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Investigation of disease occurrence and pathogenicity test of *Aspergillus niger* causing collar rot of groundnut in Rajasthan

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Abstract

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop. A large number of diseases play a major role in limiting the yield of groundnut. Among all the soil born diseases, collar rot caused by *Aspergillus niger* is an important pathogen. This disease is wide spread and causes serious losses. So, a survey was conducted in groundnut growing areas of Sikar and Jaipur districts of Rajasthan during *kharif* season 2014-2015. Comprehensive knowledge on incidence and severity of collar rot disease is essential in devising area-wise control measures to this hitherto difficult to manage disease. Samples were collected from different fields for isolation of pathogen and further studies conducted. The disease incidence in different Tahsil of Sikar and Jaipur district varied from (13.09 to 52.00%). The incidence of collar rot was more in sandy soil (52.00%) followed by sandy loam (43.00%). Pathogenicity test were proved by using Koch's Postulate. Further, the seed inoculum and soil inoculum levels were tested for disease pressure on groundnut plant. The Percent Disease Incidence (PDI) showed that the soil inoculum caused maximum disease pressure compare to seed inoculum.

Keywords: collar rot, *Aspergillus niger*, *Arachis hypogaea* L., pathogenicity

Introduction

Groundnut (*Arachis hypogaea* L.), is an important oilseed crop and it is also known as the 'King' of oilseeds. It is an important food and cash crops of our country. Groundnut is cultivated in more than 90 countries of the world [20]. Among them, Asia with (63.4%) area produces (71.7%) of world groundnut production. In Asia, leading producers of groundnut are India, China and Indonesia. The groundnut is grown over 18, 00,000 hectare of land throughout the world [13]. In India the total coverage area under this crop is about 5.5 million ha and production 9.5 million tones and average productivity 1723 kg/ha [1]. Major growing states of India are Gujarat, Andhra Pradesh, Rajasthan, Tamil Nadu and Punjab. Cultivation of groundnut crop in Rajasthan about 0.46 million hectares with annual production 0.90 million tones and productivity 1950 kg/ha [2]. Rajasthan stands seventh in terms of area and production both and the major groundnut growing districts are Bikaner, Jaipur, Sikar, Churu, Dausa, Udaipur and Nagaur. However, the yield of groundnut is reducing day by day because of various biotic and abiotic factors. The fungal pathogens being most predominant and the collar rot disease of groundnut is seed and soil borne disease and cause both pre and post-emergence death of groundnut seedling. This disease was first reported by [6]. However, first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India [7]. The maximum disease incidence (25-50%) was recorded in Rajasthan [3] and around (55-85%) crop loss as recorded in India with Blackening symptoms that travel from roots to stem affecting the vascular system, followed by shredding at root-stem internodes in complete wilting and plant death [19]. Therefore, Keeping in view of the severity of disease and the importance of the crop among oilseeds, grown in India, it was selected for present study and detailed investigations for determine the percent disease incidence in growing area of Sikar and Jaipur districts of Rajasthan and disease pressure by using seed and soil inoculum technique [8].

Materials and Methods

An extensive survey was conducted in groundnut fields where the crop was particularly grow in rainy season during the year, 2014-2015 in fields of Sikar and Jaipur districts (fig.-1). In both these districts, four predominant groundnut growing tahsil, from each tahsil four villages (four fields in each village) were chosen for disease assessment.

