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Rhizoctonia bataticola: A serious threat to chickpea production

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Abstract

Rhizoctonia bataticola (Taub.) Butler {Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid} is a soil inhabiting fungus which is a serious threat to more than 500 plant species. Although considerable research related to ecology of *Rhizoctonia* has been done, it still appears to be a potential pathogen causing severe losses in various crops. Further research is required to have a better identification and characterization of genetic variability among different isolates collected from different ecological zones. Better understanding of variation among populations of pathogen for avirulence genes for will definitely aid in designing improved management strategies to combat *R. bataticola* attack. This study will enable readers to have a more clear picture of the dry root rot pathogen, *R. bataticola* in respect of variability, distribution, pathogenicity and economic impact on different plant species. Various techniques have been developed for diagnosis of the pathogen at the initial stages. The development of molecular techniques for better identification and detection of the fungus will certainly help in minimizing the soaring crop losses to a considerable extent.

Keywords: *Rhizoctonia bataticola*, variability, diagnosis, dry root rot, pathogenicity

Introduction

Rhizoctonia bataticola (Taub.) Butler is a very important soil-inhabiting, fungus posing a serious threat to a wide range of crops. It is known to incite different types of diseases viz., stem blight, seedling blight, leaf blight, wilt, seedling decay, root rot, stalk rot, fruit rot, and charcoal rot in crop plants (Dhingra and Sinclair, 1978) [25]. The genus *Rhizoctonia* is believed to be a heterogeneous group of filamentous fungal taxon which produces asexual spores and share a number of similar features in their anamorphic states (Garcia *et al.* 2006) [53]. The members of this genus are generally soil borne fungi, mostly associated with roots, although there are few reports of their saprophytic ability in a few taxa. The Fungi belonging to these genera are cosmopolitan in nature and are distributed worldwide in both agricultural and forest soils and include some of the most economically important plant pathogens, causing foliar and root rot diseases of major crops. *R. bataticola* is important member in the genus that causes seedling blight and root rot in many legumes when the plants are weakened due to some other stress factors (Hawang *et al.* 2003) [37]. When main host crop is absent, the pathogen survives as a saprophyte on dead organic matter. The survival and saprophytic activity of the pathogen is influenced by several biotic and abiotic factors. Dry root rot (DRR) of chickpea caused by *R. bataticola* is a serious threat to the global chickpea production (Pande and Sharma, 2010) [84]. The estimated crop losses due to DRR have been estimated around 10-25% (Pandey and Singh, 1990) [66]. A critical analysis of weather data over the years revealed that incidence of DRR is high in areas where average temperature exceeds 33°C (Sharma *et al.* 2010) [84]. Rising incidences of DRR at numerous sites over years suggests that the disease development is highly influenced by climate change. According to Savary *et al.* (2011) [79], DRR is an acute-emerging disease which has an irregular occurrence, both spatially and temporally and cause enormous losses covering new areas.

The isolates of *R. bataticola* illustrate a great variability irrespective of their isolation from different hosts or plant parts of a single host (Prameela and Singh 1998; Meena *et al.* 2006) [71, 54]. Sundravadana *et al.* (2011) [99] described the variability among various isolates of *R. bataticola* from seeds, roots, and leaves of several pulse crops (black gram, green gram, red gram, soybean, and cowpea) based on morphological, genetic and pathogenic features. Sixty four isolates of *M. phaseolina* from sunflower and cotton have been divided into three Categories viz., highly virulent, virulent and poorly virulent (Manici *et al.* 1992; Monga *et al.* 2004) [52, 57].

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The pathogen produces various hydrolytic enzymes such as cellulase, hemicellulase, pectinase, protease etc which acts as a primary pathogenic tool of the fungus to incite infection (Amadioha and Oladiran, 1993) ^[9]. However, Singh and Mehrotra (1980) have reported that increased root exudation by chickpea is a major factor governing pre-emergence damping-off losses by *R. bataticola* at extreme temperatures. The study regarding the mode of action will definitely enable a better understanding of the pathogen and will facilitate development of advanced management practices.

