STUDIES ON RHIZOME ROT OF GINGER CAUSED BY *Fusarium oxysporum f.sp. zingiberi* IN MAHARASHTRA STATE

By

Mr. Gangawane Hanumant Dnyandeo
(Reg. No. 014 /41)

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI – 413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE, INDIA

in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
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2020

CANDIDATE'S DECLARATION

I hereby declare that, this thesis or part
thereof has not been submitted
by me or other person to any
other University or Institution
for a Degree or
Diploma

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This is to certify that the thesis entitled, "**STUDIES ON RHIZOME ROT OF GINGER CAUSED BY *Fusarium oxysporum f.sp. zingiberi* IN MAHARASHTRA STATE "** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar (M.S.) in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY,** embodies the results of a piece of *bonafide* research work carried out by **Mr. GANGAWANE HANUMANT DNYANDEO,** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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Place: M.P.K.V., Rahuri. **(Gangawane H.D.)**

Date:

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LIST OF ABBREVIATIONS/SYMBOLS

%	:	Per cent
**	:	1 % level of significance
/	:	Per
@	:	At the rate of
µg	:	Microgram (s)
µm	:	Micrometer
µml	:	Micromilliliter (s)
AFLP	:	Amplified Fragment Length Polymorphism
C.D.	:	Critical difference
C.V.	:	Coefficient of Variation
CRD	:	Completely Randomized Design
cv.	:	Cultivar
EC	:	Emulsified concentration
et al.	:	and others *et alia*
etc.	:	Et cetera (and so forth)
EW	:	Emulsion, oil in water
Fig.	:	Figure (s)
g	:	gram (s)
ha	:	Hectare
hr.	:	Hour (s)
i.e.	:	That is, id est
in vitro	:	In Laboratory
in vivo	:	Under field
kg	:	Kilogram (s)
M.S.	:	Maharashtra State
ml	:	Milliliter
ml	:	Micro Litre (s)
mm	:	Millimeter (s)
MT	:	Metric Tonne (s)
No.	:	Number
^{o}C	:	Degree Celsius

PCR	:	Polymerase Chain Reaction
PDC	:	Per cent Disease Control
PDI	:	Per cent Disease Incidence or Per cent Disease Index
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphisms
RH	:	Relative humidity
RNase	:	Ribonuclease enzyme
rpm	:	revolution per minute
S.E.	:	Standard error
spp.	:	Species
SSR	:	Simple Sequence Repeats
t	:	Tonne (s)
Temp.	:	Temperature
viz.	:	Videlicet (namely)
WP	:	Wettable powder
WS	:	Wettable sulphur
f.sp.	:	Formae species

ABSTRACT

STUDIES ON RHIZOME ROT OF GINGER CAUSED BY *Fusarium oxysporum f.sp. zingiberi* IN MAHARASHTRA STATE

By

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A candidate for the degree

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DOCTOR OF PHILOSOPHY (AGRICULTURE)

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2020

Research Guide : Dr. B.M. Ilhe

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Ginger (*Zingiber officinale* Rosc) Rhizome rot caused by the fungus *Fusarium oxysporum f.sp. zingiberi* is one of the most important diseases causing considerable losses in Ginger. Keeping in view the economic importance of the crop and losses caused by rhizome rot disease, the present investigation was undertaken to study the aspects like survey for the disease incidence in the major ginger growing areas of Western Maharashtra and Marathwada regions, isolation, pathogenicity test, Molecular variability studies, *In vitro* evaluation of fungicides, bioagents and integrated disease management.

During survey, it was revealed that, the disease was severe in all the districts during *Rabi* 2015-16 and 2016-17. Mean disease incidence ranged from 30 to 46 per cent, in different districts surveyed. The highest incidence (48 %) of Rhizome rot of ginger (*F. oxysporum f.sp. zingiberi*) was noticed in farmer's fields of Andheri village in Aurangabad district, whereas least (30 %) incidence of the disease was recorded at Shirasgaon village in Nashik district. The symptoms of Rhizome rot of ginger (*F. oxysporum f.sp. zingiberi*) included pale brown discoloration of the vascular strands that invades the rest of the rhizome that become brown and dry.

The above ground symptoms were the water-conducting portion of symptomatic rhizomes was discoloured brown and had a black dry rot of the cortex tissue. The Mycelium was white, uniform, fluffy light pinkish tinge in the fungal colony. *F. oxysporum f.sp. zingiberi* produces three types of sexual a spores, micro-conidia, macro-conidia and chlamydospores. The 3.9 - 14.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 septation were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells gradually pointed, curved edges, varied in size from 3.5 x 20 µm with 3-4 septation. Fusarial basal rot can survive in the soil for long period of time through Chlamydospores.

The pathogenicity of the pathogen was proved and the pathogen was confirmed as *Fusarium oxysporum f.sp. zingiberi.* Pathogenicity tests were carried out to establish that the fungus thus isolated is capable of producing typical symptoms of *F.oxysporum* under artificial inoculation conditions on Ginger cv. Mahim and also to prove Koch's postulates of the pathogen.

Significant variation was present in isolates from Ahmednagar, Pune, Satara, Kolhapur, Solapur, Sangli, Nashik and Aurangabad districts in respect of the cultural characteristics *viz.*, colour of the colony, mycelial growth, colour of conidia, shape of conidia, beak and length of conidia produced by *Fusarium oxysporum f.sp. zingiberi.* The mycelium was white, uniform, fluffy, light pinkish tinge in colony of *F. oxysporum f.sp. zingiberi.*

DNA profiling revealed high degree of polymorphism amongst the isolates collected from eight districts of Western Maharashtra and Marathwada region. UPGMA cluster clearly delineated variability among *Fusarium oxysporum f.sp. zingberi* isolates grouping most of them in two clusters *viz.*, I and II. During the molecular variability analysis among the isolates FOZ-2 (Pune) and FOZ-8 (Aurangbad) more divergence i.e. minimum similarity coefficient about 0.426. Maximum similarity coefficient about 0.755 was present in between FOZ-2 (Pune) isolates and and FOZ-1 (Ahmednagar) isolate indicating that these isolates were less divergent from each other.

Among the different fungicides tested, the maximum 100 per cent inhibition was found in Carbendazim, Carbendazim + Mancozeb and Benomyl, and the least inhibition (21.55 per cent) was recorded with Metalaxyl + Copper oxychloride.

Among the differance bioagents tested, the out of 75.66 per cent inhibition was found in *Trichoderma viride*, followed by *Trichoderma harzianum* and *Trichoderma koningii*, and the least inhibition was recorded with the Bacterial bioagents *Bacillus subtilis* with 43.18 per cent**.**

The pot with Rhizome dip treatment 30 min. and soil drenching after one month of sowing with Carbendazim (0.1 %) + Mancozeb (0.2 %) was most effective in managing the disease with per cent disease incidence of 8.33 per cent, and the next best treatment was Carbendazim (0.1 %) alone with PDI of 13.88 per cent, followed by Benomyl (36.11 %), the maximum PDI was recorded with control (77.77 %). Among the bioagents tested, the combination of *T. viride* (3 g) + *Pseudomonas fluorescencs* (3 g/litre) with PDI of 27.77 per cent was effective in managing the r

hizome rot of Ginger caused by *F. oxysproum f.sp. zingiberi* disease in Maharashtra.

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1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belonging to the family Zingiberaceae and is an important spice crop grown for its aromatic rhizomes which are used as a spice and also as medicine (Sharma and Pandey, 2014). It is obtained from the underground stems or rhizome. Ginger originated in South- East Asia, probably in India (Burkill, 1966, Purseglove *et al*., 1981). It is usually grown as an annual spice. The whole plant is refreshingly aromatic, but it is the underground rhizome (raw or processed) which is valued as spice. Its medicinal value is increasingly being recognized a now days.

The Sanskrit name 'Singabera' has given rise to Greek '*zingiberi'* and later the generic name *zingiber*. Ginger is cultivated in several parts of the world and the most important countries are being India, China, Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica and Nepal. Among them, India and China are the dominant exports of to the world market. In terms of quality, Jamaican and Indian ginger are considered of superior quality followed by West African type. Jamaican ginger rhizomes possesses delicate aroma and flavour and is sometimes considered as first grade. Indian ginger entering the world market is known as 'Cochin' and 'Calicut'. The refreshing pleasant aroma, biting taste and carminative property of ginger make it an indispensable ingredient of food processing throughout the world. Fresh ginger, ginger powder from dry ginger, oleoresin and oil are all used for this purpose. Fresh ginger is unique for its flowery flavour and spicy taste. It is also used in jams and marmalades. The syrup in which ginger is preserved is valued for pickle and sauce making. It is also used in the production of ginger paste (Pruthi, 1993).

At present, India is the largest producer of ginger and is accounting for about one-third of the total world output and followed by Thailand and Japan. The area under ginger in India is 1,35,250 ha with a total production of 6,82,630 Mt and productivity of 4,870 kg/ha (Anon, 2014-15). The area under ginger in Maharashtra is 1060 ha with a total production 1040 Mt). In India, a large portion of the ginger produced is consumed domestically as green ginger or dried ginger. It is cultivated in many States and major producers are Assam, West-Bengal, Meghalaya, Kerala, Arunachal Pradesh, Orissa, West Bengal and Mizoram.

India is considered as '*The land of spices'* and enjoys from time immemorial a unique position in the production and export of ginger. This crop is

cultivated for its underground rhizomes, which are used in many ways. Ginger is used as flavoring agent, a preservative, used in pickle and ginger oil in soft drinks, Ginger is commonly used to treat various types of "stomach problems," including motion sickness, morning sickness, colic, upset stomach, gas, diarrhoea, nausea caused by cancer treatment, nausea and vomiting after surgery, as well as loss of appetite.

Among the major constraints for growing this crop is the rhizome rot. Even though, important foliar diseases do exist in this crop, rhizome rot is most important in view of severe crop losses.

A roving survey was conducted during *Kharif* 2003-2005 in major ginger growing areas of Karnataka. Rhizome rot incidence was noticed in all the locations surveyed with a range from 5.50 to 45.60 per cent (Sagar *et al.,* 2008). It occurs in several parts of India wherever this crop is grown. The term rhizome rot is loosely used for all the diseases affecting the rhizome, irrespective of pathogens involved, since the ultimate result is the partial or total loss of rhizome caused by *Pythium spp, Fusarium oxysporum f.sp. zingiberi*, *F. solani* and *Pseudomonas solanacearum* (Dake and Edison,1989). The pathogens involved, decide the nature of damage and also symptoms expression. The major diseases identified are the soft rot resulting in wet rot, yellows and bacterial wilt.

Purpose of this study was to examine the ginger samples from different areas of Maharashtra and to identify *Fusarium oxysporum f.sp. zingiberi*, the causal organism and its management with the following objectives.

1. Survey and collection of disease samples from different ginger growing districts in Maharashtra.
2. To isolate, identify the pathogen and to prove the pathogenicity of organisam associated with the rhizome rot.
3. To study the molecular variability of different isolates.
4. *In vitro* evaluation of fungicides against pathogen causing rhizome rot.
5. *In vitro* evaluation of bioagents against pathogen causing rhizome rot.
6. To find out integrated disease management module against pathogen associated with the rhizome rot of ginger.

2. REVIEW OF LITRATURE

2.1 History and occurrence of rhizome rot of ginger

Uppal *et al.* (1935) carried out systematic comparative studies between different *Fusarium* isolates from different parts of the Madras presidency (Tamil Nadu). In Queensland first time reported ginger yellows, a serious stem and rhizome rot by Simmonds (1955).Trujillo (1963) also reported that ginger yellow disease caused due to *Fusarium oxysporum* f.sp. *zingiberi* in Hawaii. Correll *et al.* (1991) concluded that *Fusarium oxysporum* is considered as one of the world's most harmful pathogen. Elliott (2003) observed rhizome rot disease of ginger in Jamaica, West Indies.

Dohroo (2005) reported that ginger crop of Kerala and Tamil Nadu is sometimes almost totally destroyed by rhizome rot.

Perveen and Bokhari (2010) first time reported that *Fusarium* wilt of paper flower (Bouga in villeaglabra) caused by *Fusarium oxysporum* from Italy.

Li *et al.* (2014) first time reported that, ginger rhizome rot caused by *Fusarium oxysporum* in China.

2.2 Survey for disease incidence

Sagar *et al*. (2008) reported that, the prevalence of rhizome rot in Karanataka. A survey was conducted during *Kharif* 2003-2005 in major ginger growing areas of Karnataka. Rhizome rot incidence was noticed in all the locations surveyed with a range from 5.50 to 45.60 per cent. The maximum disease incidence of 23.70 per cent was recorded in Shivarmogga district followed by Kodagu (22.90 %), Uttarkannada (20.28 %), Chickmagalur (18.99 %) and Bidar (13.00 %).

Ahmed (2010) was conducted survey for the assessment of chickpea wilt disease in February-March 2005 and 2006 of major chickpea growing areas of Pakistan. During survey, maximum disease prevalence (92.58 ± 3.69), incidence (82.52 ± 5.14) and severity (7.00 ± 0.48) was recorded in the districts Khushab, Bhakkar, Layyah, Mianwali and Sargodha. The lowest disease prevalence (29.70 ± 3.19), incidence (9.50 ± 1.72) and severity (2.1 ± 0.10) was recorded in districts Jafarabad, Dera Allah Yar, Attock, Peshawar and Islamabad

Patil (2010) was conducted survey for the assessment of wilt disease in 2008 and 2009 of chickpea growing areas of Marathwada and noted maximum wilt

incidence in the Nanded (19.6 %) followed by Parbhani (19.5 %) district. The moderate incidence of wilt in Beed (15.3 %), Hingoli (16.2 %) and Latur (17.4 %) and significantly least incidence in Aurangabad (10.3 %), Osmanabad (13.7 %) and Jalna (13.9 %) districts.

Nagare (2011) was carried out survey for the assessment of safflower wilt disease in the Latur district and revealed that average wilt incidence was varied from 7.16 per cent to 13.99 per cent in ten tahsils of Latur district. Maximum average safflower wilt incidence (13.99 %) was recorded in Ausa followed by Ahmedpur (13.33 %), Nilanga (12.33 %), Renapur (11.99 %), Deoni (11.66 %), Shirur Anantpal (10.99 %) and Udgir (10.33 %) while, minimum average wilt incidence was recorded in Jalkot 7.16 %), followed by Latur (8.33 %) and Chakur (9.99 %).

Kohire *et al.* (2012) carriedout a survey during 2008-2009 of chickpea wilt in the Jalana district revealed that average wilt complex to the tune of 8.43 per cent. Tehsil wise survey report indicated maximum wilt increase in tahsil Partur (11.69 %) fallowed by Ghansawangi (10.31 %), Jalana (10.22 %), Bhokardan (9.91 %), Badnapur (7.10 %), Ambad (6.77 %), Mantha (6.15 %) and Jafarabad (5.53 %).

Magar (2012) was conducted field survey of chickpea growing districts of Marathwada region during the month of December and February, 2010-11 and 2011-12 to record incidence and noticed that wilt incidence was maximum in Nanded (23.77 %) followed by Parbhani (22.02 %), Latur (19.05 %), Hingoli (17.16 %), Jalna (16.51 %), Beed (15.73 %) and Aurangabad (13.9 %), whereas; minimum incidence of wilt was observed in Osmanabad (13.58 %) district.

Awachar (2014) isolated the roots of twenty three wilted chickpea samples collected from different locations of Ahmednagar district of Maharashtra. Among them, 10 samples showed the growth of *Fusarium oxysporum f.sp. ciceri*; while, 4 and 9 samples yielded *Sclerotium rolfsii* and *Rhizoctonia bataticola*, respectively.

Sharma *et al.* (2014) reported that, rhizome rot of ginger caused by fungi *Pythium spp.* and *Fusarium oxysporum f.sp. zingiberi* and bacterium *Ralstonia solanacearum* is a major problem all over India. Disease has complex etiology, and hence, little work has been done.

2.3 Symptomatology

Patel and Prasad (1963) described the symptoms of *Fusarium* wilt of cumin.

Trujillo (1963) and Teakle (1965) reported *Fusarium* rot (*Fusarium sp*.) can cause serious problems, symptoms include pale brown discolouration of the vascular strands that invades the rest of the rhizome that become brown and dry.

Everts *et al.* (1985) *Fusarium oxysporum* has been isolated frequently from transplant brought into colarodo fusarium basal rot of onion.

Pegg and Stirling (1994) and Stirling (2004) reported that Australian ginger growers normally assume that patches of yellowing plants with rotting rhizomes are affected by *Fusarium* yellows (caused by *Fusarium oxysporum f.sp. zingiberi*).

Elliott (2003) isolated *Fusarium* spp. from rotted ginger.

Kulkarni (2011) isolated four causal organisms of rhizome rot of ginger from different areas of Karnataka. These organisms are viz., *Pythium aphanidermatum, Fusrarium solani, Ralstonia solanacearum* and *Sclerotium rolfsii*.

2.4 Isolation and Pathogenicity

Yang *et al.* (1988) isolated the fungi *Fusarium* and *Pythium* causing rhizome rot to ginger plant from collected seed gingers and diseased plants in Chonbuk province in S. Korea and pathogenicity proved by them.

Dake and Edison (1989) isolated *F. solani* of ginger rhizome rot and proved pathogenicity of the organism on ginger plant

Nagare (2011) proved the pathogenicity test by using soil inoculation and water culture method and confirmed after development of symptoms and reisolation of pathogen proved it to be as *F. oxysporum f.sp. carthemi*. Pre-emergence mortality recorded with all the test isolates of FOC was ranged from 20 to 40 per cent whereas; post-emergence wilting was ranged from 66.66 to 100.00 per cent and caused 100.00 per cent wilting in safflower cv. Nira within 5 days by using, respectively two methods.

Kadam (2012) proved the pathogenicity test by using soil inoculation method and confirmed after development of symptoms and reisolation of pathogen proved it to be as *F. oxysporum f.sp. ciceri*, caused wilting and death of the seedling in 15-22 days (Akola, Pune, Dhule, Satara and Parbhani). Other isolates showed

seedling mortality on 22-30th days after inoculation (Nashik, Washim, Buldhana, Amravati and Jalgaon isolates).

Magar (2012) proved the pathogenicity test by different inoculation methods *viz.*, soil inoculation and seed inoculation and confirmed after development of symptoms and reisolation of pathogen proved it to be as *F. oxysporum f.sp. ciceri*. In soil inoculation technique wilting per centage at 60 and 90 DAS were 88.52 and 93.17 per cent, respectively. While by seed inoculation techniques the wilting per centage at 60 and 90 DAI was 82.82 and 85.90 per cent, respectively.

Atram et al. (2015) were obtained the total 7 isolates of *Fusarium oxysporum f.sp. ciceri* (FOC) from wilted chickpea plants from Wardha, Washim, Lonar, Pune, Nashik , Rahuri, and Ahmednagar agro climatic regions of Maharashtra and proved to be pathogenic to susceptible cultivar JG-62, of which 4 isolates were highly pathogenic (FOC-2, FOC-3, FOC-5 and FOC-6), one was 26 strongly pathogenic (FOC-1) and two were moderately pathogenic (FOC-4 and FOC-7).

2.5 Cultural variability of *F. oxysporum.*

Patel and Prasad (1963) studied the growth of *Fusarium* on different media and observed that the growth of fungus was maximum on Richards agar medium followed by Czapeck's dox agar medium fusarium wilt of cumin.

Jhamaria (1972), Raghuwanshi (1995) and Mandhare (1997) studied *Fusarium oxysporum* on different media they found luxurious growth and sporulation of *F. oxysporum* on PDA medium followed by Richard's and Czapeck's Dox Agar media.

