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Collar rot (*Aspergillus niger*) a serious disease of groundnut, its present status and future prospects

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Abstract

Collar rot disease of groundnut is one of the most serious, destructive diseases in and yield losses range from 13 to 52% and can be as high as 93.6% in some areas. Collar rot nature is often seed and soil borne. Being mainly a soil-inhabiting pathogen, many environmental and soil factors are responsible for the development of disease. No systematic research related to the biology, ecology and epidemiology of collar rot in groundnut has been conducted so far. To combat this problem, research is needed to improve the identification and characterisation of variability within its epidemiological and pathological niches and development of disease resistant groundnut hybrids. Moreover, various chemical and biological control methods have been developed but major emphasis is on development of groundnut cultivars with genetic resistance to for environment friendly control of the collar rot disease. The current paper reviews the information on distribution, impact of the disease, symptoms, variability, host resistance and Management approaches has been enumerated to understand the present status of knowledge about collar rot and will try to focus on the future perspectives available to improve collar rot management.

Keywords: collar rot, groundnut, variability, *Aspergillus niger*, management

Introduction

Groundnut (*Arachis hypogaea* L.), one of the important oil seed crops of the world is believed to be a native of South America. It was introduced to East Asia from South America and from there to India by Spaniards during the 19th Century. India gradually became a major groundnut producing country of the world within a span of 5-6 decades. This crop assumed great significance in the present day economy. It is mainly grown as oil seed *kharif* crop in India. The kernels are widely acknowledged as a rich and cheap source of vegetable protein. Groundnut is cultivated in more than 90 countries of the world (Virmani and Singh, 1985) [62]. 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. In India, the major groundnut growing states are Gujarat, Andhra Pradesh, Rajasthan, Tamilnadu and Punjab (Kumari, M. and Singh, M., 2016) [23]. A major limiting factor in profitable cultivation of this crop in Rajasthan is the attack of several diseases mainly caused by fungi, which takes heavy loss of the crop at all the stages of growth right from sowing to harvest and storage.

Table 1: List of fungal disease of groundnut

	Name of diseases	Pathogenic fungal spp.
1	Alternaria leaf spot	Alternaria arachidis
2	Collar rot	<i>Aspergillus niger</i>
3	Leaf spot, early	Cercospora arachidicola
4	Leaf spot, late	Cercosporidium personatum
5	Powdery mildew	Oidium arachidis
6	Rust	Puccinia arachidis
7	Charcoal rot	Macrophomina phaseolina

Economic importance and occurrence of disease

Collar rot disease in groundnut prominently is distributed in countries with tropical and sub-tropical climates where high temperature prevails during the rainy season. Collar rot disease of groundnut (*Arachis hypogaea* L.) caused by *Aspergillus niger* van Tiegham is an important disease in several temperate countries (Carina *et al.*, 2006) [6].

However, in India, Jain and Nema (1952) ^[22] at Nagpur first reported the *Aspergillus* blight of groundnut caused by *A. niger* and worked out the morphology of the pathogen. The primary source of the inoculum of collar rot pathogen has been shown to be mycelium and spores carried on the seeds and organic debris in the soil (Nema *et al.*, 1955) ^[41]. Collar rot is more serious problem in sandy soil (Gibson 1953; Chohan 1965; Kumari and Singh 2016) ^[17, 23]. Annual world yield loss caused by disease is more than 10% (Pande & Rao, 2000) ^[43] and is more prevalent in soils with low moisture content and high temperature, approximately 30 °C (Kishore *et al.*, 2007, Kumari, M. and Singh, M. 2016) ^[30, 23]. Diseases attacking in very devastating on stem tissues near ground surface and causing rot, wilting and plant death (Pande & Rao, 2000, Kuamri M. and Singh M. 2017) ^[43, 25]. Similarly, Ghewande *et al.* (2002) ^[16] reported that losses in terms of mortality of plants due to collar rot range from 28 to 50 per cent. In Nilanga (50 to 80%), Coimbatore (93.6%) (Kadam *et al.*, 2011). It is estimated that over five lakh hectare groundnut fields were infected with this pathogen and yield losses of more than 25 per cent have been reported (Mayee and Datar, 1988) ^[36]. The most of the groundnut cultivars are susceptible to this disease. It is emerging as a major and wide spread problem in Rajasthan. This disease was first reported by Jochem (1926) ^[21]. It is an important disease in the major groundnut growing areas of the Rajasthan state. The maximum disease incidence (25-50%) (Bakhetia, 1983) ^[5] and (13-52%) (Kumari, M. and Singh M., 2016) ^[23], Andhra Pradesh (Raju, 2001) ^[48], was recorded in Rajasthan and around (55-85%) crop loss as recorded in India (Sharma *et al.*, 2012) ^[53]. The present review focuses on the current status of collar rot disease of groundnut with special reference to its occurrence and collection of disease samples. Additionally, a detailed account of infection, variability and management strategies for collar rot are also included.

