Study of symptoms, cultural characteristic and isolation of *Cercospora* species causing tikka disease of ground in Ranchi district of Jharkhand

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Abstract
Groundnut (Arachis hypogea L.) is one of the most popular oilseeds crops widely grown in India. This crop is severely affected by leaf spot disease caused by Cercospora species leading to brown to black coloured spots on the leaves which hinder the market quality of the groundnut. The spotting is due to the attack of Cercospora personata (Berk. and Curt.) Ell. and Ever. and Cercospora arachidicola Hori. The present study concentrated on the study of symptoms, cultural characteristic and isolation of Cercospora species causing tikka disease of ground.

The first visible symptoms appeared on the leaflets of lower leaves as dark spots which at a later stage, were surrounded by yellow rings. Cultural studies of both Cercospora species grown on PDA media showed that potato dextrose agar is good for the growth. Both the fungus Cercospora arachidicola and Cercosporidium personatum showed quite faster growth in the media used and comparatively same radial growth on potato dextrose agar. However, fungi failed sporulate on the medium. Temperature requirement of the fungus was found 25°C where good growth was observed.

Keywords: *Arachis hypogeae*, *Cercospora*, symptom study, isolation, cultural characteristic.

Introduction

Groundnut or peanut (*Arachis hypogea*) originated in South America. It is grown throughout the tropical, subtropical and warm temperate region of the world. It is one of the important oilseeds crops in the world often known for its global economic significance not only for its wide spread distribution but also for the even wider areas of processing and consumption[1]. The edible oil economy of India is primarily dependent on groundnut which occupies approximately 45 percent of total cropped area and contributes nearly 55 percent of total major oil seed production.

Even though, in India, ground nut is being cultivated under large area, the potential yield has still not been achieved. There are several constraints which contribute to the low productivity of the crop. The most important constraints in the production of the crop are the wide spread occurrence and severity of several diseases and insect pests[2]. The groundnut crop suffers from many diseases caused by fungi, bacteria, viruses and nonparasitic diseases. Among the fungal diseases, rust (*Puccinia arachidis*), early leaf spot (*Cercospora arachidicola*), late leaf spot (*Cercosporidium personatum*) are more prevalent and destructive in nature[3]. The early and late leaf spot diseases are together referred as tikka disease of groundnut. Tikka disease of groundnuts is caused by two species of Cercospora: *Cercospora personata* (Berk. and Curt.) Elle and Eve., now known as *Cercosporidium personatum* (Berk. and Curt.) Deighton and *Cercospora arachidicola* Hori. The two form-species differ from each other with respect to the size, shape and colour of necrotic lesions they produce, conidia formation and the nature of the mycelium.

The tikka disease damages the plant by reducing the total available photosynthetic area by lesion formation and by stimulating leaflet abscission. This disease of groundnut is very destructive on a worldwide scale as evident from maximum yield losses ranging from 10 to 50 percent. Without the foliar application of fungicides, the disease could cause up to 100 percent defoliation prior to harvest and losses in excess of 50 percent of potential yield. But the loss varies from locality to locality and also between seasons.

In India, losses up to 70 percent yield due to combined infection of rust and leaf spot pathogen have been reported. The combined infection of rust (*P. arachidis*) and leaf spots (*C. arachidicola* and *C. personatum*) causes losses up to 52.65 percent in yield, 27.25 percent in 100 kernel weight and 45.76 percent in dry weight. In addition to losses in yield, this disease also affects the yield and quality of haulm which is nutritious fodder.

Tikka disease of groundnut is widely studied disease in the world. And a number of management approaches viz. development of partial resistant varieties, cultural practices, application of fungicides, biological control measures, were evaluated and recommended[4]. In spite of all these measures, the tikka disease is still a big constraint in the production of groundnut.

Distribution and Economic Importance: Groundnut (*Arachis hypogea* L.) also known as peanut or earthnut or money nut is a member of family Papilionaceae, largest and most important of the three divisions of Leguminosae. The botanical name of groundnut, *Arachis hypogaea* L. is derived from two Greek words, *Arachis* (arachos) meaning a 'weed' and *hypogaea* meaning 'below ground'. According to botanists, a more popular name for groundnut would be

ground pea because groundnut is a pea and not a nut. The term 'nut' has perhaps been added, since the pea has a shell and flavour similar to the shells of many true nuts[5]. It is native to South America, originated between Southern Bolivia and Northern Argentina, from where it spread throughout the new world.