Molecular techniques are widely used for identification of pathogen species and assessing genetic variation among various populations. Random amplified polymorphic DNA (RAPD) proves to be a promising, versatile and informative molecular tool to identify genetic diversity among plant pathogen population (Chiocchetti *et al.* 1999) ^[21]. RAPD analyses are widely used to characterize genetic diversity of different isolates of *M. phaseolina* (Almeida *et al.* 2003) ^[7]. The molecular character variability is used to determine resistant cultivars and to evaluate germplasm resistant line (Thirumalaisamy *et al.* 2006; Shekhar *et al.* 2006) ^[102, 86]. This review entirely focuses on taxonomic status, symptomatology, and mode of action and management practices of *R. bataticola* which will help in a clear understanding of the fungus.

Taxonomic Position

The taxonomic status of *R. bataticola* has not been described by scientists, however, there are various reports regarding the taxonomy of its pycnidial stage *Macrophomina phaseolina* (Ref). The genus "Rhizoctonia" (meaning "root killer") belongs to the class Agaricomycetes which comprises of all anamorphic fungi that lack their sexual stage. Rhizoctonia belongs to order Cantharellales and family Ceratobasidiaceae. *Rhizoctonia* sp. are usually saprophytic in nature; however some of them act as facultative parasite causing important plant diseases. Augustin Pyramus de Candolle (1815), coined the genus "Rhizoctonia" for those plant pathogenic fungi which produce both hyphae and sclerotia. Subsequently, over 100 additional names were added to the genus by different authors. Made a comprehensive survey on the genus and repositioned it. According to them, *R. bataticola* is used as a synonym to *Macrophomina phaseolina* (Tassi) Goid. Currently, *M. phaseolina* is formally accepted as the correct taxonomic name (CMI description of pathogenic fungi and bacteria No. 275) whereas its sclerotial phase is referred as *R. bataticola*.

Morphological variation within *R. bataticola* isolates

R. bataticola possess a great variation amongst its isolates. The isolates collected from different agroclimatic zones demonstrate huge variation in their morphological and culture parameters like, growth pattern, growth rate, colony color, mycelia characters, morphology of the sclerotia and sclerotia initiation time (Sharma *et al.* 2004) ^[84]. The morphological and culture variation in *R. bataticola* has been exhibited in different hosts viz., sunflower, cowpea, pearl millet, groundnut, and bean (Ndiaye, 2007; Fernandez *et al.* 2006; Suriachandraselvan and Seetharaman, 2003; Atiq *et al.* 2001; Okwulehie, 2001 Rantoo *et al.* 1997) ^[60, 26, 100, 11, 64, 73] The pathogenic variability of this fungus has been reported in soybean and sunflower, which is believed to be due to hyphal fusion, mutation and mitotic recombination (Dhingra and

Sinclair, 1978; Jimenez *et al.* 1983) ^[25, 42]. The fast growth habit or mycelial spread and the abundant presence of sclerotia may also contribute to pathogen variability (Jimenez *et al.* 1983) ^[42]. Significant relationship between various parameters has been observed such as time required for sclerotial initiation- sclerotial intensity and disease severity of the isolates.

Hooda and Grover (1988) ^[38] reported a direct relationship between sclerotial intensity and pathogenicity, which lead to the fact that pathogenic isolates leads to more sclerotial production. In contrast to it, Manici *et al.* (1992) ^[52] reported that there is no such positive correlation. However, a negative type of correlation between cottony type and sclerotial production was reported by Simosa and Delgado (1991) ^[26]. The morphological features of *R. bataticola* vary considerably with respect to different isolates and age of the culture (Sharma *et al.* 2004) ^[84].

Symptomatology

Dry root rot disease of chickpea usually occurs during reproductive phase of the crop, around flowering and podding time as scattered dried plants (Sharma *et al.* 2015) ^[80]. The seedling stage may be affected by the disease but the old plants are more susceptible towards infection (Sharma and Pande, 2013) ^[81]. The most favorable temperature for incidence of disease is found to be 30 °C. The affected plants dry suddenly with straw coloured leaves, while in few cases the stem and lower leaves show a characteristic brown colour. Drooping of leaflets and petioles are confined at the top of the plant. In some cases, when the rest of the plant gets dried, the topmost leaves become chlorotic.

