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COMPETITIVE COLONIZATION OF WHEAT STRAW BY *TRICHODERMA* SPECIES AND *SCLEROTIUM ROLFSII*

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Abstract

Trichoderma harzianum and *T. pseudokoningii* inhibited the growth of *Sclerotium rolfsii In vitro* and produced coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae. *Trichoderma polysporum* and *T. virens* also inhibited the growth of *S. rolfsii* but there was no coiling around its mycelium. *In vitro* interactions of *S. rolfsii* with microbial antagonists in dual culture assay corroborate well with competitive colonization of wheat straw by *Trichoderma* spp., and *Sclerotium rolfsii*. *Trichoderma* spp., colonized the straw more rapidly than *S. rolfsii,* over grew the straw pieces colonized by *S. rolfsii* and gradually eliminated the pathogen from the straw pieces.

Introduction

Plant diseases play a direct role in the destruction of natural resources in agriculture. Fungal phytopathogens are wide spread in the global ecosystem. Some of the soilborne plant pathogenic fungi produce sclerotia in order to survive for long periods under unfavourable conditions. These sclerotia show high resistance to chemical and to biological degradation (Coley-Smith & Cooke, 1971; Agrios, 2005). *Sclerotium rolfsii* Sacc., is an important soilborne pathogen. Ahmed *et al*., (1984) made the first report of *S. rolfsii* from Pakistan on maize (*Zea mays* L.). The fungus was subsequently reported from oat (*Avena sativa* L.) and mash bean (*Vigna mungo* (L.) Hepper) by Shahzad & Ghaffar (1995), apple (*Malus sylvestris* L.) by Jahangir *et al*., (1995) and sugarbeet (*Beta vulgaris* L.) by Ruqia (2001). Yaqub & Shahzad (2005) reported the pathogenicity of *S. rolfsii* on various crops in artificially infested soil.

Chemical compounds are commonly used to control plant diseases, however, chemical methods are not economical in the long run because they pollute the atmosphere, damage the environment, leave harmful residues and can lead to development of resistant strains among the target organisms with repeated use (Naseby *et al*., 2000). Of the various methods used for the control of plant diseases, biocontrol agents have provided a very promising and less hazardous method for the control of plant diseases. *Trichoderma* spp., common saprophytic fungi found in almost any soil and rhizosphere, have been investigated as potential biocontrol agents because of their ability to reduce the incidence of diseases caused by plant pathogenic fungi particularly many common soil-borne pathogens (Spiegel & Chet 1998; Elad, 2000; Freeman *et al*., 2004; Ashrafizadeh *et al*., 2005; Dubey *et al*., 2007; Vinale *et al*., 2008). The present report gives an account of the competitive colonization of wheat straw by *Trichoderma* species and *Sclerotium rolfsii*.

Materials and Methods

Cultures of *Sclerotium rolfsii* (isolated from sugarbeet) and *Trichoderma* species *viz*., *T. harzianum T. pseudokoningii, T. polysporum* and *T. virens* (isolated from soil) were obtained from Karachi University Culture Collection (KUCC). Pure cultures of microorganisms were prepared on potato sucrose agar (PSA).

Interaction of *S. rolfsii* with *Trichoderma* species in agar plates: In dual culture plate assays, a 5 mm diam., inoculum disc of *S. rolfsii* was placed near the edge of a Petri dish containing (PSA) medium. A similar inoculum disc of a test fungus was placed at the opposite end of the Petri dish. There were 6 replicates of each treatment. Plates were incubated at 25°C and colony diameters of the pathogen and the test organisms were recorded at 24 h intervals.

Interaction of *S. rolfsii* with *Trichoderma* species on wheat straw: Wheat straw was cut into six inch long pieces and soaked in water for 1 hour. Each piece was transferred separately into an 18x180 mm test tube and sterilized at 15 psi for 20 minutes. For each *Trichoderma* species, test tubes were divided into 3 sets of 21 test tubes each. In one set, mycelium of *S. rolfsii* was inoculated at one end of each straw piece using a flame sterilized inoculation needle. The second set was inoculated with a *Trichoderma* species, whereas, in the 3rd set, *Sclerotium rolfsii* was inoculated at one end and the *Trichoderma* species at the opposite end of the same straw piece. The straw pieces were incubated at room temperature and after each 24 h interval, 3 test tubes for each treatment were used to assess the growth of the pathogen and microbial antagonist on wheat straw. Each wheat straw piece was cut into 1cm long pieces and plated on water agar amended with rose bengal @ 0.1 g L^{-1}. The plates were incubated at room temperature for 5 days and extent of colonization of wheat straw by *S. rolfsii* and *Trichoderma* species were recorded.

