STUDIES ON VARIABILITY OF ISOLATES OF *Fusarium oxysporum* f. sp. *ricini* AND INTEGRATED DISEASE MANAGEMENT OF CASTOR WILT

MANASA U.R
B.Sc. (Ag.)

MASTER OF SCIENCE IN AGRICULTURE

(Plant Pathology)

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BY

MANASA U R

B.Sc. (Ag.)

THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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(Plant Pathology)

CHAIRPERSON: Dr. G. UMA DEVI

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
RAJENDRANAGAR HYDERABAD -500030

PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY

2018

DECLARATION

I, **Ms. MANASA U R,** hereby declare that the thesis entitled "**STUDIES ON VARIABILITY OF ISOLATES OF *Fusarium oxysporum* f. sp. *ricini* AND INTEGRATED DISEASE MANAGEMENT OF CASTOR WILT"** submitted to **Professor Jayashankar Telangana State Agricultural University,** Hyderabad for the degree of '**Master of Science in Agriculture'** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

(MANASA U R)
I.D. No. RAM/2016-63

Place:
Date:

CERTIFICATE

Ms. MANASA U R has satisfactorily prosecuted the course of research and the thesis entitled "**STUDIES ON VARIABILITY OF ISOLATES OF *Fusarium oxysporum* f. sp. *ricini* AND INTEGRATED DISEASE MANAGEMENT OF CASTOR WILT"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

(G. UMA DEVI)
Chairperson

Place:
Date:

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON VARIABILITY OF ISOLATES OF *Fusarium oxysporum* f. sp. *ricini* AND INTEGRATED DISEASE MANAGEMENT OF CASTOR WILT"** submitted in partial fulfilment of the requirements for the degree of '**Master of Science in Agriculture'** of the **Professor Jayashankar Telangana State Agricultural University, Hyderabad** is a record of the bonafide original research work carried out by **Ms. MANASA U R** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

(G. UMA DEVI)
CHAIRPERSON

Thesis approved by the Student Advisory Committee

Chairperson : **Dr. G. UMA DEVI**
Professor and Univ. Head
Department of Plant Pathology
College of Agriculture, Rajendranagar ------------------------------
Hyderabad-500030

Member : **Dr. M. SANTHA LAKSHMI PRASAD**
Principal Scientist
Plant Pathology ------------------------------
ICAR-IIOR, Rajendranagar
Hyderabad- 500030

Member : **Dr. T. UMA MAHESWARI**
Professor
Department of Entomology
College of Agriculture, Rajendranagar --------------------------------
Hyderabad-500030

Date of final viva-voce: ______________________

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(*Manasa U R*)

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
^{0}C	:	Degree Celsius
et al	:	And others people
etc.	:	And so on
No.	:	Number
viz.,	:	Namely
a.i.,	:	Active ingredient
cm	:	Centimetre
Fig	:	Figure
g	:	Gram
g/l	:	Gram per litre
ml/ha	:	Milli litre per hecctare
hrs	:	Hours
Kg	:	Kilogram
g/kg	:	Gram per kilograms
Kg ha^{-1}	:	Kilograms per hectare
m	:	Metre
Sq. mt	:	Square metre
mm	:	Milli metre
ml	:	Milli litre
Max.	:	Maximum
Min.	:	Minimum
RH	:	Relative humidity
Temp	:	Temperature
DAS	:	Days after sowing

sp.	:	Species
EC	:	Emulsifiable concentrate
SC	:	Soluble concentrate
WG	:	Water dispersible granules
SC	:	Suspension concentrate
SG	:	Water soluble granule
cfu ml^{-1}	:	Colony forming units per millilitre
DOR	:	Directorate of oil seed research
IIOR	:	Indian Institute of Oilseed research
e.g.	:	for example
PDA	:	Potato Dextrose Agar
psi	:	pounds per square inch
SEm	:	Standard Error of mean

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ABSTRACT

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Castor (*Ricinus communis* L.) is an important non-edible oilseed crop and plays a vital role in Indian vegetable oil economy. The crop is extensively grown in Mahaboobnager, Wanaparty and Nagarkurnool districts of Telangana and chitaduraga district of Karnataka.

Thirteen isolates collected from different castor growing areas of Telangana and Karnataka were studied for their cultural, morphological, pathogenic and molecular variability. The study revealed that all the isolates varied in their cultural characteristics in colony diameter which varied from 9 cm to 8.5 cm, fluffy to sparse fibrous colony growth, from rough to smooth texture, round to irregular wavy colony shape, entire growth to concentric margin and white to dark purple colour of colony. Morphological variation was observed in macro-conidia size and septation. Isolates varied in their pathogenic reaction. Highly virulent isolates *viz., For* 6, *For* 7, *For* 8, *For* 9 *and For* 10 were pathogenic to all the cultivars whereas less virulent isolates *viz., For* 1, *For* 2, *For* 5 and *For* 12 were pathogenic only to susceptible cultivar. Molecular variation was also observed among the isolates. These variations were not related to place of collection of isolates.

Evaluation of botanicals, soil amendments and fungicides against *F. oxysporum* f. sp. *ricini* in *in vitro* conditions revealed that among botanicals neem (51.7, 56.6 and 62.2 % colony inhibition), garlic (49.8, 54.7, 61.4 % colony inhibition) and tulsi (37.5, 44.9, 55.1 % colony inhibition) were superior over other botanicals, in inhibiting growth of test fungus at 5, 10, and 15 per cent concentrations respectively. Vermicompost was best among other soil amendments inhibiting mycelial growth by 28.0, 35.5 and 44.9 per cent at 5, 10 and 15 per cent respectively. Among the fungicides tested carbendazim 12 %+ Mancozeb 63 %, carbendazim and difenconazole inhibited 100 % colony growth at all the concentrations tested.

Botanicals, soil amendments and fungicides were tested for their efficancy in reducing disease incidence of castor wilt under glass house conditions and among botanicals garlic, neem and tulsi were superior over other botanicals in reducing disease incidence by 50.0, 49.3 and 47.3 per cent respectively. Among soil amendments vermicompost was best treatment by reducing disease incidence by 47.5 percent. Among fungicides tested carboxin 37.5% + thiram 37.5 %, carbendazim 12 %+ Mancozeb 63 % and carbendazim were most effective in reducing the disease incidence by 76.5, 71.1, 63.7 per cent respectively.

Integrated management of castor wilt in field condition revealed that soil solarisation alone and in combination of seed treatment with carbendazim, seed treatment and soil application of *Trichoderma harzianum*, soil application of carbofuran were found most effective compared to other main treatments and combinations employed.

Enumeration of microflora population in castor wilt sick plot revealed that soil solarisation effectievely reduced pathogen population by 73.91 per cent by end of crop season followed by neem cake application (47.82%). Neem cake treatment significantly increased *Trichoderma* and actinomycetes population in soil.

Effect of main treatments on soil physico-chemical properties revealed that neem cake significantly increased soil available nitrogen and phosphorous.

Chapter - I

INTRODUCTION

Chapter – I

INTRODUCTION

Castor (*Ricinus communis* L.), is an important non-edible oilseed crop grown in tropical and sub-tropical climate of India, China and Brazil. India occupies about 68% of the world castor area with production of about 76 % and ranks first both in area and production of the world. In India, major castor growing states are Gujarat, Telangana, Andhra Pradesh, Rajasthan, Karnataka, Tamil Nadu and Orissa with an area of 1089.3 lakh hectares and seed production of 1421 million tonnes during 2016-17 (INDIASTAT 2016-17).

In Telangana state castor crop has occupied 0.47 lakh hectares in the year 2016-17 which was 8.51% less than the previous year. Accordingly, castor seed production in the state is estimated as 0.38 lakh tones during the year 2016-17 which is 18.46% less than the previous year. Gadwal, Wanaparty, Mahabubnagar, Nagarkurnool, Karimnagar and Nizamabad are the major castor growing districts in the state. (*Kharif* 2017-18 pre-sowing price forecast of castor).

With the introduction and release of input responsive high yielding hybrids, castor is now established as a commercial crop. However, the productivity is very low, as castor is vulnerable to many pest and diseases, which include mostly fungi, bacteria and nematodes but only few pathogens cause economic losses to crop at different growth stages depending upon the seasonal conditions (Raoof and Rao, 1996), which are responsible for over 25% losses and sometimes, total crop loss. Castor is attacked by several diseases like wilt, root rot, gray mold, seedling blight, bacterial blight, *Alternaria* blight etc. Wilt is an important seed and soil borne disease of castor. It appears at all crop stages but more conspicuous during flowering and spike formation stage.

Castor wilt caused by *Fusarium oxysporum* f. sp. *ricini* disease was first time recorded in Morocco (Reiuf, 1953) and in India, it was recorded from Udaipur, Rajasthan by Nanda and Prasad (1974) and later from Gujarat during 1980-81. Losses in yield were observed in all cultivated castor hybrids in Gujarat and as high as 85 per cent wilt incidence was reported under North Gujarat conditions (Dange, 2003).

Chemical control of wilt is not feasible and economical, because of the soil as well as seed-borne nature of the pathogen. The most effective and practical method for management of *Fusarium* wilt is the use of resistant cultivars (Agrios 1988; Kraft *et al.*, 1994). However, the efficiency of resistant cultivars in disease management can be seriously limited by variability occurring in pathogen populations, (Jimenez *et al.,* 2004). Knowledge on the nature of variation and distribution of pathogenic races is required for the efficient disease management through the use of resistant cultivars (Jimenez *et al.,* 2001). Several resistant hybrids have been developed against wilt. Yet all cultivars are not performing well in all the areas.

With the growing awareness of harmful effects of pesticides, use of disease tolerant cultivar, crop rotation or sanitation practices, bioagents and botanicals are gaining importance in recent years. Keeping in view the importance of disease the current investigation was planned and carried out by encompassing the following objectives.

OBJECTIVES

1) To study wilt reaction with different isolates of *Fusarium oxysporum* f. sp. *ricini* on castor cultivars
2) To study the diversity among the isolates of *F. oxysporum* f. sp. *ricini* with ISSR primers
3) To evaluate botanicals and new fungicides against *F. oxysporum* f. sp. *ricini* (*in vitro* and *in vivo*)
4) To study the integrated disease management of castor wilt

Chapter-II

REVIEW OF LITERATURE

Chapter – II

REVIEW OF LITERATURE

2.1 Castor

Castor (*Ricinus communis* L.) is one of the important non-edible oil seeds having immense industrial and medicinal value and India is the major producer of castor. During recent years, castor has emerged as a commercial crop with export potential earning valuable foreign exchange. Gujarat, Rajasthan, Andhra Pradesh and Telangana are the major castor growing states in India.

Castor crop has occupied 8.39 lakh hectares in the year 2016-17 which was 24 per cent less than the previous year. Castor seed production in Telangana state is estimated as 0.38 lakh tones during 2016-17 which is 18.46 per cent less than the previous year. Reduction in production of castor in Telangana state may be due to various biotic and abiotic factors of which, wilt of castor caused by *Fusarium oxysporum* f. sp. *ricini* is one of the most important disease in castor growing areas of Telangana.

2.2 *Fusarium* wilt the major production constraint

In India Nanda and Prasad (1974) reported castor wilt disease from Udaipur and Sirohi districts of Rajasthan and Palampur district of Gujarat. They established the causal organism as *F. oxysporum f. sp. ricini* .

Pushpavati *et al.* (1998) reported that, yield loss depends on the stage at which wilt affects the crop, 77% at flowering stage, 63% at 90 days and 39% at later stages on secondary branches. Monocropping resulted in the endemic development of wilt and this became the limiting factor for castor cultivation in India.

The disease is reported to be serious in Andhra Pradesh, Gujarat and Rajasthan states in India (AICORPO, 2001). Currently the disease is prevalent in Russia, India, Brazil, Taiwan and Nepal (Dange, 2003).

2.3 Causal organism

Nanda and Prasad (1974) proved pathogenicity of *F. oxysporum* f. sp. *ricini* by isolating the fungus from the affected castor plant roots and stem and inoculating 4-6 week old castor plant by mixing inoculum in soil around root zone and observed initial wilt symptoms from inoculated plants 15 days after inoculaion.

The fungus produces white fluffy mycelial growth on potato dextrose agar which turns pinkish by keeping in day light. Microconidia are formed which are hyaline, round to ovoid, single celled or one septate. One celled type measure 3.66×6.4 µm and the two celled conidia measure 15.29×3.76 µm in size. Macroconidia are less in number, 3 septate, straight, spindle or sickle shaped and measure 29.68×3.93 µm. Both terminal and intercalary chlamydospores are present and measure 8.7×4.44 µm. Generally, sporodochia develop in two week old cultures.

Sviridov (1988) studied morphology of *F. oxysporum* f. sp. *ricini* isolates characterised by white mycelial growth. Micro-conidia are cylindrical or oval with or without septa. Macro-conidia are with 3-5 septa. Chlamydospores are produced singly terminal or intercalary or in chains.

Dange *et al.* (2006) reported that the fungus produces abundant white mycelial growth on potato dextrose agar and turns to pink on incubation under fluorescent light and forms micro conidia and macro conidia. Micro conidia are hyaline, round to oval, aseptate or single septate and macroconidia are hyaline, 2–6 septate, straight, spindle or sickle shaped.

2.4 Wilt symptomatology

Nanda and Prasad (1974) reported yellowing, sickly appearance of wilt infected plant and marginal necrosis, which later covers the leaves completely. They also reported shrivelled leaves, lower ones drop away leaving few top leaves followed by irreversible wilting of the plant leading to sudden death. Transverse and longitudinal sections of the affected roots revealed the presence of fungus in vascular tissues and in xylem parenchyma. Formation of tylose was also observed in xylem vessels of infected roots.

Andreeva (1979) described that wilt disease appears at all growth stages of castor crop but becomes more prominent and severe at the time of flowering and spike formation. The favourable temperature for infection was 13-15 ^{0}C but for symptom expression it was 22-25 ^{0}C. The wilt infected stem tissues showed intercellular mycelium in vessels. Hypertrophy of xylem parenchymatous cells was reported by Piplani *et al*. (1981a). Futher the pathogen also produced pectinolytic and cellulolytic enzymes for tissue disintegration (Piplani *et al*., 1981b).

Moshkin (1986) described the symptoms produced by *F. oxysporum* f. sp. *ricini* in seedling stage and adult plants of castor. In diseased seedlings the colour of the hypocotyls gets a dull tinge and the apex leaves loose their turgor. Within 2-3 days leaves wilt and dry out without any change in their green colour. A dark stripe is formed throughout the height of the stem upto the infected leaf. In adult plants individual branches or the entire plant wilts. Diseased parts acquire a dark violet tinge and during humid weather luxurious mycelium of the fungus develops near the collar of the root.

Sviridov (1988) described the external symptoms of castor wilt as drying up of seedlings, stoppage of growth, wilting and appearance of dark and blue - violet patches on stems of adult plants. The affected plants die and yield less with damaged seeds.

Chaudhari and Patel (1992) reported that wilting occurred under field conditions at the initial stage of infection and within a week the leaves along with petioles drooped down and infected plants dried completely within a fortnight.

Hillocks (1992) reported that symptoms of *Fusarium* wilt can appear at any stage of crop development depending on inoculum density, temperature and host susceptibility and it may be killed at the seedling stage when infection initiates from the seed to plants due to high inoculum density.

F. oxysporum f. sp. *ricini* caused sudden or gradual wilt of castor plants. It has a short seedling stage infection and protracted adult stage infection at all stages of growth. The infected plants are with reduced height resulting stunted plant growth (AICORPO Research Highlights, 1994).

In mildly affected or older plants, lower leaves show symptoms and survives, with reduced vigour. The most diagnostic symptoms of *Fusarium* wilt is the loss of turgidity resulting in wilt and a brown discoloration of the vascular tissue. This discoloration is localized in the cortical tissue of the vascular system of the upper tap root and lower stem when cut at a diagonal or in cross-section (Davis *et al.*, 2006).

Nagesh *et al.* (2015) reported that clusters of purple coloured sporodochia develop on stem under humid weather conditions. When stem is split open brownish discoloration and white cottony mycelia growth is seen in the pith region.

2.5 Disease cycle and epidemiology

The disease appears at all growth stages of the crop but becomes more prominent and severe at the time of flowering and spike formation. Favourable temperature for infection is 13-15 ^{0}C and for symptom expression is 22-25 ^{0}C (Andreeva, 1979).

The pathogen is primarily soil-borne and survives in the form of micro-conidia, macro-conidia and chlamydospores. Seed borne nature of the pathogen has also been reported. Seeds from wilted castor plants carried inoculum at the micropylar end in 2-19 per cent seeds and seed infection was confined to testa, tegmen and endosperm (Naik, 1994).

Chattopadhyay (2000) reported seed borne nature of *F. oxysporum* f. sp. *ricini* found that 10.8 per cent seeds were infected when collected from wilted plants of castor variety Aruna.

Seeds from infected area play an important role in dissemination of pathogen to new areas and also play role in perpetuation and spread of pathogen (Dange, 2003).

Chukunda *et al.* (2015) studied seed borne microflora of castor bean, the fungal species isolated and identified from the seeds were *Aspergillus flavus* (56%), *Aspergillus niger* (48%), *Aspergillus tereus* (40%), *Penicilium* sp. (35%), *Fusarium oxysporum* (30%), *Rhizopus stolonifer* (25%), *Curvularia lunata* (25%) and *Botrytis cinerea* (20%).

2.6 Effect of disease on growth parameters

Stevenson (1947) studied the seedling infection by *Fusarium* spp. and noticed that the cotyledonary injury of castor plants had a pronounced effect on the subsequent plant growth and yield. This was indicated by a reduction in height, seed yield and general vigour of the plant.

Infection due to *Fusarium* spp. in castor plays a significant role in reducing fresh shoot height and weight, fresh root weight and also dry shoot weight (AICORPO, 1984).

Vaidehi *et al.* (1985) reported that the culture filtrates of *F. oxysporum* reduced germination, root and shoot elongation to a greater extent in castor. Svirido (1989) reported that *F. oxysporum* f. sp. *ricini* affected castor bean seedlings, mostly young plants at the budding stage are severely affected. In comparison to healthy plants, yield of diseased plants was reduced by 1.9 to 3.6 times, weight of 1000 seeds by 8 to 14.6 per cent and oil content by 1.2 to 1.7 per cent. Plants of susceptible varieties died by the time they reached the flowering stage.

2.7 Inoculation methods

Prasad *et al.* (2008) studied three methods of inoculation in castor namely root dip, soil drenching with inoculum and soil infestation. In soil infestation method/sick pot method, the fungus was grown on autoclaved sorghum seeds for 10-15 days. The inoculum was mixed with soil and left for 7- 10 days. Then castor seeds were sown in the infested soil.

Rajan *et al.* (2016) evaluated four different methods of artificial inoculation namely seed soaking, soil drenching, root dip and sick pot for castor in glasshouse condition for their efficiency using a panel of eight genotypes with known disease reaction. The results showed that 'sick pot method' was the most ideal for accurate identification of resistance or susceptibility in plants in a short time with relative ease.

2.8 Cultural, morphological and Pathogenic variability among different castor cultivars for different isolates of pathogen.

Nanda and Prasad (1974) studied morphology and taxonomy of *F. oxysporum* f. sp. *ricini.* They observed large variability in growth characters, chlamydospore size and sporulation (conidial measurements) among different isolates of *F. oxysporum f. sp. ricini*. Sviridov (1988) studied the cultural and morphological characters along with conidial characters such as size, shape and septation of *F. oxysporum f. sp. ricini*.

Existence of physiological races with in isolates *F. oxysporum f. sp. ricini* castor was observed when variable disease reaction was recorded across locations in multi location tests of breeding materials by Directorate of Oil Seeds Research (D.O.R); under the All India Coordinated Research Project (AICORPO, 2001).

Twelve isolates collected from different parts of Gujarat and Andhra Pradesh were tested for their pathogenic variation on differential cultivars and were grouped into five pathogenic groups based on disease reaction (AICORPO, 2002).

Desai *et al.* (2003) studied the variability in 15 isolates of *F. oxysporum f. sp. ricini* with respect to cultural and morphological characters. They found the existence of variability in six isolates produced moderate to profuse fluffy white mycelium, while nine isolates produced thin, flat to slight fluffy pinkish mycelium. Variation was also observed in colony diameter; mycelia dry weight, sporulation ability and size of macro conidia among the isolates. Wilt incidence among these 15 isolates ranged from 49.97 to 100.00 per cent in cultivars VP-1 and VI-9, but cultivar 48-1 remained free from wilt.

Prasad *et al.* (2008) studied variability among 29 isolates of *F. oxysporum f. sp. ricini*. They found variation in mycelial growth, pigmentation, sporulation and size of conidia among these isolates. These isolates also showed variation in toxin production, and thereby in wilt incidence. They isolated two virulent isolates from resistant hybrid GCH-4 which has become wilt susceptible and reported that DCS-9 a resistant variety was found susceptible to three isolates. Based on the reaction of 29 isolates, high variability in virulence was observed and the isolates were grouped into five pathotypes.

Reddy *et al.* (2010) studied variability among 62 isolates of *F. oxysporum* f. sp. *ricini* from different castor growing areas of country on four castor cultivars and reported Haritha and DCS-9 resistant cultivars have shown wilt incidence of up to 100 per cent to highly virulent isolates, found high variability among isolates studied and grouped isolates into 10 pathogenic groups, also reported high cultural and morphological variation among isolates, Isolates varied in pigmentation, radial colony growth, sporulating ability, septation of macroconidia, size and number of septa, size of macroconidia.

2.9 Molecular diversity among the isolates of *F. oxysporum* f. sp. *ricini* with ISSR primers.

Houda *et al.* (2007) used Inter-simple Sequence Repeats (ISSRs) technique to assess genetic diversity and relationships within the Mediterranean *Hedysarea* species belonging to the two genus *Sulla* and *Hedysarum*. Eight ISSR primers generated a total of 96 polymorphic markers and revealed high polymorphism among the studied species. The genetic relationship results exhibited the distinction of *Hedysarum membranaceum* and *Hedysarum aculeolatum* from Sulla species. In addition, in agreement with morphological taxonomy, the two *Sulla spinosissima* and *Sulla capitata* species diverge molecularly.

Dubey *et al.* (2008) studied the variability among the isolates of *F. oxysporum* f. sp. *ciceri* using ISSR markers. They found that ISSR primers were suitable to distinguish the isolates. ISSR 11 and ISSR 12 showed good polymorphism and were found suitable for distinguishing the isolates of *F. oxysporum* f. sp. *cicero*

Arif *et al*. (2008) used DNA based molecular marker Inter Simple Sequence Repeats (ISSRs) for the study of DNA polymorphism in *Fusarium solani* associated with shisham wilt. Isolation and analysis of genetic diversity was conducted among 22 isolates of *F. solani* collected from wilted tissues using molecular marker technology. A total of 10, out of 25 ISSR primers were selected. Amplification of genomic DNA of 22 isolates, using ISSR primers, yielded 98 fragments that could be scored, of which 96 were polymorphic, with an average of 9.6 polymorphic fragments per primer.

Prasad *et al.* (2008) studied genetic variation of isolates of *F. oxysporum* f. sp. *ricini* by random amplified polymorphic DNA analysis. The isolates were grouped into five clusters based on molecular polymorphism generated by RAPD primers. The grouping of isolates based on pathogenic variation has no correlation with grouping based on RAPD analysis with most of the test isolates.

Sudisha *et al.* (2009) assessed the DNA polymorphism among 22 isolates of *Sclerospora graminicola*, the causal agent of downy mildew disease of pearl millet using 20 inter simple sequence repeats (ISSR) primers. The 19 functional ISSR primers generated 410 polymorphic bands and revealed 89% polymorphism and were able to distinguish all the 22 isolates. Polymorphic bands used to construct an unweighted pair group method of averages (UPGMA) dendrogram based on Jaccard’s co-efficient of similarity and principal coordinate analysis resulted in the formation of four major clusters of 22 isolates.

Gayatri *et al.* (2009) demonstrated the synergistic use of gene-specific markers, ITS-RFLP, ISSR and AFLP for distinguishing Indian *F. oxysporum* f. sp. *ciceris* races. The internal transcribed spacer region-restriction fragment length polymorphism (ITS-RFLP) approach along with intersimple sequence repeat (ISSR) method used to differentiate different races.

Baysal *et al.* (2010) used ISSR and RAPD markers to characterize *F. oxysporum* f. sp. *melongenae* isolates collected from eggplant fields in southern Turkey. Pathogens were identified by their morphology, and their identity was confirmed by PCR amplification using the specific primer PF02-3. The isolates were classified into groups on the basis of ISSR and RAPD fingerprints, which showed a level of genetic specificity.

Reddy *et al*. (2010) studied genetic diversity among *F. oxysporum* f. sp. *ricini* using ISSR, RAPD, SSR and ITS-RFLP markers reported high genetic variation among the isolates and classified the isolates based on fingerprints. They reported there was no relationship between their clustering in dendrograms and geographic origin or virulence of the isolates.

Amaral *et al*. (2013) studied 30 *F. oxysporum* f. sp. *lycopersici* isolates representative of the three races were analysed by RAPD, ISSR and RFLP of intergenic spacer using DNA bulks for each race. Ten RAPD primers and 20 ISSR primers were selected generating a large number of polymorphic bands.

Rakhonde *et al.* (2017) collected and analysed eighteen isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea representing nine states and eight Agro climatic region of India for their virulence and genetic diversity. Isolates were grouped based on their pathogenic variability from highly pathogenic to moderately pathogenic, the same set of isolates was used for molecular characterization with inter simple sequence repeats (ISSR) - polymerase chain reaction (PCR). Unweighted paired group method with arithmetic average grouped the isolates into five categories at a genetic similarity ranging from 50 to 94 per cent.

Altinok *et al*. (2017) conducted a study to determine genetic diversity and population structure of *Fusarium oxysporum* f. sp. *melongenae* isolates obtained from Turkey with 16 ISSR primers. The study revealed 31 loci belonging to 202 *Fusarium oxysporum* f. sp. *melongenae* isolates and 14 of them were found to be polymorphic.

2.10 Evaluation of botanicals, soil amendments and fungicides against *F. oxysporum* f. sp. *ricini*

2.10.1 Botanicals

Plant species viz., *Aloe barbadensis, Datura stramonium, Zingiber officinale, Murraya koenigii, Azadirachta indica* and *Brassica juncea* extracts and *Menttha piperita* oil showed considerable activity against *A. niger*, *F. oxysporum* f. sp. *pisi, Macrophomina phaseolina*, *Alternaria alternata* in pea through poisoned food and seed inoculation techniques (Pankaj *et al.*, 2003).

Irum (2007) studied the antifungal effect of aqueous extracts of four plants species *viz., A. indica*, *Datura metel* L., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. under *in vitro*. It was found that all the plant extracts at 40% concentration were effective in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceri*. Among these plants extracts, *A. indica* and *D. metel* inhibited fungal growth by 80%. Even at 10% concentration, both plants extract had inhibitory effect, while *O. sanctum* extracts showed low inhibition (60%) as compared to other plant extracts. Among the chemical treatments Benomyl (50WP) and Carbendazim (50WP) were most effective against *F. oxysporum* f. sp. *ciceri*. Results indicated that plant extracts had equal potential as fungicides for the reduction of pathogen growth.

Babu *et al*. (2008) studied *in vitro* efficacy of different plant extracts viz., *A. indica, Artemessis annua, Eucalyptus globulus, O. sanctum* and *Rheum emodi* at concentrations 5, 10, 15 and 20 per cent concentrations against *F. solani* f. sp. *melongenae* causing brinjal wilt. Among the different extracts tested 20% of *A. indica* was found most superior followed by *R. emodi, E. globulus, A. annua and O. sanctum* against test pathogen.

Riaz *et al.* (2008) tested antifungal activity of different concentrations of leaf extracts of wheat, maize, sunflower, chilies, onion, and marigold against *in vitro* growth of *F. oxysporum* f. sp. *gladioli* causing corms of gladiolus. Extract of marigold, sunflower and chillies were found highly effective where all the concentrations significantly reduced fungal biomass by 54-79%, 33-85% and 45-57%, respectively.