The pathogen: *Aspergillus niger*

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. Genus *Aspergillus* which includes a set of fungi that are generally considered asexual,

although perfect forms (forms that reproduce sexually) have also been reported. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould"). A number of morphologically similar species were recently described by Samson *et al.* (2004) ^[49]. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grains, compost piles and other decaying vegetation. Microscopically, its conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (1.38-4 µm diameter), dark brown to black and rough-walled (Kumari and Singh 2016) ^[23]. It is known to create increased amount of pathogenicity in various species of plants, which can be treated by antibiotics, chemicals and antibiosis (Sharma, 2012) ^[53]. *A. niger* is a filamentous fungus growing aerobically on organic matter. In nature, it is found in soil and 5 litter, in compost and on rotten plant material. Reiss (1986) ^[45] collected data on the influence of temperature, water activity and pH on the growth of diverse *Aspergilli*. *A. niger* is capable to grow in the wide temperature range of 6-47 °C with a relatively high temperature optimum at 35-37 °C.

External manifestation of collar rot disease

The collar rot disease of groundnut showed two type symptoms i.e. pre-emergence and post emergence (Kumari, M. and Singh, M. 2016) ^[23] (picture-1) Subrahmanayam *et al.* (1992) ^[51] observed that pre-emergence seed rot infected seeds failed to germinate and were covered with masses of black conidia which imparted them a sooty appearance. Seeds become infected during the last days of maturation in the soil and during harvesting, handling and particularly during shelling (Subrahmanyam and Rao, 1977) ^[50]. Crown rot was the most serious disease of groundnut in early stage of growth and they further indicated that the emerged seedlings were affected at collar region causing yellowing of lower leaves, slow death due to blighting effect on the shoot, finally leading to death of crown portion, Suzui and Makino, 1980) ^[54].



Fig 1: The cultural, morphological and pathogenic variability of pathogen

Morphological and cultural variability of pathogen

The necrotrophic fungus *A. niger* exists in an anamorph stage in soil and on crop residues. Under in vitro conditions, the fungus grows rapidly on potato dextrose agar (PDA) and produces white coloured mycelium that become darker with age and produce black spore. The shape, colour and size of colony within Petri plates were recorded after 3, 5 and 7 days of incubation, respectively. Microscopic variation viz: size of conidia, colour of conidia, size of conidiophore, size of collumella and type of hyphae were recorded by using ocular microscope, stage micrometer and compound microscope. The different isolates of *A. niger* showed different growth characters, i.e. colour of fungus mycelium (super white, pale yellow, yellowish white and dull white), colony spread (Rapid, medium moderate to rapid and slow), margin of fungus colony (entire with cut and entire), size of colony (28.00 to 55.34 mm), type of hyphae (branched septate), length of conidiophores (180-360 µm), diameter of conidia (1.38-4 µm) and fruiting body cleistothecia are present in each isolate (Kumari, M. and Singh, M. 2016, Gautam and Bhadauria, 2012)^[23, 13]. Culturally, *Aspergillus* species require 1-3 weeks for growth. The colonies begin as a white dense mycelium, which later assume a variety of colours according to the species based on the colour of their conidia. This observation is in consonance with the findings of Disalvo (2000)^[9]. Mislivec (2000)^[35] observed some of the conidia of *A. niger* species isolated were hyaline, pigmented, smooth or rough with single or double wall. (Gilman, 2001, Diba *et al.* 2007)^[14, 11] examined of species differentiation basis on their cultural features like colony colour, texture and margins, as well as microscopic such as size of conidia and conidiophores and their arrangements of *A. niger*. (Sharma *et al.* 2012)^[53] reported that conidiophores of *A. niger* are smooth-walled, hyaline or turning dark towards the vesicle and conidial heads are bi seriate with the phialides borne on brown, often septate, dark brown to black and rough-walled.

Pathogenic variability of pathogen

In the pathogenic variability of *A. niger* on groundnut showed in various effect on seed germination, incidence of seed and collar rot under artificial conditions. The pathogenic variability in *A. niger* is assumed to be due to various sexual like process. However, many workers reported various incidence of collar rot of groundnut in different agro-ecological zones (Ramakrishna and Kolte, 1989, Sahu and Mishra, 1994, Mohapatra and Sahoo, 2011, Thomidis *et al.* 2012, Gautam *et al.*, 2011, Abdela 2009, Tefera and Tana, 2002)^[46, 56, 32, 12, 60]. Groundnut collar rot samples collected from various groundnut grown in various agro-ecological zones of Rajasthan (Kumari, M. and Singh, M. 2016)^[23]. The isolates varied in cultural and morphological characters like colony colour, growth pattern. Recently, on the basis of virulence study of 4 isolates of *A. niger* on groundnut susceptible cultivar. In all the test isolates, two pathotypes isolated from Sikar District of Rajasthan were reported more virulent at 15 days after sowing and decline gradually with the increase in plant age. All the test isolates were able to incite collar rot symptoms to varying degrees during the period of investigation (Kumari, M. and Singh, M. 2016)^[23].

