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Influence of Physico-Chemical and Microbiological Properties of Soil on Root Rot of Sugarbeet caused by *Sclerotium rolfsii.*

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A survey was conducted on the occurrence of sugarbeet root rot caused by *Sclerotium rolfsii* in Tamil Nadu (India) during 2008-2009. Soil samples collected from conducive and suppressive soils were tested under pot culture conditions inoculated with sclerotia of *S. rolfsii*. Sugarbeet grown in disease conducive soils such as Ooty, Thondamuthur, Pollachi, and Bhavanisagar were observed with root rot incidence of 45.9, 54.2, 37.5 and 50 per cent respectively; whereas sugarbeet grown in the experimental plot of TNAU (suppressive soil) was free from disease. The physico-chemical properties of soils showed that the conducive soils were sandy loam to sandy clay loam, non calcareous with normal pH, while the suppressive soil were clay loam, calcareous with higher pH. Actinomycete population was dominant in the suppressive soil than conducive soils.

Key words: Actinomycetes, conducive soil, microbiological and physico-chemical properties, *Sclerotium rolfsii*, suppressive soil.

Sclerotium rolfsii Sacc. (teleomorph, *Athelia rolfsii* (Curzi) Tu & Kimbrough) is a devastating soil-borne fungus with a wide host range (over 500 plant species belonging to 100 families) (Aycock, 1966). It forms brownish sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane. Among the methods employed to manage *S. rolfsii viz*., fungicide applications, soil solarization, use of antagonistic microorganisms, deep ploughing, crop rotation and incorporation of organic and inorganic residues (Rakh *et al.,* 2011), fungicides are normally recommended which are not economical and often causing pollution in the ecosystem. Because of the worsening problems in control of soil-borne diseases, a serious search is needed to identify alternative methods for plant protection. The phenomenon of disease suppressive soils has fascinated plant pathologists for decades, observed in many locations around the world, Suppressive soils are those in which a specific pathogen does not persist despite favorable environmental conditions, the pathogen establishes but doesn't cause disease, or disease occurs but diminishes with continuous monoculture of the same crop species. Both abiotic and biotic factors are responsible for the plant disease suppression (Janvier *et al.,* 2007; Ghorbani *et al*., 2008). Positive correlation between soil pH and disease suppressiveness was observed by many researchers (Sullivan, 2001; Hoper *et al.,* 1995; Jones *et al*., 1989). Application of lime (calcium carbonate) increased soil pH and reduced incidence of cavity spot (*Pythium* spp.) in carrot (Hiltunen and White, 2002). The objective of the present study includes analysis of physico-chemical and microbiological properties of soils suppressive and conducive to sugarbeet root rot caused by *Sclerotium rolfsii.*

Materials and Methods

Survey for the occurrence of sugarbeet root rot

A survey was conducted for the occurrence of root rot caused by *S. rolfsii* in sugarbeet growing areas of Tamil Nadu (India) during 2008-2009. that included Ooty, Thondamuthur, Pollachi, Bhavanisagar and the experimental plot of Tamil Nadu Agricultural University (TNAU), Coimbatore. The disease incidence was calculated by the formula

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Soil in a specific area without root rot disease was designated as suppressive soil whereas, soil observed with higher percentage of root rot disease was designated as conducive soil. Soil samples were collected separately from these soils during fallow period and brought to the laboratory for further analysis and experiments.

Pot culture experiment

Twenty centimeter dia pots were filled separately with disease suppressive soil and disease conducive soils. Upper five cm soil was challenge inoculated with sclerotia of *S. rolfsii* as described by Thilagavathi *et al.* (2012). After 90 days, the seeds of susceptible sugarbeet *cv* . Indus was sown @ 10 seeds per pot. After ten days, three seedlings per pot were maintained. Uniform soil moisture was maintained through out the study. Four replications were

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maintained for each soil. Observations were recorded for root rot incidence until 120 days. The whole experiment was repeated twice.

