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Studies on the critical factors governing the infection and disease development in groundnut

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Orissa is one of the six groundnut growing states in India and the crop has become popular and remunerative among the farmers. Diseases are major constraints to groundnut production in different parts of the state. The warm humid condition of the coastal ecosystem favours the initiation, development and subsequent spread of the disease. In the present investigation, an extensive survey was made to asses the occurrence of different minor fungal foliar diseases of groundnut. Three diseases were detected, the causal agents isolated and identified as *Leptosphaerulina crassiasca, Phoma microspora* and *Fusarium equiseti.* After identification of the casual fungi, a study was conducted on the effect of temperature, relative humidity and hydrogen ion concentration on the mycelial growth of the above three fungi. The response of the three test fungi varied when exposed to different temperatures, *L. crassiasca* and *F. equiseti* could grow best at 30°C whereas the optimum temperature requirement for *P. microspora* was 25°C. On the effect of relative humidity on growth, the growth of the three test fungi was maximum at relative humidity of 90-100 per cent. However, *F. equiesti* could grow well even at 75.1% relative humidity. The effect of pH on the growth of test fungi suggested that all the three pathogens could produce the maximum growth in terms of dry weight of mycelial mat at pH 6.0.

Key words : Groundnut, *Leptosphaerulina crassiasca, Phoma microspora, Fusarium equiseti,* temperature, pH, relative humidity

INTRODUCTION

Groundnut is most important oilseed crop in Orissa and its cultivation is widely distributed in all the districts of the state. In recent years with intensification of cultivation, occurrence of different types of diseases become a serious problem, causing substantial economic loss of the crop. Minor disease has like *Alternaria* leaf spot was found very destructive (40 to 50% damage to the foliage) in the Pubjab (Aulakh, 1969), Phoma blight causes considerable yield losses of groundnut in many countries (Porter *et al.,* 1982). Balasubramanian and Narayansami (1991) have reported that the optimum temperature and pH requirement of *Phoma microspora* in laboratory conditions were 25°C and 7.0 respectively. Systematic information is not available on the effect of temperature, pH and relative humidity on fungus causing minor diseases of irradiant in Orissa State. In this context a study has been conducted to asses the effect of the above critical factors governing the occurrence and develpment of such diseases.

MATERIALS AND METHODS

In order to determine the optimum temperature required for fungal growth of three fungi causing minor diseases in groundnut like *Leptosphaerulina crassiasca, Phoma microspora* and *Fusarium equiseti* 20 ml of the melted and sterilized PDA medium was poured into each Pertiplate and cooled down to 40°-45°C for solidification under aseptic conditions. 5 mm mycelial disc of each test fungus was cut from the growing margin of 5 day — old pure Petri plate culture by means of a sterilized cork

borer and transferred carefully to the centre of each Petri plate with the help of sterilized inoculating needle under aseptic condition. Three such Perti plates were maintained for each temperature regime and all the plates were incubated in BOD incubators at 10°, 15°, 20°, 25°, 30°, 35° and 40°C for 5 days. Linear growth by measuring the diameter in fixed directions were recorded in mm with the help of a transparent plastic scale. The colony was measured in crosswise directions and the average was calculated.

To study the optimum relative humidity for mycelial growth, the test fungi were grown at different ranges (percentage) of relative humidity (RH) maintained inside desiccator. For different percentage of RH, saturated solution of different salts as mentioned below were prepared in 250 ml of sterilized distilled water.

Relative humidity	Saturated solution of the corresponding chemical
100.0	Sterile water
90.0	Potassium nitrate (KNO_3)
84.0	Potassium chloride (KCl)
80.0	Ammonium sulphate $(NH_4)_2SO_4$
75.1	Sodium chloride (NaCl)
71.4	Sodium acetate (CH_3COONa)

The salt solutions were poured in to the bottom chamber of pre-sterilized desiccators to maintain humidity at different ranges. The radial growth of the fungus was studied by inoculating Potato dextrose agar Petri plate with 5 mm mycelial disc of 5-days old cultures of the fungus in the upper chamber of the desiccator. The Petri plates were kept opened inside the upper chamber of the desiccator and its lids were sealed with grease to maintain proper humidity. Three replications were maintained for each treatment. The radial growth of each fungus was measured after 5 days.

