

STUDIES ON COLLAR ROT OF TUBEROSE

A THESIS SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)

BY

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1995

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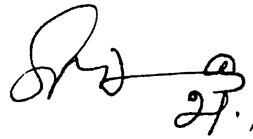
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Certified that the thesis entitled "Studies on Collar rot in Tuberose", submitted in partial fulfilment for the award of degree of MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY), College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of the bonafide research work carried out by Sri Santosh Kumar Kar under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation has been duly acknowledged.


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MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)

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Santosh Kumar Kar
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Dated 21st December 1996,

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ABSTRACTS

Title of the Thesis	Studies on Collar rot of tuberose
Author	Santosh Kumar Kar Adm. No. 68 PPT/93
Chairman	Dr. S.R.Das Associate Professor Department of Plant Pathology College of Agriculture, O.U.A.T. Bhubaneswar
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Collar rot is a serious disease of tuberose (Polianthes tuberosa L.) caused by Sclerotium rolfsii Sacc. reported for the first time in Orissa. Out of seven inoculation methods compared, artificial inoculation was highly successful, when inoculum mixed in top 2-3 cm soil and covered with leaf debris of tuberose. Groundnut shell and Sorghum grain media supported maximum Sclerotial and mycelia growth of the fungus. Laboratory tests proved that clayey soil is favourable collar rot incidence. On the contrary the least collar rot infection was in sandy soil. Superiority of triazole fungicides such as Tilt (propiconazole) and Opus (epoxiconazole) at 0.1% behaved far better than others in controlling the disease *in vitro* and *in vivo*. Cold aqueous root extract of drum stick (Moringa oleifera) and seed extract of soapnut (Sopindus trifoliat) were found completely inhibitory to collar rot fungus *in vitro*. Weedcide Ronstar (Oxidiazon) fully inhibited the mycelial growth of S.rolfsii *in vitro*. Other weedcides such as Stomp (pendimethalin) and Basalin (fluchloralin) were drastically affected mycelial growth. Similarly granular insecticide Furadon (carbofuran) was found moderately effective against mycelial growth of S.rolfsii in petriplates. The non-target effect of this weedcides/insecticides could be utilised for the control of collar rot disease in tuberose in addition to control of weeds or insects as the case may be.

CHAPTER—I

INTRODUCTION

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) has gained considerable importance among bulbous ornamentals for the prettiness elegance, and sweet fragrance of its flowers. It has tremendous economic potential in Cut-flower trade and perfumery industry. The flowers remain fresh for pretty long time and stand long distance transportation. The flowers are used for making garlands, floral ornaments bouquets and button holes. The long flowering spikes are excellent for table decoration arranged in bowls and vases. Variegated type is very attractive and suitable for the garden for beautification. Oil extracted from tuberose flowers is used as raw material for making most expensive perfumes. Its fragrant flowers are added alongwith, stimulants or sedatives to be favourite beverage prepared from chocolate and served either cold or hot.

The bulbs are reported to contain an alkaloid lycorine, which causes vomiting. Hecogenin and a small amount of tigogenin two steroidal sapogenins and a poly fructosan have been isolated from bulb of tuberose. Presence of a transfructosidase has also been observed. The bulbs are considered diuretic and emetic. They are rubbed with turmeric and butter and applied as a paste over red inflammatory pimples of infants (Kirtikar and Basu, 1935). Dried tuberose bulb powder is used as a remedy for gonorrhoea.

Tuberose is a native of Mexico. During 16th century it spread to different parts of the world. The name tuberose is derived from tuberosa, this plant being the tuberous hyacinth as distinguished from the bulbous hyacinth.

Sadhu and Bose (1973) have reported the existence of 4 cultivars, viz. 'Single Double', 'Semi-double and Variegated'. The cultivar 'Single' appears to be commercially more promising than others.

Tuberose has gained considerable importance due to its varied uses. It is cultivated on commercial scale in France, Italy, South Africa, North Carolina, U.S.A. and in many tropical and sub-tropical countries including India. Cut-flowers of tuberose has great demand for indoor decoration especially in the metropolitan cities like Delhi, Calcutta, Madras, Bombay and Bangalore. Tuberose cultivation on a commercial scale is mainly confined to Midnapore and Nadia districts of West Bengal, Darahalli and Bangalore of Karnatak, Pune and Thane districts of Maharashtra, Coimbatore district of Tamilnadu and Lucknow of Uttar Pradesh. At present the total area under tuberose cultivation in India is estimated to be about 20,000 ha. However, there exists a considerable gap between demand and supply.

Recently, commercial cultivation of tuberose has gained importance in Orissa for its uses as garlands, bouquets, cut-flowers and decorating marriage functions through the year. The area under tuberose is rapidly expanding by bringing new areas under cultivation around big cities and towns.

Like any other crops, tuberose also suffers from several diseases. Of these Collar-rot is one. The disease often known as stem-rot, sclerotial-rot or sclerotial wilt. This destructive disease of tuberose was for the first time described from West Bengal of India (Das, 1961). The causal fungus was reported to be *Sclerotium rolfsii* Sacc. Its occurrence was also recorded from Ranchi area of Bihar (Dutta, 1975).

In Orissa, the malady is rapidly gaining momentum inflicting heavy damage to this valuable commercial ornamental crop in city suburbs of Bhubaneswar in recent years. The disease causes rotting of leaves, bulbs and flowering shoot leading to mortality of entire clump. Govt. of Orissa has recently implemented new agricultural policy which provides ample incentives for promoting commercial floriculture.

With the increase of area and intensive cultivation of susceptible varieties, the disease is likely to be accentuated in near future. Hence a detail study is necessary to assess various aspects of it to manage the disease.

In view of the growing importance of the disease and non-availability of adequate information it was thought necessary to investigate on the following aspects.

- i) A detailed study of symptomatology was undertaken to help the farmers and other workers for correct diagnosis of the disease.
- ii) Attempt was made to study the morphology and cultural characteristic of the fungus *in vitro* and *in vivo*.
- iii) Different methods of inoculations were compared to prove the pathogenicity of the fungus and to find out a suitable method for evaluation of varietal resistance.

- iv) Studies were initiated to find out suitable culture media which would support optimum mycelial growth and sclerotial production. Such information would be helpful for undertaking basic studies on the fungus.
- v) Certain aspects of epidemiological studies such as amount of inoculum and soil types which have direct bearing on disease incidence and development were also taken up. The knowledge would be helpful to manage the disease skillfully.
- vi) Investigations were also carried out to evaluate a number of systemic and contact fungicides *in vitro* and *in vivo* to control the disease.
- vii) Certain plant extract were assessed against the pathogen in laboratory. The results would be of much help in controlling the disease without polluting environment.
- viii) Attempts were also initiated to evaluate certain weedcides and granular insecticides to assess their non-target effect on disease control.

CHAPTER—II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The first record of a disease caused by *Sclerotium rolfsii* sacc. was the description of tomato blight by Peter Honry Rolfs, (1892) in Florida. Rolfs (1893) recognised the small round sclerotia of his fungus as outstanding morphological characteristic of the organism. In recognition of Rolf's understanding that the fungus belong to the group of non-spore producing fungi and designated Sclerotium. Saccardo (1911) honoured him by naming the organism *Sclerotium rolfsii* Sp.n.

The fungus *S.rolfsii* was for the first recorded on tuberose plant (*Polyanthes tuberose* L.) causing rotting of leaves and stems in Nadia, Midnapore, Hoogley and 24-Parganas districts of West Bengal in India (Das, 1961). The fungus was isolated from infected plant materials as well as from soil in malt agar sucessfully. Its pathogenicity was proved and details of symptoms described.

Subsequently incidense of sclerotial wilt of tuberose caused by *S.rolfsii* was also reported in severe form during rainy and summer season in Ranchi area Bihar state (Dutta, 1975).

This disease is seen to be common occurence in India and other Tube rose growing countries of the world and can menance to Tuberose cultivation in certain areas. Studies have been made various aspects of this is pathogen concerning the nature of disease, pathogenecity, varietal reaction, host range, control, grpwth of artificial media, morphology and imporatance of fungal metabolites on the host physiology in different countries on various crops.

But keeping in view the knowledge of disease on Tuberose plant not much work has been done. Convincing knowledge on some of the aspects are available, however, only few reports are available concerning the growth of the fungus in different nutrient sources, pathogenicity, manipulation for sclerotia productions, host range, sclerotia germination, toxic metabolites during pathogenesis. In the following section an attempt has been made to review the recontributions, of various investigators on some related aspects dealt in this thesis.

SOURCES OF ANTIFUNGAL PLANT PRODUCT

Chaudhury *et.al.*(1982) reported that benzene extract from *Piper nigrum* shared considerable fungitoxic activity against *Sclerotium rolfsii*. The extract was more inhibitory to mycelial growth than to sclerotial germination.

Singh *et.al.* (1987) screened 5 plant oil, *in vitro* against *Sclerotium rolfsii*. They found that oils from *Eucalyptus alobulus* and *Ocimum canum*, *O.americanum* were most effective.

Prithiviraj *et.al.* (1996) observed that the effect of methanol extract of leaves of *Aegle murelus* was seen on mycelial, (7th and 14th day) and decrease in the number of sclerotia was observed at high conc. of the extract.

EFFECT OF PLANT EXTRACTS ON GROWTH OF PHYTOPATHOGENIC FUNGI

Singh *et.al.* (1980) reported that growth inhibiting effect of aqueous extract from leaf, trunk, bark, fruit pulp and oil of neem (*Azadiracta indica*) against

Fusarium oxysporum f. Sp. *cleri*, *Rhizoctonia*, *Sclerotium rolfsii* and *Sclerotium sclerotiorum*, inciting wilt and rot in gram.

Rao (1983) reported that castor cake extract inhibited the growth of *Sclerotium rolfsii* when incorporated in Rechar's medium at 3% and higher conc. while its extracts increased the mycelial growth of Pathogen over control at lower conc.

Singh *et.al.* (1987) reported that hyphal dry weight and sclerotial production of *Sclerotium rolfsii* sacc. a foot rot pathogen of barley was significantly reduced by bark extracts of *Acacia arabica*, bulb and leaf extracts of garlic and onion, leaf extracts of *Rauvolfia serpentina*, *Calotropis procera*, *Eucalyptus globus* and *Embellica officinalis* and rhizome extracts of turmeric and ginger.

Sivakadacham *et.al.* (1988) showed that leaf extracts of *Adhatoda vasica* suppressed mycelial growth of *Phytophthora* and *Pythium* Spp. and inhibited sexual production. Extracts of *Azadirachta indica* enhanced oospore production in *Pythium butleri* and inhibitory to *Rhizoctonia solani* against *Adhatoda vasica* and *Cullen corylifolium* were suppressive to *sclerotium rolfsii*.

Handique *et.al.* (1990) reported that essential oil of Lemon grass at 100 ppm, decreased the growth of *Rhizoctonia solani* by 62 percent and that to be ineffective against *Sclerotium rolfsii*. At 1000 ppm it totally inhibited growth of first two organisms and caused 80 percent inhibition of *Sclerotium rolfsii*.

Singh *et al.* (1990) claimed that volatile and non-volatile fractions of leaves of neem and blue gum against *Sclerotium rolfsii* sacc. - a foot rot pathogen of Barley. Neem oil was not effective followed by distillates of both plants. Viability of sclerotia was only 20% and 8% when treated with blue gum leaf distillate and neem oil respectively.

