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Factors Affecting Growth and Sporulation of *Alternaria brassicicola*

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The conidia of *Alternaria brassicicola* were nutrient independent and 100% germination occurred in distilled water. Arabinose, free amino acids and aqueous extracts of non-host plant leaves were inhibitory to the germination of the conidia. The germination of conidia was high at high RH (%) values, neutral pH and at temperature range of 25-35°C. Germination of conidia was 100% irrespective of intensity and quality of light conditions. Significant percentage of germination of conidia, however, occurred at RH values 48% or less and at osmotic stress conditions of -15 bar. The germination of conidia showed high tolerance to allyl-isothiocyanate present in the seeds of oil seed brassicas.

Key words : *Alternaria brassicicola*, Leaf spot, Oil seed brassicas, Spore germination, Nutritional factors, Environmental factors, Allyl-isothiocyanate.

INTRODUCTION

Leaf spot incited by *Alternaria brassicicola* (Schw.) Wiltshiree is a major disease of cruciferous oilseed and vegetable crops in West Bengal (Vasudeva, 1958 ; Mukherjee, 1982). In the plains of India including West Bengal cruciferous oilseed crops such as mustard (*Brassica juncea*), brown sarson (*B. campestris* var. *Brown Sarson*), and yellow sarson (*B. campestris* var. *Yellow Sarson*) are cultivated as *Rabi* crops in winter. The widespread occurrence of *A. brassicicola* all over India indicates successful adaptation of the pathogen despite cool temperature and low moisture conditions in air and soil during the winter months.

Nutritional and certain environmental factors optimal for growth and sporulation

of the pathogen had been studied earlier (Changsri and Weber, 1963). Temperature and humidity conditions optimum for the germination of the conidia of the organism have also been reported (Sarkar and Sen Gupta, 1978). Epidemiological studies concerning the germination of the conidia of the pathogen are insufficient. The objectives of this work were to examine the nutritional and environmental factors affecting the germination of conidia of the pathogen.

MATERIALS AND METHODS

A virulent culture of *Alternaria brassicicola* was locally isolated from infected leaves of brown sarson (*Brassica campestris* var. *Brown Sarson*). The organism was maintained on potato-dextrose agar slopes at 20°C with monthly subculturing. The organism produced abundent conidia when grown on PDA in Petridishes and incubated at 28°C in the derk. Conidial suspensions were prepared following the method of Tuite (1969) and adjusted to 10^6 conidia/ml using a haemocytometer.

Drops of spore suspension were placed in cavity slides with or without different treatments and incubated in moist chambers. After 24 hrs percentage germination of conidia were determined. All procedures were conducted in aseptic conditions.

Relative humidity chambers were prepared using aqueous glycerin solution following the method of Johnson (1940). For pH studies buffer solutions were prepared following Gomori (1955). For water stress conditions, PDA plates were prepared by mixing required amount of mannitol with PDA (w/v) or water to obtain germination media with different water potentials of -5, -15, -25, or -50 bars, For the study of nutrient effects, aqueous solutions of different concentrations of carbon and nitrogen nutrients and host extracts were prepared, sterilized and used.

RESULTS AND DISCUSSION

Spores were exposed to different concentrations of carbon, nitrogen and combination of carbon and nitrogen sources. From the results (Table 1) it is evident that the spores of the pathogen were nutrient independent since the washed spores germinated 100% in distilled water. It is also apparent that the presence of test carbohydrates except arabinose, inorganic or organic nitrogen sources in the germination medium had little or no effect on the germination of the conidia of the pathogen. The monosaccharide arabinose and all the test

amino acids were significantly inhibitory. Asparagine and urea were not inhibitory to the germination *in vitro*.

Table 1 : Effect of carbon and nitrogen sources on germination of conidia of *A. brassicicola*

Nutrients used	Germination[a] (%) in concentration 0.1%	0.5%	1.0%
Arabinose	34.70	33.20	11.70
Glucose	95.30	95.50	92.30
Galactose	100.00	100.00	100 00
Maltose	64.50	86.20	85.70
Starch	100.00	94.50	92.60
Sodium nitrate	100.00	98.90	94.50
Ammonium sulphate	97.90	98.30	95.30
Glycine	74.40	42.87	39.35
Valine	50.00	39.50	43.07
Glutamic acid	46.15	45.30	68.58
Asparagine	100.00	97.58	95.50
Methionine	42.10	38.40	17.80
Peptone	100.00	83.07	96.10
Urea	92.92	100.00	—
Water control	100.00	100.00	100.00

[a] Percentage germination was based on slide germination bioassay technique.