Karim *et al.* (2010) screened antimicrobial activity of *Manilkara zapota* (L.). Bioassays for antimicrobial activities were carried out using ethyl acetate extracts of both stem bark and leaves of *M. zapota* against some pathogenic bacteria and fungi. These extracts were found effective in inhibiting growth of *Aspergillus flavus*, and *Fusarium* sp. and other bacterial pathogens. Which may be due to terpenoids, flavonoids and glycosides.

Sharma *et al*. (2011) studied the effect of plant extracts *viz*., *A. indica*, *C. procera*, *Cyperus rotundus*, *D.alba*, *E. globulus*, *P. hysterophorus*, *O. sanctum*, *Tagetes erectus*, *Euphorbia hirta* and *Polyalthia longifolia* against *F. oxysporum* f. sp. *lycopersici*. All the botanicals inhibited the spore germination and radial growth of the pathogen.

Shalini *et al*. (2014) tested 26 plant aqueous extract against *F. oxysporum* f.sp. *ricini* at 5% and 10% using poisoned food technique and found that neem, tulsi and garlic extracts effectively inhibited mycelium growth over control.

2.10.2 Soil amendments

Yelmane *et al*. (2010) used the extracts of different organics of neem cake, mustard cake, FYM, groundnut cake, poultry manure, press mud, castor cake and coconut cake against *F. solani* by poisoned food technique *in vitro*. Least growth of the pathogen was recorded in the extracts of neem cake with excellent inhibitory effect (59.8 %) against *F. solani* followed by mustard cake (52.61 %), FYM (49.40 %), groundnut cake (44.80 %), poultry manure (42.29 %), and least by other cakes.

Mahalakshmi *et al.* (2013) tested the effect of oil cakes and organic manures on the growth of wilt pathogen under *in vitro* conditions. The extracts of different oil cakes and organic manures were tested against *F. oxysporum* f. sp. *dianthi* by poisoned food technique *in vitro*. The results indicated that vermicompost significantly reduced mycelial growth but other oil cakes extracts were not effective.

Chandel *et al.* (2015) evaluated nine different oil cakes viz., neem cake, cotton cake, mustard cake, castor cake, soybean cake, apricot seed cake, olive cake, sunflower cake and dry pine needle on *Fusarium oxysporum* f. sp. *dianthi in vivo*, Neem cake and pine needles out of nine organic amendments were found statistically superior in reducing the disease by giving 41.39 and 28.46 per cent disease control, whereas mustard cake and cotton seed cake gave 47.42 and 37.75 per cent disease control respectively.

Mukesh *et al.* (2017a) conducted trials with various organic amendments viz., Neem oil cake, Groundnut cake, Castor cake, Mustard cake and Poultry manure and four dates of sowing. All the organic amendment tested reduced wilt incidence of coriander significantly over check. Neem oil cake was found significantly superior over all other treatments and resulted maximum disease control (58.95%) followed by Groundnut cake (54.05%), Castor cake (50.86%) and Mustard cake (44.15%). Poultry manure was least effective in reducing wilt incidence (41.15%).

Dhivya *et al.* (2017) evaluated five oil cakes *viz*., neem (*Azadirachta indica*), coconut (*Cocos nucifera*), gingelly (*Sesamum indicum*), cotton (*Gossypium hirsutum*) and mahua (*Madhuca latifolia*) *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease of tomato. The result showed that neem cake extract (10%) recorded maximum (80.77) per cent reduction of mycelia growth over control followed by Mahua cake (10%) extract (76.33) per cent and minimum (62.11) per cent reduction was observed in coconut cake.

Patra (2017) evaluated various oil seed cakes *viz.,* Sesame cake, Mustard cake, Neem cake, Groundnut cake, Vermi-compost and Spent Mushroom Substrate (SMS) on wilt disease of chickpea. Out of different treatments neem cake showed minimum disease incidence (11.23%) followed by vermi-compost (12.06 %).

2.10.3 Fungicides

Kishore and Singh (2008) reported that systemic fungicides *viz.,* bavistin, benlate and thiophanate methyl were more effective in reducing wilt of linseed by about 82.4%, 69.0%, and 53.5% respectively against *F. oxysporum* f. sp. *lini.*

Raju *et al.* (2008) tested the efficacy of carbendazim, captan, zineb, thiophanate methyl and thiram against *F. oxysporum* f. sp. *udum* in *vitro*. Among all these fungicides, carbendazim completely inhibited the growth of the pathogen at all concentrations (100, 250 and 500 ppm) tested.

Nasir *et al.* (2011) tested the Fungitoxic effect of six fungicides on *Fusarium oxysporium* f.sp. *ciceris viz.,* Benomyl, Derosal, Ridomil, Cabrio Top, Vitavax and Prevent at four concentrations, 5, 10, 20 and 50 ppm through poisoned food technique. Cabrio Top was the least effective. Derosal and Benomyl exhibited 100% reduction in disease incidence while Vitavax and Cabrio Top exhibited 96.33 and 88.37% in disease incidence, respectively.

Sintayehu *et al.* (2011) evaluated five fungicides Prochloraz 50 WP, Tebuconazole 25 EC, Penncozeb 80 WP, Seed plus 30 WS and Matalaxyl-m+ mancozeb 68 WG as seed bulb dressing and bulb dip treatments against basal rot in the field and storage. Bulb dressing with these fungicides resulted in a significant reduction in severity of basal rot affected cull bulbs on shallot.

Taskeen-Un-Nisa *et al.* (2011) tested different systemic fungicides *in vitro* against *F. oxysporum* at different concentrations significantly inhibited the mycelial growth of *F. oxysporum.* Hexaconozole at highest concentration (1000ppm) caused highest reduction of mycelial growth (8.80 mm) followed by carbendazim (9.40 mm), bitertanol (18.60 mm) and myclobutanil (20 mm) at the same concentration.

Patil *et al.* (2012) tested systemic fungicides and found that among all fungicides, Tebuconazole at 0.025, 0.05 and 0.1 per cent inhibited the growth of *F. oxysporum* f. sp. *cepae* completely. Next best systemic fungicide in inhibiting the mycelial growth is Carbendazim at 0.1 per cent which inhibited complete growth of the fungus, followed by Thiophanate Methyl (87.68%), Propiconazole (82.87%) and Hexaconazole (82.51%).

Anitha *et al*. (2014) evaluated four fungicides *viz.*, Mancozeb, Saaf, Carbendazim and Cuprozin in three different concentrations and *Trichoderma viride* against mycelial growth of the casual pathogen *Fusarium oxysporum* f. sp. *cubense* in *in vitro*. Among the chemical fungicides Carbendazim was most effective at all concentrations against the pathogen followed by Saaf.

Bhaliya *et al.* (2014) evaluated different contact, systemic and combination fungicides against *Fusarium solani in vitro*. Out of six systemic fungicides, carbendazim was found best with 98.68% mycelial growth inhibition followed by propiconazole (85.27%) and difenoconazole (75.53%). Carbendazim showed complete inhibition of mycelial growth of the test fungus at 500 ppm concentration followed by same fungicide with 250 ppm (99.46%) and 100 ppm (98.12%). Among the fungicides combination of carbendazim + mancozeb and Tricyclazole + mancozeb gave 100% growth inhibition at all concentration followed by carboxin + thiram with 98.79%.

Patil *et al.* (2016) tested six systemic fungicides evaluated, maximum inhibition of mycelial growth of *F. oxysporum* was observed with propiconazole (93.52%) followed by carbendazim (91.54%). Among the six combi-product fungicides evaluated, hundred per cent inhibition of mycelial growth of *F. oxysporum* was observed with carbendazim 12 % + mancozeb 63 % (Saaf), carbendazim 25 % + mancozeb 50 % WS (Sprint), carboxin 37.5 + thiram 37.5 (Vitavax power) at all tested concentrations (0.1, 0.15, 0.20).

Ravichandran and Hegde (2017) conducted a lab experiment to evaluate six combiproduct, five contact and four systemic fungicides against *Rhizoctonia bataticola* causing dry root rot in chickpea. Among combiproducts evaluated, carbendazim 25% + mancozeb 50% (Sprint), carboxin 37.5% + thiram 37.5% (Vitavax power 75% WP) and carbendazim 12% + mancozeb 63% (Saaf) were found to be most effective with complete inhibition of mycelial growth of *R. bataticola* at all the concentrations tested. Out of four systemic fungicides evaluated carbendazim, difenconazole and tebuconazole were best with cent per cent inhibition of mycelial growth at all concentrations tested.

Mukesh *et al.* (2017b) studied effect of fungicide on *Fusarium oxysporum* f.sp. *corianderii*, among all the tested fungicides, complete inhibition of the fungal growth was recorded with Bavistin (carbendazim) at all tested concentrations. The next best fungicides in order of fungal growth inhibition were Carbendazim + Mancozeb (companion), Topsin-M (thiophanate methyl), Vitavax (carboxin + thiram) and Benomyl (benlate) which inhibited the fungal growth by Carbendazim was found significantly superior at 200 and 500 ppm with (100%) inhibition of mycelial growth followed by Companion and Topsin- M at 500 ppm and with (100%). Seed treatment with Bavistin (carbendazim) resulted in lowest wilt incidence (10.39%), seed dressing with Topsin-M and Vitavax that showed wilt incidence of 14.33 and 21.55 per cent, respectively.

2.11 Integrated disease management

Raoof and Rao (1996) studied the effect of soil solarisation on castor wilt in a wilt sick plot by covering the low density transparent polythelene sheet (200 gauge) and observed maximum reduction in wilt incidence (50 %).

Chattopadhyay and Varaprasad (2001) studied the effect of ten different *Trichoderma* isolates from different sources along with carbendazim, leaf extract of *A. indica* as seed treatment and pre-sowing soil application of carbofuron on castor wilt incidence in wilt sick plot and reported significant lower wilt incidence in all the treatments compared to control.

Singh and Goswami (2001) conducted an experiment to find the efficacy of neem cake and carbofuran for management of disease complex caused by *M. incognita* and *F. oxysporum* on cowpea and found that application of both neem cake and carbofuran significantly increased plant growth and reduced disease incidence. These results suggest the potential use of neem cake with carbofuron for sustainable management of disease- complex on cowpea.

Desai *et al*. (2003) conducted a study in field naturally infested with *F. oxysporum* f. sp. *ricini* by covering polythene sheets for three weeks during summer. This reduced the population of castor wilt pathogen by 67.25% wilt incidence by 38.43% and increased seed yield by 124.68% as compared to non-solarized plots.

Dange *et al*. (2006) suggested that application of bio agents like *Trichoderma sp* and chemical like carbendezim as seed treatment as well as use of resistant cultivars along with proper cultural practices like soil solarisation provides an effective control of the Fusarium wilt of castor

Sharma *et al.* (2005) studied integrated management of *Fusarium* yellows of gladiolus under pot culture and polyhouse conditions, the results of an integrated approach using pots treated with neem cake, carbendazim and *Trichoderma harzianum* revealed the highest disease control. This approach enhanced corm yield and improved plant health.

Haseeb *et al.* (2007) used chemicals like carbofuran, bavistin, botanicals (*Azadirachta indica*) seed powder and *Murraya koenigii* leaf powder and biological control agents (*T. virens*) and *P. fluorescens* against root knot nematode, *M. incognita* and wilt fungus, *F. oxysporum* disease complex of field pea, *Pisum sativum* and found all the treatments significantly improved the growth of plants compared to untreated inoculated plants and they also found carbofuran and bavistin showed maximum suppression of root knot development and fungal infection, respectively, while *A. indica* seed powder and *T. virens* were found effective against both the pathogens.

Nasreen and Ghaffar (2010) found complete eradication of seed borne infection of *F. solani* in bitter gourd by carbendazim and gave maximum reduction in cucumber and bottle gourd. They also reported that *F. solani* infested seeds of bottle gourd, cucumber and bitter gourd reduced seedling mortality and root infection when sown in mustard and neem cake amended soil. They concluded that mustard cake was found most effective at all ratios followed by neem and castor cake.

Kamdi *et al.* (2012) evaluated two antagonists, two fungicides and two botanical extract against *F. oxysporum* f. sp. *ciceri* causing chickpea wilt. Field studies revealed that carbendazim seed treatment gave minimum wilt incidence (26.38%) and maximum yield of chickpea.

Meghwal *et al.* (2014) studied the antagonistic potential of five species of *Trichoderma* against castor wilt pathogen. Among all five species *T.harzianum* has maximum potential to reduce disease incidence.

Shalini *et al*. (2014) conducted pot culture studies with biocontrol agents and chemicals for the management of wilt disease complex of castor caused by *F. oxysporum* f. sp. *ricini* and nematode, *Rotylenchulus reniformis*. Combination treatment of carbofuran + carbendazim was found most effective in reducing wilt incidence and reniform nematode population.

Bharathi *et al*. (2015) conducted a study on different combinations of seed treatment and furrow application with bio agent and fungicides in solarised and non solarised soils and concluded that solarisation is best option for management of castor wilt disease.

Chandel and Deepika (2015) studied effect of organic amendments, biological control agents and soil solarization alone and in combination with antagonists against Fusarium wilt of carnation caused by *Fusarium oxysporum* Schledit. f. sp. *dianthi*. The results revealed that neem cake and pine needles out of nine organic amendments gave 77.49 and 72.49 per cent disease control with minimum disease incidence. Combined application of two antagonists *Trichoderma viride* and *T.harzianum* after 60 days of soil solarization practices registered minimum incidence (16.25%) of the disease followed by *T. viride* and *T. harzianum.*

Salim and Sobita (2017) studied soil solarization and integrated approaches with neem cake and Carbendazim against *Fusarium oxysporum* f. sp. *lycopersici* in the green house conditions. Sick soil of *Fusarium oxysporum* f. sp. *lycopersici* was made before solarization. After solarization FYM , Neem cake powder and carbendazim were applied in pot. The disease intensity (%) of *Fusarium oxysporum* f. sp. *lycopersici* at 60 days after germination was significantly reduced in treatments solarized soil + neem cake + *F.o,* solarized soil + carbendazim + *F.o* and Solarized soil + neem cake + carbendazim compared to Unsolarized soil + carbendazim and Solarized soil alone.

Milica *et al.* (2017) reported that Soil borne pathogens can survive for many years in the absence of a host plant by forming persistent structures such as microsclerotia, sclerotia, chlamydospores or oospores. Consequently, soil borne diseases are particularly difficult to predict, detect, diagnose and successfully control. There is no single solution for the many problems posed by growers. Non-chemical options, such as soil solarisation, crop rotation, biological control, soil amendments or steaming may be considered too risky and/or uneconomical when used alone.

Patra *et al.* (2017) studied effect of different soil amendments on chickpea wilt caused by fungal pathogen *Fusarium oxysporum* f. sp. *ciceri*. Out of different treatments neem cake showed minimum disease incidence.

Singh *et al.* (2017) followed integrated disease management practices like seed treatment with Carbendazim, *Trichoderma harzianum* and intercropping with linseed, alone and in combination on lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis.* The results indicated that seed treatment with *Trichoderma harzianum* + Carbendazim + intercropping with linseed found minimum wilt incidence, maximum disease control and higher yield.

2.11.1 Soil physico- chemical parameters and enumeration of soil microflora

Sharma *et al.* (2002) studied effect of soil solarisation on microflora of soil, total microbial population including fungi, bacteria and actinomycetes decreased. Chemical characteristics of soil such as available nitrogen, potassium, organic carbon contents, electrical conductivity and pH increased after solarisation, however, the phosphorus content decreased.

Elnasikh *et al.* (2011) studied impact of neem cake on soil Microflora and some soil properties and reported that neem seed cake had positive effect on actinomycetes and had negative effect on pathogenic microflora, significantly increased soil available nitrogen and electronic conductivity.

Garba and Oyinlola (2014) studied the influence of neem seed cake and inorganic fertilizer amendments for sustained productivity of maize and reported that the organic matter in the neem seed cake has the ability to improve the physical, chemical and biological properties of the soil.

Sofi *et al.* (2014) conducted field experiment to study effect of soil solarisation on physical, chemical and biological properties of soil in cauliflower crop. Solarization significantly increased electrical conductivity, organic carbon, nitrogen and potassium over pre solarized soil. The mean pH, EC, Ca, Mg, N, P, K and C recorded in solarized soil was higher than in non-solarized. Soil solarization reduced the population of fungi, bacteria from, actinomycetes, and reduction in population was recorded even after 90 days, when compared with non-solarized soil.

Gupta *et al.* (2017) studied effect of soil solarization on physio chemical properties of soil under protected cultivation revealed that solarization resulted in slight change in the pH and EC of the soil, the status of nitrogen, potassium and phosphorus were raised substantially.

Oyinlola *et al.* (2017) studied effect of neem seed cake and inorganic fertilizer on yield of tomato and soil properties and reported that the values of soil organic carbon and nitrogen increased over the years.

Chapter-III

MATERIAL AND METHODS

Chapter – III

MATERIALS AND METHODS

This chapter includes all the material used and methods adopted in the investigation and the techniques are detailed under respective headings.

3.1 Location of work

The present investigation was carried out at ICAR- Indian Institute of Oilseed Research (IIOR), Rajendranagar, Hyderabad and Department of Plant Pathology, College of Agriculture, PJTSAU.

3.2 Laboratory techniques

The general laboratory techniques followed for the present study were those described by Nene and Thapliyal (1993), Dhingra and Sinclair (1995) and Aneja (2003) for preparation of media, sterilization, isolation and maintenance of fungal and bacterial cultures with slight modification wherever necessary.

3.2.1 Glassware

Glassware of Borosil was used throughout the investigation. The glassware used in present study were Petri plates (90 mm diameter), conical flasks (250, 500, 1000 ml), measuring cylinders (25, 250 and 500 ml), test tubes, pipettes (0.1, 1.0, 2.0, 5.0 ml and 10 ml), micro pipettes (10, 100 and 1000 µl) and beakers (50, 100, 500 and 1000 ml).

3.2.2 Cleaning of Glassware:

The glassware were first washed with a detergent followed by thorough cleaning with the tap water and then placed in cleaning solution for 24 hrs, later thoroughly washed with tap water and finally rinsed with distilled water for 3-4 times. They were air dried before use. The cleaning solution prepared with following composition.

Potassium dichromate ($K_2Cr_2O_7$) : 60 g
Concentrated sulphuric acid (H_2SO_4): 60 ml
Distilled water : 1000 ml

3.2.3 Sterilization of glassware

Glassware used for present investigation were wrapped in thick paper and were sterilized in hot air oven at 180 °C for 1 hour before use. Surface of Laminar Air Flow chamber was sterilized by wiping with cotton swab dipped in alcohol. Inoculation loop, cork borer and scalpel were sterilized by dipping in alcohol and heated to red hot using a spirit lamp.

Culture media and distilled water were sterilized in an autoclave at 15 lb *psi* for 15 minutes, while soil was sterilized at 20 lb *psi* for 1 hour, three times in alternate days. Soil sterilized by formaldehyde and autoclave was used in the present investigation.

3.2.4 Disinfestation of pots

Plastic pots were washed, cleaned and disinfested before use, by rinsing them through four per cent formalin solution. The formalin was allowed to evaporate before use for experiments.

3.2.5 Equipment and Apparatus used

Compound microscope (10x, 40x and 100x magnifications) was used for observing the fungi. Hot air oven and autoclave were used for sterilization of glassware and media, respectively. BOD incubators were used for incubating test materials and cultures at different temperatures. The cultures were stored in a refrigerator. Weighing of media and chemicals were done on a single pan electronic balance with a sensitivity of 0.001 g. Pipettes and micro pipette were used for serial dilution of soil and transferring the suspension on to the media plate under sterile condition, loading gel and also for poisoned food studies with botanicals, chemicals, centrifuge for extraction of botanical and oil cakes extraction.

Other tools which were used in the present investigation for various purposes included camel brush, inoculation needles, compound microscope, plastic pots, rose can, plastic covers and rubber bands.

3.3 Plant material

Seeds of castor cultivars Haritha, 48-1, DCS-107, GCH-4, DCS-9, Kranthi and JI-35 were collected from IIOR and used for pathogenicity studies.

3.4 Fungal isolates

Thirteen isolates of *F. oxysporum* f. sp. *ricini* (Table 3.1) isolated from diseased samples collected from different castor growing areas of Telangana and Karnataka.

3.5 Preparation of media

3.5.1 Potato Dextrose Agar medium

Potato Dextrose Agar (HIMEDIA)	:	39.0 g
Distilled water	:	1.0 litre

Potato Dextrose Agar (PDA) medium was prepared by adding 39.0 g of PDA to one litre of distilled water and sterilized by autoclaving.

3.5.2 Preparation of PDA Slants

PDA slants were prepared by transferring 8.0 ml of the medium to culture tubes. The tubes were plugged with non-absorbent cotton and sterilized in an autoclave. After sterilization, the tubes were removed from the autoclave when they were still in hot condition (*i.e*. approximately 40 °C) and kept in a slanting position to allow medium to solidify. After solidification, the slants were kept in refrigerator for further use.

3.6 Isolation and Identification of pathogenic isolates

3.6.1 Isolation and purification of isolates of *F. oxysporum* f. sp. *ricini*

The pathogen was isolated from diseased root/stem samples of castor as per methods described by Dhingra and Sinclair (1985). Infected portion of root/stem adjoining with small healthy tissue were cut into small pieces of 2 mm size and washed thoroughly in sterilized water to remove the dirt. The washed root/stem pieces were surface sterilized with 1 % sodium hypochlorite solution for 30-60 seconds and subsequently rinsed in sterile distilled water 2-3 times for 2 minutes to remove the traces of sodium hypochlorite. Surface sterilized bits (4-5) were then aseptically placed in sterilized Petri dishes containing pre-sterilized potato dextrose agar (PDA) medium.

Inoculated Petri dishes were incubated at 27±1°C in BOD incubator. As soon as the fungal growth was visible around the inoculated pieces, it was transferred to another Petri dishes containing sterilized PDA medium. After 2-3 days, they were transferred to fresh PDA slant and incubated at 27±1°C.

3.6.2 Identification of the isolates

The isolated culture of the fungus associated with wilt diseased specimen was identified based on micro and macro conidial characters and the presence of prominent hook at the base of macroconidia and other spore characters with the help of monograph entitled “The *Fusarium”* written by Booth (1975), *Illustrated Genera of Imperfect Fungi* (Barnett and Hunter, 1992) and CMI descriptions.

3.6.3 Purification

The cultures were purified by single spore isolation method (Toussoun and Nelson, 1976) and pure cultures were maintained on PDA slants refrigerator at 4 °C.

Table 3.1. Isolates of *Fusarium oxysporum* f. sp. *ricini* collected from Telangana and Karnataka states.

S. No	Designation of names to isolates	Places of collection
1	*For* 1	Marikanive, Hiriyur, Karnataka
2	*For* 2	Narkuda, Telangana
3	*For* 3	Narkuda, Telangana
4	*For* 4	Harthikote, Hiriyur, Karnataka
5	*For* 5	Uduvalli, Hiriyur, Karnataka
6	*For* 6	Pollepalli, Bijanapalli, Telangana
7	*For* 7	Gangaram, Bijanapalli, Telangana
8	*For* 8	Regional Agricultural Research Station, Palem, Telangana
9	*For* 9	Pollepalli, Bijanapalli, Telangana
10	*For* 10	IIOR, Rajendranagar, Hyderabad, Telangana
11	*For* 11	Lakshmipalli, Devarkadra, Telangana
12	*For* 12	Hajilapuram, Devarkadra, Telangana
13	*For* 13	Basanaypalli, Devarkadra, Telangana

3.7 Cultural and morphological variability

In order to study cultural characteristics, 5 mm mycelia bits of each isolates were taken from the actively growing cultures and centrally placed on 90 mm Petri plates containing sterilized PDA medium. After inoculation Petri plates were incubated at 25 ± 1 ^{0}C for 7 days. Each plate was replicated three times. After seven days radial growth of pathogen was recorded. Others characteristics *viz;* pigmentation, sporulation of different isolates was recorded by observing culture plate after complete growth of the mycelium which showed slight pinches of colour.

Morphological studies were carried out by taking small amount of mycelium from fourteen day old pure culture plates using a sterile needle and transferred onto a cleaned glass slide under asceptic conditions. The culture was taken from five different positions of the culture plate, four from adjacent side and one from middle. The mycelium was stained with lactophenol cotton blue (0.1%) and observed under compound microscope at 10 x. Spores size was measured by software Y W Camera.

3.8 Pathogenic variability

The standard sick pot inoculation technique was used to test the isolates for pathogenicity on castor plants (Prasad *et al.,* 2016). Pure cultures of *Fusarium oxysporum* f. sp. *ricini* isolates were mass multiplied on sorghum grains separately. The sorghum grains were soaked in 2% sucrose solution over night and boiled in fresh water for 30 minutes and excess water was drained and transferred to conical flasks and autoclaved at 15 *psi* for 15 minutes. The sterilized flasks were inoculated with 7 day old culture of *F. oxysporum* f. sp. *ricini* grown on potato dextrose agar and flasks were incubated for 10-14 days at 25 ^{0}C. Plastic pots with capacity of 4 kg were filled with sterilized soil (3 parts of red and 1 part of black soil) and inoculated with *F. oxysporum* f. sp. *ricini* (4g/kg soil). Immediately after inoculation, the pots were sprinkled with water and kept in glass house for 2-3 days. Seeds of seven different castor cultivars were sown in infested soil along with susceptible (JI-35) and resistant (48-1) checks to confirm uniform spread of innoculum.

The experiment was laid out in completely randomized block design and replicated thrice. Optimum temperature (25 ^{0}C) was maintained in a green house and after germination the seedlings were observed daily for wilt symptoms. The data on total number of seedlings and seedlings affected were recorded after germination upto 30days after sowing and per cent disease incidence was calculated as per the formula given below and the cultivars were categorized as per the disease reaction.

$$\text{Wilt Incidence (PDI)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

On the basis of reaction to the disease (PDI), the genotypes were grouped into following categories (Lakshminarayana and Raoof, 2005)

Table 3.2 Categorisation of wilt incidence (%)

Wilt incidence (PDI)	Category
0.0	Highly resistant
0.1-20.0	Resistant
>20.0%	Susceptible

3.9 Molecular variability

3.9.1 Fungal multiplication

Potato dextrose broth (PDB) medium was used for obtaining mycelial mats of the fungal isolates for DNA extraction. Hundred ml of medium was dispensed in 250 ml conical flasks and sterilized at 121.6°C at 15 lb pressure for 20 min. Each flask was inoculated with 5 mm mycelial disc of the fungal isolates, which were taken from the actively growing (7 day old) cultures on the PDA plates. The inoculated flasks were incubated for fifteen days at 27±1°C in shaker incubator. After incubation, the mycelial mats were harvested by filtering through sterilized Whatman filter paper No.1 and the mycelial mats were wrapped in aluminium foil and stored in refrigerator at -20°C for further isolation of fungal DNA.

3.9.2 Extraction of Fungal DNA

- DNA extraction was done by Cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980; Doyle and Doyle, 1990; Connolly *et al.*, 1994; Loganathan *et al.,* 2013).
- Around 300 mg of mycelial mats was weighed and ground to fine powder in liquid nitrogen by using pre-chilled mortar and pestle.
- The powdered mycelia was transferred into sterilized centrifuge tubes containing 800 µl of pre-heated (65 °C) CTAB extraction buffer (2 %) to make a slurry. These tubes were incubated at 65 °C for 30 min in a water bath and after incubation, equal volume of Chloroform: isoamyl alcohol (24:1, v/v) was added to the slurry and gently mixed.

- The tubes were then centrifuged at 14,000 rpm for 10 minutes at 4 °C. Upper aqueous phase containing nucleic acid was precipitated with 0.6 volumes of ice cold isopropanol and the nucleic acid was further collected by centrifugation at 14,000 rpm for 10 minutes at 4 °C.
- The pellet obtained was washed twice with ethanol (70 %) and air dried. Then the pellets were solubilized in 20-50 µl chilled TE buffer (10 mM Tris-HCI, 1 mM EDTA, pH- 8.0) and the quantification of DNA was done by running over 1% agarose gel and finally the extracted DNA was stored at -20 °C.