Groundnut was introduced in India by around 16th century by the Portuguese. It is grown under a wide range of environmental conditions encompassing latitudes between 40° South and 40° North of the equator. In India, it is grown over an area of 8 million ha with an annual production of 7.5 million tonnes. There are a few economically important foliar fungal diseases such as early and late leaf spots, commonly called as tikka diseases and groundnut rust which are commonly present wherever groundnut is grown. As the area under groundnut is pre-dominant in kharif (rainy) season (81%), the foliar diseases like late leaf spot and rust may cause yield losses up to 50% in the semi-arid tropics. In India, late leaf spot is more severe than early leaf spot[6]. It causes severe defoliation and reduces pod yields by more than 50% if the crop is not protected with chemicals. The fungicides are the most common tools for controlling groundnut (*Arachis hypogea* L.) diseases.

Disease Cycle: Primary infection takes place by soil borne conidia which is in the soil and on seeds in the shells. The spread of the disease during the season is by means of wind disseminated conidia. Penetration is directly through the epidermis and also by way of stomata. Chiefly it occurs through the upper epidermis. However, there is possibility of infection through both the leaf surfaces.

Material and Methods

Symptomatology and causal organism: An attempt was made to identify the early leaf spot and late leaf spot symptoms caused by *Cercospora arachidicola* and *Cercosporidium personatum* respectively. The groundnut sown in the farm was regularly visited for the identification and characterization of the symptoms of the tikka disease caused by the two pathogens. The growth of the pathogen was also measured in accordance with respect to time (table 1). The disease severity (%) and parameters corresponding to the sowing date were recorded (table 2). In the beginning, small chlorotic spots were noticed on the leaves around 30 to 35 days after sowing. Due to excessive spotting on leaves, there was gradual weakening of the foliage, which later resulted in defoliation. First symptoms of both the leaf spots were the appearance of pale areas on the upper surface of older leaves. As the lesions developed, the two species were distinguished. The infection sites on the shrivelled leaves are excellent sources of conidia for the airborne dispersion of this organism whenever the relative humidity is high. Our isolate was obtained from a site of this type.

Isolation of the fungi, Isolation of the pathogen (*C. personatum* and *C. arachidicola*) and culture study: The isolates used in this study were obtained from infected leaves of the groundnut, variety "local spreading" grown in Ranchi district of Jharkhand. The leaves of groundnut showing typical symptoms of early and late leaf spots caused by *Cercospora arachidicola* and *Cercosporidium personatum* respectively were collected from the farm. Standard tissue isolation method was followed to obtain *Cercospora arachidicola* and *Cercosporidium personatum* from the infected ground nut leaves showing the typical leaf spot symptoms. Identification of the fungus was made based on the morphological characters of the isolated fungus.

Round the research period, following steps were carried out with a view to isolate the *C. personatum* and *C. arachidicola*, the causal organism of tikka disease of groundnut. Infected leaf of *Arachis hypogea* was brought to the laboratory from the field for the isolation of the pathogen. The infected leaf was then washed thoroughly in running tap water and the infected part was removed by a scissor or knife and quickly transferred to the sterilising solution.

Then the excised leaf segment was placed aseptically into the prepared slant for culture of the pathogen. The slant was then incubated at a required temperature for 3-5 days.

Basal medium used for growth and sporulation of the fungus was PDA (Potato Dextrose Agar) media having composition peeled and sliced potato 100 g, dextrose 10.00 g, agar-agar 10.00 g and water to make 1000 ml. The media was sterilized at121°C (15lb) for 15 min. To carry out the study, 20 ml of the medium was poured in Petri plates. Such Petri plates were inoculated with 5 mm mycelial disc cut from periphery of actively growing culture and incubated at 25°C. Each treatment was replicated thrice. Observations were taken when both the fungus covered complete Petri plate in the media. The cultural characteristics of both the fungi were studied on the media potato dextrose agar.

The cultural characteristics like colony characters, colour of the colony and kind of mycelium on both the fungi were observed. The colony diameter were recorded as well as margin was also recorded. The data on radial growth were analysed. Thirty ml of the medium was added to each of 100 ml flasks. All the flasks were sterilized at 121° C (15lb) for 15 min. Inoculum disc of 5 mm size was inoculated to all flasks and incubated at 27±1°C for 16 days. Each treatment was replicated thrice. Mycelial discs measuring 5 mm were taken from culture plates and inoculated into potato dextrose agar. For each treatment, three replications were maintained. Isolation was repeated several times to obtain the pure culture. The pure culture was maintained in the slant and also was preserved for further use.

