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Recent Advances in The Management of Devastating Fungus *Sclerotium Rolfsii*

M.K. Pandey[1], A.B. Rai, R.N. Prasad and P.S. Naik*

Sclerotium rolfsii Sacc. (Teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) is one of the devastating, cosmopolitan, ubiquitous, noxious, notorious, serious soil-borne and omnivorous pathogen with a diversified host range including both monocotyledonous and dicotyledonous plants encompassing more than 500 host species (Aycock, 1966; Punja, 1985). Blclcl *et al.*, 1994 reported that *S. rolfsii* prevail at 95 % in the pea nut fields and produced on average 8.6-21.9 sclerotia/700 g soil samples, and 2.8-3.5 loci/30 mitre (m) peanut rows in Adana province of Turky. Sclerotium (pl. sclerotia), similar word 'sclerosis' is used in medical sciences which is of greek origin word 'sklçrôsis' means 'abnormal hardening of body tissues'. Sclerotium is a hard, compact, dark coloured resting body resistant to unfavourable environmental conditions, which may remain dormant for long periods of time and germinate on the return of favourable conditions. Sclerotium is formed by many fungi like, *Rhizoctonia, Sclerotinia* and *Claviceps* etc., whereas formation of Sclerotium (pl. sclerotia) is a typical/basic character in *Sclerotium* for which the same word has been given to this genus. The major difference between sclerotia of *Rhizoctonia solani* and *S. rolfsii* is differentiation, differentiation of *R. solani* into a rind and a medulla while, differentiation of sclerotia of *S. rolfsii* into a rind a cortex and a medulla (Singh, 1982). This

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fungus is mostly facultative parasite, growing saprophytically after killing their host. The pathogen has white thread like mycelium and tan to dark brown sclerotia produced on the colony (fig.). The pathogen does not produce inoculum for secondary infection in the neighboring plants in the same season (Fry, 1982). It become severe only when large inoculum is present in the soil. Root diseases caused by *S. rolfsii* generally become severe in moist soil at warm temperature (Cook and Papendick, 1972). Due to wide range, crop rotation is not an adaptable cultural practice that is usually recommended for other soil-borne diseases. Sclerotia formed on undecomposed tissues in the field are capable for initiating infection and serve as the primary inoculum of the disease in the field (Punja and Grogan, 1982).

Peter Henry Rolfs in 1892 first time reported this fungus on tomato in Florida causing more than 70 % loss (Aycock, 1966). While, the binomial of Rolfs fungus (*Sclerotium rolfsii*) was first time ascribed by Saccardo (1913) to include those fungi with no known sexual state but formed small, tan to dark-brown spherical sclerotia (0.5 to 2 mm diameter) comprised of rind, cortex and medulla. The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years associated with the prolific growth rate of the fungus (2-3 cm per day in culture) make it well-suited facultative and a pathogen of major importance throughout the world. Despite work on several aspects of this fungus for over hundred years, many basic facts about the pathogen remained to be understood such as biology and role of basidiospores of this fungus in disease development, role of different parts of *Cyperus rotundus* and compound from *C. rotundus* which is responsible for basidial stage induction. Recently, some workers (Harlton *et al.*, 1995; Nalim *et al.*, 1995; Okabe *et al.*, 1998) have studied the variability in this fungus taking mycelia as the experimental tool, but involvement of basidiospores in variability of this fungus has not yet been thoroughly explored. In addition to collar rot infection, report on this fungus causing spotted leaf rot disease (fig.) in many taxonomically unrelated plant species is also available in literature (Singh and Pavgi, 1965). Several hypothesis exist on the possible functions and use of sclerotial exudate during sclerotial development without conclusive

reasons. Polyphenoloxidase (PPO) production by the pathogen is reported earlier (Punja and Damiani, 1996). But its involvement in pathogenicity affecting the other plant phenolic is not clearly understood which unequivocally needs further study.