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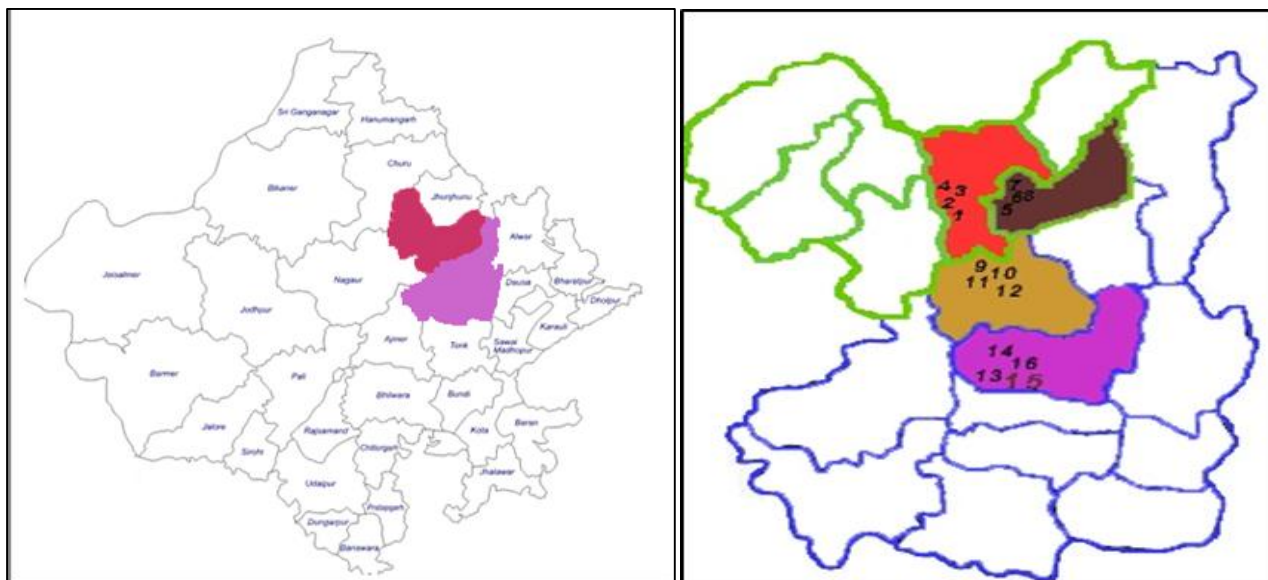


Fig 1: Locale of study area (Tehsil of Sikar and Jaipur district)

The per cent disease incidence (PDI) in these areas was calculated by following formula

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Isolation from infected tissues of leaves was done under aseptic condition on PDA medium and the culture was purified with single spore and sub culturing was done in culture tubes suggested by [18]. Pathogenicity of different isolates was tested by using Koch's Postulate (1883).

The disease pressure on groundnut plants was noticed by soil inoculation technique suggested by [8], Soil inoculation technique suggested by [15, 17]. In the seed inoculation technique healthy surface sterilized groundnut seeds were rolled, on 7 days old sporulating culture of *A. niger*. Inoculated seeds were sown 5 cm depth in earthen pots (pre-sterilized and having autoclaved soil) @ of 4 seeds/pot with 4 replication of each isolate. The un-inoculated apparently healthy seeds served as control. In soil inoculation technique the each fungal isolate (*A. niger*) grown on sorghum grain medium at $25 \pm ^\circ\text{C}$ for 10 days use as the soil inoculums and pots were inoculated with fungal inoculum multiplied on sorghum grain medium. Four healthy surface sterilized groundnut seeds were sown in each pot with four replication of each isolate with un-inoculated sterilized soil served as control. Observation on seed germination and pre and post emergence mortality of seeds were recorded under both conditions of each isolate. The affected collar region become shredded, is soon covered with profuse and black growth of the fungus (spore) and this vary character was also observed to confirm the identification of four isolates of *A. niger* were established from infected plant materials collected during survey of various districts of Rajasthan.

