STUDIES ON STORAGE ROT OF GLADIOLUS (*Gladiolus* sp) CAUSED BY *Fusarium solani* (Mart) Sacc.

By

SANJIVKUMAR RAJARAM INGLE
B.Sc. (Agri.)

A THESIS SUBMITTED TO THE

MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI

DISTRICT : AHMEDNAGAR
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF SCIENCE (AGRICULTURE)
IN
PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY
COLLEGE OF AGRICULTURE
PUNE - 411 005.

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DISTRICT : AHMEDNAGAR

In Partial Fulfillment Of The Requirements For
The Degree Of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

Approved By The Advisory Committee

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CANDIDATES DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other university or institute for a degree or diploma.

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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON STORAGE ROT OF GLADIOLUS (Gladiolus sp.) CAUSED BY Fusarium solani (Mart) Sacc." submitted to the faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra State) in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY embodies the results of a piece of bonafide research work carried out by Mr. Sanjivkumar Rajaram Ingle under my guidance and supervision and that no part of this thesis has been submitted for any other degree or publication.

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Date :

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Associate Dean,
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Pune - 411 ØØ5

Date : 21 FEB 1994

(Dr.V.M. KHAIRE)

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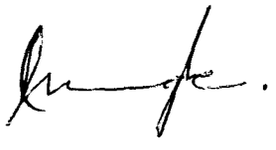

(S.R. Ingle)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURE

.....

A B S T R A C T

STUDIES ON STORAGE ROT OF GLADIOLUS (*Gladiolus* sp.) CAUSED BY *FUSARIUM SOLANI* (MART) SACC.

BY

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Research Guide	:	Smt. V. C. Kadam
Department	:	Plant Pathology and Agricultural Microbiology

Gladiolus is one of the most important flower crops in today's floriculture industry, grown primarily for cut flowers, and also as matchless contributor towords fillings of herbaceous borders, beddings, rockeries and as a pot plant but at the time of storage the corms get affected by storage rot which is a serious problem. Studies were therefore undertaken on this disease with a view to find out a suitable chemical control.

The affected samples were collected from All India Coordinated Floriculture Improvement Project, Ganesh khind, Pune. After isolation they yielded fungal isolate which was obtained in pure culture and used for further studies. The pathogenic isolate was found to be highly virulent causing quick rot of corms of gladiolus.

The organisam was studied in detail for morphological, cultural and physiological characters. The mycelium was strilate, sparse to dense and flocuose greyish-white with persistant arial growth. The organism produced abandant conidia and conidiophores. Conidia were elongated and sparsely branched which merged to form effuse sporodochia. Chlamydospores develop after 7-14 days. They were globose to oval, smooth to rough walled and formed intecalary or on short lateral branches .

It grew well and sporulated in abundance on Potato Dextrose Agar and French Bean Agar extract. Among all other carbon sources Richard's medium gave the best results. Potassium Nitrate and Urea were the best inorganic nitrogen sources when compared with organic nitrogen sources.

An optimum pH for the growth and sporulation was 4.ØØ to 5.ØØ with tolerance range between 2-1Ø. The optimum temperature was $29^{o}C \pm 1^{o}C$ with $18^{o}C$ to $35^{o}C$ as the growth range. A hundred percent relative humidity favoured the rapid development of the diseases.

The organism was proved to be pathogenic on 12 hosts including corms of Gladiolus, Apple, Ashgourd, Banana, Brinjal, Bottle gourd, Capsicum, Colocasia, Muskmelon, Potato, Sweet potato, Zinger .

Bavistin and Benomyl were found to be very effective for controlling the storage rot.

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INTRODUCTION

INTRODUCTION

Gladiolus (*Gladiolus* sp) is one of the most important flower crop in today's floriculture industry, grown primarily for cut flowers, and also as a matchless contributor towards filling of herbaceous borders, beddings, rockeries and as a pot plant. Its name is derived from the Latin word "Gladius" meaning "Sword" on the account of its foliage. Other synonyms include sword lily and corn flag.

Gladiolus is a member of family Iridaceae and it is a tender herbaceous perennial. Leaves are sword shaped. Phyllods and flowers are borne on a spike in to two whorls with six perianth segments, sessile and zygomorphic. In Africa, parts of gladiolus like corm is used as food while the flower may be consumed as an uncooked salad after nipping the anther. Some species contribute to the medicinal field as effective curative against diarrhoea and headache.

Gladiolus can be grown over a wide range of edaphic conditions, preferably slightly acidic, deep, well drained sandy to clay loam rich in organic matter and nutrients. A moderate climate is preferred.

In the international cutflower trade, gladiolus occupies the fourth place. In Holland and other European countries, it is the most popular after tulip. It has been noticed that Gladiolus contributes greatly to the revenue of Holland. A similar trend has been noticed in Israel too. In India gladiolus occupies an area of 359 ha while its distribution in the state of Maharashtra is 100 ha. The negligible level of its production may be

influenced by a number of factors, one of them being production. The Gladiolus is propagated by corms. Cormel production in cluster on stolons between mother and daughter corms are the important source of maximising the multiplication. These corms and cormels are selected carefully, cured and treated and then stored. Storage helps to break the dormancy period, which is a typical physiological barrier forced by the plant. Many time during storage, the corms are attacked by many pathogens which greatly deteriorate and spoil the corms. Since it is these corms which later on serve as a store house, nourishing the plant and the future flower, it's deterlorated condition will lead to total failure of the crop. A wide range of diseases are housed by the gladiolus corms induding Dry rot or core rot (Fusarium sp.) Dry rot or neck rot (Stromotina gladiali), core or spongy rot (Botrytis gladioliorum),Curvulariablight (Curvularia trifoli f. sp. glodioli) storage rot (Penicillium sp) and leaf spot (Alternaria fareiculata). A great deal of loss has been incurred due to storage rot. Two species of Fusarium have been reported to cause the rot viz. Fusarium oxysporium f. sp. gladioli and Fusarium solani. The typical symptoms include corm splitting into two halves, showing radiating dark coloured streaks extending from the corm base through flesh. In severe case entire corm turns black and rots. Well marked red brown lesions baring clear and concentric markings with well defined margins are found. In the case of Fusarium solani, dry rot is followed by mumification. To find a solution for tackling the storage rot problem.

Following objectives were included in the study of the problem :

1. Isolation of causal agent of storage rot disease of gladiolus.
2. To study pathogenecity of the casual agent.
3. To study the morphological physiological and cultural characteristics of pathogen.
4. Study of varietal screening.
5. Host range.
6. Effect of environment on disease development.
7. Evaluation of the fungicides for control of diseases.
8. To study the interrelationship if any among the different casual organisms.

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

The casual organism Fusarium belongs to

Subdivision : Ascomycotina

Class : Pyrenomycetes

Order : Sphaeriales

Family : Hypocreaceae

The genus has an important position as it causes wide range of infection on commercial plants like wilts, damping off and rots.

2.1 Isolation , pathogenicity and symptology:

McCullouch (1943) studying the vascular disease of gladiolus caused by Fusarium stated that isolation could be made from different parts of the corm viz. the core, radiating vascular bundles, the husk and node lesions. She also stated that the organism showed less growth in culture. The disease manifested itself as brown coloured lesions, which developed and discoloured the cores and radiating vascular strands proceeding inward at a rapid rate resulting in the rot of entire corm.

Tandon and Bhargava (1963) reported that Fusarium solani was the causal agent. However Singh reported that Fusarium oxysporium f. sp.gladioli was the pathogen to be infesting the corms. This observation tallied with a multitude of other workers including McCullouch (1943) and Gould and Miller (1970). Similar results were obtained by Magie (1971). When Friendship corms were held in storage, they were found to be infected by Fusarium culumurum,Fusarium roseum, Fusarium sanbucinusm and Fusarium

solani to be the chief destructors leading to reduction in yield of flower and corm production (Woltz and Magie 1973).

2.2 Morphology of Fungus

McCulloch (1943) reported that the aerial mycelium of the fungus was submerged, white, with persistant aerial growth.

Booth, while studying the morphology of fungus reported that macro and microconidia developed in the culture. Macroconidia measured 35-55 x 4.5-6 u for one strain while the microconidia measured 45-100 x 5-8 u. Chlamydospores developed in 7-19 days and were globose to oval, smooth to rough walled 9-12 x 8-10 u and formed on short lateral branches or were intercalary.

2.3 Cultural Characters

Reddy and Chaudhari (1985) showed that growth was maximum in Richard's medium.

Prasad and Patel (1964) studied the growth of Fusarium on different media and observed the fungal growth was the best on Richard's medium.

Among the nonsynthetic media McCulloch (1943) reported Potato Dextrose Agar to be superior to other media. Reddy and Chaudhari (19[illegible]) also agreed to the same.

2.4 Physiological characters

2.4.1 Utilization Of Carbon Compounds :

Tandon and Bhargava (1963), showed that sucrose was the most favourable medium for carbon source. However, Bhargava, in two different experiments conducted in 1973 and 1980, found that maltose and starch favoured the growth of the fungus.

2.4.2 Utilization Of Nitrogen Compounds :

Bhatnagar et.al (1967-69) showed that the poor growth of the organism occured or was seen on Calcium nitrate and Ammonium sulphate. Similar findings were found by Godage (1979) and they also showed that the effect of different nitrogenous compounds and that growth and sporulation of Fusarium sp. to be abundant on Potassium nitrate and good on Sodium nitrate and Urea.

2.4.3 Temperature :

Nielsen and Moyer (1979) studying the effect of Fusarium sp. on root rot of sweet potato concluded that 29 °C was the optimum temperature for the disease development. They also postulated that 12 °C, 28 °C and 38 °C to be the minimum, optimum and maximum ambient temperatures.

2.5 Host Range :

Gould and Miller (1970) concluded from their experiment that Fusarium caused basal rot of both bulbous Iris and Narcissus.

Rath et al (1978) reported Fusarium solani to be causing rhizome rot of Ginger during storage.

Garge and Gupta (1979) found that host like tomato to be potential host and also concluded that apple, cucumber, egg plant, pepper, squash and potato to be among the hosts harbouring Fusarium.

Nielsen and Moyer (1979) concluded from their studies that rot of stored sweet potato was caused by Fusarium solani.

Chakrabarti et al (1977) reported Fusarium solani to be the casual agent of fruit rot of banana.

2.6 **Fungicidal Bioassay** :

Gould and Miller (197Ø) reported that Benzimidazole fungicide increased yield of healthy bulbs better than Mercurial fungicides without causing phytotoxic effect when used to treat Fusarium infected stocks of Narcissus and Iris.

Forsberg (197Ø) comparing the effect of Thirum and Benomyl reported that Benomyl was more effective in efficiently controlling corm rot resulting in more economic yield.

Magie (1971) reported Benomyl to be much more effective in controlling Fusarium rot of gladiolus. Magie and Wilfret (1974) stated that Benomyl was effective in controlling Fusarium diseases, but when used twice yearly on mother and doughter corms during a two year period, tolerant strains of fungus developed and caused severe corm rotting.

Wani et al (1982) stated that it was better to use fungicides in combination for effective control of rot rather than using

them alone.

2.7 **Varietal Screening** :

Kaur et al while screening 50 cultivars against Fusarium reported that Snow Princess, Melody, Camelia, Mayur, Sylvia to be highly resistant. Varieties Rose supreme, Pusa Suhagini, Suchitra and Ratna butterfly to be resistant. Cultivars showing suseptibility were Sapna, Friendship, Happy End, Royal Jublice, White Oscar, Spic and Span, while Poonam, Apple blossom, Setting sun were found to be highly suseptible. Anonymous (1992) .

MATERIALS AND METHODS

3 MATERIALS AND METHODS

The present studies were conducted in the Department of Plant Pathology, College Of Agriculture, Pune - 411 ØØ5. The study included the isolation of organism from the rotting corms, their inoculation, followed by reisolation to prove the pathogenecity. This was followed by testing its effect on a wide range of hosts, reactions of different varieties and finally their control with the help of different fungicides.