3.9.3 Inter-Simple Sequence Repeat (ISSR) analysis

Molecular variability was assessed for *F. oxysporum* f. sp. *ricini* isolates with four ISSR primers (Dubey *et al.,* 2008). The reaction mixture contained 2µl of genomic DNA, 2µl of 10X assay buffer, 0.8µl dNTPs, 1µl primer, 0.4 µl unit of Taq DNA polymerase to a final volume of 20µl reaction by adding 13.8µl of sterile distiled water. The reactions were amplified using the following PCR program in Bio-Rad thermo cycler, under the following conditions: thermal cycler. 4 min for initial denaturation at 94 ^{0}C for one cycle, 20 sec for cyclic denaturation at 92 ^{0}C, 45 sec for annealing at 52 ^{0}C and 2 min for extension at 72 ^{0}C for 38 cycles. Final extension was continued for an additional 7 min, and then reactions were held at 4 ^{0}C.

Table 3.3 List of primers used for ISSR analysis

Sl. No	Primer name	Sequences
1	ISSR-1	5'-(AC)8 G-3'
2	ISSR-2	5'-(AC)8 T-3'
3	ISSR-826	5'-(AC)8 C-3'
4	ISSR-836	5'-(AG)8 YA3'

3.9.4 Gel electrophoresis

3.9.4.1 Preparation 50 x TAE buffer

Tris base	: 242.0 g
Glacial acetic acid	: 57.1 ml
0.5 M EDTA (pH 8.0)	: 100 ml

Two hundred and forty two gram of Tris base was dissolved in 700 ml distilled water, 57.1 ml of glacial acetic acid, 100 ml of 0.5 M EDTA was added, final volume was made up to 1 litre and was adjusted to pH 8.5 with acetic acid.

3.9.4.2 Preparation of loading dye

0.5 M EDTA	: 20 µl
1 M Tris	: 100 µl
50 % Glycerol	: 880 µl

A pinch of Bromophenol blue mixed thoroughly and kept in Eppendorf tubes for use.

3.9.4.3 Agarose gel electrophoresis

Two ml of 50X TAE was added to 98 ml of distilled water to prepare 1X TAE and 1.5g of agarose was added to the solution. Contents were boiled in a microwave oven till the solution was clear. 5µl of ethidium bromide was added when the agarose solution is ready to solidify and poured immediately into comb inserted gel-casting tray after making it leak proof. Care was taken to avoid air bubbles in the gel. After solidification the gel was then kept in electrophoresis tank filled with 1x TAE buffer; comb and leak proof tapes were removed carefully.

Two microlitre of loading dye was placed on a strip of parafilm, 10 µl of PCR product was mixed thoroughly by repeated pipetting. Samples were loaded in wells slowly with pipette, suitable marker DNA (λ 100 bp) was also loaded along with samples for knowing size of the band and then electrophoretic assembly was connected to power supply unit. Samples in well were allowed to separate for 1 hour at 120 V in 1X TAE. The gel was observed in gel doc and gel profiles were documented and profiles were further used to measure the genetic diversity among the isolates.

3.9.4.4 Scoring and analysis of Data

Bands with the same migration were considered homologous fragments, independently of their intensity (Perez *et al*, 2012).

Bands were scored as either 1 or 0 on the basis of presence or absence of the bands. Data was analysed to obtain dendrogram for the isolates using NTSYspc version 2.02i (Rohlf *et al*, 1993)

3.10 Evaluation of soil amendments, botanicals and chemicals against *F. oxysporum* f. sp. *ricini*

3.10.1 Evaluation of botanicals extracts

Five botanicals *viz.,* garlic, sunflower, sapota, neem and tulsi which are easily available for farmers were selected to know their inhibition of the *F. oxysporum* f. sp. *ricini*. These extracts were tested by using poisoned food technique at 5, 10 and 15 per cent concentrations, respectively, (Nene and Thapliyal, 1973). The list of botanicals used in the present study is given in Table 3.4.

3.10.1.2 Preparation of plant based products

Preparation of cold aqueous extract of each test plant were collected and washed first in tap water and then in distilled water. Hundred grams of each fresh sample was crushed in a surface sterilized pestle and mortar by adding 100 ml sterile distilled water (1:1 w/v) the macerate was filtered through double layered muslin cloth. The extract was used as stock solution.

Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium respectively so as to get 5, 10 and 15 per cent concentrations. The medium was thoroughly shaken for uniform mixing of the extract.

3.10.1.3 Evaluation of botanicals *in vitro*

Twenty ml of poisoned medium was poured into each of the 90 mm sterilized Petri plates. A 5 mm mycelial disc taken from the periphery of fungal culture was placed at the centre of the petriplate. The disc was placed upside down in the centre of the Petri plate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration. Control treatment was maintained without adding any botanical to the medium and the plates were incubated at $27\pm1\ ^{0}C$ in an incubator.

Four replications were maintained for each treatment and the plates were incubated at 27 ± 1ºC. Radial mycelial growth was recorded after complete growth of the test fungus in control plates. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} X\ 100$$

Where,

I = Per cent inhibition of mycelium growth
C = Radial growth of mycelium in control
T = Radial growth of mycelium in treatment

3.10.2 Evaluation of soil amendment extracts

The soil amendments *viz.,* vermicompost, groundnut cake, cotton cake, coconut cake and safflower seed cake were used in the present study to evaluate the inhibition on *F. oxysporum* f. sp. *ricini* with these soil amendments. These extracts were tested by using poisoned food technique at 5, 10 and 15 per cent concentrations, respectively. (Nene and Thapliyal, 1973)

3.10.2.2 Preparation of extract of soil amendment

The extract of each soil amendment was prepared as per the procedure of dubey and Patel (2000). Fifty grams of each soil amendment was made into fine powder and soaked in sterile distilled water @ one gram in 1.25 ml of water separately and kept overnight. Then the material was ground using pestle and mortar and filtered through a muslin cloth and the filtrate was centrifuged at 10000 rpm for 15 min. The supernatant served as the standard extract solution (100%).

Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium respectively so as to get 5, 10 and 15 per cent concentrations. The medium was thoroughly shaken for uniform mixing of the extract.

Table 3.4 List of botanicals tested against *F. oxysporum* f. sp. *ricini*

S. No.	Common name	Scientific name	Plant Part used	Quantity used (g)
1	Garlic	*Allium sativum*	clove	100
2	Sunflower	*Helianthus annuus*	Leaf	100
3	Sapota	*Manilkara zapota*	Leaf	100
4	Neem	*Azardirachta indica*	Leaf	100
5	Tulsi	*Ocimum sanctum*	Leaf	100

Table 3.5 List of soil amendments and tested against *F. oxysporum* f. sp. *ricini*

S. No.	Name of soil amendments	Quantity used (g)
1	Vermicompost	25
2	Groundnut cake	25
3	Cotton seed cake	25
4	Coconut cake	25
5	Safflower seed cake	25

3.10.2.3 Evaluation of soil amendments extract *in vitro*

Twenty ml of poisoned medium was poured into each of the 90 mm sterilized Petri plates. A 5 mm mycelial discs taken from the periphery of fungal culture was placed at the centre of the plate. The disc was placed upside down in the centre of the Petri plate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration. Control treatment was maintained without adding any soil amendment to the medium and the plates were incubated at 27±1 ^{0}C till test fungus covered entire plate in control plate.

Four replications were maintained for each treatment and plates were incubated at 27 ± 1°C. Radial mycelial growth was recorded after complete growth of the fungus in control plates. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} X\ 100$$

Where,

I = Per cent inhibition of mycelium growth
C = Radial growth of mycelium in control
T = Radial growth of mycelium in treatment

3.10.3 Evaluation of fungicides *in vitro*

Efficacy of ten commonly available fungicides as mentioned in Table.3.6 were tested against *F. oxysporum* f. sp. *ricini* using poisoned food technique (Nene and Thapliyal, 1973) at 500, 1000 and 2000 ppm concentrations respectively.

Required quantity of individual chemical was added separately into flask containing sterilized molten and cooled potato dextrose agar medium so as to get the desired concentration of the fungicide. Twenty ml of poisoned medium was poured into each of the 90 mm sterilized Petri plates. A 5 mm mycelial disc taken from the periphery of fungal culture was placed at centre of plate. The disc was placed upside down in the centre of the Petri plate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration. Control treatment was maintained without adding any fungicides to the medium. The plates were incubated at 27 ± 1 ^{0}C in an incubator. Three replications were maintained for each treatment, radial mycelial growth was recorded after complete growth of test fungus in control plates. The per cent inhibition of the radial mycelial growth of the pathogen over control was calculated by using the formula given by Vincent (1947)

$$I = \frac{(C - T)}{C} X\ 100$$

Where,

I = Per cent inhibition of mycelium growth
C = Radial growth of mycelium in control
T = Radial growth of mycelium in treatment

3.10.4 Evaluation of botanicals against *F. oxysporum* f. sp. *ricini in vivo*

The standard sick pot inoculation technique was conducted as per procedure of Prasad *et al.,* 2016. Pure culture of *Fusarium oxysporum* f. sp. *ricini* was mass multiplied on sorghum grains. The sorghum grains were soaked in 2 % sucrose solution over night and boiled in fresh water for 30 minutes and excess water was drained and transferred to conical flasks and autoclaved at 15 *psi* for 15 minutes. The sterilized flasks were inoculated with 7 day old culture of *F. oxysporum* f. sp. *ricini* grown on potato dextrose agar and flasks were incubated for 10 days at 25 ^{0}C. One kg capacity pots were filled with sterilized soil (3 parts of red and 1 part of black) and inoculated with *F. oxysporum* f. sp. *ricini* (4 g/kg soil). Immediately after inoculation, the pots were sprinkled with water and kept in glass house for 2-3 days.

Seeds of GCH-4 castor cultivar soaked overnight in 15 per cent botanical extract were sown separately in each pot, followed by drenching in each pot with 15 % of extract when initial symptoms were observed in treated pots. The experiment was conducted in completely randomized block design and replicated four times along with pathogenic control without any treatment (sterilized soil + inoculum) and healthy check was maintained with seeds sown in sterilized soil. Observations on wilt incidence were recorded regularly and per cent disease incidence was calculated.

$$\text{Wilt Incidence (PDI)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

3.10.5 Evaluation of soil amendments against *F. oxysporum* f. sp. *ricini in vivo*

The standard sick pot inoculation technique was used to test the isolates for pathogenicity on castor plants (Prasad *et al.,* 2016). Pure culture of *Fusarium oxysporum* f. sp. *ricini* was mass multiplied on sorghum grains. The sorghum grains were soaked in 2 % sucrose solution over night and boiled in fresh water for 30 minutes and excess water was drained and transferred to conical flasks and autoclaved at 15 *psi* for 15 minutes. The sterilized flasks were inoculated with 7 day old culture of *F. oxysporum* f. sp. *ricini* grown on potato dextrose agar and flasks were incubated for 10-14 days at 25 ^{0}C. One kg capacity pots were filled with sterilized soil (3 parts of red and 1 part of black) and inoculated with *F. oxysporum* f. sp. *ricini* (4 g/kg soil). Immediately after inoculation, the pots were sprinkled with water and kept in glass house for 2-3 days.

Fifteen gram of powdered soil amendments were mixed in soil before sowing of GCH-4 cultivar seed separately in different pots. The experiment was conducted in completely randomized block design and replicated four times along with pathogenic control without any treatment (sterilized soil + inoculum) and healthy check was maintained with seeds sown in sterilized soil. Observations on wilt incidence were recorded regularly and per cent disease incidence was calculated.

$$\text{Wilt Incidence (PDI)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

3.10.6 Evaluation of fungicides *in vivo*

The efficacy of fungicides were tested *in vivo* using standard sick pot inoculation technique was used to test the isolates for pathogenicity on castor plants (Prasad *et al.*, 2016). Pure culture of *Fusarium oxysporum* f. sp. *ricini* was mass multiplied on sorghum grains. The sorghum grains were soaked in 2 % sucrose solution over night and boiled in fresh water for 30 minutes and excess water was drained and transferred to conical flasks and autoclaved at 15 *psi* for 15 minutes. The sterilized flasks were inoculated with 7 day old culture of *F. oxysporum* f. sp. *ricini* grown on potato dextrose agar and flasks were incubated for 10-14 days at 25 ^{0}C. One kg capacity pots were filled with sterilized soil (3 parts of red and 1 part of black) and inoculated with *F. oxysporum* f. sp. *ricini* (4 g/kg soil). Immediately after inoculation, the pots were sprinkled with water and kept in glass house for 2-3 days.

Seeds of GCH-4 castor cultivar were treated with recommended dosage with each of the test fungicides, followed by drenching in each pots with recommended dosage of fungicides when initial symptoms were observed in treated pots. The experiment was conducted in completely randomized block design and replicated thrice along with pathogenic check without any treatment (sterilized soil + inoculum) and healthy control was maintained with seed sown in sterilized soil. Observations on wilt incidence were recorded regularly and per cent disease incidence was calculated.

$$\text{Wilt Incidence (PDI)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

Table 3.6 List of fungicides tested against *F. oxysporum* f. sp. *ricini*

S. No.	Common name	Trade name	Recommended dosage
1	Propineb 61.3 %+ Iprovalicarb 5.5 %	Melody duo	2.25 g/l of water
2	Pyraclostrobin 5 % + metiram 55 % WG	Cabrio Top	2 g/l of water
3	Carbendazim 12 % + Mancozeb 63 % WP	Saaf	3-5 g/l of water
4	Tebuconazole + trifloxystrobin	Native 75WG	0.4 g/l of water
5	Hexaconazole+ captan	Taquat 70%WP	0.5-1 g/l of water
6	Carboxin 37.5 %+ Thiram 37.5 % WP	Vitavax Power	2-3 g/Kg seed
7	Thiophanate methyl 70% WP	Roko	2-3 g/Kg seed
8	Difenoconazole	Score 25 % EC	1ml/l of water
9	Propiconazole	Tilt 25 % EC	1ml/l of water
10	Carbendazim	Bavistin 50 %WP	2 g/Kg seed

3.11 Integrated disease management of castor wilt

The experiment was carried in wilt sick plot at ICAR-IIOR, Rajendranagar, Hyderabad in strip plot design with four main treatments and four sub treatments. Soil solarisation was done with white polythene cover of 200 micron size in hot summer months of the year (April- May). Sowing was done with castor cultivar GCH-4 in total field area of 31x25 m^2 divided into plot size of 7x2 m^2 with three replications with spacing of 60 x 45 cm.

The main treatments and sub treatments imposed to the plots,

Main treatments : Mt_1: Soil solarisation

Mt_2: Application of dazomet (30-40 g/m^2)

Mt_3: Application of neem cake (1.78 kg/m^2)

Mt_4: Control.

Sub treatments : Sbt_1: Seed treatment with *T. harzianum*, Th 4d WP 10g/kg and soil application of *T.harzianum,*Th 4d WP 1 kg/100 kg FYM.

Sbt_2: Seed treatment with carbendazim 2g/kg seed

Sbt_3: Soil application of carbofuron 2kg a.i./ha

Sbt_4 : Control.

Neem cake (1.78 kg/m^2) and dazomet (30-40 g/ m^2) were applied 2 days before the date of sowing and *Trichoderma harzianum* (Th 4d) (10g/ kg) and fungicide carbendazim (2 g/kg) were applied as seed treatment before sowing. *T. harzianum* (Th 4d) mixed with FYM (1kg/100 kg FYM) and Carbofuron (2kg a.i./ha was applied to soil at the time of sowing.

3.11.1 Data collection

Data on per cent disease incidence was recorded at 30, 60, 90 and 120 days after sowing. Wilt incidence was calculated as per the formula,

$$\text{Wilt Incidence (PDI)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

3.12 Enumeration of *Fusarium, Trichoderma* and Actinomycetes from wilt sick plot of castor

3.12.1 Collection and preparation of soil samples

Soil samples were drawn at random from 0-15 cm soil depth from the experimental field from each main treatment plots before solarisation, 30 DAS and after harvesting of crop. The soil was mixed thoroughly and samples of about 500 g were obtained by quartering technique and stored in neatly labelled polythene bag for soil analysis.

3.12.2 Composition of Media

3.12.2.1 Fusarium specific media

Sodium nitrate	-	2.00 g
Dipotassium phosphate	-	1.00 g
Magnesium sulphate	-	0.50 g
Potassium chloride	-	0.50 g
Ferrous sulphate	-	0.01g
Sucrose	-	30.00 g
Yeast	-	2.00 g
Agar	-	20.00 g
PCNB	-	500 µg
Streptomycin	-	1.40 g
Malachite green	-	0.025 g
Distilled water	-	1000 ml

3.12.2.2 Trichoderma specific media

Magnesium sulphate	-	0.20 g
Dipotassium phosphate	-	0.90 g
Ammonium nitrate	-	1.00 g
Potassium chloride	-	0.15 g
D-glucose	-	3.00 g
Metalaxyl	-	0.30 g
PCNB	-	0.20 g
Chloramphenical	-	0.25 g
Agar	-	18.00 g
Distilled water	-	1000 ml
Rose Bengal	-	0.15g

3.12.2.3 Actinomycetes specific media (SRL)

Sodium carbonate	-	2.00 g
L-Asparagine	-	1.00 g
Sodium propionate	-	4.00 g
Dipotassium phosphate	-	0.50 g
Magnesium sulphate	-	0.10 g
Ferrous sulphate	-	0.001g
Agar	-	15.00 g

3.12.3 Preparation of media

3.12.3.1 Fusarium specific media

All the chemicals of required quantity except malachite green and streptomycin were mixed in 1 litre distilled water, which was boiled to dissolve the agar-agar.

The medium was transferred to 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 121.6 °C at 15 lbs pressure for 20 minutes. After cooling the required quantity of malachite green and streptomycin were added to the medium just before pouring in the sterilized petri dishes.

3.12.3.2 Trichoderma specific media

All the chemicals except Chloramphenicol, Rose Bengal and were mixed in 1 litre distilled water, which was boiled to dissolve the agar-agar.

The medium was transferred to 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 121.6 °C at 15 lbs pressure for 20 minutes. After cooling the required quantity of Chloramphenicol, Rose Bengal and was added to the medium just before pouring in the sterilized petri dishes.

3.12.3.3 Actinomycetes specific media

Actinomycetes specific media 21.7 g was added to 1 liter distilled water. To which glycerol 0.5 g of was added to it gently heated to bring it to boiling. Two hundred and fifty millilitre of the medium was distributed into 500 ml conical flasks then the medium was sterilized in an autoclave at 15 *psi* at 121 ^{0}C for 20 minutes.

3.12.4 Preparation of water blanks

Nine ml of distilled water was added to clean 25ml test tubes; this was sterilized in an autoclave at 15 *psi* at 121 ^{0}C for 20 minutes.

3.12.5 Determination of *Fusarium* population in soil by serial dilution and pour plant method

One gram of soil sample was suspended in 9 ml of sterilized water blanks. Ten folds serial dilutions were prepared. From 10^{-1} to 10^{-5}, one ml of suspension from 10^{-2} to 10^{-4} fold dilutions was transferred in sterilized Petri plates. To these plates sterilized Fusarium specific medium at 45 °C temperature was poured in each Petri plates and mixed with the aliquot gently. After solidification of the medium, plates were kept at 25±2 °C in incubator for 4-5 days. Three replications were carried for each dilution. The Fusarium population per gram of soil was enumerated using following formula.

$$\text{Number of colony per gram soil} = \frac{\text{Average plate count}}{\text{Weight of soil}} \times \text{dilution}$$

3.12.6 Determination of *Trichoderma* population in soil by serial dilution and pour plate method

One gram of soil sample was suspended in 9 ml of sterilized water blanks. Ten fold serial dilutions were prepared from 10^{-1} to 10^{-5}, one ml of suspension from 10^{-2} to 10^{-4} dilutions were transferred in sterilized Petri plates. To which sterilized luke warm *Trichoderma* specific medium was poured in each Petri plate and mixed with aliquot gently. After solidification of medium, plates were kept at 25±°C temperature in incubator for 4-5 days. Three replications were carried for each dilution. Following formula was used to enumerate the *Trichoderma* population.

$$\text{Number colony per gram of soil} = \frac{\text{Average plate count}}{\text{Weight of soil}} \times \text{dilution}$$

3.12.7 Determination of actinomycetes population in soil by serial dilution and pour plant method

One gram of soil sample was suspended in 9 ml of sterilized water blanks. Ten fold serial dilutions were prepared from 10^{-1} to 10^{-5}, one ml of suspension from 10^{-3} to 10^{-4} dilutions were transferred in sterilized Petri plates. Luke warm sterilized actinomycetes specific medium was poured in each Petri plate and mixed with aliquot gently. After solidification of medium, plates were kept at 25±°C temperature in incubator for 2-3 days. Three replications were carried for each dilution. Following formula was used to enumerate the actinomycetes population.

$$\text{Number of colony per gram of soil} = \frac{\text{Average plate count}}{\text{Weight of soil}} \times \text{dilution}$$

3.13 Determination of Physio – chemical and chemical properties soil samples

3.13.1 Physio – chemical properties soil samples

S. No	Particulars	Value	Method / Reference
1	P^H [1: 2.5 soil: water]	8.0	ELICO, LI 612 pH analyser (Jackson, 1973)
2	Electrical conductivity [dS m^{-1}] [1:2:5 soil : water]	0.16	SYSTRONICS Conductivity - TDS meter 308 (Jackson, 1973)
3	Organic carbon (%)	0.38	Walkley and Black's modified method (Jackson, 1967)

3.13.2 Chemical properties soil samples

S. No	Particulars	Value	Method / Reference
1	Available nitrogen (kg ha^{-1})	160	Alkaline permanganate method using KELPLUS SUPRA UL N – analyser (Subbaiah and Asija, 1956)
2	Available phosphorus (kg ha^{-1})	32	Olsen's method for extraction and Ascorbic acid method for estimation by using UV- VIS UV5704SS Spectrophotometer at 420 nm (Olsen's *et al.*, 1954)
3	Available potassium (kg ha^{-1})	343	Neutral normal ammonium acetate method using ELICO CL361 Flame photometer (Piper, 1966)

3.14 Statistical analysis:

The data obtained from different experiments was transformed using Arc sin and square root transformations, where ever necessary (Panse and Sukhatme, 1978) and was statistically analysed using CRD, Strip plot analysis and two way anova as per procedures suggested by Snedecor and Cochran (1967).

Chapter- IV

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

Castor wilt caused by *Fusarium oxysporum* f. sp. *ricini* is an economically important and major disease in castor growing areas of Telangana. The present study was undertaken to determine the variability among the isolates of *Fusarium oxysporum* f.sp. *ricini* causing wilt in castor (*Ricinis communis* L.), screening of castor cultivars for Fusarium wilt, evaluation of botanicals, soil amendments, fungicides, and evaluating different combination of disease management methods under field conditions at Indian Institute of Oilseeds Research, Hyderabad during 2017-2018. The results of experiments are presented hereunder.

4.1 Collection, isolation, purification and identification of pathogen

4.1.1 Collection of diseased samples

The diseased samples were collected from Hiriyur, Karnataka and four different castor growing regions of Telangana *viz.*, Narkuda, Palem, Bijanapalli, Rajendranagar and Devarkadra during *Kharif* 2017. The characteristic symptoms like stunting, yellowing, bronzing, marginal necrosis of leaves and black streaks on the stems of infected plant, vascular discoloration, wilting and death of the plants (Plate. 3) were observed. These infected root/stem samples were washed thoroughly in tap water and dried between folds of the filter paper, kept in paper bags for further studies.

4.1.2 Isolation, purification and identification of pathogen

Thirteen isolates of *Fusarium oxysporum* f. sp. *ricini* were isolated from diseased root/stem samples of castor (Plate. 4) as mentioned in the materials and methods (Section 3.6.1). The cultures were purified by single spore isolation method (Toussoun and Nelson, 1976) and these were further maintained on PDA slants at 4 ^{0}C. White aerial mycelium which turned pinkish/purplish/yellowish when kept in day light was observed and growth of the colony ranged from 80-90 mm. Microconidia are 0-1 septate, hyaline, round to ovoid, one celled. Macroconidia are few in number, 3-5 septate, straight, spindle as well as sickle shaped. Based on morphological characters the fungus associated with disease was identified as *F. oxysporum* f. sp. *ricini*.

4.2 Cultural variability

The data presented in Table 4.1 revealed that all the thirteen isolates of *F. oxysporum* f. sp. *ricini* exhibited a variation with respect to variation in their cultural characteristics in colony diameter, growth, texture, shape, margin, colour and details presented in Plate. 5. The colony of isolates *For* 1, *For* 2, *For* 3, *For* 4, *For* 5 and *For* 13 were fast growing while isolates *For* 6, *For* 7, *For* 8, *For* 9 and *For* 10 were slow in their growth which was initiated from 3rd day onwards. Only two isolates *For* 1 and *For* 3 showed radial growth of 9 mm and completely filled the plate while the rest of the isolates showed variation in their growth. Isolate *For* 4 showed extreme variations in its growth pattern which was not uniform and showed irregular growth pattern with distinct sectoring and fluffy growth. Similarly isolate *For* 5 also showed sectoring with fluffy growth.

Most of the isolates produced abundant fluffy aerial mycelium. Isolate *For* 6 and *For* 13 produced scanty fibrous growth of mycelium which was not abundant. In case of isolates *For* 7, *For* 8, *For* 9 and *For* 10 aerial growth was suppressed. Isolates of *For* 1, *For* 2, *For* 4, *For* 5, *For* 6, *For* 9, *For* 10, *For* 11, *For* 12 and *For* 13 colonies lacked fineness and appeared rough. While, two isolates *For* 7 and *For* 8 appeared smooth surface on media. Variation in shape of colony among the isolates was observed. Most of the colonies had round colony shape (*For* 1, *For* 2, *For* 3, *For* 11, *For* 12 and *For* 13), isolates *For* 7, *For* 9 and *For* 10 showed irregular wavy growth, while sectoring / saltation was observed in isolates *For* 4 and *For* 5. The colonies also varied in their growth margins. Isolates *For* 1, *For* 2, *For* 3, *For* 11 and *For* 12 showed uniform growth on media, whereas isolates *For* 6, *For* 7, *For* 9, *For* 10 and *For* 13 were grown in concentric circles showing many rings in colony. Isolates *For* 6 and *For* 8 had filamentous growth which spread towards periphery.

Variation in the mycelium colour was observed among the isolates on PDA medium. Pigmentation of the colony was observed as white in isolates *For* 1, *For* 4, *For* 5 and isolate *For* 13, light buff in isolate *For* 2, *For* 3 and *For* 12, yellow in isolate *For* 11, light pinkish purple in isolate *For* 8, light purple in isolate *For* 7 and pink in isolate *For* 6, purple in isolate *For* 9 and dark purple *For* 10 among the isolates of *F. oxysporum* f. sp. *ricini*. Pigmentation was restricted to centre of the colony and periphery remained white in isolates *For* 6, *For* 7, *For* 8, *For* 9, *For* 11 and *For* 12. Pigmentation was uniform in isolates *For* 3 and *For* 10 in whole colony. Pigmentation in colonies started from fourth day after inoculation to sixth day. Similar variation in cultural and morphology of *Fusarium* isolates was observed by Prasad *et al*, (2008) among 29 isolates of *F. oxysporum* f. sp. *ricini* collected from different castor growing areas of country. Colony pigmentation varied from light pink to dark purple and orange, mycelial growth varied from profuse to scanty thin growth. Singh (2016) observed large variation in colonies among 12 isolates of *F. oxysporum* and 11 isolates of *F. solani* causing wilt in chilli crop. Isolates produced circular or irregular shaped colony with rough, smooth, or rugose texture in *F. oxysporum* and *F. solani* species. Appearance of pigmentation in colonies varied from 3-5 days after inoculation. Desai *et al.* (2003) observed that among 15 isolates of *F. oxysporum* f. sp. *ricini*, six isolates produced moderate to profuse fluffy white mycelia, while nine isolates produced thin flat to slight fluffy pinkish mycelia. Reddy *et al.* (2010) studied colony characteristics of 146 isolates of *F. oxysporum* f. sp. *ricini* and reported three types of pigmentation when isolates incubated for 8 days on PDA medium. Among the three types of pigmentation, pink pigmentation was high (108), followed by purple (26) and brick red (12). Three types of mycelia, moderately fluffy (91 isolates); fluffy (44 isolates) and sparse (11 isolates) were seen. Colony radial growth was of two types; fast growing (80 isolates) and slow growing (66 isolates). Sporulating ability was of two types: low sporulating (63 isolates) and high sporulating (83 isolates).