Analysis of physico-chemical properties of soils

The disease suppressive and conducive soil samples collected from different locations of Tamil Nadu were analyzed for their physical and chemical properties. Soil texture was analysed by the method described by Piper (1966). Soil pH and electrical conductivity (EC) were estimated with a glass electrode using a soil to water ratio of 1:1. Available N was extracted with 2M kcl for 1 h and determined by Kjeldahl method (Waring and Bremner, 1964). Available P was extracted with Olsen reagent (0.5 M NaHCO3) (pH 8.5) at soil-extractant ratio of 1:20, shaken for 30 min and quantified by molybdenum-blue colorimetry (Olsen *et al*., 1954). Available K was extracted with neutral normal ammonium acetate (pH 7.0), shaken for 25 min and measured by flame photometry (Hanway and Heidel, 1952). Organic carbon was analysed by the method described by Walkley and Black (1934).

Isolation of beneficial microbes encouraged by conducive and suppressive soils

Each disease suppressive and conducive soils were serially diluted upto 10^{-6}, finally transferred into King's B, nutrient agar and Kenknight's agar medium for the isolation of *Pseudomonas* sp., *Bacillus* sp. and actinomycetes respectively. Three replications were maintained. Colonies were identified based on their morphological characters. The number of colony forming units (cfu) were recorded.

Antagonistic potential of actinomycete isolates in suppressive soils

Actinomycete isolates of suppressive soil were tested *in vitro* against *S. rolfsii*. A nine mm mycelial disc from actively growing colony of *S. rolfsii* was placed at the centre of the Petri plate containing Kenknights medium. After 12 h of incubation, sterile Whatman No. 40 filter paper discs with six mm dia were placed equidistance at four sides centering around the fungal disc. The actinobacterial isolates were grown in nutrient broth and about 25 µl of cell suspension from 72 h old culture broth was dropped over the filter paper discs. Sterile water was used as a check (Pande and Chaube, 2004). Four replications were maintained for each isolate. Observations were made after complete growth of pathogen in the control plate. Efficiency of actinobacterial isolate on the inhibition of mycelial growth of *S. rolfsii* was assessed using the formula, Per cent inhibition over control = (C – T) / C) x100. Where, C- mycelial growth of pathogen in control, T- mycelial growth of pathogen in dual plate.

Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines.

Results and Discussion

The results from the survey revealed that the sugarbeet grown in the experimental plot of TNAU, Coimbatore was free from root rot disease, this soil was designated as suppressive soil, whereas Ooty, Thondamuthur, Pollachi and Bhavanisagar areas recorded root rot incidence of 38.8, 42.5, 26.8 and 36.3 per cent respectively, these soils were designated as conducive soils. Soil samples collected from disease suppressive and conducive soils were tested under pot culture conditions challenge inoculated with *S. rolfsii*. The results of the pot culture experiment revealed that sugarbeet grown in disease conducive soil samples of Ooty, Thondamuthur, Pollachi and Bhavanisagar registered mean root rot incidence of 45.9, 54.2, 37.5 and 50 per cent respectively, whereas sugarbeet grown in soil sample from the experimental plot of TNAU was free from root rot disease (Table 1). The analysis of physico-chemical properties showed that significant differences were observed for the soil pH, calcium level and soil texture than others. Conducive soils were sandy loam to sandy clay loam soil texture, non calcareous with pH range of 6.71 to 7.43. Whereas suppressive soil were clay loam, calcareous with high soil pH 8.30 (Table 2). Increasing soil pH or calcium levels may be beneficial for disease management in many other crops (Sullivan, 2001). Direct correlation between adequate calcium level, and/or higher pH and decreasing disease occurrence was established for tomato, cotton and melons (Jones *et al*., 1989). In the present study, disease suppressive and conducive soils varied in their soil texture, pH and calcium level (lime). So these three factors might be responsible for the disease suppressiveness or conduciveness of particular soil. The other factors not did significantly. Results from analysis of beneficial microbes in soils revealed that the actinomycetes were dominant in the suppressive soil than conducive soil (Fig.1). A total of 20 different actinobacterial isolates abtained from disease suppressive soils were screened *in vitro* against *S. rolfsii*. The results revealed that