3.1 **Materials** :

3.1.1 **Sources of Isolates** - The sample of infected corms of gladiolus were collected for isolation from All India Coordinated Floriculture Improvement Project, Ganesh Khind Pune, in the month of August- September, 1991.

3.1.2 **Corms** - Healthy corms of gladiolus were obtained from All India Coordinated Floriculture Improvement Project Ganesh Khind Pune, to study the susceptibility of other hosts. Fruits, rhizomes and bulbs of related species were also obtained from market. Their list is as given below :

1) Gladiolus	Gladiolus sp.
2) Beet root	Beta vulgaris
3) Carrot	Daucus carota
4) Capsicum	Capsicum frutescens
5) Cucumber	Cucumis sativus
6) Groundnut	Arachis hypogea
7) Ginger	Zingiber officinale
8) Onion	Allium cepa

9) Potato — Solanum tuberosum

1Ø) Tomato — Lycopersicon esculentum

11) Brinjal — Solanum melangena

12) Tuberose — Polianthes tuberosa

3.1.3 **Fugicides** : The following fungicides were tried firstly in the laboratory using different concentrations by poison food technique against Fusarium corm rot.

No.	Fungicidal formulation with	Chemical Name	Concentration
1)	Bavistin 50 % BASF India Ltd., Bombay.	Methyl 1-H Benzimidazole -2-yl Carbamate	0.05 0.10 0.20
2)	Blitox 50 % Rallis India Ltd., Banglore.	Copper oxy--chloride	0.10 0.20 0.30
3)	Benomyl 50 % Bharat Pulvurising Mills, Pvt. Ltd., Bombay.	Methyl-H 1-(Butylcarbutnol) -2 Carbamate	0.05 0.10 0.20
4)	Caftaf Rallis India Ltd. Bombay	CAS-N-1,1,2,2 Tetrachloroethyl (Thio)Cyclohexen 1,2 dimethylmorphaline	0.10 0.20 0.30
5)	Dithane M-45 % Indofils Chemicals Ltd., Bombay.	Manganese Ethyl Bisdithiocarb--amate + Zinc	0.20 0.25 0.30
6)	Dithane 2 78 % Indofils Chemicals Ltd.,Bombay.	Zinc Ethyl Bisdithiocarb--amate	0.20 0.25 0.30
7)	Folpet	N -Trichloro--methyl thio-phthalimide	0.10 0.20 0.30
8)	Topsin Moti Pesticides India Pvt. Ltd., Masani, Mathura.	Diethyl 4,4-0-Phenylenebis (3-Thioallophanate)	0.50 0.10 0.20

3.1.4 **Laboratory Instruments And Equipments** : Various laboratory instruments which were used during the study were autoclave, incubator, hot air oven, research microscope, refrigerator. pH meter, waterbath, shaker, Top-pan balance, Digital balance. Among the other petty items used included moist

chamber, automizer, inoculating needle, cork borer, pair of scissors, scalpel, spirit lamp, various glassware like conical flasks, petri plates, beakers, funnels, slides, filter papers and chemicals required for sterilization and preparation of media.

3.1.5 **Culture Media** : The following semi synthetic and non synthetic solid culture media were used to study the growth and cultural characters of fungus.

Semi Synthetic Media	Non Synthetic Media
1) Nutrient Agar	1) Host leaf extract with Agar
2) Yeast Extract Agar Agar	2) Host extracts of corm with Agar
3) Czapek's Agar	3) Extracts of corms + leaf with Agar
4) Richard's Agar	4) Cluster bean meal Agar
5) Ashby's Agar	5) French bean meal Agar
6) Jensen's Agar	6) Potato Dextrose Agar
	7) Oat meal Agar
	8) Corn meal Agar

In the study of physiological aspects, Richard's Agar medium was used as basal medium, while Potato Dextrose Agar was used as a basal medium in the poison food technique.

3.2 **Methods**

Isolation, Pathogenecity and Reisolation.

3.2.1 **Isolation** : The samples of affected corms of gladiolus were obtained from All India Co-ordinated Floriculture Improvement Project, Ganesh khind, Pune. They were washed thoroughly under tap water and were allowed to dry in air. With sterile scalpel, affected corm parts were cut into smaller pieces and surface sterilized with 1:1000 mercuric chloride solution for a minute. They were washed in sterilized water for three to four times to remove the traces of corrosive sublimate. These pieces were then transferred aseptically to sterilized plates poured with potato dextrose agar. The plates were then incubated in inverted fashion in the incubator at 25±1 °C. The organism developed profusely within three to four days and grew vigorously. It was then repeatedly subcultured on potato dextrose agar slant which was maintained in pure culture for further studies.

3.2.2 **Pathogenicity** : The pathogenicity of the isolated pure organism obtained from affected gladiolus corms was tested on healthy corms in the laboratory. The healthy corms were first washed under the tap water and disinfected with 1:1000 mercuric chloride solution. They were slightly injured with the help of sand paper. Then the spore suspension was made from the organism cultured on potato dextrose agar, and, with the help of sterilized cotton wool, the suspension was applied only with sterilized water. Cotton wool soaked in sterilized water was kept on the corms to prevent the drying

of the inoculated corm portion.

These corms were securely placed in a moist chamber at ambient room temperature i.e. 27±1 °C, for a period of 30 days. Observations were recorded daily for the development of the infection.

3.2.3 **Reisolation** : The organism was reisolated from artificially inoculated corms described in pathogenicity experiment showing black and brown rotting symptoms. The culture obtained in the reisolation was transferred on potato dextrose agar slants for comparison with original culture.

3.3 **Morphology of the Fungus**

Observations on morphological characters viz. shape, size and septation in seven days old cultures were from the culture grown in potato dextrose agar at ambient room temperature i.e. 27±1 °C and that of mycelial growth on gladiolus corms. Slides were prepared hygenically in the mounting medium. Lactophenol added with cotton blue was used to compare with each other and to examine under research microscope.

3.4 **Growth and cultural characters**

The fungal culture thus isolated was grown on different solid synthetic and non synthetic media in order to study its growth characters and its ability to sporulate on different synthetic and non synthetic media. The different synthetic and non synthetic media were used as mentioned earlier. For this study

fresh medium was prepared following standard methods in the laboratory with meticulous care. For each treatment sterilized plates were poured with media in triplicate and were inoculated with mycelium of fresh growing culture of Fusarium solani(mart) Sacc. The plates were incubated at ambient room temperature i.e. 27±1 °C and observations were recorded for mycelial characters, colony colour, growth substrate, aerial mycelium and sporulation after seven days of incubation.

3.5 **Physiological characters**

3.5.1 **Utilization of carbon compounds** : The ability of the fungus to utilize different carbon compounds was studied using modified Richard's broth (without sucrose) as a basal medium. The stock solution of the basal medium was distributed in aliquots of 100 ml seperately in 250 ml Erlenmeyer flasks, to which, adequate quantity of a carbon compound calculated on the basis of the carbon content equal to those of Richard's medium without sucrose, served as control. The carbon compounds under study were as follows -

1) Glucose
2) Galactose
3) Fructose
4) Sucrose
5) D - mannitol
6) Lactose
7) Starch

The medium was prepared, sterilized as usual, and poured in sterilized petri plates and inoculated at room temperature i.e. 27±1 °C for a period of seven days. Observations were recorded after seven days of incubation period, noting

mycological characters mentioned earlier.

3.5.2 **Utilization Of Nitrogen Compounds** :

3.5.2.1 **Utilization of Inorganic Nitrogen Compounds** : The experiment was conducted in order to study the effect of various inorganic nitrogenous compounds on the growth and sporulation of the fungus, modified Richard's medium (broth) without nitrogen source (Potassium nitrate) was used as a basal medium. The solution of basal medium was distributed in aliquots of 100 ml seperately in 250 ml Erlenmeyer flasks. To each of these flasks adequate quantity just equivalent to the same amount of nitrogen as is Potassium nitrate in the original basal medium was added. The inorganic compounds under study were as follows -

1) Sodium nitrate
2) Potassium nitrate
3) Ammonium nitrate
4) Calcium nitrate
5) Ammonium sulphate
6) Peptone
7) Urea

Richard's broth medium without potassium nitrate served as a control. After sterilization of medium, sterilized plates were poured in for each compound and the same were inoculated with a mycelial bit of growing inoculum which was seven days old. They were incubated at ambient room temperature i.e. 27 ± 1^{o} C for seven days. Observations were recorded after seven days of incubation period and mycological characters were studied as mentioned earlier.

3.5.3 **Effect of H - ion concentration** : The studies on the rate of growth of the fungus as influenced by the hydrogen ion concentration of the medium were made in Richard's solution. Richard's solution was prepared and adjusted to different pH values in Erlenmeyer flask with a glass electrode by adding an appropriate quantity of Ø.1N Sulphuric acid or Ø.1N Sodium hydroxide. Thus the pH was adjusted to the following levels -

1)	2.ØØ	8)	7.5Ø
2)	3.ØØ	9)	8.ØØ
3)	4.ØØ	1Ø)	8.5Ø
4)	5.ØØ	11)	9.ØØ
5)	6.ØØ	12)	9.5Ø
6)	6.5Ø	13)	1Ø.ØØ
7)	7.ØØ	14)	1Ø.5Ø

The test tubes were prepared for each pH value and were sterilized along with the flasks at 1.54 kg/cm^2 for 15 minutes. The final pH of the medium in the flask after sterilization was determined by testing the sample solution from the tubes. The flasks containing 1ØØ ml medium were inoculated with a piece of young culture of the fungus and the flasks were incubated at ambient room temperature i.e. $27 \pm 1^{\circ}C$ for 21 days. The mycelial mat formed in the medium gained a constant weight at $37^{\circ}C$. In each treatment the mycelial mats were weighed and the weight was accurately recorded.

3.5.4 **Growth and Sporulation on solid medium** : Richard's solution was prepared and the same was distributed in aliquots of 1ØØ ml each in a 25Ø ml Erlenmeyer flask. The pH values of these

solutions were adjusted as described in the previous experiment. Two test tubes for each pH value were also sterilizd at 1.54 kg/cm^2 for 15 minutes, along with the flasks in which suitable quantity of agar was added for solidification of the medium. The pH of medium was adjusted as per given norms. The pH of the medium after sterilization of flasks was determined by testing the sample solutions from the tubes. After sterilization of flasks containing medium, duplicate plates were poured with the same for each of the pH values. The center of these plates inoculated and incubated at the ambient room temperature i.e. 27±1 °C. Observations on colony diameter were recorded after the incubation period of seven days as described earlier.

3.5.5 **Effect of different temperatures on growth and sporulation** :

The effect of different levels of temperature on the growth of the fungus was studied on potato dextrose agar which was sterilized at 1.54 kg/cm^2 for 15 minutes. The sterilized petriplates were poured in duplicate with the agar and were inoculated with a mycelial bit of a young growing culture nearly Ø.5 cm in diameter and incubated at different levels of temperature viz. Ø °C, 5 °C, 13 °C, 14 °C, 22 °C, 26 °C, 29 °C and 35 °C for an incubation period of seven days. Observations on colony diameter, growth characters and sporulation were noted to find out the ability of the fungus to grow.

3.5.6 **Host Range** : An in vitro study on the ability of the fungus to infect other types of vegetables was undertaken. The list of vegetables included in the study has been enlisted earlier. The vegetables in mature condition were selected. They were first washed under tap water and then surface sterilized with 1:1000 mercuric chloride solution for one minute. The vegetable was rewashed with sterilized water for two to three times and were then allowed to dry in the air. Then a little injury was made by using new sand paper. A thin spore suspension of seven days ~~young~~ old growing culture was prepared in distilled water and was applied on the injured skin of the vegetable with the help of cotton wool, aseptically. The sterilized moist cotton wool was kept on the vegetable in order to keep the surface of the vegetable moist. These vegetables were kept in sterilized moist chamber in which 100 per cent humidity was maintained for seven days at ambient room temperature i.e. $27 \pm 1^{\circ}$ C. Uninoculated vegetables were applied only with sterilized water which served as a control for each case.

