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Isolation, Identification and Pathogenicity of Collar Rot of Groundnut (*Arachis hypogaea*) Caused by *Aspergillus niger* van Tieghem

Govind Junjadia[1]*, J.R. Verma[2], Dama Ram[3], M.M. Kumawat[4], Mithilesh Kumar[5] and Surendra Kumar[6]
[1]Research Scholar, Department of Plant Pathology, CoA, Agricultural University, Jodhpur (Rajasthan), India.
[2]Professor, Department of Plant Pathology, CoA, Agricultural University, Jodhpur (Rajasthan), India.
[3]Assistant Professor, Department of Plant Pathology, CoA, Agricultural University, Jodhpur (Rajasthan), India.
[4]Professor, Department of Entomology, CoA, Baytu, Agricultural University, Jodhpur (Rajasthan), India.
[5]Associate Professor, Genetics and Plant Breeding, ARS, Mandor (Rajasthan), India.
[6]Associate Professor, Department of Agronomy, CoA, Agricultural University, Jodhpur (Rajasthan), India.

(Corresponding author: Govind Junjadia)*
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ABSTRACT: Groundnut (*Arachis hypogaea* L.) is an economically important legume crop in India and many other countries worldwide. Groundnut cultivation is affected by many biotic and abiotic stresses. Among biotic stresses, groundnut is attacked by many fungal, bacterial and viral pathogens. The most harmful fungal diseases are Collar rot, stem rot, rust and other soil borne diseases. Collar rot caused by *Aspergillus niger* is one of the most important disease of groundnut extensive in India and worldwide. In this study, symptomatic infected plant samples were collected from different locations in Rajasthan, Isolated fungal species were identified on the basis of morphological characterization. The groundnut plant had common symptoms, including the first appearance of seedling rotting in the cotyledon and hypocotyl areas following germination, followed by drying and wilting of lateral branches and whole plant died. Microscopic examinations of the temporary mounts prepared from *Aspergillus niger* affected groundnut plant specimens and pure culture of *A. niger* revealed mycelia were septate, hyaline and conidiophores were long with spherical vesicles at the apex, conidia were globose, brown to black in color. The pathogenicity of A. niger was evaluated under pot house circumstances on the GJG-19 variety of groundnut using seed and soil inoculation methods, thereby confirming Koch's Postulate. The artificial inoculated plants exhibited pre-emergent rot of seed and post-emergent rot with appearance of a circular, brownish spot on the cotyledons. The discolored area rapidly became soft and rotten and spread on to the stems and hypocotyls which become yellow, soft, rotten and collapse. Greyish white mycelium and black fructifications of the pathogen appeared on the surface of the affected parts.

Keywords: Isolation, inoculation, microscopic, Koch’s Postulate.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume grown in more than 80 countries in tropical, subtropical and warm temperate regions (Bajaya *et al*., 2022). Apart from being rich in calcium, thiamine, and niacin, as well as having 26% protein, 48% edible oil, 20% carbohydrates and 3% fiber, groundnut oil is used as an edible oil and for the production of soap, margarine and other products like butter and sweets. The shells can be used as manure, animal feed, fuel as an energy source and as a raw material for a variety of products (Vankatanarayana, 1952). It is primarily used as an oil seed crop. The remaining oil cake is utilized as fertilizer and has significant levels of potassium, phosphorus and nitrogen. Its leaves and haulms provide a plentiful supply of cow feed and basic materials for silage production. Because groundnuts add a significant quantity of nitrogen to the soil, their cultivation contributes to increased soil fertility. The crop is severely harmed by a variety of diseases, the majority of which are brought on by fungus, at every stage of growth, from sowing to harvest and storage. Groundnut are susceptible to a variety of diseases produced by fungi and other microorganisms. These diseases can result in many tissue damages, interfere with the photosynthetic process and result in large output losses.

