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EFFECT OF DIFFERENT MEDIA ON GROWTH AND SPORULATION OF *CERCOSPORA ARACHIDICOLA* CAUSING EARLY LEAF SPOT OF GROUND NUT

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ABSTRACT
The pathogen of ground nut early leaf spot, *Cercospora arachidicola* was inoculated on Potato sucrose agar, Oatmeal agar, Potato dextrose agar, V8-juice agar, Leaf extract agar, Carrot juice agar and Peanut hull extract agar. Out of all the media tested, the maximum growth was supported by Potato Sucrose Agar followed by Oat Meal Agar at 23 ± 1ºC and more than 90% relative humidity. Maximum sporulation of *C. arachidicola* was obtained in Potato Sucrose Agar at 6 per cent sucrose followed by 5 per cent.

INTRODUCTION

Presently, Groundnut (*Arachis hypogaea* L.) is grown in all the six continents as an important oil and food crop in approximately 23.95 million hectares in over 100 countries. It is the third major oil seed crop of the world next to soybean and cotton. India occupies first in area (5.31 million ha) and second in production (5.77 million tons) in 2012 (FAO Statistics. 2013). In Andhra Pradesh, it is grown in 13.01 lakh ha with 0.85 m.t production. In Uttar Pradesh, it is cultivated in 0.90 lakh hectors with a production of 0.60 lakh tones and yield of 670 kg/ha in 2009-10 (Directorate of Economics and Statistics, 2013).

The major constraints of groundnut production are diseases. Among the fungal diseases of groundnut, the early leaf spot caused by *Cercospora arachidicola* is the most serious disease wherever groundnut is grown and also responsible for yield losses due to this disease could be as high as 30-50% (Subrahmanyam *et al.*, 1980, Damicone *et al.*, 1999; Mohammed, 2004). Premature defoliation can occur in severe cases and petioles and stems may also become infected (Pretorius, 2006).

Maximum germination of conidia occurred by 24-48 hrs at temperature of 16-25ºC and 100% relative humidity (Alderman and Beute. 1986). *Cercospora arachidicola* produces sporulation on unsoaked Peanut Hull Extract Agar (PHEA) than in soaked-PHEA and Potato Oatmeal Agar (Starkey 1980). Zhenglong *et al.* (2011) reports indicated that mycelia growth of *Cercospora zeae-maydis* was very slow on the media, with an average of 0.33 mm per day.

The information on growth and sporulation of *Cercospora arachidicola* is scanty due to their slow growth and sparsely production of spores. Moreover, there is no suitable medium to study the growth and sporulation of *C. arachidicola* on synthetic medium. Hence, there is need to find out a suitable medium for growth and sporulation of *C. arachidicola*. Keeping this in view, the present experiment has been carried out to optimize the conditions for growth and sporulation of *C. arachidicola* causing early leaf spot of groundnut.

MATERIALS AND METHODS

Isolation, purification and identification of the pathogen

The pathogen, *Cercospora arachidicola* was isolated from infected groundnut leaves by tissue segment method (Rangaswami and Mahadevan, 2006). The affected portion along with a portion of healthy tissue was cut and surface sterilized with 0.1% $NaHCl_2$ solution for 30 seconds followed by three changes of sterile distilled water. These leaf bits were then inoculated in a petri plate on Potato Dextrose Agar medium (PDA) using a sterile inoculation needle, incubated at 25°c and observed periodically for the growth of the fungus (Kumari *et al.*, 2013).

The culture was purified by single hyphal tip method and maintained on Potato Dextrose Agar medium by periodical

transfer throughout the present investigation for further studies. The fungus isolated from early leaf spot samples of groundnut was identified based on descriptions given by Kolte (1984) and Mc Donald *et al*. (1985).