EFFECT OF PLANT EXTRACT ON SPORULATION, GROWTH AND GERMINATION OF SPORES OF PHYTOPATHOGENIC FUNGI

Narain and Satapathy (1977) reported that aqueous extracts of leaf flower, stem and roots from 2 varieties of *Vinca rosea* (*Cathaventhus roseus*) inhibited spore germination, sporulation and mycelial growth of *Helminthosporium nodulosum*, *Sclerotium rolfsii*, *Pestalotia* Sp., *Fusarium oxysporum*, *Colletotrichum* sp. and *Aspergillus niger* parasitizing on ridge, green gram brinjal, tobacco and ground nut respectively. Leaf extract were more antifungal than extracts from flower stem and root.

Banarjee *et al.* (1989) reported that germination of sclerotia of *Sclerotium* (*Corticium rolfsii*, *Rhizoctonia* (*Cratobasidium*, *Oryzae-sativae* and *Sclerotium bydrophyllum*) soaked in 5% oil emulsion of *Azadirachta indica* and *Cymbopogon nardus* was strongly inhibited, while selective effectiveness of *Pongamia glabra* *P. oinnata* was observed against *Corticium rolfsii* and *C. oryzae-sativae*.

ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS IN VIVO

Singh *et al.* (1979) showed that garlic leaf extract treated seeds sown in soil infested with *Fusarium oxysporum* f. *Ciecri* and *Sclerotinia gelerotiorum* singly and

Intermixed produced with free seedlings in gram *Cicer arietinum*. Whereas all the seedlings from untreated seed showed wilt symptom.

Rao *et.al.* (1983) reported that castor cake and bean leaves were more effective among three types of plant materials (ground nut, bean and Maize leaves) and three types of oil cakes (Castor, neem and ground nut) amended to soil (1%) separately against *Sclerotium rolfsii* causing smut and root rot of wheat.

Dutta *et.al.* (1986) claimed that Urea, ZnSO_4 and leaf extracts of *Eupatorium adenophorum* suppressed growth and sclerotial germination of *Sclerotium rolfsii* and also tended to reduce the microbial population in the rhizosphere on soil.

Polakshappa (1989) reported that out of 6 organic amendments tested in pot against *S.rolfsii*, groundnut cake and safflower cake were the best, both in sterile and unsterile amended soil.

Sundarbabu *et.al.* (1990) conducted experiment with cakes of neem, castor, mahua and ground nut @400 kg/ha for control of root knot nematode on tuberose in comparison with phorate and showed that neem cake was at par with phorate 10 G for control of nematodes and also promoted good growth of the plants.

Sugha *et.al.* (1993) conducted an experiment during rainy and winter seasons of 1990-91 and studied the effect of inoculum load, types of soil, seedling age and fungal antagonists on incidence of collar rot of chickpea caused by *Sclerotium rolfsii* sacc. An increase in inoculum load of the pathogen increased disease incidence. The mortality of seedlings was the highest 94% in clay soil. The disease development faster in younger than in older seedlings.

Wokocha *et.al.* (1986) evaluated six fungicides viz. Aatopam-N, Aldrex T, Calixin M, PCNB, Captan and Captafol at 200 mg a.i. for their effectiveness in reducing basal stem rot disease incited by *Sclerotium rolfsii* in tomato crop in pre- and post inoculation soil drenched in glass house. The result showed that only PCNB effectively reduced the disease seriously when applied 10 days before inoculation.

EFFECT OF CHEMICALS IN VITRO AND IN VIVO

Dutta (1975) reported control of sclerotial wilt in tuberose by soil drenching of Brassicol (1%) or Zineb(0.3%) in pot experiment at Ranchi. Galkawad *et.al.* (1991) screened Nine chemicals including systemic fungicides, antibiotics and plant extracts for their toxicity against *Sclerotium rolfsii*. Among these chemicals systemic fungicides viz. Bavistin at 0.1% was found most effective which comparatively checked the growth of mycelium, *in vitro* the drenching with Bavistin 0.1% was found best method in controlling collar rot of sesame as compared to seed dressing foliar spray alone *in Vivo*.

Sahid *et.al.* (1990) reported that captan and Ridomil were the most effective; Bayleton, Cupravit and Daconil were least effective while Bavistin and Polyram combination exhibited intermediate effectiveness in inhibiting the mycelial growth and sclerotial production of *Sclerotium rolfsii*. The germination of sclerotia of this fungus was checked most effectively by Benelate and Ridomil. Dithare M-45 was least effective and Captan displayed intermediate action.

MANAGEMENT

Rodriguez-Kabana *et.al.* (1991) reported that crop rotation of 'Flonunner' Pea-nut *Arachis hypogaea*) with the soyabean (*glycine max*) cultivar was studied for 6 years to evaluate long term effects in management of *Melodogyne arenaria* and *Sclerotium rolfsii* in peanut production.

Tiwari (1995) reported that contaf SEC (hexaconazole) at conc. of 0.1% and 0.2% was tested with Bavistin 50 WP (0.2%) and Topsin-M 80 (0.2%) as soil treatment in vitro (infestation of *S.rolfsii* on sorghum grains) and in vivo gram and sun flower) in Rabi 1991 and 1992 contaf was found highly effective in reducing the infestation on sorghum grains and sclerotial germination as well as in controlling root rot in gram and sunflower. The effectiveness of contaf was followed by Baviskin and Topsin-M.

Baker (1988) reported that collar rot (*Sclerotium rolfsii*) is predominantly a seedling disease of chickpea resulting substantial seedling mortality. Seeds of Chickpea treated with six fungicides was sown in inoculated soil in earthen pots placed in open atmosphere. Pre and post emergence mortality was recorded. It was found that seed dressing with vitavax 200, Thiride-75 and captan had significant effect in controlling the collar-rot disease of chickpea. While Bavistin Benlate and Ridomil treatment didnot differ significantly from the control.

Calicdon (1986) studied the protective and therapeutic activity of crude plant extract against *Sclertium rolfsii* (Sacc.), the causal organnism of damping off disease of Tomato. With ethanol as extracting medium all the crude plant extracts were active as protectant and therapeutant.

Wokocha *et.al.* (1986) reported that six fungicides Atopam-N, Aldrex T, Callix M, PCNB, Captan and Captafol were evaluated at 200 mg a.i 1 (-1) for their effectiveness in reducing basal stemrot disease invited by *Sclerotium rolfsii* on Tomato in pre and post inoculation soil drenches in glass house.

Gotoman *et.al.* (1994) reported that tagetes crude extract was as effective as Maneb in inhibiting the growth of *Curvularia lunata* and *Rhizopus stolonifer*. However, results based on arbitrary rating scale showed that TCE was as effective as maneb in inhibiting mycelial growth of *Botryodiplodia theobromae*, *Helminthosporium maydis*, *Sclero rolfsii*, and *R. stolonifer*. In vivo evaluation revealed that TCE exhibited a very effective pretreatment action against *B. theobromae*, *R. stolonifer*, *S. rolfsii* and *H. pathogens*.

BIOLOGICAL CONTROL

Mukharjee (1993) reported that one isolate of *Trichoderma* (NP-1) was effective against *Sclerotium rolfsii* in laboratory and green house study. One isolate each of *Trichoderma* (27), *Aspergillus* (67) and *Penicillium* (Ant.2) reduced dry root rot in the laboratory tests. Non provided protection against wilt in sick soil with JG62 as test variety. However, some of the isolates of fluorescent pseudomonas delayed the wilting.

Sollorin *et.al.* (1990) determined the efficiency of *Trichoderma* sp. at various inoculum density levels against *Sclerotium rolfsii*. The percentage of damping off caused by *S. rolfsii* ranged from 25.33 to 86.33%. Disease control by *Trichoderma*

sp. on the other hand was from 25.67 to 71.00%. *Trichoderma* sp. thus has good potential as biological control agent against *S.rolfsii*.

Deb *et.al.* (1991) studied the Biological control of *Sclerotium rolfsii* Sacc. with antagonistic micro-organisms. Antagonism between eleven rhizoporene fungal isolated from Soyabean and *S.rolfsii* was observed *in vitro*. A clear inhibition concentration was produced by *Trichoderma viride* and to some extent by *Fusarium solani* while others suppressed in growth of the test pathogens by overgrowing *S. aspergillus* flavus. In pathogens infested soil, *Trichoderma harzianum* and *T.Konigiri* amendments gave comparatively better control of foot rot disease of Soyabean i.e, 45% and 38% respectively. Population *S.rolfsii* decreased considerably (i.e, reduced population 50%) whereas the antagonistic (*Trichoderma harzianum* and *T. Konigiri* culture was separately added to infected soil, which could be co-related with the decrease in disease severity.

Bashir *et.al.* (1987) found in experimental plots at NARC Islamabad 1984 that Lentil seedlings were wilted and rotting from collar region downward the roots of the plant were associated with white fungal mycelia. The infected roots were cut into small pieces and surface sterilized and plated in PDA medium and kept in room temp (24-28 °C). A white colony of the fungus appeared on the medium. The mycelia were sterile, hyaline and septate. The fungus was identified *S.rolfsii*.

Howare *et.al.* (1994) reported that a rapid and reliable greenhouse method was developed to screen chickpeas for resistance to collar-rot caused by *Sclerotium rolfsii* sacc. Groundnut shells were superior to sorghum straw, sand-maize meal and potato dextrose both in supporting the growth and formation of

sclerotia of the pathogens. Soil infested with sclerotial inoculum developed on groundnut shells was used to study the reaction of 12 chickpea cultivars to the collar rot pathogens.

Das (1961) discusses of Rajanigandha (*Polyanthes tuberosa* L) and Larkspur *Dalium ajacis* L caused by *Sclerotium rolfsii* sacc.

Dutta (1975) discussed *Sclerotium* wilt of *Polyanthes* and *Caladium* and their control.

CHAPTER—III

MATERIALS AND METHODS

MATERIALS AND METHODS

The materials used and the techniques followed for carrying out the various experiments have been described foregoing sections following that of earlier workers wherever felt necessary.

COLLECTION OF PLANT SAMPLES

The Plant samples collected from farmers field near Phulnakhara (Bhubaneswar-Cuttack) road and Satsangh Bihar at Bhubaneswar during the month of July 1995. Each sample was labelled properly and taken into laboratory for examination of incidence of collar rot caused by *Sclerotium rolfsii*. The symptom was studied and was noted down for rotting.

ISOLATION OF PATHOGENS

To isolate the fungus the moist blotter method as recommended by ISIA (1953, 1961) was followed. The diseased plant samples collected were washed and diseased collar parts were cut into pieces which were then disinfected with 1: 1000 (0.1%) mercuric chloride solution. These were transferred to PDA slants after several washing in sterile water and incubated at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The cultures were maintained by sub-culturing time to time in PDA slants.

The pure culture was obtained by transferring a young imature white *Sclerotium* from culture tube to a fresh PDA slant and incubated for 9-10 days. From this culture a young white *Sclerotium* was again transferred to a sterilised

PDA slant. Thus, a pure culture was obtained and was maintained by sub-culturing throughout the course of investigation.

STERILISATION OF GLASSWARE

All the glassware including petridish, pipettes, glass rods, watch glasses, cavity slides, corkborer etc. were sterilised in hot air oven at 160°C for 2 hours.

PREPARATION OF DIFFERENT MEDIA:

Potato dextrose medium was prepared in following manner. 200 g of peeled sliced potato was boiled in 500 ml of distilled water. The water was decanted when the potato was soft. In another 500 ml distilled water 20 g of agar agar was added and stirred properly, then add 20 g dextrose and the final volume was adjusted to 1 lit by adding potato decanted solution.

Different natural media like sorghum grain medium (SGM), wheat grain medium (WGM), rice straw medium (RSM) and sand maize meal medium (SMM) were prepared by taking 100 g of grain, shell or straw, soaked in water for about 6 hrs. These were sterilized separately in 250 ml Erlenmeyer flask at 121°C for 45 minutes.

Sand maize meal medium was prepared as above taking 90 g of fine sand, 10 g of maize meal in which 20 ml. of water was added.