Aspergillus niger van Teighem, a fungus that causes groundnut collar rot, also known as seedling blight, is one of the major diseases that are spread via soil and seeds. Groundnut collar rot is prevalent in almost every groundnut-growing region of the world, but it is more visible in countries with tropical and subtropical climates when high temperatures are recorded during the rainy season. Jochem (1926) from Java was the first to report this disease, But Jain and Nema (1952) in India were the first to document the *Aspergillus* blight on groundnut caused by *A. niger*. According to Bakhetia (1983), the disease incidence in Rajasthan might potentially reach as high as 50 percent. According to Dighule *et al.* (2018), crop losses in Maharashtra due to *Aspergillus niger* van Teighem-caused groundnut collar rot range from 28.00 to 50.00 percent. The first sign of the disease, according to Jain and Nema (1952) was the development of round, brownish patches on the cotyledons. The portion that was discoloured quickly turned soft and rotten, spreading to the stem and hypocotyls which similarly turned yellow, deteriorated, collapsed and the damaged stem breaks off. The surface of the affected regions showed black fructifications of the pathogen and greyish white mycelia (Pandey and Chakraborty 2023). Mycelia and spores borne by seeds and debris in the soil have been shown to be the main source of the collar rot pathogen inoculum (Nema *et al.*, 1955). In sandy soil it is a more severe and yield-reducing under biotic stress condition (Gibson, 1953, Chohan, 1965 ; Kona *et al.*, 2024; Jat *et al.*, 2024). Since, the disease has become a serious problem due to soil and seed borne nature as well as huge economical losses caused by collar rot pathogen, the accurate identification of the fungal species responsible for groundnut collar rot disease might serve as a helpful reference for its efficient management.

MATERIALS AND METHODS

Isolation and purification. Collar rot-infected plant samples were collected from the fields with the highest disease incidence during the study. On PDA media, the pathogen was subsequently isolated. After cutting the impacted sample section into small (3–4 mm) pieces, it was surface sterilized by immersing it in a 1% sodium hypochlorite solution for one minute. It was then dried on sterile blotter paper after being treated three times with distilled water to remove any last traces of disinfectant. The pieces were aseptically put in PDA-containing Petri plates and subsequently incubated in a BOD incubator at 27 ± 1°C. After 72 hours, a pure culture of the fungus was obtained using the hyphal tip culture method (Riker & Riker 1936; Rangaswami, 1971). A fungal colony disc was cut with a cork borer, put in the center of a Petri plate with on to agar and left to incubate for two days. The Petri Plate was examined under a dissecting microscope and the pathogen mycelial thread were observed at a high magnification. Using a cork borer, the hyphal thread was cut at its apical end (about 1 mm from the end) removed and placed on a different agar plate.

Identification. The cultural and morphological features of the associated fungus were used to identify it. The hyphae of the pathogen were septate, hyaline, or less yellow in color, while the colonies were black in color and usually dull white or colorless on the back. Conidiophores were hyaline, smooth, septate, or non-septate, and their length and diameter varied greatly. When grown on culture media containing submerged mycelium, *A. niger* grows vegetatively rather quickly. Conidiophores range greatly in length and diameter, measuring 200–400 × 7–10 µm and 20 µm, respectively. They can be smooth, septate, or non-septate. Most of them come directly from the substratum. Conidial heads can range in size from tiny, nearly columnar masses of a few conidial chains to the common globes or radiate heads, which can be up to 300, 500, or 1000 µm long. They are fuscous, blackish-brown, purple brown, or carbonous black in all shades. The sizes of globose vesicles range from 20 to 50 µm to 100 µm. Conidia are globose to sub-globose in diameter (3.5-5.0 µm), have rough walls, and are dark brown to black in color (Gilman, 2001; Raper and Fennel 1965).

Pathogenicity test. Using seed inoculation methods proposed by Kataria and Grover (1976); Radhakrishnan and Sen (1985); Sen and Kapoor (1975), the pathogenicity of *A. niger* was evaluated in pot conditions. Utilize the two techniques given below to demonstrate pathogenicity. Observations of both healthy and diseased plants were noted, and PDI was calculated by

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Seed inoculation technique. Surface-sterilized groundnut seeds that appeared to be in good condition were used for pathogenicity test. *A. niger* were grown on PDA-containing Petri Plates and the seeds were rolled and allowed to sporulate for seven days. Inoculated seeds were planted at a depth of 5 cm in pre-sterilized earthen pots with autoclaved soil, with ten seeds per pot and four replications. The control group consisted of the seemingly healthy un-inoculated seeds. These pots were routinely watered and housed in cages.