3.5.7 **Fungicidal Bioassay** :

3.5.7.1 **Poison Food Technique** - Preliminary trials were undertaken to see if any fungicide could be found effective in checking the growth of the fungus in vitro culture to control the disease causing organism. Appropriate quantities of the fungicides in three different concentrations for each fungicide as given

were added to previously sterilized 100 ml of potato dextrose agar, seperately for each concentration. The flasks were then shaken well to ensure uniform distribution of fungicide in the potato dextrose agar medium. Previously sterilized petriplates were poured with this medium in duplicate for each concentration and were inoculated with seven days old culture and then incubated at ambient room temperature i.e. 27±1 °C for 10 days.

3.5.8 **Fungicidal Control In vitro** : Fungicidal control of the rot of gladiolus corms under laboratory conditions for the corms which were taken out of the storage conditions were used. The corms showed rot disease on them caused by Fusarium solani. The trial was conducted in the laboratory of the Department Of Plant Pathology of Agriculture College, Pune - 411 005, during rabi season of 1990. The local variety of gladiolus was tested.

For this trial eight fungicides were selected. The corms of gladiolus were dipped in the spore suspension of Fusarium solani for 2 minutes. After 24 hrs of inoculation of corms, the same were sprayed with the fungicides with the given concentrations, from observations of poison food technique. Subsequent fungicidal sprays were given after two days interval. The untreated inoculated corms served as control. The observations were recorded regarding disease index after 10 days of inoculation. The disease indices were

worked out as per the following scale grading followed by judging disease intensity.

Ø	healthy corms
1-1-25	percent infection on corms
2-26-5Ø	percent infection on corms
3-5-75	percent infection on corms
4-76-1ØØ	percent infection on corms

The disease index percentage was calculated by using the following formula

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of corms assessed}} \times \frac{1ØØ}{\text{Maximum rating}}$$

EXPERIMENTAL RESULTS

4 EXPERIMENTAL RESULT

4.1 Isolation, pathogenicity and symptomology

4.1.1 Isolation :

Isolates were obtained from the samples collected at All India Co-ordinated Floriculture Improvement Project, Pune. From affected corms of gladiolus the mycelium was aerial, striate sparse to dense and flocose greyish - white. The micro-conidio which were developed after 2-3 days were born on lateral conidiophores. The macro-conidia were developed after seven days. The chlamydospores tended to develop after 7-14 days.

4.1.2 Pathogenecity And Symptomology :

In the inoculation experiment it was seen that the fungus could infect the corm skin at the point of injury. The rotting symptoms were observed 31 days after inoculation. The first symptoms that appeared at the base of the corm were as rather shallow, hard, rough spots dark brown to black in colour. Red brown lesions having clear and concentric markings with well defined margins were found to be on the corms. The tissues which appeared as water soaked areas turned brown. Rotting extended rapidly to cover approximately half of the surface of the corm within a period of 30 days. Pinpoint infection on the surface of the corms was found to have developed to spots which further expanded to coales with each other within a period of 20-30 days. Under high humid moist conditions the disease was developed to the extent of practicaly 100 per cent. The brown spots showed the

PLATE-I

A) D - A naturally affected corms of gladiolus showing a typical symptoms of corm rot

H - Healthy corms of gladiolus

B) - Symptems produced by an artificial inoculation with Fusariam solani isolate V/S healthy gladiolus corms.


D
H


CONTROL
INOCULATED

presence of white mycelial growth having conidia and conidiophore within the period of 28-30 days.

4.1.3 **Symptoms Of Corms Rot On Naturally Affected Host** :

The naturally affected, corms during storage showed symptoms like corms splitting into two halves, radiating dark coloured streaks extending from the base into the flesh and in severe cases the center of the entire corm became black and rotten. Brown lesions having clear and concentric markings with well defined margins were found on the corms. Some times in severe infections the patches became dark black, many a time the rotten corms were soft or hard. The affected corms did not show buds and such corms were not fit for planting.

4.2 **Reisolation**

The organism was reisolated from the inoculated corms. The fungus obtained was found to be identical with the original culture and was used for inoculation in all respect.

4.2.1 **Morphology Of The Fungus** : The mycelium of Fusarium solani was aerial striate, sparse to dense and flocose greyish white. Typically developed culture was also blue to bluish to brown althuogh occasionally a brownish vinaceous pigmentation may be present.

Macro-conidia developed abundantly in fresh isolates after 2-3 days. They were formed from lateral conidiophores which initially may have been merely elongated lateral phalides which narrowed slightly toward the apex. They later produced micro-

conidophores which were elongated and seperately branched radiating up to 400 u in length, each branch usually terminating into a single cylindrical basically subclavate philades which measured 45-80 x 2.5-3 u. These were in marked contrast to short micro-conidiophores with numerous philades formed in Fusarium oxysporum. Micro-conidia of Fusarium solani were also rather broader and more oval in shape with some-what thicker walls. They were 8.16 x2-4 u and may have a single septation.

Macro-conidia developed after 4 to 7 days from initially simple but, later from short multibranched conidiophores which soon merged to form effuse sporodochia. Marco-conidia were equilaterally furiod with many of the spores having the widest diameters. Many had rounded foot cells similar to Clyindrocarpon species. The apical cells were pointed and somewhat beaked. chlamydospores tended to develop abundantly on weak media after 7-19 days. They were globose to oval, smooth, to rough walled 9-12 x 8-10 u and formed intercalary or terminal on short lateral branches. As stated by Nash (1963) Fusarium solani occures in the form of chlamydospores in naturally infected parts.

4.2.2 **Growth and cultural characters:**

Observation on growth and cultural characters of the fungus on the various synthetic and non synthetic media was recorded. The results obtained from observations have been presented in the Table No.1 and Table

The test organism was cultured on six synthetic and eight non synthetic media. The fungal growth was measured in the form of radial diameter which ranged from 2Ø to 9Ø mm on synthetic media whereas on non synthetic it was 3Ø to 45 mm. among the six synthetic media tested the test organism grew well on Richard's medium as far as mycelial growth and sporulation was concerned. Therefore it was used as a basal medium for further isolations. The growth on nutrient agar and Czapek's Dox agar medium was good while that on yeast extract agar was moderate. The growth of colonies on Ashby's medium and Jensens medium, however was poor.

Among the eight non synthetic media used growth was excellent on potato dextrose agar. The growth on french bean extract was good while that on an extract of gladiolus corms +leaves and gladiolus leaves was moderate. The growth on oat meal agar was poor while that on gladiolus corms, cluster bean extract and corn meal agar was very poor.

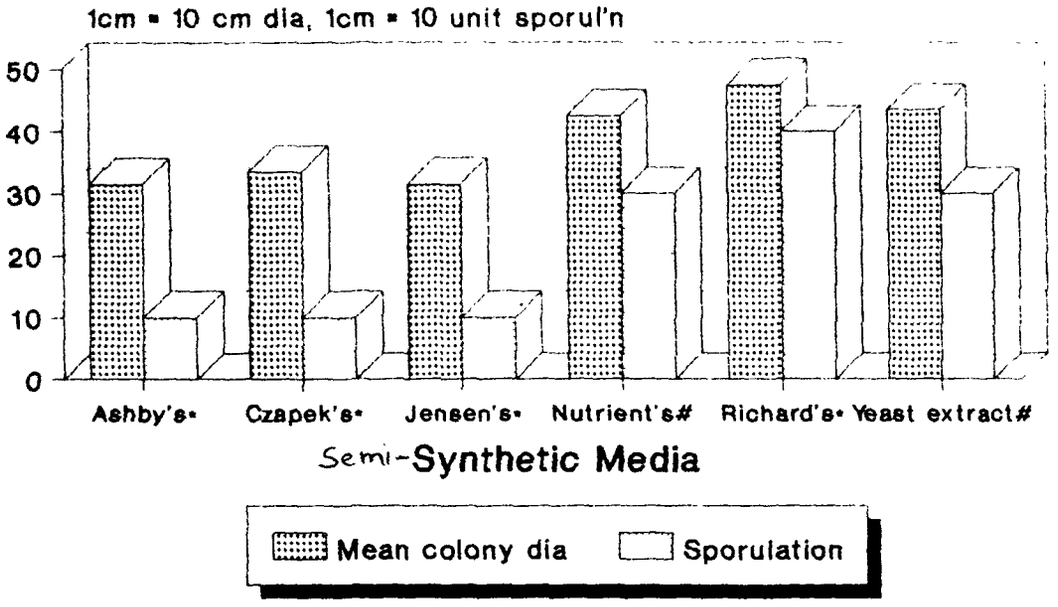
TABLE NO. 1

Colony and growth character of *Fusarium solani* isolate on different synthetic media

S.No.	Name of the synthetic media	Mean colony dia.	Sporulation	Colony and growth character
1.	Ashby's medium	30-33	+	Scanty growth mainly centred around the point where mycelial bit was placed. Mycelial growth minimum with scanty sporulation.
2.	Czapek's medium	32-35	+	Scanty to no growth, centred around point of inoculation Brownish center with white mycelium covering it on the boundary, scanty sporulation.
3.	Jenson's medium	30-33	+	Scanty to no growth centered around point of inoculation. Brown spot some what circular with scanty sporulation.
4.	Nutrient Agar	40-45	+++	Very good growth, circular colony with mycelium profusly grown, white in colur and good sporulation.
5.	Richard's medium	45-50	++++	Abundant growth, colonies circular with entire margin mycelium well developed and profusely grown, snowy white in colur and abundant sporulation.
6.	Yeast extract agar	42-45	+++	Very good growth, colonies circular with entire margin, mycelium well developed concentric rings very prominant.

Rating Nil - Good +++
Scantly + Abundant ++++

Colony and growth character of *Fusarium solani* isolate on Semi-Synthetic media



• = medium, # = Agar
0 = nil, 10 = scanty, 20 = moderate
30 = good, 40 = abundant

PLATE -II

Growth of Fusarium Solani Isolates on different semi synthetic media

A) PETRIPLATE NO.	NAME OF CULTURE MEDIA
1	Ashby's medium
2	Czapek's medium
3	Jensons medium
4	Nutrient agar
5	Richard's agar
6	Yeast extract agar