Managing of collar rot disease

Chemical control

Seed treatment by fungicides is effective to some extent in reducing losses caused by *A. niger* in crops which are particularly vulnerable at the seedling stage. Companion

(carbendazim + mancozeb) 71.83% followed by Bavistin 69.16% and Vitavax power 65.80% found most effective by seed treatment (Kumari, *et al.* 2016)^[23]. Mishra *et al.* (1992)^[37] reported the effectiveness of fungicides like Bavistin (0.2%), Saaf (0.2%), Benlate (0.2%) and Thiram (0.3%) against collar rot disease of groundnut. Sheth *et al.* (2010)^[57] noted complete inhibition of mycelial growth of *A. niger* infecting citrus fruits by treating with Carbendazim, Benomyl and Carbendazim + Mancozeb at 500 & 1000 ppm. Vitavax power (Carboxin 37.5% + Thiram 37.5%) was found highly effective against collar rot of groundnut by using as seed dresser (Shivpuri *et al.* 2011)^[59]. Mohapatra & Beher *et al.* (2012)^[33] recorded minimum collar rot incidence (4.66%) in seeds treated with bavistin @ 2.0 g/kg of seed. Nandeesh *et al.* (2013)^[38] recorded minimum collar rot incidence (4.66%) in seeds treated with bavistin followed by Saaf (5.33%), Thiram and Benlate (6.33%). Nathawat B.D.S. and Mahadra partap (2014) recorded cent per cent mycelial growth inhibition of *A. niger* by tabuconazole and Propiconazole at 100 to 1000 ppm. Singh (2006)^[55] found that Carbendazim and Mancozeb completely inhibited the fungal growth *in vitro* at higher concentration (1000 ppm). Sharma *et al.* (2012)^[53] observed that Carbendazim and Mancozeb completely inhibited the mycelium growth of *A. niger* at 500 and 1000 ppm. Wani and Kuruchave (2004)^[64] reported inhibited 100% inhibition of mycelium growth of *A. niger* and *A. flavus* by thiram 0.2% and combined fungicides Carbendazim + Mancozeb. Carboxin and thiram at 1000 and 1500 ppm concentration also inhibit cent per cent mycelium growth of test fungus (Gangopadhyay, *et al.*; 1996, Karthikeyan, 1996)^[19, 28]. Patel *et al.* (2008)^[44] recorded significantly reduced the disease severity of Anola fruit rot caused by *A. niger* at 1000 ppm of Carbendazim + Mancozeb Raju and Naik (2006) reported that pre-harvest spray of Carbendazim (0.1%) gave the most effective control of black mold of onion caused by *A. niger*. Yadav *et al.* (2007)^[65] proved Carbendazim (0.1%) most effective against *A. niger* caused fruit rot in Aonla.

Biological control

Seed treatment with biocontrol agents like *Trichoderma viride*, *T. harzianum* has shown some benefits in managing the collar rot of groundnut (Kumari, M. and Singh, M. 2017)^[25] (Rao Sitaramaih 2001)^[47] (Gangwar *et al.*, 2014)^[20] (Pratibha, 2012)^[42]. Effectiveness of organic amendments (neem cake, mahua cake, castor cake, farm yard manure, sheep manure and poultry manure), biocontrol agent (*T. harzianum*) and fungicides (carbendazim and thiram) to control collar rot pathogen. The lowest disease incidence was recorded when carbendazim was applied as a seed treatment whereas *T. harzianum* was found most effective as soil treatment Karthikeyan (1996)^[28] Kumari, M. and Singh, M. 2017)^[26]. Charitha *et al.* (2009)^[7] recorded the efficacy of *Trichoderma* spp. and *P. fluorescens* (*in vitro*) against *A. niger*. *T. viride* was observed more effective in inhibiting the pathogen in pot culture. Combined effect of seed treatment with *T. viride* and captan significantly reduced the collar rot in groundnut. Gajera *et al.* (2011)^[15] observed antagonistic effect of 12 isolates of 3 *Trichoderma* strains (*T. virens*, *T. viride* and *T. harzianum*) against the collar rot pathogen and observed that *T. viride* most effective. Latha (2013)^[31] evaluated different fungal bacterial bio- agents against *A. niger* and found that maximum mycelium growth inhibition of *A. niger in vitro*. They also conducted field trials and reported least (20.45%) disease incidence with bio-formulation mixture of Pf1+Tv1+ neem cake + FYM as

against (60.0%) in untreated control. Nandeeshya *et al.* (2013)^[38] evaluated the efficacy of 14 isolates of *Trichoderma* spp. and isolates TAG-2, TAG-13 and TAG-10 showed maximum mycelium inhibition. Shama and Saha (2013) Nathawat B.D.S., and Mahadra partap (2014), Kishore *et al.* (2001)^[29] reported that the *T. viride* and *T. harzianum* were found to be effective in reducing the radial growth of *A. niger* *in vitro*.