Soil inoculation technique. *A. niger* were grown on sorghum grain at 27 ± 1°C for 10 days to use as the soil inoculum. Prior to sowing, pots were sterilized with copper sulphate solution and filled with sterilized soil. These pots were inoculated with fungal inoculum multiplied on sorghum grain. Ten apparently healthy and surface sterilized groundnut seeds were sown in each pot with four replications. Surface sterilized seed sown in un-inoculated sterilized soil served as control. These pots were kept in cage house and watered as and when required and maintained under identical condition. Observation on seed germination and pre and post emergence mortality of seeds were recorded under both conditions.

Seed cum soil inoculation technique. This method combined the two previously mentioned methods, applying the pathogen inoculum to the soil and rolling the seeds with a seven-day-old fungal culture. The

surface sterilized seeds were then planted in uninoculated sterilized soil as a control.

RESULTS

Isolation of the pathogen. Plants showing typical symptoms of collar rot disease were collected during survey from every surveyed field of five districts *viz*., Jodhpur, Nagaur, Phalodi, Jalore, Sirohi and brought to the laboratory.

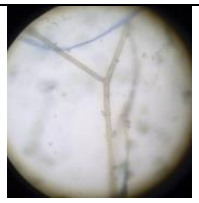
In order to isolate the causing fungus under aseptic conditions, a few samples of diseased crops were incubated in BOD at 27 ± 1°C in petri plates that contained Potato Dextrose Agar (PDA) medium. The hyphal tip approach was used to obtain a pure culture of the pathogen. The hyphae were hyaline, septate, and yellow in colour. The colonies were typically colorless on the back and black on the front. Hyaline, smooth, septate, or non-septate conidiophores were all present. Conidia had rough walls, were dark brown to black, and were globose to subglobose.

Microscopic examinations. Microscopic examinations of the temporary mounts prepared from *Aspergillus niger* affected groundnut plant specimens and pure culture of *A. niger* revealed Cultural and Morphological characters of the pathogen which describe below. Cultural characters of *A. niger* were studied on Potato Dextrose Agar (PDA) medium. The study revealed that the fungus grew well on PDA medium and covered an area of 90 mm on petri plates within seven days when incubated at 27 ± 1°C. The fungus initially produced white to yellowish felt- like mat of mycelia on potato dextrose agar medium. Later the fungal colony turned black mycelial growth with black center. Reverse colony color was pale yellow. The colony topography was smooth and elevation was slightly raised. The fungus produced circular colony with regular margin and showed concentric zonation which were easily and clearly visible from lower side of the Petri Plate. Microscopic examinations of the temporary mounts prepared from *A. niger* affected groundnut plant specimens and pure culture of *A. niger* revealed mycelia were septate, hyaline and conidiophores were long with spherical vesicles at the apex, conidia were globose, brown to black in colour.

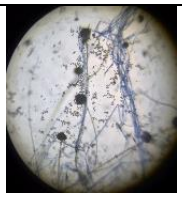
Identification. After the pathogen's purification, its cultural and morphological characteristics were examined in order to identify it. After being sent to the Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi, for identification, the pure culture was identified as *Aspergillus niger* Van Tieghem (ID No. 11,311.20).

Pure culture of *Aspergillus niger*

PDA Slants of *Aspergillus niger*



Hypheal branching



Conidiophore and conida

Plate 1. Pure culture, PDA slants and microscopic pictures of *Aspergillus niger*.

Pathogenicity test. The fungus isolated from the affected part of groundnut plants was isolated using the hyphal tip culture technique and evaluated for pathogenicity to cause groundnut collar rot. Through the use of seed and soil inoculation techniques, the pathogenicity of the isolated fungus was demonstrated. The pathogen was utilized as a seed and soil inoculum (10g/pot) and grew on sterilized sorghum grains. The seed shown pre-mortality after 7 days of planting, and after 15 days, the young seedlings displayed black fungal development on the ground-level stem, girdling the entire base of the young stem at the collar region. Observations of pathogenicity of *A. niger* are presented in Table 1. Highest per cent disease incidence (75.79 %) was observed in seed + soil inoculation technique followed by seed inoculation (66.96 %) while minimum per cent disease incidence (48.15 %) was observed in soil inoculation technique. Re-isolations from these diseased seedlings yielded the culture of the fungus and identical to original one. The re-isolation culture was again found to produce the disease.

Table 1: Pathogenicity test of collar rot of groundnut against *Aspergillus niger*.

