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BIOLOGICAL CONTROL OF *SCLEROTIUM ORYZAE* CATT., THE CAUSE OF STEM ROT OF RICE. 1. POPULATION AND VIABILITY OF SCLEROTIA IN SOIL.*

S.M. Haroon Usmani and Abdul Ghaffar

Department of Botany, The University, Karachi, Pakistan.

Abstract

A floatation technique was developed for the recovery and enumeration of sclerotia of *Sclerotium oryzae* from soil. During a study of the population of sclerotia of *S. oryzae* in soil of rice-wheat-rice or rice-fallow-rice rotations a greater number of sclerotia were observed in soil under wheat as compared to rice or fallow fields. More sclerotia were found on the soil surface with gradual decline in numbers at 6" and 9" depths. Viability of sclerotia on rice stubbles and in rice fields was much higher than the sclerotia isolated from soil under wheat crop or fallow field. Decreased viability of sclerotia at greater depths was related to a predominant colonization of sclerotia by bacteria.

Introduction

Sclerotium oryzae Catt., the cause of stem rot of rice (*Oryza sativa* L.) is known to produce considerable damage to the paddy crop. Losses as high as 72-75% have been recorded in Pakistan (Shafi, 1970) and Arkansas (Cralley, 1936) with estimates of 5-10% for the rest of the rice growing world. The damage is due to increased tillering, unfilled panicles, chalky grain and widespread lodging of the plants (Misawa & Kato, 1962, Tisdale, 1921, Cralley, 1936). The fungus survives in the form of sclerotia which are produced in large numbers on the stem of infected plants at the water level and subsequently deposited in soil during harvest or tillage operation. Survival of these sclerotia for varying periods, 133 days to 6 years have appeared in literature (Park & Bertus, 1932; Tullis & Cralley, 1941). Considering the sclerotia as infective propagules, the population of sclerotia in soil and their viability was tested. This is reported below.

Experimental Results

Population of sclerotia in soil:

Soil samples were collected from the Rice Experimental Research Station, Kala Shah Kaku near Lahore, Pakistan. Only one crop of rice is sown in a year where rice-wheat-rice and rice-fallow-rice crop rotations are generally practiced. Soil samples were obtained in March, 1973 when mature wheat crop was in the field and in November, 1973 when mature rice crop was in the field. The soil was a clay loam with a pH of 7.3-8.6. Random samples were taken from 2", 6" and 9" of the soil depths from the centre and corner of the fields.

Five g air dried soil was suspended in 500 ml of distilled water in a beaker and thoroughly stirred. After an hour the soil suspension was washed through a 30 mesh sieve on to a 80 mesh sieve to retain the sclerotia which measure 183-323u in diameter. The material collected on the 80 mesh screen was washed with a wash bottle into a 500 ml beaker, the side wall of which was lined with a strip of filter paper. After 10-15 minutes

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the heavier particles settled leaving the sclerotia floating on the surface. Instead of separating the sclerotia under vacuum (Krause & Webster, 1972), the sclerotia being hydrophobic and buoyant adhered on the strip of filter paper. After siphoning out a little water the strip of paper was removed and the number of sclerotia counted under a dissecting microscope at X20 magnification.

Number of sclerotia varied from 2-35/g of soil (Table 1). More sclerotia were found in the upper 2 inch layer of soil with a gradual decline at 6" and 9" depths. Sclerotial population was higher in soil under wheat (rice-wheat-rice rotation) as compared to soil under a standing crop of paddy and fallow (rice-fallow-rice rotation). No significant differences in the number of sclerotia were found in the centre or corner of the fields.

Viability of sclerotia:

Viability of the sclerotia collected was determined. Sclerotia were surface sterilized with 40/100, w/v Calcium hypochlorite solution for 5 minutes and rinsed in sterile distilled water were placed on 10 mm diam. disks of Potato Dextrose Agar containing Penicillin and Streptomycin each at 3000 p.p.m. They were incubated at $30 \pm 3°C$ under constant white flourescent light. The sclerotia germinated within 48 hrs. It may be mentioned that unlike Krause & Webster (1972) the production of conidophore and conidia by the germinating sclerotia was not observed.

TABLE 1. Average population of sclerotia of *Sclerotium oryzae* Catt., per g. of soil under different crop rotations.

Depth (inches)	Rotations		
	* Rice-Wheat-Rice	* Rice-Fallow-Rice	* Rice-Wheat-Rice
Corner of the field			
2	26	15	4
6	16	12	3
9	10	10	1
Centre of the field			
2	35	12	4
6	17	07	02
9	13	06	0.2

Analysis of variance

Source of variation	d.f.	M.S.
Rotations	2	1036. 78**
Position in the same rotation	3	23.64 n.s.
Depth of same position	12	155.17 **
Observations of same depths (Error)	18	27.03

* Standing crop of wheat, rice or fallow fields.

** Significant at .01 level of probability.

n.s. Not significant.

Of the sclerotia present in soil, the viability ranged from 7-18% in the wheat field, 4-10% in rice fallow field and 03-65% in the rice field (Table 2). The viability of sclerotia was low at the 6-9 inches depth as compared to those obtained from the surface. In contrast to these observations the sclerotia from rice stubbles showed 92% viability.