Smith (1988) reported that the *Fusarium oxysporum* has varying appearance like the aerial mycelium first appears white and they may change to variety of colours from violet to dark purple in potato dextrose agar medium.

Dohroo and Sharma (1992) studied different isolates of *Fusarium oxysporum f.sp. zingiberi* on PDA medium. They found violet black and peach yellow pigmentation.

2.6 Morphological variability of *F. oxysporum*

Chattopadhyay and Gupta (1967) studied the morphology of *Fusarium spp*. of 20 days old culture. He reported that when grown on potato dextrose agar the size of conidia varies from 9.3-29.7 x 2.7-6.0 µm.

Agrios (1988) reported that *F. oxysporum* produces three types of a sexual spores, micro-conidia, macro-conidia and chlamydospores.

Dohroo and Sharma (1992) reported the morphological characters of *Fusarium oxysporu*m *f.sp. zingiberi* and further stated that 3.9 - 11.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 Septation and 13.6 - 31.2 x 2.9-4.9 µm size of macro conidia with 3-4 Septation.

Delahaunt and Stevenson (2004) reported that Fusarial basal rot can survive in the soil for long period of time through Chlamydospores.

Awachar (2014) studied the cultural characteristics in ten isolates of *Fusarium oxysporum f.sp. ciceri*. Isolates FOC-4 (Rahuri-a), FOC-5 (Shrirampur), FOC-6 (Rahata), FOC-16 (Akole), FOC-17 (Newasa) and FOC-19 (Sangamner) showed circular, raised, cottony colony with entire margins, isolates FOC-15 (Shevgaon) and FOC-23 (Rahuri-b) showed circular, flat, smooth colony with entire margin. In FOC-18 (Ahmednagar) circular, flat, smooth colony with filiform margin was observed. On the basis of colour of mycelium isolates FOC5, FOC-15, FOC-19, FOC-21 and FOC-23 showed white colour, isolates FOC-4, FOC-6, FOC-16 and FOC-17 showed creamy white colour. In FOC-18 light purple colour was observed.

Y. Li *et al.* (2014) reported that *Fusarium oxysporum* has two types of asexual spores i.e. micro-conidia and macro-conidia, the micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells with gradually pointed, curved edges, varied in size from 3.5 x 19-36 µm.

2.7 Molecular variability of pathogen

Jimenez-Gasco *et al.* (2003) reported that specific primers and Polymerase Chain Reaction (PCR) assays to identify *Fusarium oxysporum f.sp. ciceri* and primer were developed for each of the *Fusarium oxysporum f.sp. ciceri* pathogenic races 0, 1A, 5 and 6. *Fusarium oxysporum f.sp. ciceri* and race- specific random amplified polymorphic DNA (RAPD) markers identified in a previous study were cloned and sequenced and Sequenced Characterized Amplified Region (SCAR) primers for specific PCR were developed. Each cloned RAPD marker was characterized by Southern hybridization analysis of EcoR1 digested genomic DNA of a subset of *Fusarium oxysporum f.sp. ciceri* and nonpathogenic *Fusarium oxysporum* isolates. All except two cloned RAPD markers consisted of DNA sequence that were found highly repetitive in the genome of all *Fusarium oxysporum f.sp. ciceri* isolates representing eight reported races from a wide geographic range, non-pathogenic *Fusarium oxysporum* isolates, isolates of

Fusarium oxysporum f.sp. lycopersici, Fusarium oxysporum f.sp. melonis, Fusarium oxysporum f.sp. niveum, Fusarium oxysporum f.sp. phaseoli, Fusarium oxysporum f.sp. pisi and isolates of 47 different *Fusarium* spp. were tested using the SCAR markers developed. The specific primer pairs amplified a single 1503 bp product from all *Fusarium oxysporum f.sp. ciceri* isolates; and 40 single 900 and 1000 bp products were selectively amplified from race 0 and race 6 isolates, respectively. The specificity of this amplification was confirmed by hybridization analysis of the PCR products. A race 5 specific identification assay was developed using a touchdown-PCR procedure. A joint use of race 0 and race 6 specific SCAR primers in a single-PCR reaction together with a PCR assay using the race 6 specific primer pair correctly identified race 1A isolates for which no RAPD marker had been found previously. All the PCR assays described herein detected upto 0.1 ng of fungal genomic DNA. The specific SCAR primers and PCR assays developed in this study clearly identify and differentiate isolates of *Fusarium oxysporum f.sp. ciceri* and of each of its pathogenic races 0, 1A, 5 and 6. Hence the present study is undertaken to know the presence of race type of *Fusarium oxysporum f.sp. ciceri* and the extent of the molecular variation present in the isolates of *Fusarium oxysporum f.sp. ciceri* for its exploitation in wilt resistance breeding and wilt management in future.

Jimenez *et al. (*2004) reported that the isolates of *Fusarium oxysporum f.sp. ciceri* representative of all pathotype races and geographic origin assessed using RAPD primers gave two main cluster groups. That cluster correlated with the yellowing and wilting pathotypes respectively. Further the yellowing isolates were divided into two distinct sub clusters corresponding race 1 and 1B/C.

Lakhdar *et al.* (2004) collected 32 isolates of *Fusarium oxysporum f.sp. lentis* from wilted lentil plants from different lentil growing areas in Algeria and performed pathogenic test for all isolates. Results indicated that the *Fusarium oxysporum f.sp. lentis* isolates represent a single race but differ in their aggressiveness on the susceptible lines. The amount of genetic variation was evaluated by polymerase chain reaction (PCR) amplification with a set of 6 RAPD primers and 3 AFLP primers. All amplification revealed scorable polymorphism among the isolates and total of 8 polymorphic fragents were scored for RAPD primers and 93 for AFLP for primers.

Pappalardo *et al.* (2009) reported that Genetic variations among 29 isolates of *Fusarium oxysporum f.sp. zingiberi* (*Foz*) collected from diseased ginger

rhizome in production regions throughout Queensland was analysed using DNA amplication fingerprinting (DAF). Eight isolates of other *Fusarium* species and/or *formae speciales* were included for comparative analysis. Within the Foz isolates, three haplotypes were identified based on 17 polymorphic bands generated with five primers. Two groups showed very little genetic variation (98.6 % similarity), whereas the third single isolate was quite distinct in terms of its molecular profile (77.2 % similarity). Genetic similarity among the *Fusarium solani*, *Fusarium oxysporum f.sp. Lycopersici*, *Fusarium oxysporum f.sp. cubence* races 1, 3 and 4 isolates compared.

Meena Gupta *et al.* (2014) reported that 19 isolates of *Fusarium oxysporum f.sp. zingiberi* causal organism of *Fusarium* yellow in ginger were collected from different ginger growing areas of Himachal Pradesh and designated as I1 to I19. Morphological variations with respect to mycelia colour, conidial size and formation of chlamydospores and pathogenic variation in terms of disease incidence among different isolates was studied these isolates. The mycelia colour varied from white to dull white with slightly pinkish tings. The microcondial size varied from 5.20 to 4.00 um (I8) to 4.70 um (I7) to 32.0 to 5.70 um (I4). Chlamydospore dimensions also varied in all the nineteen isolates of the test pathogen. All isolates formed chlamydospore on PDA medium. Pathogenic variability revealed that lesions size varied from 8.50 to 18.00 after 10 days of inoculation whereas, incubation period varies from 11 to 19 days. Genetic variation was also analysed by using forty 10-meroilgonucleotide RAPD primer, out of which 2 primers yielded informative, strong and reproducible DNA Amplicons of *Fusarium oxysporum f.sp*. *zingiberi* and thus were selected for studying the variation among isolates. The dendrogram of DNA fingerprints revealed 0 to 80 per cent variation among isolates. All isolates were grouped into two different major groups each comprising of ten and nine isolates, respectively.

Atram *et al.* (2015) were studied the genetic diversity among the 7 Isolates of *Fusarium oxysporum f.sp. ciceri* by using RAPD primers of OPF series. Off which, 16 primers produced, 108 scorable bands. Out of 108 bands, 101 bands were polymorphic and level of polymorphism was 93.18 per cent. The primer OPF-1 amplified maximum of 35 bands within the size 3627bp to 434bp while, primer OPF-12 amplified minimum of 7 bands within the size 1969bp to 722bp. The Wardha isolates (FOC-4) was found to have higher value of similarity coefficient (0.74); whereas, Rahuri isolates (FOC-2) was found to have lower value of similarity

coefficient (0.49). The two major clusters were obtained on the basis of analysis. The Cluster-A, includes 3 isolates Nashik , Pune, Rahuri and Cluster-B which includes isolates from Wardha, Lonar, Karanjalad, Tisgaon. The Cluster-A shows isolates from Nashik (FOC-1), Rahuri (FOC-2) shows around 0.64 similarity and one isolates from Pune (FOC-3) shows 0.63 similarities. The Cluster-B shows isolates from Wardha (FOC-4), Lonar (FOC-5) shows around 0.74 similarities and isolates Karanjalad (FOC-5) shows around 0.69 similarities, isolates Tisgaon (FOC-6) shows around 0.68 similarities.

Debberma *et al.* (2015) were studied the genetic diversity among the 4 races (6 isolates) of *Fusarium oxysporum f.sp. ciceri* Race-1 (Hydrabad and Akola); Racc-2 (Kanpur); Race-3 (Gurdaspur); Type Racc-4 (New Delhi and Jabalpur) by using 9 SSR of MB series. Out of 9, eight primers produced 118 scorable bands with an average of 14 bands per primer. Out 18 bands, 99 bands were polymorphic and average level of polymorphism was 84.8 per cent. The 49 primer MB-2 amplified of maximum 21 bands within the size 44 bp to 748 bp, while primer MB -5 amplified minimum of 4 bands within the size 67 bp to 289 bp respectively. The seven SSR primers MB-2 (555 bp, 475 bp. 412 bp, 323 bp, 298 bp. 273 bp and 247 bp); MB -5 (289 bp); MB-9 (494 bp. 407 bp, 340 bp, 129 bp and 70 bp); MB-10 (174 bp); MB-13 (230 bp): MB-17 (407 bp, 270 bp and 107 bp) and MB-18 (259 bp) showed 19 monomorphic bands. The Race-3 (Gurdaspur) was found to have lower value of similarity coefficient (0.4322034); whereas, Race-1 (Akola isolate) was found to have higher value of similarity coefficient (0.8559322). The Race-1, (Hyderabad and Akola isolate) showed the higher similarity index and were grouped under cluster-A. Racc-2 (Kanpur) and Racc-4, (New Delhi and Jabalpur) were group under cluster-B. However, Race-3 (Gurdaspur) showed distinct out-group which is least similar to other races of *Fusarium oxysporum f.sp. ciceri*. Thus from this it is clear that Race-3 (Gurdaspur) is distinct from other races of *Fusarium oxysporum f.sp. ciceri*.

2.8. *In vitro* evaluation of fungicide against *F. oxysporum*

Jones and Miller (1982) reported some effective means of controlling *Fusarium oxysporum* which includes disinfection of the soil and planting material with fungicides chemicals.

Mathur *et al*. (1984) found that seed treatment of Metalaxyl MZ-72 reduced disease incidence of ginger rhizome rot.

Das *et al.* (1990) reported the effect of seed treatments with captan, captafol and mancozeb at 0.2, 0.2 and 0.3 % on pre-emergence rhizome rot ginger caused by fungi *Pythium* spp. All these fungicides controlled pre-emergence rhizome rot of ginger.

Chauhan and Patel (1990) reported that Metalaxyl MZ-72 and Bordeaux mixture was effective against *Pythium spp.* and *Fusarium solani in vitro* test.

Elliott (2003) found rhizome dip treatment of ginger with Ridomil MZ (0.2 %) for 20 minutes was effective in reducing the incidence of disease.

Singh and Jha (2003) evaluated seven fungicides *viz.*, Thiram, Bavistin, Blitox, Captaf, Indofil M-45, Ridomil MZ and Kitazin against chickpea wilt *in vitro* and *in vivo* and proved that Thiram and Carbendazim were the most suitable fungicides in inhibiting the growth of *F. oxysporum f.sp. ciceri in vitro* and reduced the wilt incidence *in vivo*.

Meena and Mathur (2003) observed that fungicidal mixture of Ridomil MZ and Bavistin was effective in treating seed rhizomes and soil individually and in combination for the suppression of rhizome rot of ginger.

Poddar *et al.* (2004) evaluated four systemic fungicides *viz.*, Carbendazim, Propiconazole, Tebucanazole, Thiophonate Methyl and found the Carbendazim to inhibit maximum growth of *Fusarium oxysporum* in *in vitro.*

Chavan (2004) tested five fungicides at three concentrations 500, 1000 and 1500 ppm against *Fusarium oxysporum f.sp. ciceri* and most effective fungicides CM-75 (100 %) followed by Carbendazim (76.42 %), Mancozeb (66.29 %) and Carzet (61.23 %) at all concentration; whereas, Benomyl was least effective for pathogen. Poor sporulation was observed in Carzet and Mancozeb at higher concentration. There was no sporulation with CM-75 at all the concentration.

Dohroo (2006) concluded that rhizome treatment with fungicide Mancozeb + Carbendazim (0.3 + 0.1 %) was effective against rhizome rot of ginger.

Kishore and Kulkarni (2008) evaluated Carbendazim + Mancozeb (98.88 % inhibition) was at par with Carbendazim + Iprodione, followed by Mancozeb for wilt of Carnation.

Mulik (2009) tested five fungicides at three concentrations 500, 1000 and 1500 ppm against *Fusarium oxysporum f.sp. ciceri* and most effective fungicides Carbendazim (72.75 %) followed by Mancozeb (66.85 %), Captan (64.76 %) and

Thiram (64.38 %) at all concentration; Whereas, Benomyl (16.66 %) was least effective for pathogen. Good sporulation was observed in Benomyl at higher concentration. There was no sporulation with Carbendazim at all the concentration

Chavan *et al.* (2009) reported that the systemic fungicides, Carbendazim and Carbendazim + Mancozeb to give 100 per cent inhibition of mycelial growth at all the concentrations tested against *Fusarium solani*, the causal agent of patchouli wilt.

Nisa *et al.* (2011) reported that the systemic fungicide Hexaconazole at higher concentration (1000 ppm) caused highest reduction in mycelial growth of *Fusarium oxysporum*.

Korde (2011) tested five fungicides at two concentrations 1000 and 2000 ppm against *Fusarium oxysporum f.sp. ciceri* and most effective fungicides Carbendazim (89.81 %) followed by Thiram (81.29 %), Difenconazole (66.81 %) and Hexaconazole (59.67 %) at all concentration; whereas, Propiconazole (56.82 %) was least effective for pathogen

Subhani *et al.* (2011) were tested six fungicides *viz.*, Benomyl, Derosal, Ridomil, Cabrio Top, Vitavax and Prevent at four concentrations, 5, 10, 20 and 50 ppm *in vitro* against *Fusarium oxysporum f.sp. ciceri* and most effective fungicides Derosal (100 %), Vitavax (95.81 %), Benomyl, (93.80 %), Cabrio Top (70.96 %), Ridomil (31.75 %) and Prevent (10.68 %) over reduction in mycelial growth, respectively at 500 ppm concentration.

Raghu and Ravikumar (2011) reported drenching of Metalaxyl MZ-72 1 g/lit and drenching showed less disease incidence as compared to control.

Singh (2011) reported that seed treatment with Ridomil MZ-72 WP and combination product Metalaxyl MZ-728 % + Mancozeb 64 % (1.25 g/ litre) were effective followed by *T. harzianum.*

Shanmugan *et al.* (2013) studied polyhouse evaluation of selected antagonists for rhizome rot disease management. Rhizome rotting was assessed on 0-4 scale.

Sharma and Pandey (2014) reported that rhizome treated with Metalaxyl MZ-72+ Mancozeb gave good rhizome germination and lowest disease incidence.

2.9. *In vitro* evaluation of bioagents against *F. oxysporum*

Philippe and Claude (1991) reported biological control of *Fusarium* disease by *Pseudomonas fluorescens*.

Amara *et al.* (1996) reported that the mutant of *Pseudomonas fluorescens* in relation to growth regulators to be useful in biological control of tomato wilt.

Ram *et al.* (1997) reported soil application of biocontrol agents *Trichoderma harzianum* and *Pseudomonas fluorescens* in sufficient number suppressed *Fusarium* and disease intensity.

Meena and Mathur (2003) evaluated individual application of bio-agents not to be effective against pathogen *Fusarium solani* (Mart).

Francisca *et al*. (2010) observed that the colonies of *Trichoderma harzianum* inhibited the strains of *Fusarium culmorum* in different environmental conditions.

2.10 Integrated disease management module against pathogen associated with the rhizome rot of ginger

Ravikumar *et al.* (2012) found *Trichoderma harzianum* and *Pseudomonas fluorescens* to be effective against rhizome rot of ginger.

Dohroo *et al.* (2012) applied *Trichoderma harzianum* with FYM and periodic drenching of copper oxycholoride @ 0.3% during rainy season in field condition and found control of soft rot disease in ginger.

Dama and Patil (2014) reported that *Trichoderma harzianum* to be competent antagonist against post harvest tomato rots *in-vitro* and highest mycelial growth inhibition was observed.

3. MATERIAL AND METHODS

During the present investigations on Rhizome rot of ginger (*Zingiber officinale* Rosc.), caused by *Fusarium oxysporum f.sp. zingiberi* various experiments were conducted at the Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri and SLBC, M.P.K.V., Rahuri during 2015-2016 and 2016-2017 to fulfill the objectives defined. The details of the materials used and methods followed for various experiments are described here in the following paragraphs.

3.1 Materials

3.1.1 Experimental site

Laboratory (plate and pot culture) and field experiments were conducted at the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri.

3.1.2 Glassware

The common glasswares (Borosil, J-sil and Corning make) *viz.*, Petridishes, test tubes, conical flasks, volumetric flasks, measuring cylinders, glass rods, beakers, funnel, pipettes, etc. were obtained from the Department of Plant Pathology and Agril. Microbiology, M.P.K.V, Rahuri.

3.1.3 Equipments

The laboratory equipments *viz.*, Autoclave, Hot air oven, Laminar airflow Cabinet, BOD Incubator, Refrigerator, Binocular research Microscope, Electronic balance, pH meter, Phase contrast Microscope, Distillation assembly, Vortexer (REMI), Deep freezer (New Brunsaik), Horizontal electrophoresis unit, Microwave oven (BPL Ltd), Variable volume Micropipette, Water bath (REMI), Gel documentation system (Alpha Innotech), Thermocycler (Eppendorf), Hemocytometer, Refrigerated orbital shaker (REMI) available at the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri were utilized, as and when required.

3.1.4 Chemicals

Standard chemicals, reagents, TE buffer (pH 8), 100 mM Tris HCL, 1 mM disodium EDTA, 0.5 mM NaCl, 1 per cent SDS, 7.5 M ammonium acetate, 99 per cent ethanol, RNase, agarose, 6 X loading dye, electrophoresis buffer, sterile water, PCR buffer, dNTPs, tag DNA polymerase enzyme, MgCl2, operon primer, 1

Kb DNA ladder etc. required for the experimentation were obtained from the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri.

3.1.5 Disease samples

Ginger exhibiting typical symptoms of Rhizome rot (*Fusarium oxysporum f.sp. zingiberi*) disease were collected in the paper bags from the ginger fields, surveyed during Rabi 2015 and Rabi, 2016. The diseased samples were brought to the laboratory and subjected aseptically to tissue isolation on Potato dextrose agar medium.

3.1.6 Seeds

Disease free and apparently healthy ginger rhizomes were collected from farmer's fields and were used for pot experiment.