Pathogenicity test under *in vitro* and *in vivo*

The susceptible groundnut variety "Narayani" was used for this experiment. Pathogenicity of *C.arachidicola* was proved based on modified detached leaf method under *in vitro* (Foolad *et al*., 2000) and in field condition. Under *in vitro*, the leaf bases of surface sterilized leaflets were dipped in the plastic boxes (20x10x10 cm) which contain water and sprayed with mycelial bits of *C.arachidicola* on the leaflets. The boxes were lined with sterilized absorbent cotton for maintenance of high relative humidity. The boxes were kept in an incubator at $23 \pm 1°C$ and periodically observed for development of disease symptoms.

Under field condition, pathogenicity test was conducted by atomizing the distilled water containing mycelial bits of *C.arachidicola*. After treatment, the inoculated plants were covered with a polythene cover for 5 days to maintain high humidity of > 90 per cent. Uninoculated plant served as control. The inoculated plant was examined for the development of leaf spot symptoms (Wagh *et al*. 2013).

Growth of *Cercospora* on different media

Efforts were made to study the effect of seven different media varied in the nutrient composition like Potato sucrose agar, Oatmeal agar, Potato dextrose agar, V8-juice agar, Leaf extract agar, Carrot juice agar and Peanut hull extract agar on the growth of *Cercospora arachidicola* (Zhenglong *et al* 2011, Zhang *et al*., 2001, Smith 1971, Starkey, 1980).

Effect of sucrose concentration on Sporulation of *Cercospora arachidicola* on PSA

The medium which found to be suitable for the growth of *C.arachidicola* was selected for optimization of conditions for sporulation. The Potato Sucrose Agar (200g peeled potato, 20g sucrose, 15g agar, distilled water; 1 liter) medium was supplemented with different concentrations of sucrose i.e., from 1-6% (10g to 60g). The pathogen inoculated on different concentrations of sucrose in PSA was incubated at $23 \pm 1°C$ and 90% relative humidity to observe the sporulation.

A small amount of *C.arachidicola* obtained from ten days old culture was placed on the slide and teased thoroughly with lactophenol to obtain uniform spread. A cover slip was placed over it. The length and breadth of 10 conidia were measured under compound microscope and the average sizes were calculated with the help of ocular and stage micrometer (Adhikary *et al*., 2013).

RESULTS AND DISCUSSION

The samples infected with leaf spot disease of groundnut were collected from S.V.Agriculture College Farm, Tirupati, Chittore (Dt) of Andhra Pradesh and Allahabad School of Agriculture Farm, SHIATS, Allahabad, U.P. The pathogens were isolated and maintained pure culture on PDA at 28°C. The color of the colonies was light brown to black and exhibited fried egg shape like appearance. Initially, the mycelial growth on PDA was thin, septate, light yellow in color and hypha growth was parallel to each other. Moreover, the knots like structure were observed on the hyphae of the some cells (Fig 1). The morphological characters are identical as reported by El-Ghall *et al*., 1982.

Among the seven media, the maximum growth was observed in potato sucrose agar followed by peanut oatmeal agar upto 30 days. The least growth was recorded in PDA, where there was no sign of mycelium even after 10 days of incubation. In fresh peanut hull extract agar, carrot juice agar and leaf extract agar media; the growth was slow when compared to oatmeal agar and potato sucrose agar. Based on the growth of the mycelium, the media was divided into four categories.

Growth rate	Duration (days)	Media
Fast	8-10	Potato sucrose agar, Oatmeal agar
Slow	15-20	Leaf extract agar, carrot juice agar, peanut hull extract agar
Very slow	25-30	Potato dextrose agar
Absent	-	V8 juice agar

The present study results show that potato sucrose agar found to be suitable for optimum growth of the pathogen during the periodical observations and for further investigations (Fig 2). Present findings supported the results of Zhao Zhenglong *et al.,* (2011) who reported that spores were abundantly produced in Oatmeal Agar and Potato Sucrose Agar and Abdou *et al.,* (1974) who stated that light was not required for sporulation of *C.arachidicola*. The results are not supported the work of Zhang *et al*., (2001) who stated that *Cercosporidium personatum* growth was observed on V8 juice agar.