The differential colour of the isolates may be assigned to the presence of specific pigment produced as by-product and involved in the enzymatic activities *viz*., javanicin, bostrycoidin, solanione and lycopersin (Booth,1971)

4.3 Morphological variability

Variation in size of both macro- and micro-conidia was observed among the isolates of *F. oxysporum* f. sp. *ricini* (Table 4.2, Plate. 6 (a), (b) and (c)). A significant variation in the size of macro-conidia was observed among the isolates of *F. oxysporum* f. sp. *ricini*. The biggest sized macro conidia were observed in isolates *For* 4, *For* 5 (31.46x2.86 µm), isolate *For* 13 (30.11x2.17 µm) and isolates *For* 11, *For* 12 (29.41x2.15µm), respectively, whereas, the smallest sized macro-conidia were observed in isolates For 7 (25.71x1.83 µm), isolates, *For* 2, *For* 3, *For* 8, *For* 6 (25.74x2.86 µm) and *For* 10 (26.72x2.17 µm), *For* 9 (26.72x2.18 µm) and *For* 1 (27.17x1.43 µm) respectively. The *F. oxysporum* f. sp. *ricini* isolates revealed variations in number of septations in the micro and macroconidia. The most frequent conidial septations observed in most of the isolates was 2 (isolates- *For* 1, *For* 2, *For* 3, *For* 6, *For* 7, *For* 8, *For* 9, *For* 10, *For* 11 and *For* 12) and in case of isolates *For* 4, *For* 5 and *For* 13, four septate macro conidia was observed. In case of micro conidia only isolates *For* 3, *For* 4 and *For* 13 had one septate micro conidia, remaining all isolates micro conidia were a septate. Chlamydospores were found in 15 day old cultures, which were globose, thick walled found either singly or in pairs and produced either terminally or intercalary. However significant differences was not observed in the production of chlamydospores.

Similar results were reported by Reddy *et al,* (2010), Prasad *et al,* (2008) and Desai *et al,* (2003) with regard to differences in size and septation of micro and macro conidia of *F. oxysporum* f. sp. *ricini* isolates.

4.4 Pathogenic variability

Knowledge on nature of variation among the races of pathogen is essential for effective disease management strategy through resistant cultivars. The pathogenic variability on seven castor cultivars *viz.*, Haritha, Kranthi, JI-35, 48-1, GCH-4, DCS-107 and DCS-9 was carried out with thirteen isolates of *F. oxysporum* f. sp. *ricini* and the results are presented in Table 4.3

Perusal of the data indicated that all the cultivars varied in their disease reaction to the isolates of *F. oxysporum* f. sp. *ricini.* Significant variation in disease incidence was observed in the castor cultivars by completely killing the plant showing cent per cent disease incidence while in cultivars 48-1 and DCS-107 there was no incidence of the disease.

The cultivar JI-35 was susceptible to all the isolates of *F. oxysporum* f. sp *ricini.* Cultivars DCS-107 and 48-1 showed highly resistant to resistant reaction to all the isolates of *F. oxysporum* f. sp. *ricini.* Cultivar DCS-9 showed highly resistant reaction to isolates *For* 1, *For* 2, *For* 3, *For* 4, *For* 5 and *For* 12, resistant reaction to *For* 11 and *For* 12 and was susceptible to remaining isolates *For* 6, *For* 7, *For* 8, *For* 9, *For* 10. Cultivar GCH-4 showed highly resistant reaction to *For* 2, *For* 5, *For* 11 and *For* 12, resistant reaction to *For* 1 and *For* 13 and showed susceptible reaction to *For* 3, *For* 4, *For* 6, *For* 7, *For* 8, *For* 9 and *For* 10. Cultivar Kranthi was susceptible to most of the *F. oxysporum* f. sp. *ricini* isolates (*For* 3, *For* 4, *For* 5, *For* 6, *For* 7, *For* 8, *For* 9, *For* 10, *For* 11 and isolate *For* 13), but showed highly resistant reaction to *For* 1, *For* 2 and resistant reaction to *For* 12. Cultivar Haritha was highly resistant to isolates *For* 1, *For* 2 and *For* 12, showed resistant reaction to isolates *For* 5, *For* 11 and *For* 13, whereas it showed susceptible reaction to *For* 6, *For* 7, *For* 8, *For* 9 and isolate *For* 10.

Based on per cent disease incidence the cultivars were grouped as highly resistant, resistant and susceptible and the data is presented in Table 4.4. Based on the virulence reaction of the isolates they were classified into four groups (Table 4.5). Group I consists of highly virulent isolates *For* 10, *For* 9, *For* 8, *For* 7 and *For* 6 which were pathogenic to all the cultivars except 48-1 and DCS-107. Group II consisted of virulent isolates *For* 3 and *For* 4 which were pathogenic to cultivars Haritha, Kranthi, JI-35 and GCH-4 and cultivars 48-1, DCS-107 and DCS-9 were resistant to these isolates. Group III consisted of moderately virulent isolates *For* 11 and *For* 13 which caused susceptible reaction only in cultivars Kranthi and JI-35. Isolates *For* 12, *For* 5, *For* 2 and *For* 1 were pathogenic only to JI-35 which is highly susceptible cultivar and belonged to group IV. The grouping of isolates according to pathogenic reaction is given in Table 4.4. Plate 7(a), (b), (c) and (d).

Pathogenicity data was converted to binary data, by denoting pathogenic reaction as "1" and no reaction as "0" and generated a dendrogram (Fig 4.1). From the dendrogram generated the isolates were grouped into two major clusters (Table 4.6), cluster I and cluster II, which were further divided into two sub groups. Sub group I of cluster I included isolates *For* 6, *For* 7, *For* 8, *For* 9, *For* 10 and Subgroup II of cluster I consisted of isolates *For* 3 and *For* 4. Sub group I of cluster II consisted isolates *For* 11 and *For* 13 and subgroup II of cluster II consisted isolates *For* 1, *For* 2, *For* 5 and *For* 12. From the dendrogram groping it is inferred that Isolates belonging to cluster I are more virulent compared to isolates of cluster II.

Similar results were found from studies conducted by Reddy *et al.* (2010) with sixty two isolates of *F. oxysporum* f. sp. *ricini* which showed high variation in virulence among isolates of *Fusarium*, and also reported that place of collection of isolates had no correlation with pathogenicity of isolates. The highly virulent isolates caused wilt reaction in Haritha and DCS-9. Prasad *et al.* (2008) studied variation among 29 isolates *F. oxysporum* f. sp. *ricini* collected from different parts of country and reported large variation in pathogenicity of the isolates. Highly virulent isolates caused wilt in resistant cultivars Kranthi and DCS-9 and less virulent isolates gave resistance reaction to all the cultivars and also reported virulence may not be dependent on area of collection. Lakshminarayana and Raoof, (2005) and Desai *et al,* (2003) also reported high variation in virulence reaction among *F. oxysporum* f. sp. *ricini* tested.

This variation in pathogenicity among isolates may be due to genetic makeup isolates. The variability in these isolates might be brought about by either natural mutation, natural selection, genetic drift, gene flow or mating system (McDonald and Linde, 2002). The variation in the *F. oxysporum* isolates, in turn can occur by means of natural mutations, through parasexuality or heterokaryosis (Buxton, 1962; Kuhn *et al*., 1995).

4.5 Molecular variability

Molecular markers are an important tool for studying genetic variation and identification of the pathogen. In the present investigation a powerful and extremely sensitive DNA based ISSR marker was used to study the variation among the isolates of *F. oxysporum* f. sp. *ricini*.

All the thirteen isolates of *F. oxysporum*. f. sp. *ricini* were compared and categorized by ISSR markers. Out of 20 primer sets tried four primers ISSR-1, ISSR-2, ISSR-826 and ISSR-836 gave scorable banding patterns. PCR was performed with all the isolates using these four primers and amplified DNA products were separated by gel electrophoresis on 1.5% agarose gel. Gel profiles (Figure 4.2) were used to measure the genetic diversity among the isolates. Bands with different electrophoretic mobility were scored as either 1 or 0 on the basis of presence or absence of the band. A binary similarity data was analysed to obtain dendrogram for the thirteen isolates using NTSYSpc version 2.02i. (Figure. 4.3).

From the dendrogram generated the isolates of *F. oxysporum* f. sp. *ricini* were divided into four groups (Table 4.7). Group I consists of isolates *For* 11, *For* 12, which were collected from Devarkadra. Group II included *For* 13, *For* 4 collected from Devarkadra and Hiriyur, Group III consisted of only one isolate *For* 10 from IIOR. Isolates *For* 3, *For* 9, *For* 8, *For* 7, *For* 6, *For* 5, *For* 2, and *For* 1 in Group IV which were collected from Narkuda (*For* 3 and *For* 2), Palem (*For 8*), Bijanapalli (*For 6, For 7, For 9*), Hiriyur (*For 5 and For 1*). This ISSR analysis showed high genetic variation among the isolates of *F. oxysporum* f. sp. *ricini*. Grouping of isolates according to ISSR primers could not be correlated with area of collection, pathogenicity of isolates or colony characteristics.

Similar results were reported by Reddy *et al.* (2010) with genetic variation among isolates of *F. oxysporum* f. sp. *ricini* but observed no relationship between their grouping according to ISSR analysis, and geographic origin or virulence of the isolates or morphology of isolates. Prasad *et al.* (2008) studied genetic variation among 29 isolates of *F. oxysporum* f. sp. *ricini* using RAPD markers, reported that genetic variation among the isolates do not reflect variation in area of collection, pathogenicity or morphology of isolates.

4.6 Evaluation of botanicals, soil amendments and fungicides against *F. oxysporum* f. sp. *ricini in vitro*

4.6.1 Evaluation of botanicals

Five botanicals *viz.*, garlic, sunflower, sapota, neem and tulsi were tested for their efficacy against *F. oxysporum* f. sp. *ricini,* at 5, 10, and 15% per cent concentrations and the results are presented in Table 4.8, Plate. 8 and Figure 4.4

All the treatments were significant in inhibiting the mean radial growth of *F. oxysporum* f. sp. *ricini* at all the concentrations tested. Among the treatments maximum inhibition of mycelial growth was observed with neem leaf extract treatment which showed 51.7, 56.6, and 62.2 per cent inhibition at 5, 10, and 15 per cent concentrations.

The next best treatment was garlic clove extract which inhibited the colony growth by 49.8, 54.7 and 61.4 per cent at 5, 10 and 15 per cent concentration. Tulsi leaf extract inhibited the growth of the pathogen by 37.5, 44.9 and 55.1 per cent at 5, 10 and 15 per cent respectively. Sunflower leaf extract inhibited the growth of colony by 30.7, 31.8 and 35.6 per cent at 5, 10 and 15 per cent concentration respectively. Least colony inhibition was observed in sapota leaf extract treatment with 25.1, 29.6 and 31.1 per cent colony inhibition at 5, 10 and 15 per cent concentrations.

Similar results were recorded by Shalini *et al.* (2014) against *F. oxysporum* f. sp. *ricini* and reported inhibition of colony by neem, garlic and tulsi extracts. Vahunia *et al.* (2017) reported inhibition by neem and tulsi extracts on *F. oxysporum* f. sp. *ricini*. Anil *et al.* (2015) reported inhibition by neem leaf extract against *Fusarium oxysporum* f. sp. *lycopersici*. Riaz *et al.* (2008) reported sunflower leaf extract effectively inhibited colony growth of *Fusarium oxysporum* f.sp. *gladioli*. Karim *et al.* (2008) reported inhibitory effect of sapota leaf extract on *Fusarium sp.*

4.6.2 Evaluation of soil amendments

Five soil amendments namely vermicompost, groundnut cake, cotton cake, coconut kernel cake and safflower cake were tested for their effect against *F. oxysporum* f. sp. *ricini* and the results are presented in Table 4.9, Plate. 9 and Figure 4.5

All the treatments significantly inhibited mycelial growth of the fungus at all the concentrations tested. Vermicompost showed highest colony inhibition of 28.0, 35.5, 44.9 per cent at 5, 10, 15 per cent concentrations followed by cotton cake extract with an inhibition of 14.9, 17.2, 19.5 at 5, 10, 15 per cent respectively. Coconut cake extract inhibited the growth of colony by 13.11, 14.9, 16.5 percent at 5, 10 and 15 per cent respectively and was third best treatment, ground nut cake extract inhibited colony by 12.3, 13.8, 18.0 per cent at 5, 10 and 15 per cent respectively and the least effective treatment was safflower seed cake extract which inhibited colony growth by 12.7, 16.1 and 17.2 per cent at 5, 10 and 15 per cent respectively.

Mahalakshmi *et al.* (2013) reported that vermicompost considerably inhibited colony growth, oil cakes showed least colony inhibition. Yelmane *et al*. (2010) reported inhibitory effect of soil amendments against *F. solani*.

4.6.3 Evaluation of fungicides

Ten fungicides *viz.*, Propineb 61.3 %+ Iprovalicarb 5.5 %, Pyraclostrobin 5 % + metiram 55% WG, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole + trifloxystrobin 75 WG, Hexaconazole 5% + captan 70%, Carboxin 37.5% + Thiram 37.5% WP, Thiophanate methyl 70% WP, Difenconazole, Propiconazole, Carbendazim were evaluated under *in vitro* condition against *F. oxysporum* f. sp. *ricini.* The data on per cent inhibition of radial growth of the fungus is presented in Table 4.10, Plate. 10 and Figure 4.6.

Among ten fungicides evaluated, all the fungicides were significant in inhibiting the pathogen at all the concentration tested. Three fungicides *viz* Carbendazim, Carbendazim 12% + Mancozeb 63% WP, Difenconazole inhibited hundred per cent colony growth at all the concentrations, followed by Tebuconazole + trifloxystrobin 75 WG, which inhibited the growth of the mycelium by 89.4 per cent at 500 ppm concentration, 100 per cent at 1000 ppm and 2000 ppm respectively. Carboxin 37.5% + Thiram 37.5% WP inhibited colony growth by 88.2, 89.0, 100 per cent at 500 ppm, 1000 ppm, 2000 ppm. Similarly, Propiconazole inhibited the pathogen by 89.0, 90.2 and 97.0 per cent at 500 ppm, 1000 ppm and 2000ppm respectively, while Hexaconazole 5% + Captan 70% inhibited the growth by 81.4, 85.6 and 88.3 percent at all the concentrations. Thiophanate methyl 70% WP inhibited colony growth by 73.8, 81.8, 90.2 per cent at 500 ppm, 1000 ppm, 2000 ppm respectively. Least per cent inhibition was recorded by Pyraclostrobin 5% + Metiram 55% WG by 51.0, 62.1, 68.2 per cent, Propineb 61.3% + Iprovalicarb 5.5% by 47.5, 51.1, 60.2 per cent at 500 ppm, 1000 ppm, 2000 ppm respectively.

Similar results were obtained by of Ravichandran and Hegde (2015) against *Fusarium oxysporum* f. sp. *ciceri*, Baliya and Jadeja, (2014) *Fusarium solani,* Singh (2016) against *Fusarium oxysporum* and *Fusarium solani,* Patra and Biswas (2016) against *Fusarium oxysporum* f. sp. *ciceri.*

4.7 Evaluation of botanicals, soil amendments and fungicides against *F. oxysporum* f. sp. *ricini* in glass house

4.7.1 Evaluation of botanicals

A pot culture experiment was conducted to study the effect of botanicals *viz.*, garlic, sunflower, sapota, neem and tulsi against *Fusarium* wilt of castor in cultivar GCH-4 in glass house by combination of seed treatment and soil drenching. The experiment was conducted in randomised block design and replicated four times. Data on per cent disease incidence was collected and the results are presented in Table 4.11, Plate. 11 and Figure 4.7

Among the five treatments garlic clove extract; neem leaf extract and tulsi leaf extract were statistically on par with each other and recorded lowest disease incidence of 42.7, 43.3 and 45.0 per cent respectively with reduction in disease incidence by 50.0, 49.3 and 47.3 per cent respectively over control. Treatments *viz.*, sunflower leaf extract and sapota leaf extract were also statistically on par with each other and recorded disease incidence of 65.5 % and 66.2 % respectively and showed 23.4 and 22.5 per cent reduction in disease over control.

Magar *et al*, (2014) reported neem and garlic clove extracts were effective in reducing Fusarium wilt of chickpea significantly. Nwachukwu and Umechuruba (2001) reported neem and tulsi leaf extract effectively reduced disease caused by *Fusarium moniliforme* on African yam bean both under *in vivo* and *in vitro* conditions. Singh (2016) reported that soil application of garlic extract effectively reduced Fusarium wilt in chilli. Riaz *et al*, (2008) found that sunflower leaf extract reduced Fusarium wilt incidence of gladiolus under glass house condition.

Higher plants are large reservoirs of antifungal compounds, being biodegradable, are considered valuable in disease resistance (Okigbo and Ajalie, 2005; Siva *et al*., 2008). Flavonoids, isoflavonoids, glycosides, tanins, coumarins, terpenes, alkaloids and phenolic compounds are secondary metabolites synthesized by plants which have toxic effect on pathogenic micro organisms and induce systemic resistance response in plants against pathogens. In the present study, inhibitory effect of botanicals against *F. oxysporum* f. sp. *ricini* may be due to the presence of antifungal compounds in the plant extracts of neem, garlic and tulsi. Inhibitive and disease suppression action of *A. sativum* bulb extract has been attributed to a component allicin having strong antimicrobial activity against plant diseases.

4.7.2 Evaluation of soil amendments

Soil amendments *viz.,* Vermicompost, groundnut cake, cotton cake, coconut kernel cake and safflower cake were used in the present study to find out the effect of soil amendments in inhibiting the pathogen *F. oxysporum* f. sp. *ricini* on cultivar GCH-4 by under glass house conditions and the experimental results are presented in Table 4.12, Plate. 12 and Figure 4.8.

Among the treatments vermicompost was highly effective in reducing the disease incidence by 47.5 per cent, followed by cotton cake 60 per cent. The rest of amendments groundnut cake, coconut kernel cake and safflower cake were statistically on par with each other and recorded disease incidence of 62.7, 63.2 and 64.0 per cent, respectively by reducing disease incidence to 26.6, 26.0 and 25.1 per cent respectively over control.

Similar results were obtained by Magar *et al.* (2014) and reported cotton cake and ground nut cake were effective in reducing Fusarium wilt of chickpea in field conditions. Patra *et al.* (2017) reported that vermicompost and groundnut cake were effective in reducing Fusarium wilt of chickpea.

Organic amendments are known to produce volatile and non volatile substances during their decomposition which in turn reduce the disease incidence and also stimulate resident and introduced antagonists (Lumsden *et al.,* 1983).

4.7.3 Evaluation of fungicides

The efficacy of ten fungicides *viz.,* Propineb 61.3 %+ Iprovalicarb 5.5 %, Pyraclostrobin 5 % + metiram 55% WG, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole + trifloxystrobin 75 WG, Hexaconazole 5% + captan 70%, Carboxin 37.5% + Thiram 37.5% WP, Thiophanate methyl 70% WP, Difenconazole, Propiconazole, Carbendazim was tested under glass house conditions by seed treatment and soil drenching at recommended dosage and results are presented in Table 4.13, Plate. 13 and Figure 4.9.

All the treatments were found effective in reducing disease incidence. Least disease incidence was recorded in Carboxin 37.5% + Thiram 37.5% WP with 20.3 per cent disease incidence and reduction in disease incidence by 76.5 per cent over control. Carbendazim 12% + Mancozeb 63% WP was found to be the next best treatment recording disease incidence of 25.1 per cent with 71 per cent reduction of disease over control. Carbendazim, Hexaconazole 5% + captan 70%, propiconazole and Thiophanate methyl 70% WP treatments showed similar disease incidence of 31.5, 32.1, 33.2 and 33.0 per cent respectively and exhibited 63.7, 63, 61.7 and 61.9 per cent reduction in disease incidence over control. Fungicide difenconazole could reduce the disease incidence by 54.4 per cent recording disease incidence of 39.5 per cent. Maximum disease incidence was recorded by Propineb 61.3 %+ Iprovalicarb 5.5 %, Pyraclostrobin 5 % + metiram 55% WG, with 60.2 and 58.9 % respectively with only 30.6 and 32.1 % reduction of disease over control.

Similar results were recorded by Singh (2016) against *Fusarium oxysporum* and *Fusarium solani*, both under field and glass house conditions. Similarly, Mukesh *et al*. (2017b) reported against *Fusarium oxysporum* f. sp. *corianderii*, Patra and Biswas. (2016) against *Fusarium oxysporum* f. sp *ciceri.*

Effectiveness of these fungicides is attributed to their mode of action which is different from one another Carbendazim and Thiophanate methyl are benzimidazole group of fungicides; they interfere with energy production and cell wall synthesis of fungi. According to Davidse (1986) carbendazim induced nuclear instability by disturbing the mitosis and meiosis. The effectiveness of the triazole fungicides propiconazole, hexaconazole, defenconazole and tebuconazole is attributed to their interference with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. A similar study was reported for the effectiveness of triazoles, which inhibit the sterol biosynthesis pathway in fungi (Nene and Thapliyal, 1973). Vitavax belongs to oxanthiin group and is known to inhibit succinic dehydrogenase, an enzyme important for mitochondrial respiration (Agrios, 2005)

4.8 Integrated disease management of castor wilt

4.8.1 Evaluation of combination of different management practices on Fusarium wilt under field conditions

An integrated disease management approach was formulated to manage the Fusarium wilt of castor by employing soil solarisation, dazomet application (30-40 g/m^2) and neem cake application (1.78 kg/m^2) as main treatments. Seed treatment (10g/kg) and soil application (1 kg/100 kg FYM) of *Trichoderma harzianum* Th4d WP, seed treatment with carbendazim (2g/kg seed) and soil application of carbofuron (2kg a.i./ha) were used as sub treatments in the present study and the results are presented in Table 4.14 and Plate. 14

Perusal of the data showed that all the main treatments *i.e.* soil solarisation, dazomet application (30-40 g/m^2) and neem cake application (1.78 kg/m^2) to soil were significant in reducing the disease incidence of castor wilt with seed treatment (10g/kg) and soil application (1 kg/100 kg FYM) of *Trichoderma harzianum* Th4d WP, seed treatment with carbendazim (2g/kg seed) and soil application of carbofuron (2kg a.i./ha) were applied when compared to control.

However, at 30 DAS it was observed that though there was a reduction in disease incidence the interaction among the main treatment and sub treatment were not significant.

Significant effect of the treatment was observed from 60 DAS onwards. Seed treatment of carbendazim was highly significant in reducing the disease incidence in soil solarised plots with lowest wilt incidence of 6.4 per cent, followed by seed treatment and soil application of *T. harzianum* Th4d WP (7.8 %) and soil application of carbofuron granules (9.2 %) while control recorded 20.3 per cent wilt incidence. In case of plots fumigated with dazomet seed treatment of carbendazim, seed treatment and soil application of *T. harzianum* Th4d WP recorded similar disease incidence of 24.9 and 24.8 per cent, while soil application of carbofuron granules recorded wilt incidence of 40.2 per cent. The experimental plots applied with neem cake recorded lowest wilt incidence in seed treatment and soil application of *T. harzianum* Th4d WP (16.7 %) and seed treatment with carbendazim (17.7 %). Soil application of carbofuron recorded wilt incidence of 23.9 per cent where as in control wilt incidence of 32.4 per cent was observed. In plots maintained as control for main treatments and applied with only sub treatments, seed treatment with carbendazim recorded lowest wilt incidence of 45.4 per cent, followed by seed treatment and soil application of *T. harzianum* Th4d WP (47.1 %) and soil application of carbofuron (49.5 %) when compared to control (51.9 %).

Similar trend was observed in soil solarised plots at 90 DAS recording wilt incidence in seed treatment with carbendazim (11.7 %), followed by seed treatment and soil application of *T. harzianum* Th4d WP (14.4 %) and soil application of carbofuron (25.4 %). In case of plots fumigated with dazomet least disease incidence among all sub treatment was observed. Seed treatment with carbendazim recorded wilt incidence of 34.0 per cent followed by seed treatment and soil application of *T. harzianum* Th4d WP with 39.7 per cent disease incidence and soil application of carbofuron granules recorded 64.7 per cent wilt incidence. In plots applied with neem cake lowest wilt incidence was recorded in case of seed treatment and soil application of *T. harzianum* Th 4d WP (20.9 %), followed by seed treatment with carbendazim 23.7 per cent wilt incidence, soil application of carbofuron with wilt incidence of 38.1 per cent and control recorded 42.6 per cent. The control plot recorded lowest wilt incidence in seed treatment with carbendazim (58.3 %), followed by seed treatment and soil application of

T. harzianum Th4d WP with 62.7 per cent, followed by soil application of carbofuron 65.8 per cent and control recorded 74.7 per cent wilt.

Similarly, after harvesting of in case of soil solarised plots seed treatment with carbendazim and seed treatment and soil application of *T. harzianum* Th4d WP recorded wilt incidence of 21.9 and 22.4 per cent respectively, followed by soil application of carbofuron with 33.7 per cent and control 36.7 per cent wilt incidence. In case of plots fumigated with dazomet seed treatment with carbendazim recorded lowest wilt incidence of 46.8 per cent, followed by seed treatment and soil application of *T. harzianum* Th4d WP with 33.6 per cent wilt, soil application of carbofuron recorded 50.3 per cent and control 54.4 per cent wilt. In control plot seed treatment with carbendazim recorded lowest wilt incidence of 60.9 per cent, followed by seed treatment and soil application of *T. harzianum* Th4d WP with 68.5 per cent wilt, soil application of carbofuron with 86.9 per cent and control 96.4 per cent.

It is inferred from above results that lowest wilt incidence was recorded in combinations of soil solarisation with seed treatment with carbendazim, seed treatment and soil application of *T. harzianum* (Th4d), soil application of carbofuron and soil solarisation alone compared to other main treatments. Among sub treatments soil solarisation with carbendazim was found most effective when compared to *T. harzianum* (Th4d) and carbofuron, followed by seed treatment and soil application of *T. harzianum* (Th4d). Soil solarisation along with application of carbofuron also reduced disease incidence effectively. Soil application of neem cake alone and in combination was effective in controlling the disease. Neem cake in combination with seed treatment and soil application of *Trichoderma* was best combination, followed by neem cake application and seed treatment of carbendazim. Soil application of carbofuron along with neem cake application also controlled disease considerably. Soil fumigation with dazomet alone and in combination was comparatively less effective. Dazomet application with seed treatment with carbendazim was best among the combinations followed by seed treatment and soil application of Trichoderma.

Studies of Chakraborty *et al*. (2009) reported that soil solarisation with low doses of bavistin (Carbendazim) was best treatment and gave maximum disease control of Fusarium wilt of egg plant. Raj and Kumar (2009) reported that combination of soil solarisation with *Trichoderma* gave superior control of Fusarium wilt of gladiolus when compared to treatments applied singly. Saravanan *et al.* (2003) also reported that *Trichoderma viride* applied as soil or sucker dipping or their combinations or along with the neem cake also had a significant reduction in Fusarium wilt disease of Banana.

