Japan) for germination of urediospore. The per cent urediospore germination was worked out from the data using the formula as described earlier under effect of temperature on urediospore germination.

Statistical analysis

Analysis of variance (ANOVA) test was used to analyse the data. The percentage values were subjected to *arc-sine* transformation to normalize the data. The treatment means were compared using least significant difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Temperature on urediospore germination

The percentage germination of urediospore of coffee leaf rust fungus as influenced by different level of temperature is presented in Table 1.

Table 1. Effect of temperatures (°C) on germination (in %) of urediospore of *H. vastatrix*

Temperatures	Germination
18	33.00 (34.76)^{b}
20	35.40 (36.13)^{b}
22	40.80 (38.17)^{ab}
24	48.60 (43.79)^{a}
26	36.20 (35.27)^{b}
SEM	2.96
CD (P = 0.05)	8.63

Figures in parentheses are *arc-sine* transformed values.In a column, means followed by same letter(s) are not significantly different at P=0.05 as per LSD.

The results indicate that urediospore germination was maximum (48.60%) at a temperature of 24°C followed by 22°C (40.80%). Significant reduction in spore germination was observed at 18, 20 and 26°C with germination percentage of 33.00, 35.40 and 36.20% respectively which were on par with each other.

Nutman and Roberts (1963) found that a temperature of 22°C was optimum for germination of urediospore on water agar medium. It was observed that 23°C was the optimum temperature for urediospore germination of coffee rust fungus (Akutsu, 1981). Montoya and Chaves (1974) worked out a regression equation for calculating urediospore germination of *H. vastatrix*. In the present study, it was observed that under *in-vitro* conditions, a temperature of 24°C was the optimum temperature for maximum germination of urediospore of the coffee leaf rust fungus.

Relative humidity on urediospore germination

The results obtained from the studies on influence of relative humidity on percentage urediospore germination of coffee leaf rust pathogen are presented in Table 2.

Table 2. Influence of relative humidity (in %) on germination (in %) of urediospore of *H. vastatrix*

Humidities	Germination
50	31.60 (33.83)^{bc}
60	37.40 (37.40)^{ab}
70	40.80 (39.45)^{a}
80	29.00 (32.55)^{c}
90	26.40 (30.75)^{c}
SEM	2.63
CD (P = 0.05)	7.68

Figures in parentheses a re *arc-sine* transformed values. In a column, means followed by same letter(s) are not significantly different at P = 0.05 as per LSD.

At 70% relative humidity, urediospore germination of the rust fungus was found to be maximum (40.80%), while the spore germination (26.40%) was minimum at 90% relative humidity. Statistically, no significant difference in the spore germination was observed at RH 80% (29.00%) and RH 90% (26.40%). An extent of 31.60% urediospore germination was observed at 50% relative humidity where as at RH 60%, the spore germination was 37.40%. The observations from the experiment indicate that relative humidity ranging from 60 to 70% was suitable for germination of urediospores, while optimum relative humidity was found to be 70% under *in-vitro* conditions. Ward (1882) has mentioned that for the germination of spores of coffee rust fungus, water is a pre-requisite. The dew drops formed during the winter season also plays a major role in the disease epidemic (Mayne, 1932). Kushalappa *et al*. (1983) has opined that the urediospore of coffee rust fungus do not germinate, even at high relative humidity, if free water is absent. The results obtained in the present study also indicate that germination of spores of the coffee rust fungus was reduced at 80 and 90% relative humidity levels which are in confirmatory with the statement of Kushalappa *et al.* (1983).

The present study revealed that maximum spore germination at 24°C and at 70% relative humidity indicating the optimum temperature and RH required for urediospore germination of *H. vastatrix* under *in-vitro* conditions. The leads from the experiments throw light on the possible infection process (epidemic) of the rust fungus in the field. It could be a favourable abiotic factor for the development of coffee rust disease, if the temperature between 22-24°C and RH 60-70% prevails under field conditions.

This is the first report from India, on evaluation of the influence of abiotic factors (temperature and relative humidity) on germination of urediospore of the coffee leaf rust disease fungus *Hemileia vastatrix.*

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