In Plant-extracts, *in vitro*, Garlic clove extract at 10 and 15% concentration showed maximum growth inhibition of fungus followed by Neem and Datura leaves extract and *in vivo* maximum disease control was recorded in Garlic clove extract 40.60% followed by Neem leaves 34.78% at 15% concentration by seed soaking method (Kumari *et al.* 2016)^[23] (Nathawat and Partap 2014)^[39]. (Mohamed *et al.* 2012)^[34] (Sheth *et al.* 2010)^[57] observed that garlic cloves, onion and neem leaf extracts have the ability to cause reduction in the mycelial growth of *A. niger*. Shakil *et al.* (2012)^[58] evaluate the efficiency of aqueous extracts of five plant species, aqueous bulb extracts of *A. sativum* and *A. cepa* and the leaf extract *procera* showed better disease management. Kumar *et al.* (2012)^[42] evaluated 17 plant extract and completely inhibited the mycelium growth of *A. niger* *in vitro* and also proved the extract of neem leaf most effective in inhibiting mycelium growth as well as in preventing of spore formation of *A. niger* (Vishwapal *et al.*, 2013)^[63]. Wani and Kuruchere (2014) reported garlic bulbs extract inhibited 100% mycelium growth *in vitro* of *A. niger* and *A. flavus*.

Host plant Resistant

Fourteen varieties were screened against *A. niger* under artificial conditions. None of variety was found immune or resistant to collar rot. Entries RG-425, CSNG-19-1, SNG-69, GG-21 and RG-559-3 were observed moderately resistance, RG-578, RG-378, RG-582, M-13, Girnar-2 and SNG-123 were found susceptible and RG-382, RG-510 and Chitra were highly susceptible (Kumari *et al.* 2016)^[23]. Aulakh *et al.* (1970)^[2] studied twenty groundnut varieties for resistant against *A. niger* under artificial conditions and only one exotic variety, U4-47-7 (EC 21115) from Sudan was found to be significantly resistant to the disease as compared with the other varieties. Dasgupta *et al.* (1997)^[10] conducted in field screening trials of groundnut germplasm against soil and seed isolates of *A. niger* in sandy soil. They observed that lines C421 and C1780 were field resistant to both seed and soil isolates. To evaluate resistance in groundnut against soil borne pathogen field screening is an effective method (Brenneman *et al.*, 1990. Shokes *et al.*, 1992)^[4]. Similarly different genotype exhibited different response to stem rot (Branch & Csinos. 1987., Gorbet. 2004)^[3, 18]. Nathawat, *et al.* (2014)^[39] evaluates five varieties screened against collar rot of groundnut caused by *A. niger* and only GG 2 was found tolerant to collar rot, while GG 5, GG 7, GG 20 and GG 37 were susceptible to highly susceptible and Maximum collar rot incidence was observed in 10 days old plants followed by 15 days old plants.

Future prospects

Collar rot diseases of groundnut pose a threat to sustainable groundnut cultivation. The current understanding of the collar rot- *A. niger* interaction has provided significant information and paved a way for working on this emerging disease. We now have evidences that climate has a significant role in collar rot emergence as frequent high temperature and low moisture conditions lead to increased incidence in groundnut. More studies are needed to determine the temporal and spatial

distributions of the pathogen. Proper and rapid characterisation of symptoms of the disease for timely scouting is needed. Pathogenic characterisation based on morphology and genetic makeup will provide ample opportunities to understand the population genetics. Additionally, information on biology and epidemiology of collar rot will further strengthen the screening procedures required to identify resistant sources. This in turn will assist breeders in optimising breeding strategies that will enable long-term resistance over broader geographical areas. The pathogenic and morphological study has been described earlier; so genetic variability of the pathogen also be done. Despite the extensive investigations in other hosts, the infection process of *A. niger* on groundnut has not been studied. Understanding the genetics, behaviour of host and pathogen in the process of disease development and host-pathogen relationship are crucial for reliable breeding programmes for disease resistance. There is also a need to map the genetic profile of the few groundnut accessions that have shown traces of resistance to collar rot. This will identify the putative molecular marker to identify the gene (s) responsible for the resistance. Further, knowledge of infection process and host defence mechanisms will help in devising effective management strategies to collar rot.

To the best of our knowledge, this is the first review on collar rot of groundnut which describes the overall status on disease occurrence, symptomatology, variability and available information on management of the disease.

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