Results and Discussion

The results of the investigation undertaken caused by *Cercosporidium personatum* and *Cercospora arachidicola* during the study period are presented as follows:

Symptomatology and Causal Organism: Figure 1 shows healthy plant leaves of *Arachis hypogeae* prior to occurrence of tikka disease. Figure 2 and figure 3 are showing leaves with early symptoms and severely infected leaves of *Arachis hypogeae* from tikka disease of ground respectively. Symptoms appeared when the plants were 1-2 months old. Due to excessive spotting on leaves, there was gradual weakening of the foliage, which later resulted in defoliation. In the beginning, small chlorotic spots were noticed on the leaves around 30 to 35 days after sowing. First symptoms of both the leaf spots were the appearance of pale areas on the upper surface of older leaves.

As the lesions developed, the two species were distinguished. These spots in *C. arachidicola* later developed into black lesions on the upper surface and were surrounded by chlorotic halo whereas the corresponding lesions on the lower surface exhibited light brown colour. The yellow halo was not found on the lower leaf surface. In case of late leaf spot caused by *C. personatum*, lesions were smaller, more in number, almost circular and darker in colour than those of *C. arachidicola*. On the abaxial surface, the lesions were tan or carbon black and slightly rough in appearance.

The lesions were also observed on petioles, stem and pegs. The lesions were oval, elongate and with more distinct margins than the leaflet lesions. In severe cases, affected leaflet became necrotic, coalesced, leading to shedding of leaves. In very severe conditions, extensive defoliation was noticed. Conidiophores of *C. arachidicola* were brown, continuous or 1 to 2 septate, unbranched and geniculate. The stromata were dark brown. Conidia were hyaline, pale yellow, obclavate. In case of *C. personatum*, the conidiophores were brown and continuous, sometimes septate, unbranched and geniculate on the black stroma. Conidia were obclavate to cylindrical, light coloured, with bluntly rounded end.

***C. arachidicola*:** The leaf spots were comparatively larger in size, irregularly circular in outline and 4-10 mm in diameter. The leaf spots were often confluent and necrotic lesions occurred on both the surfaces. Those on the upper surface were reddish brown to black and on the lower surface were light brown. There was a yellow halo around each spot but halos on the lower surface were less distinct. It had both external and internal hyphae.The internal hyphae were both intercellular and intracellular. The haustoria was absent. The unbranched conidiophores arise scattered from a deep brown stroma (25-100 p in dia.).

They were geniculate, yellowish brown and 15 to 45 p long and 3-5 p broad and were either aseptate or septate with 1 to 2 septa. The conidiophores usually occurred on the upper surface of the host leaf but occasionally was found on the lower surface as well and thus described as amphigenous. They are sparse and do not occur. The conidia were obclavate hyaline or pale yellow to slightly olivaceous, 38-108µ long and 6µ broad. They were septate with 4-12 septa and have rounded to distinctly truncate base and sub-acute apex.

***C. personatum*:** Leaf spots were small, more circular and about 1-6 mm in diameter. The necrotic lesions appeared on both the leaf surfaces and changed from dark brown to dark in colour. Young spots lacked bright yellow halo which, however, developed around the older ones. The mycelium consisted of hyphae which were entirely internal. The septate hyphae ramify in the intercellular spaces and obtain nutrition by sending branched haustoria into the mesophyll (both spongy and palisade) cells. They were geniculate, olivaceous brown, 24-54 p long and 5-8 µ broad. The unbranched conidiophores arose in tufts from a dense, globular and brown to black stroma (23-30µ in dia.) and were either aseptate or septate with 1-2 septa.

The conidiophores were confined to the lower surface of the host leaf and are seen arranged in concentric circles in the tuft. The conidia were obclavate or cylindrical, light coloured, 18-60 µ in length and 6-11 µ in breadth. They were septate with 1-7 septa and had bluntly rounded ends. The symptoms caused by the two pathogens also differed. The disease is also called leaf spot of groundnut because it causes spots on leaves.

Earyl leaf spots symptoms (by *Cercosporidium personatum*): Formation of circular, irregular reddish brown lesions was bigger on lower surface. The lesions were surrounded on the upper leaf surface by a narrow yellow halo. The lesions were approximately (1-10mm) in diameter.

Late leaf spots symptoms (*C. personatum*): Leaf spots were mainly circular and darker than early leaf spots. They rarely had haloes and were abundant on leaves. Spots were smaller (1-6mm) in diameter.

Figure 1: Healthy plant leaves of *Arachis hypogeae* prior to occurrence of tikka disease.

Figure 2: Leaves of *Arachis hypogeae* showing early symptoms of tikka disease.