INOCULATION IN THE MEDIA

Each flask was seeded with a mycellal disc (5mm) from a 7 day old culture of *S. rolfsii* grown in PDA and incubated at $28 \pm 1^{\circ}\text{C}$ for 3 weeks. Three flask was used for each medium and each flask was considered as one replication.

SCLEROTIAL COUNT

The entire medium was mixed in 1 lit. of water and stirred in magnetic stirrer for about 1 minute. The floating sclerotia were sieved and counted under a stereoscopic microscope.

SYMPTOMATOLOGY

Survey was conducted during kharif 1995 in and around Bhubaneswar to study the incidence symptom at various stages of disease development.

Specimens sowing different types of symptoms were collected and examined in the labouratory for its pathogen, symptoms were also noted after artificial inoculation with the fungus. Photographs of the symptoms were taken.

PATHOGENICITY

To prove the pathogenicity of the fungus seven inoculation methods were tested such as sclerotia mixed in entire srol, sclerotia mixed in top 2-3 cm srol, sclerotia mixed in 2-3cm srol and covered with leaf debris, sclerotia spread on

surface soil, sclerotia spread on surface soil and covered with leaf debris. Mycelial propagule placed in plant collar region and mycelial propagule placed in plant collar region and covered with leaf debris and compared with suitable check without any inoculation. Per cent infection/mortality was counted and the incubation period was noted. The plants were raised in 15 cm pots filled with sterilized soil autoclaved at 121 °C for 45 minutes for consecutive two days. Five bulbs were planted in each pot and two pots were taken as one replicate.

FACTOR INFLUENCING DISEASE DEVELOPMENT

a) Inoculum load

To study the effect of inoculum load of *S. rolfsii* on disease development plants were raised in 15cm pots filled with sterilized soil. Five plants were transplanted in each pot. Ten days after establishment of the plants, the top portion of the soil was worked out and 15g of mycelial propagules maintained in Sorghum grain was mixed in top 2-3cm soil and regularly irrigated with sterile water. Symptom development, disease incidence and mortality was recorded.

b) Soil Types

Various soil types such as sandy loam, loam, clay, clayey loam, sandy and laterite soil were collected from different parts of Puri district. These soils were sterilized separately and filled in 15 cm pots. These pots were inoculated with *S. rolfsii* grown in sorghum grain medium in top 2-3 cm soil and watered for about 3-4 days. Five hundred sorghum grains soaked in 2% sucrose solution were spread on the soil. Per cent of grain infection was counted at 5th, 10th and 15th day after at five days interval.

DISEASE MANAGEMENT

a) Evaluation of fungicide in *in vitro* (mycelial growth)

To evaluate the relative fungitoxicity of 6 fungicides at different concentration viz. 50, 100, 200, 500, 1000 $\mu\text{g/ml}$ on the growth of the fungus was determined by following poison food technique (Schmita, 1930). The fungicide used were Tilt (propiconazole), OPUS (epoxiconazole), Calxin (tridemorph), Indofil M-45 (mancozeb), Blitox-50 (Copper oxychloride) and Bavistin (Carbendazim).

The required concentration of the individual fungicide was prepared as follows 1g a.i. of the fungicide dissolved in 100ml of sterile water gives 10000 $\mu\text{g/ml}$ stock solution of the compound. From the stock solution required quantity of the solution was added to the medium to make the final volume 100ml. and thus the desired concentration were prepared.

A seven day old culture of *S. rolfsii* previously maintained in PDA was used for inoculation. Agar disc of 5mm size was cut in sterilized cork borer and was transferred to the middle of the prepared media. The diameter of the fungus colony was measured when in untreated medium there was full growth.

b) *In vitro* evaluation of fungicide on Sclerotial germination

In order to find out the efficacy of different fungicide against sclerotial germination of *S. rolfsii*, sclerotia were produced in mass in groundnut shell medium. Fungicides like Tilt, Opus, Calxin, Indofil M-45, Blitox-50 and Bavistin

were diluted to desired concentration. Young sclerotia were dipped in such fungicidal solution separately in culture tube for 1, 6, and 24 hr. Sterilized moist chambers were prepared lined with filter papers. In each petridish 20 nos. of sclerotia were taken by means of camel hair brush and moistened with 2% sucrose solution. Germination was recorded after 48 hr. of incubation and the germinated sclerotia were counted. Each petridish was considered one replicate. The experiment was repeated twice.

EVALUATION OF PLANT EXTRACT (mycelial inhibition)

Plant extracts of Basanga leaf (*Adhatoda vasica*), Moringa root (*Moringa oleifera*), Neem leaf (*Azadirachta indica*), Manjuati root (*Lawsonia inermis*), Neem seeds (*Azadirachta indica*), Soapnut seeds (*Sapindus trifoliata*), Patalgarud leaf (*Rauwolfia serpentina*), Karanja leaf (*Pongamia glabra*), Lantana leaf (*Lantana camara*) were prepared as follows.

100g of fresh leaves/root/seeds from each plant was collected, washed in sterile water, grinded in a mixer and filtered through double layer muslin cloth and again by Whatman No. 1 filter paper. The cold aqueous extracts were diluted to desired concentration by adding in PDA. Before adding in PDA media the aqueous extract was boiled at 45 °C for about 15 minutes to make the extract contaminant free. The mycelial disc of *S. rolfsii* was inoculated as described earlier.

IN VIVO EVALUATION OF FUNGICIDE

Performances of 6 fungicides evaluated *in vitro* were also tested *in vivo*. To conduct the *in vivo* test, tuberose bulbs were planted in pots filled with sterilized

soil. After the establishment of the plant, the top soil of about 2-3 cm was worked out and 15g of mycelial propagule grown in sorghum grains were mixed and covered with soil. Watering was done after 48 hr. of inoculation. Different fungicidal solution of desired concentration was added @ 100ml/pot twice at 15 days interval. Disease incidence and mortality of plants due to collar rot caused by *S. rolfsii* was recorded.

NON TARGET EFFECT OF WEEDICIDES/INSECTICIDES

In vitro evaluation of weedlcides such as Basalin, Stump, Delchloro, Ronstar and Insecticide Furadon were conducted. Four concentration like 0.5, 1.0, 1.5, 2.0 % were prepared and tested following poison food technique.

CHAPTER—IV

EXPERIMENTAL FINDINGS



D - Disease H - Healthy

Fig. 1 Photograph of tuberose plant showing the symptoms of collar rot caused by *sclerotium rolfsii*

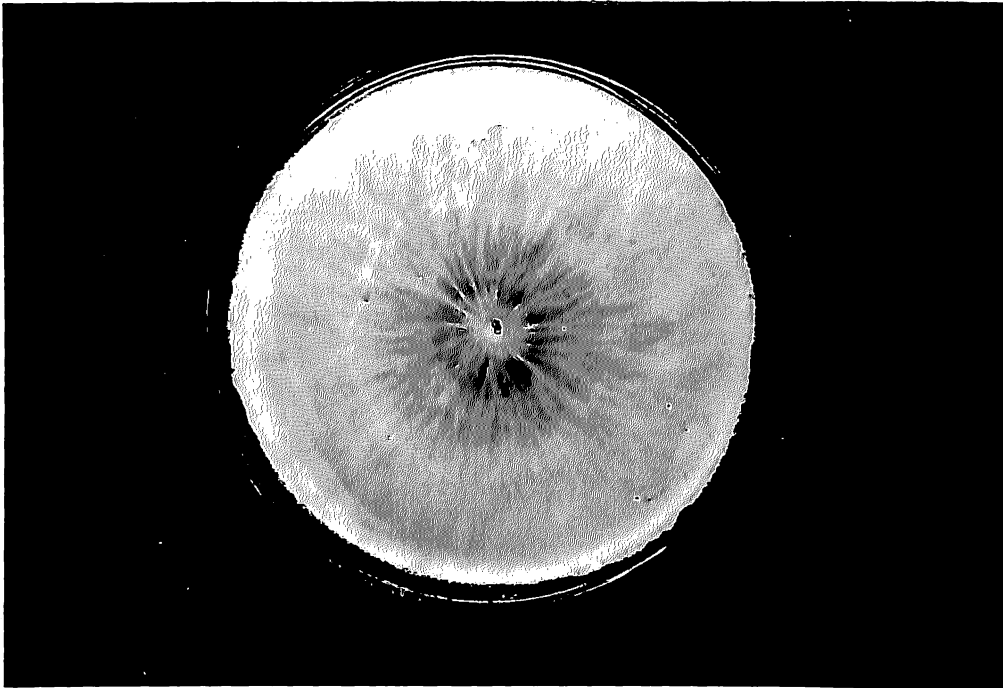


Fig. 2 Photograph showing Mycelial growth of *S.rolfsii* on PDA plate



Fig. 3 Photograph showing the fumation of sclerotia of *S.rolfsii* on PDA Plate

sclerotia. The young vigourously growing mycelium from the culture was white (Fig.2). The hyphae were hyaline, septate, stout. In the early stage of growth the mycellal growth looked silky white which became dull by loosing its lustre. It grew very first producing radiating aerial mycelia. Hyphae aggregated forming thick strands or rhizomorph-like structures. Individual hyphae were hyaline, thin walled, sparsely septate when young. The cells were 60-330 μm long and 2-8 μm wide. Older hyphae contained numerous granules. Clamp connections were very common. Mostly broader hyphae showed clamp connections. Sclerotial initials were formed from the hyphal strands as minute mycellal knots. Sclerotia were round small like mustard size hard and they were white in the beginning, but soon became dark coloured (Fig.3). They measured 0.8 to 1.5 mm (average 1.1 mm) in diameter.

On host tissues mycelium was seen clinging as dense white cotton like threads on leaf surface and collar region of stem. Hyphae were septated wide apart containing barrel shaped cells and branched in an acute angle manner. Sclerotia were found in mycellal mat formed either on leaf surface or on decayed stem tissues.

PATHOGENICITY

In order to prove the pathogenicity of the fungus of *S.rolfsii*, Koch's postulate were done. Seven methods of inoculation were tried to test the effectiveness of inoculation. Data on infection per cent and incubation period are presented in Table 1.

Table 1: Effect of different methods of inoculation of *Sclerotium rolfii* in causing collar-rot in tuberosse

Method of inoculation	Plant infection (%)	Incubation period (days)
Sclerotia mixed in entire soil	26.7 (30.99)	17
Sclerotia mixed in top 2-3 cm soil.	83.3 (70.07)	9
Sclerotia mixed in top 2-3 cm soil and covered with leaf debris of tuberosse.	100.0 (90)	7
Sclerotia spread on surface Soil	36.7 (37.22)	15
Sclerotia spread on surface soil and covered with leaf debris of tuberosse.	70.0 (57.00)	11
Mycelial propagules placed in plant collar region.	72.0 (58.05)	11
Mycelial propagules placed in collar region and covered with leaf debris of tuberosse.	84.0 (70.07)	9
Control	0 (0)	0
SE(m) $\pm$	(5.88)	
C.D. (0.05)	(18.11)	

Results showed that all the methods of inoculation tested successfully caused infection and expressed disease symptoms on inoculated host plants. However, no disease symptoms were seen in un-inoculated control plants until end of the experiment. Maximum infection of 100% was achieved where sclerotia were mixed in top 2-3 cm soil and covered with leaf debris of tuberoses. It was followed by seventh and second method where mycelial propagules were placed in collar region of plants (84.0%) and sclerotia mixed in top 2-3 cm soil (83.3). All other methods of inoculation produced satisfactory infection except method 1 (sclerotia mixed in entire soil) and 4 (sclerotia spread on surface soil). The least infection was in the first method where sclerotia were mixed in entire soil (26.7%).