Pathogenicity of the fungus was established by inoculation and reisolation of *C.arachidicola* from artificially inoculated groundnut plants. In the present investigation, pathogenicity test of *C.arachidicola* was studied by detached leaf method under *in vitro* and in field condition. The detached leaf method results show that circular necrotic lesions were observed on the upper leaflet surface of inoculated leaf at 8 to 10 days after inoculation of the pathogen, indicating initiation of infection. Initially the spots were smaller, mostly circular in shape, and dark brown in color with yellow halo (Fig 3a).

Under field condition, symptoms first appeared on lower leaves as small chlorotic spots on the upper leaflet surface 10 days after inoculation of the pathogen. The spots then developed into mature, sporulating lesions. The observed spots were mostly circular in shape and dark brown in color surrounded by yellow halo (Fig 3b). The pathogen was reisolated on PSA medium from the lesion area and was found to be the same as the original culture thereby proving the Kotch's postulates.

Table 1: Effect of sucrose percentage in PSA on sporulation of *Cercospora arachidicola*

S.No.	Percentage of PSA	Average Number of conidia/microscopic field*	Conidial measurement (μm) Length (μm)	Width (μm)
1	1%	5.2	25-57.5	2.5-5.0
2	2%	6.4	25-62.5	2.5-5.0
3	3%	7.2	30-75	2.5-5.0
4	4%	11.3	30-82	2.5-5.0
5	5%	17.6	36-92	2.5-5.0
6	6%	21.8	50-105	2.5-5.0

* Average of 10 microscopic fields (40 xs) in each of three replication.

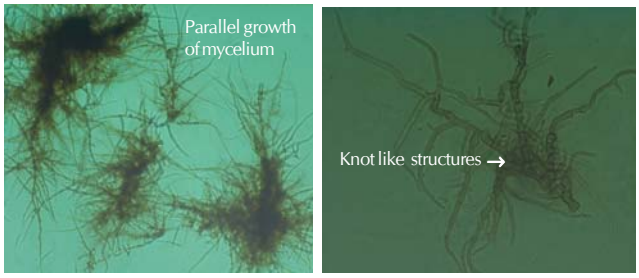

Figure 1: *Cercospora arachidicola* mycelium on PSA medium

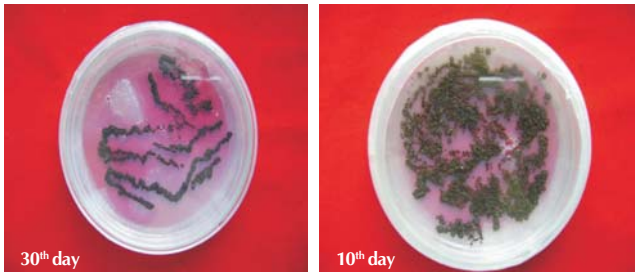

Figure 2: Growth of *Cercospora arachidicola* on potato sucrose agar

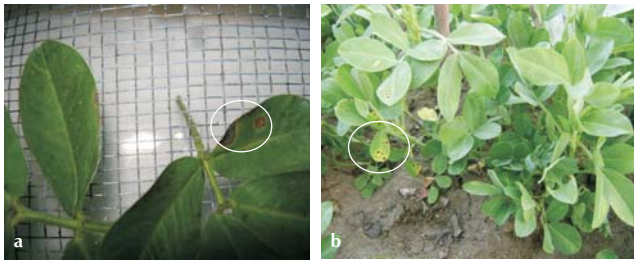

Figure 3: Pathogenicity test of *Cercospora arachidicola* by (a) detached leaf method under *in vitro* and (b) under *in vivo* on susceptible groundnut variety "Narayani"

The results show that the pathogen produced symptoms within 7-8 days of inoculation. Subrahmanyam *et al.*, (1990) used similar method to study the pathogenicity of *C.arachidicola* on groundnut plants in which they used conidial suspension. Kishore *et al.,* (2005) used harvested conidial suspension of *C.arachidicola* from a single-lesion culture maintained on detached groundnut leaves for artificial inoculation of 30 day old ground plants. In the present study, result indicated that the mycelial bits of *C.arachidicola* can also be used for artificial inoculation of groundnut plants in future. This may be the pioneer work proving the pathogenicity with mycelial bits and as such it is highly useful for the pathogens like *C.arachidicola* in which sporulation is rather difficult.