3.1.7 Culture media

Potato dextrose Agar (PDA) was used as basal culture medium for isolation, purification and maintenance of the pure culture of *Fusarium oxysporum f.sp. zingiberi*. Sand : maize (1:2) medium was used for mass multiplication of the pathogen. For studying cultural characteristics of *Fusarium oxysporum f.sp. zingiberi*, synthetic readymade (make: Hi media) and non-synthetic (prepared) media were used. The synthetic media and ingredients of non-synthetic media were obtained from the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri.

3.1.8 Fungicides

The total Thirteen fungicides (systemic and contact) *viz.*, Carbendazim + Mancozeb, Carbendazim 50WP, Metalaxyl 35 WS, Captan 50WP, Hexaconazole + Mancozeb, Propaconazole 25 EC, Copper oxychloride 50 WP, Hexaconazole 5 EC, Mancozeb 75 WP, Bordeaux mixture, Metalaxyl + Copper oxychloride, Benomyl 50 WP and Hexaconazole + Captan were used for *in vitro* and *in vivo* (Pot culture) experiments conducted during present studies.

3.1.9 Biocontrol agents

Pure culture and talk based formulations of biocontrol agents *viz.*, *Trichoderma viride, T. harzianum, T. hamatum, T. virens, T. koningii, Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the Spawn Production-cum-Biocontrol Laboratory, Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri; were maintained and multiplied on appropriate culture media and used for present studies.

3.1.10 Miscellaneous

Earthen pots (30 cm dia.), Mortar and pestle, plant protection appliances, inoculation needle, forceps, blotter papers, paper bags, polythene bags, spirit lamp, mercuric chloride, formalin, labels, scale, sand, soil, FYM, screen house etc. were used during the course of present investigation.

3.1.11 Composition of the media

The composition and preparation of the above mentioned synthetic and semi synthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Hawksworth *et al*. (1983). The composition of the media is given below.

1. Potato dextrose agar medium

For all the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for culturing *F. oxysporum.*

The composition of PDA used is given below.

Materials	Quantity
Peeled and sliced potato	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml (to make up the volume)

Two hundred grams of peeled potatoes were cut into small pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. Each of 20 g of dextrose and agar-agar were dissolved in potato extract and the final volume was made up to 1000 ml by adding distilled water. A known quantity of such medium was dispensed into number of conical flasks and plugged with non-absorbent cotton and finally wrapped with paper. The flasks containing dispensed medium were sterilized at 1.1 kg cm^{-2} pressure for 20 min.

Methodology

3.2 Survey and Disease incidence

A roving survey was conducted during the *Rabi* 2015-2016 and *Rabi* 2016-2017 seasons in the ginger growing areas of the seven districts of Western Maharashtra and one district of Marathwada region to assess rhizome rot disease incidence. Ginger growing pockets/fields were identified from the records available at the office of Sub-Divisional Agriculture Officer of the districts to be surveyed. The field visits were undertaken during months of September and October. The incidence

of disease was recorded by random throwing of quadrant (l m^2) in five place of a field. The number of healthy and diseased plants were counted in a quadrant and per cent of disease incidence was estimated by

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants in quadrant}}{\text{Total number of plants in the quadrant}} \times 100$$

Table 3.1 Survey of Rhizome rot of Ginger on farmer's fields

Districts	Taluka	Village	Area (Acre)	Variety
Ahmednagar	Rahuri	Vambori	2 acre	Mahim
	Shrirampur	Padegaon	1 acre	Mahim
	Akole	Akole	1 acre	Mahim
Pune	Indapur	Hingangaon	1 acre	Satari local
	Baramati	Katewadi	2 acre	Mahim
Satara	Koregaon	Rahmatpur	3 acre	Mahim
		Borgaon	0.5 acre	Satari local
		Nagthane	1 acre	Mahim
Kolhapur	Hatkanangle	Bhadole	1 acre	Mahim
Sangali	Miraj	Kasbe digraj	2 acre	Mahim
Solapur	Karmala	Kander	1 acre	Mahim
Nashik	Yeola	Shirasgaon	0.5 acre	Mahim
Aurangabad	Sillod	Andheri	1 acre	Mahim
	Kannad	Andhaner	2 acre	Satari local

3.2.1 Collection of Disease samples

Ginger exhibiting typical symptoms of Rhizome rot (*Fusarium oxysporum f.sp. zingiberi*) disease were collected in the paper bags from the ginger fields, surveyed during Rabi 2015-2016 and Rabi,2016- 2017. The 14 diseased samples were brought to the laboratory and subjected aseptically to tissue isolation on Potato dextrose agar medium.

3.3 Isolation of the fungus

Naturally infected ginger plants with typical symptoms of rhizome rot collected during survey were brought to the laboratory. These were washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm). These bits were surface sterilized with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for two minutes, washed by giving three successive changes with sterile distilled water in glass Petri plates to remove traces of mercuric chloride and blot dried. These sterilized bits were further inoculated

aseptically on cooled PDA medium in sterilized glass Petri plates under aseptic conditions of Laminar-air-flow cabinet and incubated in BOD incubator at 28 $\pm$ 2^0C temperature. Within 2-3 days of incubation, whitish mycelial mat developed and within next 10-12 days, numerous conidia developed in the plates. The test pathogen was purified by applying hyphal tip isolation technique and through frequent sub-culturing. The pure culture was maintained on agar slants in test tubes in refrigerator. Applying the same procedure, a total of eight isolates of *Fusarium oxysporum f.sp. zingiberi*, one of each representing seven districts of the Western Maharashtra and one district of Marathwada region were isolated, purified, and maintained in refrigerator for further studies.

3.3.1 Hyphal tip isolation

Ten ml of clear filtered two per cent water agar was poured into the sterile Petri plates and allowed to solidify. Diluted spore suspension was prepared in sterilized distilled water from 10 and 12 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at 28 ± 2^0C for 12 and 24 hrs. Then such plates were examined under microscope so as to locate germination of conidia. Single isolated germinated conidium was marked with ink on the lower surface of the plates. The growing hyphal tip portion was cut with a cork borer and transferred to fresh PDA slants under aseptic conditions and incubated at 28 ± 2^0C. Such obtained pure culture tubes were used for further studies.

3.3.2 Maintenance of the culture

The fungus was sub cultured on PDA slant and allowed to grow at 28 ± 1^0C for 10 and 12 days. Such slants were preserved in refrigerator at 5^0Cand renewed once in a month. This pure culture was used for future study.

3.3.3 Identification of the fungus

The morphological characters of the fungus such as cultural characters, mycelial growth, hyphal arrangement, mode of conidial production, conidiophore, length and breadth of conidia were studied by using microscope.

3.3.4 Mass multiplication of *Fusarium oxysporum f.sp. zingiberi* inoculum

Sand : maize medium was used for mass multiplication of *Fusarium oxysporum f.sp. zingiberi* Sand : maize medium (1 part partially broken maize grains + 2 part sand + distilled water to moisten the medium) was prepared, filled (200 g / flask) into the conical flasks (250 ml cap.) and also in polypropylene bags (200 g /

bag) and autoclaved at 20 lbs pressure for 30 min., for two consecutive days. After cooling at room temperature, these flasks/bags containing sand: maize medium were inoculated with mycelial discs (8 to 10) of the test pathogen obtained from a week old culture and incubated at room temperature for two weeks. This mass multiplied inoculum was used for preparation of sick soil / potting mixture in earthen pots that required for various pot culture studies (Plate 4c).

3.3.5 Proving the pathogenicity

Pathogenicity tests were carried out to establish that the fungus thus isolated is capable of producing typical symptoms of *F. oxysporum* under artificial inoculation conditions on Ginger cv. Mahim, and also to prove Koch's postulates of the pathogen.

Pure culture of *Fusarium oxysporum f.sp. zingiberi* fungus was maintained separately on sand-maize meal medium (sieved fine river sand 80 g, maize meal 20 g and 50 ml water) for 10 days in conical flasks. After 10 days incubation, mass culture of the fungus was uniformly mixed with sterilized soil and FYM in proportion of 3:1.

Six earthen pots were treated with 5 per cent copper sulphate ($CuS0_4$) solution. Out of six pots, four pots were filled with *F. oxysporum* inoculated soil and remaining two pots were kept as control which were filled with sterilized uninoculated soil. The inoculated soil in the pots was incubated for 15 days at room temperature, frequently stirred and watered so that, the fungus could colonise in the soil, then two rhizomes of ginger were planted in each pot and observations recorded daily for disease development and continued till complete rotting of the plant.

Observations were made at regular interval for the symptom development. The organism was re-isolated from the artificially inoculated rhizome showing *Fusarium oxysporum* symptoms and thus obtained culture was compared with the original culture for the similarities in the morphology and colony characteristics.

3.4 Study the variability among the isolates of *F. oxysporum*

3.4.1 Morphology of the fungus

All the 14 isolates were subjected to check morphological variability like colony character, topography of the colony, conidial length, width and beak length were calculated under microscope by using Image scope instrument. Among 14

isolates collected 8 isolates were selected for molecular studies based morphology and virulence.

Table 3.2. List of isolates of *Fusarium oxysporum f.sp. zingiberi*

Sr. No.	Districts	Foz Isolates
1.	Ahmadnagar	Foz - 1
2.	Pune	Foz - 2
3.	Satara	Foz - 3
4.	Kolhapur	Foz - 4
5.	Sangli	Foz – 5
6.	Solapur	Foz – 6
7.	Nashik	Foz - 7
8.	Aurangabad	Foz - 8

3.4.2 RAPD Primers

11 RAPD primers commercially synthesized by Siga Company were used for the present study. The list of primers used for amplification of genomic DNA with their sequences is given in Table 3.3 as under.

Table 3.3. List of primers screened against the *Fusarium oxysporum f.sp. zingiberi*

Sr. No.	RAPD Primers	Primer Sequence 5'-3'	Anneling temperature (^{0}C)
1	RFu 1	CAGGCCCTTC	27
2	RFu 2	TGCCGAGCTG	29
3	RFu 3	AGTCAGCCAC3	27
4	RFu 4	AATCGGGCTG3	27
5	RFu 5	AGGGGTCTTG	27
6	RFu 6	GGTCCCTGAC	27
7	RFu 7	GAAACGGGTG	29
8	RFu 8	GTGACGTAGG	27
9	RFu 9	GGGTAACGCC	27
10	RFu 10	GTG ATCGC AG	29
11	RFu 11	CAATCGCCGT	27

The random amplified polymorphic DNA (RAPD) sequence analysis was used to detect the variations among the isolates of *Fusarium oxysporum f.sp. zingiberi.* The standardized protocols of isolation of DNA and RAPD reaction (Meena Gupta *et al.*, 2014) were adopted for this study.

3.4.3 Genomic DNA extraction

The genomic DNA was isolated from mycelial mat of all *Fusarium oxysporum f.sp. zingiberi* isolates through method standardized by Meena Gupta *et al.* (2014) as described below. The liquid culture of each isolate was raised in conical flask. PDB (Potato dextrose broth) 100 ml was inoculated with 7 mm bit of culture disc cut from edge of 5 days old culture of each isolate grown in Petri dish. The inoculated broth was incubated at 28 ± 2^{0}C for 7 days. The mycelial mat was filtered

through Whatman no. 1 filter paper and dried at room temperature. The dry mycelium was transferred to sterile mortar and pestle and ground with glasswool. The DNA extraction buffer and a mixture of buffer saturated phenol/chloroform/isoamyl alcohol (25:24:1) was added per 0.5 gm of starting tissue and the solution was ground thoroughly. The mixture was then transferred into several microfuge tubes and centrifuged at 16000 Xg for 5 min at room temperature to pellet tissue debris and the glass wool, pellet to the bottom of tube. The aqueous phase was transferred to a new tube. The DNA in each tube was precipitated with 0.6 volume of iso-propanol by incubating the mixture at room temperature for 10 min. The DNA was recovered by centrifugation. The pellet was rinsed with 70 per cent ethanol, air dried briefly and resuspended in 340 µl TE buffer containing RNase A at 20 µl /ml. The extracted DNA was resolved on 0.8 per cent Agarose gel. The quantification was done by spectrophotometer and stored at -20^{0}C until further use.

3.4.3.1 RNase treatment

To degrade the RNA present in isolated DNA sample 1.2 µl of RNase A (10 mg/ml) was added to the microfuge tube containing DNA and incubated at 37^{0}C for 1 hour in water bath.

3.4.3.2 Quantification of DNA

l µl DNA diluted with 49 µl of distilled water of each isolated sample was taken against blank. The quantity of DNA was determined by monitoring the absorbance at 260 nm in a biophotometer. The A260/A280 ratio was checked for quality of DNA.

3.4.3.3 RAPD DNA fingerprint analysis

The PCR protocol for RAPD reaction was optimized with various PCR components (Table 3.4) and thermal cycler programme (Table 3.5).

Master mixture (24.0 µl) containing all the above reactants, except template DNA were dispensed in autoclaved PCR tubes (0.2 ml). Genomic DNA of each isolates of *Fusarium oxysporum f.sp. zingiberi* was added to the individual tubes containing the master mixture. The contents of each tube were mixed by tapping with fingers followed by a brief spin to collect contents at the bottom of the tube. The tubes were placed in Thermocycler and subjected to PCR according to the protocol standardized below (Table 3.5).

Table 3.4 PCR components were used for detection of *Fusarium oxysporum f.sp. zingiberi isolates*

PCR Components	Required Concentration	Volume/reaction
PCR Buffer (10X)	1 X	2.5 µl
$MgCl_2$ (25 m M)	1.5 mM	1.7 µl
dNTPs (2.5 mM each)	0.25 mM	2.0 µl
Taq DNA Polymerase (10X)	1.25 U	1.5 µl
Primer (10 uM)	0.4 µM	1.5 µl
Template DNA (10 µl)	30 ng /µl	1.0 µl
Distilled water	---------	16.0 µl
Total		**25** µl

Table 3.5. Standardized PCR protocols for amplification of DNA

Step No.	Temp. (^{0}C)	Duration (Min)	No. of Cycles	Function
1	94	4		Initial Denaturation
2	94	1	40	Denaturation
3	35	1		Annealing
4	72	2		Extention
5	72	10		Final Elongation

The amplified RAPD product was separated on 1.2 per cent agarose gel, stained with ethidium bromide and visualized under Gel documentation system. The polymorphism was detected by comparing RAPD product of all *Fusarium oxysporum f.sp. zingiberi* isolates.

3.4.3.4 Scoring and data Analysis

RAPD markers across the eight isolates were scored for their presence '1' or absence '0' of bands for each primer. By comparing the banding patterns of isolates for a specific primer, isolate's specific bands were identified and faint or unclear bands were not considered. The binary data so generated were used for diversity analysis. The data were entered into binary matrix and subsequently analyzed using NTSYS-pc version 2.10. Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYS-pc.

Relationships between the eight isolates of *Fusarium oxysporum f.sp. zingiberi* were graphically represented in the form of dendrograms. The cophenetic correlation analysis was carried out using the COPH function of NTSYS-pc. In this method the dendrogram and similarity matrix were correlated to find the goodness of fit of the dendrogram constructed based on the similarity coefficients. The matrix comparison was carried out using the MXCOMP function in the NTSYS-pc version 2.02

3.4.3.5 Polymorphism percentage

The polymorphic percentage of the obtained bands was calculated by using the following formula,

$$\text{Polymorphism percent} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.5 *In vitro* evaluation of fungicides against *F. oxysporum*

Poisoned Food Technique : Efficacy of 13 test fungicides was evaluated *in vitro* against *Fusarium oxysporum f.sp. zingiberi* (FOZ-8 isolate), applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium. Based on active ingredient, requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^0C) PDA medium separately in conical flasks (250 ml cap.) to obtain desired concentrations of the test fungicides. Fungicide amended PDA medium was then poured (20 ml / plate) aseptically in glass Petri plates (90.33 mm dia) and allowed to solidify at room temperature. For each of the test fungicide and its test concentrations, three plates / treatment/replication were maintained and each test fungicide with various concentrations was replicated thrice. After solidification of the medium, all the plates were inoculated aseptically with a 7 mm culture disc obtained from a week old actively growing pure culture of *Fusarium oxysporum f.sp. zingiberi.* The culture disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 28 + 2^0C. Petri plates filled with PDA (without any fungicide) and inoculated with the culture disc of *Fusarium oxysporum f.sp. zingiberi* were maintained as untreated control.

Experimental details

Design : C.R.D.

Replications : Three

Treatments : Thirteen

Table 3.6. List of fungicides tested against the *Fusarium oxysporum f.sp. zingiberi*

Tr. No.	Fungicides Name	Trade name	Concentrations (%)
T_1	Hexaconazole	Contaf 5% EC	0.1
T_2	Copper oxychloride	Blitox 70% WP	0.2
T_3	Carbendazim	Bavistin 50% WP	0.1
T_4	Mancozeb	Indofil M-45 75% WP	0.2
T_5	Metalaxyl	Togron 35 WS	0.2
T_6	Captan	Captaf 50 WP	0.2
T_7	Propiconazole	Tilt 25% EC	0.1
T_8	Bordeaux mixture	Kwicbordo	0.1
T_9	Benomyl	Benlate 50 WP	0.1
T_{10}	Control		

Observations on radial mycelial growth were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Percent inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying following formula (Vincent, 1927).

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of the test fungus in untreated control plate

T = Growth of the test fungus in treated plate

3.6 Evaluation of bioagents against *F. oxysporum*

Dual Culture Technique: Five fungal antagonists *viz., Trichoderma viride, T. harzianum, T. hamatum, T. virens, T. koningii,* and two bacterial antagonists *viz., Pseudomonas fluorescens, Bacillus subtilis* were evaluated *in vitro* against *Fusarium oxysporum f.sp. zingiberi* (FOZ- 8 isolate), applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old culture of the test bioagents and the test pathogen (*Fusarium oxysporum f.sp. zingiberi* (FOZ-8 isolate) were used for the study. Culture discs (7 mm dia.) of the test pathogen and bioagents (7 mm dia.)were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed aseptically at equidistance and exactly opposite

with each other on solidified PDA medium in Petri plates and plates were incubated at 28 $\pm$ 2^0C. Three plates/ treatment/replication were maintained. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Experimental details

Design : CRD
Replications : Three
Treatments : Eight

Table 3.7. List of bioagents tested against the *Fusarium oxysporum f.sp. zingiberi*

Tr. No.	Treatments details
T_1	*Trichoderma viride*
T_2	*Trichoderma harzianum*
T_3	*Trichoderma koningii*
T_4	*Trichoderma hamatum*
T_5	*Trichoderma virens*
T_6	*Pseudomonas fluorescens*
T_7	*Bacillus subtilis*
T_8	Control

Observations on linear mycelial growth of the test pathogen and bio-agent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Percent inhibition of the test pathogen by the bioagent over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978).

$$\text{Percent Growth Inhibition} = \frac{\text{Linear mycelial growth in control plate} - \text{Linear mycelial growth in intersecting plate}}{\text{Linear mycelial growth in control plate}} \times 100$$

3.7 Integrated disease management module against pathogen associated with the rhizome rot of ginger under Pot culture.

Healthy ginger rhizomes were injured with the help of needle and were given dip treatment for 30 minutes and then planted in plastic pot containing sick soil. One ginger rhizome was planted in each pot. For evaluation of one treatment, nine ginger plants were used. After 30 days, soil drenching treatments of Fungicide and bioagents were done. Observations were recorded after death of control plants as per scale and formula of Shanmugan *et al.* (2013).

Scale:-

Rhizome rotting was assessed on 0-4 scale:

0 = No disease.

1 = 1-25 % Infected area.

2 = 26-50% Infected area.

3 = 51-75 % Infected area.