The disease symptoms appeared in shortest time of 7 days in third method viz. sclerotia mixed in top 2-3cm soil and covered with leaf debris. The disease appeared in 9th day in second and seventh method. However symptom expression was very much delayed in first and fourth method which took 17 and 15 days respectively for typical disease reproduction. Incubation period in other methods varied in between 9-15 days.

Symptoms of the disease and morphological characters of the fungus were same as observed in natural infection (Fig.4). The fungus was reisolated and showed identical characters.

PHYSIOLOGICAL STUDIES

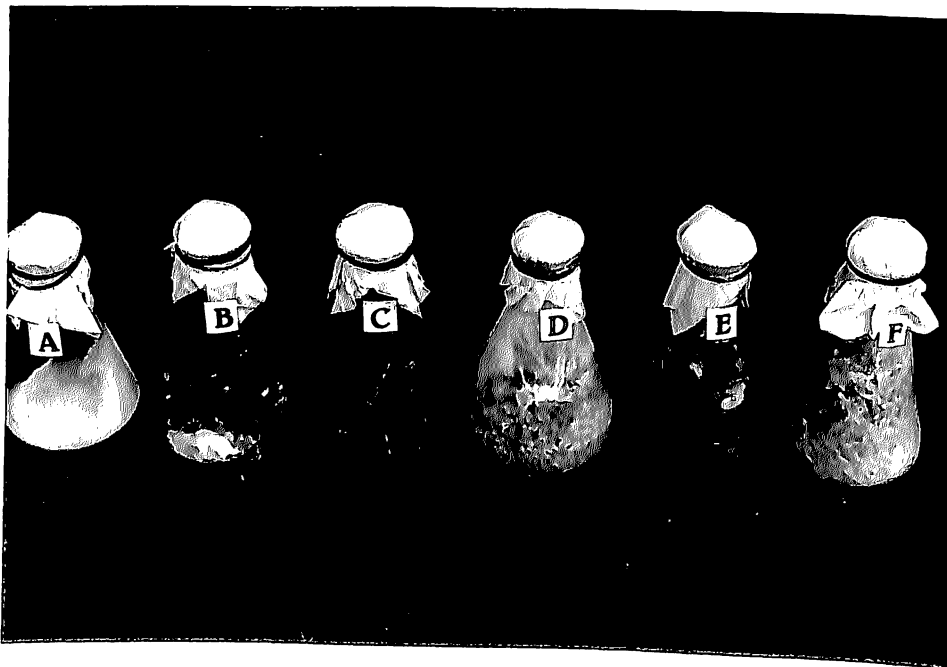
Growth and sclerotia production in culture media

The data on the effect of 6 different nutritional solid media on mycelial growth and sclerotia production are furnished in Table 2 and depicted in Fig.5.



Fig. 4 Photograph showing the infection after inoculation with *S.rolfsii*

NI - Non-inoculated I-Inoculated



A - Potato dextrose media C - Paddy straw media E - Groundnut shell media
B - Sand maize meal media D - Jowar grain media F - Wheat grain media

Fig. 5 Phtograph showing growth and sclerotial production in different culture media

Table 2: Growth of *Sclerotium rolfsii* in different culture media

Culture Media	Number of Sclerotia (per / 100g)	Mycelial growth
Sorghum grain medium (SGM)	656.66 (6.48)	+++
Wheat grain medium (WGM)	210.00 (5.34)	+++
Groundnut shell medium (GSM)	2044.00 (7.62)	++
Rice straw medium (RSM)	403.33 (5.99)	+
Sand-Maize meal medium (SMM)	1367.33 (7.21)	+
Potato dextrose medium (PDA)	746.66 (6.61)	+++
SE (m) $\pm$	(0.18)	
C.D. (0.05)	(0.12)	

Figures in parenthes represent log transformed values

Potato dextrose agar was found to be the best it supported maximum linear mycelial growth viz. 70.7 mm and sclerotia followed by melt agar. The rate of growth per day was found highest in PDA followed by oatmeal agar. From the above data on Table it was observed that there was significant difference among culture media on sclerotial production of *S. rolfsii*.

Out of six culture media, Groundnut shell medium (GSM) supported the maximum sclerotial production (2044/100g medium) which was found significantly superior to all other media in respect of sclerotial growth. Sand maize meal medium (SMM) was seen the next best medium in supporting sclerotial production. Sorghum grain medium (SGM) induced the highest mycelial growth followed by wheat grain and potato dextrose agar. Wheat grain medium produced poorest sclerotial growth followed by rice straw medium.

FACTORS INFLUENCING DISEASE DEVELOPMENT

Inoculum load

Mortality per cent and days taken for mortality by *S. rolfsii* causing collar-rot in tuberose plants are summarised in Table-3. It was evident from the data that there was significant differences among inoculum loads of *S. rolfsii* in inducing mortality in tuberose. Highest mortality (100%) of tuberose plants was recorded when 12.5 to 15g of sclerotia were used as inoculum. These two treatment were followed by 10g of sclerotia in which 96.6% mortality was observed. Amount of inoculum viz. 15.00 and 12.5g sclerotia were found significantly better than other treatment. The least mortality per cent was however noted in 1g of sclerotia used as

Table 3: Effect of quantity of inoculum (Sclerotia) of *S.rolfsii* inducing mortality (%) in tuberose plants

Inoculum quantity (g)	Mortality (%)	Days to mortality (day)
1.0	10.0 (15.00)	0
2.5	23.3 (28.77)	30
5.0	46.7 (43.07)	30
7.5	76.7 (61.22)	23
10.0	96.6 (77.70)	16
12.5	100.0 (90.00)	13
15.0	100.0 (90.0)	9
Control	0	0
SE (m) +	(4.02)	
C.D. (0.05)	(12.19)	

Figures in parentheses are transformed angular values

Inoculum. It was further observed that mortality of plants was quicker when mortality of planto was quicker when higher amount of sclerotia were used. It took only 9 days to kill a plant at 15g but the mortality was delayed up to 30 days at 2.5g. There was no incidence of the disease and practically no mortality at low rate of sclerotia viz. 1g of inoculum used.

Soil Types

The data on sorghum grain infection by *S. rolfsii* in different soil types at with 5th and 15th day after inoculation have been presented in Table 4 and Fig.6.

A close look to the data indicated that highest grain infection was in clay soil (391.11) and the lowest was in sandy soil. In general grain infection was maximum after 15th day of inoculation. There was statistical significance difference among soil types in inducing grain infection by *S. rolfsii*. There was potential infection of grain in all other types of soil. But sandy soil significantly reduced chances of *S. rolfsii* infection of grain. Among the dates of observation a long gap of 15 days was found more conducive for maximum infection which was statistically significant to other day of observations.

DISEASE MANAGEMENT

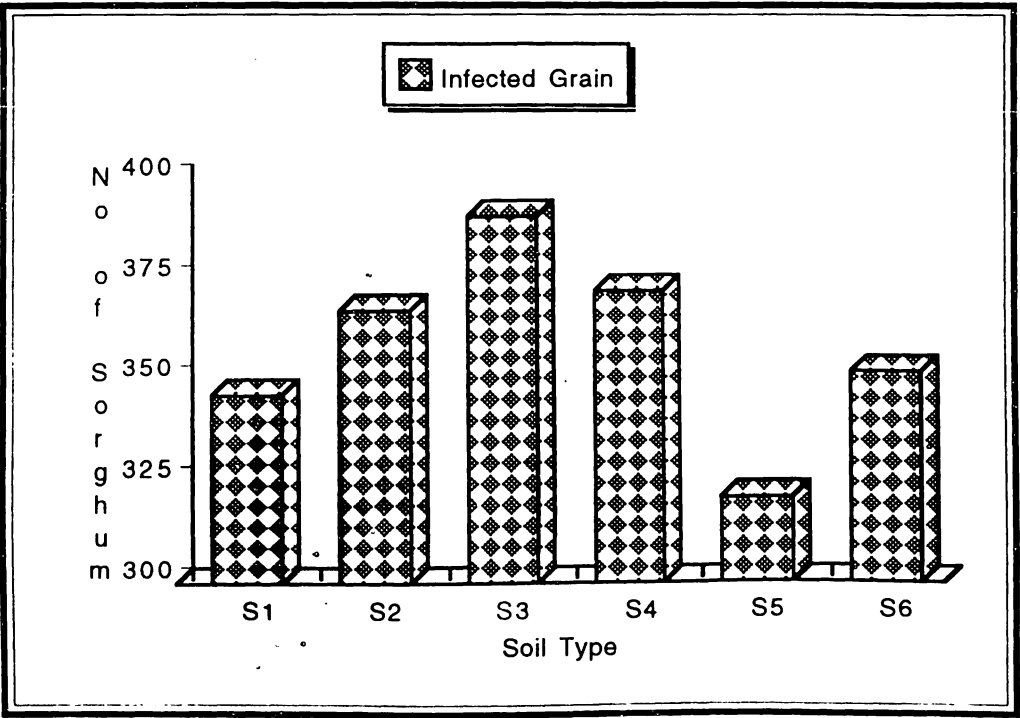
Evaluation of fungicides *in vitro* (Mycelial growth)

Inhibition of radial mycelial growth of *S. rolfsii* recorded on PDA after six days of incubation in 90 mm petriplates impregnated with different doses of fungicides maintained at $28 \pm 1^{\circ}\text{C}$ are presented in Table 5 and illustrated in Fig.7.

Table 4: *n vitro* assesment of soil types on ifection of *S.rolfsii* enducing collar-rot of tuberose.

Soil type	Number of Sorghum Grain media infected
Sandy loam Soil	346.667
Loam Soil	367.778
Clayey soil	391.111
Clay loam ssoil	372.556
Sandy Soil	320.667
Lateritic soil	352.222
SE (m) $\pm$	4.669
C.D. (0.05)	13.402
Time period	
D ₁ - 5th day	269.389
D ₂ - 10th day	377.500
D ₃ - 15th day	428.611
SE (m) $\pm$	3.301
C.D. (0.05)	9.475
Soil x Time period	
SE (m) $\pm$	8.087
C.D. (0.05)	23.213

Fig. 6: *In vitro* assesment of soil types on infection of *S.rolfsii*



S1 = Sandy loam soil, S2 = Loam soil, S3 = Clayey soil, S4 = Clayey loam soil, S5 = Sandy soil, S6 = Lateritic soil