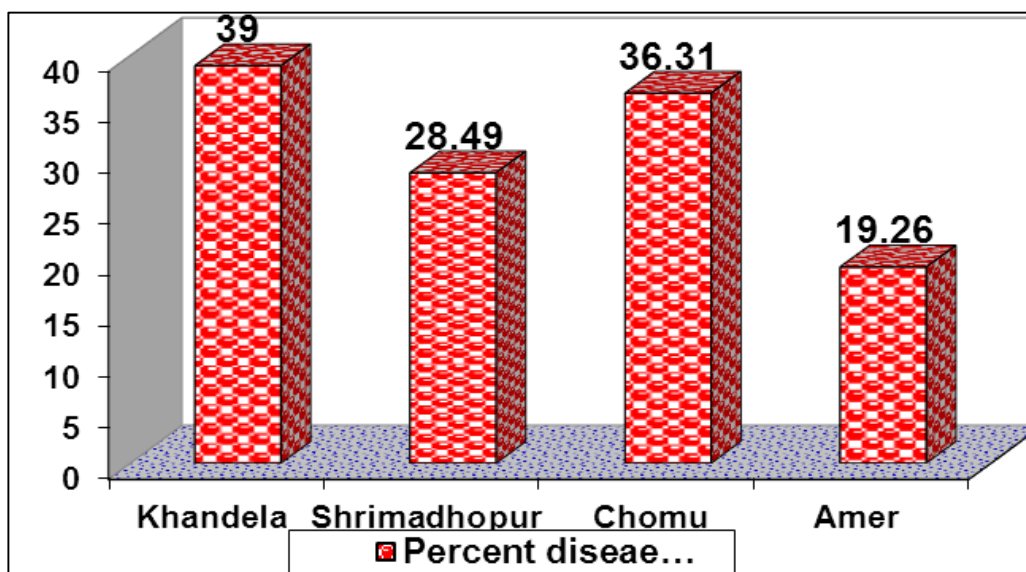
Results and Discussion

The collar rot incidence varied from (13.09 to 52.00%) in different tahsils of Sikar and Jaipur district. The result of

survey indicated that the maximum per cent incidence was observed in Khandela tahsil of Sikar district (39.00%) followed by Chomu tahsil of Jaipur district (31.61%) (Table-1, fig-1). The disease incidence ranged from 16.98 per cent (Surdarsanpura) to 45.00 per cent (Singod) in villages of both tahsil of Jaipur and in Sikar disease incidence ranged from 20.71 per cent (Bhopatpura) to 52.00 per cent (Bawari) was noticed in both tahsil. Similarly, collar rot disease was observed up to 20.00 per cent yield losses in chittoor district of AP during *kharif* (2012) [12, 14] reported the pathogen cause 26 per cent damage in different groundnut genotypes. [4] Observed 40 to 50 per cent losses in terms of mortality of plants due to *A. niger* in India. The typical symptoms were appears in two phases viz, pre-emergence and post-emergence phase. In the pre-emergence sooty black masses of spores are rapidly killed emerging hypocotyls and in post-emergence phase, spherical light brown lesions appear on the cotyledons and in later the lesions are converted into light brown color. The seedlings then collapse and die due to the rotting of the succulent hypocotyls (Plate: 2) similar as described by [5, 11]. Isolations of four isolates (AN-01, AN-02, AN-03 and AN-04) were made from diseased plant samples. Fungus colony with black growth started appearing after 3 days. Later on, the growth was very fast which covered the entire Petri-plate within 5 days (Plate -1). Carbon black or dark brown spores were observed under microscopic study. Similarly [16], were also reported. The results of inoculum levels study were summarized in (Table 2 and fig.2). In both techniques highest per cent disease incidence (76.99%) was observed in soil inoculation technique in isolate AN-01 followed by soil inoculation (68.24%) in isolate AN-03. while minimum per cent disease incidence (42.06%) was observed in seed inoculation technique in isolate AN-04, respectively. Similarly, reported that seed inoculation inoculum levels of *A. niger* [10] and through soil inoculation has also been reported by [7]. Re-isolations from these diseased seedlings yielded the culture of the fungus of each isolate and identical to original one. The re-isolation culture was again found to produce the disease.

Table 1: Infected plant materials collected during survey of groundnut growing areas of two districts (Sikar and Jaipur) of Rajasthan in Kharif 2014-15

Sample No.	Isolate No.	Districts	Tahsil	Village	Disease incidence (%)	Average
1.	AN-01	Sikar	Khandela	Thikariya, Malikpura Palsana, Bawari	29.00% 35.00% 40.00% 52.00%	39.00%
2.	AN-02	Sikar	Shrimadhopur	Parthvipura Patwari ka bas Bhopatpura Ringus	41.56% 25.00% 20.71% 26.71%	28.49%
3.	AN-03	Jaipur	Chomu	Singod Nagal bharda Alisar Khejroli	45.00% 21.71% 23.45% 36.31%	31.61%
4.	AN-04	Jaipur	Amer	Partapura Raithal Devgodha Surdarsanpura	31.23% 15.76% 13.09% 16.98%	19.26%

**Fig 2:** Infected plant materials collected during survey of groundnut growing areas (tehsil) of two districts (Sikar and Jaipur) of Rajasthan in Kharif 2014-15**Table 2:** Pathogenicity of the isolates of *Aspergillus niger* on groundnut