4 = 76-100 % Infected area.

$$\text{Percent disease Index} = \frac{\Sigma \text{ of all disease ratings}}{\text{Total number of Plant rating} \times \text{Highest numerical rating}} \times 100$$

Experimental details

Location	:	MPKV main campus, Rahuri
Crop	:	Ginger (*Zingiber officinale* Rosc.)
Variety	:	Mahim (Satari local)
Design	:	C.R.D.
Replications	:	Three
Number of plants per pot	:	9
Date of sowing	:	1 June 2016
Treatments	:	Eight

Table 3.8. Treatments of fungicides and bioagents under pot culture condition

Treatment No.	Rhizome dip treatment (30 minutes)	Soil drenching after one month of sowing
1.	Carbendazim @ (0.1 %)	Carbendazim @ 0.1 %
2.	Benomyl (0.1 %)	Benomyl (0.1 %)
3.	Carbendazim @ (0.1 %) + Mancozeb @ (0.2 %)	Carbendazim @ (0.1 %) + Mancozeb @ (0.2 %)
4.	*Trichoderma viride* @ 3 g/lit + *Pseudomonas fluorescens* @ 3 g/lit	*Trichoderma viride* @ 3 g/lit +*Pseudomonas fluorescens* @ 3 g/lit
5.	*Trichoderma viride* @ 5 g/lit	*Trichoderma viride* @ 5 g/lit
6.	Hexaconazole @ (0.1 %)+ Captan @ 0.2 %	Hexaconazole @ (0.1 %) + Captan @ 0.2 %
7.	*Pseudomonas fluorescens* @ 5 g/lit	*Pseudomonas fluorescens* @ 5 g/lit
8.	Control	

4 .RESULTS AND DISCUSSION

Present studies on the Rhizome rot of ginger caused by *Fusarium oxysporum f.sp. zingiberi* were undertaken during *Rabi*, 2015-16 and 2016-2017 at Department of Plant Pathology and Agril. Microbiology, MPKV Rahuri on the aspects *viz.*, survey, isolation, pathogenicity test, molecular variability studies, *in vitro* evaluation of fungicides, bioagents and integrated disease management (pot culture). The results obtained on all these aspects are presented in the following paragraphs.

4.1 Survey for the disease incidence of rhizome rot of ginger

A random survey was carried out for recording the incidence of rhizome rot of ginger during *Rabi,* 2015-16 in seven major ginger growing districts of Western Maharashtra (Ahmednagar, Pune, Satara Kolhapur, Sangli Solapur, Nashik ,) and one Marathwada region (Aurangabad) of Maharashtra. The village wise disease incidence is presented in Table 4.1 and Plate 1.

From the survey, it was revealed that the disease was severe in all the districts during *Rabi* 2015-16 and mean percent disease incidence ranged from 30 to 46 per cent, respectively in different parts of the districts surveyed. The highest incidence of (48 %) rhizome rot of ginger (*F. oxysporum*) was noticed in farmer's fields of Andheri village in Aurangabad district, whereas least (30 %) incidence of the disease was recorded at Shirasgaon village in Nashik district.

In Satara district, the highest disease incidence was recorded at Naghtane (46 %) village of Satara taluka, whereas least disease incidence was recorded at Borgaon (42 %) in Satara taluka. In Pune district, the highest disease incidence was observed at Hingangaon (40 %) in Indapur taluka, whereas lowest at Katewadi (38 %) of Baramati taluka. In Sangli district, the highest disease incidence was recorded at Kasbe digraj (42 %) village of Miraj taluka and in Solapur district, the lowest disease incidence was recorded at Kander (40 %) village in Karmala Taluka. In Aurangabad district, the highest disease incidence was recorded at Andheri (48 %) village of Sillod taluka, whereas least disease incidence was recorded at Andhaner (46 %) in Kannad taluka. In Ahmednagar district, the highest disease incidence were recorded at Vambori (42 %) village in Rahuri taluka, whereas least incidence was observed at Akole (32 %) village in Akole taluka.

The disease normally appeared during last week of June or last week of July, maximum number of infected plants were recorded during the months of August

and September. The initial symptoms of rhizome rot were recorded on the above ground parts in the form of slight paleness at the tip of terminal leaves, the infected leaves ultimately withered. The infected rhizome becomes discoloured and later showed rotting (Dohroo *et al.*, 2012). The symptoms of the disease in different farmer's fields during field survey are presented in Plate 2.

From the survey of different ginger fields, it was seen that rhizome rot disease was predominant in all the districts, the percent disease incidence ranged between 30 to 48 per cent. From data collected, it was observed that in spite of regular fungicide and biocontrol agents treatment of rhizome and drencing, rhizome rot occurred. It was noticed that the some fields had less incidence where good agriculture practices and regular fungicide rhizome treatment and drenching were followed. On the other hand, some neglected fields were found severely infected with rhizome rot and there was more yield loss.

Survey data were available on the incidence of rhizome rot of ginger in different districts of Maharashtra. However, reports from other parts of India were reviewed. The results of the present investigation correlated with results of earlier workers like Sagar *et al*. (2008) who reported the prevalence of rhizome rot in Karanataka in a survey conducted during *Kharif* 2003-2005 in major ginger growing areas of Karnataka. Rhizome rot incidence was noticed in all the locations surveyed with a range from 5.50 to 45.60 per cent. The maximum disease incidence of 23.70 per cent was recorded in Shimoga district followed by Kodagu (22.90 %), Uttar Canada (20.28 %), Chickmagalur (18.99 %) and Bider (13.00 %). Sharma *et al.* (2015) reported that rhizome rot of ginger caused by fungi *Phytium spp.* and *Fusarium oxysporum f.sp. zingiberi* and bacterium *Pseudomonas solanacearum* are the major problems all over India. The has a disease has a complex etiology and hence little work has been done.

Table 4.1. Survey for the disease incidence of Rhizome rot of ginger in farmer fields of different districts of Western Maharashtra and Marathwada regions in Maharashtra

Districts	Taluka	Village	Area (Acre)	Variety	Previous crop	Soil type	Incidence (%)
Ahmednagar	Rahuri	Vambori	2 acre	Mahim	Watermelon	Red	42.00
	Shrirampur	Padegaon	1 acre	Mahim	Onion	Black	38.00
	Akole	Akole	1 acre	Mahim	Pomogranate	Black	36.00
Pune	Indapur	Hingangaon	1 acre	Satari local	Maize	Black	40.00
	Baramati	Katewadi	2 acre	Mahim	Onion	Black	38.00
Satara	Koregaon	Rahmatpur	3 acre	Mahim	Sugercane	Red	44.00
		Borgaon	0.5 acre	Satari local	Ginger	Black	42.00
		Nagthane	1 acre	Mahim	Groundnut	Black	46.00
Kolhapur	Hatkanangle	Bhadole	1 acre	Mahim	Banana	Black	44.00
Sangali	Miraj	Kasbe digraj	2 acre	Mahim	Watermelon	Black	42.00
Solapur	Karmala	Kander	1 acre	Mahim	Groundnut	Red	40.00
Nashik	Yeola	Shirasgaon	0.5 acre	Mahim	Tomato	Red	30.00
Aurangabad	Sillod	Andheri	1 acre	Mahim	Tomato	Red	48.00
	Kannad	Andhaner	2 acre	Satari local	Onion	Red	46.00

4.1.1 Symptomatology

The symptoms of rhizome rot of ginger (*F. oxysporum f.sp. zingiberi*) included pale brown discolouration of the vascular strands that invades the rest of the rhizome that becomes brown and dry (Plate 3). Trujillo (1963) and Teakle (1965) reported *Fusarium* rot (*Fusarium sp*.) can cause serious problems, symptoms include pale brown discolouration of the vascular strands that invades the rest of the rhizome that become brown and dry. The above ground symptoms were the water-conducting portion of symptomatic rhizomes was discoloured brown and had a black dry rot of the cortex tissue.

Pegg and Stirling (1994) and Stirling (2004) reported that Australian ginger growers normally assume that patches of yellowing plants with rotting rhizomes are affected by *Fusarium* yellows (caused by *Fusarium oxysporum f.sp. zingiberi*).

4.2 Isolation of the fungus.

Among 14 diseased sample isolated and 8 isolates were selected for molecular studies based morphology and virulence .Isolation was made from infected rhizome of ginger which yielded a growth of *Fusarium oxysporum*. The growth of fungus from infected tissue was distinctly visible after three to four days in Petriplates containing the potato dextrose agar medium. The pure culture of fungus was obtained by single spore isolation method on Potato Dextrose Agar (PDA) in plates and was maintained on PDA slants in tesmolt tubes for further investigation (Plate 4). Smith (1988) reported that the *Fusarium oxysporum* has varying appearance like the aerial mycelium first appears white and they may change to variety of colours from violet to dark purple in potato dextrose agar medium. Dohroo and Sharma (1992) reported the morphological characters of *Fusarium oxysporu*m *f.sp. zingiberi* and further stated that 3.9 - 11.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 Septation and 13.6 - 31.2 x 2.9-4.9 µm size of macro conidia with 3-4 Septation. Y.Li *et al.* (2014) reported that *Fusarium oxysporum* has two types of asexual spores i.e. micro-conidia and macro-conidia, the micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells with gradually pointed, curved edges, varied in size from 3.5 x 19-36 µm.

4.2.1 Identification of the fungus

Based on typical symptoms of rhizome rot produced on the plants of naturally and artificially diseased ginger plants, morphological and cultural characteristics, and pathogenicity test; the pathogen under investigation was identified as *F. oxysporum f.sp. zingiberi.* It was further confirmed by the Division of Mycology, Agharkar Research Institute, Pune, Maharashtra (Plate 4a and 4b).

The mycelium was white, uniform, fluffy light pinkish tinge in colony of *F. oxysporum f.sp. zingiberi* which produced three types of a sexual spores, micro-conidia, macro-conidia and chlamydospores. The 3.9 - 14.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 septation micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells gradually pointed, curved edges, varied in size from 3.5 x 20 µm with 3-4 septation. Fusarial basal rot can survive in the soil for long period of time through Chlamydospores (Plate 4).

4.2.2 Pathogenicity test

Healthy growing, 45 days old seedlings of ginger cv. Mahim were spray inoculated with the spore suspension (5 x 10^4 spores/ml) of the test pathogen and incubated in the screen house; where high relative humidity (80 to 90 %) and optimum temperature (28 $\pm$ 1^0C) were maintained for further development of rhizome rot disease symptoms. Pathogenicity of *Fusarium oxysporum* was proved on cv. Mahim in screen house. After one week of incubation, typical symptoms of rhizome rot were observed on leaves of artificially inoculated ginger plants. The test pathogen was reisolated from artificially diseased rhizome, on PDA medium and morphological and cultural characteristics studied were found similar to that of the test pathogen isolated from naturally infected ginger plants. Thus, pathogenicity of the test pathogen was proved (Plate 5) and the pathogen was confirmed as *Fusarium oxysporum.* Pathogenicity tests were carried out to establish that the fungus thus isolated is capable of producing typical symptoms of *F. oxysporum* under artificial inoculation conditions on ginger cv. Mahim, and also to prove Koch's postulates of the pathogen.

Pure culture of *Fusarium oxysporum f.sp. zingiberi* fungus was maintained separately on sand-maize meal medium (sieved fine river sand 80 g, maize meal 20 g and 50 ml water) for ten days in conical flasks. After ten days incubation,

mass culture of the fungus was uniformaly mixed with sterilized soil with already mixed FYM in proportion of 3:1 (Plate 4c).

Six earthen pots were treated with 5 per cent copper sulphate ($CuSO_4$) solution. Out of six pots, four pots were filled with *F. oxysporum* inoculated soil and remaining two pots were kept as control which were filled with sterilized uninoculated soil. The inoculated soil in the pots was incubated for 15 days at room temperature, frequently stirred and watered so that, the fungus could colonise in the soil, then two rhizomes of ginger were planted in each pot and observations were recorded daily for disease development and continued till complete rotting of the plant.

Observations were made at regular interval for the symptoms development. The organism was re-isolated from the artificially inoculated rhizome showing *Fusarium* symptoms and thus obtained culture was compared with the original culture for the similarities in the morphology and colony characteristics (Plate 5).

Isolation, pathogenicity and identification *Fusarium oxysporum f.sp. zingiberi* causing blight in Rhizome rot of Ginger and other vegetable crops was successfully attempted and reported by several earlier workers Trujillo (1963) and Teakle (1965); Pegg and Stirling (1994) and Stirling (2004).

4.3 Variability among the isolates of *F. oxysporum*

4.3.1 Morphology of the fungus

Results (Table 4.2) revealed that significant variation was present in isolates from Ahmednagar, Pune, Satara, Kolhapur, Solapur, Sangli, Nashik and Aurangabad districts in region of the cultural characteristics *viz.*, colour of the colony, mycelial growth, colour of conidia, shape of conidia, beak and length of conidia produced by *Fusarium oxysporum*. Fusarial basal rot can survive in the soil for long period of time through Chlamydospores isolated from different locations were studied *in vitro* using potato dextrose agar medium, and it was observed that there is variation in different isolates (Plate 6).

The Mycelium was white, uniform, fluffy light pinkish tinge in colony of *F. oxysporum f.sp. zingiberi* which produced three types of a sexual spores, micro-conidia, macro-conidia and chlamydospores. The 3.9 - 14.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 septation .micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells gradually pointed, curved edges, varied in size from 3.5 x 20 µm with 3-4 septation (Plate 7).

Variability in cultural characteristics of *Fusarium oxysporum* were studied and reported earlier by Meena Gupta et al. (2014) studied the mycelia colour varied from white to dull white with slightly pinkish tings. The microcondial size varied from 5.20 to 4.00 um (I8) to 4.70 um (I7) to 32.0 to 5.70 um (I4). Chlamydospore dimensions also varied in all the nineteen isolates of the test pathogen. All isolates formed chlamydospore on PDA medium. Pathogenic variability revealed that lesions size varied from 8.50 to 18.00 after 10 days of inoculation whereas, incubation period varies from 11 to 19 days.studied the morphology of *Fusarium spp*. of 10 days old culture.

Agrios (1988) reported that *F. oxysporum* produces three types of a sexual spores, micro-conidia, macro-conidia and chlamydospores.

Dohroo and Sharma (1992) reported the smorphological characters of *Fusarium oxysporu*m *f.sp. zingiberi* and stated that micro-conidia were of 3.9 - 11.7 µm x 1.9-3.9 µm size with 1 or 2 septation, and macro conidia 13.6 - 31.2 x 2.9-4.9 µm size with 3-4 septation.

Table 4.2 Variability in colony characters among different isolates of *Fusarium oxysporum f.sp. zingiberi* (Rhizome rot of Ginger) collected from different district in Maharashtra

Isolate Districts	Name of the Village	Mean of colony diameter (mm)	Colony Colour	Colours at reverse	Colony character	Shape	Size of conidia
Ahmednagar	Vambori	90.00	Creamy white	White to yellow	Mycelium white, dense, uniform and fluffy	Regular	11.1-13.4 x 4.4-5.6µm
	Padegaon	88.40	Creamy white	White to yellow	Mycelium white, dense, uniform and fluffy	Regular	11.0-13.5 x 4.3-5.5µm
	Akole	88.20	Creamy white to yellow	yellow to white	Mycelium white, dense, uniform and fluffy	Regular	12.2-13.6 x 3.1-4µm
Pune	Hingangaon	88.40	Dark gray	Dark gray with creamy center	Mycelium white , fluffy, uniform and dense	Regular	10.0-12.5 x 4.1-5.3µm
	Katewadi	90.000	Dark gray	Dark gray with creamy center	Mycelium white, uniform in centre and suppressed at periphary	Regular	6.5-7.3.5 x 3.7-4µm
Satara	Rahmatpur	90.00	Creamy white	Creamy yellow	Mycelium white, uniform and fluffy	Regular	6.4-7..3 x 3.5-4.0µm
	Borgaon	92.00	Dark white	Dark white to yellow	Mycelium white , fluffy, yellow tings in the colony	Regular	11.5-14.8 x 3.5-4.0µm
	Nagthane	90.00	Dark white	Dark white to yellow	Mycelium white , fluffy, yellow tings in the colony	Regular	12.3-14.2 x 3.6-4.6µm
Kolhapur	Bhadole	88.80	Creamy yellow	Creamy yellow	Mycelium white, uniform raised and dense	Regular	8.0-12.5 x 4.1-8.3µm
Sangli	Kasbe digraj	88.33	White to light yellow	Creamy yellow	Mycelium white, uniform raised and dense	Regular	9.0-12.5 x 4.1-5.3µm
Solapur	Kander	88.00	Creamy white	. White to yellow	Mycelium white, dense, uniform and fluffy	Regular	10.0-12.5 x 4.1-5.3µm
Nashik	Shirasgaon	88.10	Dark white	. Dark white to yellow	Mycelium white , fluffy, yellow tings in the colony	Regular	7.0-15.5 x 4.1-4.3µm
Aurangabad	Andheri	94.00	Creamy white	White to pinkish	Mycelium white, uniform , fluffy light pinkish tinge in colony	Regular	6.0-20x 6.1-8.3µm
	Andhaner	92.00	pinkish	Pinkish to white	Mycelium white, dense, uniform pinkish and fluffy	Regular	12.0-13.5 x2.5-5.3µm

4.3.2 Molecular variability

For the purpose of studing molecular variability of *F. oxysporum f.sp. zingiberi* collected from different district of Maharashtra, a total of 8 samples were selected for extraction of genomic DNA from all different districts. The genomic DNAs of the above 8 samples were isolated by using Hi media fungal DNA isolation kit and subjected to PCR amplification using 11 RFu series primers. Among 11 primers employed in the present investigation, 10 primers amplified the genomic DNAs of the *F. oxysporum f.sp. zingiberi*

Among RFu1 – 11, primer, all the primers amplified the test genomic DNAs except RFu6. All the 10 primers yielding polymorphic amplicons were considered for data analysis. Maximum numbers of amplicons were produced by primers RFu5 and RFu10, whereas least number of amplicons was generated by primer RFu3.

A total number of 131 amplicons were generated by amplification of 10 RAPD primers out of which, 94 amplicons were polymorphic with average polymorphism 92.85 per cent. Each primer thus produced on an average 4.67 amplicons. The size of amplification product ranged from 0.2 kb to 1.3 kb. The RAPD analysis results revealed that a total of 92.85 per cent polymorphism was found between the isolates, indicating there is a molecular variability among the isolates. Information on the amplicons generated for all the primers was used to determine genetic distance between the isolates and to construct a dendrogram by using un-weighted pair group arithmetic mean method (UPGMA).

4.3.2.1 DNA extraction

DNA of eight isolates of *Fusarium oxysporum f.sp. zingiberi* was extracted from their mycelial mat grown on PDB by using protocol standardized by Meena Gupta *et al.* (2014) with some modifications and yielded 350- 400 ng/µl quantity of DNA which was amenable to PCR amplification.

4.3.2.2 RAPD analysis of *Fusarium oxysporum f.sp. zingiberi* isolates

The isolates of *Fusarium oxysporum f.sp zingiberi* causing rhizome rot of ginger from eight districts of Western Maharashtra and Marathwada regions of Maharashtra were used for molecular characterization study.

The eleven primers *viz.*, Rfu -1 to Rfu -11 series primers were used for screening eight isolates of *Fusarium oxysporum f.sp. zingiberi*. Among these, 10 primers gave highly reproducible and scorable amplifications and thus, they were exploited as most informative markers for molecular characterization study. The RAPD-PCR fingerprint pattern revealed that ten RAPD primers generated a total 131 amplicons. Among 131 amplicons, 94 amplicons were found polymorphic and 35 amplicons were monomorphic. The Primer Rfu- 5 and Rfu- 10 could produce highest number of bands (16) followed by Rfu -2 and Rfu -11 each having 14 bands. However, the primer Rfu -3 and Rfu -4 showed lowest number of bands 10 (Table 4.3, Plate 8 and 9). The primers used in this study have shown polymorphism in the range of 36.36 per cent to 92.85 per cent (Table 4.3). The primers Rfu -8 and Rfu -11 showed maximum percent polymorphism each (92.85 %). Rest of primers showed percent polymorphism in the range from 66.66 to 81.81 percent. The primer Rfu -3 showed minimum percent polymorphism (36.36 %).