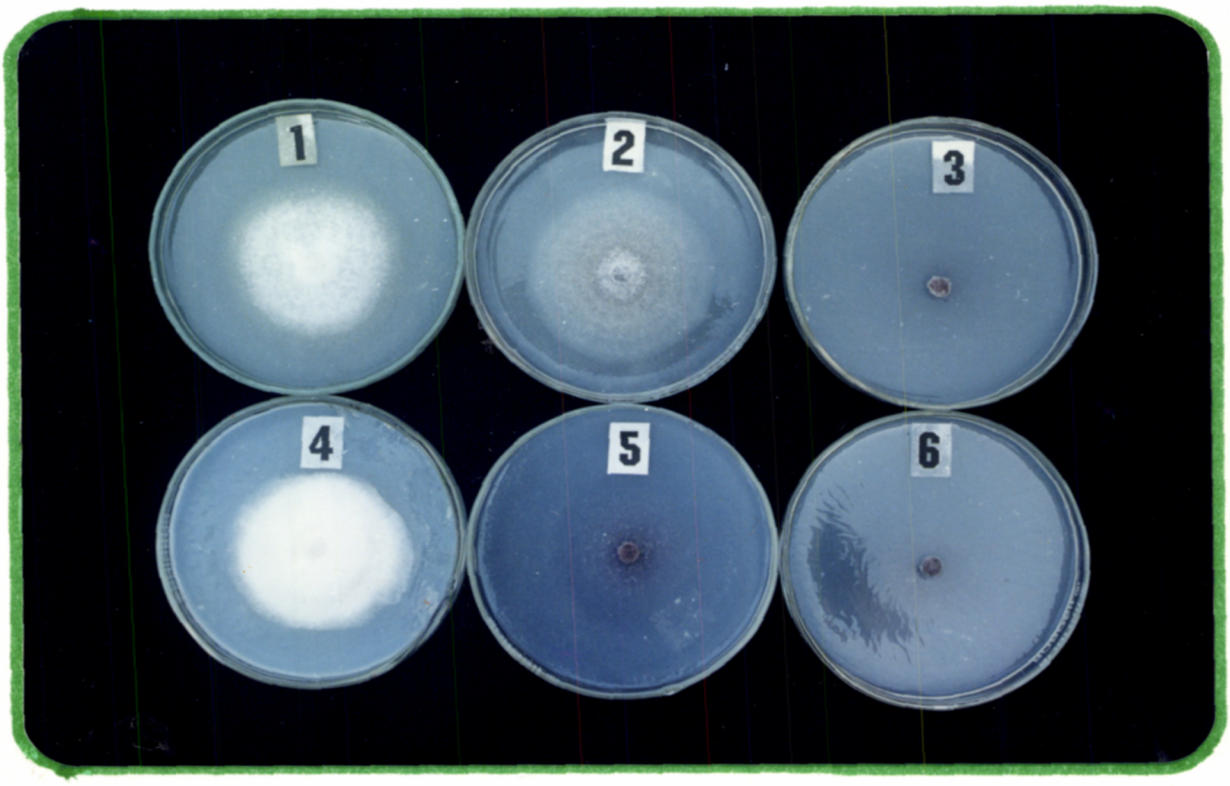

1
2
3
4
5
6

TABLE NO. 2

Colony And Growth character of *fusarium solani* isolate On Non-synthetic Media

Sr. No.	Name Of The Non-synthetic Medium	Mean Colony Dia.	Sporulation	Colony And Growth Characters
1.	Gladiolus Corm + Leaf Extract with agar	51 mm	++	Moderate growth. Colonies circular with concentric rings, the growth was poor at center while good growth was seen at the margin, the colour of colonies was white. Moderate sporulation.
2.	Cluster bean	40 mm	+	Very poor growth, the growth rate was very slow. The mycelium was snow white in colour but sporulation was scanty.
3.	Corn meal agar	40 mm	--	Very poor growth. The growth was considerably slow. No growth of mycelium.Sporulation was nil.
4.	Gladiolus leaves	51 mm	++	Moderate growth. Colonies were circular. The colour of the mycelium was dull white. Moderate sporulation.
5.	Gladiolus corms	42 mm	++	The growth was thick at the center. The concentric rings developed but were not clear. The colonies were moderate and circular in shape and white in colour. Good sporulation.
6.	French bean	60 mm	+++	Very good growth, colonies circular, mycelium cottony. The colonies were round in shape with concentric rings. Moderate sporulation.
7.	Oat meal agar	48 mm	++	The growth was thick but not circular in shape. The rate of growth was very slow. Mycelium white in colour with moderate sporulation.
8.	Potato dextrose agar	63.65 mm	++++	Abundant growth in the center and very profuse growth around it. Concentric rings were observed. Mycelium was white in colour. Growth took,place at a rapid rate. Abundent sporulation.

N. B. Rating Of Sporulation No growth -- Scanty + Moderate ++
Good +++ Abundent ++++

Note:- X axis Denotes Non Synthetic media

C+L # Corm + Leaf extract with agar

CB # Cluster bean agar

CM # Corn meal agar

CL # Gladiolus leaves agar

GC # Gladiolus corms agar

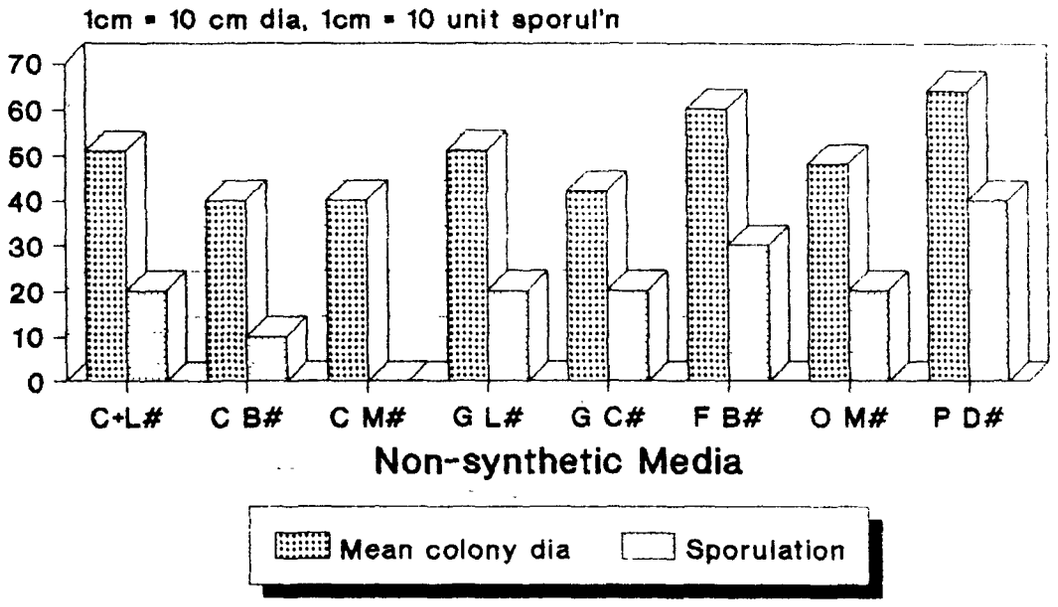
FB # French bean agar

OM # Oatmeal agar

PD # Potato dextrase agar

Note:- Y axis Denotes colony diameter in cm

Colony and growth character of *Fusarium solani* isolate on Non-synthetic media

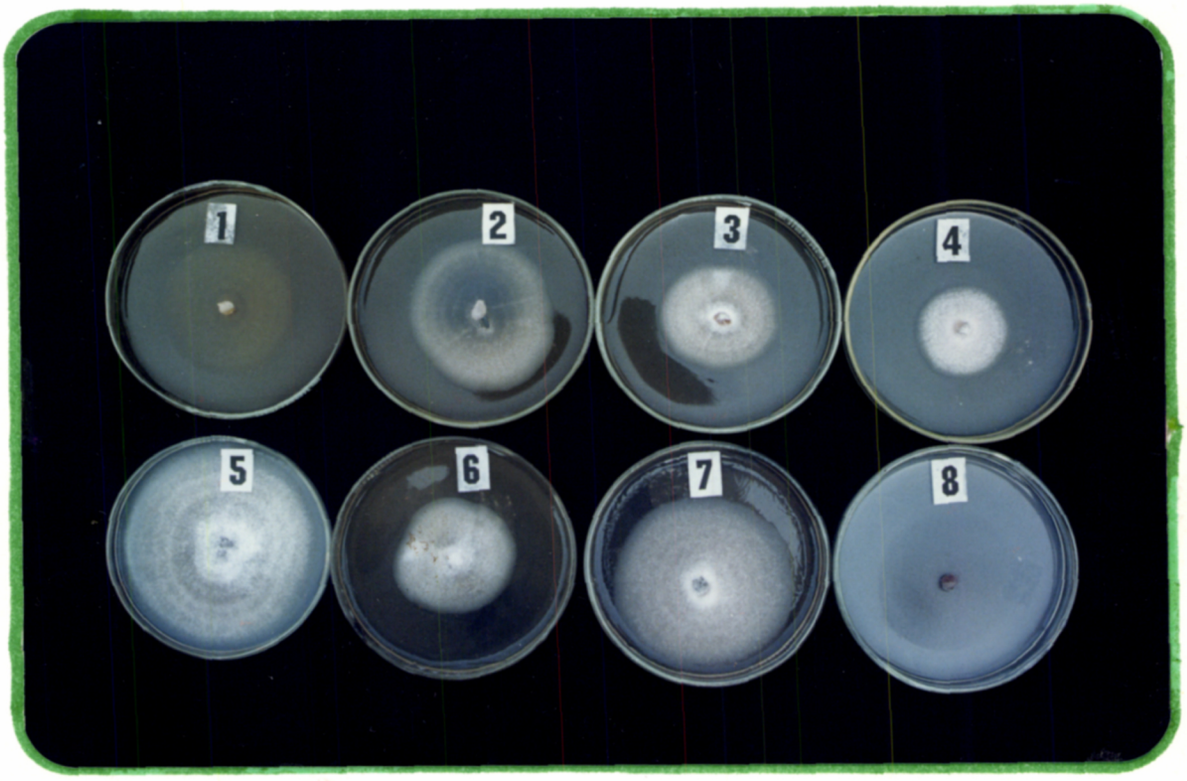


# = Agar
0 = nil, 10 = scanty, 20 = moderate
30 = good, 40 = abundant

PLATE -III

Growth of Fusarium solani islate on diffrent non sysnthetic media.

A) PETRIPLATE NO.	NAME OF CULTURE MEDIA
1	Corms leaf extract with agar
2	Cluster bean agar
3	Corn meal agar
4	Gladiolus leaves agar
5	Gladiolus corms agar
6	French bean agar
7	Oatmeal agar
8	Potato dextrose agar



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4.3 Physiological characters

4.3.1 Utilization Of Carbon Compounds :

The ability of the isolate to utilize seven different carbon compounds in Richard's broth was studied. The observations recorded for dry mycelial weight after 21 days of inoculation have been presented in table No. 3.

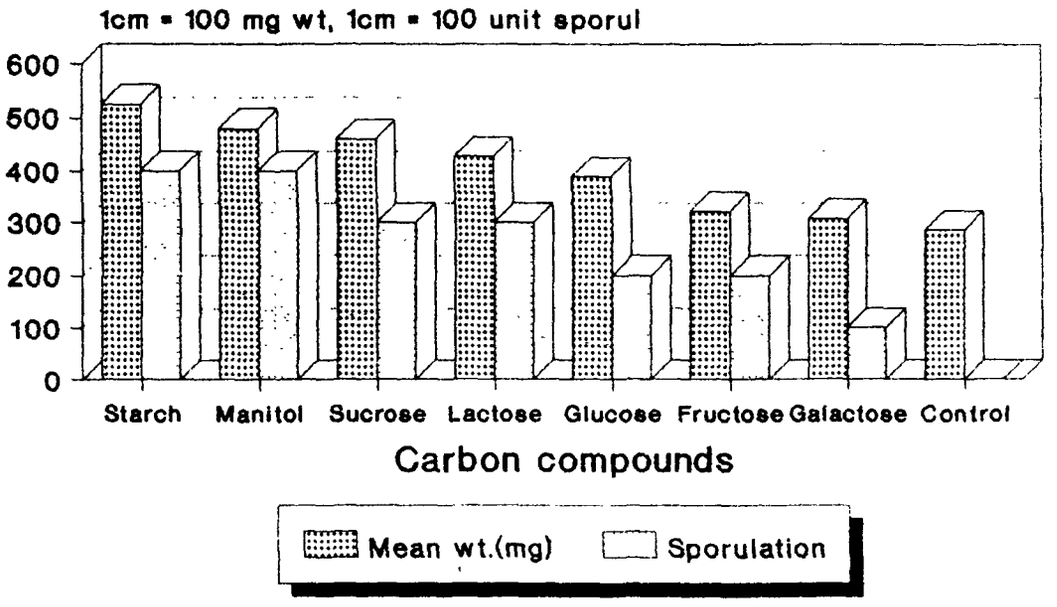
TABLE NO. 3

Utilization of Carbon Compounds By *Fusarium solani* Isolate

Sr. No.	Name of compound	Mean Wt. of mycelial mat (mgs)	Sporulation
1.	Starch	525	++++
2.	Manitol	48Ø	++++
3.	Sucrose	459	+++
4.	Lactose	426	+++
5.	Glucose	388	++
6.	Fructose	322	++
7.	Galactose	3Ø8	+
8.	Control	287	-

Rating Nil - Good +++
Scantly + Abundant ++++

Utilization of Carbon compounds by *Fusarium solani* isolate



0 = nil, 100 = scanty, 200 = moderate
300 = good, 400 = abundant

From the results presented in Table No. 3, it was revealed that all the isolates could luxuriantly utilize the carbon compounds. The carbon compound manitol/starch recorded the highest mycelial dry weight (480.00 mg, 525.00 mg) followed by sucrose (459.0 mg). The minimum mycelial dry weight was noticed in the control treatment 287 mg. The sugars lactose, glucose and galactose appeared to be at par with each other in producing the dry mycelial weight. The sporulation of the isolates on starch/manitol was abundent, sporulation on sucrose, and lactose was good and sporulation on glucose and fructose was moderate where as in the case of galactose there was poor sporulation while no sporulation occured in the control (no sugar).