Animisha *et al*. (2012) found that combination of carbendazim and neem cake was effective in controlling Fusarium wilt of chickpea. Slusarski and Pietr (2009) combination of dazomet and *Trichoderma asperellum* was effective in controlling *Verticillium* wilt of bell pepper.

4.8.2 Seed yield

Combination of treatments and sub treatments significantly influenced seed yield of crop. Soil solarisation and seed treatment with carbendazim recorded highest yield of 4036.7 Kg ha^{-1}, followed by soil solarisation with seed treatment and soil application of *T. harzianum* recorded (3291.7 Kg ha^{-1}). Yield of 2435 and 2428.3 Kg ha^{-1} was recorded from plots treated with soil solarisation + soil application of carbofuron, Neem cake application + Seed treatment soil application of *T. harzianum* respectively, (Table 4.15). Similar results were recorded from studies of Raoof and Rao (1997) and Desai and Dange (2003) who recorded highest yield in soil solarised treatment in wilt sick plot of castor.

4.8.3 Effect of main treatments on population of Fusarium, *Trichoderma* and Actinomycetes

To determine the effects of main treatments on soil microflora employed in integrated disease management soil samples were collected from their respective plots at three intervals *i.e.* before imposing the treatments, 30 days after sowing and after harvesting.

4.8.3.1 Enumeration of *Fusarium* colonies (cfu x 10^3 g^{-1} soil)

The data pertaining to Table 4.16 and Plate 15 indicated that white coloured *Fusarium* colonies were enumerated on FSM. Among all the treatments maximum reduction in *Fusarium* colonies was found in plots employed with soil solarisation and soil application of neem cake. Initial colonies of 2.3 cfu x 10^3 g^{-1} soil was recorded in both the plots before application of treatments. But after 30 days after sowing there was drastic reduction in *Fusarium* colonies to 0.3 cfu x 10^3 g^{-1} soil was observed. However, after harvesting of crop there was slight increase in number of colonies in both the treatments soil solarisation (0.6 cfu x 10^3 g^{-1} soil) and neem cake treatments (1.2 cfu x 10^3 g^{-1} soil). Significant reduction in *Fusarium* colonies was also observed in soil application of dazomet treatment from 2.3 cfu x 10^3 g^{-1} before application of treatment to 1.8 cfu x 10^3 g^{-1} soil at 30 days of crop and *Fusarium* colonies increased to 1.2 cfu x 10^3 g^{-1} soil after crop harvesting. In control plot initial *Fusarium* colony count was 2.5 cfu x 10^3 g^{-1} soil which increased to 2.9 cfu x 10^3 g^{-1} soil at 30 days of crop and further increased to 3.6 cfu x 10^3 g^{-1} soil after harvesting of crop.

It is inferred from above results that in case of soil solarisation treatment pathogen population was reduced by 73.91 per cent by end of crop season followed by neem cake treatment by 47.82 per cent. In case of dazomet treatment only 4.34 per cent reduction in pathogen population was observed after harvesting of crop. Hence, the disease incidence was very high and appeared very early in crop season. In control plot 30 the increase in pathogen population was recorded as 30.55 per cent by end of crop season.

The results obtained were in correspondence with results of Raoof and Rao, (1997), who reported 35 per cent reduction in population of *Fusarium* by soil solarisation of wilt sick plots of castor by end of crop season. Similarly, Desai and Dange (2003) reported the reduction in *Fusarium* population in wilt sick soils of castor. The suppressive ability of neem cake in inhibiting growth of soil borne pathogens was reported by Patra *et al*, (2017).

Soil covering with transparent polyethylene films, combined with abundant watering, makes it possible to heat soil by sun irradiation to temperatures lethal for many soil-borne pathogens; this destroys resting spores (chlamydospores) of Fusarium thus reducing innoculum density in soil. The suppressive ability of neem organic amendment is through competition, antibiosis due to increase of soil beneficial microbial populations.

4.8.3.2 Enumeration of *Trichoderma* colonies (cfu x 10^3 g^{-1} soil)

Green coloured colonies of *Trichoderma* were enumerated on TSM (Plate. 15). All the treatments showed increase in *Trichoderma* colonies when used as seed treatment and soil application as one of the sub treatment (Table 4.17).

In case of soil solarisation employed plot the initial colony count from 0.2 x 10^3 cfu g^{-1} soil increased to 2.6 x 10^3 cfu g^{-1} soil and there after the colonies were reduced to 1.3 x 10^3 cfu g^{-1} soil after the harvesting of the crop. The colony count of *Trichoderma* in neem cake treated plot 0.8 x 10^3 cfu g^{-1} soil was increased to 4.8 x 10^3 cfu g^{-1} soil initially at 30 days crop stage. But slight decrease in number of colonies was recorded (3.8 x 10^3 cfu g^{-1} soil) after crop harvesting. Similar trend was observed in dazomet applied plots where in the number of colonies increased from 0.4 x 10^3 cfu g^{-1} increased to 1.9 x 10^3 cfu g^{-1} soil at 30 days stage of the crop but later reduced to 0.7 x 10^3 cfu g^{-1} soil after crop harvesting. In control initially, *Trichoderma* colonies increased from 0.2 x 10^3 cfu g^{-1} soil to 1.2 x 10^3 cfu g^{-1} soil, drastic reduction to 0.2 x 10^3 cfu g^{-1} soil was observed after crop harvesting.

It is inferred from above results that highest increase in *Trichoderma* colonies was observed in soil solarisation treatment up to 92.3 per cent at 30 days of crop stage and it was reduced by 50 per cent after crop harvest. In case of neem cake treatment 83.3 per cent increase in *Trichoderma* colonies were observed initially at 30 days of crop and 20.8 per cent reduction in colonies were observed after crop harvest. In case of dazomet treatment initial increase of 78. 9 per cent at 30 days of crop and these colonies were reduced by 63.1 per cent after harvesting of crop.

Increase in colonies of *Trichoderma* may be due to application of *Trichoderma* as seed treatment and soil application along with FYM as one of the sub treatment in all the main treatments employed. But further decrease in *Trichoderma* colonies in all the treatments was in line with findings of Elena and Tjamos (1992) who reported that the failure of the fungal antagonist could be justified by the high inoculum level of the pathogen in the wilt experimental field.

4.8.3.3 Enumeration of Actinomycetes colonies (cfu x 10^3 g^{-1} soil)

White depressed colonies resembling bacterial colonies were enumerated on actinomycetes specific media (Plate. 15). Among the treatments improved increase in number of actinomycetes colony was observed in neem cake treatment from initial 16.8 x 10^3 cfu g^{-1} soil to 26 x 10^3 cfu g^{-1} soil after harvesting of crop. In case of soil solarisation treatment decrease in actinomycetes colonies was observed from initial 18 x 10^3 cfu g^{-1} soil to 8.3 x 10^3 cfu g^{-1} soil after harvesting of crop. In case of dazomet and control treatments actinomycetes population remained unaffected. Details of experiment are given in Table 4.18.

Increase in actinomycetes in case of neem cake treatment was in line with findings of Krishnakumar *et al.* (2005) and Parag (2011). Decrease in actinomycetes colonies in soil solarisation treatment was similar with findings of Sofi (2014) and Gamliel and Katan (1990).

Neem cake contains more amounts of secondary and micronutrients which help in increase in microbial population.

4.8.4 Effect of main treatments on soil physio-chemical properties.

Soil samples collected from main treatment plots were analysed for their physical and chemical properties.

Among physical and chemical soil properties tested soil available nitrogen and phosphorous showed significance difference among treatments as given in Table 4. 19 (a) and 4.19 (b). In case of neem cake applied plot there was an increase in both soil available nitrogen and phosphorous. In case of soil solarisation significant increase in soil available nitrogen was observed but there was significant decrease in soil available phosphorous.

The present findings in line with of Kumar *et al.* (2017), Radwan and Mohammad (2011) and Sharma *et al.* (2002) who reported increase in soil available nitrogen by neem cake and soil solarisation treatments in different Indian soils and reduction in phosphorous content of soil by soil solarisation.

Neem cake a soil amendment is rich in N, P, K and other micro nutrients. Elnasikh *et al.* (2011) reported role of neem cake in increasing population of *Nitrosomonas* and *Nitrobacter*. Solarization may increase the levels of available mineral nutrients in soils by breaking down soluble organic matter.

Chapter- V

SUMMARY AND CONCLUSION

Chapter V

SUMMARY AND CONCLUSIONS

The outcome of the present investigation about the variability in *F. oxysporum* f.sp *ricini* isolates associated with the disease and its management is summarized here.

To assess the variability, diseased samples were collected from castor growing locations of Karnataka (Hiriyur) and Telangana (Palem, Bijanapalli, Narkuda, Rajendrnagar). Pathogen isolates were identified as *Fusarium oxysporum* f.sp. *ricini* based on cultural, morphological characters. Variability among collected isolates was studied through cultural, morphological characters, pathogenicity test and using molecular marker (ISSR).

Cultural and morphology studies revealed that variation among isolates of *F. oxysporum* f. sp. *ricini*. Biggest sized macro conidia were produced by isolates *For* 4, *For* 5 (31.46x2.86 µm), smallest was by *For* 1 (27.17x1.43 µm). Two isolates *For* 1 and *For* 3 showed radial growth of 9 mm and completely filled the plate while the rest of the isolates (*For* 2, *For* 6, *For* 7, *For* 8, *For* 9, *For* 10, *For* 11, *For* 12 and *For* 13) showed variation in their growth. Isolate *For* 4 and *For* 5 showed extreme variation in growth pattern, distinct sectoring was observed. Only *For* 6 and *For* 13 produced scanty fibrous growth of mycelium remaining isolates were grown by producing abundant fluffy aerial mycelium. Isolates *For* 1, *For* 2, *For* 4, *For* 5, *For* 6, *For* 9, *For* 10, *For* 11, *For* 12 and *For* 13 colonies appeared rough. While, two isolates *For* 7 and *For* 8 appeared smooth surface on media. Variation in shape was observed as round (isolates *For* 1, *For* 2, *For* 3, *For* 11, *For* 12 and *For* 13), irregular wavy (*For* 7, *For* 9 and *For* 10) sectoring / saltation (*For* 4 and *For* 5). The colonies also varied in their growth margins with uniform spread (*For* 1, *For* 2, *For* 3, *For* 11 and *For* 12), concentric circles of mycelial growth showing many rings in colony (*For* 6, *For* 7, *For* 9, *For* 10 and *For* 13) and Isolates *For* 6 and *For* 8 had filamentous growth which spread towards periphery. Variation in the mycelim colour was observed among the isolates. The pigmentation of the colony was observed as white (*For* 1, *For* 4, *For* 5, *For* 13), light buff (*For* 2, *For* 3 and *For* 12), yellow (*For* 11), light pinkish purple (*For* 8), light purple (*For* 7) and pink in (*For* 6), purple (*For* 9) and dark purple (*For* 10). Pigmentation was restricted to centre of the colony in isolates *For* 6, *For* 7, *For* 8, *For* 9, *For* 11 and *For* 12 and pigmentation was uniform in isolates *For* 3 and *For* 10.

According to the pathogenic reaction of the isolates to different castor cultivars, isolates were classified into four groups. Group I consists of highly virulent isolates *For*

10, *For* 9, *For* 8, *For* 7 and *For* 6 which were pathogenic to all the cultivars except 48-1 and DCS-107. Group II consists of virulent isolates *For* 3 and *For* 4 were pathogenic to cultivars Haritha, Kranthi, JI-35 and GCH-4 and cultivars 48-1, DCS-107 and DCS-9 were resistant to these isolates. Group III consists of moderately virulent isolates *For* 11 and *For* 13 which caused susceptible reaction only in cultivars Kranthi and JI-35. Isolates *For* 12, *For* 5, *For* 2 and *For* 1 were pathogenic only to JI-35 which is highly susceptible cultivar and belonged to group IV, indicating the existence of variability in the pathogenic behavior of the isolates.

Genetic variability among 13 isolates of *F. o. ricini* was studied using four ISSR primers. Isolates were grouped into four groups, Group I consists of isolates *For* 11, *For* 12, which were collected from Devarkadra. Group II included *For* 13, *For* 4 collected from Devarkadra and Hiriyur, Group III consisted of only one isolate *For* 10 from IIOR. Isolates *For* 3, *For* 9, *For* 8, *For* 7, *For* 6, *For* 5, *For* 2, and *For* 1 in Group IV which were collected from different places Narkuda (*For* 3 and *For* 2), Palem (*For 8*), Bijanapalli (*For 6, For 7, For 9*), Hiriyur (*For 5 and For 1*). This ISSR analysis showed high genetic variation among isolates of *F. oxysporum* f.sp *ricini*. Grouping of isolates according to ISSR primers could not be correlated with area of collection, pathogenicity of isolates or colony characteristics.

In vitro efficacy of botanicals, soil amendments and fungicides was studied on inhibition of mycelial growth of *F. oxysporum* f. sp *ricini* among botanicals neem (51.7, 56.6, and 62.2%), garlic (49.8, 54.7 and 61.4%) and tulsi (37.5, 44.9 and 55.1 %) were found most effective followed by sunflower (30.7, 31.8 and 35.6 %) and sapota (25.1, 29.6 and 31.1 %) inhibiting mycelial colony by per cent inhibition at 5, 10, and 15 per cent concentrations. Vermicompost showed colony inhibition of 28.0, 35.5, 44.9 per cent at 5, 10, 15 per cent concentrations was the best treatment among soil amendments used, followed by cotton cake (14.9, 17.2, 19.5 %), Coconut cake (13.11, 14.9, 16.5 %) groundnut cake extract (12.3, 13.8, 18.0 %) at 5, 10 and 15 per cent respectively. Least effective was safflower seed cake (12.7, 16.1 and 17.2 %) at 5, 10 and 15 per cent respectively. Among ten fungicides evaluated, three fungicides *viz* Carbendazim, Carbendazim 12% + Mancozeb 63% WP, Difenconazole completely inhibited the colony growth at all the concentrations, followed by Tebuconazole trifloxystrobin 75 WG, which inhibited 89.4 per cent at 500 ppm concentration and 100 per cent at 1000 ppm and 2000 ppm respectively. Carboxin 37.5% + Thiram 37.5% WP inhibited colony growth by 88.2, 89.0, 100 per cent at 500 ppm, 1000 ppm, 2000 ppm. Similarly

Propiconazole (89.0, 90.2 and 97.0 %), Hexaconazole 5% + captan 70% (81.4, 85.6 and 88.3 %), Thiophanate methyl 70% WP (73.8, 81.8, 90.2 %) at 500 ppm, 1000 ppm and 2000ppm respectively, Least per cent inhibition was recorded by Pyraclostrobin 5% + metiram 55% WG (51.0, 62.1, 68.2 %) and Propineb 61.3% + Iprovalicarb 5.5% (47.5, 51.1, 60.2 %) at 500 ppm, 1000 ppm, 2000 ppm respectively.

Botanicals, soil amendments and fungicides were tested for their efficacy in reducing disease incidence of castor wilt. Among botanicals garlic clove extract; neem leaf extract and tulsi leaf extract recorded lowest disease incidence of 42.7, 43.3 and 45.0 per cent respectively and were effective in reducing disease. Least effective were sunflower and sapota leaf extracts with disease incidence of 65.5 % and 66.2 % respectively. Among soil amendments vermicompost was highly effective and recorded lowest disease incidence of 47.5 per cent, followed by cotton cake 60 per cent. The rest of amendments groundnut cake, coconut kernel cake disease incidence of 62.7, 63.2 and 64.0 per cent respectively. Among the fungicides tested least disease incidence was recorded in Carboxin 37.5% + Thiram 37.5% WP with 20.3 per cent disease incidence followed by Carbendazim 12% + Mancozeb 63% WP (25.1 %). Carbendazim, Hexaconazole 5% + captan 70%, propiconazole and Thiophanate methyl 70% WP treatments showed similar disease incidence of 31.5, 32.1, 33.2 and 33.0 per cent respectively. Fungicide difenconazole recording disease incidence of 39.5 per cent. Maximum disease incidence was recorded by Propineb 61.3 %+ Iprovalicarb 5.5 %, Pyraclostrobin 5 % + metiram 55% WG, with 60.2 and 58.9 % respectively.

Study conducted on integrated disease management revealed that lowest wilt incidence was recorded in combinations of soil solarisation with seed treatment with carbendazim, seed treatment and soil application of *T. harzianum* (Th 4d), soil application of carbofuron and soil solarisation alone compared to other main treatments. Among subtreatments soil solarisation with carbendazim was found most effective when compared to *T. harzianum* (Th 4d) and carbofuron, followed by seed treatment and soil application of *T. harzianum* (Th 4d). Soil solarisation along with application of carbofuron also reduced disease incidence effectively. Soil application of neem cake alone and in combination was effective in controlling the disease. Neem cake in combination with seed treatment and soil application of Trichoderma was best combination, followed by neem cake application and seed treatment of carbendazim. Soil application of carbofuron along with neem cake application also controlled disease considerably. Soil fumigation with dazomet alone and in combination was comparatively less effective. Dazomet application with seed treatment with carbendazim was best among the combinations followed by seed treatment and soil application of Trichoderma.

Maximum reduction in pathogen population was recorded in soil solarisation treatment by 73.91 per cent, followed by neem cake treatment by 47.82 per cent. In control plot 30.55 per cent increase pathogen population was recorded by end of crop season. Highest increase in Trichoderma colonies was observed in soil solarisation treatment up to 92.3 per cent at 30 days of crop stage and it was reduced by 50 per cent after crop harvest. In case of neem cake treatment 83.3 per cent increase in Trichoderma colonies were observed initially at 30 days of crop and 20.8 per cent reduction in colonies were observed after crop harvest. In case of dazomet treatment initial increase of 78.9 per cent at 30 days of crop and these colonies were reduced by 63.1 per cent after harvesting of crop. Increase in number of actinomycetes colony was observed in neem cake treatment from initial 16.8 cfu x 10^3 g^{-1} soil to 26 cfu x 10^3 g^{-1} soil after harvesting of crop. In case of soil solarisation treatment decrease in actinomycetes colonies was observed from initial 18 cfu x 10^3 g^{-1} soil to 8.3 cfu x 10^3 g^{-1} soil after harvesting of crop. In case of dazomet and control treatments actinomycetes population remained unaffected.

In case of neem cake applied plot there was an increase in both soil available nitrogen and phosphorous. Increase in soil available nitrogen was also observed in soil solarised plots.

In conclusion, the present study indicated the presence of intra species variation in isolates of *F. oxysporum* f.sp *ricini,* morphological and cultural characteristics among the isolates will contribute in the identification of pre-existing or any evolved pathogenic forms and will also be helpful in their taxonomic classification. The information regarding pathogenic variability among the isolates will be helpful in understanding the distribution of most prevalent races/pathotypes in India. DNA banding pattern of ISSR markers and diversity analyses will be further useful for identification and differentiation of the *F. oxysporum* f. sp. *ricini* isolates. The management study indicated soil and seed borne pathogen *F. oxysporum* f. sp. *ricini* can be effectively managed by integration of physical, cultural, biological and chemical disease management practices.

Table 4.1 Cultural variability among isolates of *F. oxysporum* f. sp. *ricini* on PDA medium

Colony characteristics 8 DAI*								
Isolates	**Places of collection**	**Mean colony diameter (cm)**	**Colony growth**	**Texture of colony**	**Colony shape**	**Colony margin**	**Pigmentation**	**Days to pigmentation**
For 1	Marikanive, Hiriyur, Karnataka	9.0**	Fluffy	Rough	Round	Entire	White	-
For 2	Narkuda, Telangana	8.9	Fluffy	Rough	Round	Entire	Light buff	7
For 3	Narkuda, Telangana	9.0	Fluffy	Smooth	Round	Entire	Light buff	7
For 4	Harthikote, Hiriyur, Karnataka	9.0	Fluffy	Rough	Sectored	-	White	-
For 5	Uduvalli, Hiriyur, Karnataka	8.5	Fluffy	Rough	Sectored	-	White	-
For 6	Pollepalli, Bijanapalli, Telangana	8.5	Scanty fibres	Rough	Round	Filamentous	Pink	6
For 7	Gangaram, Bijanapalli, Telangana	8.5	Supressed	Smooth	Wavy	Concentric	Purplish	6
For 8	Regional Agricultural Research Centre, Palem, Telangana	8.6	Supressed	Smooth	Round	Filamentous	Light purple	6
For 9	Pollepalli, Bijanapalli, Telangana	8.6	Supressed	Rough	Wavy	Concentric	Purplish	5
For 10	IIOR, Rajendranagar, Hyderabad, Telangana	8.7	Supressed	Rough	Wavy	Concentric	Dark purple	5
For 11	Lakshmipalli, Devarkadra, Telangana	8.8	Fluffy	Rough	Round	Entire	Yellowish	7
For 12	Hajilapuram, Devarkadra, Telangana	8.8	Fluffy	Rough	Round	Entire	Light buff	7
For 13	Basanaypalli, Devarkadra, Telangana	8.8	Scanty fibers	Rough	Round	Concentric	White	-

*Days after inoculation ** mean of three replications

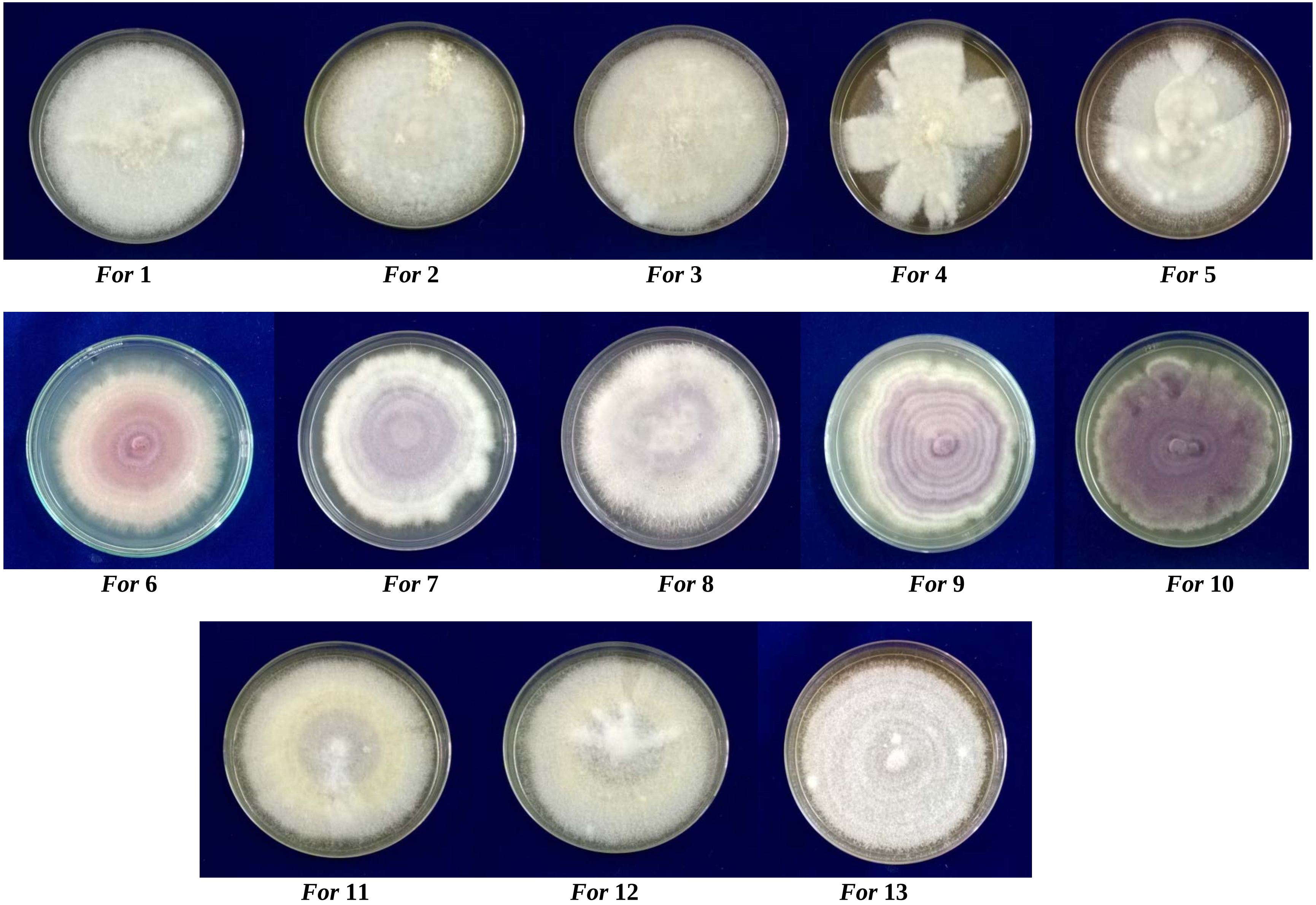


Plate 5. Pure cultures of *F. oxysporum* f. sp. *ricini* on PDA medium

Table 4.2 Morphological variability among isolates of *F. oxysporum* f. sp. *ricini*

Isolate	Microconidia size (length x width in µm)	Number of septa in Microconidia	Macroconidia size (length x width in µm)	Number of septa in Macroconidia
For 1	6.38x1.43	0	27.17x1.43	2
For 2	6.38x1.43	0	25.74x2.86	2
For 3	6.15x1.43	1	25.74x2.86	2
For 4	6.25x1.43	1	31.46x2.86	4
For 5	5.90x1.39	0	31.46x2.86	4
For 6	5.91x1.43	0	25.74x2.86	2
For 7	6.24x1.44	0	25.71x1.83	2
For 8	5.58x1.40	0	25.74x2.86	2
For 9	5.15x1.40	0	26.72x2.18	2
For 10	5.15x1.43	0	26.72x2.17	2
For 11	5.80x1.42	0	29.41x2.15	2
For 12	5.72x1.43	0	29.41x2.15	2
For 13	5.72x1.43	1	30.11x2.17	4

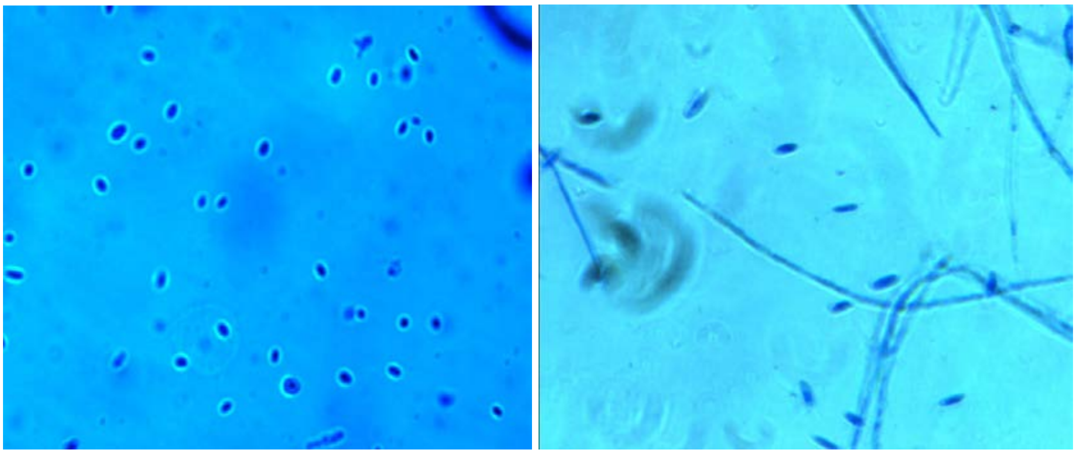


Plate 6 (a) Micro-conidia produced by isolates of *F. oxysporum* f. sp. *ricini*

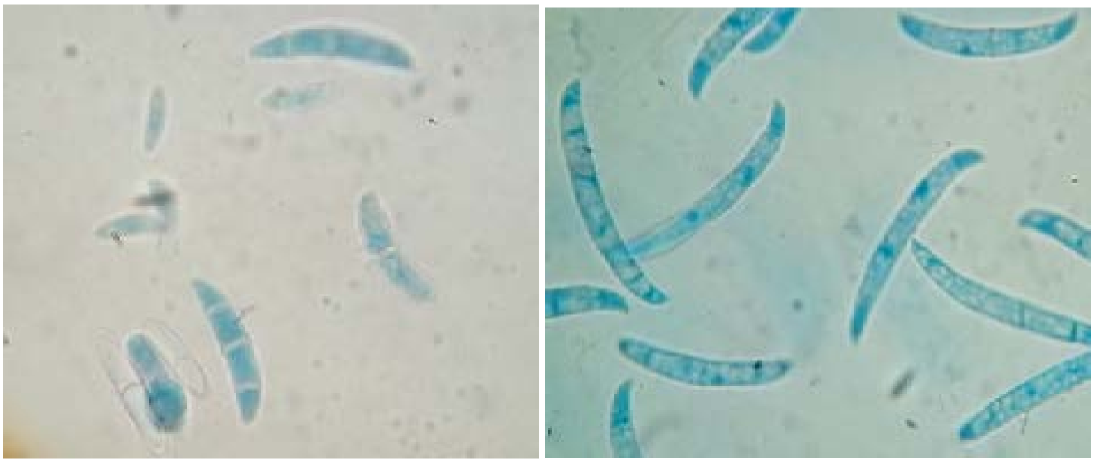


Plate 6 (b) Macro-conidia produced by isolates of *F. oxysporum* f. sp. *ricini*

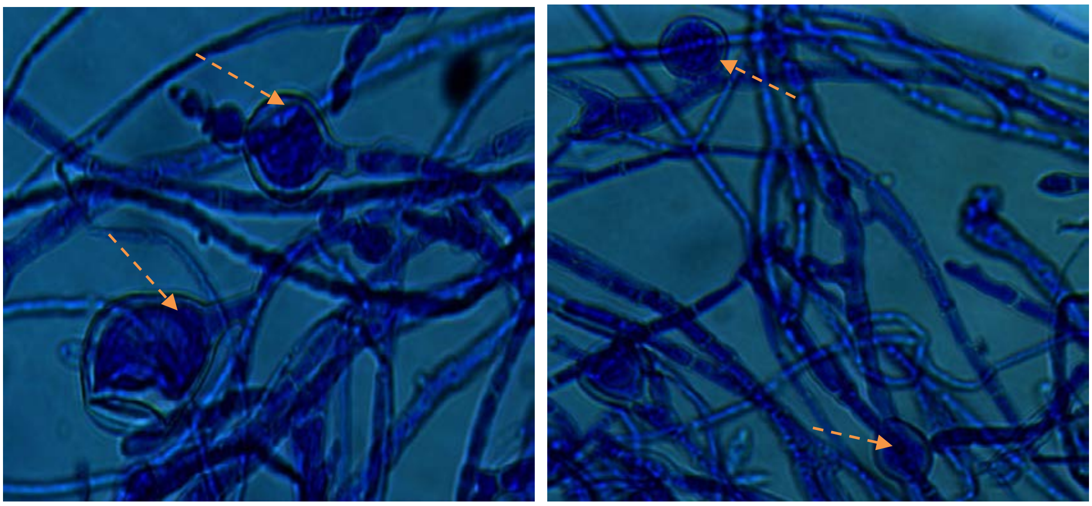


Plate 6 (c) Chlamydospors produced by isolates of *F. oxysporum* f. sp. ricini

Table 4.3 Evaluation of pathogenic variability among *F. oxysporum* f. sp. *ricini* isolates on castor cultivars by sick pot technique.