The data generated by eleven RAPD primers were used to establish the genetic relationship among eight isolates of *Fusarium oxysporum f.sp. zingiberi* by using the Jaccard's similarity coefficient (Jaccard, 1908).

Table 4.3. Per cent polymorphism among isolates of *Fusarium oxysporum f.sp. zingiberi* on the basis of RAPD analysis

Sr. No.	RAPD primers	Sequence of primers 5' -------- 3'	Total number of bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)
1	Rfu – 1	5' CAGGCCCTTC3	12	4	8	66.66
2	Rfu – 2	5' TGCCGAGCTG3	14	5	7	50
3	Rfu – 3	5' AGTCAGCCAC3	11	7	4	36.36
4	Rfu – 4	5' AATCGGGCTG3	13	6	7	53.84
5	Rfu – 5	5' AGGGGTCTTG3	16	3	13	81.25
6	Rfu – 7	5' GAAACGGGTG3	11	2	9	81.81
7	Rfu – 8	5' GTGACGTAGG3	14	1	13	92.85
8	Rfu – 9	5' GGGTAACGCC3	10	2	8	80
9	Rfu – 10	5' GTG ATCGCAG3	16	4	12	75
10	Rfu – 11	5' CAATCGCCGT3	14	1	13	92.85
	Total		**131**	**35**	**94**	**71.75**

Statistical analyses were performed using NTSYS-pc version 2.21c (Exeter Software, NY, USA). Dendrogram was generated by vising the SAHN (Sequential Agglomerative Hierarchical Nested) programme of NTSYS and matrix comparison was done using COPH and MXCOMP programmes and correlated with the original distance matrices in order to test for the association between the cluster in the dendrogram and the Dice matrix. The un-weighted pair group method with arithmetic mean (UPGMA) cluster analysis method was followed for construction of phylogenetic tree.

Table 4.4. Divergent analysis of eight isolates of *F. oxysporum f.sp. zingiberi* by RAPD primers.

Similarity matrix								
	Foz 1	**Foz 2**	**Foz 3**	**Foz 4**	**Foz 5**	**Foz 6**	**Foz 7**	**Foz 8**
Foz 1	1							
Foz 2	0.755	1						
Foz 3	0.593	0.561	1					
Foz 4	0.643	0.582	0.66	1				
Foz 5	0.621	0.648	0.636	0.66	1			
Foz 6	0.685	0.509	0.554	0.545	0.526	1		
Foz 7	0.554	0.577	0.537	0.528	0.566	0.538	1	
Foz 8	0.426	0.491	0.431	0.5	0.566	0.429	0.49	1
	Foz 1	**Foz 2**	**Foz 3**	**Foz 4**	**Foz 5**	**Foz 6**	**Foz 7**	**Foz 8**

4.3.2.3 Genetic diversity analysis of *F. oxysporum f.sp. zingiberi* isolates based on RAPD primers

Genetic diversity is commonly measured by genetic distance or genetic similarity both of which imply that there are either similarities or differences at genetic level.

The pair wise similarity coefficient ranged from 0.426 to 0.755 implying that a large part of genome is dissimilar among isolates. The isolates Foz-2 (Pune) and Foz-8 (Aurangabad) showed more divergence i.e. minimum similarity coefficient of about 0.426. Maximum similarity coefficient of about 0.755 was present in between Foz-2 (Pune) isolate and Foz- 1 (Ahmednagar) isolates indicating that these isolates were less divergent from each other.

The phylogenetic relationship among the isolates was examined by UPGMA cluster analysis and depicted in Fig. 4.2. The isolates used in present investigation could be grouped into two main clusters *viz.*, clusters I, II and four sub cluster.

4.3.2.4 Cluster analysis of eight *F. oxysporum f.sp. zingiberi* isolates

Two major group:-

Groups	Clusters		No. of isolates	Name of isolates
I	-	-	1	Foz 8
II	IIA	-	1	Foz 7
	IIB	IIBa	5	Foz 1, Foz 2, Foz 3, Foz 4, Foz 5
		IIBb	1	Foz 6

The dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA after analysis of banding patterns generated by eight *F. oxysporum f.sp. zingiberi* isolates with 10 RAPD primers. The dendrogram separated *F. oxysporum f.sp. zingiberi* isolates into two main clusters, *viz.*, I and II. Cluster I having only one isolate which was collected from Aurangabad (Foz 8). The other group II is divided into 2 sub groups IIA is having the isolate FOZ 7 collected from Nashik, and Sub group II divided into IIBa and IIBb, the isolates FOZ 1, FOZ 2, FOZ 3, FOZ 4 and Foz 5 were included under the group IIBa and the isolates FOZ6 are placed under IIBb (Fig. 4.2).

The cluster analysis showed the variability among the 08 isolates collected from different districts of Maharashtra. The genetic diversity in a pathogen directly reflects the pathogen's ability to adapt to surrounding environment. Thus, the diversity observed could help in understanding the extent of adaptability of pathogen. In our study, we observed a fair amount of genetic diversity in *F. oxysporum f.sp. zingiberi* which leads to the assumption that the *F. oxysporum f.sp. zingiberi* has acquired its ability to adapt to different climatic condition as well as fungicidal resistance ability.

In present study, it was observed that the eight *F. oxysporum f.sp. zingiberi* isolates stood into two main clusters, *viz.*, I and II. Cluster I having only one isolate reported by Meena Gupta *et al.* (2014).

Pappalardo *et al*. (2009) reported that genetic variations among 29 isolates of *Fusarium oxysporum f.sp*. *zingiberi* (*Foz*) collected from diseased ginger rhizome in production regions throughout Queensland. There were analysed using DNA amplication fingerprinting (DAF). Eight isolates of other Fusarium species and/or *formae speciales* were included for comparative analysis. Within the Foz isolates, three haplotypes were identified based on 17 polymorphic bands generated with five primers. Two groups showed very little genetic variation (98.6 % similarity), whereas the third single isolate was quite distinct in terms of its molecular profile (77.2 % similarity). Genetic similarity among the *Fusarium solani*, *Fusarium oxysporum f.sp. lycopersici*, *Fusarium oxysporum f.sp. cubence* races 1, 3 and 4 isolates compared.

Meena Gupta *et al.* (2014) reported variability among 19 isolates of *Fusarium oxysporum* f.sp. *zingiberi,* causal organism of *Fusarium* yellows in ginger, collected from different ginger growing areas of Himachal Pradesh and designated as I1 to I19. Morphological variations with respect to mycelia colour, conidial size and formation of chlamydospores and pathogenic variation in terms of disease incidence among different isolates was studied. The mycelia colour varied from white to dull white with slightly pinkish tinge. The microcondial size varied from 5.20 to 4.00 µm (I8) to 4.70 µm (I7) to 32.0 to 5.70 µm (I4). Chlamydospore dimensions also varied in all the nineteen isolates of the test pathogen. All isolates formed chlamydospore on PDA medium. Pathogenic variability revealed that lesions size varied from 8.50 to 18.00 µm after 10 days of inoculation whereas, incubation period varied from 11 to 19 days. Genetic variation was also analysed by using 40. 10- mer oilgonucleotide RAPD primer, out of which 2 primers yielded informative, strong and reproducible DNA amplicons of *Fusarium oxysporum f.sp. zingiberi* and thus were selected for studying the variation among isolates. The dendrogram of DNA fingerprints revealed o to 80 per cent variation among isolates. All isolates were grouped into two different major groups, each comprising of ten and nine isolates, respectively.

4.4. *In vitro* evaluation of fungicide against *F. oxysporum f.sp. zingiberi*

4.4.1 *In vitro* evaluation of fungicides

A total of 13 fungicides *viz.*, Hexaconazole 5 EC, Copperoxychloride 50 WP, Carbendazim 50 WP, Metalaxyl 35 WS, Carbendazim + Mancozeb, Metalaxyl +

Copper oxychloride, Mancozeb,Hexaconazole + Captan ,Propaconazole 25 EC, Bordeaux mixture, Benomyl 50 WP, Hexaconazole + Mancozeb and Captan 50 WP were evaluated *in vitro* against *F. oxysporum,* applying Poisoned Food Technique and using Potato Dextrose Agar as basal medium. Effects of these fungicides on radial mycelial growth and inhibition of test pathogen were recorded. All the treatments were replicated three times and a suitable untreated control (without fungicides) was also maintained.

4.4.2 Radial mycelial growth

Results revealed that all the fungicides tested recorded a wide range of radial mycelial growth (colony diameter) of the test pathogen. The mycelia growths ranged from 0.00 to 39.16 mm mean colony diameter (Plate 10).

Table 4.5. *In vitro* evaluations of fungicides at different concentration on radial mycelial growth and inhibition of *Fusarium oxysporum f.sp. zingiberi*

Tr. No.	Fungicides	Concentration percent used	Mean colony diameter (mm)* (after 7) days of inoculation	Percent inhibition of growth
1	Hexaconazole	0.15	38.23 (38.19)	57.52
2	Copper oxychloride	0.30	39.16 (38.74)	56.48
3	Carbendazim	0.1	0.00 (0.00)	100.00
4	Metalaxyl	0.2	40.40 (39.46)	55.11
5	Carbendazim + Mancozeb	0.1+0.2	0.00 (0.00)	100.00
6	Metalaxyl + Copper oxychloride	0.2 +0.10	70.60 (57.16)	21.55
7	Mancozeb	0.25	38.03 (38.07)	57.74
8	Hexaconazole + Captan	0.15+0.20	12.40 (20.61)	86.22
9	Propaconazole	0.15	18.30 (25.32)	79.66
10	Bordeaux mixture	0.1	24.16 (29.44)	73.15
11	Benomyl	0.1	0.00 (0.00)	100.00
12	Hexaconazole+ Mancozeb	0.15+0.20	10.10 (18.53)	88.77
13	Captan	0.20	20.16 (26.68)	77.60
14	Control		90.00 (75.82)	-
	CV		0.34	00.00
	S.E. $\pm$		0.058	
	CD at 1%		0.167	

*=Average of three replications
Figures in parenthesis are angular transformed values

It was observed from the data given in table 4.4 that Carbendazim (0.1 %), Carbendazim (0.1 %) + Mancozeb (0.2 %) and Benomyl (0.1 %), completely inhibited the growth of the pathogen *Fusarium oxysporum* on potato dextrose agar medium. This showed that the

fungicides Carbendazim , Carbendazim + Mancozeb and Benomyl, in the given concentration, were 100 per cent effective against *F. oxysporum.*

Hexaconazole + Mancozeb (10.10 mm), Hexaconazole + Captan (12.40 mm), Propaconazole (18.30 mm) and Captan (20.16 mm) followed them in order of efficacy. (Fig. 4.3).

Highest mean radial growth (90.00 mm) was observed in untreated control plates.

4.4.3 Mycelial inhibition

Results (Table 4.3 and Fig. 4.3) revealed that all the fungicides tested significantly inhibited mycelial growth of the test fungus over untreated control (00.00 %). The percentage mycelial growth inhibition ranged from 21.55 per cent (Metalaxyl + Copper oxychloride) to 100 per cent (Carbendazim, Carbendazim + Mancozeb and Benomyl). However, highest percentage of mycelial growth inhibition was recorded with Carbendazim, Carbendazim + Mancozeb and Benomyl (100 %). This was followed by the fungicides, Hexaconazole + Mancozeb (88.77 %), Hexaconazole + Captan (86.22 %), Propaconazole (79.66 %) and Captan (77.60 %). Least mycelial growth inhibition was recorded with Bordeaux mixture (73.15 %) which was followed by Mancozeb (57.54 %), Hexaconazole (57.52 %), Copper oxychloride (56.48 %) and Metalaxyl (55.11 %).

Fungicides *viz.*, Hexaconazole, Copperoxychloride, Carbendazim, Metalaxyl, Carbendazim + Mancozeb, Metalaxyl + Copper oxychloride Mancozeb, Hexaconazole + Captan, Propaconazole, Bordeaux mixture, Benomyl, Hexaconazole + Mancozeb and Captan were reported to cause significant inhibition of mycelial growth of *Fusarium oxysporum*.

Chauhan and Patel (1990) evaluated that Metalaxyl MZ-72 and Bordeaux mixture effective against *Pythium spp* and *Fusarium solani in vitro* test.

Elliott (2003) found rhizome dip treatment of ginger with Ridomil MZ (0.2 %) for 20 minutes to reduce the incidence of disease.

Meena and Mathur (2003) observed that fungicidal mixture of Ridomil MZ and Bavistin was effective in treating seed rhizomes and soil individually and in combination for the suppression of rhizome rot of ginger.

Poddar *et al.* (2004) evaluated four systemic fungicides *viz.*, Carbendazim, Propiconazole, Tebucanazole, Thiophonate Methyl and found the Carbendazim to inhibit maximum growth of *Fusarium oxysporum* in *in vitro.*

Dohroo (2006) concluded that rhizome treatment with fungicide Mancozeb + Carbendazim (0.3 + 0.1 %) to be effective against rhizome rot of ginger.

4.5 *In vitro* evaluation of bioagents against *F. oxysporum*

Five fungal (*viz., Trichoderma viride, T. harzianum, T. koningii, T. hamatum* and *T. virens*) and two bacterial (*Pseudomonas fluorescens* and *Bacilllus subtilis*) antagonists were evaluated *In vitro* against *F.oxysporum*, applying Dual Culture Technique (Dennis and Webster, 1971) and using PDA as basal medium as detailed under (Plate 11).

Table 4.6. *In vitro* evaluation of different bioagents on linear mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *zingiberi*

Sr. No.	Biocontrol agents	Mean colony diameter (mm)* (after 7 days)	Per cent (%) inhibition
1	*Trichoderma viride*	22.00 (27.96)	75.66
2	*Trichoderma harzianum*	23.20 (28.79)	74.31
3	*Trichoderma koningii*	25.43 (30.28)	72.11
4	*Trichoderma hamatum*	28.50 (32.26)	68.70
5	*Trichoderma virens*	32.22 (34.58)	64.33
6	*Pseudomonas fluorescens*	42.19 (40.50)	52.29
7	*Bacillus subtilis*	51.32 (45.76)	43.18
8	Control	90.33 (71.88)	00.00
	S.E. ±	0.35	
	C.D. at 1%	1.06	
	CV %	1.57	

* = Average of three replications.
Figures in parenthesis are angular transformed values

Results (Table 4.5) revealed that all the bioagents evaluated exhibited fungistatic/fungitoxic activity against *F. oxysporum* and significantly inhibited mycelial growth of the test pathogen over untreated control. Of the seven antagonists tested, *T.*

viride was found most effective and recorded least linear mycelial growth (22.00 mm) with highest mycelial growth inhibition (75.66 %) of the test pathogen as compared to untreated control (90.33 mm and 00.00 %). The second and third best antagonists found were *T. harzianum* and *T. koningii*, which recorded mycelial growth of 23.20 mm and 25.43 mm, respectively and inhibition of 74.31 and 72.11 per cent, respectively. This was followed by *T. hamatum* (col. dia.: 28.50 mm and inhibition: (68.70 %). The fungal antagonist *T. virens* was found least effective which recorded 32.22 mm and 64.33 per cent linear mycelial growth and inhibition, respectively. Bacterial antagonists *P. fluorescens* (col. dia.: 42.19 mm and inhibition: 52.29 %) and *B.subtilis* (col. dia.: 51.32 mm and inhibition: 43.18 %). were also found least effective in regand of per cent linear mycelial growth and inhibition, respectively (Fig. 4.4).

Thus, all the fungal and bacterial antagonists/bioagents evaluated *In vitro* were found fungistatic/fungitoxic against *F.oxysporum* and caused significant reduction in the linear mycelial growth of the test pathogen over untreated control.

The inhibitory effect of *Trichoderma* spp. and *P. fluorescens* and *B. subtilis* against *F. oxysporum* may be attributed to one or more of biocontrol the mechanisms *viz.,* antibiosis, lysis, mycoparasitism, competition and production of volatile and non-volatile substances.

Results of the present study on antagonistic effects of the *Trichoderma* spp., *Pseudomonas fluorescens,* and *Bacillus subtilis* against *F. oxysporum* are in conformity with those reported earlier by several workers (Philippe and Claude (1991); Amara *et al.* (1996); Ram *et al.* (1997) ; Meena and Mathur (2003)

4.6 Effective integrated disease management module against pathogen associated with the rhizome rot of ginger *in vivo* (Pot culture)

Evaluation of fungicides and effective biocontrol agent studies were carried out in glasshouse under pot culture. Nine plants were used for one treatment, three replications were maintained. Results of fungicides and biocontrol agents are given in Table 4.6. Plastic pots were filled with sick soil, and seed rhizomes were treated with following rhizome dip treatments. Then planting of ginger rhizome in pots was done (Plate 12). Then after 30 days of planting, soil drencing application of fungicides and

effective biocontrol agentin pots were done. After 45 days of planting, drenching treatment was done.

According to Shanmugam formula

$$\text{Percent disease Index} = \frac{\Sigma \text{ of all disease ratings}}{\text{Total number of plant rating} \times \text{Highest numerical rating}} \times 100$$

Table 4.7. Integrated disease management module against pathogen associated with the rhizome rot of ginger under pot culture condition

Treatment No.	Rhizome dip treatment (30 minutes)	Soil drencing after 1 month of sowing	Percent disease incidence (%)
1.	Carbendazim @ (0.1 %)	Carbendazim @ (0.1 %)	13.88 (21.82)
2.	Benomyl (0.1 %)	Benomyl (0.1 %)	36.11 (36.92)
3.	Carbendazim @ (0.1 %) + Mancozeb @ (0.2 %)	Carbendazim @ (0.1 %) + Mancozeb @ (0.2 %)	8.33 (16.72)
4.	*Trichoderma viride* @ 3 g/lit + *Pseudomonas fluorescens* @ 3 g/lit	*Trichoderma viride* @ 3 g/lit + *Pseudomonas fluorescens* @ 3 g/lit	*27.77* (31.73)
5.	*Trichoderma viride* @ 5 g/lit	*Trichoderma viride* @ 5 g/lit	38.88 (38.55)
6.	Hexaconazole @ 0.1 % + Captan @ 0.2 %	Hexaconazole @ 0.1 % + Captan @ 0.2 %	33.33 (35.25)
7.	*Pseudomonas fluorescens* @ 5 g/lit	*Pseudomonas fluorescens* @ 5 g/lit	*47.22* (43.40)
8.	Control	Control	77.77 (61.95)
	S.E. ±	-	1.277
	C.D. at 1%	-	3.830
	CV %	-	6.18

* = Average of three replications.
Figures in parenthesis are angular transformed values

Results (Tables 4.6) reveled that control plants showed total wilting and rotting of rhizomes after 80 days of plantings. The pot with Rhizome dip treatment 30 min and soil drenching after one month of sowing with Carbendazim (0.1 %) + Mancozeb (0.2 %) was most effective in managing the disease with per cent disease incidence of 8.33 percent and the next best treatment was Carbendazim (0.1 %) alone with PDI of 13.88 per cent, followed by Benomyl (36.11 %). The least PDI was recorded with control (77.77 %). Among the bio agents tested, the combination of *Trichoderma*

viride (3 g) + *Pseudomonas fluorescence* (3 g/litre) with PDI of 27.77 percent showed effectiveness in managing the disease. Ravikumar *et al.* (2012) found *Trichoderma harzianum* and *Pseudomonas fluorescens* to be effective in lowering the percent diseases incidence against rhizome rot of ginger.