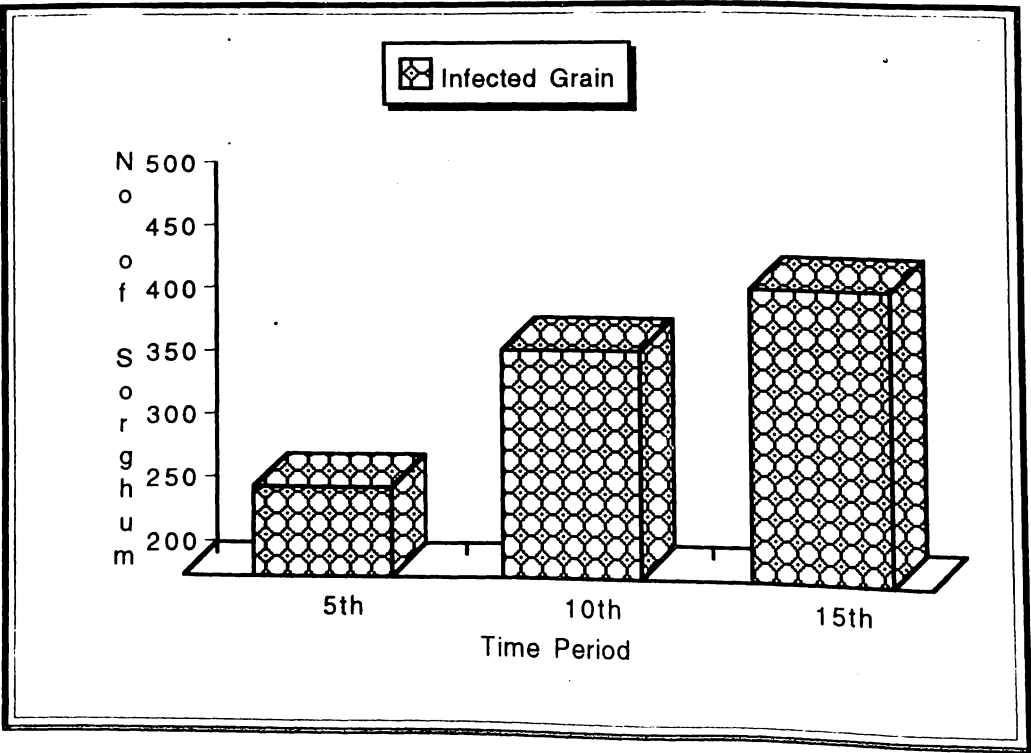
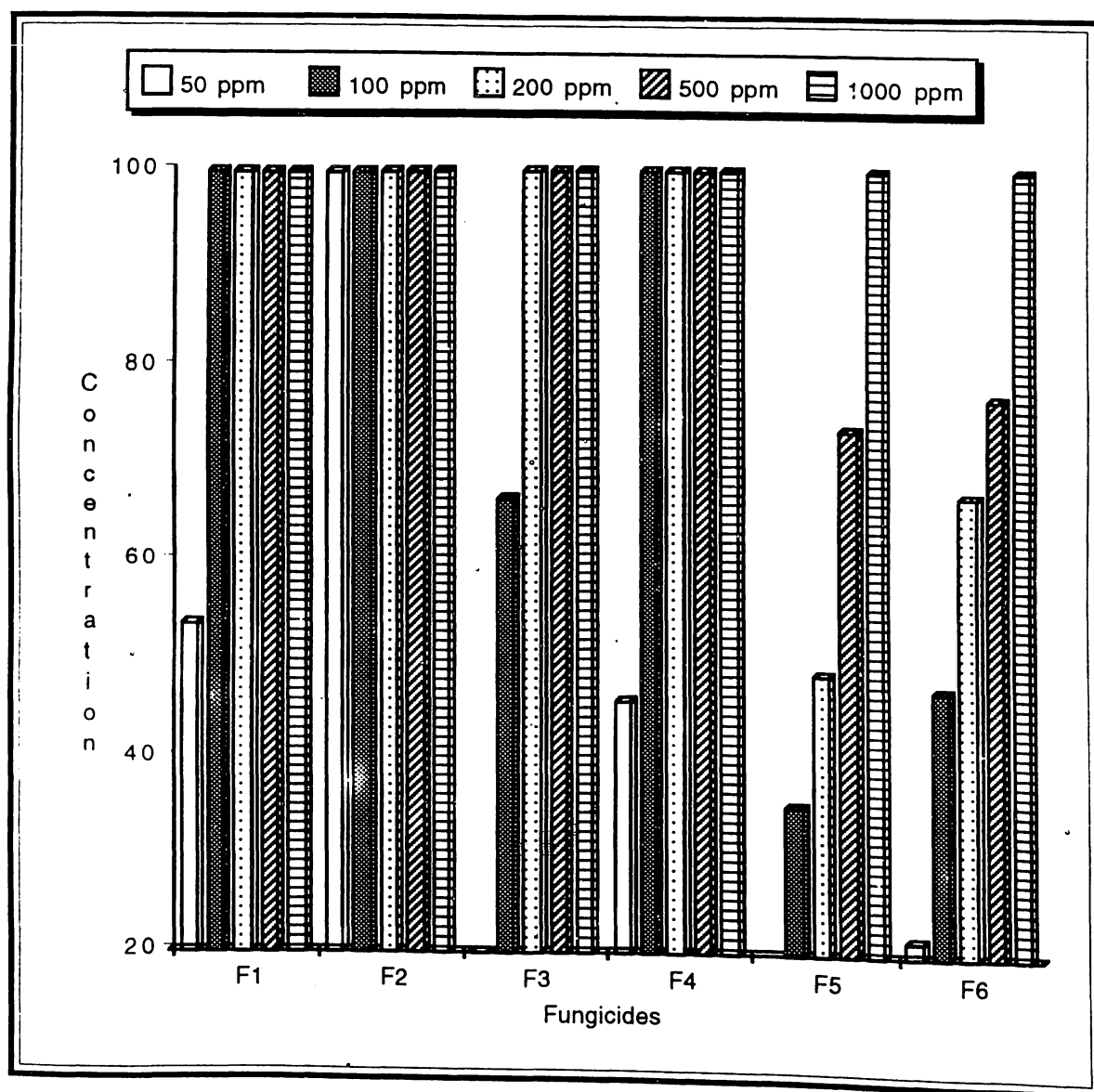


Table 5: Effect of different fungicides on per cent inhibition of radial mycelial growth of *S.rolfsii* inciting collar-rot of tuberoses

Fungicides	Concentration (mg/ml)				
	50	100	200	500	1000
Tilt (propiconazole)	53.6 (47.11)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Opus (Epoiconazole)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Calixin (Tridemorph)	20.0 (26.45)	66.6 (54.58)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Indofile M-45 (Mancozeb)	45.6 (42.50)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Blitox-50 (Copper Oxychloride)	16.6 (24.03)	35.0 (36.24)	48.3 (44.03)	73.3 (58.93)	100.0 (90.00)
Bavistin (Carbendazim)	21.6 (27.71)	46.6 (43.08)	66.6 (54.78)	76.6 (61.14)	100.0 (90.00)
SE (m) $\pm$	(1.67)	(1.21)	(1.32)	(0.64)	(N.S)
C.D. (0.05)	(5.15)	(3.73)	(4.07)	(1.97)	

Figures in parentheses are angular transformed values



F1 = Tilt, F2 = Opus, F3 = Calixin, F4 = Indofil, F5 = Blitox, F6 = Bavistin

Fig. 7: Effect of different fungicides on per cent inhibition of radial mycelial growth of *S.rolfsii*

From the above data (Table 5) it was indicated that there was significant difference among fungicides in inhibiting linear mycelial growth of the fungus at all the concentration used except at 1000 $\mu\text{g/ml}$. At the lowest concentration (50 $\mu\text{g/ml}$) Cent percent inhibition of mycelial growth was achieved in epoxiconazole. The least inhibition of 16.6% was noted in copper oxychloride at this dose. At (100 $\mu\text{g/ml}$) mancozeb became effective with 100 % reduction of growth. But when the dose increased to 200 ($\mu\text{g/ml}$) tridemorph completely inhibited the entire linear growth of the fungus. At 500 ($\mu\text{g/ml}$) substantial growth reduction was seen in copper oxychloride (73.3%) and carbendazim (76.6). At 1000 ($\mu\text{g/ml}$) all the fungicides successfully reduced the entire mycelial growth. In general with the increase of concentration of fungicide there was decrease of growth. Thus it was found that systemic fungicides viz. epoxiconazole and propiconazole were comparatively more effective against *S. rolfsii*.

***In vitro* evaluation of fungicides on sclerotial germination**

Data on sclerotial germination dipped in different fungicides for various time period incubated at $28 \pm 1^\circ\text{C}$ as described earlier are summarised in Table 6.

Critical examination of data (Table-6) revealed that there was significant reduction of sclerotial germination of *S. rolfsii* in different fungicides dipped for different time period. At one hour dipping of sclerotia in propiconazole germination was found drastically reduced (26.7%). Carbendazim affected sclerotial germination moderately but mancozeb could not reduce the germination

Table 6: Effect of various fungicidal dip in different time period (hr) on sclerotial germination (%) of *S.rolfsii*.

Fungicides	1 hr.	6 hr.	24 hr
Tilt (Propioconazole, 0.1%)	26.7 (30.99)	16.7 (23.85)	0 (0)
Opus (Epoxiconazole, 0.1%)	66.7 (54.78)	40.0 (39.23)	6.7 (12.48)
Calixin (Tridemorph, 0.05%)	76.7 (61.22)	50.0 (45.00)	0 (0)
Indofil M-45 (Mancozeb, 0.3%)	100.0 (90.00)	96.7 (83.85)	86.7 (68.85)
Blitox-50 (Copper Oxychloride, 0.3%)	96.7 (77.71)	73.3 (59.01)	56.7 (48.85)
Bavistin (Carbendazim, 0.1%)	56.7 (48.85)	36.7 (37.22)	0 (0)
Control (Sterile water)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
SE (m) +	(4.67)	(5.23)	(2.58)
C.D. (0.05)	(8.65)	(9.39)	(7.94)

Figures in parentheses are angular transformed values

to any extent and behaved like water. All the fungicides except mancozeb were found moderately effective in reducing sclerotial germination. When the period of dipping in fungicides increased to 24hr. there was no germination of sclerotia of *S. rolfsii* in propiconazole, carbendazim and in tridemorph as against cent percent germination in control. Thus it indicates, these systemic fungicides viz. propiconazole (Tilt), tridemorph (Calixin) and Carbendazim may be used in the control of sclerotial disease of the crop in future.

***In vivo* evaluation of fungicides**

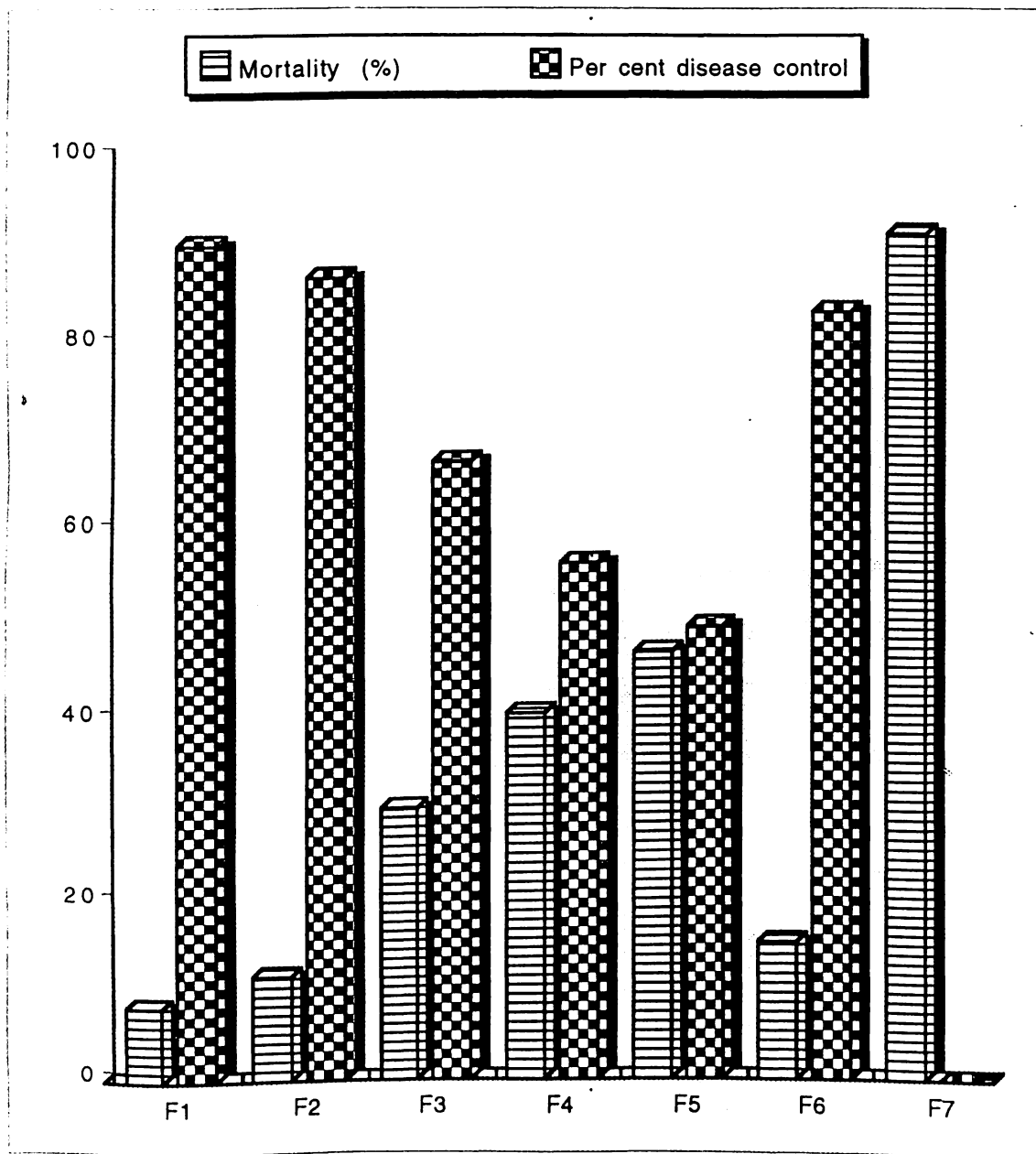
Mortality of potted tuberose plants artificially inoculated with *S. rolfsii* (multiplied on sorghum drenched with different fungicides @ 100 (ml/pot), as described earlier has been furnished in Table 7 and Fig.8.