Table 1. Root rot incidence of sugarbeet in soils of Tamil Nadu under natural field and pot culture conditions

Location	Root rot incidence of sugarbeet			
	Natural field	Pot culture conditions		
	Conditions	Trial 1	Trial 2	Mean
Ooty	38.8_{d} (38.5)	50.0_{c} (45.0)	41.7_{c} (40.2)	45.9
Thondamuthur	42.5_{c} (40.7)	58.3_{d} (49.8)	50.0_{d} (45.0)	54.2
Pollachi	26.8_{b} (31.1)	41.7_{b} (40.2)	33.3_{b} (35.2)	37.5
Bhavanisagar	36.3_{d} (37.0)	41.6_{b} (40.2)	58.3_{e} (49.8)	50.0
Coimbatore (Experimental plot of TNAU)	0.00_{a} (0.0)	0.00_{a} (0.0)	0.00_{a} (0.0)	00.00

Values are mean of four replications. Means followed by a common letter are not significantly different at 5% level by DMRT. Data in parantheses are arcsine transformed values

Table 2. Physico-chemical properties of disease suppressive and conducive soils of Tamil Nadu

Location of soil samples	pH	EC (dSm^{-1})	Texture	Lime	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	Organic C (%)
Disease conducive soil								
Ooty	6.71 (N)	0.10(NS)	Scl	NC	233.42(Low)	46.21 (High)	498.07 (High)	0.53 (Medium)
Thondamuthur	7.27 (N)	0.12(NS)	Sl	NC	128.06(Low)	50.57 (High)	317.64 (High)	0.23 (Low)
Pollachi	6.82 (N)	0.16(NS)	Scl	NC	196.45(Low)	50.32 (High)	387.11 (High)	0.27(Low)
Bhavanisagar	7.43 (N)	0.12(NS)	Sl	NC	199.63(Low)	74.91 (High)	403.20 (High)	0.39 (Low)
Disease suppressive soil								
Coimbatore (Experimental plot of TNAU)	8.30 (MA)	0.13 (NS)	Cl	C	219.00 (Low)	17.00 (Medium)	717.33 (High)	0.28 (low)

N-Normal; MA-Moderately alkaline; NS-Non-saline; Sl-Sandy loam; Cl-Clay loam; Scl-Sandy clay loam; C-Calcareous; NC-Non-Calcareous

among the different isolates, AM2 isolate recorded a maximum per cent inhibition of mycelial growth (77.8 %) followed by AM1, AM4, AM10, AM11 and AM3 with 75.2, 74.0, 72.8, 72.7 and 72.2 per cent inhibition of mycelial growth over control. Other isolates also

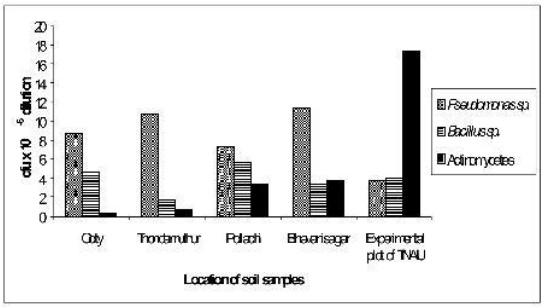

Fig. 1. Microbiological properties of different soils of Tamil Nadu

inhibited the mycelial growth in the range of 68.6 to 6.1 per cent. Similarly, maximum inhibition zone was observed in the AM2 isolate (2.0 cm) which was on par with AM1 (1.89 cm). Most of the isolates exhibited