On the other hand, when the diseased plants were uprooted, the tap root appears black in color and lower portion generally remains in soil, while the number of lateral and fine roots reduces. The dead root is quite fragile and appears shredding of the bark. The dark tiny sclerotial bodies get exposed or remain inside the wood or bark (Nene *et al.* 1991) ^[62]. On vertical split of dried stem of the collar region, sparse mycelium or tiny sclerotia can be observed. Sclerotial survivability is directly dependent on the soil moisture level; however, its survival is reduced in wet soil compared to dry soil.



Fig 1: Comparison of dry root rot infected chickpea plants with control

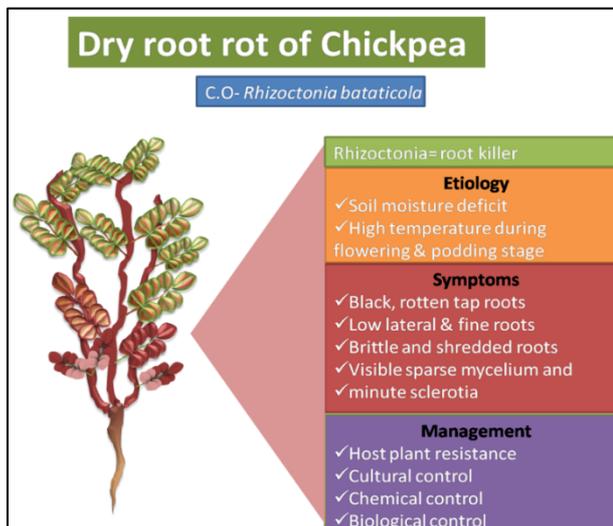


Fig 2: A brief overview of dry root rot of Chickpea

Epidemiology

Epidemiology is the study of outbreak of an infectious disease. For occurrence of any disease, four factors play a key role and is termed as disease tetrahedron. Disease tetrahedron is usually the association of virulent pathogen, susceptible host, conducive environmental conditions for pathogen and the time period for which all these factors are interacting.

Microsclerotia act as the chief source of inoculum and have the ability to be active in the soil for about 15 years (Short *et al.* 1980)^[87]. They are generally found in clusters on the soil surface and reported up to the depth of 0-20 cm in soil (Alabouvette, 1990; Campbell and Van der Gaag, 1993)^[5, 17] with survival ability under adverse environmental conditions (Short *et al.* 1980)^[87].

The conducive temperature range for germination of microsclerotia is around 28–35 °C (Mihail, 1989)^[55]. The germ tube leads to formation of appressorium which indirectly penetrates through the host epidermis by secreting cell wall degrading enzymes, although there have been reports of indirect penetration of the fungi through natural openings also (Bowers *et al.* 1999; Mayek-Perez *et al.* 2002)^[15, 53]. The mycelium grows through the cortex and enters the xylem vessel and ultimately colonizes the vascular tissue (Abawi and Pastor-Corrales, 1990)^[1]. Inside vascular tissues, the fungus spreads through the tap root and plugs the vessels resulting in wilting of the plant (Wyllie, 1988)^[87]. The sclerotial production ability of *R. bataticola* is dependent on the host and the particular nature of the fungal isolate that will establish the epidemiological role of conidia in the disease cycle (Ahmed and Ahmed, 1969)^[69].

The survival of *R. bataticola* has been reported from 2-15 years and is highly affected by environmental parameters irrespective of association with the host tissues (Short, 1980; Baird *et al.* 2003)^[87, 18]. There are reports of survival of the fungus in sorghum and corn residues, cucurbit roots under dry soil conditions for up to 10, 16 and 18 months, respectively (Ghaffar and Akhtar, 1968; Cook *et al.* 1973)^[30, 22]. Repeated freezing and thawing of soil, low C: N ratio and soil moisture content are critical factors affecting microsclerotia survival (Dhingra and Sinclair, 1975)^[23]. Under low soil moisture condition production of microsclerotia gets enhanced whereas high soil moisture has negative effect on sclerotial production (Dhingra and Sinclair, 1977; Olaya and Abawi, 1996)^[65].