S. No	Isolates	Per cent disease incidence*						
		Castor cultivars						
		Harita	Kranthi	JI-35	48-1	DCS-107	GCH-4	DCS-9
1	*For* 1	0.0 (4.0)**	0.0 (4.0)	28.9 (32.3)	0.0 (4.0)	0.0 (4.0)	17.8 (24.9)	0.0 (4.0)
2	*For* 2	0.0 (4.0)	0.0 (4.0)	28.8 (31.5)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
3	*For* 3	35.5 (36.5)	37.8 (37.9)	37.7 (37.9)	0.0 (4.0)	0.0 (4.0)	24.4 (29.4)	0.0 (4.0)
4	*For* 4	53.3 (46.9)	72.2 (58.4)	77.8 (62.1)	11.1 (17.4)	11.1 (17.7)	47.8 (37.9)	0.0 (4.0)
5	*For* 5	16.7 (24.1)	26.7 (30.7)	38.9 (38.5)	5.60 (10.7)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
6	*For* 6	65.0 (53.8)	100.0 (85.9)	100.0 (85.9)	17.8 (24.9)	20.0 (26.5)	68.9 (52.1)	43.3 (41.1)
7	*For* 7	66.7 (54.9	100.0 (85.9)	100.0 (85.9)	12.3 (18.2)	17.8 (24.9)	70.0 (53.4)	55.5 (48.2)
8	*For* 8	75.6 (60.5)	82.2 (65.0)	100.0 (85.9)	17.8 (24.9)	13.3 (19.0)	82.2 (65.0)	38.8 (38.4)
9	*For* 9	55.6 (48.2)	100.0 (85.9)	94.4 (79.2)	16.7 (24.1)	18.9 (25.7)	83.3 (65.8)	30.5 (33.4)
10	*For* 10	47.8 (43.6)	72.2 (58.4)	93.3 (78.4)	17.8 (24.9)	6.7 (11.5)	88.9 (72.5)	27.7 (31.6)
11	*For* 11	17.8 (24.9)	38.7 (36.5)	66.7 (54.7)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	17.7 (24.9)
12	*For* 12	0.0 (4.0)	11.1 (17.4)	61.1 (51.5)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
13	*For* 13	12.2 (18.2)	24.4 (29.4)	55.5 (48.7)	0.0 (4.0)	0.0 (4.0)	18.9 (25.7)	18.8 (25.7)
	S.Em ±	3.08	2.80	4.30	3.30	3.49	2.15	1.50
	CD at 5%	8.97	8.16	12.52	9.61	10.15	6.26	4.36

*Mean of three replications

**Figures in the parentheses are angular transformed values

Table 4.4. Reaction of castor cultivars for different isolates of *F. oxysporum* f. sp. *ricini*

Isolates	Harita	Kranthi	JI-35	48-1	DCS-107	GCH-4	DCS-9
For 1	HR	HR	S	HR	HR	R	HR
For 2	HR	HR	S	HR	HR	HR	HR
For 3	S	S	S	HR	HR	S	HR
For 4	S	S	S	R	R	S	HR
For 5	R	S	S	R	HR	HR	HR
For 6	S	S	S	R	R	S	S
For 7	S	S	S	R	R	S	S
For 8	S	S	S	R	R	S	S
For 9	S	S	S	R	R	S	S
For 10	S	S	S	R	R	S	S
For 11	R	S	S	HR	HR	HR	R
For 12	HR	R	S	HR	HR	HR	HR
For 13	R	S	S	HR	HR	R	R

Table 4.5. Grouping of isolates based on disease reaction to different castor cultivars

Groups	Isolates
Group I	*For* 10, *For* 9, *For* 8, *For* 7, *For* 6
Group II	*For* 3, *For* 4
Group III	*For* 13, *For* 11
Group IV	*For* 12, *For* 5, *For* 2, *For* 1

Table 4.6 Grouping of isolates according to dendrogram generated for wilt reaction to different castor cultivars.

Groups	Sub groups	Isolates
Cluster I	Sub group I	*For* 10, *For* 9, *For* 8, *For* 7, *For* 6
	Sub group II	*For* 3 and *For* 4
Cluster II	Sub group I	*For* 12, *For* 5, *For* 2, *For* 1
	Sub group II	*For* 13 and *For* 11

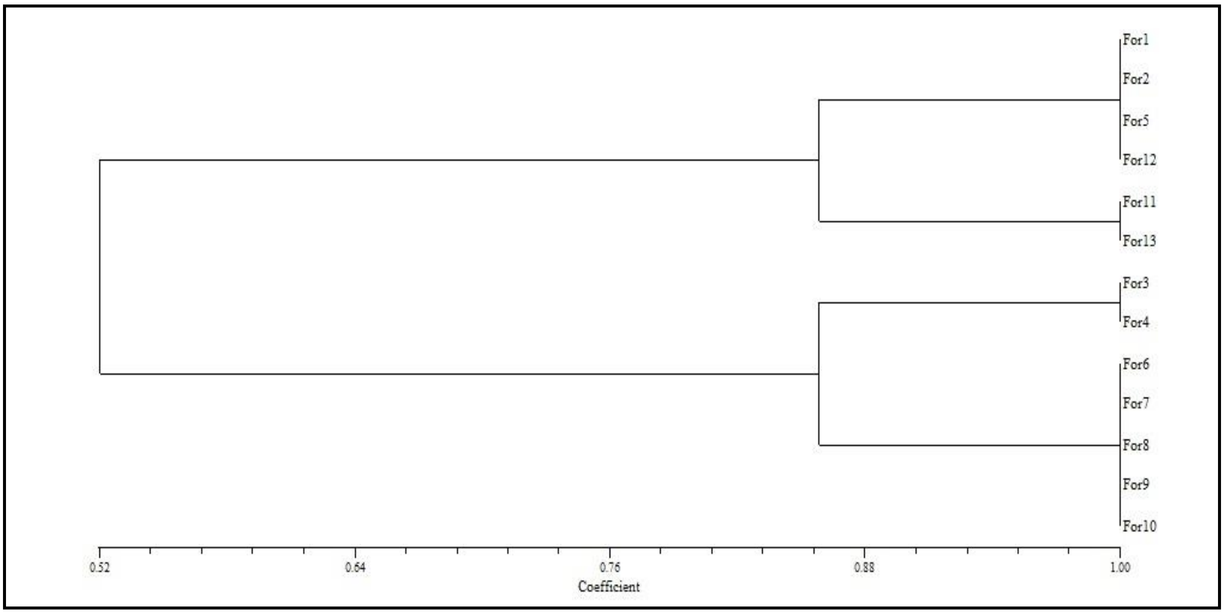


Figure 4.1 Dendrogram generated from wilt reaction to different castor cultivars.

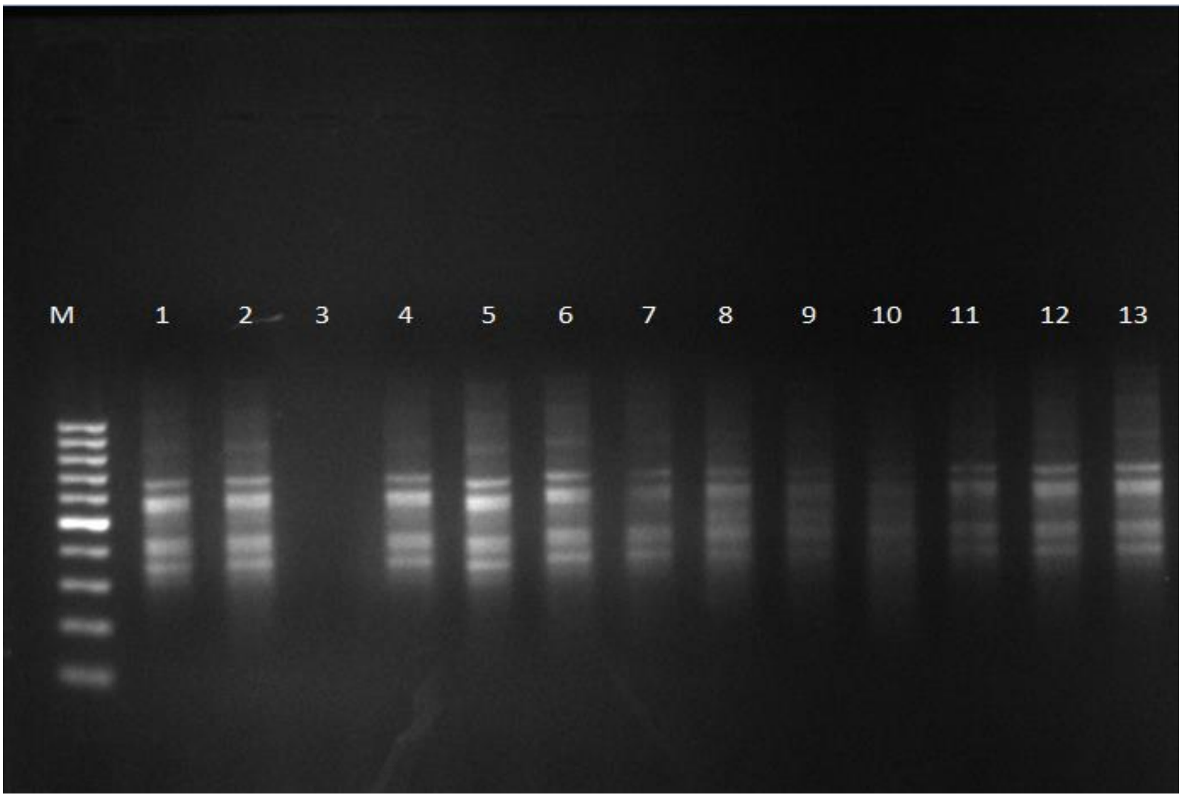


Figure 4.2 ISSR profile of *F. oxysporum* f. sp. *ricini* isolates with ISSR-1 primer.

M- Marker. numbers, 1 to 13- isolates of *F. oxysporum* f. sp. *ricini*

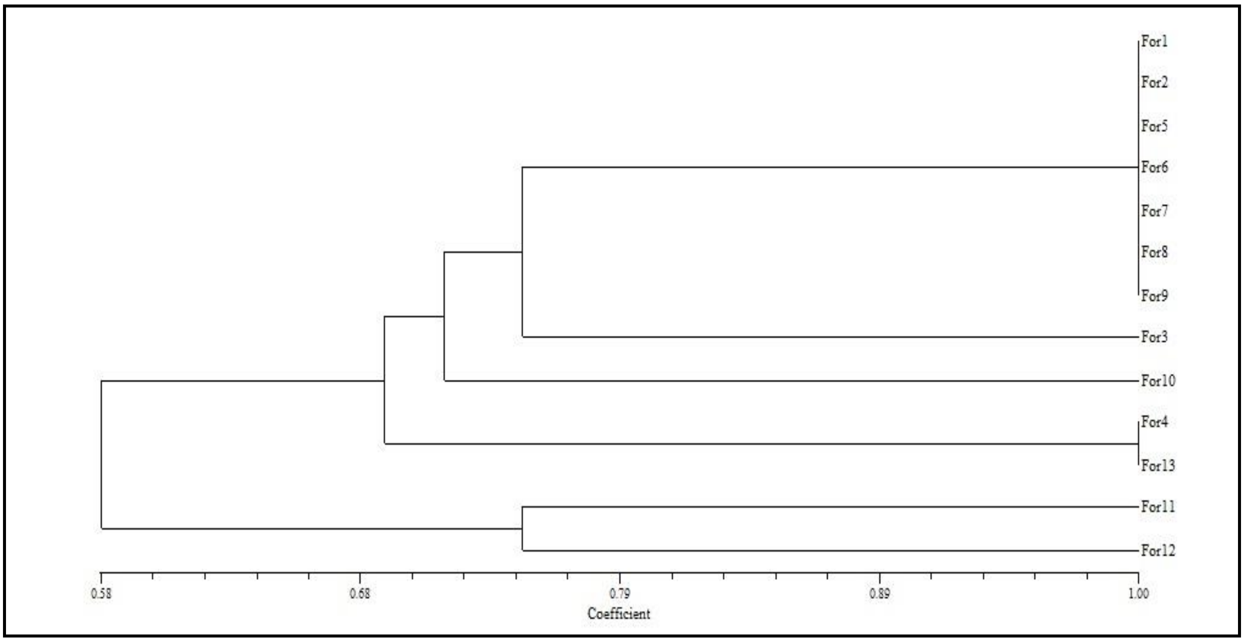


Figure 4.3 Dendrogram generated for 13 *F. oxysporum* f. sp. *ricini* isolates using four ISSR primers.

Table 4.7 Grouping isolates based on dendrogram

Groups	**Isolates**
Group I	*For* 11, *For* 12
Group II	*For* 13, *For* 4
Group III	*For* 10
Group IV	*For* 3, *For* 9, *For* 8, *For* 7, *For* 6, *For* 5, *For* 2, *For* 1

Table 4.16 Effect of main treatments on population of *Fusarium*

Treatments	Fusarium (cfu x 10^3 g^{-1} soil)			
	BTA	30 DAS	After crop harvest	Mean
Soil solarisation	2.3* (1.6)	0.3 (0.9)	0.6 (1.0)	1.0 (1.2)
Application of dazomet	2.3 (1.6)	1.8 (1.5)	2.2 (1.6)	2.1 (1.6)
Application of neem cake	2.3 (1.6)	0.3 (0.9)	1.2 (1.3)	1.2 (1.3)
Control	2.5 (1.7)	2.9 (1.8)	3.6 (2.0)	3.0 (1.9)
SEm±	0.06			
CD (p=0.05)	0.18			

BTA - Before treatment application. *Mean of three replications
Figures in the parentheses are square transformed values

Table 4.17 Effect of main treatments on population of Trichoderma

Treatments	Trichoderma (cfu x 10^3 g^{-1} soil)			
	BTA	30 DAS	After crop harvest	Mean
Soil solarisation	0.2* (0.8)	2.6 (1.7)	1.3 (1.3)	1.3 (1.3)
Application of dazomet	0.4 (0.9)	1.9 (1.5)	0.7 (1.1)	1.0 (1.2)
Application of neem cake	0.8 (1.3)	4.8 (2.3)	3.8 (2.0)	3.1 (1.9)
Control	0.2 (0.7)	1.2 (1.3)	0.2 (0.8)	0.5 (1.0)
SEm±	0.06			
CD (p=0.05)	0.18			

BTA- Before treatment application. *Mean of three replications.
Figures in the parentheses are square transformed values

Table 4.18 Effect of main treatments on population of Actinomycetes

Treatments	Actinomycetes cfu x 10^3 g^{-1} soil			
	BTA	30 DAS	After crop harvest	Mean
Soil solarisation	18.0* (4.3)	9.7 (3.1)	8.3 (2.9)	12.0 (3.5)
Application of dazomet	16.6 (4.1)	14.3 (3.8)	12.0 (3.5)	14.3 (3.8)
Application of neem cake	16.8 (4.1)	27.0 (5.2)	26.0 (5.5)	23.2 (4.9)
Control	16.0 (4.0)	15.0 (3.9)	14.0 (3.8)	15.0 (3.9)
SEm±	0.08			
CD(p=0.05)	0.22			

BTA- Before treatment application. *Mean of three replications.
Figures in the parentheses are square transformed values

Table 4.19 (a) Effect of main treatments on Soil Physio – chemical properties soil samples and chemical properties of soil

Treatments	Soil available nitrogen kg ha^{-1}				Soil available phosphorous kg ha^{-1}				Soil available potassium kg ha^{-1}			
	BTA	30 DAS	120 DAS	Mean	BTA	30 DAS	120 DAS	Mean	BTA	30 DAS	120 DAS	Mean
Soil solarisation	174.1*	180.6	179.0	177.9	24.5	20.7	18.9	21.3	497.2	516.3	511.8	508.4
Application of dazomet	164.4	165.7	163.5	164.5	20.9	19.6	18.6	19.7	521.1	511.8	501.4	511.4
Application of neem cake	175.1	182.5	179.1	178.9	20.5	27.0	25.0	24.1	516.3	532.5	520.4	523.0
Control	174.0	168.2	163.8	168.6	22.3	19.5	17.9	19.9	507.3	502.0	498.0	502.4
			S.Em±	1.13			S.Em±	0.46				NS
			CD(p=0.05)	3.32			CD(p=0.05)	1.37				NS

*Mean of three replications.

Table 4.19 (b) Effect of main treatments on Soil Physio – chemical properties soil samples and chemical properties of soil

Treatments	Soil organic carbon kg ha^{-1}				Soil electronic conductivity ds m^{-1}				Soil p^H			
	BTA	30 DAS	120 DAS	Mean	BTA	30 DAS	120 DAS	Mean	BTA	30 DAS	120 DAS	Mean
Soil solarisation	0.50*	0.53	0.55	0.52	0.42	0.44	0.41	0.42	7.7	7.9	8.0	7.8
Application of dazomet	0.50	0.52	0.53	0.51	0.43	0.43	0.42	0.42	7.7	7.8	8.0	7.8
Application of neem cake	0.51	0.54	0.57	0.54	0.43	0.43	0.41	0.42	7.7	7.8	7.8	7.7
Control	0.49	0.51	0.53	0.51	0.43	0.43	0.43	0.43	7.8	7.9	7.9	7.8
				NS				NS				NS
				NS				NS				NS

*Mean of three replications.

Plate 7(a) Reaction of cultivar 48-1 and JI-35 to different isolates of *F. oxysporum* f. sp. *ricini*

Plate 7(b) Reaction of cultivar DCS-9 to isolate *F. oxysporum* f. sp. *ricini*

Plate 7 (c) Reaction of cultivars DCS -107 and GCH-4 to isolates of *F. oxysporum* f. sp. *ricini* .

Plate 7(d) Reaction of Haritha and Kranthi cultivars to isolates of *Fusarium oxysporum* f. sp. *ricini*

Table 4.8 Evaluation of botanicals against *F. oxysporum* f. sp. *ricini in vitro*

Botanicals extracts	Colony diameter (cm)			Inhibition of mycelialgrowth (%)			Mean mycelial growthinhibition
	Concentrations (%)			Concentrations (%)			
	5	10	15	5	10	15	
Garlic clove extract	4.5*	4.0	3.4	49.8 (44.9)	54.7 (47.7)	61.4 (51.6)	55.3
Sunflower leaf extract	6.2	6.1	5.7	30.7 (33.6)	31.8 (34.3)	35.6 (36.6)	32.7
Sapota leaf extract	6.7	6.3	6.1	25.1 (30.0)	29.6 (32.9)	31.1 (33.9)	28.5
Neem leaf extract	4.3	3.9	3.4	51.7 (45.9)	56.6 (48.7)	62.2 (52.0)	56.8
Tulsi leaf extract	5.6	4.9	4.0	37.5 (37.7)	44.9 (42.1)	55.1 (47.9)	45.8
Control	8.9	8.9	8.9	-	-	-	-
	Chemicals		Concentrations		Chemical x Concentration		
Sem±	1.22		1.58		0.70		
CD(p=0.01)	4.67		6.03		1.90		

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.9 Evaluation of soil amendmentsagainst *F. oxysporum* f. sp. *ricini in vitro*

Soil amendments	Colony diameter (cm)			Inhibition of mycelial growth (%)			Mean mycelial growth inhibition
	Concentrations (%)			Concentrations (%)			
	5	10	15	5	10	15	
Vermicompost	6.3*	5.7	4.9	28.8 (32.5)	35.5 (36.6)	44.9 (42.1)	36.4
Groundnut cake	7.8	7.7	7.3	12.3 (20.5)	13.8 (21.8)	18.0 (25.1)	14.7
Cotton cake	7.6	7.4	7.2	14.9 (22.7)	17.2 (24.5)	19.5 (26.2)	17.2
Coconut kernel cake	7.7	7.6	7.4	13.11 (21.2)	14.9 (22.78)	16.5 (23.9)	14.8
Safflower cake	7.8	7.5	7.4	12.7 (20.9)	16.1 (23.6)	17.2 (24.5)	15.3
Control	8.9	8.9	8.9	-	-	-	-
	Chemicals		Concentrations		Chemical x Concentration		
Sem±	1.32		1.71		0.76		
CD(p=0.01)	5.04		6.50		2.91		

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.10 Evaluation of fungicides against *F. oxysporum* f. sp. *ricini in vitro*

Chemicals	Mean colony diameter(cm)			Inhibition of mycelial growth(%)			Mean mycelial growth inhibition
	Concentrations (ppm)			Concentrations (ppm)			
	500	1000	2000	500	1000	2000	
Propineb 61.3 %+ Iprovalicarb 5.5 %	4.6*	4.3	3.5	47.5 (43.6)	51.1 (45.6)	60.2 (50.9)	52.9
Pyraclostrobin 5 % + Metiram 55 % WG	4.3	3.3	2.8	51.0 (45.57)	62.1 (52.0)	68.2 (55.6)	60.4
Carbendazim 12 % + Mancozeb 63 % WP	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Tebuconazole +Trifloxystrobin	0.9	0.0	0.0	89.4 (71.0)	100.0 (90.0)	100.0 (90.0)	96.4
Hexaconazole+ Captan	1.6	1.3	1.0	81.4 (64.5)	85.6 (67.7)	88.3 (70.0)	85.1
Carboxin 37.5 %+ Thiram 37.5 % WP	1.0	0.9	0.0	88.2 (70.0)	89.0 (70.7)	100.0 (90.0)	92.4
Thiophanate methyl 70% WP	2.3	1.6	0.9	73.8 (59.2)	81.8 (64.8)	90.2 (72.4)	81.9
Difenoconazole	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Propiconazole	1.0	0.9	0.3	89.0 (70.7)	90.2 (71.7)	97.0 (72.4)	92.0
Carbendazim	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Control	8.8	8.8	8.8	-	-	-	

	Chemicals	Concentrations	Chemical x Concentration
Sem±	1.27	2.32	0.73
CD(p=0.01)	4.80	7.67	1.96

*****Mean of three replications. Figures in the parentheses are angular transformed values

Table 4.11Effect of botanicals on castor wilt in cultivar GCH-4 under glass house condition

Botanicals extracts	Disease incidence (%)	Per cent Disease reduction over control
Garlic	42.7* (40.8)	50.0
Sunflower	65.5 (54.0)	23.4
Sapota	66.2 (54.5)	22.5
Neem	43.3 (41.1)	49.3
Tulsi	45.0 (42.1)	47.3
Control	85.5 (67.6)	-
Sem±	0.73	
CD(p=0.05)	2.19	

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.12Effect of soil amendments on castor wilt in cultivar GCH-4 under glass house condition

Soil amendments	Disease incidence (%)	Per cent Disease reduction over control
Vermicompost	47.5* (44.4)	44.4
Groundnut cake	62.7 (53.0)	26.6
Cotton cake	60.0 (52.8)	29.8
Coconut kernel cake	63.2 (53.3)	26.0
Safflower cake	64.0 (56.3)	25.1
Control	85.5 (67.6)	-
Sem±	0.59	
CD(p=0.05)	1.78	

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.13 Effect of fungicides on castor wilt in cultivar GCH-4underglass house condition

Chemicals	Disease incidence (%)	Per cent Disease reduction over control
Propineb 61.3 %+ Iprovalicarb 5.5 %	60.2* (50.9)	30.6
Pyraclostrobin 5 % + Metiram 55 % WG	58.9 (50.2)	32.1
Carbendazim 12 % + Mancozeb 63 % WP	25.1 (30.1)	71.0
Tebuconazole +Trifloxystrobin	40.0 (39.3)	53.9
Hexaconazole+ Captan	32.1 (34.5)	63.0
Carboxin 37.5 %+ Thiram 37.5 % WP	20.3 (26.8)	76.5
Thiophanate methyl 70% WP	33.0 (35.1)	61.9
Difenoconazole	39.5 (39.0)	54.4
Propiconazole	33.2 (35.2)	61.7
Carbendazim	31.5 (34.2)	63.7
Control	86.8 (68.8)	-
Sem±	0.72	
CD(p=0.05)	2.12	

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.8 Evaluation of botanicals against *F. oxysporum* f. sp. *ricini in vitro*

Botanicals extracts	**Colony diameter (cm)**			**Inhibition of mycelialgrowth (%)**			**Mean mycelial growthinhibition**
	Concentrations (%)			**Concentrations (%)**			
	5	**10**	**15**	**5**	**10**	**15**	
Garlic clove extract	4.5*	4.0	3.4	49.8 (44.9)	54.7 (47.7)	61.4 (51.6)	55.3
Sunflower leaf extract	6.2	6.1	5.7	30.7 (33.6)	31.8 (34.3)	35.6 (36.6)	32.7
Sapota leaf extract	6.7	6.3	6.1	25.1 (30.0)	29.6 (32.9)	31.1 (33.9)	28.5
Neem leaf extract	4.3	3.9	3.4	51.7 (45.9)	56.6 (48.7)	62.2 (52.0)	56.8
Tulsi leaf extract	5.6	4.9	4.0	37.5 (37.7)	44.9 (42.1)	55.1 (47.9)	45.8
Control	8.9	8.9	8.9	-	-	-	-
	Chemicals		Concentrations		Chemical x Concentration		
Sem±	1.22		1.58		0.70		
CD(p=0.01)	4.67		6.03		1.90		