Despite continuous research over the past one century, this pathogen continues to plague growers causing considerable economic loss. Management efforts have often met with limited success, due to the extensive host range, prolific growth and ability to produce large number of sclerotia that may persist in the soil for several years. Furthermore, control measures effective for a particular crop or area may not be adaptable elsewhere due to regulatory or economic constraints. Several fungicides effectively controlled this pathogen on various crops in the field (Brown and Hendrix, 1980, Punja *et al*., 1982), but the major limitations to their widespread use to control this pathogen are requirement of large amounts of chemicals, variable effects on different crops, and variation in results in different seasons. In this chapter we only discussed about management aspect of this fungus since other aspects like ultrastructure and morphogenesis of sclerotia, soil biology, pathogenesis and disease development have been reviewed by (Aycock, 1966, Punja, 1985, Sarma *et al*., 2002 and Arunasri *et al*., 2011). The management of this fungus classified into i. Cultural management, ii. Biological management, iii. Chemical management, iv. Integrated management.

Cultural management

Fertilizer and manure

In case of fertilizer, mechanism of nitrogen (N) action, both in form and level, was studied *in vitro* and it was shown that lower levels of NO_{3-} or NH_4 (50 ppm) were inhibitory, while higher levels were directly toxic to sclerotial germination. Effect of NO_{3-} were not dependent on soil pH. A wide variety of nitrogenous substances including inorganics like NH_3, NH_4NO_{3-}, $Ca(NO_3)_2$ and organics like chitin, peptone and ammonium acetate affect germination. Such inhibition of germination was attributed to a) build up of antibiotic producing bacteria in the mycosphere, b) release of NH_3 that temporarily raised soil pH to above 8.5 and caused direct toxicity and c)

direct toxicity of the ions (Sen, 1983). Use of farm yard manure reduces root rot of *Cyamopsis psoraloides* and chick pea. While Sahni *et al.*, 2008 found significant protection of chick pea from *S. rolfsii* after use of vermicompost.

Aqueous extract *in vitro* and some crop residue in soil appeared to inhibit the growth of the pathogen in soil. Alfalfa hay distillate containing volatiles like methanol, acetaldehyde, isobutyraldehyde and isovalerldehyde stimulated germination-lysis in the absence of the host. Decomposition of some crop residue like alfalfa hay, legume residue and oilcakes like those of groundnut and *Sesamum indicum* released NH_3 that was directly toxic to sclertia.

Use of micro-elements and macro-elements

Management of diseases caused by *S. rolfsii* through application of micro element calcium and macro element, nitrogen in different forms has been described by many workers (Punja, 1989). The earliest observations on the influence of nitrogenous fertilizers on development of *S. rolfsii* were made by Leach and Davey (1942) who showed that heavy post-plant applications of anhydrous ammonia and ammonium sulphate reduced disease on sugar beet from 30% to 7.6 and 9.2%, respectively. Similarly, Punja and Grogan (1982) have shown that extremely low doses of ammonium nitrogen compounds provided a high level of control on the disease in golf greens. Reduced susceptibility of hosts to the pathogen following application of calcium compounds is mediated through host as calcium forms crystals of insoluble calcium oxalate results from the sequestering of oxalic acid produced by fungus (Punja *et al.*, 1985). Among the various calcium compounds, calcium nitrate proved effective as it reduced disease intensity up to 77% in carrot (Punja *et al.*, 1986). However, under high inoculum levels and disease pressure, calcium nitrate was found to be ineffective (Worley and Morton, 1964).

Soil solarisation

Soil solarisation show reduction in inoculums of several pathogens including *S. rolfsii*. Perhaps, Jones *et al*., 1966 were first to demonstrate the covering soil with black plastic film of 1.5 mm thickness reduced southern blight of tomato. Mulching

with polythene, grass cuttings, coconut fronds and stones appear to reduce root rot in beans, but for some treatments disease incidence increases at a later stage. It was also shown that alternate wetting and drying of soil led to the release of nutrients from sclerotia that were later colonized by bacteria inhibitory to their germination.