Perusal of data (Table 7) showed that all the fungicides tested minimised the mortality of tuberose due to collar-rot infection by *S.rolfsii* significantly as compared to untreated control. However, the test fungicide differed in their efficacy among themselves. The least mortality was recorded in propiconazole (8.3%) followed by epoxiconazole (11.6%) and carbendazim (15.0%). Maximum mortality was observed where no fungicide was applied (91.6%). These three fungicides were found statistically *at par* with each other and significantly superior to rest of the fungicides in their efficacy in containing the disease. Propiconazole proved to be the best fungicide providing 90.9% disease control. The next best fungicide was epoxiconazole which protected the crop to the extent of 87.3%. These three fungicides are recommended for the control of collar-rot of tuberose in view of better disease control ability.

Table 7: Effect of different fungicides as soil drench on the mortality of tuberose plants caused by *S. rolfii*

Fungicides	Dose (%)	Mortality of Plants due to Collarot (%)	Per cent disease Control (PDC)
Tilt (Propiconazole)	0.1	8.3 (16.21)	90.9
Opus (Epoiconazole)	0.1	11.6 (19.30)	87.3
Calixin (Tridemorph)	0.05	30.0 (33.16)	67.2
Indofil M-45(Mancozeb)	0.3	40.0 (39.31)	56.3
Blitox-50(Copper Oxychloride)	0.3	46.6 (43.07)	49.1
Bavistin (Carbendazim)	0.1	15.0 (22.59)	83.3
Control (Sterile water)		91.6 (76.25)	Nil
SE (m) $\pm$		(3.60)	
C.D. (0.05)		(11.09)	

Figures in parentheses are angular transformed values.



F1 = Tilt, F2 = Opus, F3 = Calixin, F4 = Indofil, F5 = Blitox,
F6 = Bavistin, F7 = Control

Fig. 8: *In vivo* effect of different fungicides on controlling collar-rot in tuberose

Evaluation of plant extracts (Mycelial inhibition)

Inhibition of linear mycelial growth of *S.rolfsii* recorded on PDA impregnated with different aqueous plant extracts at various concentrations maintained at $28 \pm 1^{\circ}\text{C}$ are presented in Table 8 and depicted in Fig. 9.

Critical examination of data (Table 8) clearly revealed that there was significant differences among plant extracts in inhibiting radial mycelial growth. Root extract of *Moringa oleifera* inhibited mycelial growth even at 10 per cent concentration. It was found significantly better than any other plant extract in respect of mycelial inhibition of *S. rolfsii*. At this lower concentration viz, (10%) *soapnut* (*sopindus trifoliat*) also behaved nicely in inhibiting 82.7% mycelial growth. In general, the efficacy of all the plant extracts increased with the increase of concentration. At 20 percent concentration both *M. oleifera* and *S. trifoliat* extract inhibited complete mycelial growth showing their superiority over others. Root extracts of *Azadirachta indica* and *Lawsonia inermis* acted moderately to inhibit mycelial growth.

NON TARGET EFFECT OF WEEDICIDES/INSECTICIDES

Evaluation of weedicides/insecticides *in vitro*:

Data on per cent mycelial inhibition as influenced by different weedcides and insecticides are presented in Table 9. Close examination of data (Table 9 and Fig.10 A, B, C & D) indicated that there was significant differences among

Table 8: Effect of different plant extracts on radial mycelial inhibition (%) of *S.rolfsii* the incitant of Collar-rot in tuberose.

Plant extract	Concentration(%)		
	10	20	50
Basang leaf	19.6	22.3	40.0
(<i>Adhatoda vasika</i>)	(26.31)	(28.18)	(39.23)
Moringa root	96.6	100.0	100.0
(<i>Moringa oleifera</i>)	(83.85)	(90.00)	(90.00)
Neem leaf	25.7	47.0	87.7
(<i>Azadirachta indica</i>)	(30.39)	(43.27)	(69.50)
Manjuati root	33.7	62.0	78.0
(<i>Larsonia inermis</i>)	(35.46)	(51.95)	(62.04)
Neem seed	28.7	40.0	51.7
(<i>Azadirachta indica</i>)	(32.37)	(3.23)	(45.96)
Soapnut seed	82.7	100.0	100.0
(<i>Sopindus trifoliat</i>)	(65.43)	(90.00)	(90.00)
Patal garuda leaf	26.3	40.7	84.7
(<i>Rauwolfia serpentina</i>)	(30.87)	(39.62)	(60.96)
Karanja leaf	36.7	51.7	67.3
(<i>Pongamia glabra</i>)	(34.85)	(45.96)	(55.15)
Lantana leaf	38.7	46.7	59.0
(<i>Lantana camera</i>)	(38.45)	(43.09)	(50.22)
Control	-	-	-
SE(m)+	(2.21)	(1.14)	(1.06)
C.D.(0.05)	(6.57)	(3.39)	(3.15)

Figures in parentheses are angular transformed values

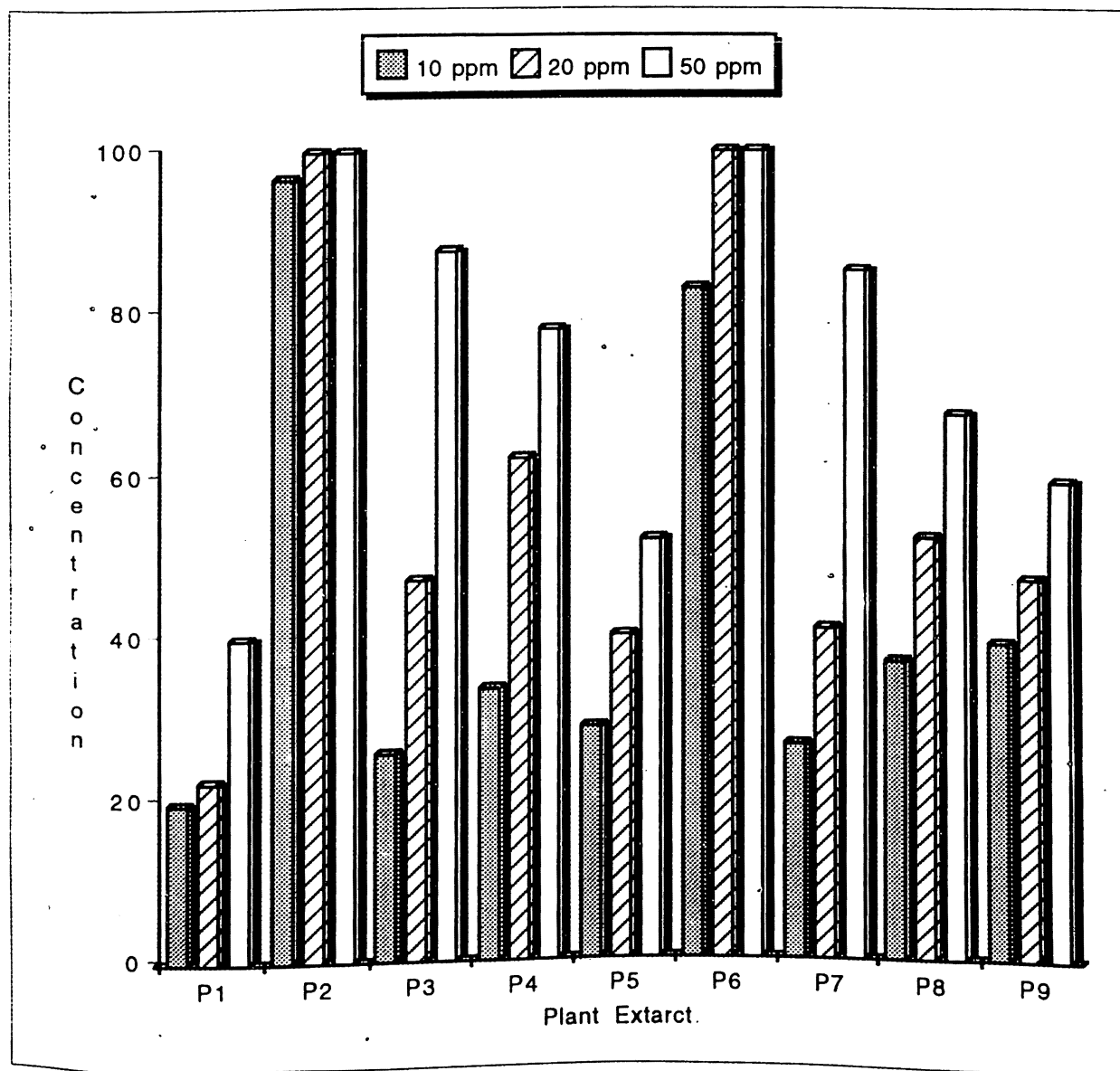


Fig. 9: Effect of different plant extracts on radial mycelial inhibition (%) of *S.rolfsii*

Table 9. Effect of certain weedcides and granular insecticides on percent inhibition of radial mycelial growth of *S.rolfsii* at different concentration

Treatments	Mycelial inhibition
Basalin (Fluchlorolinz)	94.0 (76.10)
Stomp (Pendimethalin)	96.0 (78.76)
Delchloro (Butachlor)	89.0 (70.49)
Ronstar (Oxadiazone)	100 (90.00)
Furadon (Carbofuran)	84.0 (66.30)
SE(m) $\pm$	0.45
CD(0.05)	1.27
Concentration	
0.5	86 (67.99)
1.0	93 (74.07)
1.5	95 (77.39)
2.0	99 (85.90)
SE(m) $\pm$	0.40
CD (0.05)	1.14
SE(m) $\pm$	0.89
CD (0.05)	2.55