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.9 Evaluation of soil amendmentsagainst *F. oxysporum* f. sp. *ricini in vitro*

Soil amendments	Colony diameter (cm)			Inhibition of mycelial growth (%)			Mean mycelial growth inhibition
	Concentrations (%)			Concentrations (%)			
	5	10	15	5	10	15	
Vermicompost	6.3*	5.7	4.9	28.8 (32.5)	35.5 (36.6)	44.9 (42.1)	36.4
Groundnut cake	7.8	7.7	7.3	12.3 (20.5)	13.8 (21.8)	18.0 (25.1)	14.7
Cotton cake	7.6	7.4	7.2	14.9 (22.7)	17.2 (24.5)	19.5 (26.2)	17.2
Coconut kernel cake	7.7	7.6	7.4	13.11 (21.2)	14.9 (22.78)	16.5 (23.9)	14.8
Safflower cake	7.8	7.5	7.4	12.7 (20.9)	16.1 (23.6)	17.2 (24.5)	15.3
Control	8.9	8.9	8.9	-	-	-	-
	Chemicals		Concentrations		Chemical x Concentration		
Sem±	1.32		1.71		0.76		
CD(p=0.01)	5.04		6.50		2.91		

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.10 Evaluation of fungicides against *F. oxysporum* f. sp. *ricini in vitro*

Chemicals	Mean colony diameter(cm)			Inhibition of mycelial growth(%)			Mean mycelial growth inhibition
	Concentrations (ppm)			Concentrations (ppm)			
	500	**1000**	**2000**	**500**	**1000**	**2000**	
Propineb 61.3 %+ Iprovalicarb 5.5 %	4.6*	4.3	3.5	47.5 (43.6)	51.1 (45.6)	60.2 (50.9)	52.9
Pyraclostrobin 5 % + Metiram 55 % WG	4.3	3.3	2.8	51.0 (45.57)	62.1 (52.0)	68.2 (55.6)	60.4
Carbendazim 12 % + Mancozeb 63 % WP	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Tebuconazole +Trifloxystrobin	0.9	0.0	0.0	89.4 (71.0)	100.0 (90.0)	100.0 (90.0)	96.4
Hexaconazole+ Captan	1.6	1.3	1.0	81.4 (64.5)	85.6 (67.7)	88.3 (70.0)	85.1
Carboxin 37.5 %+ Thiram 37.5 % WP	1.0	0.9	0.0	88.2 (70.0)	89.0 (70.7)	100.0 (90.0)	92.4
Thiophanate methyl 70% WP	2.3	1.6	0.9	73.8 (59.2)	81.8 (64.8)	90.2 (72.4)	81.9
Difenoconazole	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Propiconazole	1.0	0.9	0.3	89.0 (70.7)	90.2 (71.7)	97.0 (72.4)	92.0
Carbendazim	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0
Control	8.8	8.8	8.8	-	-	-	

	Chemicals	Concentrations	Chemical x Concentration
Sem±	1.27	2.32	0.73
CD(p=0.01)	4.80	7.67	1.96

***Mean of three replications. Figures in the parentheses are angular transformed values

Table 4.11Effect of botanicals on castor wilt in cultivar GCH-4 under glass house condition

Botanicals extracts	Disease incidence (%)	Per cent Disease reduction over control
Garlic	42.7* (40.8)	50.0
Sunflower	65.5 (54.0)	23.4
Sapota	66.2 (54.5)	22.5
Neem	43.3 (41.1)	49.3
Tulsi	45.0 (42.1)	47.3
Control	85.5 (67.6)	-
Sem±	0.73	
CD(p=0.05)	2.19	

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.12Effect of soil amendments on castor wilt in cultivar GCH-4 under glass house condition

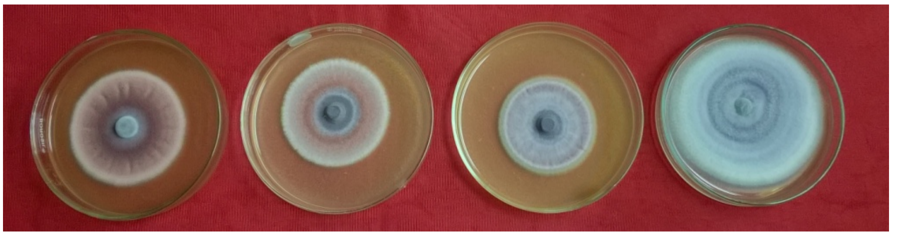
Soil amendments	Disease incidence (%)	Per cent Disease reduction over control
Vermicompost	47.5* (44.4)	44.4
Groundnut cake	62.7 (53.0)	26.6
Cotton cake	60.0 (52.8)	29.8
Coconut kernel cake	63.2 (53.3)	26.0
Safflower cake	64.0 (56.3)	25.1
Control	85.5 (67.6)	-
Sem±	0.59	
CD(p=0.05)	1.78	

*Mean of four replications. Figures in the parentheses are angular transformed values

Table 4.13 Effect of fungicides on castor wilt in cultivar GCH-4underglass house condition

Chemicals	Disease incidence (%)	Per cent Disease reduction over control
Propineb 61.3 %+ Iprovalicarb 5.5 %	60.2* (50.9)	30.6
Pyraclostrobin 5 % + Metiram 55 % WG	58.9 (50.2)	32.1
Carbendazim 12 % + Mancozeb 63 % WP	25.1 (30.1)	71.0
Tebuconazole +Trifloxystrobin	40.0 (39.3)	53.9
Hexaconazole+ Captan	32.1 (34.5)	63.0
Carboxin 37.5 %+ Thiram 37.5 % WP	20.3 (26.8)	76.5
Thiophanate methyl 70% WP	33.0 (35.1)	61.9
Difenoconazole	39.5 (39.0)	54.4
Propiconazole	33.2 (35.2)	61.7
Carbendazim	31.5 (34.2)	63.7
Control	86.8 (68.8)	-
Sem±	0.72	
CD(p=0.05)	2.12	

*Mean of four replications. Figures in the parentheses are angular transformed values

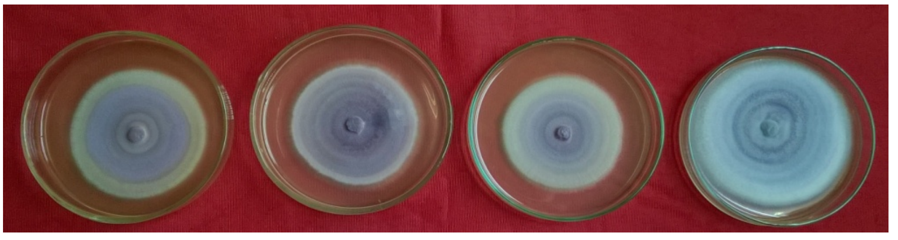


5% 10% 15% Control

Garlic clove extract

5% 10% 15% Control

Sunflower leaf extract



5% 10% 15% Control

Sapota leaf extract

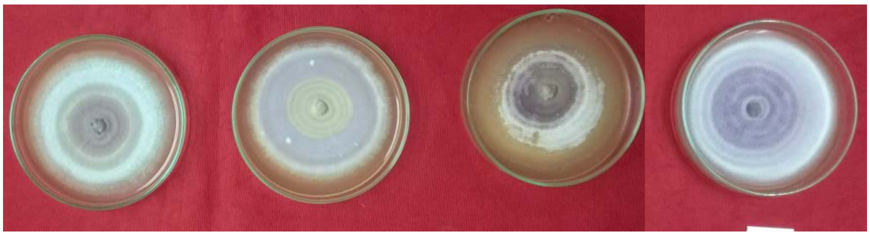
5% 10% 15% Control

Neem leaf extract

5% 10% 15% Control

Tulsi leaf extract

Plate 8: **Effect of botanicals against isolate *For 10 F. oxysporum* f. sp. *ricini***

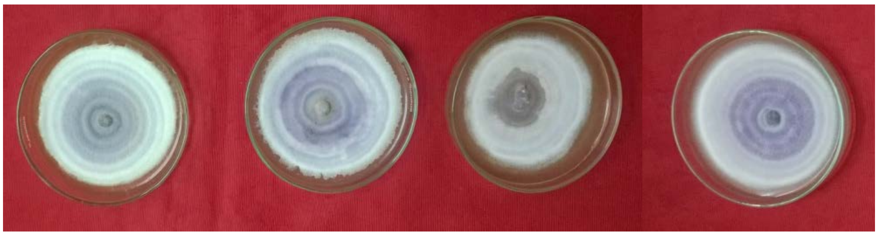


15% 10% 5% Control

Vermicompost

15% 10% 5% Control 1

Ground nut cake

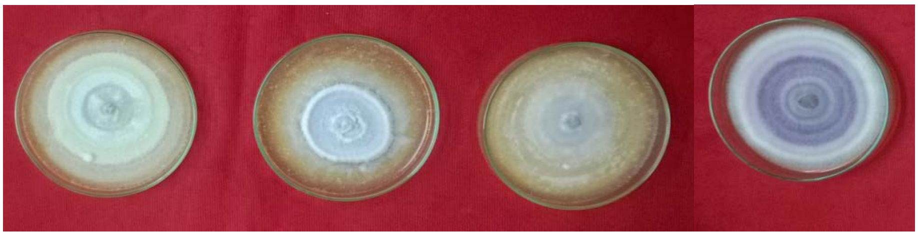


15% 10% 5% Control

Cotton cake

15% 10% 5% Control

Coconut kernel cake



15% 10% 5% Control

Safflower seed cake

Plate 9: Effect of different soil amendments on isolate *For 10 F. oxysporum* f. sp. *ricini*

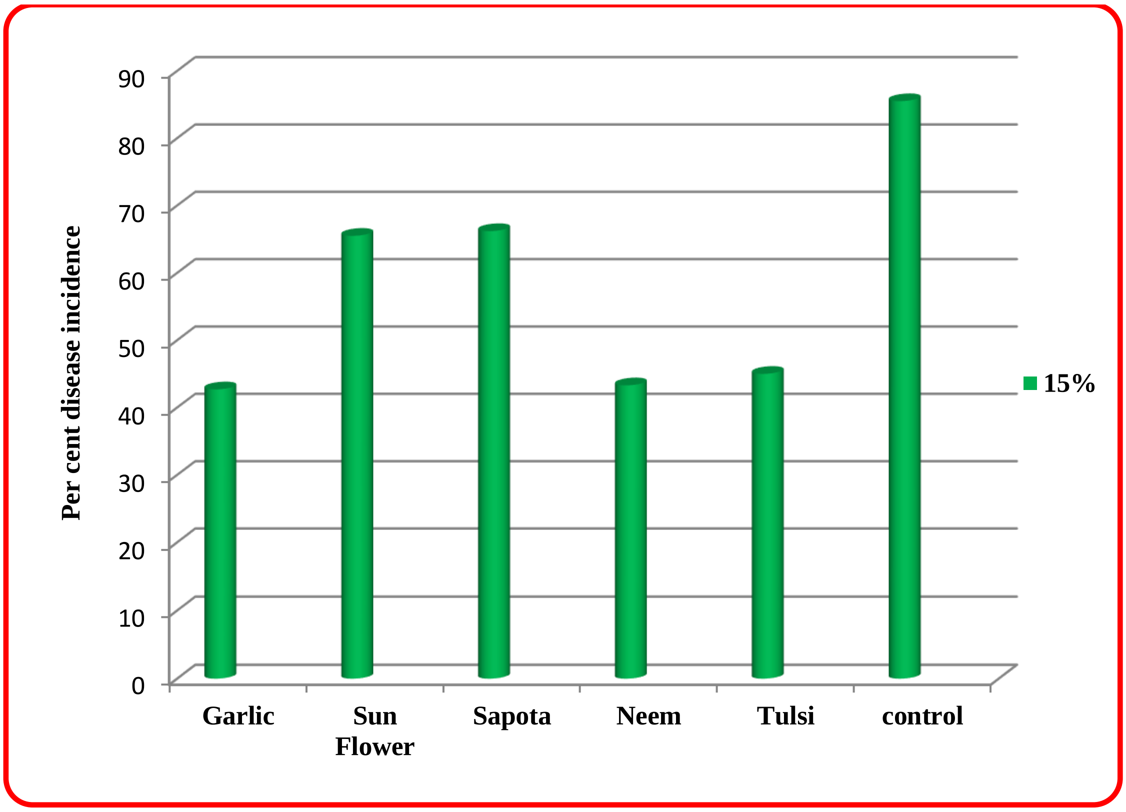


Figure 4.7 Effect of botanical extracts on per cent disease incidence of castor wilt *in vivo* on cultivar GCH-4.

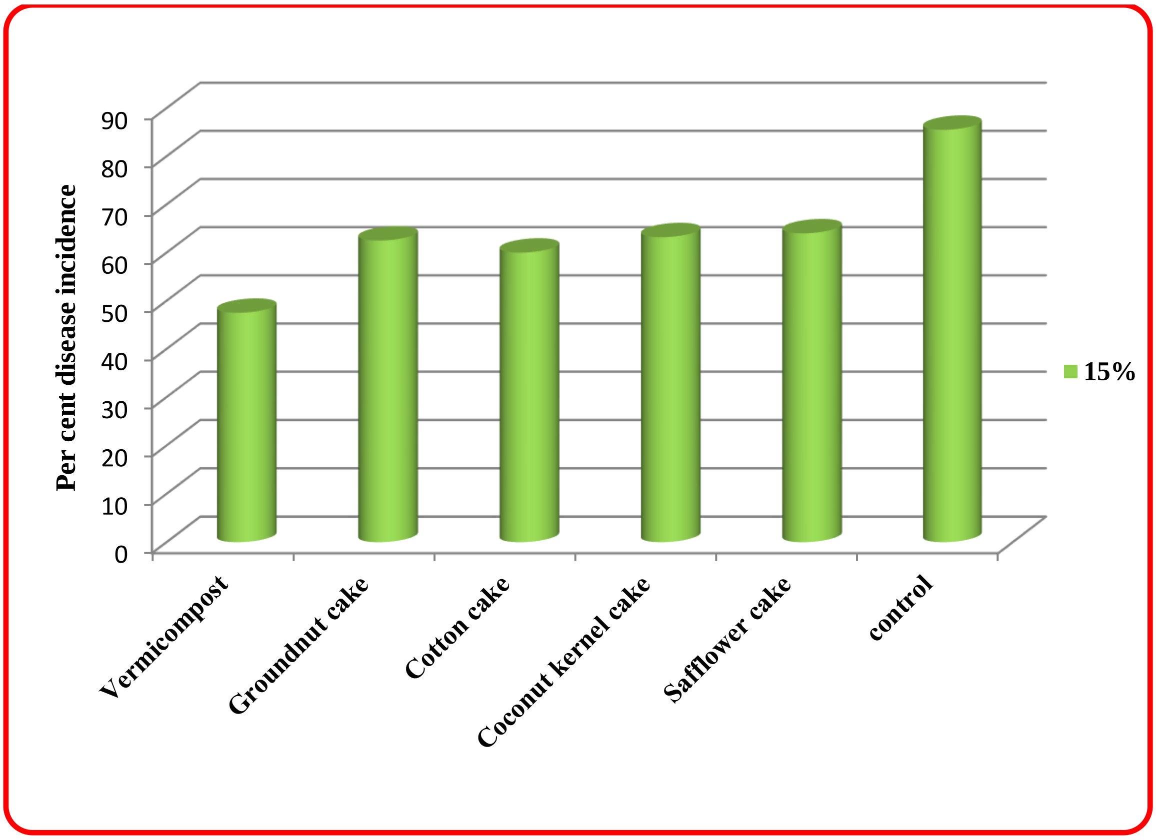


Figure 4.8 Effect of soil amendments extracts on per cent disease incidence of castor wilt*in vivo* on cultivar GCH-4.

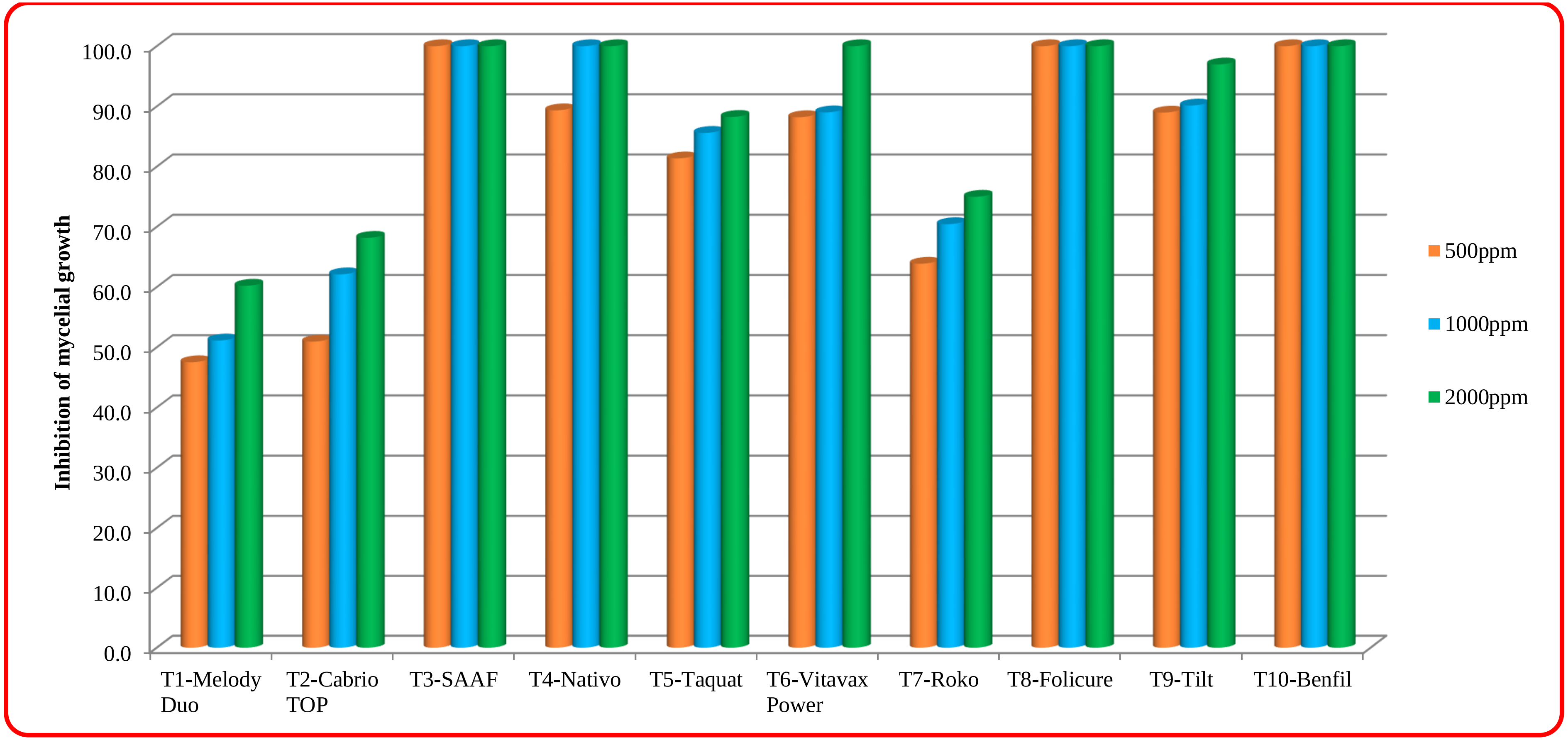


Figure 4.6 Effect of fungicides on mycelial growth of *F. oxysporum* f.sp. *ricini in vitro*

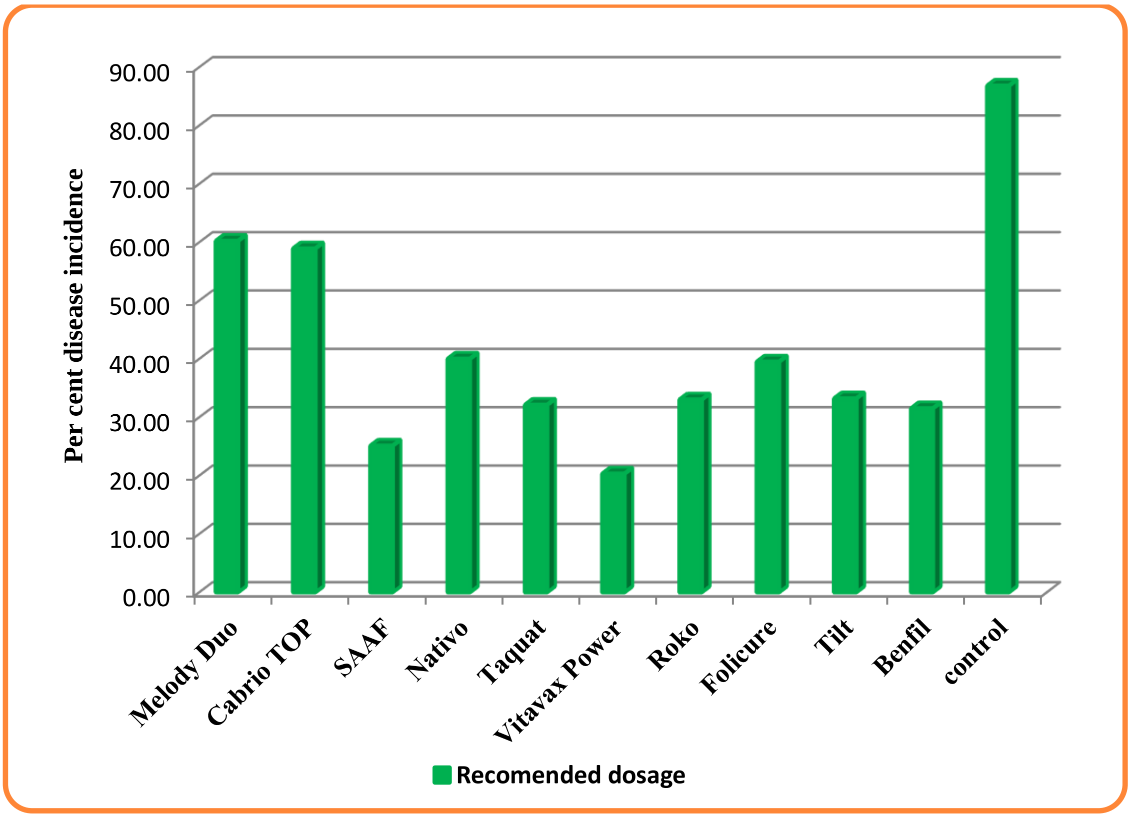


Figure 4.9 Effect fungicidess on per cent disease incidence of castor wilt *in vivo* on cultivar GCH-4.

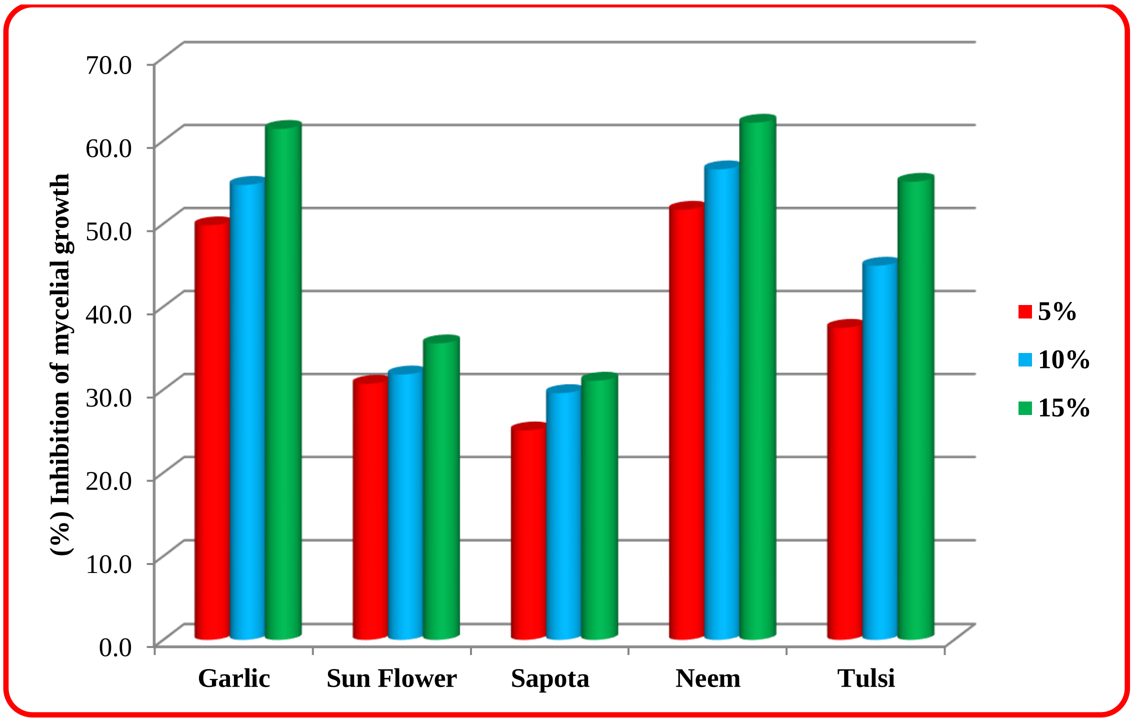


Figure 4.4 Effect of botanical extracts on mycelial growth of *F. oxysporum* f. sp. *ricini in vitro*

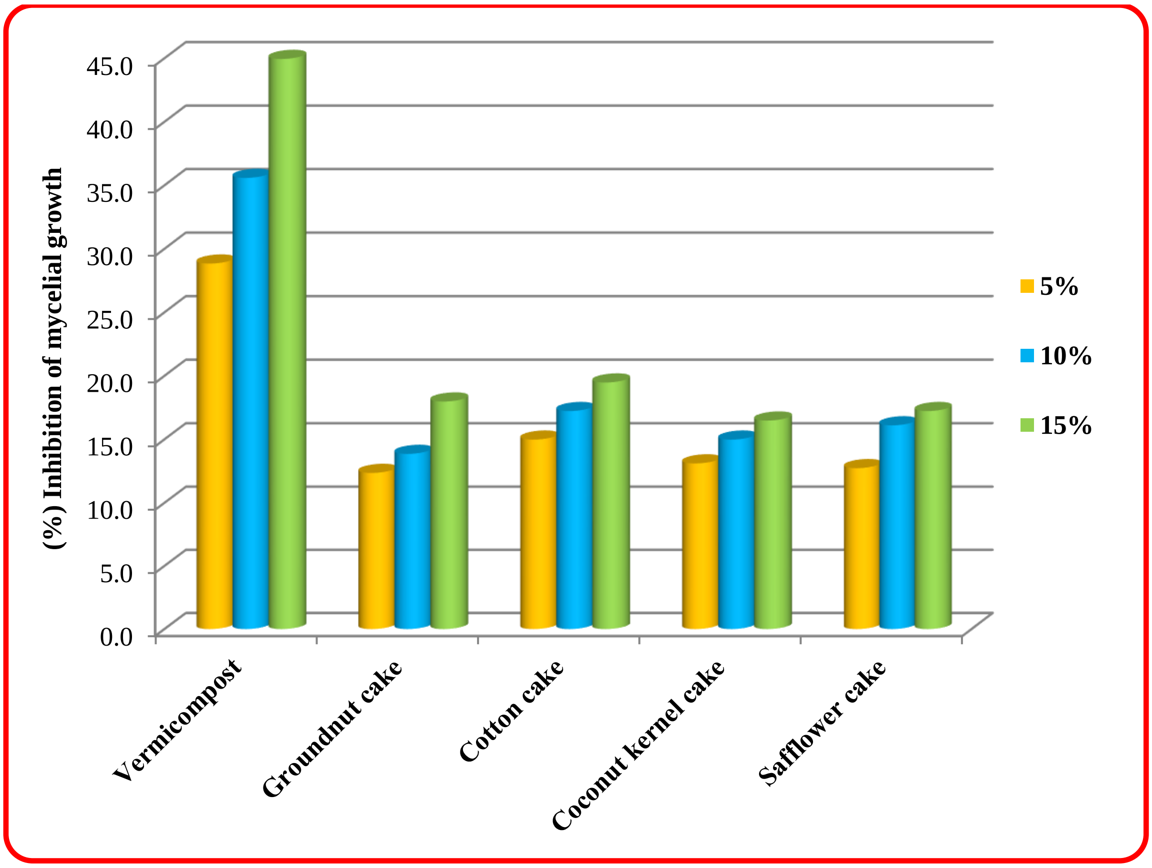


Figure 4.5 Effect of soil amendments extracts on mycelial growth of *F. oxysporum* f. sp. *ricini in vitro*