In order to determine the optimum pH requirement for growth of the fungi Richards medium was adjusted to different pH levels by adding standard HCl and NaOH and the measurement was made with the help of 'ELICO' digital pH meter. One hundred ml each of such medium was poured into each of 250 ml conical flasks and sterilized. The flasks were allowed to cool and aseptically inoculated with 5 mm mycelial disc of the fungus obtained with the help of a sterilized cork borer from the margin of an actively growing 5-day-old culture. Triplicates were maintained for each treatment. The mycelial mats were harvested 10 days after inoculation, duly filtered, dried in oven and weighed.

RESULTS AND DISCUSSION

Experiment was conducted to find out the optimum temperature required for the growth of three test fungi namely, *L. crassiasca, P. microspora and F. equiseti* under laboratory conditions and the mean radial growth of the fungi at seven temperature regimes are presented in Table 1.

Table 1 : Effect of temperature on mycelial growth of three test fungi

Temperature	Radial growth of the fungi (mm)		
(°C)	*L. crassiasca*	*P. microspora*	*F. equiseti*
10	8.33	11.33	9.33
15	16.33	23.66	22.00
20	26.33	8.33	51.33
25	44.33	64.00	71.66
30	59.00	56.66	81.00
35	57.66	34.33	80.66
40	49.66	20.66	70.33
C.D (P=0.05)	3.30	2.72	3.00

L. *crassiasca* exhibited maximum growth (59.00 mm) at 30°C. The mycelial growth (57.66 mm) obtained at 35°C was at par with obtained at 30°C indicating that the fungus could grow well within the temperature range of 30° to 35°C. Beyond this range, the growth rate declined. The mycelial growth of *P. microspora* was found to be maximum at 25°C beyond which it declined drastically. Likewise, the maximum growth (80.00) of *F. equiseti* was achieved at 30°C which was statistically at par with that obtained at 35°C (80.66 mm). *F. equieseti* preferred a temperature range of 30° to 35°C beyond which growth declined. This indicated the ability of the fungi to survive, grow and reproduce at wide rage of temperature, the optimum being 30°C in case of *L. crassiasca* and *F. equieseti and 25°C in case of P. microspora.* Behera *et al.* (1989)

investigated the climatic requirements of *Fusarium pallidoroseum* and found that the optimum temperature for growth was 28°C.

The mean radial growth of the test pathogens in terms of colony diameter as influenced by six different levels of relative humidity are presented in Table 2.

Table 2 : Effect of relative humidity on mycelial growth of three test fungi

Relative humidity (%)	Radial growth of the fungi (mm)		
	L. *crassiasca*	*P. microspora*	*F. equiseti*
100.0	65.00	73.33	76.33
90.0	64.33	73.33	76.66
84.5	52.00	68.66	76.66
80.0	49.66	64.33	65.66
75.1	37.33	54.00	65.00
71.4	35.66	50.66	51.33
C.D (P=0.05)	3.01	3.23	2.37

It was observed that the growth rate of the fungi increased as the relative humidity increased from 71.4% to 100%. In case of *L. crassiasca* and *P. microspora,* mycelial growth was observed almost equal at both 90 and 100% relative humidity but below 90% it declined considerably. However, *F. equiseti* exhibited similar growth from 84.5% to 100% relative humidity. At relative humidity lower than this range the growth declined. The result indicated that there was an increase in growth of the fungi when there was rise in the level of relative humidity from 71.4% to 90% and no corresponding increase in the growth when relative humidity was increased further from 90 to 100% Yen *et al.* (1956) reported that highest ascospore germination in *L. crassiasca* was found at 100% relative humidity.

Mean data recorded on dry weight of mycelial growth of the three test pathogens under the influence of seven pH levels are presented in the Table 3.

Table 3 : Effect of hydrogen ion concentration on mycelial growth of three test fungi

pH level	Dry weight of mycellial mat (mg)		
	L. crassiasca	*P. microspora*	*F. equiseti*
2.0	199.33	172.66	174.33
3.0	205.33	202.00	198.00
4.0	228.00	219.33	209.66
5.0	233.33	236.66	224.33
6.0	237.00	257.00	231.00
7.0	223.33	250.66	212.00
8.0	207.33	240.00	181.33
C.D (P=0.05)	6.55	6.76	5.09

It was observed that *L. crassiasca* preferred a pH of 6.0 for exhibiting the maximum mycelial growth (237.00 mg) which was statistically at par with that obtained at pH 5-0 (233.33 mg). Likewise, both *P. microspora* and *F. equiseti* could grow well at pH 6.0 with the resulting mycelial dry weights of 257.00 mg and 231.00 mg respectively. The growth declined steadily either on increasing or decreasing the pH levels from the optimum levels. Thus better growth and sporulation were observed at slightly acidic condition as was evident in the investigation.

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