Ram *et al.* (1997) reported soil application of biocontrol agents *Trichoderma harzianum* and *Pseudomonas fluorescences* to be effective in suppressing *Fusarium* and disease intensity.

Singh and Jha (2003) evaluated seven fungicides *viz.*, Thiram, Bavistin, Blitox, Captaf, Indofil M-45, Ridomil MZ and Kitazin against chickpea wilt *in vitro* and *in vivo* and proved that Thiram and Carbendazim were the most suitable fungicides in inhibiting the growth of *F. oxysporum f.sp. ciceri in vitro* and reduced the wilt incidence *in vivo*.

Chavan *et al.* (2009) reported that the systemic fungicides, Carbendazim and Carbendazim + Mancozeb to give 100 per cent inhibition of mycelial growth at all the concentrations tested against *Fusarium solani*, the causal agent of patchouli wilt.

5. SUMMARY AND CONCLUSION

The investigations on rhizome rot of ginger in Maharashtra were carried out at Department of Plant Pathology, PGI, M.P.K.V., Rahuri, during the years 2015-2016 and 2016-2017. Rhizome rot of ginger is a fungal disease caused by *Fusarium oxysporum f.sp zingiberi* which is one of the most important diseases causing considerable losses in Ginger. Keeping in view the economic importance of the crop and losses caused by the disease, present study on the aspects like survey, isolation, pathogenicity test, molecular variability studies, *in vitro* and *in vivo* evaluation of fungicides, bioagents and integrated disease management (pot culture) were undertaken. The results obtained during the course of this study are summarized here as under :

The survey of ginger on rhizome rot in districts of Ahmednagar, Pune, Satara, Kolhapur, Solapur, Sangli, Nashik and Aurangabad districts revealed the prevalence of the disease in all the seven districts of Western Maharashtra and one district of Marathwada regions. Among the various districts surveyed, the disease incidence exhibited almost a similar trend. The highest disease incidence was recorded at Andheri (48 %) village of Sillod taluka in Aurangabad district, whereas least disease incidence was recorded at Shirasgaon (30 %) of Yeola taluka in Nashik district.

In Satara district, the highest disease incidence was recorded at Nagthane (46 %) village of Satara taluka, whereas, least disease incidence was recorded at Borgaon (42 %) in Satara taluka. In Pune district, the highest disease incidence was observed at Hingangaon (40 %) in Indapur taluka, whereas lowest at Katewadi (38 %) of Baramati taluka. In Sangli district, the highest disease incidence was recorded at Kasbe digraj (42 %) village of Miraj taluka and in Solapur district, the lowest disease incidence were recorded at Kander (40 %) village in Karmala taluka, whereas least in Aurangabad district, the highest disease incidence was recorded at Andheri (48 %) village of Sillod taluka, whereas least disease incidence was recorded at Andhaner (46 %) in Kannad staluka. In Ahemadnagar district, the highest disease incidence were recorded at Vambori (42 %) village in Rahuri taluka, whereas least incidence was observed at Akole (32%) village in Akole taluka.

The test pathogen (*Fusarium oxysporum*) was isolated successfully on the basal culture medium Potato dextrose agar, from the plant showing typical symptoms of

Fusarium rot. The pathogen produced brown to circular pinkish colour colony fungal growth on Potato Dextrose Agar

Based on typical symptoms of rhizome rot produced on the plants of naturally and artificially diseased ginger plants, morphological and cultural characteristics, and pathogenicity test; the pathogen under investigation was identified as *F. oxysporum f.sp. zingiberi.* It was further confirmed by the Division of Mycology Agharkar Research Institute, MACS Pune, Maharashtra state.

Fusarium oxysporum has two types of asexual spores i.e. micro-conidia and macro-conidia, the micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells with gradually pointed, curved edges, varied in size from 3.5 x 19-36 µm. fungal growth on Potato dextrose agar.

The pathogenicity of *F. oxysporum f.sp. zingiberi* was proved on ginger cv. Mahim grown in earthen pots and maintained in screen house. The typical symptoms of rhizome *rot* on plant of the artificially inoculated ginger seedlings were produced. The test pathogen was reisolated on PDA from the artificially diseased ginger seedlings, which exhibited similar morphological and cultural characteristics as that obtained from naturally diseased ginger plants.

Significant variation was present in isolates from Ahmednagar, Pune, Satara, Kolhapur, Solapur, Sangli, Nashik and Aurangabad districts in respect of the cultural characteristics *viz.,* colour of the colony, mycelial growth, colour of conidia, shape of conidia, beak and length of conidia produced by *Fusarium oxysporum.* The mycelium was white, uniform , fluffy light with pinkish tinge in colony of *F. oxysporum f.sp. zingiberi.*

Further, DNA was extracted by modified protocol and subjected for genetic diversity analysis by using RAPD primers. Initially 11 primers were screened, among which 10 primers had generated reproducible and scorable banding pattern. Hence, genetic diversity was analyzed based on data obtained by 10 RAPD primers. These primers were found 36.36 to 92.85 per cent polymorphic in nature. All primers had amplified total number of 129 bands among which 94 polymorphic and 35 monomorphic, respectively. Primer Rfu-5 and Rfu-10 had produced each 16 bands with 81.25 and 75 percent polymorphism, respectively. The primer Rfu-3 had shown lowest

per cent polymorphism (36.36. %). The pair wise similarity coefficient ranged from 0.426 to 0.755 implying that a large part of genome is dissimilar among isolates. The isolates Foz-2 (Pune) and Foz-8 (Aurangabad) showed more divergence i.e. minimum similarity coefficient of about 0.426. Maximum similarity coefficient of about 0.755 was present in between Foz-2 (Pune) isolate and Foz- 1 (Ahmednagar) isolates indicating that these isolates were less divergent from each other. The dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA after analysis of banding patterns generated by eight *F. oxysporum f.sp. zingiberi* isolates with 10 RAPD primers. The dendrogram separated *F. oxysporum f.sp. zingiberi* isolates into two main clusters, *viz.*, I and II. Cluster I having only one isolate which was collected from Aurangabad (Foz 8). The other group II is divided into 2 sub groups IIA is having the isolate FOZ 7 collected from Nashik, and Sub group II divided into IIBa and IIBb, the isolates FOZ 1, FOZ 2, FOZ 3, FOZ 4 and Foz 5 were included under the group IIBa and the isolates FOZ6 are placed under IIBb.

A total of 13 fungicides were evaluated *in vitro* against *F. oxysporum*, applying Poisoned Food Technique and using Potato dextrose agar as basal medium. Effects of these fungicides on radial mycelial growth and inhibition of test pathogen were recorded. All the treatments were replicated three times and a suitable untreated control (without fungicides) was also maintained. It was observed that the Carbendazim (0.1 %), Carbendazim (0.1 %) + Mancozeb (0.25 %) and Benomyl (0.1 %), completely inhibited the growth of the pathogen *Fusarium oxysporum* on potato dextrose agar medium. This showed that, the fungicides Carbendazim, Carbendazim + Mancozeb and Benomyl, in the given concentration were 100 per cent effective against *F. oxysporum.*

Five fungal antagonists were evaluated *in vitro* against *F. oxysporum*, applying Dual Culture Technique (Dennis and Webster, 1971) and using PDA as basal medium. All the bioagents evaluated exhibited fungistatic/fungitoxic activity against *F. oxysporum* and significantly inhibited mycelial growth of the test pathogen over untreated control. Of the seven antagonists tested, *T. viride* was found most effective and recorded least linear mycelial growth (22.00 mm) with highest mycelial growth inhibition (75.66 %) of the test pathogen as compared to untreated control (90.33 mm and 00.00 %).

Evaluation of fungicides and effective biocontrol agent studies were carried out in glasshouse under pot culture The pot with rhizome dip treatment 30 min and soil drenching after one month of sowing with Carbendazim (0.1 %) + Mancozeb

(0.2 %) was most effective in managing the disease with disease incidence of 8.33 percent and the next best chemical was Carbendazim (0.1 %) alone with PDI of 13.88 per cent, followed by Benomyl (36.11 %), the least PDI was recorded with control (77.77 %). Among the bio agents tested, the combination of *Trichoderma viride* (3 g) + *Pseudomonas fluorescens* (3g/litre) with PDI of 27.77 per cent shows effectiveness in managing the disease.

Conclusion

1. Survey was carried out for recording the incidence of rhizome rot of ginger during *Rabi*, 2015-16 in major ginger growing districts of Western Maharashtra (Ahmednagar, Pune, Satara Kolhapur, Sangli, Solapur, Nashik) and Marathwada regions (Aurangabad) of Maharashtra. From the survey it was revealed that the disease was severe in all the districts during *Rabi* 2015-16 and 2016-17. Disease incidence ranged from 30 to 48 per cent, in different parts of the districts surveyed. The highest, incidence (48 %) of rhizome rot of ginger (*F. oxysporum*) was noticed in farmers fields of Andheri village in Aurangabad district, whereas least (30 %) incidence of the disease was recorded at Shirasgaon village in Nashik district.

2. The symptoms of Rhizome rot of ginger (*F. oxysporum*) appeared as can cause serious problems, symptoms include pale brown discolouration of the vascular strands that invades the rest of the rhizome that become brown and dry. The above ground symptoms were the water-conducting portion of symptomatic rhizomes was discoloured brown and had a black dry rot of the cortex tissue.

3. The mycelium was white, uniform, fluffy light with pinkish tinge in colony of *F. oxysporum f.sp. zingiberi* which produces three types of sexual a spores, micro-conidia, macro-conidia and chlamydospores. The 3.9 - 14.7 µm x 1.9-3.9 µm size of micro-conidia with 1 or 2 septation .micro-conidia were most abundantly produced spores and were oval, elliptical or kidney shaped and produced on aerial mycelium, macro-conidia 3-5 cells, gradually pointed, curved edges, varied in size from 3.5 x 20 µm with 3-4 Septation. Fusarial basal rot can survive in the soil for long period of time through Chlamydospores.

4. Pathogenicity of *Fusarium oxysporum f.sp. zingiberi* was proved on cv. Mahim in screen house. After one week of incubation, typical symptoms of rhizome rot were observed on leaf of artificially inoculated ginger plants. The test pathogen

was reisolated from artificially diseased rhizome, on PDA medium and morphological and cultural characteristics studied were found similar to that of the test pathogen isolated from naturally infected ginger plants. Thus, pathogenicity of the test pathogen was proved and the pathogen was confirmed as *Fusarium oxysporum f.sp. zingiberi.*

5. The variability in cultural characteristics of *Fusarium oxysporum f.sp. zingiberi* has two types of asexual spores i.e. micro-conidia and macro-conidia, the mycelium white, dense,uniform and fluffy varied in size from 12.0-13.5 x 2.5-5.3 sµm.

6. DNA profiling revealed high degree of polymorphism amongst the isolates collected from eight districts of Western Maharashtra and Marathwada region. UPGMA cluster clearly delineated variability among *Fusarium oxysporum f.sp. zingberi* isolates grouping most of them in two clusters *viz.,* I and II. During the molecular variability analysis among the isolates FOZ-2 (Pune) and FOZ-8 (Aurangbad) more divergence i.e. minimum similarity coefficient about 0.426. Maximum similarity coefficient about 0.755 was present in between FOZ-2 (Pune) isolates and and FOZ-1 (Ahmednagar) isolate indicating that these isolates were less divergent from each other.

7. Among the different fungicides tested, the maximum of 100 per cent inhibition was found 21.55 % in Carbendazim, Carbendazim + Mancozeb and Benomyl and the least inhibition was recorded with the (Metalaxyl + Copper oxychloride).

8. Among the different bioagents tested, the maximum of 75.66 percent inhibition was found in *T. viride*, followed by *T. harzianum* and *T. koningii*, and the least inhibition 43.18 % was recorded with the bacterial bioagent *B. subtilis.*

9. The pot culture experiments with rhizome dip treatment 30 min and soil drenching after one month of sowing with Carbendazim (0.1 %) + Mancozeb (0.2 %) was most effective in managing the disease with per cent disease incidence of 8.33 per cent and the next best treatment was Carbendazim (0.1 %) alone with PDI of 13.88 per cent, followed by Benomyl (36.11 %), the least PDI was recorded with control (77.77 %). Among the bio agents tested, the combination of *Trichoderma viride* (3 g) + *Pseudomonas fluorescens* (3 g/litre) with PDI of 27.77 per cent showed effectiveness in managing the disease.

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8. VITA

GANGAWANE HANUMANT DNYANDEO

DOCTOR OF PHILOSOPHY (AGRICULTURE)

IN

PLANT PATHOLOGY

2020

Title of Thesis		**:**	**Studies on rhizome rot of ginger caused by *Fusarium oxysporum f.sp. zingiberi* in Maharashtra state**
Major field		**:**	Plant Pathology
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Educational	**Bachelor Degree Obtained**	**:**	B.Sc. (Agri.)
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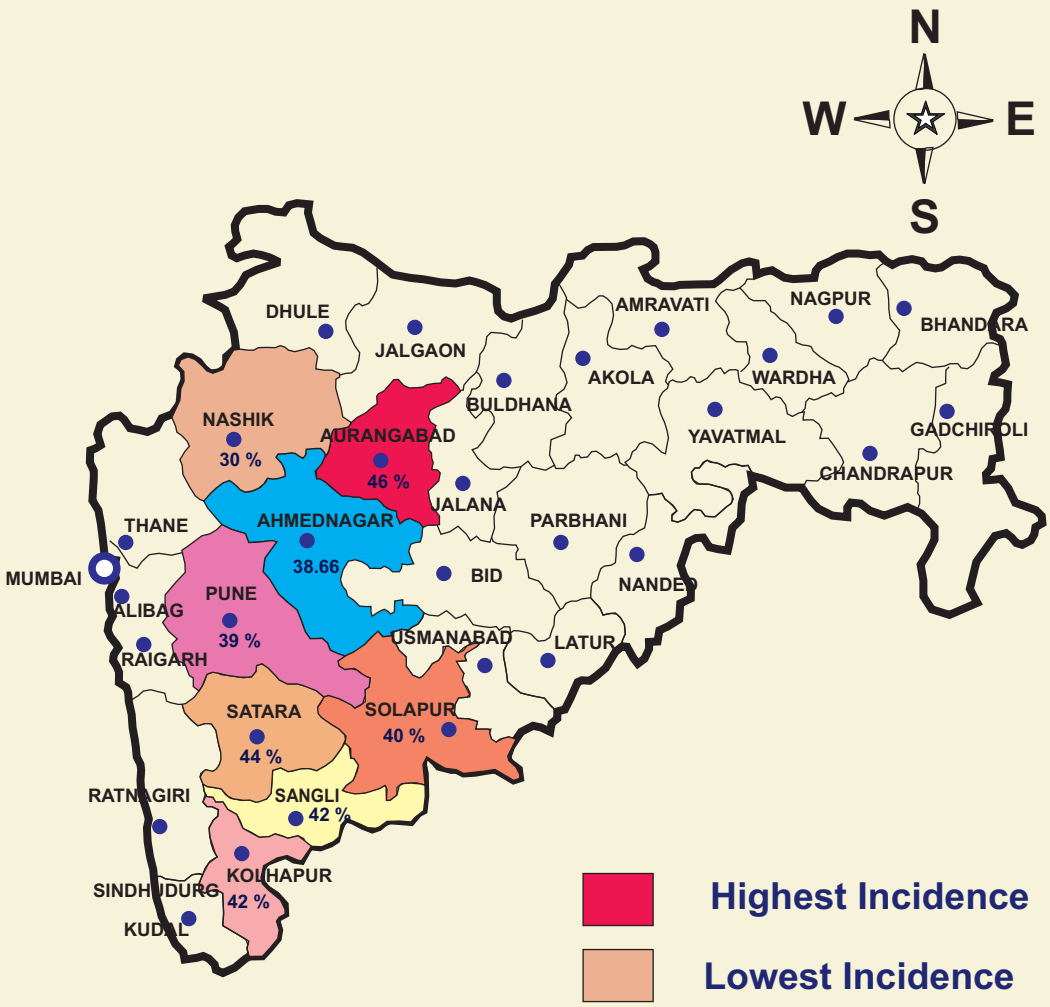


Plate 1. **Map represents the major Ginger growing districts of Western Maharashtra and Marathwada regions selected for survey and mean per cent disease incidence of rhizome rot of ginger and locations of samples collected for variability study.**

Ahmednagar 38.66 %

Pune 39%

Satara 44 %

Kolhapur 42 %

Sangli 42 %

Solapur 40 %

Nashik 30 %

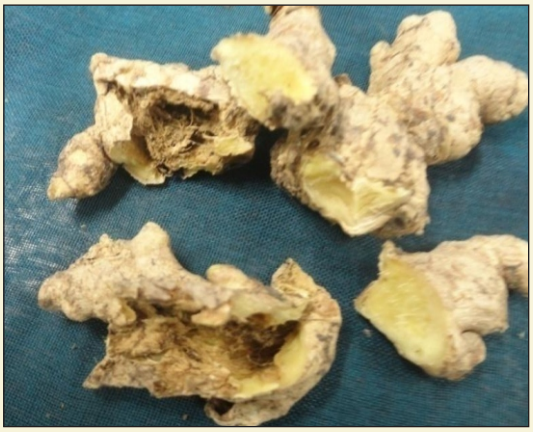
Aurangabad 46 %

Plate 2. Symptoms and Disease incidence of rhizome rot of ginger observed during different field in districts of farmers survey of Western Maharashtra and Marathwada region.

Initial symptoms

Rhizome rot of ginger

Advanced stage

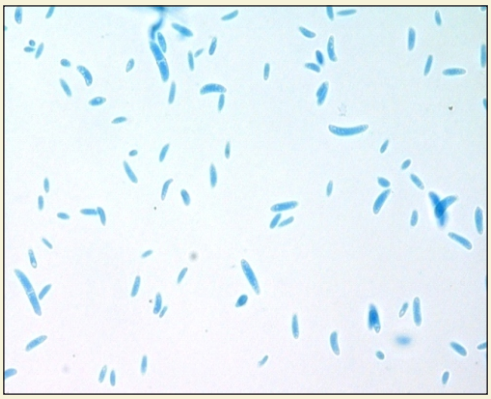


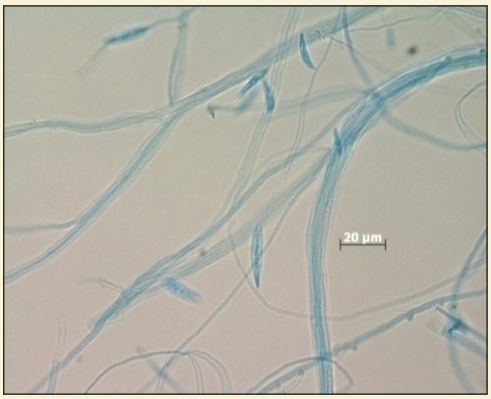
Diseased Rhizome

Healthy Rhizome

Plate 3. Symptoms of Rhizome rot of Ginger.

a. Pure culture of *Fusarium oxysporum f. sp. zingiberi*



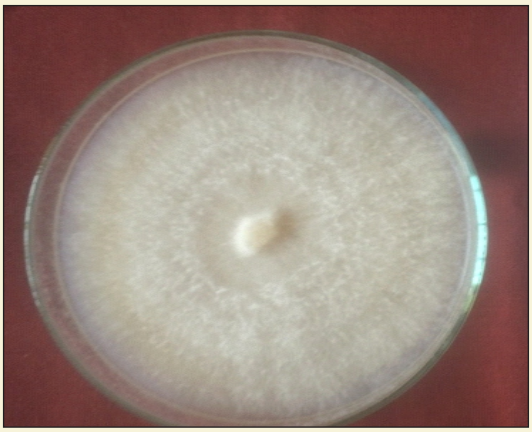


b. Microphotograph of *Fusarium oxysporum f. sp. zingiberi* mycelium and conidia

c. Mass multification, Purification and maintenance of isolated cuture

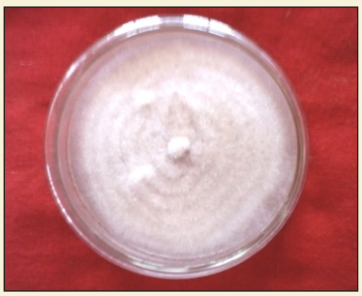
Plate 4. a., b., c.