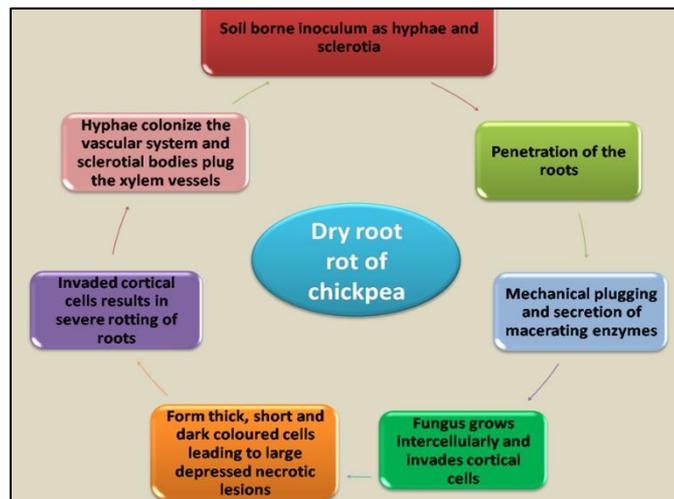


Fig 3: Disease cycle of dry root rot of chickpea (Sharma *et al.* 2016)

Mode of action of *R. bataticola*

R. bataticola is known to produce cell wall degrading enzymes like cellulase and pectinase. *R. bataticola* produces both pectin-methyl-esterase (PME) and polygalacturonase (PG) *in vivo* and *in vitro*, with the latter releasing more reducing materials in reaction mixtures. Amadioha and Oladiran (1993)^[9] reported that the filtrates from culture of *R. bataticola* and *Rhizoctonia*-infected tissues of *S. tuberosum* contain PME and PG. The two pectinases produced in culture varies and do not act on their substrates in the same manner, as those associated with the diseased tissues.

R. bataticola is a cellulolytic fungus capable of producing cellulases in culture and *Rhizoctonia*-infected plants (Amadioha, 1998)^[8]. The degradation of cellulose usually takes place by two different types of enzymes namely C_x and C_1 (Amadioha, 1993; Ikotun, 1984; Adia and Fajola, 1983)^[29, 2]. The C_1 enzyme acts on the crystalline parts of the cellulose chain and loosens the microfibrils which in turn facilitates C_x enzyme in breaking the β -1, 4-glucosidic bonds (Mandels and Stainberg, 1976)^[5]. Thus, according to the hypothesis of Rease (1956), both C_1 and C_x enzymes are produced by *R. bataticola* produces

Singh and Mehrotra (1980)^[77] through their experiments have reported that increased root exudation by chickpea is a key factor leading to increased pre-emergence damping-off of gram seedlings by *R. bataticola* at elevated temperatures. The seeds of susceptible varieties have been found to exude greater amounts of carbohydrate and amino acids compared to resistant ones. The large amount of carbohydrates and amino acids reveal that these substances play a major role in stimulating the growth of the pathogens in the vicinity of seeds, ultimately leading to the very high rate of pre-emergence damping off. The differences in susceptibility of the cultivars are attributed to the amount of carbohydrate exuded by the seeds during germination.

Other hydrolytic enzymes *viz.* hemicelluloses, amylases, phosphatidases and proteases also play a key role in disease development (Amadioha, 1998)^[8]. Proteins and phospholipids are regarded as the integral components of the biological membranes and their degradation is primary step towards initiation of pathogenesis. Mostly, cellular organelles are being targeted by these enzymes leading to alteration in their permeability. Additionally, pectinases, are also released due to compartmentalization of such organelles. However, the role of phosphatidases in expression and advancement of disease has been thoroughly studied in different cultivars of

Brassica juncea plants challenged with *M. phaseolina* (Srivastava and Dhawan, 1982). Increased activity of phosphatidase enzyme was observed only on susceptible cultivars, which might lead to a fact that expression levels of these pathogenicity factors are related to host susceptibility and could be used as a tool to assess both host resistance and virulence (Srivastava and Dhawan, 1982).