Combination of 0.5% solution of carbohydrates with 0.1% solution of nitrogen sources did not have any affect on germination. Germination of conidia in all combinations of carbon and nitrogen sources was 100%.

Extracts of different plant parts of host and non-host plants were tested. Results (Table 2) showed that the extracts from the leaf of rice or maize were significantly inhibitory to the germination of conidia of the pathogen.

Table 2 : Effects of host and non-host plant extracts on germination of conidia of *A. brassicicola*

Plants	Germination (%) in extracts[a] of Root[b]	Stem	Leaf	Seed
Yellow sarson	85.05	83.90	100.00	100.00
Brown sarson	100.00	100.00	100.00	100.00
Raddish	92.70	77.70	100.00	95.00
Rice[c]	—	—	63.02	—
Maize[c]	—	—	63.20	—

[a] Tissue sample (10 g) was extracted with 100 ml water using a mortar and a pestle.
[b] Root, stem and leaf samples were obtained from 7-day-old seedlings.
[c] Only leaf tissue extracts were used.

It is apparent that nutritional condition of the germination fluid play a significant role in the germination of the conidia of the pathogen. Thus, pentose sugar, amino acids and extracts from non-hosts were inhibitory to the germination. This observation was further supported by histopathological data. Spores placed on non-host plants like beans and rice germinated poorly under the green house conditions (results not shown here).

Effects of different temperatures on germination indicated that optimum temperature for germination of conidia was 25-35°C and germination was 100% at these temperatures. The results of this work are in confirmation of earlier workers (Changsri and Weber, 1963 ; Sarker and Sengupta, 1978).

In all the light regimes such as fluoresent white light, continuous dark, intermittent light and darkness of 12 hrs each and the different spectra of light such as violet (390-422 nm), blue (422-492 nm), green (492-535 nm), orange (535-647 nm), and red (647-760 nm), germination of conidia was 100%. Although the variation in the wavelengths of light is known to cause significant effects on the germination behaviour of several test fungi (Sussman, 1967). Results of this investigation clearly indicated that spectral variation of light did not have any effect on the germination of conidia of *A. brassicicola* and *in vitro*.

Spore suspensions were adjusted to pH 3, 4 and 5 with citrate buffer, to pH 6, 7 and 8 with phosphate buffer and to pH 9 with tris buffer. Percentage germination was calculated after 24 hrs. Results showed that (Figure 1) germination was poor at pH values less than 6 and higher than 8, the optimum was at pH 7.

There is an early report (Changsri and Weber, 1963) that optimum growth of the organism occurred at pH 7.1. The present study further indicates that the germination of the conidia of the organism is also favoured at neutral pH (pH 7). Both acidic and alkaline pH conditions were not conducive for spore germination of the pathogen.

Results concerned with the effect of different RH (%) conditions (Figure 2) showed that germination was good at high RH (%) values, but significant germination (58%) occurred at RH values 48% or less. Apparently the organism can germinate at less moisture conditions also.

Percentage germination was determined in different stress solutions prepared by using different concentration of aqueous mannitol. Results (Figure 3) showed that decrease in water potential of the germinating medium caused significant decrease of the germination of the conidia. At -15 bar the germination was approximately 50% and at -50 bar germination was completely inhibited.

It is apparent from the result that the organism favours high relative humidity

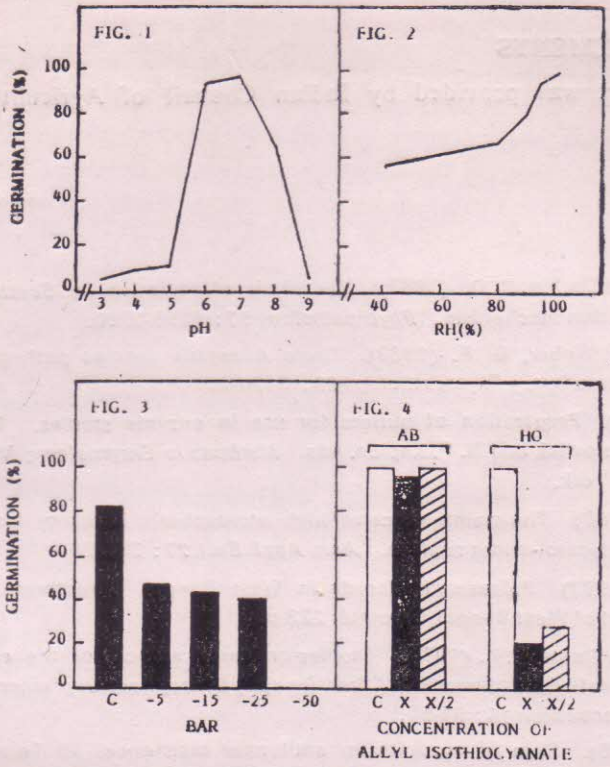

Fig. 1. Effect of *p*H values of the germination fluid on the percentage germination of conidia of *A brassicicola.*