4.3.2 **Utilization of nitrogenous compound** :

The ability of the organism to utilise seven nitrogenous compound in Richard's broth was studied. The observations were recorded on the basis of dry mycelial weight and results have been presented in the table No.4 and graphical in Fig. No.4 From the results presented in table No.4 it is noticed that potassium nitrate had recorded, the maximum average mycelial dry weight (497mg) which was significantly superior to other nitrogenous sources. It was followed by urea (474 mg). The average lowest mycelial dry weight was noticed in the control treatment (without nitrogenous compound). The nitrogenous compound viz. calcium nitrate and ammonium nitrate were moderate in their weight while sodium nitrate and peptone were at par with each other in producing average mycelial dry weight.

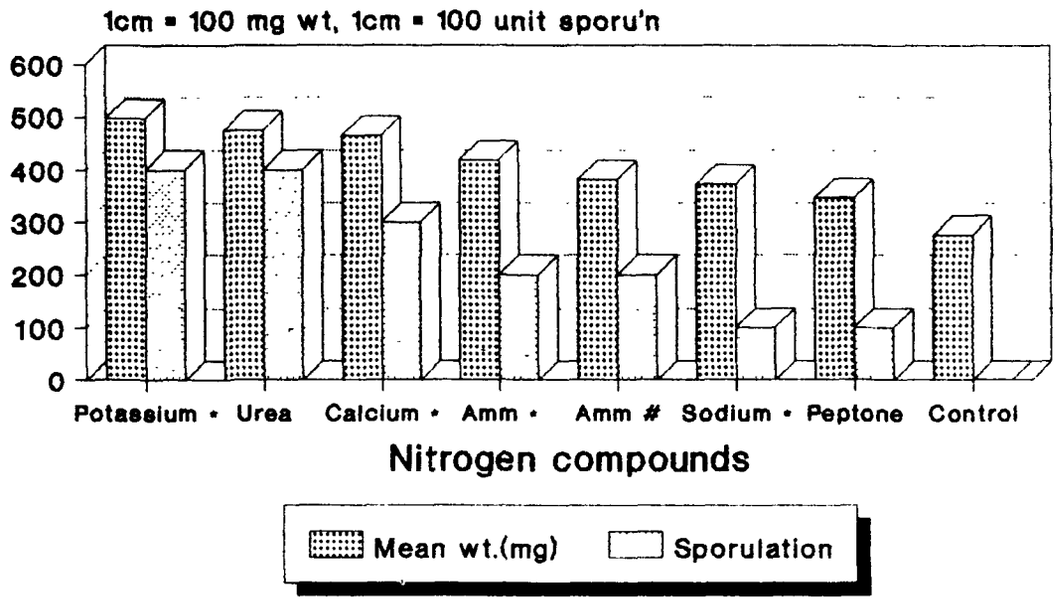
TABLE NO. 4

Utilization Of Nitrogen Compounds By *Fusarium solani* Isolate

Sr. No.	Nitrogen Compound	Mean mycelial dry weight (mgs)	Sporulation
1.	Potassium Nitrate	497	++++
2.	Urea	474	++++
3.	Calcium Nitrate	464	+++
4.	Ammonium Nitrate	418	++
5.	Ammonium Sulphate	382	++
6.	Sodium Nitrate	373	+
7.	Peptone	347	+
8.	Control	276	-

Rating : Scanty : + Moderate : ++
Good : +++ Abundant : ++++
Nil : -

Utilization of Nitrogen compounds by *Fusarium solani* isolate



• = Nitrate, # = Sulphate
0 = nil, 100 = scanty, 200 = moderate
300 = good, 400 = abundant

4.3.4 **Effect Of H-ion Concentration On Growth Of Fusarium** :

The results regarding the effect of different pH levels on the growth of Fusarium isolates have been presented in Table No. 5., and graphically in Fig. No.5. The persual of data presented in Table No. 5 indicated that the organism could tolerate a pH range from 3-8. However the average maximum dry weight was noticed at pH of 4.Ø5 (899 mg) which was considered to be optimum pH for the growth of the Fusarium. The lowest mycelial dry weight has been noticed at pH 2 (239 mg).

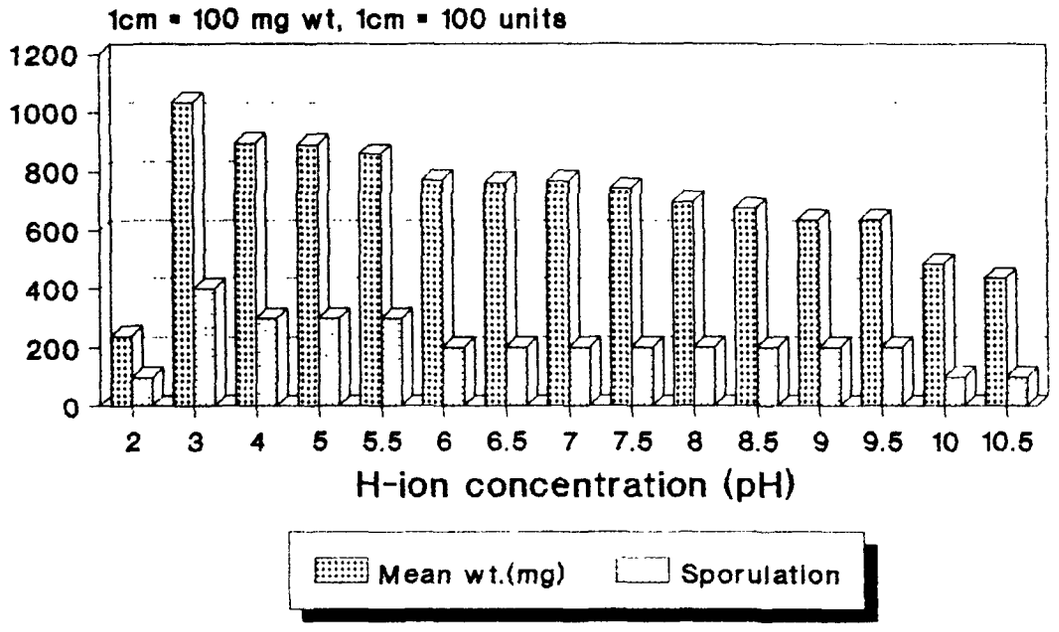
TABLE NO. 5

Effect of H-ion Concentration On Dry Mycelial Weight Of *Fusarium solani*

Sr. No.	Initial pH of Medium	pH of The Medium After Sterilization	Sporulation	Dry Weight of Mycelial mat (mgs)
1.	2.Ø	2.ØØ	+	239
2.	3.Ø	3.Ø5	++++	1Ø36
3.	4.Ø	4.Ø5	+++	899
4.	5.Ø	5.Ø5	+++	892
5.	5.5	5.5Ø	+++	861
6.	6.Ø	6.ØØ	++	773
7.	6.5	6.Ø5	++	763
8.	7.Ø	6.Ø8	++	769
9.	7.5	7.25	++	745
1Ø	8.Ø	7.5Ø	++	698
11	8.5	7.7Ø	++	678
12	9.Ø	8.ØØ	++	639
13	9.5	8.4Ø	++	64Ø
14	1Ø.Ø	9.1Ø	+	488
15	1Ø.5	1Ø.1Ø	+	437

Rating : Scanty : + Moderate : ++
Good : +++ Abundent : ++++
Nil : -

Effect of H-ion concentration on dry mycelial weight of *Fusarium solani*



0 - nil, 100 = scanty, 200 = moderate
300 = good, 400 = abundant

4.3.4 **Effect Of Temperature On Growth And Sporulation Of Fusarium** :

Studies were carried out to asses the minimum, optimum and maximum temperature requirement of the pathogen. The results have been presented in Table No. 6 and graphically in Fig. No.6 from the data, it could be seen that the organism could grow between 18°C to 29°C. Very poor or scanty growth was observed at 13°C, 5°C and below and at 35°C or higher. Maximum Sporulation of organism was observed at 22°C and 29°C where as there was little sporulation at 5°C.

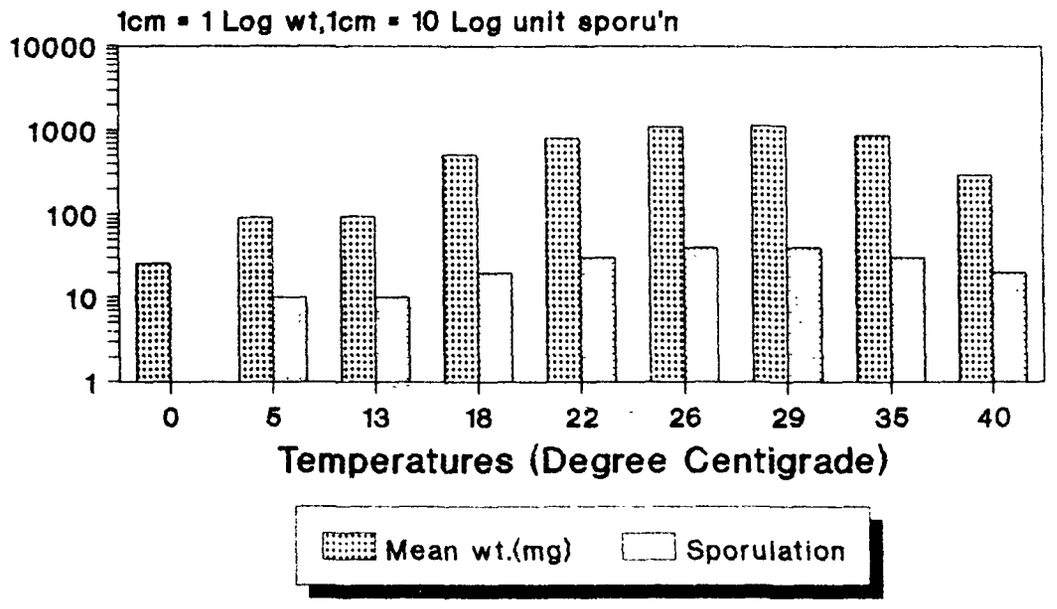
TABLE NO. 6

Effect of Temperature on Growth and Sporulation of Fusarium solani

Sr. No.	Temperature	Sporulation	Mean Mycelial Dry Wt. (mgs)
1.	Ø °C	-	26
2.	5 °C	+	94
3.	13 °C	+	96
4.	18 °C	++	5Ø7
5.	22 °C	+++	8Ø6
6.	26 °C	++++	1Ø93
7.	29 °C	++++	1141
8.	35 °C	+++	859
9.	4Ø °C	++	297

Rating : Scanty : + Moderat : ++
Good : +++ Abundent : ++++
Nil : -

Effect of temperature on growth and sporulation of *Fusarium solani*



0 = nil, 10 = scanty, 20 = moderate
30 = good, 40 = abundant

4.4 Host range

For studying the response of range of other hosts which harbour and nourish Fusarium solani inoculation on a versatile range of host species was done to test their response and reaction. The pathogen found infecting different hosts is tabulated in Table No. 7.