Biological management

Biological management of *S. rolfsii* mainly includes fungi and bacteria. Among the fungal bioagents, *Trichoderma* and *Gliocladium* species are the major antagonists which are ubiquitous in natural soil Papavizas, 1985. Antibiosis and mycoparasitism by these fungi are the major characteristics through which they reduce the inoculums of *S. rolfsii* (Elad *et al.*, 1982, Mukherjee *et al.*, 1995, Upadhyay and Mukhopadhyay, 1986). During antibiosis, both volatile and nonvolatile secondary metabolites are implicated in restricting the vegetative growth of pathogenic fungi (Claydon *et al.*, 1987, Dennis and Webster, 1971, Upadhyay and Mukhopadhyay, 1986). *Trichoderma* spp. attack the pathogen by excreting lytic enzymes including â-1,3-glucanase(s), protinase(s) and chitinase(s), enabling them to degrade host cell walls and thus reduce inoculum potential (Chet *et al.*, 1993, Geremia *et al.*, 1993, Goldman *et al.*, 1994). Elad *et al.* (1982) found that *Trichoderma* isolates produces chitinases and glucanases when grown on live mycelium of *S. rolfsii*.

Numerous reports reveals in literature citing effective control biocontrol of *S. rolfsii* by *Gliocladium virence in vitro* as well as under field conditions (Pierto *et al.*, 1993, Lumsden *et al;.*, 1992, Mukherjee *et al.*, 1995, Wilhite and Straney, 1996). The main mode of action of the biocontrol agent is parasitism and antibiosis. The biocontrol agent colonize sclerotia of *S. rolfsii* immediately when it comes in contact with sclerotia and penetrate to the inner layers of sclerotia where it sporulates (Mukherjee *et al.*, 1995). Similarly, the biocontrol agent produce chitinolytic enzymes that include chitin 1,4- â-chitobio and glucan N-acetyl1- â-D-glucosaminidase and glucan 1,3- â-glucosidase, known to be deleterious for *S. rolfsii* (Pierto *et al.*, 1993). Similarly, the biocontrol agent also produces an antibiotic, gliotoxin which is inhibitory to *S. rolfsii* at 50 µg/ml. Several compounds like some mixtures of fatty acids, viridian, dimethyl

gluiotoxin, viridiol, mixture of phenolic acids like ferulic acid are also detected in the culture filtrate of biocontrol agent. But inhibitory effect of all the compounds is not the same and depends on the interacting pathogen and the biocontrol agent isolate (Lumsden *et al*., 1992).

Anitha Chowdary (1997) isolated mycoflora from the rhizosphere of bell pepper antagonistic to *S. rolfsii* which included *Aspergillus flavus, A. niger, Cladosporium* sp., *Fusarium* sp., *Pencillium* sp., *Rhizopus* sp., and *Trichoderma viride*. The rhizosphere mycoflora of ginger *viz*., *Rhizopus* sp., *Aspergillus carneus, A. niger, A. fumigatus, A. flavus* (Sclerotial stage), *Eupenicillium javanicum*, *Eupenicillium javanicum* and four isolates of bacteria were found to be antagonistic to *Pythium aphanidermatum*. Pandey and Upadhyay (2000) isolated rhizosphere fungi of pigeon pea on Martin medium *viz*., *Aspergillus niger, A. fumigatus, Penicillium* sp., *Fusarium udum*, *Pythium* sp., *Rhizopus* sp., *Trichoderma harzianum*, *Gliocladium virens* and bacteria were isolated on soil extract agar medium which included 3 isolates B_1, B_2 and B_3. Kishore *et al*., 2005 reported *Pseudomonas aeruginosa* inhibits the plant cell wall degrading enzymes of *S. rolfsii* and reduces the severity of ground nut stem rot. He also observed 393 ground nut associated bacterial strains, applied both as seed treatment, soil amendment, for control of stem rot disease of ground nut in control environment. Twelve strains significantly reduces the incidence of stem rot, rot of which ground nut seed endophytes *P. aeruginosa* GSE 18 and GSE 19 reduces the seedling mortality by 54% and 58%, compared to control. In dual culture the 12 biocontrol strains reduces the mycelial growth of *S. rolfsii* by 32% - 74% as compared to the control. Cell free culture filtrate of *Pseudomonas aeruginosa* GSE 18 and GSE 19 inhibited the activity *in vitro* of the cell wall degrading enzyme (CWDE) polygalacturonase and cellulose by *S. rolfsii* up to a maximum of 55% and 55%, respectively, when measured 6 days after inoculation. *Pseudomonas aeruginosa* GSE 18 and GSE 19 with a known tolerance to thiram, a commonly used seed dressing fungicide suppressed the growth of *S. rolfsii* inhibited the activity of CWDE and reduced the incidence of stem rot. Anahosur (2001) isolated antagonists like *Trichoderma* sp., *Gliocladium virens* and *Pseudomonas fluorescens* from rhizosphere soil of sunflower