Fig 4: Culture of *R. bataticola*

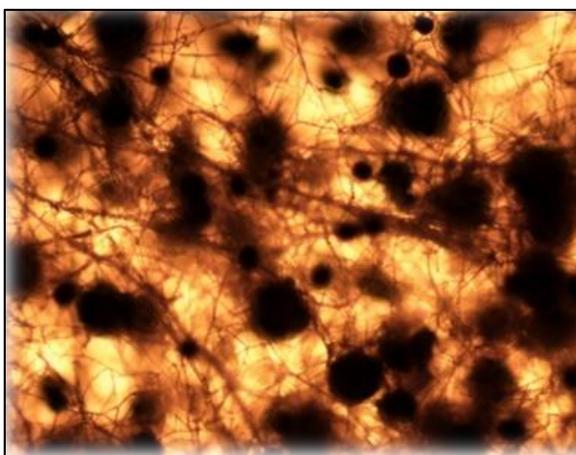


Fig 5: Microsclerotia of *R. bataticola* (10X)

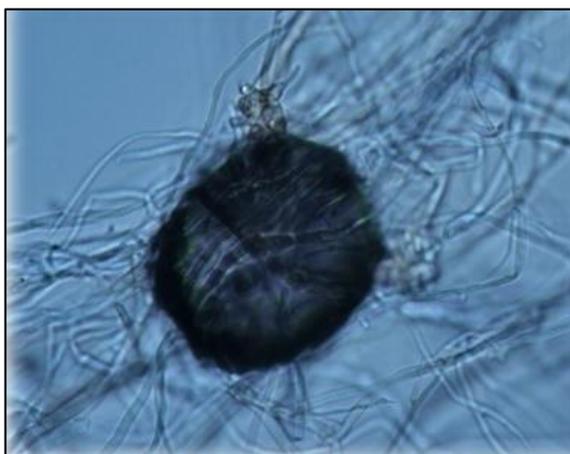


Fig 6: Enlarged view of microsclerotia (40X)

Variability among isolates of *R. bataticola*

The variation in the isolates of *R. bataticola* has been examined on the basis of several factors viz. morphological, sclerotia formation, pigmentation, pathogenic and genetic characters etc. In a study conducted, eleven isolates of *R. bataticola* from different parts of the pulse crops have shown morphological variability, including sclerotial character and pigment productions (Sundravada et al. 2011) [99]. Based on the morphological characters, the isolates were categorized into three groups such as linear, fluffy and linear growth with

fluffy mycelial growth at centre. Mycelial growth rate was also classified as fast, moderate and slow. Similarly, Sobti and Sharma (1992) [87] have categorized seven isolates of *R. bataticola* into various groups viz. black in centre and white in periphery, fluffy whitish with black periphery, charcoal black, grayish and submerged, with a black centre.

The degree of production of sclerotia is directly related with pathogenicity of isolates. Based on sclerotial formation, the isolates are categorised as abundant, moderate and less sclerotia forming isolates. In an experiment, Jain et al. (1973) [40] reported that the size of the sclerotia was found to be maximum in isolates collected from soil and stem. *R. bataticola* isolated from *Gliricidia* recorded the production of biggest sclerotia with a mean diameter of 101.51µm whereas cowpea isolate produced the smallest sclerotia with 66.88 µm mean diameter (Byadgi and Hegde, 1985) [16]. RAPD-PCR has been successfully used to identify strains and races in phytopathogenic fungi and also been used for studying inter- and intraspecific variability among populations from same and/or different geographic regions (Williams et al. 1990) [105]. The RAPD pattern analysis facilitates to identify variations at the DNA level and thus suitable for differentiation of *M. phaseolina* isolates below species level (Franco et al. 2006) [27]. PCR-based DNA fingerprinting, particularly with short oligonucleotide primers, has been used by various researchers for the analysis of genetic variation in plant pathogens (Purkayastha et al. 2006) [72].