The PSA medium was selected for optimization of conditions for sporulation, since it was proved to be the best among the different media tested under present investigation. The isolated *Cercospora* isolate was further used for optimization of conditions for sporulation. These results show that the pathogen sporulated in 7 to 10 days at 1 to 6% concentration of sucrose in PSA at 23 ± 1°C and more than 90% relative humidity. The same results are also reported by El-Ghall *et al.*, (1982) that maximum sporulation was obtained at 7-10

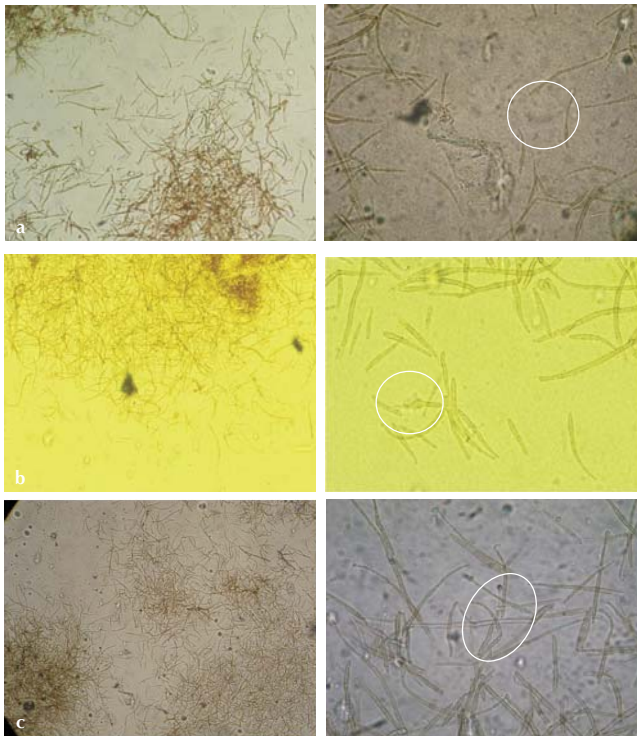

Figure 4: Sporulation of *Cercospora arachidicola* at (a) 1% PSA (b) 3% PSA and (c) 6% PSA media under 10x and 40x

days with *C.arachidicola*. Khare *et al.,* (2012) stated that the best growth and sporulation of *C. zea-maydis* was also observed at the temperature of 25°C.

Effect of sucrose percentage on sporulation of *C.arachidicola* revealed that the maximum sporulation was observed at 6% being highly prominent and significant as compared to other concentration ranges. The isolate showed minimum sporulation at 1%, which subsequently increased at 2%, 3%, 4%, 5% and 6% (Fig. 4) and gradually declined after 6%. The conidia of *C.arachidicola* measure an average length of 25-65 μm and 2.5-5.0 μm in breadth (table 1). However, maximum sizes of the conidia were observed at 6% PSA. The PSA medium supplemented with 6% sucrose not only supported the sporulation but also the size of the conidia was bigger. The maximum size of the conidia in 6% sucrose concentration was 50-105 μm length and 2.5-5.0 μm width. The least size was observed in 1% sucrose. The conidia are subhyaline or pale yellow, obclavate with 4-12 septa and often curved at the tips. Conidial size and shape of the pathogen is more or less similar to the work of Jenkins (1938) who made the conidial measurements of *C.arachidicola*.

In conclusion, the study revealed that *Cercospora arachidicola* can be grown and sporulated maximum in 6% PSA at 23 ± 1°C and > 90% R.H.

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