Among the flower crops, isolated species reported to harbour Fusarium solani was found to infect the tulips, narcissus, tuberose, lilies, all of which have a some what similar propagation methods and life cycles. Among the vegetables tomato, cucumber, capsicum, potato, sweet potato, ginger, groundnut, ashgourd, bottle gourd, colocasia and yam were other supported important hosts. Apple, pears and bananas were some of the fruits which were affected greatly by Fusarium sp. The infected hosts showed several symptoms. Initial symptoms expressed themselves as brown coloured spots. These spots increased in size & merging with the neighbouring ones to form large water soaked lesions. This was accompanied by a profuse cottony white growth of the mycelium on the host. As the growth of the organism progressed, a major transformation took place by which the mycelium changed its appearance to black from white, along with the extra softening was observed leading to complete breakdown of tissues resulting into the product which was totally unfit for any use.

TABLE 7

Symptomps shown by different hosts inoculated with *Fusarium solani*

Host Name	Symptoms
Apple	The brown black spots initially on the rind of fruits which later on coalased to form water soaked lesion which grew in size and resultng into total breakdown of cell leading to complete rotting.
Ashgourd	Developed initialy dark brown coloured rot symptoms, the spots merged with each other and formed big spot or patch on the fruit rind. This later covered the entire surface of the fruit which was completely rotten.
Banana	Development of circular necrotic lesions with superficial concentric rings radiating from the point of infection. The infection progressed and central deep with the pulp and destroyed the fruit.
Brinjal	There was development of small circular nectrotic spot on superficial areas. Later on the spots increased in size and merged leading to roting of fruit.
Bottle gourd	Small dark brown spot developed on the fruit. The spot grew in its size and then imerged with each other and as the result of which big patches were observed in the fruit ultimately resulting soft into rotting of tissues leading to loss of the vegetable.
Capsicum	The small brown spots, were developed. Around the brown spot there was yelloing of the tissues which later turned it unpalatable and useless.
Colocasia	Small dark roundish spots increase in area to assume an oval or irregular apperance. A zonation pattern of brown balck colour developed which, turned soft, decayed giving out bad odour.

Gladiolus	The initial pinpoint spots later on grew in size and turned black in colour, further these spots merged with each other forming patches which were irregular and hard and later on the whole surface of corm was covered by this infection.
Musk Melon	A water soaked spot first developed esspecially when in contact with soil. There was complete break down of in tissue.
Potato	Initially necrotic lesions were formed which later on turned black. Infection spread inwards, the entire tuber turned brown balck and rotten giving out an offensive smell.
Sweet potato	Development of circular nectrotic lesion with superficial concentric rings radiating from wound infection sites. The infection progressed initially beyond the vasaclar ring resulting in entire rotting or rot.
Zinger	The first systmptom was the apperance of black spot which later became water soaked. The rhizome rotting converting into a pulpy mass giving out a putrifying smell.

PLATE - IV

Host range study of Fusarium Solani Isolate

1) Name of Host - Tomato

N.B - D: Diseased

H: Healthy

2) Name of Host - Peach

N.B - D: Diseased

H: Healthy

3) Name of Host - Capsicum

N.B - D: Diseased

H: Healthy

4) Name of Host - Brinjal

N.B - D: Diseased

H: Healthy


D
H
1
D
H
2
D
H
3
D
H
4

4.5 **Fungicidal Bioassay** :

4.5.1 **Poison Food Technique** : The observations were recorded in vitro for study of eight different fungicides with three concentrations each, on the growth characters and sporulation of the test organism which are presented in Table No. 8. The data indicated that the fungicides viz. Bavistin and Benomyl showed no growth of the test organism at the various concentrations used in the study. Bavistin was found to be the most effective at Ø.Ø5 per cent concentration followed by Benomyl. The next best fungicide controlling the organism was Ziram at high concentration. Caftaf, Topsin were also effective where as the Folpet, Blitox and Mancozeb were relatively ineffective against the control of Fusarium sp.

Among the sulphur and copper fungicides, Blitox and Mancozeb were not so effective, and at low concentration level they did not show any effect on sporulation. Where as systemic fungicide Bavistin was found to be the most effective while others such as Benomyl, Zirum, Caftaf, Topsin were found to be quite effective on sporulation. Folpet was effective on sporulation at high concentration.

TABLE NO. 8

Effect of Fungicides on Control of Corn Rot Caused By *Fusarium solani* By Poison Food Technique

Sr.No.	Name Of Fungicide	Conc code	Conc. Of Fungicide	Mean Colony Dia. (mm)	Sporulation	Symptomology
1.	Blitox	A	0.1	90.33	+	Poor growth, colonies less profuse in the center sparsely spread out. Mycelium dull white in colour. Moderate sporulation
		B	0.2	81.33	+	Poor growth,less profuse in center sparsely spread out. Mycelium dull white in colour with poor sporulation.
		C	0.3	61.67	+	Very poor growth less profuse in center sparsely spread out. Mycelium dull white in colour with poor sporulation.
2.	Mancozeb	A	0.20	61.60	++	Good growth, colonies profuse circular in shape with entire margins, aerial mycelium with colonies was snowy white. Moderate sporulation.
		B	0.25	55.00	++	Abandent growth, profuse colonies, circular with entire margins, aerial mycelium with concentric rings colony white in colour with moderate sporulation.
		C	0.30	48.30	+	Abundant growth, profuse colonies, circular with entire margins, aerial mycelium with concentric rings colony white in colour with moderate sporulation.
3.	Ziram	A	0.20	15.00	-	Very poor growth, colonies not developed, milky white in colour. No sporulation.

Table No.8 Contd...

		B	0.25	15.00	-	Very poor growth, colonies not developed, milky white in colour. No sporulation.
		C	0.30	10.00	-	Very poor growth, colonies not developed, milky white in colour. No sporulation.
4.	Bavistin	A	0.05	0 mm	-	No growth
		B	0.10	0 mm	-	No growth
		C	0.20	0 mm	-	No growth
5.	Topsin	A	0.05	25.67	-	Poor growth colonies irregular with serrated margin, myeclium poor with centre deep grown brownish in colour surrounded by only white mycelium with scanity sporulation.
		B	0.10	24.67	-	Poor growth colonies irregular with serrated margine, mycelium poorly grown with centre deep grown brownish in colour surro--unded by only white mycelium with no sporulation.
		C	0.20	20.45	-	Very poor growth; colonies irregular with serrated margin mycelium poor with centre deep grown brownish in colour surrounded by only white mycelium with no sporulation.
6.	Caftaf	A	0.10	19.00	+	Scanty growth; colonies irregular with serrated margin with raised centre, greenish in colour scanty sporulation.
		B	0.20	16.70	+	Scanty growth; colonies irregular with serrated margines with raised centre greenish in colour seanty sporulation.

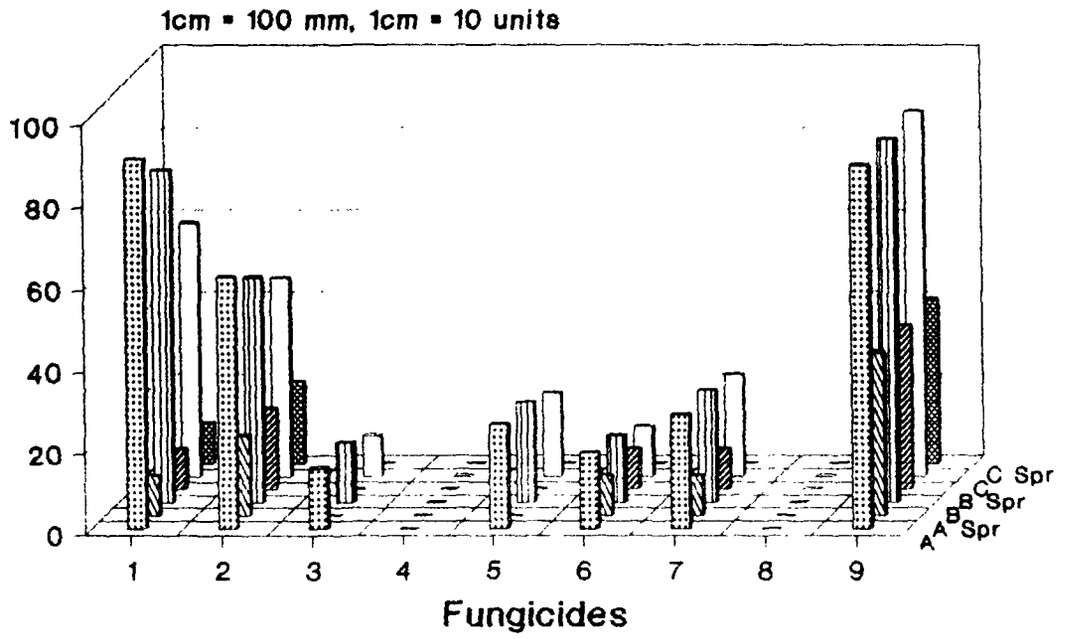
Table No.8 Contd...

		C	0.30	12.30	-	Scanty growth; colonies irregular with serrated margines with raised centre greenish in colour no sporulation.
7.	Folpet	A	0.10	28.33	+	Poor growth colonies with spongy centre aerialmycelium irregular in shape with serrated margine colony colour was snowy white sparse sporulation.
		B	0.20	27.67	+	Poor growth colonies with spongy centre aerial mycelium irregular in shape with serrated margine colony colour was snowy white in colour no sporulation.
		C	0.30	25.00	-	Poor growth; colony with spongy centre, irregular in shape with serrated margin colony colar was snow white in colour. No sporulation.
8.	Bonomyl	A	0.20	0	-	No growth
		B	0.25	0	-	No growth
		C	0.30	0	-	No growth
9.	Control			89 m	++++	Abundant growth, colonies circular with entire margin mycelium prfuse and show white in colour Abandant sporulation.

Rating :

Nil	-
Scanty	+
Moderate	++
Good	+++
Abundant	++++

Effect of fungicides on control of corm rot caused by *Fusarium solani*



0 - nil, 10 = scanty, 20 = moderate
30 = good, 40 = abundant

PLATE - V

POISION FOOD TECHNIQUE

Growth of Fusarium Solani Isolate on diffrent fungicides at different le of concertation

A) Sr.No	Petri Plate No	Name of Fungicide	Concertration %
1	1 A	Blitox	Ø.1
2	1 B	Blitox	Ø.2
3	1 C	Blitox	Ø.3
4	2 A	Mancozeb	Ø.2Ø
5	2 B	Mancozeb	Ø.25
6	2 C	Mancozeb	Ø.3Ø
7	Central Plate (Control No.9)	With out fungicide	Nill

B) Sr.No	Petri Plate No	Name of Fungicide	Concertration %
1	3 A	Ziram	Ø.2Ø
2	3 B	Ziram	Ø.25
3	3 C	Ziram	Ø.3Ø
4	4 A	Bavistin	Ø.Ø5
5	4 B	Bavistin	Ø.1Ø
6	4 C	Bavistin	Ø.2Ø
7	Central Plate (Control No.9)	With out Fungicide	Nil