Results and Discussion

Of the microbial antagonists tested in dual culture plate assays, *T. harzianum* and *T. pseudokoningii* showed inhibition in growth of *S. rolfsii* and produced coiling around its mycelium. *T. polysporum* and *T. virens* also inhibited the growth of *S. rolfsii* but no further growth and coiling were observed. There are reports where the growth of *S. rolfsii* was inhibited by *T. harzianum* (Henis, 1984; Adandonon, 2000). *T. koningii* (Latunde-Dada, 1993), *T. longibrachiatum* (Sreenivasaprasad & Manibhushanrao, 1990) and *T. virens* (Sreenivasaprasad & Manibhushanrao, 1990; Mukherjee & Raghu, 1997). Results of the present studies appear to be the first report of inhibition of *in vitro* growth of *S. rolfsii* by *T. polysporum.*

Results of competitive colonization of wheat straw by *S. rolfsii* and *Trichoderma* spp., were correlated with the interaction of microbial antagonists with the pathogen in dual culture assays. *T. harzianum, T. pseudokoningii, T. polysporum* and *T. virens* showed 100% colonization of wheat straw after 5 days when inoculated alone (Fig. 1). Similarly, *S. rolfsii,* when inoculated alone colonized the straw pieces after 6 days incubation (Fig. 1).