Average of four replications

Isolate	Germination (%)		Pre-emergence mortality (%)		Post-emergence mortality (%)		Percent disease incidence	
	Seed Inoculation	Soil Inoculation	Seed Inoculation	Soil Inoculation	Seed Inoculation	Soil Inoculation	Seed Inoculation	Soil Inoculation
AN-01	68.75 (56.01)	62.51 (52.25)	31.25 (33.98)	35.49 (36.57)	35.04 (36.30)	41.50 (40.11)	65.00 (53.73)	76.99 (61.34)
AN-02	81.25 (64.36)	75.00 (60.01)	18.75 (25.66)	25.00 (29.95)	27.13 (31.39)	30.24 (33.36)	45.88 (42.64)	55.24 (48.01)
AN-03	75.00 (60.01)	68.75 (56.02)	25.13 (30.09)	31.25 (33.98)	30.28 (33.39)	37.29 (37.64)	55.41 (48.11)	68.24 (55.70)
AN-04	87.50 (69.78)	81.25 (64.54)	12.50 (20.68)	18.75 (25.65)	18.50 (25.47)	23.31 (28.87)	31.25 (33.99)	42.06 (40.43)
Control	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm±	1.11	0.84	0.52	0.36	0.39	0.34	0.45	0.49
CD(p=0.05)	3.41	2.59	1.60	1.09	1.21	1.05	1.38	1.50

Figures in parentheses are angular transformed values

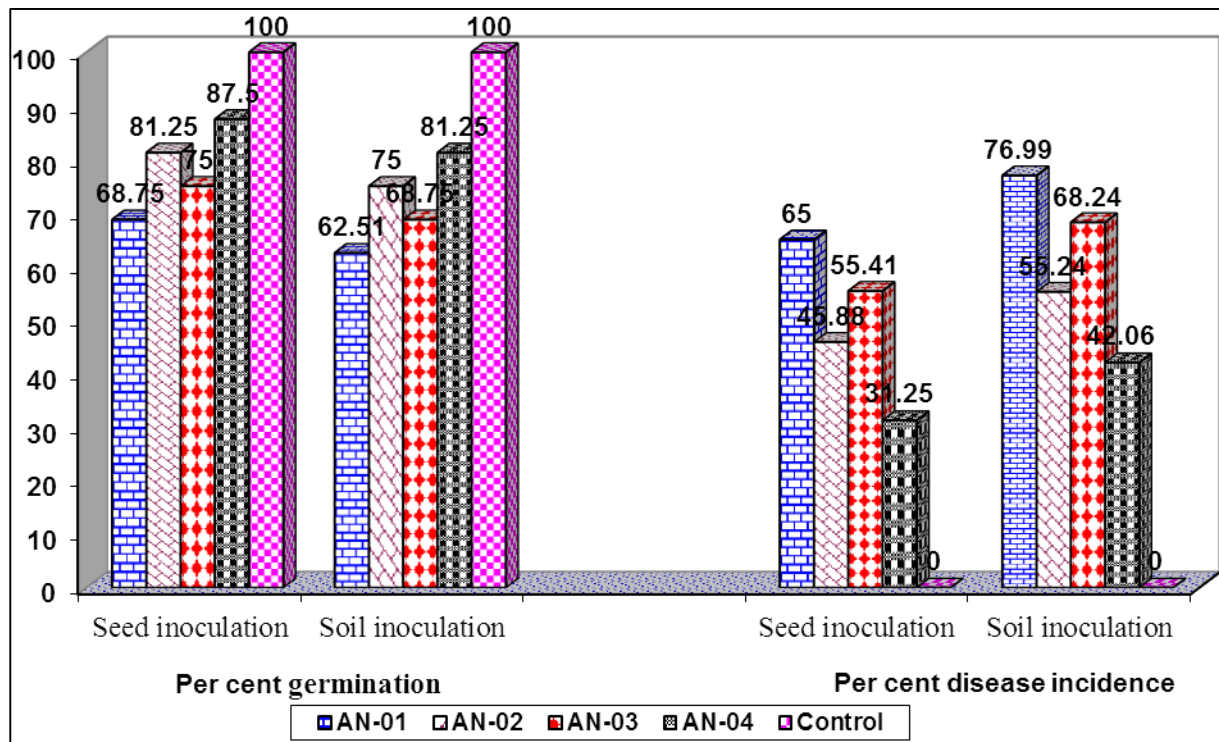


Fig 3: Inoculum levels of the isolates of *Aspergillus niger* on groundnut



Fig 4

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