A
A
2
9
1
B
B
C
C

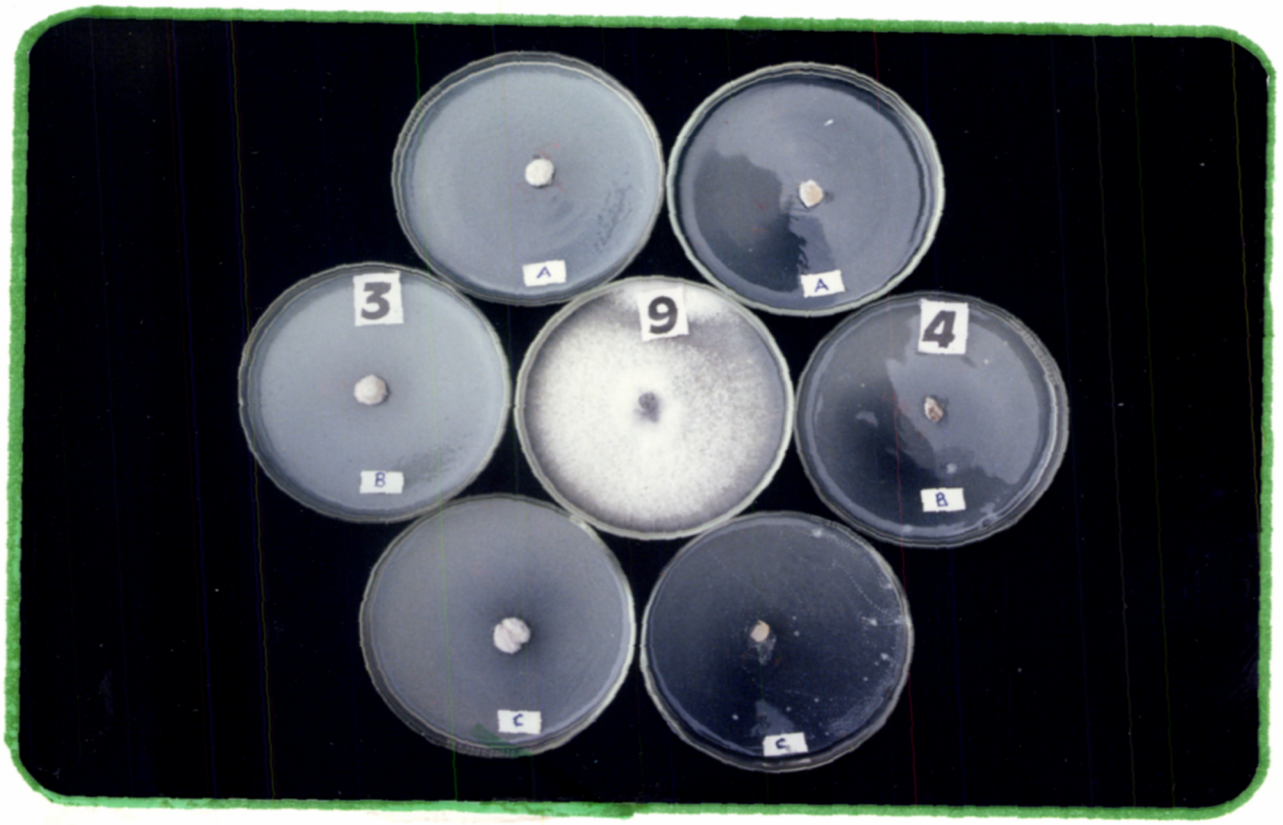

A
A
3
9
4
B
B
C
C

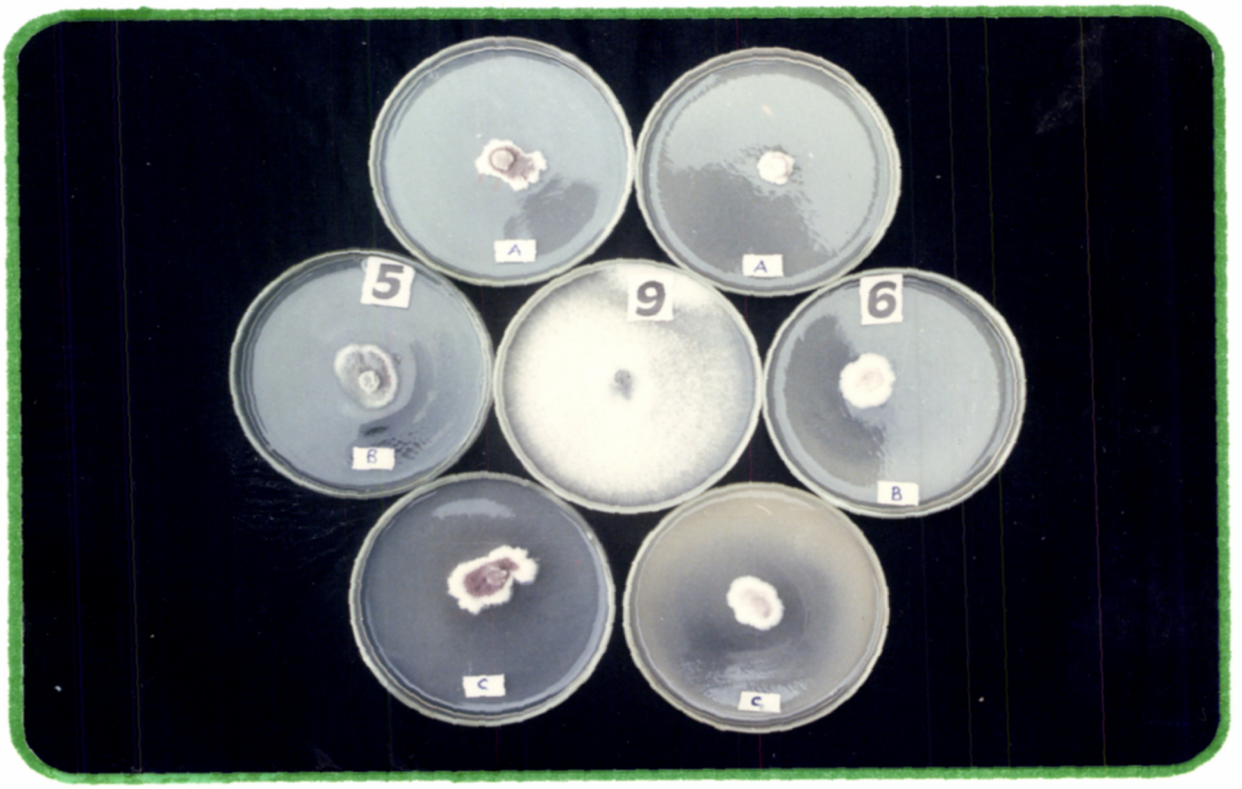
PLATE - VI

POISION FOOD TECHNIQUE

Growth of Fusarium Solani Isolate on diffrent fungicides at diffrent level of concertation

A) Sr.No	Petri Plate No	Name of Fungicide	Concertratio
1	5 A	Topsin	Ø.Ø5
2	5 B	Topsin	Ø.1Ø
3	5 C	Topsin	Ø.2Ø
4	6 A	Caftaf	Ø.1Ø
5	6 B	Caftaf	Ø.2Ø
6	6 C	Caftaf	Ø.3Ø
7	Central Plate (Control No.9)	With out fungicide	Nill

B) Sr.No	Petri Plate No	Name of Fungicide	Concertratio
1	7 A	Folpet	Ø.1Ø
2	7 B	Folpet	Ø.2Ø
3	7 C	Folpet	Ø.3Ø
4	8 A	Benomyl	Ø.2Ø
5	8 B	Benomyl	Ø.25
6	8 C	Benomyl	Ø.3Ø
7	Central Plate (Control No.9)	With out Fungicide	Nil


A
A
5
9
6
B
B
C
C

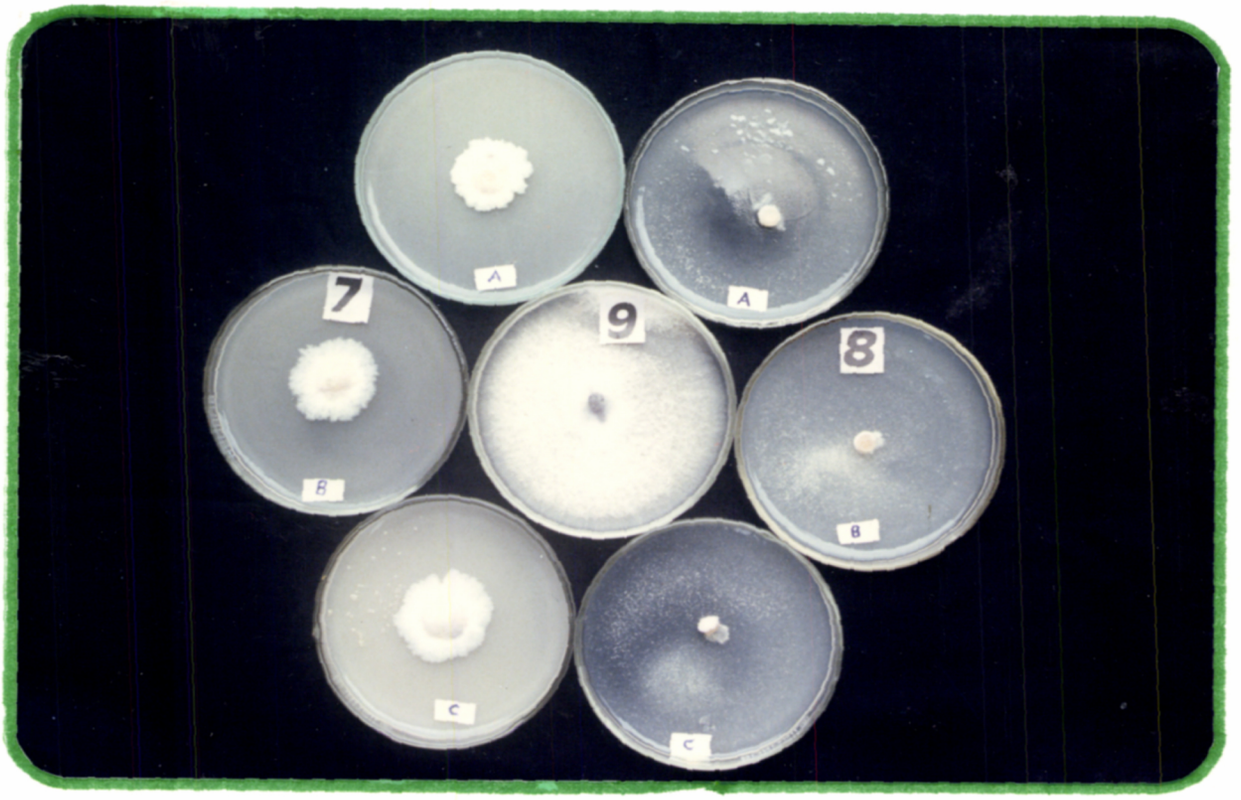

A
A
7
9
8
B
B
C
C

TABLE NO 9

Fungicidal control of corms by treatment to corms

Name of fungicide	No of corm taken	No of corms rotted	% of Infection	Rating
Blitox	10	9	90	4
Mancozeb	10	8	80	4
Ziram	10	2	20	1
Bavistin	10	0	0	0
Topsin	10	5	50	2
Caftaf	10	4	50	2
Folpet	10	6	60	3
Benomyl	10	0	0	0
Control	10	10	100	4

Rating : 1 - 125% infection on corms.
2 - 26-50% infection on corms.
3 - 50-75% infection on corms.
4 - 76-100% infection on corms.

4.6 **Varietal Screening** :

To test the response of different varieties, varietal studies were carried out. During the course of this experiment the corms of different varieties and of uniform size, shape and other qualitative characters were carefully selected.

Inoculation with Fusarium culture was carried out. The process was carried in two lots. In the first instance, pricks were made on the skin of the corms. In the second inoculant was directly applied into the skin with the help of cotton wool. The third was left as control.

As seen from the table No.16 among the various varieties used Oscar, Poonam, Friendship, King Lear and Spic and Span were found to be very suseptible. The rotting took place at a rapid rate and to the extent of 50% or more. The varieties Sapna and Happy End showed 25-60% rotting symptoms. Suchitra, Sylvia, Tropical Seas and Selection-1 took more time for developing the rotting symptoms and the incidence was less in the range of 11-25%.They could be grouped into resistant varieties. Varieties showing no suseptibility were Snow Princess, Melody and Mayur and could be grouped under highly resistant.

TABLE NO 1Ø

Varietal Screening

Name of variety	Percentage of rating of infection	Reaction
Friendship	5Ø%	Highly suseptible
Oscar	5Ø%	Highly suseptible
King Lear	5Ø%	Highly suseptible
Poonam	5Ø%	Highly suseptible
Happyend	26-5Ø%	Suseptible
Sapna	26-5Ø%	Suseptible
Suchitra	11-25%	Resistant
Sylvia	11-25%	Resistant
Selection-1	11-25%	Resistant
Tropic seas	11-25%	Resistant
Mayur	-	Highly resistant
Melody	-	Highly resistant
Snow princess	-	Highly resistant

DISCUSSION

5 DISCUSSION

Gladiolus is gaining the status in the flower market as a commercial cut flower. One of the main obstacle in its production has been the out-break of the rot of the corms in storage. Since gladiolus corms are required to be stored because of the dormancy. This problem manifests itself mainly during storage. It has also been noted that rough handling and delay in placing the plant into curing condition, resulted in higher incidence of rot. Also the prevailing environmental conditions during harvest and storage may indirectly be affecting the presence and virulence of the pathogen or the susceptibility of the cultivars.