Isolate of Sillod (Aurangabad)

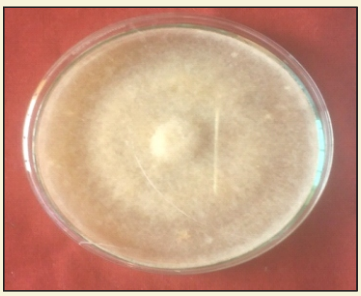


Isolate of Borgaon (Satara)

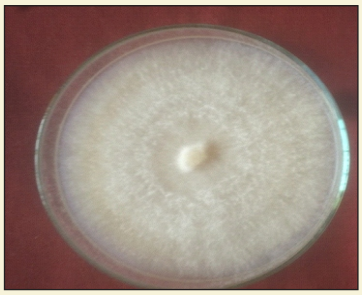
Plate 5. **Pathogenicity test of *F.oxysporum f. sp. zingiberi* on Ginger cv. Mahim.**



FOZ -1 Ahmednagar (Vambori)



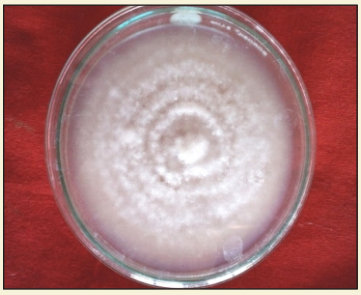
FOZ- 2 Pune (Hingangaon)



FOZ- 3 Satara (Borgaon)

FOZ- 4 Kolhapur (Bhadole)

FOZ- 5 Sangli (Kasbe Digraj)

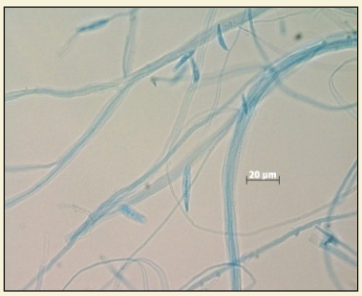


FOZ-6 Solapur (Kandar)

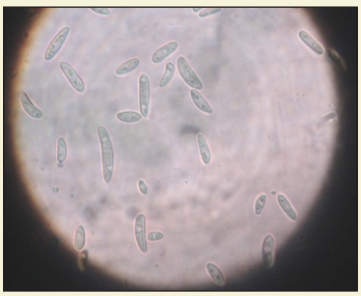
FOZ- 7 Nashik (Shirasgaon)

FOZ- 8 Aurangabad (Andheri)

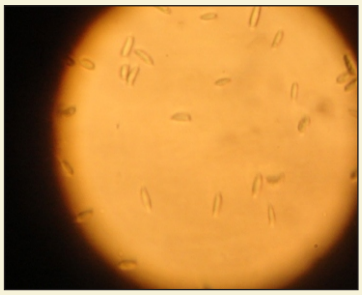
Plate 6. **Cultural variability of *Fusarium oxysporum f. sp. zingiberi* isolates from Maharashtra.**



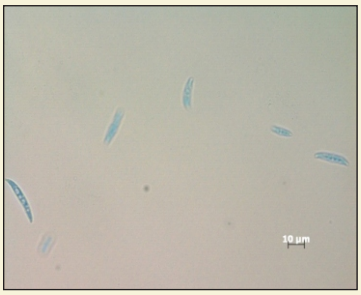
FOZ -1 Ahmednagar
(Vambori)



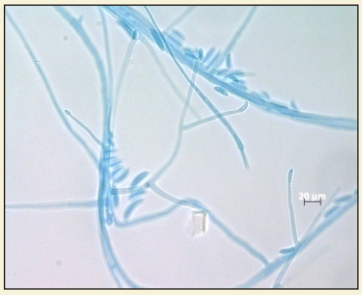
FOZ- 2 Pune
(Hingangaon)



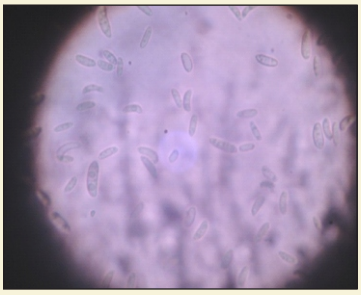
FOZ- 3 Satara
(Borgaon)



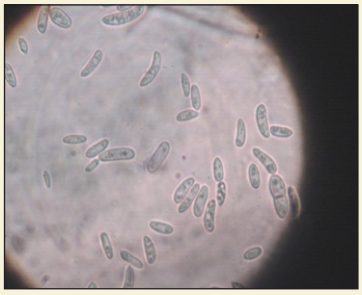
FOZ- 4 Kolhapur
(Bhadole)



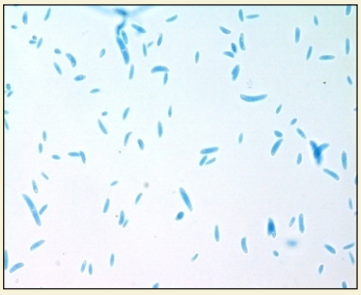
FOZ- 5 Sangli
(Kasbe Digraj)



FOZ-6 Solapur
(Kandar)

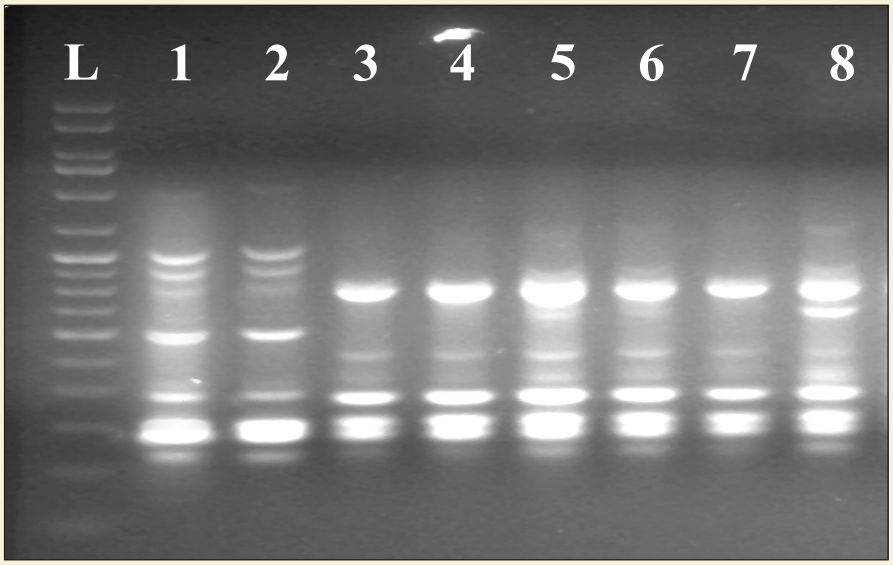


FOZ- 7 Nashik
(Shirasgaon)

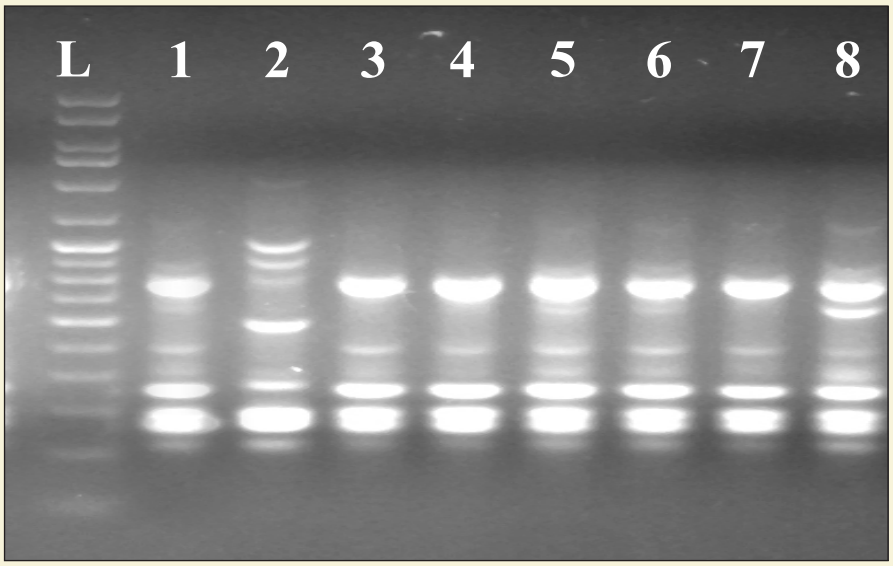


FOZ- 8 Aurangabad
(Andheri

Plate 7. **Morphological variability of *Fusarium oxysporum f. sp. zingiberi* isolates from Maharashtra.**



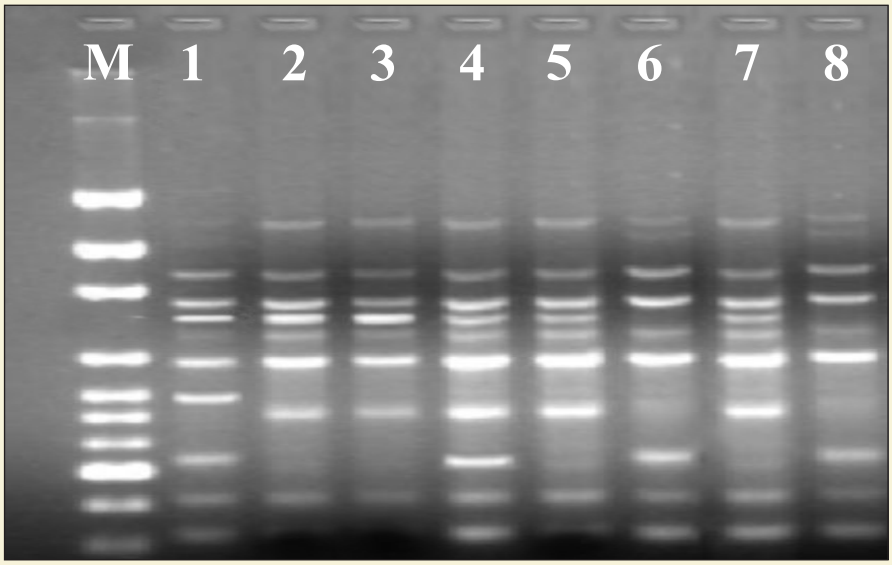
Rfu1



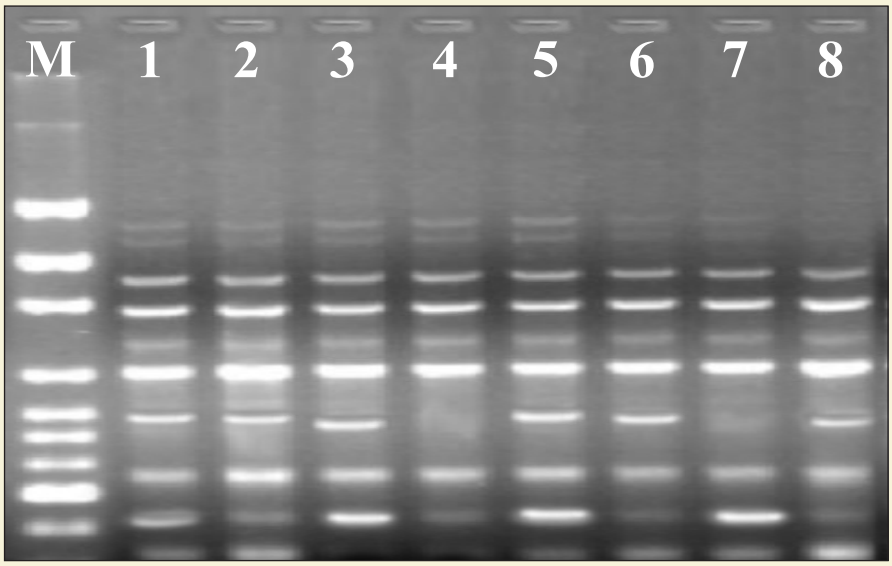
Rfu5

1. Foz 1
2. Foz 2
3. Foz 3
4. Foz 4
5. Foz 5
6. Foz 6
7. Foz 7
8. Foz 8

Plate 8. **RAPD profiles of 8 isolates of *F. oxysporum f.sp. zingiberi* obtained with primers RFu 1 (5'- CAGGCCCTTC3) and RFu 5 (5'- AGGGGTCTTG3).**



Rfu8



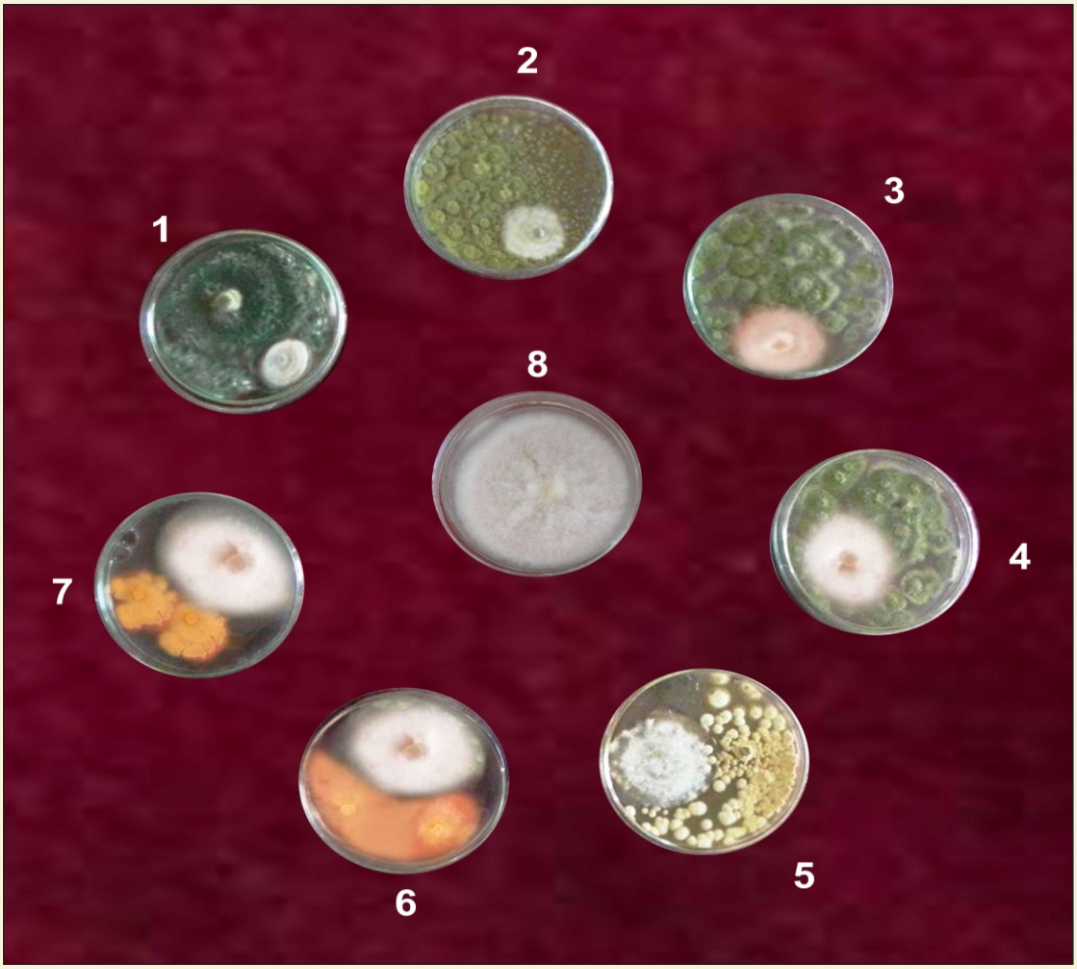
Rfu10

1. Foz 1
2. Foz 2
3. Foz 3
4. Foz 4
5. Foz 5
6. Foz 6
7. Foz 7
8. Foz 8

Plate 9. **RAPD profiles of 8 isolates of *F. oxysporum f.sp. zingiberi* obtained with primers RFu 8 (5'- GTGACGTAGG3) and RFu 10 (5'- GTGATCGCAG3).**

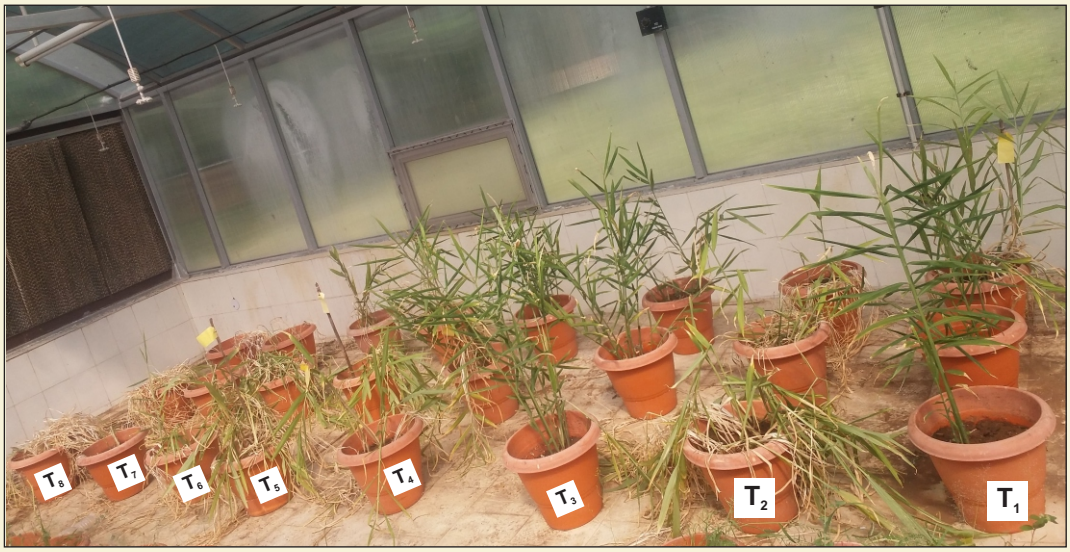
T1- Hexaconazole,
T2- Copper oxychloride,
T3- Carbendazim,
T4- Metalaxyl
T5- Carbendazim + Mancozeb
T6- Metalaxyl + COC
T7- Mancozeb
T8- Hexaconazole + Captan
T9- Propaconazole
T10- Bordeaux mixture
T11- Benomyl
T12- Hexaconazole+ Mancozeb
T13- Captan
T14-Control (Untreated)

Plate 10. ***In vitro*** **evaluations of fungicides at different concentration on radial mycelial growth and inhibition of** ***Fusarium oxysporum f. sp. zingiberi*****.**



T1- *Trichoderma viride*
T2- *Trichoderma harzianum*
T3- *Trichoderma koningii*
T4- *Trichoderma hamatum*
T5- *Trichoderma virens*
T6- *Pseudomonas fluorescens*
T7- *Bacillus subtilis*
T8- Control (Untreated)

Plate 11. ***In vitro*** **evaluation of different bioagents on linear mycelial growth and inhibition of** ***Fusarium oxysporum f. sp. zingiberi.***



Treatment No.	Rhizome dip treatment (30 minutes)	Soil drencing after 1 month of sowing
1.	Carbendazim@ (0.1%)	Carbendazim@ (0.1%)
2.	Benomyl (0.1%)	Benomyl (0.1%)
3.	Carbendazim @ (0.1%) + Mancozeb @ (0.2%)	Carbendazim @ (0.1%) + Mancozeb @ (0.2%)
4.	*Trichoderma viride* @ 3 g/lit + *Pseudomonas fluorescens* @ 3 g/lit	*Trichoderma viride* @ 3 g/lit + *Pseudomonas fluorescens* @ 3 g/lit
5.	*Trichoderma viride* @ 5 g/lit	*Trichoderma viride* @ 5 g/lit
6.	Hexaconazole @ (0.1%)+ Captan @ (0.2%)	Hexaconazole @ (0.1%)+ Captan @ (0.2%)
7.	*Pseudomonas fluorescens* @ 5 g/lit	*Pseudomonas fluorescens* @ 5 g/lit
8.	Control	Control

Plate 12. Integrated disease management module against pathogen associated with the rhizome rot of ginger *in vitro* pot culture.