Figure 3: Severely infected leaves of *Arachis hypogeae* from tikka disease of ground.

Table 1
Showing growth of the pathogen in accordance with respect to time.

Species	Size of Spots / Lesions (mm)				
	1st Week	4th Week	6th Week	8th Week	10th Week
Cercospora arachidicola	0.00	1.50	3.25	6.08	8.10
Cercosporidium personatum	0.00	0.00	2.70	2.80	4.80

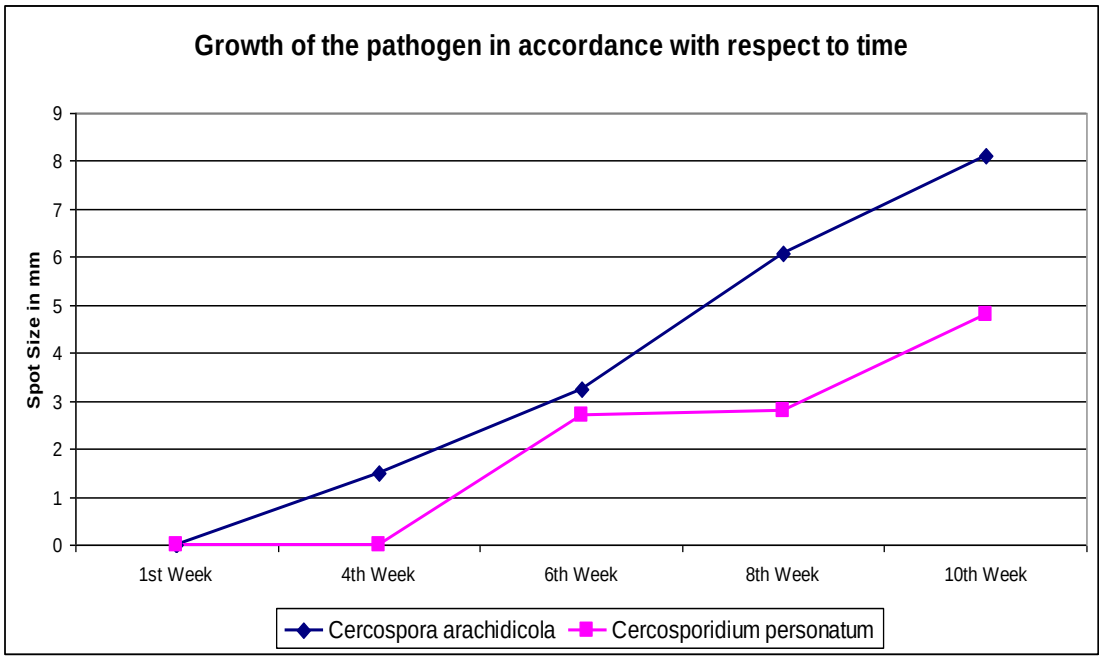


Table 2
Showing Disease Severity (%) and whether parameters corresponding to the sowing date

Days after sowing	Disease Severity in %	Max. Temp °C	Max. Relative Humidity	Rainfall
30 Days	20	25 °C	54%	No
60 Days	45	23 °C	72.67%	Heavy
80 Days	70	23 °C	70%	Little

Causal Organism: On the basis of morphological and cultural features it was concluded that the causal organism was *Cercospora* species. Morphological characteristic of both species had been described as follows:

Some characteristics of both the pathogen

Characters	*Cercospora arachidicola*	*Cercosporidium peesonatum*
Mycelium	Intra-cellular	Inter-cellular
Haustria	Absent	Present
Conidiphores	1 – 2 Septate	Aseptate
Conidia	1 – 2 Septate	3 – 4 Septate

Isolation of the fungi and Culture Studies: Standard tissue isolation technique was followed to obtain the pathogen from the infected groundnut leaves showing typical tikka leaf spots symptoms. However, fungi failed sporulate on the medium. Growth characters of both the fungi were studied. Consequently, a comparison was made of the type of culture produced by both species of cercospora on potato dextrose agar (PDA) media. The cultural characteristics like colony characters, colour of the colony and kind of mycelium were observed. Figure 4 illustrates the difference in colony type and diameter after 16 days incubation at 22 C. The colony of *C. arachidicola* was whitish brown with concentric rings and has raised outgrowth at the centre with regular margins. The mycelium was fluffy. In the case of *Cercosporidium personatum* the colony on potato dextrose agar was deep pink with regular margins having concentric rings. The mycelium was slightly fluffy.