Fig. 2. Effect of Relative Humidity on the percentage germination of conidia of *A. brassicicola.*

Fig. 3. Effect of medium with different osmotic potential on the percentage germination of conidia of *A. brassicicola*.

Fig. 4. Germination of conidia of *A. brassicicola* (AB) and *H. oryzae* (HO) in presence of water (C) and different concentration of allyl isothiocyanate (X or X/2).

and 0 to -5 bar for optimum germination. However, when the moisture level was less than the optimum conditions, the organism showed significant percentage of germination. The nearly 50% germination of the conidia even under water stress conditions (-15 or -25 bar) clearly indicated good adaptation of the organism to dry atmospheric conditions and stress.

The effects of toxic matabolites such as allyl-isothiocyanate commonly present in the brassica cells on the germination of conidia were investigated. The water extracts of mustard oil containing allyl-isothiocyanate was serially diluted and tested on the germination of conidia of *H. oryzae* and *A. brassicicola.* Results (Figure 4) indicated that water extracts of mustard oil containing allyl-isothiocyanate were not inhibitory to the germination of *A. brassicicola* but were inhibitory to the germination of *H. oryzae.* The data suggested good adaptation of *A. brassicicola* to the toxic metabolities present in the cell saps of brassicas. Tolerance to the constitutive toxic biochemicals of the host by several phytopathogens have been reported earlier (Turner, 1961 ; Arneson and Durbin, 1967 ; Schonbeck, 1967 ; Schlosser, 1975).

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REFERENCES

Arneson, P. A., and Durbin, R. D. (1967). Hydrolysis of tomatine by *Septoria lycopersici* : A detoxification mechanism. *Phytopathology* 57 : 1258-1260.

Changsri, W., and Weber, G. F. (1963). Three *Alternaria* species pathogenic on certain cultivated crucifers. *Phytopathology* 53 : 643-648

Gomori, G. (1955). Preparation of buffers for use in enzyme studies. Pages 138-146. *In* : S. P. Colowick and N. P. Kaplan. eds. *Methods in Enzymology*, Vol. I, Academic Press, New York.

Johnson, C. G. (1940). The maintenance of high atmospheric humidity for entomological work with glycerol-water mixture. *Ann. Appl. Biol.* 27 : 295-299.

Mukherjee, D. K. (1982). Pulses and Oilseeds in West Bengal. Directorate of Agriculture, Government of West Bengal, Calcutta. 223 p.

Sarkar, B., and Sen Gupta, P. K. (1978). Studies on some aspects of the epidemiology of *Alternaria* leaf blight of mustard (*Brassica* sp.) *Beitrage zur tropischen Landwirtschaft und Veterinarmedizin* 16 : 91-96.

Schlosser, E. (1975). Role of saponins in antifungal resistance. III Tomatin dependent development of fruit rot organisms on tomato fruits. *Z. Pflanzenkrankh. Pflanzenschutz* 82 : 476-484.

Schonbeck, F. (1967). Untersuchungen uber Bluteninfektionen V. Unter-suchungen an Tulpen. *Phytopathol. Z.* 59 : 205-224.

Sussman, A. S. (1976). Activators of fungal spore germination. Pages 101-137. *In* : D. J. Weber and W. M. Hess. eds. *The Fungal Spore, Form and Function*. John Wiley and Sons Ins. U. S. A. 895 p.

Tuite, J. (1969). *Plant Pathological Methods*. Burgess Publishing Co., Minneapolis Minn.

Turner, E. M. C (1961) An enzymic basis of pathogenic speci icity in *Ophiobolus graminis*. *J. Exptl. Botany*, 12 : 169-175.

Vasudeva, R. S. (1958). Diseases of rape and mustard. Pages 77-105. *In* : D. Singh ed. *Rape and Mustard*. Indian Central Oil Seed Committee, Gandhi Bhavan, Hyderabad.

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