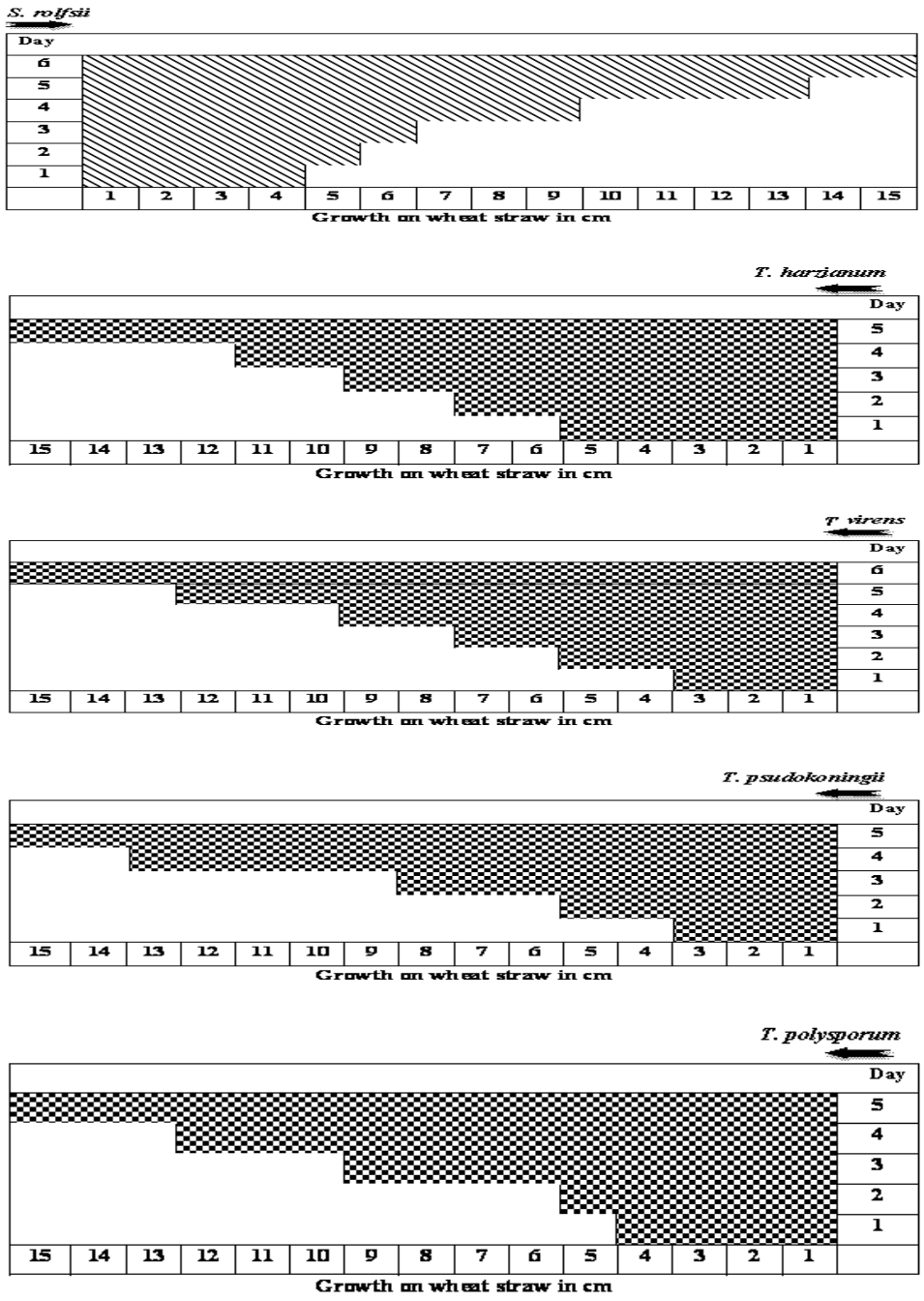

Fig. 1. Growth of *S. rolfsii* and *Trichoderma* species on wheat straw in single inoculation.

In double inoculation, *S. rolfsii* showed 26% colonization of wheat straw after 3 days of inoculation whereas *T. harzianum* colonized 53% of wheat straw. It was interesting to note that *T. harzianum* gradually colonized up to 90% of wheat straw whereas colonization of *S. rolfsii* reduced gradually with increase in growth of *T. harzianum* and after 6 days, *S. rolfsii* was isolated only form 7% of the wheat straw pieces (Fig. 2). Other species of *Trichoderma* also showed similar effect (Fig. 2). Such growth pattern supports the results of Elad *et al*., (1983a,b) who reported that *T. harzianum* colonizes *S. rolfsii*, the hyphae of which are disrupted and die when *T. harzianum* comes in contact.

Trichoderma spp., are fungi that are present in substantial numbers in nearly all agriculture soils and in other environments such as decaying wood. Among their other activities, they grow tropically toward hyphae of other fungi, coil around them in a lactin mediated reaction, and degrade cell walls of the target fungi. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. In addition to, or sometimes in conjunction with mycoparasitism, individual strains may produce antifungal compounds like gliotoxin and gliovirin (Harman *et al*., 2004). The effectiveness of *Trichoderma* lies in a combination of competition for nutrients, production of antifungal metabolites including hydrolytic enzymes and mycoparasitism (Deshpande, 1999; Henis, 1984). *Trichoderma* spp., produce extra-cellular β–(1-3)–glucanases, chitinases, lipases, proteases and β-1-4–glucanolytic enzymes when grow on cell wall of the pathogenic fungi (Suominen *et al*., 1993; Lisboa *et al*., 2002). *T. harzianum* was able to control *Botrytis cinerarea* on grapes by colonizing blossom tissue and excluding the pathogen from its infection site (Gullino, 1992). Sivan & Chet (1989) demonstrated that competition for nutrients is the major mechanism used by *T. harzianum* to control *F. oxysporum* f.sp. *melonis*. Moreover, *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes (Benitz *et al*., 2004). The mechanisms used by *Trichoderma* such as competition, antibiosis, parasitism and systemic–induce resistance are influenced by concentration and availability of nutrients like carbohydrates in lignocellolosic substances, chitin and lipids within the soil organic matter (Hoitink *et al*., 2006., Krause *et al*., 2001). It is well documented that some strains promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al*., 2004).

It has been reported that the highest CM–cellulose and β-glucasidase activity was observed in the autoclaved substrate inoculated with *T. longibrachiatum*. When *Pleurotus ostreatus* was cultivated alone, little variation in activities among substrates was noted. When *P. ostreaus* was cultured with *T. longibrachiatum*, CM–cellulase and β–glucasidase activities in the autoclaved substrate were greater than in the other heat treatments, but the CM- cellulase activity was highest in autoclaved substrates when *T. longibrachiatum* was cultured alone (Velazqez-Cedeno *et al*., 2004). In sterilized substrate, both laccase and Mn–per–oxidase activities were increased in dual culture (Savoie *et al.,* 2001). Use of *Trichoderma* species effectively reduced the viability of sclerotia of *Sclerotium rolfsii* (Atrigues & Davet, 1984; Khattabi *et al*., 2001). Chitinases are produced by many fungi where their major role is the modification which acts as a structural component of their cell wall. They are produced by *Trichoderma* spp. These enzymes are involved in penetration of a host by mycoparasites (Inbar & Chet, 1995). The chitinases are able to inhibit fungal growth *in vitro* by causing lysis of hyphal tips in combination with the activity of β-1,3-glucanase such as *Fusarium, Pythium*, *Phytophthora*, *Verticillium*, *Rhizoctonia* and *Sclerotinia* (Baker & Cook, 1974).