infected with *S. rolfsii*. The potential for the use of fungal antagonists as bio-control agents of plant diseases was suggested more than 70 years ago by Weindling (1932), who was the first to report the parasitic activity of *Trichoderma* spp. against *Rhizoctonia solani* and *S. rolfsii*. Morton and Stroube (1955) screened the antagonistic fungi bacteria and actinomycetes against *S. rolfsii* of soybean by dual culture technique. Muthamilan and Jeyarajan (1992) tested antagonism of *T. viride, T. harzianum* isolates and *Laetisaria arvalis* against *S. rolfsii* by dual culture on Potato Dextrose Agar medium. They observed a reduction of 68.7 per cent in sclerotial production in presence of *T. viride* isolate 2. In dual culture, out of 11 isolates of *T. harzianum*, three isolates viz., T8, T10 and T2 were found effective against groundnut isolate of *S. rolfsii* and they over grew the pathogen up to 79-92 per cent (Kumar and Sen, 2000). Dutta and Das (2002) studied the antagonistic potential of *T. harzianum, T. viride* and *T. koningii* against tomato isolate of *S. rolfsii* by dual culture technique. They observed that the three antagonists reduced the growth of the pathogen by 61.5, 59.1 and 57.2 per cent, respectively and sclerotial production by 94.2, 86.8 and 84.1 per cent, respectively. Rajalakshmi (2002) observed 37.5 to 41.3 per cent reduction in growth of mycelium of groundnut, tomato and crossandra isolates of *S. rolfsii* by *T. viride* and *T. harzianum*. Rajalakshmi also observed significant reduction in sclerotial production by the antagonists in dual culture. Pant and Mukhopadhyay (2001) studied the mechanisms of biocontrol viz., antibiosis, competition, mycoparasitism or other form of direct exploitation of *G. virens* and *T. harzianum* on *S. rolfsii*. They observed infrequent coiling of hyphae of *T. harzianum* around *S. rolfsii* hyphae resulting in coagulation of protoplasm and shrunk hyphae leading to lysis. Dube (2001) described the mechanisms of disease suppression by rhizobacteria. He stated that rhizobacteria antagonize soil borne pathogens through production of antibiotics or lytic enzymes (chitinase) and through competition for nutrients, notably iron as well as by inducing systemic resistance in the plant against subsequent infection by pathogens. Rakh *et al* (2011) reported effective biocontrol system for management of stem rot disease caused by *Sclerotium rolfsii* in groundnut, 11 *Pseudomonas spp.* isolated from rhizospheric soil, were evaluated for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium

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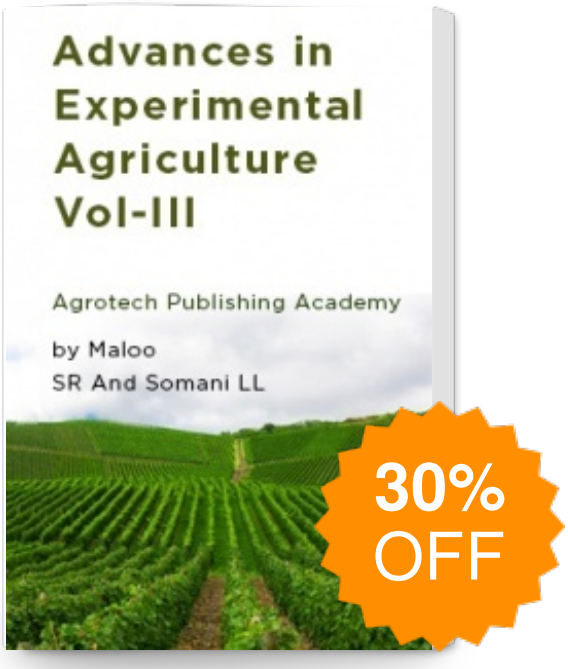

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