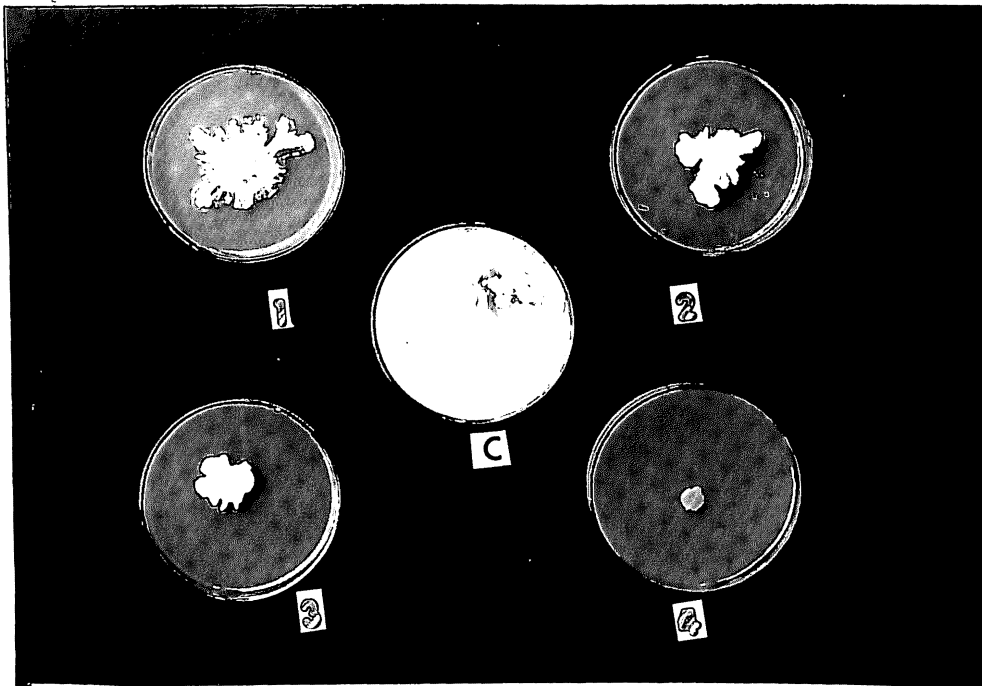


Fig. 10 A Photograph showing the mycelial growth of *S.rolfsii* in poison PDA media with baseline

- 1 - 0.5 %
- 2 - 1.0 %
- 3 - 1.5 %
- 4 - 2.0 %
- 5 - Control

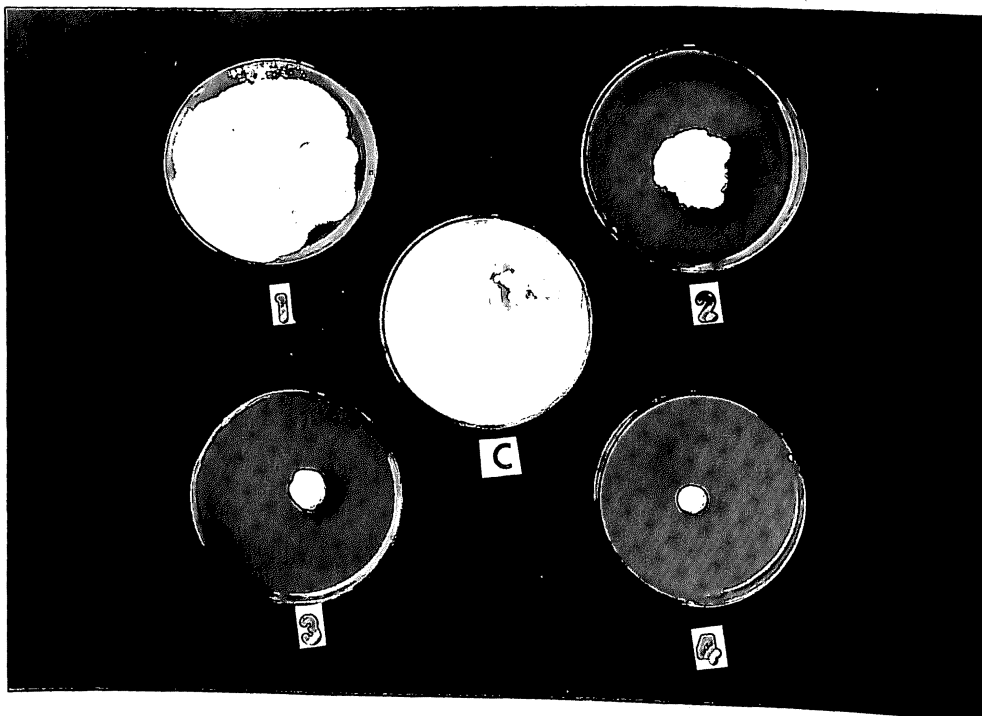


Fig. 10 B Photograph showing the mycelial growth of *S.rolfsii* in poison PDA media with stomp



Fig. 10C Photograph showing the mycelial growth of *S.rolfsii* in poison PDA media with delchlore

- 1 - 0.5 %
- 2 - 1.0 %
- 3 - 1.5 %
- 4 - 2.0 %
- 5 - Control

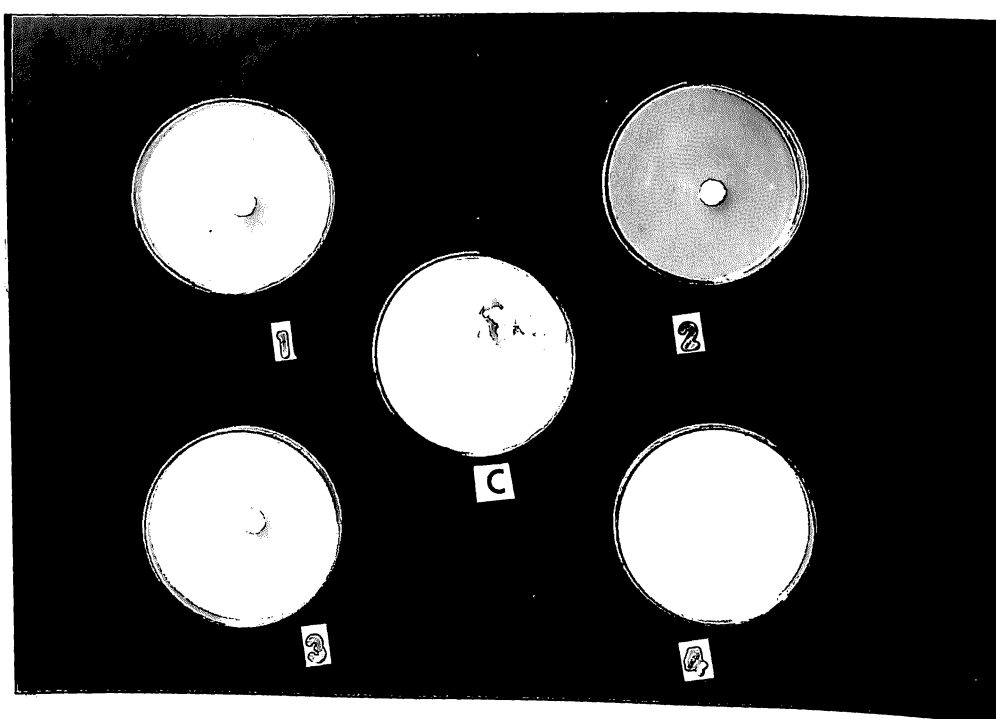


Fig. 10 D Photograph showing the mycelial growth of *S.rolfsii* in poison PDA media with ronstar

weedcides in inhibiting mycelial growth. Ronestar was found highly effective in reducing cent per cent mycelial growth of *S.rolfsii* followed by stump. There was also significant difference between concentrations. In general the rise in concentration is negatively correlated with the mycelial growth. The highest per cent of mycelial inhibition (99%) was noted in 2% concentration of weedicide followed by 1.5% averaged over all weedicide/insecticides. The interaction was also found significant.

CHAPTER—V

DISCUSSION

DISCUSSION

During the present investigation, symptoms of Collar-rot disease of tuberose was observed on leaves, collar region of stems and basal canopy of plants. The disease was characterised by the appearance of white coarse mycelial masses on the leaf surface causing rotting and detachment of such rotted leaves. Brown coloured roundish mustard like sclerotia were formed on the rotted leaves or around it. As a result of infection plants became weak and sent out few none flowering shoot in case of severe damage. Das (1961) while describing the disease from Midnapore area of West Bengal reported similar symptoms.

Drooping, yellowing followed by drying of leaves was marked as typical symptoms of collar-rot associated in tuberose. The fungus attacked roots and tuber and killed the plant incase of sever infection. These symptoms are also in agreement with that of Dutta (1975). Water soaked lesions appeared on collar region of stem and flowering shoots as a result invaded portion of stem rotted and frequent lodging of infected plant occurred in high humid weather if the soil was wet. This type of symptom associated in collar-rot of tuberose is for the first time observed in Bhubaneswar condition. Rotting of stem might have favoured due to high humid, warm temperature and wetness of the soil.

The fungus was successfully isolated on potato dextrose agar from the infected leaf and stem. The morphological characters of the fungus in diseased sample and in pure culture as regards to its mycelial habit and sclerotial nature were found to be more or less similar as described by Das (1961) and Dutta (1975). The mycelium was silky white at first but became dull with age. Mycelial

growth was radiating with much aerial hyphae and aggregated to form rhizomorph. Hyphae were hyaline, septate and thinned walled. Clamp connections were formed frequently. Branchings were at an acute angle. Sclerotia were almost round and dark coloured and found embedded in host tissues. Similar morphological characters of *S. rolfsii* was described by earlier workers (Mukhopadhyay, 1971; Saccardo, 1911) very well match with the present finding.

For proving the pathogenicity of collar-rot fungus, *S. rolfsii* seven methods of inoculation were compared. All the methods of inoculation resulted typical symptom development on the test plants with varying degree of infection. Maximum infection viz. 100% was found in sclerotia mixed into top 2-3 cm soil and covered with leaf debris. High incidence and rapid symptom development in this method of inoculation may be ascribed due to easy availability of senescent plant tissues and a warm moist environment created near the base of the plant due to moisture conservation by mulching of leaf debris.

Shew and Beute (1984) opined similar view while conducting experiment on *S. rolfsii* in groundnut. Further a thin layer covering might have facilitated aeration and moisture conservation providing congenial condition for high infection. This method may be useful in testing varietal resistance in large scale in field condition. High infection was also recorded where mycelial propagules were placed in the collar region and covered with plant debris or sclerotia mixed in top 2-3 cm soil. Placing propagules of *S. rolfsii* is a cumbersome and laborious process. This method may not be adopted for mass screening of variety for above reason Pande *et.al.* (1994) achieved success in infecting groundnut employing above methods. Chinrajeevi *et.al.* (1989) also reported the effectiveness of mixing of sclerotia of *S. rolfsii* in top soil in creating artificial epiphytotics of stem-rot in groundnut.

Out of six media compared for sclerotial production and mycelial growth of *S. rolfsii*, groundnut shell medium (GSM) supported maximum sclerotial production followed by Sand maize meal medium (SMM). Sorghum (SGM) and wheat grain medium (WGM) encouraged good mycelial growth than sclerotia production. The finding corroborates with those of Pande *et. al.* (1994) who claimed higher production of sclerotia of *S. rolfsii* in GSM and good mycelial growth in Sorghum grain medium (SGM). The ability of groundnut shell medium (SGM) to support sclerotial production compared to sorghum or wheat grain medium may be attributed to the lower weight (volume to weight ratio) of substrate as compared to sorghum grain as observed by Boyle (1961).

Experiments conducted to determine the relation of amount of inoculum and collar-rot disease development revealed that highest incidence of collar-rot (100%) was observed when 12.5-15g of inoculum were used. In general, a linear relationship was observed between the quantity of inoculum applied and collar-rot incidence. Disease expression was delayed with the reduction in the quantity of inoculum. The result is in conformity with those of Pande *et.al.* (1994).