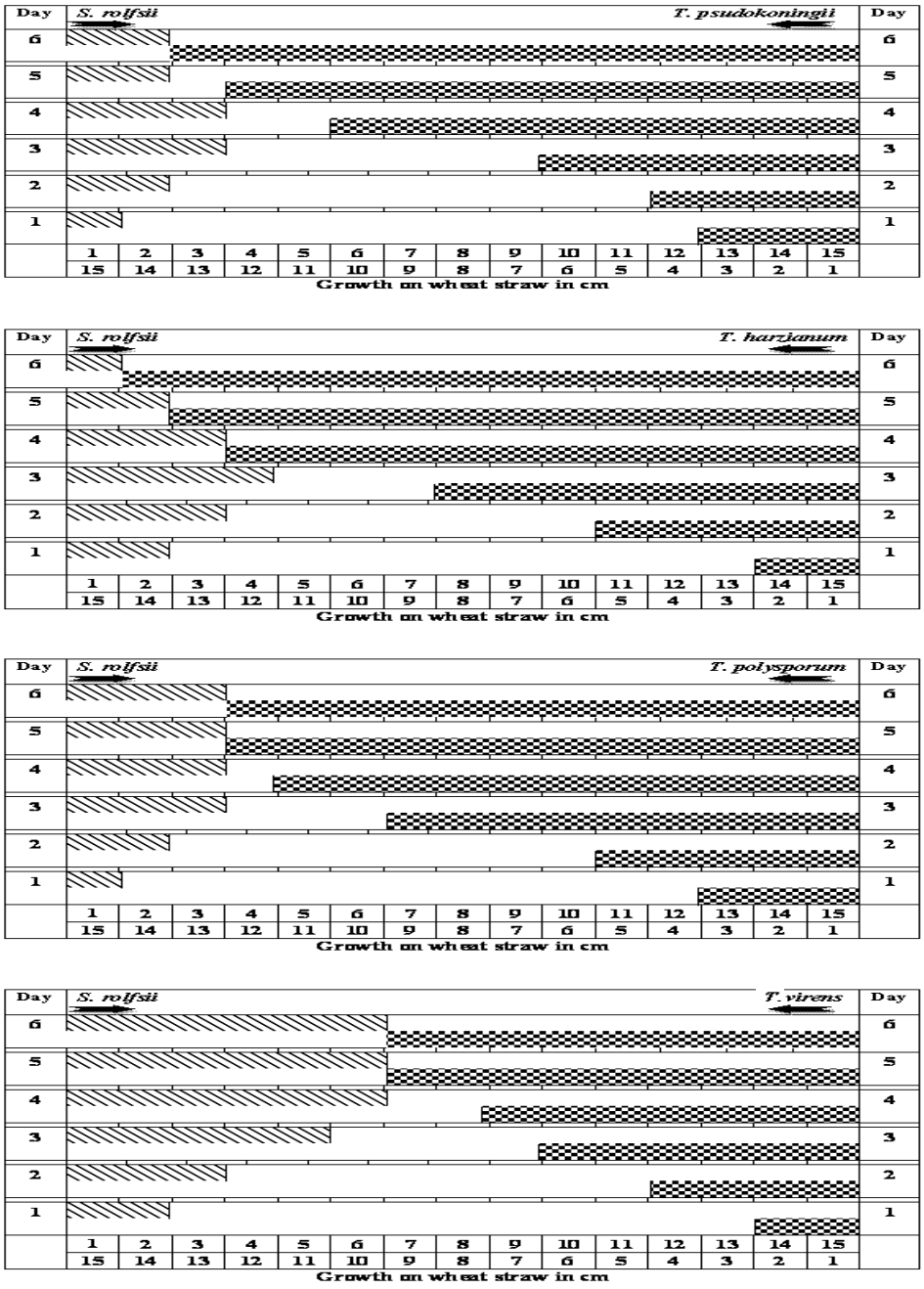

Fig. 2. Growth of *S. rolfsii* and *Trichoderma* species on wheat straw in double inoculation.

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