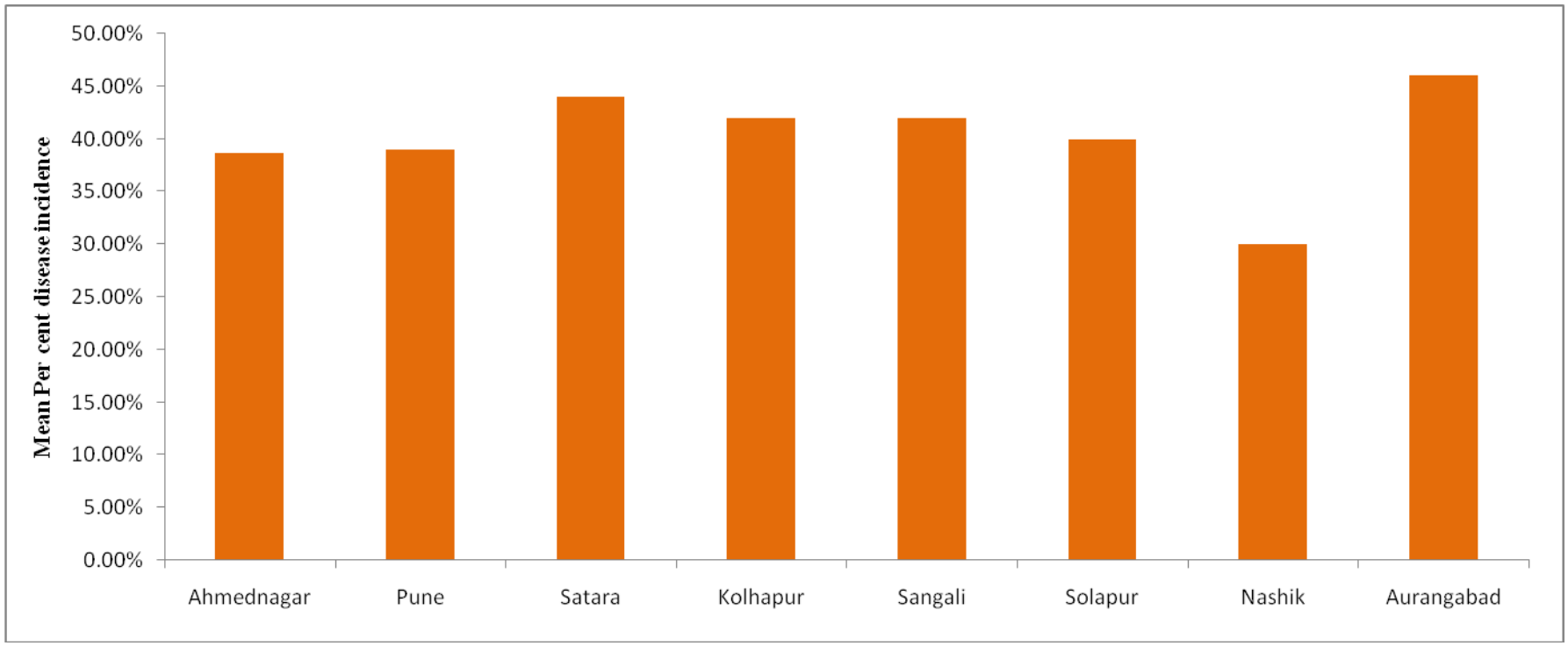


Fig 4.1. Mean per cent disease incidence in Rhizome rot of ginger in Ahmednagar, Pune, Satara, Kolhapur, Sangali, Solapur, Nashik and Aurangabaad districts.

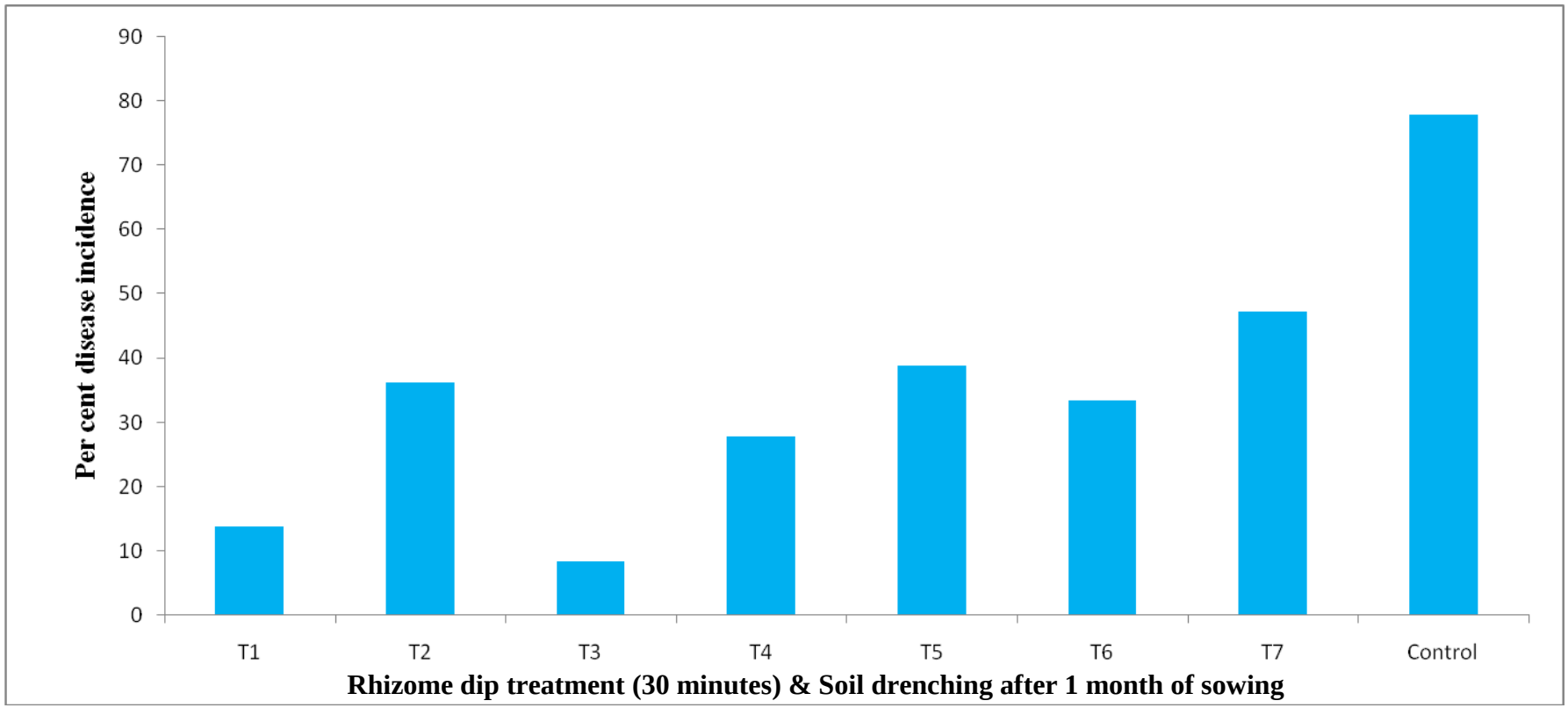


Fig 4.5. Effective integrated disease management module against pathogen associated with the rhizome rot of ginger

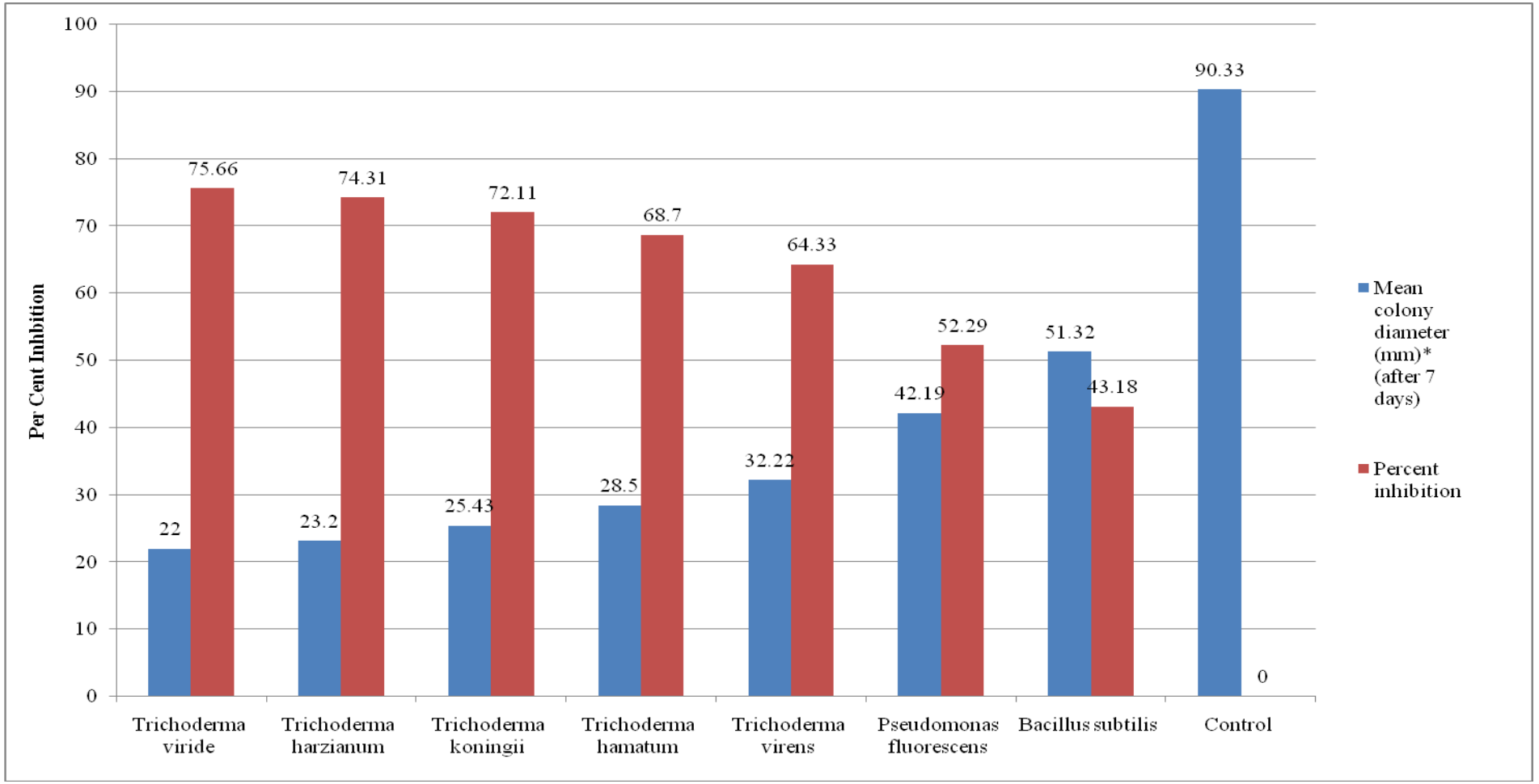


Fig 4.4. *In vitro* evaluation of different bioagents on linear mycelial growth and inhibition of *Fusarium oxysporium f.sp. Zingiberi*

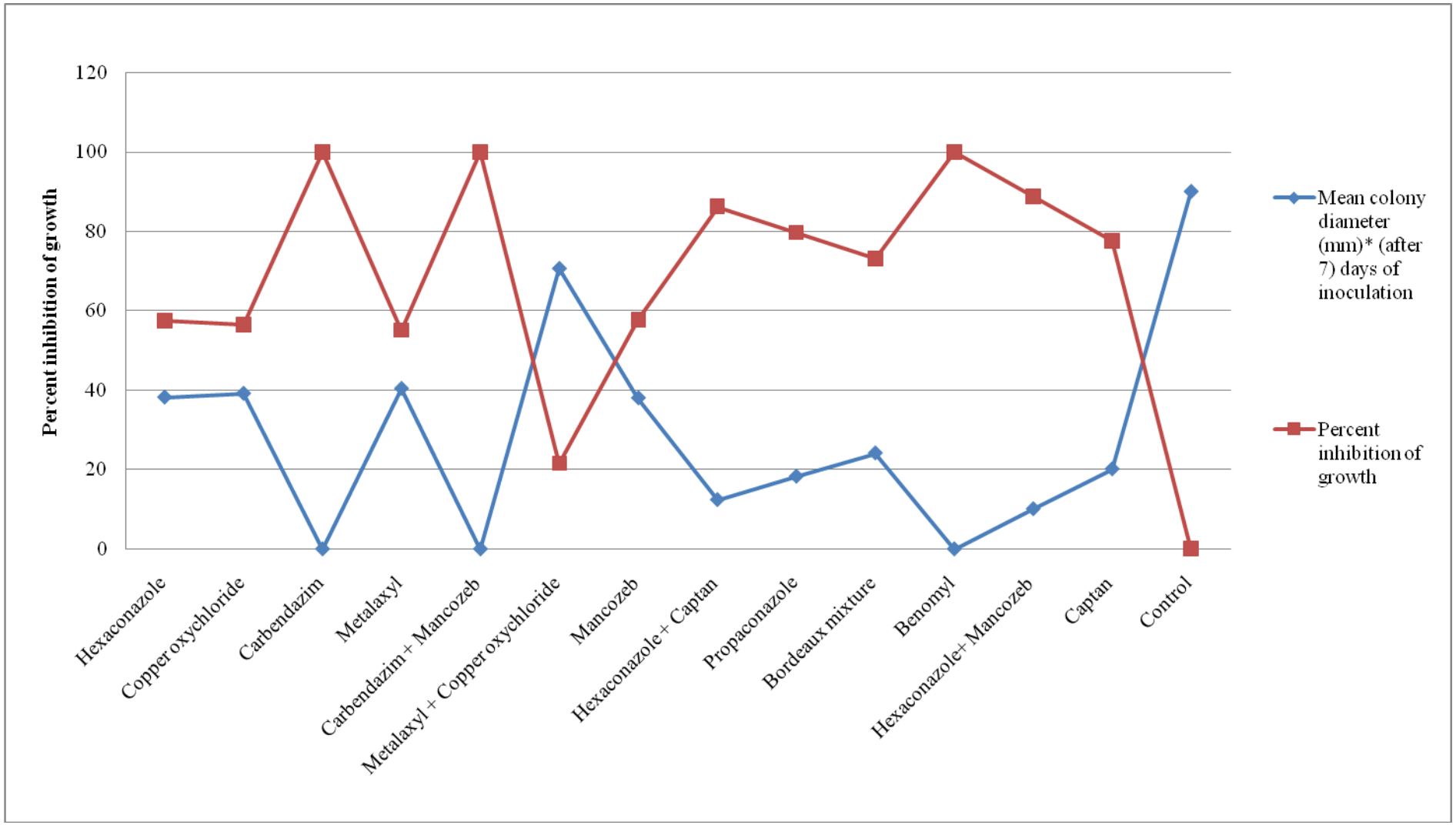


Fig 4.3. ***In vitro*** **evaluations of fungicides at different concentration on radial mycelial growth and inhibition of *Fusarium oxysporium f. sp. Zingiberi***

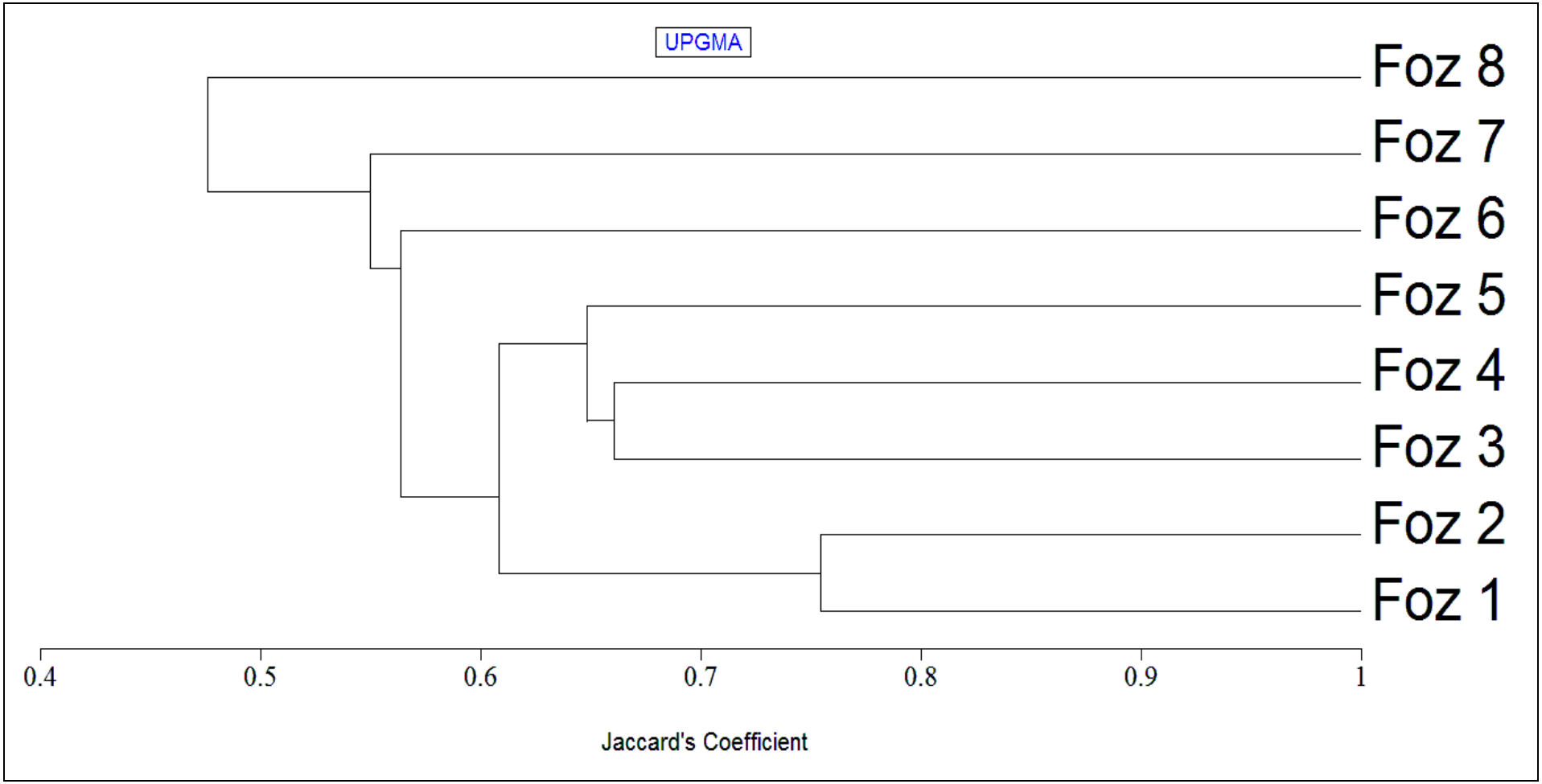


Fig.4.2. Cluster analysis of eight isolates of *F. oxysporum f.sp. zingiberi* by RAPD primers.