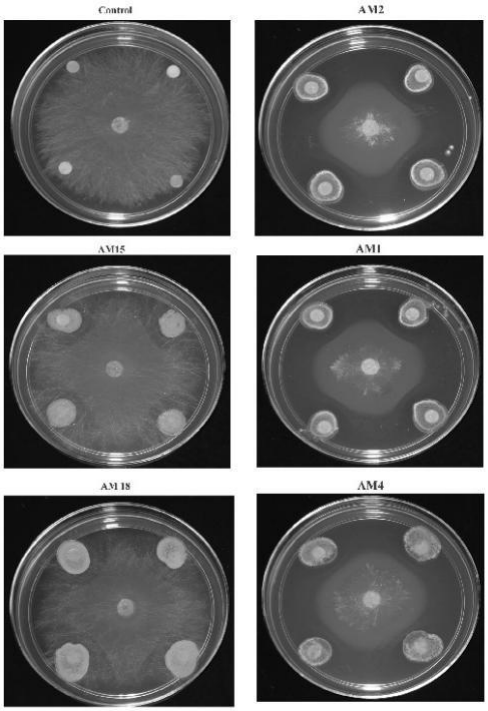

Fig. 2. Antagonistic potential of actinobacterial isolates against *S. rolfsii* under *in vitro* conditions

maximum mycelial inhibition zone against *S. rolfsii* ranging from 1.75 to 0.58 cm and some isolates did not exhibit any inhibition zone (Table 3; Fig 2). Actinomycetes, and among them many *Streptomyces* spp., are known for their antagonistic properties (Samac and Kinkel, 2001), *i.e.* by producing antifungal compounds (Chamberlain and Crawford, 1999), antibiotics and extracellular hydrolytic enzymes (Ghorbani *et al*., 2008). There were greater densities

Table 3. Antagonistic potential of actinobacterial isolates from disease suppressive soil against *S. rolfsii* under *in vitro* conditions

Actinobacterial isolates	*S. rolfsii* growth (dia in cm)	Inhibition zone (cm)	Percent inhibition over control
AM 1	2.23_{b}	1.89_{ab}	75.2_{b} (60.2)
AM 2	2.00_{a}	2.00_{a}	77.8_{a} (61.9)
AM 3	2.50_{c}	1.75_{bc}	72.2_{e} (58.2)
AM 4	2.34_{bc}	1.83_{b}	74.0_{c} (59.3)
AM 5	2.87_{d}	1.57_{d}	68.1_{g} (55.6)
AM 6	8.30_{j}	0.00_{g}	7.8_{m} (16.2)
AM 7	4.85_{f}	0.58_{f}	46.1_{i} (42.8)
AM 8	4.82_{f}	0.59_{f}	46.4_{i} (43.0)
AM 9	3.70_{e}	1.15_{e}	58.9_{h} (50.1)
AM 10	2.45_{c}	1.78_{b}	72.8_{d} (58.6)
AM 11	2.46_{c}	1.77_{b}	72.7_{d} (58.5)
AM 12	2.83_{d}	1.59_{cd}	68.6_{f} (55.9)
AM 13	7.05_{h}	0.00_{g}	21.7_{k} (27.7)
AM 14	8.40_{j}	0.00_{g}	6.7_{o} (15.0)
AM 15	6.80_{g}	0.00_{g}	24.4_{j} (29.6)
AM 16	8.45_{j}	0.00_{g}	6.1_{p} (14.3)
AM 17	8.40_{j}	0.00_{g}	6.7_{o} (15.0)
AM 18	7.35_{i}	0.00_{g}	18.3_{l} (25.4)
AM 19	8.33_{j}	0.00_{g}	7.4_{n} (15.8)
AM 20	8.38_{j}	0.00_{g}	6.9_{o} (15.2)
Control	9.00_{k}	-	-

Values are mean of four replications. Means followed by a common letter are not significantly different at 5% level by DMRT. Data in parantheses are arcsine transformed values

of actinomycetes in soils resistant to *Fusarium* wilt of banana (Peng *et al*., 1999). The present study clearly indicated that the disease suppressive soil had a dominant population of actinomycetes which was encouraged by calcium, higher pH and clay loam texture of particular soil. Hence suppressive soils with significant soil factors can be utilized for the avoidance of particular pathogen without any chemical or crop loss.

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