Inoculation techniques	Germination %	Per cent disease incidence
Soil inoculation	81.67 (64.76)	48.15 (43.91)
Seed inoculation	75.00 (60.09)	66.96 (54.94)
Seed + Soil inoculation	68.33 (55.76)	75.79 (60.72)
Un inoculated	88.33 (70.18)	0.00 (0.00)
SE(m)±	**1.31**	**1.20**
CD (p=0.05)	**3.90**	**3.55**
CV (%)	**5.12**	**7.34**

*Average of four replications.

*Figures given in parentheses are angular transformed values

Soil inoculation

Seed inoculation

Seed + Soil inoculation

Un inoculated

Plate 2. Pathogenicity test of *Aspergillus niger* by different inoculation techniques.

DISCUSSION

Samples from the surveyed fields were taken during the survey, transferred to the lab and isolated from diseased groundnut plants exhibiting the symptoms of collar rot. The symptoms were seedlings' dry, shredded appearance due to the slimy, decaying nature of the moisture. The damaged plant section became dark, withered and shredded subsequently, it was covered in masses of pathogen spores, which gave rise to the term "Kaalijad" which means "black root". The symptoms noted in the studies and throughout the current survey are consistent with what previous scientists observations (Morwood, 1945; Wadsworth and Melouk 1985; Pande and Rao 2000; Rakholiya *et al*., 2012; Divya Rani *et al*., 2017; Bajiya *et al*., 2022). Morwood (1945) had been determined that while crown rot and seedling blight emerged in the early stages, groundnut seeds did not germinate because they rotted in the soil. Black masses of fungal spores covered the rotten section of the plants, turning them dark and shredding them. Pandey and Chakraborty (2023) also observed morphological, molecular characterization and pathogenicity studies of the fungal pathogen isolated from groundnut cultivar TAG-24 growing in the Jaipur district of Rajasthan confirmed that the Collar rot disease is caused by *Aspergillus niger* H (Gene Bank accession no. ON954789 and OQ653131; NCMR-NCCS, accession number MCC 9892). Pande and Rao (2000); Rakholia *et al*. (2012); Divya Rani *et al*. (2017); Bajiya *et al*. (2022) Affected plants showed wilting and rotting immediately beneath the soil surface and symptoms such as pre- and post-emergence mortality, rotting and discoloration of the infected area have also been reported. Groundnut seeds that were infected turned black and stopped sprouting. By using a seed inoculation procedure in a pot house setting and adhering to Koch's postulates, the pathogenicity of the isolated and purified fungus *A. niger* was verified. The isolated fungus was identified as *A. niger* van. Teighem on the basis of cultural and morphological characteristics. The colonies of *A. niger* are black colored in Petri Plates and reverse usually colorless. Conidiophores generally are smooth, septate or non-septate, varying greatly in length and diameter, *i.e*., 200-400 × 7-10 and 20 µm respectively. Conidia are globose to sub-globose (3.5-5.0 µm in dia.) dark brown to black and rough-walled. Similarly, the pathogen *A. niger*, causing collar rot in groundnut crop was isolated on basic culture medium Potato Dextrose Agar, by tissue isolation technique and also proved its pathogenicity by sick soil method, earlier by several workers (Matloob and Juber 2014; Andge *et al*., 2017; Kumari *et al*., 2017; Divya Rani *et al*., 2018; Kumar *et al*., 2020).

CONCLUSIONS

The pathogen was isolated by following standard tissue isolation method and revealed the association of *Aspergillus* sp. after studying the cultural and morphological characters of the fungus show black mycelium and black fructifications conidiophores and conidia. The pathogenicity of *A. niger* was tested under pot house conditions on GJG-19 variety of groundnut by seed and soil inoculation techniques and maximum per cent disease incidence was observed in seed + soil inoculation technique followed by seed inoculation while minimum per cent disease incidence was observed in soil inoculation technique and proved Koch's Postulate and the reisolated culture of the fungus was identified as *Aspergillus niger* Van Tieghem (ID. No. 11,311.20).

FUTURE SCOPE

This study provides a foundational understanding of the pathogen *Aspergillus niger* causing collar rot in groundnut. Future research can focus on molecular characterization of pathogen strains and identification of resistant groundnut genotypes. Development of eco-friendly biocontrol methods and integration of management practices can further enhance sustainable disease control.

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