Several people have researched the genetic variability of *M. phaseolina* to work out the pathogenic and genetic patterns of diversity and genetic specialization (Jain et al. 1973; Almeida et al. 2003; Janar et al. 2003) [40, 7, 41]. Further, fine tuned characterization of *R. bataticola* and more improvement in pulse root rot resistance and varietal development strategies needed to be done. The experimental research such as morphological, pathological and genetic variability in *R. bataticola* isolates from pulse crops, significantly determines the effective, future management practices.

Management of the pathogen

Several scientists have been attempting to manage the dry root rot pathogen. However, the disease can be managed to a large extent by use of fungicides, host plant resistance, biological means integrated with other management strategies (Majumdar et al. 1996; Cardoso et al. 1997; Mandhare and Suryawanshi, 2009) [49, 18, 51].

Development of host plant resistance is an important method in combating pathogen attack. However, resistance to DRR in chickpea has been found to be ineffective till date, as none of the lines showed consistent resistant reactions to the disease. Many researchers attempted for screening various germplasm and breeding lines (Gurha et al. 2003; Ashraf et al. 2005) [62, 10]. However, only four genotypes (GBM-2, GBM-6, GCP-101, and ICCV-10) were found to be disease tolerant. Moreover, few resistant sources for DRR have also been reported by several researchers (Baker and Ahmed 1991; Gangwar et al. 2002; Prajapati et al. 2003; Pande et al. 2006; Gupta et al. 2012b; and Khan et al. 2013) [13, 39, 33, 28]. Some of the germplasm lines such as ICCV 08305, ICCV 05530, ICCV 05532 and ICCV 05529 have displayed a moderate level of resistance to DRR.

Earlier reports reveal that biocontrol agents like *Pseudomonas fluorescens* effectively colonize and reduce the germination of sclerotia of *M. phaseolina* and is considered to be a potent tool agent against dry root rot fungus (Srivastava et al. 2001) [13]. Other bioagents such as *T. viride*, *T. harzianum*, *B.*

subtilis and certain botanicals have also been found to have a profound effect in management of same pathogen in chickpea or other crops (Singh *et al.* 1998; Ahamad and Srivastava, 2000; Ray and Mukerjee, 2002; Naik *et al.* 2009).

Haram *et al.* (1996) ^[36] reported that *T. harzianum* is an efficient biological agent and is commercially produced for management of several soil borne pathogenic fungi like *R. bataticola*, *Pythium* sp. *Fusarium solani* and *F. oxysporum*. Other mechanisms have also been suggested as responsible for their biocontrol activity, which include antibiosis, mycoparasitism, competition for space and nutrients, secretion of chitinolytic enzymes, and production of inhibitory compounds.

Parakhia and Vaishnow (1986) ^[69] reported that treatment of chickpea seeds with *T. harzianum*, significantly reduced infection levels by 18%. Applying same antagonist as soil drench, reduces disease levels considerably by 28%, however addition of wheat husk bran gave infection levels of 14% compared to 70% in the untreated control. Kumar and Khare (1990) ^[40] tested the antagonistic efficacy of soybean with *R. bataticola* and *Sclerotium rolfsi*. It was found that the population level varied due to the antagonistic activity of *T. harzianum* and *B. subtilis*. Dry root rot disease can be reduced significantly by coating chickpea seeds with certain isolates of *Bacillus* and *Streptomyces* spp. (Singh and Mehrotra, 1980) ^[77]. A significant control of DRR was obtained by organic amendment of soil with various cereal straw *i.e.* wheat straw, maize straw, and sorghum straw (Singh D, 1976).

Singh *et al.* (1992) reported that integrated application of various chemicals resulted in better control of DRR in chickpea compared to single applications. Higher yield was obtained upon pretreatment of seeds with carbendazim + Thiram, followed by 2 sprays of Endosulfan or monocrotophos.

Singh *et al.* (2003) ^[34] reported the efficacy of various biocontrol agents such as *T. harzianum*, *T. viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Gliocladium virens* in controlling DRR pathogen both *in vivo* and *in vitro*. Amongst all the bioagents tested, *T. harzianum* recorded highest control of the pathogen.