Micro-organisms associated with the sclerotia of *Sclerotium oryzae*:

The sclerotia are subject to physical, chemical and microbiological factors in soil which affect their survival. There are reports of the decay of sclerotia of *Sclerotinia selerotiorum*, *S. trifoliorum*, *S. borealis*, *Botrytis cinerea*, *Claviceps purpurea*, *Sclerotium rolfsji*, *S. delphinii* after colonization by soil saprophytes (Campbell, 1947, Hino & Endo, 1940; Tribe, 1967; Curl & Hansen, 1964; Ferguson, 1953; Pohjakallio et al, 1953; Makkenan et al, 1960; Ghaffar, 1973). The reduction in viability of sclerotia of *S. oryzae* as observed in the present investigation could, therefore, be due to the effect of antagonistic micro-organisms. The association of micro-organisms with the sclerotia of *S. oryzae* in soil was therefore investigated.

Sclerotia of *S. oryzae* isolated from soil were transferred to Potato Dextrose Agar disks without surface disinfection and incubated at 30°C. PDA containing Penicillin and Streptomycin each at 3000 p.p.m. was used for the isolation of fungi and PDA adjusted to pH 7.0 was used for the isolation of bacteria and actinomycetes. Of the isolates, the bacteria and actinomycetes were found to be predominantly associated with the sclerotia

TABLE 2. Average percentage viability of sclerotia of *Sclerotium oryzae* Catt., in soil under different crop rotations.

Depth (inches)	Rotations		
	* Rice-Wheat-Rice	* Rice-Fallow-Rice	* Rice-Wheat-Rice
Corner of the field			
2	18	8	65
6	12	7	52
9	07	5	14
Centre of the field			
2	16	10	64
6	16	7	35
9	09	4	03

Analysis of variance

Source of variation	d.f.	M.S.
Rotations	2	3808.69 **
Position in the same rotations	3	44.47 n.s.
Depth of same position	12	526.30**
Observations of same depths (Error)	18	23.35

*Standing crop of wheat, rice or fallow fields

**Significant at .01 level of probability.

n.s. Not significant.

TABLE 3. Percentage colonization by micro-organisms of sclerotia of *Sclerotium oryzae* Catt., in soil.

Micro-organisms	Rice-Wheat-Rice*						Rice-Fallow-Rice*					
	Corner			Centre			Corner			Centre		
Depth (inches)	2	6	9	2	6	9	2	6	9	2	6	9
Alternaria sp.	0.6	0	2	0	1.2	0	2	1	5	5	0	0
Aspergillus flavus	9	6	4	2.6	4	5.7	2	6	1	9	8	0
Aspergillus niger	2.6	0	2	6.6	0	2	0	1	4	0	4	0
Chaetomium globosum	2.6	0	0	2	0	0	6	1	2	10	4	1
Drechslera sp.	0	2	0	0	0	0	2	4	6	4	0	10
Fusarium sp.	20.6	19.3	10.6	25.4	22	14.2	13	8	12	11	12	8
Helminthosporium spp.	0	0	0	0	0	0	1	2	3	2	4	0
Penicillim spp.	5.4	3.2	2.9	2.9	3.2	4.6	2	4	0	1	1	1
Spicaria sp.	0	0	0	0	0	0	16	22	20	27	23	24
Streptomyces spp.	25.4	20	18	26.6	14.6	8	33	24	19	35	19	10
Bacteria	42	60.6	72	39.4	58	68	35	52	60	32	40	63

*Standing crop of wheat or fallow fields.

followed by species of *Fusarium*, *Spicaria*, *Penicillium*, *Aspergillus* and *Alternaria*, (Table 3). There was an indication that bacterial colonization of sclerotia was higher with increasing depth of soil while colonization by actinomycetes was more common on the sclerotia obtained nearer to the surface. Colonization of sclerotia by species of *Spicaria*, *Chaetomium* and *Dreschlera* was more frequent on the sclerotia obtained from rice fallow fields (rice-fallow-rice rotation) than from wheat fields (rice-wheat-rice rotation).

Conclusion

Population of sclerotia of *S. oryzae* under a standing crop of wheat was higher as compared to soil under rice plantation or fallow plots. Since rice followed wheat crop in the rice-wheat-rice rotation, it would suggest that the germination of sclerotia and their infection on rice resulted in a decreased inoculum in soil. It may be mentioned that abundant black sclerotia remain embedded in greyish mycelial wefts inside the stem of infected plants until harvest .Presumably these sclerotia are dispersed in soil during tillage operation resulting in greater numbers in the following wheat crop or fallow fields. A significant increased population of sclerotia in wheat fields as compared to fallow fields would indicate its multipliaction on wheat plant as well. It is interesting to note that wheat has not been recorded as a host of *S. oryzae* although certain grasses like *Echinochloa colonum* Link., *Eleusine indica* (L.) Gaertn, *Leptochloa chinens's* (L.) Nees. and *Setaria pallide fusca* Stapf & Hubb., are known to be infected (Ou, 1972).

The present investigation has shown that abundant sclerotia of *S. oryzae* are present in the upper 2" layer of soil. Similar observations have been made by Kawai (1955). A similar situation with *Rhizoctonia* has been observed in fields in Maryland (Papavizas, 1974, personal communicaiton). Since the number of sclerotia of *S. oryzae* with low viability were observed at 6-9" depths, suggests that deep ploughing might alleviate, the damage caused by *S. oryzae*. Of the factors affecting survival of sclerotia in soil, it would appear that a predominant bacterial colonization of sclerotia at greater depths in soil may be a contributing factor for their low viability. The effects of associated micro-organisms on loss of viability of sclerotia of *S. oryzae* under different crop rotations, therefore, needs investigation.

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