Soil types sometimes influences the incidence of soil-borne disease. Therefore, six soil types were artificially inoculated with *S. rolfsii* to compare their ability in infecting sorghum grains fortified with 2% sucrose. Clay soil encouraged highest infection of sorghum grains indicating its suitability for collar-rot incidence. Sandy soil was found unfavourable to *S. rolfsii* as compared to other soil types showing least infection of grains. Heavy incidence of collar-rot in clayey soil may be attributed due to high water holding capacity and nutrient availability suitable to

S. rolfsii. Earlier suitability of clay loam and silty clay for *S. rolfsii* causing stem-rot in groundnut has been reported (Das *et.al.*, 1987). The lower incidence of grain infection by *S. rolfsii* may be ascribed due to quick depletion of soil moisture and poor source of energy. Thus heavy clay soil may be avoided for growing tuberose to escape from collar-rot infection.

Test of fungicides *in vitro* indicated that all the fungicides tested were more or less effective against *S.rolfsii* in inhibiting mycelial growth. Systemic fungicide viz. epoxiconazole and propiconazole were found highly toxic to the mycelial inhibition as evident from the complete mycelial inhibition at lower doses. In view of their toxicity *in vitro* these two fungicides may be recommended to control the disease only after *in vivo* tests. The finding partly agrees with that of Tiwari (1995) who achieved success in inhibiting infection of *S. rolfsii* causing rootrot in sunflower and gram using triazole group of fungicide(Contaf). Of the traditional contact fungicide mancozeb proved superior to others in respect to mycelial inhibition of *S. rolfsii*. Efficacy of mancozeb against *S.rolfsii* was demonstrated in other crop (Chaturvedi and Agarwal, 1975).

Tridemorph was also found effective in complete inhibition of mycelial growth of the fungus at 200 µg/ml or above that concentration. The work of Siddaramiah *et al.* (1979) supports the present finding. Although *in vitro* studies have indicated the efficacy of certain fungicides their field evaluation is further needed to confirm their bioefficacy as well as their impact on flower yield and benefit cost ratio before recommendation to tuberose growers.

Systemic fungicides such as propiconazole, carbendazim and tridemorph were proved highly toxic in inhibiting sclerotial germination at their recommended doses when sclerotia were dipped in respective fungicide for 24 hrs. Mancozeb which acted very well against mycelial inhibition did not respond in reducing sclerotial germination. The above three systemic fungicides probably penetrated into sclerotia only after a dipping period of 24 hrs. and succeeded in reducing germination fully. Ineffectiveness of mancozeb against germination of *S. rolfsii* might be due to none penetration/none absorbance of the compound into sclerotial body. Thus, above three systemic fungicides may be suggested for the control of collar rot of tuberose basing on their performances in laboratory.

All the fungicides evaluated *in vivo* against Collar-rot of tuberose minimised the incidence of the disease. However, three of those mainly systemic fungicides such as propiconazole (0.1%) epoxiconazole (0.1%) and Carbendazim (0.1%) were found more effective as compared to others in descending order of their efficacy. These three fungicides were found superior to tridemorph, mancozeb and copper oxychloride and were *at par* with each other statistically. Excellent control of the disease was achieved using them as soil drench viz. propiconazole (90.9%), epoxiconazole (87.3%) and Carbendazim (83.3%) respectively. Therefore, these three systemic fungicides are of first choice and tridemorph, Mancozeb and copper oxychloride are to be considered as second choice against control of collar-rot in tuberose. Systemic fungicides are costly, therefore, they may be used by affluent farmers growing tuberose commercially. On the other hand poor and marginal farmers may prefer the use of protectant fungicides like mancozeb or copper oxychloride for their low cost. The data from one season can not be relied upon, therefore, the trial should be repeated for one or more seasons to draw final conclusion.

Carbendazim has been reported effective against *S. rolfsii* causing root/collar-rot in different crops (Mukhopadhyay and Thakur, 1971; Siddaramiah, 1979, Lal and Nagrajan, 1983; Waraitch *et al.*, 1986; Tiwari, 1995). Siddaramiah *et al.*, (1979) claimed good mycelial inhibition of *S.rolfsii* in tridemorph isolated from groundnut. Mancozeb was suggested as seed treatment for the control of sclerotial wilt of groundnut (Dhamnikar and Peshney, 1982; Patil and Rane, 1982). Recently a triazole group of fungicide (hexaconazole) was reported successful in controlling root/collar-rot of gram and sunflower by soil drench *in vivo*. However, there is no information, regarding the performance of propiconazole and epoxiconazole against *S. rolfsii* inciting collar-rot in any Crop. Probably this forms the first new information on propiconazole and epoxiconazole for the control of *S. rolfsii* causing collar-rot in tuberose. Brassicol (PCNB) highly effective against Sclerotial wilt/rot of many crops (Ammann and Shanmugam, 1974; Sharma, *et al.*, 1974; Maiti and Sen, 1979) however, is now out of market, Thus, there two fungicides may be used as very good substitute of pentachloronitrobenzine in the control of soil borne pathogen like *S.rolfsii*.

Among nine aqueous plant extracts studied, the root extract of moringa (*Moringa Oleifera* L.) and seed extract soapnut (*Sopindus trifoliat* L.) were found highly inhibitory to *S. rolfsii*. Considerably high inhibition of mycelial growth was noted in leaf extracts of neem (*Azadirachta indica*) and Patalgaruda (*Rauwolfia serpentina*). Earlier several phyto extracts were reported to cause complete mycelial inhibition of *S. rolfsii* isolated from different crops (Nene and Kumar, 1966; Gupta and Agrawal, 1973) Singh *et.al.* (1980) demonstrated complete inhibition of mycelial growth using leaf, trunk and fruit extract of neem. Antifungal activity of

soapnut extract against *Fusarium moniliforme* was claimed by Gohil and Vala (1995). Phyto extracts of *M. oleifera* and *S. trifoliat* showing complete inhibition of *S. rolfsii* is for the time reported here. In recent years, many plant extracts are being used for the control of plant diseases. However, these products are not very much effective like fungicides. Before advocating to farmers, their efficacy should be evaluated in field condition along with commercial fungicides to assess their real potency as botanical pesticides. If search will continue no doubt some plant products may be available in near future which would replace highly toxic fungicides to control collar-rot disease with minimum risk of environmental pollution and health hazard.

Test of weedicides *in vitro* indicated none-target effect of weedicides in inhibiting mycelial growth of *S. rolfsii*. There was significant difference among weedicides in respect of mycelial inhibition. Complete mycelial growth was inhibited in Ronstar closely followed by stomp. Increase in concentration of weedicides was negatively correlated with the mycelial growth. Granular insecticides, carbofuran affected a great deal of mycelial inhibition.

In the past some weedicides including oxydiazon reported partial effective in reducing disease caused by *S. rolfsii* (Backman and Rodriguez Kabana, 1972). In the present study effectiveness of some weedicides viz. Ronstar (oxydiazon) stomp and Basalin against *S. rolfsii in vitro* are illustrated. Application of Furadon @ 2 kg.a.i./ha was reported effective in reducing root-rot caused by *S. rolfsii* in sugarbeat (Mukhopadhyay and Thakur, 1977). Weed infestation is a serious problem in tuberose. Effective weed control is desirable for maximisation of flower yield. Due to high labour cost and shortage of labourer for agricultural operations in city suburbs, weedicides are preferred now a days. The none-target effect of weedicides will thus be beneficial in reducing collar-rot of tuberose.

CHAPTER—VI

SUMMARY AND CONCLUSION

SUMMARY

Collar rot of tuberoses caused by *Sclerotium rolfsii* causing heavy damage to the crop was recorded and described for the first time in Orissa. The disease is characterised by the appearance of chlorotic rotting patches and white mycelial masses on the leaves, followed by drooping and drying. At the collar region white mycelial strands were seen deposited on the host and seen creeping to the soil. As a result of infection infected plants died. Brown mustard like bodies were also seen on these mycelium on the rotted portions of the stem or leaves.

Morphology and cultural characteristics of the fungus were described in detail. Seven methods of inoculation were tried to prove the pathogenicity of the fungus and see their effectiveness. All the methods of inoculation succeeded in inducing the disease. However, maximum infection occurred where sclerotia were mixed in to top 2-3 cm soil and covered with leaf debris. Mycelial propagules placed in plant collar region and covered with leaf debris of tuberoses and sclerotia mixed in top 2-3 cm layer of soil also proved successful in creating high disease incidence.

Six culture media were compared for the growth and sclerotial production of *S. rolfsii*. It was seen that groundnut shell medium supported maximum sclerotial production. Sand maize meal medium also encouraged good sclerotial formation. Highest mycelial growth was observed in sorghum grain medium followed by wheat grain medium. Wheat grain medium and rice straw medium were not favourable for sclerotial production.

Different amount of inoculum were tested to cause collar rot in tuberose plant. The result showed that highest incidence/mortality of tuberose plant was observed when 12.5-15 g. of inoculum were used. This amount of inoculum not only resulted heavy incidence of the disease but the incubation period was reduced considerably there by causing early mortality of plants. When the amount of inoculum was reduced to one gram there was extreme reduction of disease incidence and practically there was no mortality. Soil type some times influences the disease incidence of soil borne plant pathogen. Test conducted *in vitro* suggested that calyey soil was most favourable for stem rot infection in tuberose plant. Incidence of the disease was very low in sandy soil indicating its unsuitability for *Sclerotium rolfsii* to cause collar rot.

To choose a suitable fungicides for control of the disease *in vitro* test was conducted. Out of six fungicides, Epoxiconazole and propiconazole performed excellently in inhibiting mycelial growth of *S. rolfsii*. Among the protectant fungicides, mancozeb behaved well in reducing mycelial growth. The performance of copper oxychloride was very poor. However at very high dose, all the fungicides could inhibit the mycelial growth.

The fungus *S. rolfsii* is a soil inhabitant and frequently forms sclerotia which do not respond to protectant fungicides there by disease control some times becomes difficult. Six fungicides including some new systemic formulation were evaluated at their recommended dosages as sclerotial dip for different time period to see the sclerotial germination. In general short dipping in fungicides was found comparatively ineffective in reducing sclerotial germination. A longer dip of sclerotia for 24 hr. was highly effective in reducing sclerotial germination.

irrespective of fungicides: All the fungicides except mancozeb were found more or less effective in minimising sclerotial germination. There was absolutely no sclerotial germination after 24 hr dip of sclerotia in systemic fungicides like propiconazole, carbendazim and in tridermorph. Untreated sclerotia germinated fully. Result of *in vitro* efficacy of fungicides often falls *in vivo* or in field condition due to break down of the product by different processes and absorption/non absorption in the plant. Therefore, test *in vivo* is essential to find out the real potency of the fungicide in control of the disease. Six fungicides were tested at their recommended doses as soil drench, in pots to test their efficacy against *S. rolfsii* causing collar rot in tuberose. It was indicated that lowest incidence/infection was in propiconazole (1%) which was followed by epoxiconazole and carbendazim in order of their efficacy. Highest disease control per cent was in propiconazole, epoxiconazole and in carbendazim in descending order of their effectiveness.

Botanicals are used as ecofriendly fungicides. Out of nine aqueous plant extracts, evaluated *in vitro*, root extract of *Moringa olifera*, seed extract of *sopindus trifoliat* inhibited the growth completely at 20 percent concentrations and fairly high inhibition of fungal growth was noted in lower dose.

In recent years, weedcides are preferred than human labour for low cost and non availability of labourer in time in controlling the weeds. Some times, these weedcides and granular pesticides have some influences on soil borne plant pathogen. Four weedcides and one granular insecticide were tested *in vitro* to assess their disease control ability against *S. rolfsii* in tuberose. It was observed that ronstar (oxadiazon) inhibited mycelial growth completely at all concentration used. Pendimethalin and Fluchloralin also reduced mycelial growth to a considerable higher limit. Carbofuran also affected the mycelial growth of *S. rolfsii* to a great extent.

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