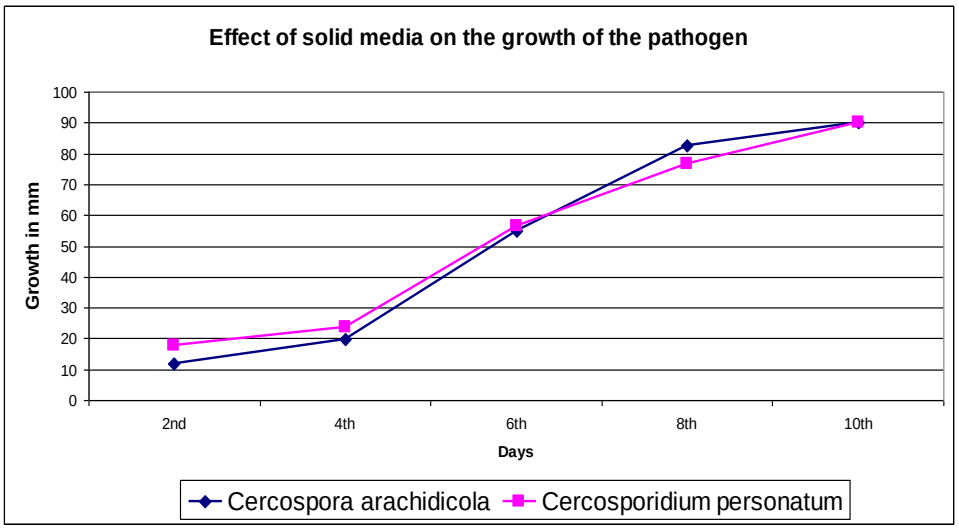
The above data also revealed that the growth of both the fungus *Cercospora arachidicola* and *Cercosporidium personatum* showed quite faster growth in the media used and comparatively same radial growth. In case of *Cercospora arachidicola*, the maximum growth of 90.00mm was recorded on 16th day of intubation on potato dextrose agar. In case of *Cercosporidium personatum*, maximum growth of ±90.00 mm was recorded on 16th day of incubation in potato dextrose agar media.

Media	Cultural characteristic	
	Cercospora arachidicola	*Cercosporodium personatum*
Potato dextrose agar media	Colony is purplish brown with concentric rings and has raised growth with regular margin	Colony is deep pink with regular margin having concentric rings. Mycelium is slightly fluffy.

Figure 4: Showing cultural growth of the pathogen in PDA media.

Table 3
Showing effect of solid media on the growth of the pathogen

Species	Radial Growth (mm) in Days				
	4th	6th	8th	12th	16th
Cercospora arachidicola	11.67	19.67	55.00	82.67	90.00
Cercosporidium personatum	17.67	23.67	56.57	76.67	90.00



Severe intensity of infection leads to a reduction in yield up to 22%. The disease caused by *Cercospora arachidicola* as early spot and caused by *Cercosporidium personatum* as late spot. The symptoms caused by the two species on groundnut and were neither the shape of the lesion nor the period of appearance of the halo is reliable criterion for distinguishing the spots produced by *Cercosporidium personatum* and *Cercospora arachidicola*. A reliable criterion which serves to easily identify the spots produced by two pathogens, is the colour of the spot in the lower surface which is light brown in the case of *Cercospora arachidicola* and carbon black in the case of *Cercosporidium personatum*. Two water soluble, nonspecific phyto toxic glycopeptides from *in vitro* and *in vivo* condition. The toxins inhibit germination of seeds of several crop plants. Nath et al[22] had stated that the intensity of sitting due to cercospora spp. decreased as the sowing was delayed. The disease caused by *Cercospora arachidicola* was characterized as early spot and caused by *Cercosporidium personatum* as late spot.

Conclusion

One or both the imperfect stages *Cercospora arachidicola* and *Cercosporidium personatum*, of these two species occur in all the main groundnut growing areas of the world depending of type of groundnut grown and also the weather conditions. Losses from the disease have been reported from 20% to 50% but can be up to 70% if the tikka disease is associated with rust (*Puccinia arachidis*). Symptoms appear when the plants are one or two months old. Due to excessive spotting on the leaves, there is gradual weakening of the foliage, which results in defoliation and consequently smaller nuts are formed.

Cercospora arachidicola forms irregular reddish brown lesions which are bigger on the lower surface on leaves of groundnut. The lesions are surrounded by yellow halo. *Cercosporidium personatum* has darker brown to black leaf spots which are smaller in diameter and almost circular. *Cercosporidium personatum* is potentially more damaging as it produces more spots. The future prospect of the study is to find out the bioremediation of these pathogens.

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