Chickpea seeds on treatment with carbendazim, thiophanate methyl and vitavax reduced the incidence and severity of DRR of chickpea significantly over untreated check (Taya *et al.* 1990; Bhardwaj 1995; Singh & Sindhan 1998; Rathore & Rathore 1999; Sharma & Gupta 2004) ^[101, 14, 89, 90, 75, 82]. The combined use of host resistance with fungicide treatment resulted in better seedling emergence and delayed the onset of root rots. Gurha *et al.* (2003) ^[34] reported that treating seeds with captan or thiram is also helpful in reducing the disease. In addition to it, seed treatment with bavistin and thiram reduced the DRR incidence in central and southern parts of India (Ghosh *et al.* 2013) ^[31].

Goyal and Mehrotra (1981) ^[32] found Benlate, Bavistin, Thiophanate methyl and Dyrene to be promising among the nine tested fungicides against *R. bataticola* in chickpea *in vitro*. Venomyl and Bavistin were reported to be most effective in inhibiting fungal growth *in vitro*. In another study, Sarwar and Raju (1985) ^[78] stated that the Topsin M-70 was highly effective at a concentration of 1000 ppm against *R. bataticola*. However, Taya *et al.* (1990) ^[101] stated that effective management of *R. bataticola* can be done by single application of carbendazim or in combination with thiram as seed treatment, soil-drench and seed treatment plus drenching.

reported that several fungicides viz. Thiram, Campton, PCNB, Mancozeb, Iprodione, Carboxine, Carbendazim, Thiabendazole etc. at concentration level of 0.2% and Tridemorph (0.07%) effectively controlled the growth and sclerotial germination of *R. bataticola in vitro*. Thiobendazole and Carbendazim were found to be most effective and inhibited sclerotial growth at concentration level of 0.006%.

Singh and Mehrotra (1982) ^[93] reported the cultivars BG-203, G-543, and Hare Chhole to show resistance against *R. bataticola* when grown in infested soil. Also, Singh and Mehrotra, (1980) ^[77] studied the effect of biological control of *R. bataticola* in chickpea and reported 4 bacteria and 6 actinomycetes isolates, proved to be antagonistic in culture. All the bacterial isolates were found to reduce the disease severity and facilitate plant growth promotion with exception to few actinomycetes.

Ved Ratan *et al.* (2010) ^[74] reported that change in sowing date could act as an efficient and economic approach towards management of dry root rot and wilt diseases of chickpea. Vijay-Mohan *et al.* (2006) ^[103] reported that DRR could be managed by using carbendazim (0.2%) and etaconazole (0.1%) as soil drenching, seed treatment and seed treatment altogether with soil drenching.

Conclusion

The agriculture in our country is constantly facing severe threat due to increasing incidence of pests and diseases. The production of foodgrains is still not sufficient to meet the demands of the growing population. In addition to it, diseases alone cause 26% of the total annual crop losses. Despite the extensive use of chemicals and various other management strategies, the losses due to diseases still withstand. So, study of the pathogen on various aspects such as morphology and genetic makeup is needed so that proper management practices could be designed to combat the losses. The pathogen illustrates a great morphological variation amongst its isolates, thus rapid and cost effective techniques should be developed for identification, characterization, screening and monitoring of pathogenic and nonpathogenic isolates. AFLP is used for study of the genetic variation of *R. bataticola* isolates. These markers have been employed to expose cryptic genetic variation of strains, or closely related species, which would be impossible to figure out with morphological or other molecular mechanisms (Sharma *et al.* 2009) ^[83].

For effective management of the pathogen, time and distribution of pathogen should be thoroughly studied so that suitable approaches may be developed prior to disease attack. The knowledge of time of pathogen attack and its overwintering will possibly facilitate the growers to apply prophylactic measures at correct time in order to minimize crop losses. Identification of traits responsible for resistance against dry root rot will enable the breeders to develop resistant varieties against the pathogen. Gene expression analysis and identification and characterization of novel pathogenicity genes in *R. bataticola* will help in understanding the pathogenesis. Study of the enzymes will definitely aid in understanding the infection process or pathogenic potential of *R. bataticola*.

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