This rot being a deep seated one, control becomes difficult, since temperature and fungicides can't reach up to vascular tissues. Use of resistant varieties, proper handling and storage procedure, pretreatment with effective fungicide are some of the efficient control measures. To elucidate all the above points a detail study was conducted which has been briefly discussed below

5.1 Isolation, Pathogenecity and Reisolation :

The affected samples of gladiolus corms were collected from All India Co-Ordinated Floriculture Improvement Project, Ganesh Khind Pune - 411 ØØ7. Isolation yielded the fungus Fusarium solani(mart) sacc. The pathogenecity test was carried out and reisolation of rotten corms yielded the fungus identical to one which was isolated from the naturally infected host. The above findings were in agreement with new disease of gladiolus Fusarium solani. The casual organism for corm rot of gladiolus (of Sarbhoy

and Agarwal(1983)) was found to be Fusarium solani infecting the corms during storage. In India Tandon and Bhargava (1963). The other workers like Magie (1971), Woltz, Magie et al (1978), McCulloch (1943), Gould and Miller (1970) have proved that Fusarium oxysporum f. sp. gladioli is also the casual organism for storage rot of gladiolus.

5.2 **Morphology Of The** Fusarium **Isolate** :

The morphological characters of seven days old culture on potato dextrose agar was recorded. The mycelium was white in colour, profusely branched and both macro and micro conidia were noticed. The mycelium was sterile, sparse to dense, flocose grey. The micro conidia were oval in shape with thicker walls measuring 8-16 x 2-4 u having only one septum. The macroconidia develop after four to seven days initially as simple but later form short multibranched conidiophores which soon merge from effuse sporodochia. The macroconidia have well marked foot cell, the apical cell being pointed and beaked. Heterothalic strains often occur in two distinct morphological forms.

Chlamydospores tend to develop abundantly on weak media after 7 to 19 days. They were globose to oval, smooth to rough walled 9-12 x 8-10 u.

5.3 **Cultural characters of the Fusarium isolate** :

While studying the cultural characters it was observed that average maximum colony diameter was on Richard's medium while the minimum growth was noticed on Jensen's medium. The above findings

were in agreement with findings of Prasad and Patel (1964), and Reddy and Chaudhari (1985), who also reported good growth of Fusarium on Richard's medium.

Among the non-synthetic media tried, the organism grew well on potato dextrose agar and French bean extract agar. Colony diameter was 63.61 u with abundant sporulation. Colonies had profuse growth in center with concentric rings white in colour. McCulloch (1943) studying vascular disease of gladiolus reported that potato dextrose agar, Bean pods agar, oat meal agar and corn meal agar were suitable media for growing Fusarium.

5.4 Physiological characters

5.4.1 Utilization of carbon compounds :

Carbon compounds play an important role in the metabolism of fungi, which exhibit a certain degree of specification in utilization of various carbon compounds.

It was observed that the Fusarium isolate luxuriantly utilized the carbon compound, starch recording the highest mycelial dry weight. This was contradictory to the findings of Tandon and Bhargava (1963) who reported sucrose to be the favourable source of carbon. In a third trial conducted . Bhargava (1979) reported that the final dry weight of mycelial mat produced on maltose or glucose was more or less similar, decreasing the enzymic activity and increasing the mycelial weight.

5.4.2 **Utilization of Nitrogen Compounds** :

It was noticed that the potassium nitrate had supported average maximum mycelial dry weight than the other nitrogenous compounds. How ever the abundant growth of the fungus was observed. Bhatnagar et.al.(1969) (1969), too reported potassium nitrate to be the nitrogen source for mycelial growth. A similar result was reported by Godage (1977). He found maximum sporulation and growth on potassium nitrate.

5.4.3 **Effect of H-ion Concentration** :

The organisam showed good tolerance to the pH range from 3 to 9. The maximum growth was observed at a pH of 3 with 1Ø36 mg as dry weight of the mycelium mat. The optimum pH ranged between 4 to 5. At pH lower than 3.Ø5 the organisam did not grow at all.

The pH value slightly greater than neutral favoured the growth of the test organisam upto 7.5. And as pH went on increasing the growth started decreasing and in a highly alkaline pH of 1Ø.5, it showed very less growth. From the observations it could be easily infered that the pathogen thrives best in the acidic range.

5.4.4 **Effect of temperature on growth and sporulation of Fusarium** :

Temperature for growth was 18 ^{o}C to 32 ^{o}C. The study made on effect of humidity on development of disease at ambient room temperature 29 ^{o}C $\pm$ 1C showed that the organism grew

effectively at hundred percent relative humidity, and it also showed abundant sporulation. Nielson and Moyer (1979) working on Fusarium root rot of sweet potato concluded 28°C to be optimum

temperature. They also reported that the minimum, optimum and maximum temperatures, were 12°C, 28°C and 36°C respectively on potato dextrose agar.

5.5 **Host range** :

Under the present host range study, a wider range of hosts were studied. The pathogen was found to infect a number of hosts which showed different intensities of infection. Some hosts showed infection in the earlier stage itself while in others, symptom development was a gradual processes. Host range studies indicated that Fusarium solani is a nonspecialized pathogen capable of infecting many hosts. The presence of this disease in many storage houses was not uncommon. Nielson and Moyer (1979) while studying root rot of sweet potato reported a wide range of hosts including apple, cucumber, egg plant, pepper, squash, tomato and potato tubers. Gould and Miller (1970) reported Iris and Narcissus to be potential hosts among the flower crops. Chakraborti et al (1977) reported Fusarium solani to be infecting Banana while Rath et al (1979) reported Fusarium solani to be the casual agent causing rhizome rot of ginger. Garg and Gupta (1979) based on their studies reported tomato to be a potential host.

5.6 **Fungicidal bioassy :**

A fungicidal trials were conducted in vitro including eight fungicides belonging to different groups.

In in vitro trials various fungicides were tested for controlling Fusarium solani. In the present study the fungicide like Bavistin stood as the best fungicide as no growth of test organism Fusarium solani at Ø.Ø5 mg/l concentration followed by Benomyl. The next best fungicide controlling the test organism was Zirum at high concentration. Caftaf, Topsin were also effective whereas the Folpet, Blitox and Mancozeb were relatively ineffective against the Fusarium sp.

Among the sulphur and copper fungicides Blitox and Mancozeb were not so effective.

5.7 **Vareital screening :**

A number of varieties of Gladiolus were tested for their reaction to corm rot. The varieties Oscar, Spic and Span, Poonam, Friendship and King-Lear were found to be highly susceptible while Sapana and Happy end were susceptible in comparison. Reslstant varieties included Suchitra, Sylvia, Tropical Seas and selection, Snow princes, Melody and Mayur were found to be highly reistant.

SUMMARY AND CONCLUSIONS

6 SUMMARY AND CONCLUSION

Gladiolus is one of the most important flower crops among the renowned commercial cut flower crops. In todays cut flower trade, gladiolus occupies a prestigous position and has a tremendous market value due to its magnificent spike and keeping quality. Day by day, it is getting more and more popular due to its availability in a magnificient of colours exhibited by its beautiful spike. The gladiolus is propogated by corms and these corms are affected by fungal diseases causing the rot of the corms. This was found to be a very serious problem during the storage.

The affected samples were collected from All India Coordinated Floriculture, Improvement Project, Ganesh khind, Pune. The pathogenecity test proved to be positive for Fusarium solani and the organism was found causing identical brown to black spots on corms in the laboratory. The morphological characters of the fungus were studied and it was noticed that the growth and sporulation of the isolate in Richard's medium was the best.

Among the seven carbon compounds tested, starch and manitol recorded the highest mycelial dry weight. Abundant sporulation was noticed in manitol.

Among the nitrogenous compounds, potassium nitrate had supported the highest average mycelial dry weight, over the other nitrogenous compounds.

The minimum and maximum temperature requirements were found to be 13 °C to 35 °C, whereas the optimum temperature for luxriant growth was found to be 28 °C.

The organism could tolerate the pH from 3-9 while the optimum pH was found to be (3.Ø5). In subsequently inceasing H-ion concentrations the growth was found to decrease. The organism showed extreme pathogenic activity on the related flowers, vegetables and fruits. In vegetables and fruits the rotting was a rapid process where as in propogating material of flowers it showed gradual symptoms. Brinjal, Capsicum, Cucumber and Tomato were very suseptible while the other vegetables used, developed the symptoms slowly.

Fungicides such as Bavistin and Benomyl at concentrations of Ø.Ø5 and Ø.2Ø respectively were found to be very effective for efficiently controlling the rot of gladiolus under strorage condition. A number of varieties were found to be susceptible when tested against Fusarium, but some verieties like Mayur, Melody were found resistant.

In vitro trials, various fungicides were tested for controlling Fusarium solani, in the present study for controlling rot disease of corms. The fungicide Bavistin gave the best action for controlling the corm rot. Bavistin at Ø.Ø5 percent was found to be effective which was the lowermost concentration of fungicide but it was highly concentrated whereas the .2 percent of Benomyl was found to be effective. This was followed by Ziram which could control the disease at a higher concentration Caftaf

and Topsin could also control the disease to a certain extent whereas Folpet, Blitox and Mancozeb were found to be relatively in-effective. When a comparison was made for the above mentioned fungicides it could be noticed that the highest concentration of Bavistin was identical to the lowest concentration of Benomyl that was Ø.2 percent. Hence it would be more economical to use Bavistin which gave precise effective control at a low concentration. Available literature was contradictory to the above findings wherein Benomyl has been found to be the most effective fungicide for controlling storage rot. This has also been reported by Magie (1971). Gould and Miller (1970) reported that Benzamidazoles were more effective and less phytotoxic than mercurial fingicides for treating Fusarium infection. Hence TBZ and Benomyl were the recomonded fingicides. Gould and Miller, also compared, application of fungicides by dusting or by soaking in aqueous solution and they concluded that dusting was the superior treatment. The differences obtained in the reaction to the fungicidal application may be due to variation in suspension temperature, the depth to which the pathogen had penetrated the bulb before treatment and also to the degree of Succulence of the bulb used. A simple result was obtained for Narcissus Forsberg (197Ø) while comparing the effect of Thirum and Benomyl concluded that Benomyl produced more flower and more corms and more rot free corms than that of Thirum. However Magie and Wolfret (1974) reported the development of tolerant strains with continued use

of Benomyl would result in severe out breaks of corm rot. Wani et al (1982) while screening fungicides in vitro against Fusarium rot concluded that use of a single fungicide for controlling a particular pathogen resulted in the development of resistant strain for that particular fungicide. However if more than one fungicide was used it delayed the development of a resistant strain. They concluded from their results that a combination of Bavistin (Ø.5 percent) and Difolaton (Ø.5 percent) mixed as a Ø.1 percent combination proved to be more effective in inhibiting the Fusarium growth.

A number of varieties of gladiolus were tested for their reaction to corm rot. Among the comparatively less varieties used, difference in reaction to the vascular parasite was ranging from highly resistant to complete susceptible. The varieties Oscar, Spic and Span, Poonam, Friendship and King Lear, were found to be highly susceptible.

While Sapna and Happy End were susceptible. In comparison the resistant varieties included Suchitra Sylvia, Tropical Seas and Selection-1. Snow Princess, Melody and Mayur were found to be highly resistant. (Anonnymous 1992).

Results obtained by Kaur et al (1981) agreed with the above. in hybridization and selection programmes there is a need to secure resistance in new varieties as it would be a more effective control measure.

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VITA

V I T A

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MASTER OF SCIENCE (AGRICULTURE)

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