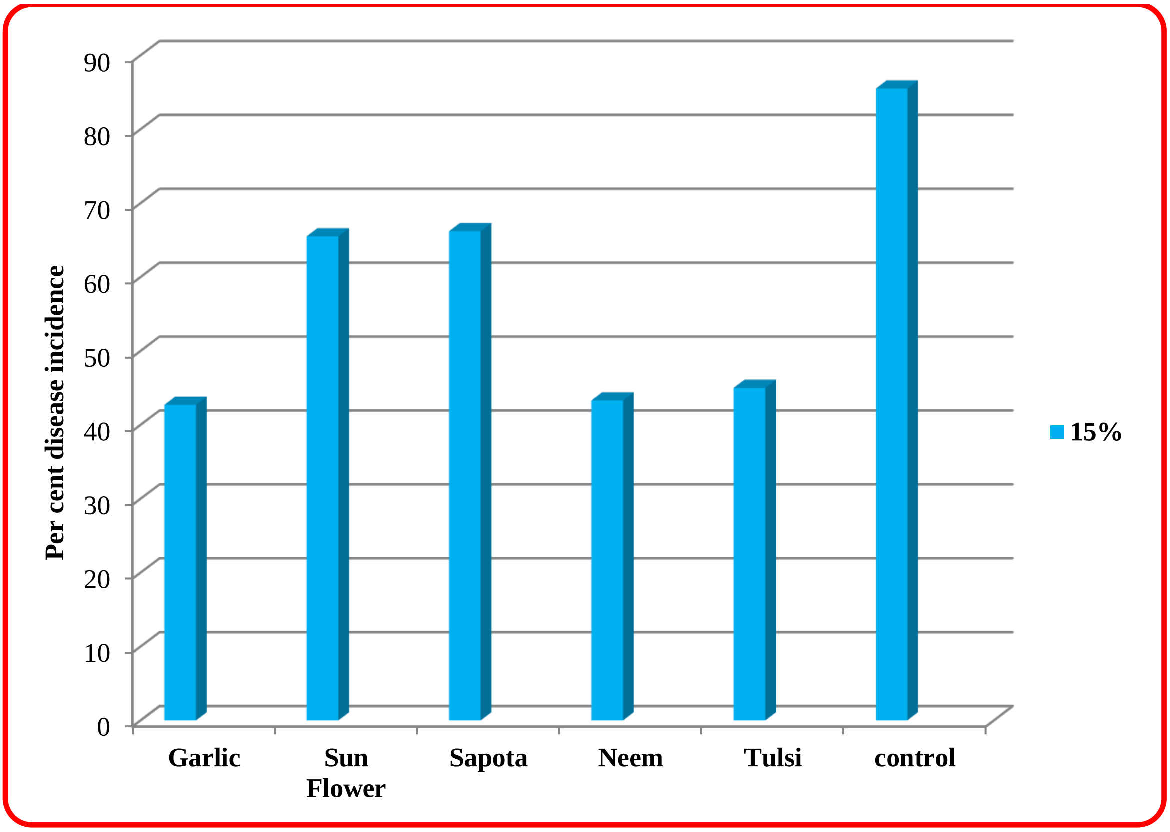
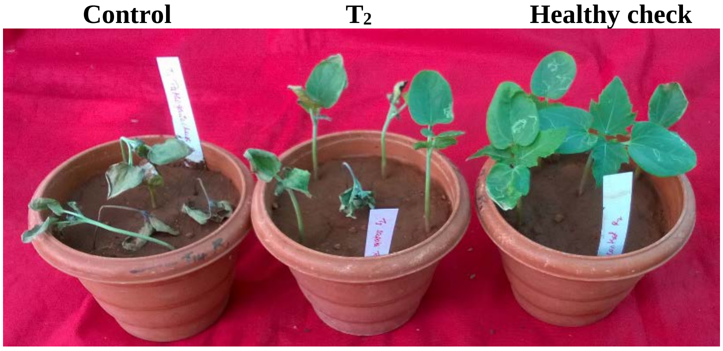


Figure 4.7 Effect of botanical extracts on per cent disease incidence of *F. oxysporum* f. sp. *ricini*

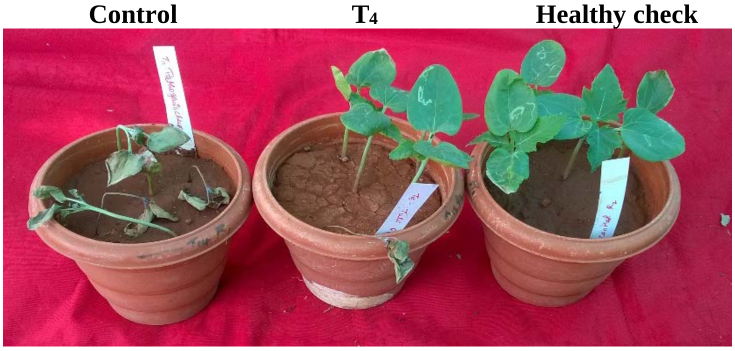
Control T_1 Healthy check

Control T_2 Healthy check



Control T_3 Healthy check

Control T_4 Healthy check



Control T_5 Healthy check

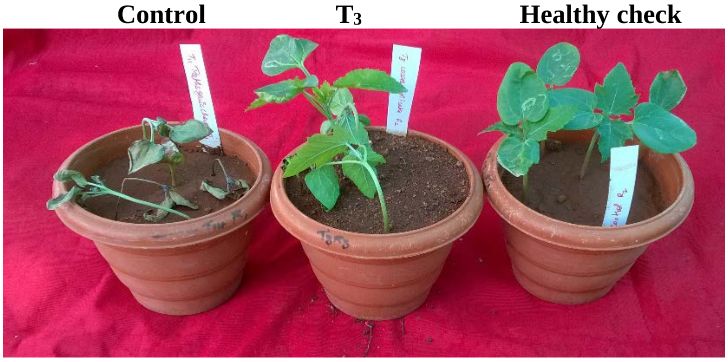
Treatments - T_1 : Garlic T_2 : Sun flower T_3 : Sapota T_4 : Neem T_5 : Tulsi

Plate 11: Effect of botanicals on castor wilt in cultivar GCH - 4 under glass house conditions

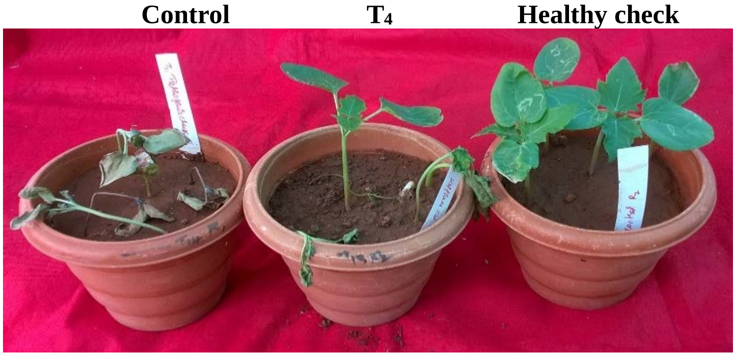
Control **T_1** **Healthy check**

Control **T_2** **Healthy check**

Control **T_3** **Healthy check**



Control **T_4** **Healthy check**



Control **T_5** **Healthy check**

Treatments - T_1 : Vermicompost T_2 : Groundnut cake T_3 : Cotton cake T_4 : Coconut kernel extract T_5 : Safflower cake

Plate 12: Effect of soil amendments on castor wilt in cultivar GCH 4 under glass house condition

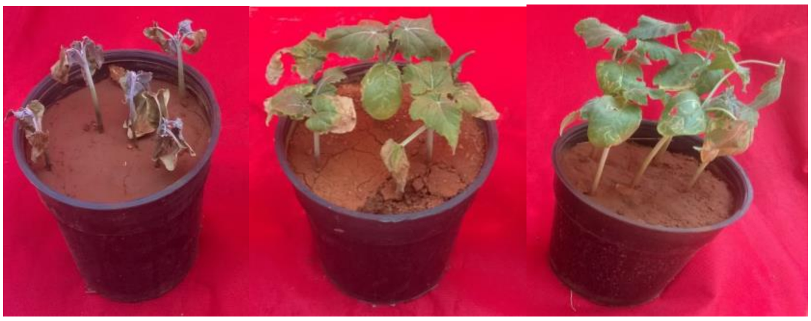
Control T$_1$

T2

T3

T4

T5



T6

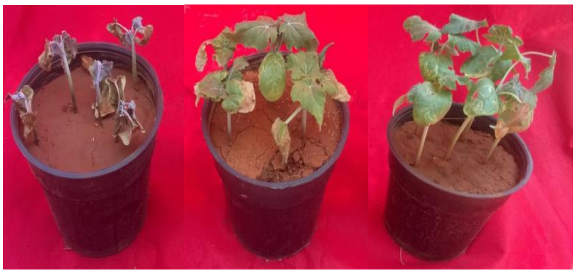
T7

T8

T9

T10

Control T_1 Healthy check



Control T_6 Healthy check

Control T_2 Healthy check

Control T_7 Healthy check

Control T_3 Healthy check

Control T_8 Healthy check

Control T_4 Healthy check

Control T_9 Healthy check

Control T_5 Healthy check

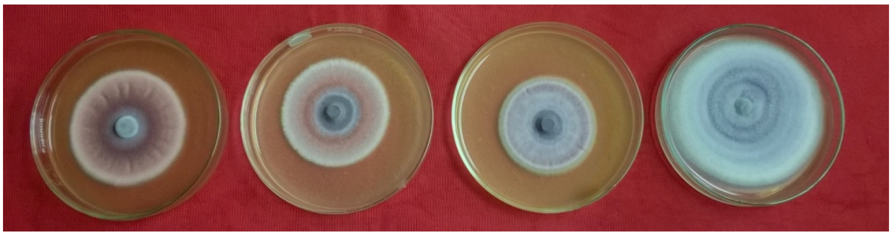
Control T_{10} Healthy check

Treatments:

T_1 : Propiconazole 61.3 % + Iprovalicarb 5.5 %
T_2 : Pyraclostrobin 5% + Metiram 55 % WG
T_3 : Carbendazim 12% + Mancozeb 63 WP
T_4 : Tebuconazole + Trifloxystrobin
T_5 : Hexaconazole + Captan

T_6 : Carboxin 37.5 % + Thiram 37.5 WP
T_7 : Thiophante methyl 70 % WP
T_8 : Difenocozole
T_9 : Propiconazole
T_{10} :Carbendazim

Plate 13. Effect of fungicides on castor wilt in cultivar GCH-4 under glass house conditions

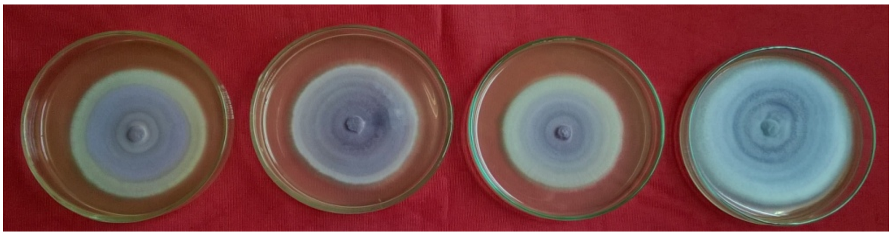


5% 10% 15% Control

Garlic clove extract

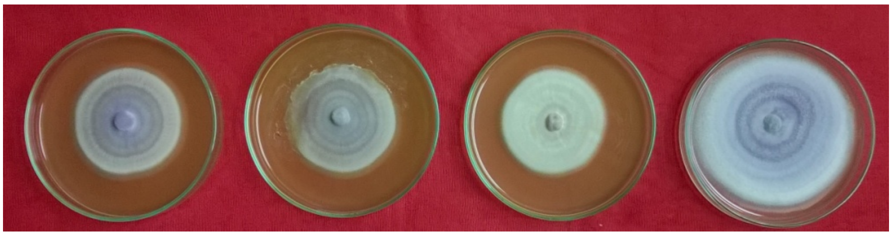
5% 10% 15% Control

Sunflower leaf extract



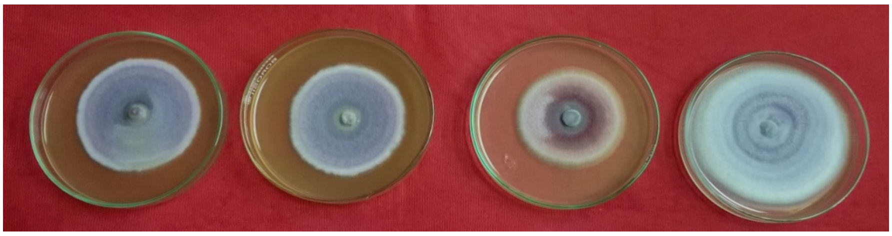
5% 10% 15% Control

Sapota leaf extract



5% 10% 15% Control

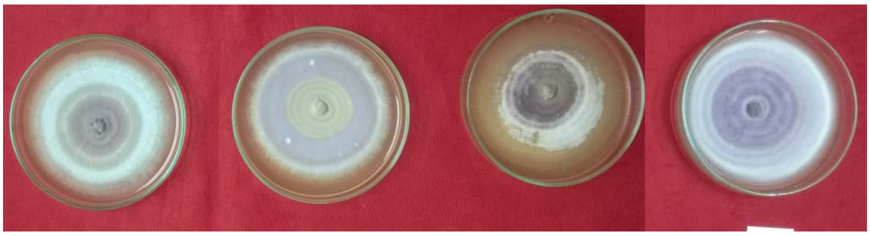
Neem leaf extract



5% 10% 15% Control

Tulsi leaf extract

Plate 8: Effect of botanicals against isolate *For 10 F. oxysporum* f. sp. *ricini*

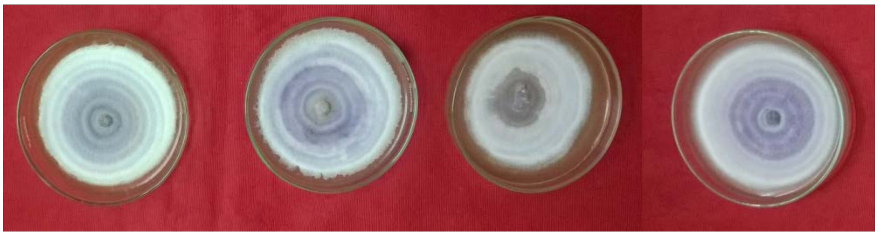


15% 10% 5% Control

Vermicompost

15% 10% 5% Control 1

Ground nut cake

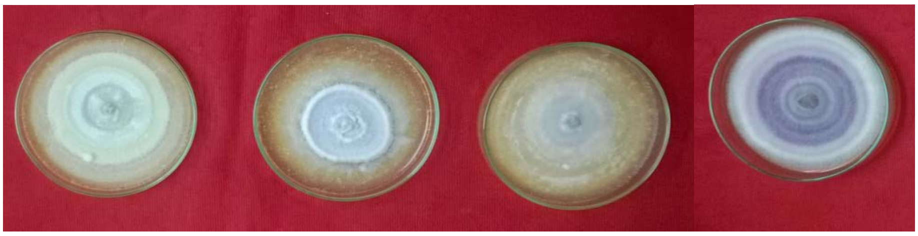


15% 10% 5% Control

Cotton cake

15% 10% 5% Control

Coconut kernel cake



15% 10% 5% Control

Safflower seed cake

Plate 9: Effect of different soil amendments on isolate *For 10 F. oxysporum* f. sp. *ricini*

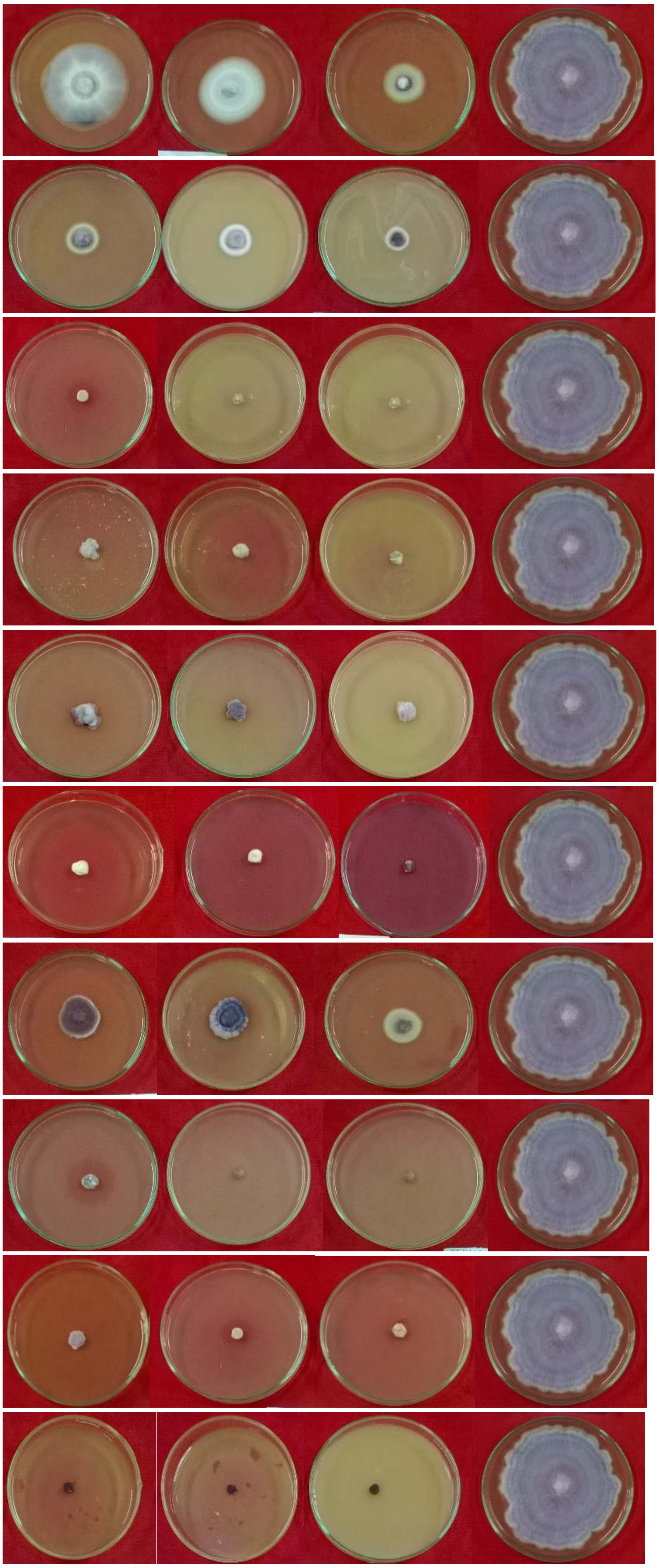
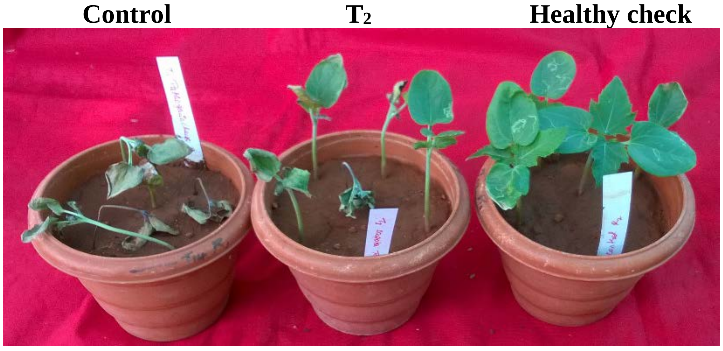


Plate 10: Effect of different fungicides on isolate *For 10 F. oxysporum* f. sp. *ricini*

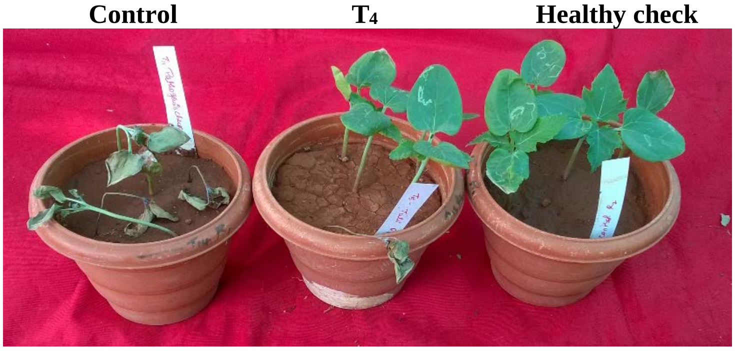
Control **T_1** **Healthy check**

Control **T_2** **Healthy check**



Control **T_3** **Healthy check**

Control **T_4** **Healthy check**

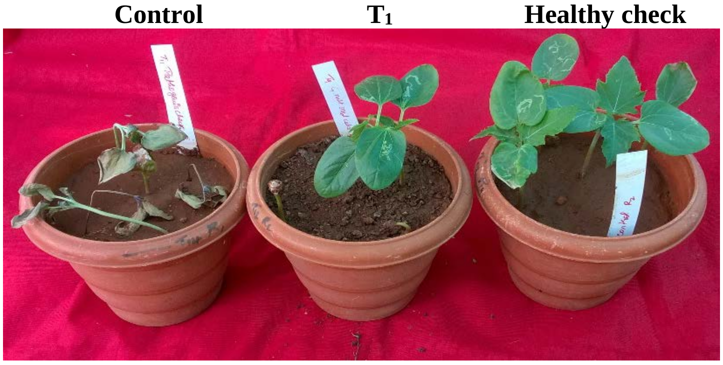


Control **T_5** **Healthy check**

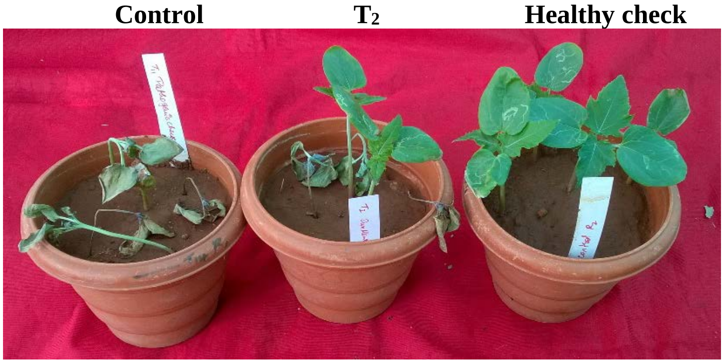
Treatments - T_1 : Garlic T_2 : Sun flower T_3 : Sapota T_4 : Neem T_5 : Tulsi

Plate 11: Effect of botanicals on castor wilt in cultivar GCH - 4 under glass house conditions

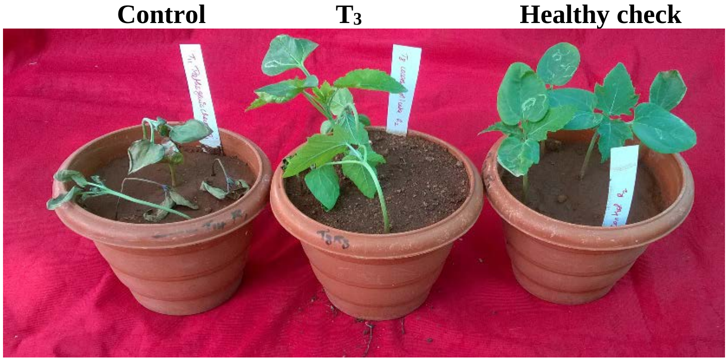
Control **T_1** **Healthy check**



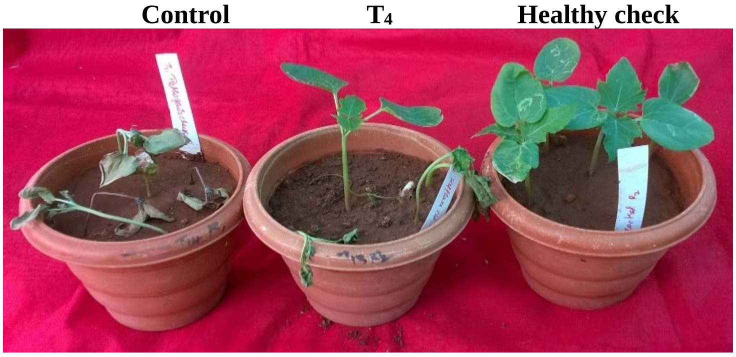
Control **T_2** **Healthy check**



Control **T_3** **Healthy check**



Control **T_4** **Healthy check**



Control **T_5** **Healthy check**

Treatments - T_1 : Vermicompost T_2 : Groundnut cake T_3 : Cotton cake T_4 : Coconut kernel extract T_5 : Safflower cake

Plate 12: Effect of soil amendments on castor wilt in cultivar GCH 4 under glass house condition

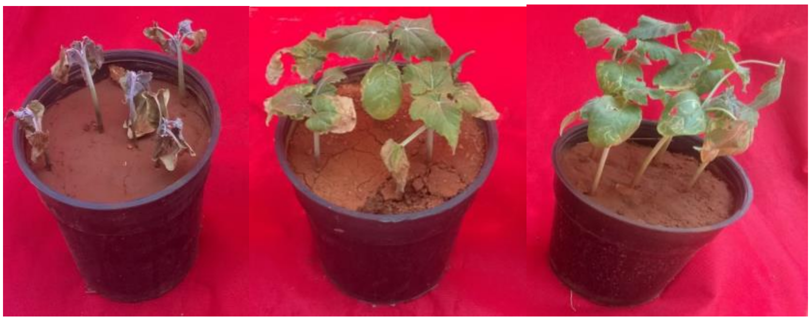
Control T_1

T2

T3

T4

T5



T6

T7

T8

T9

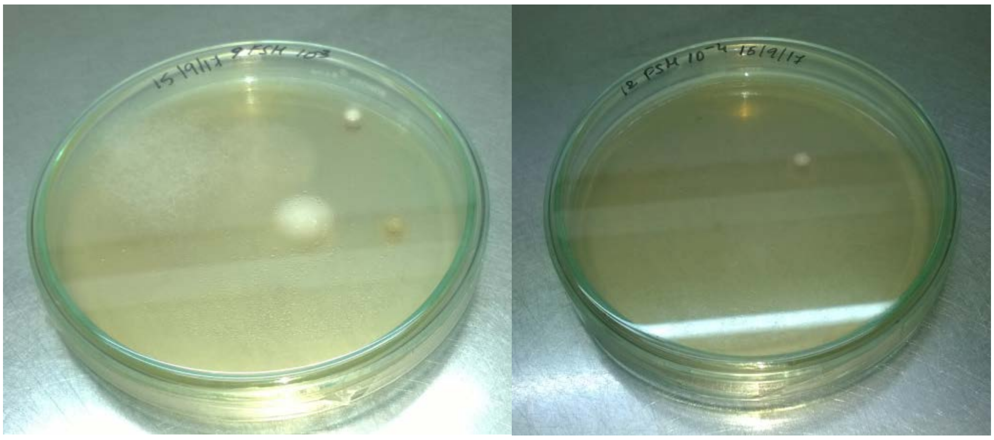
T10

Treatments:

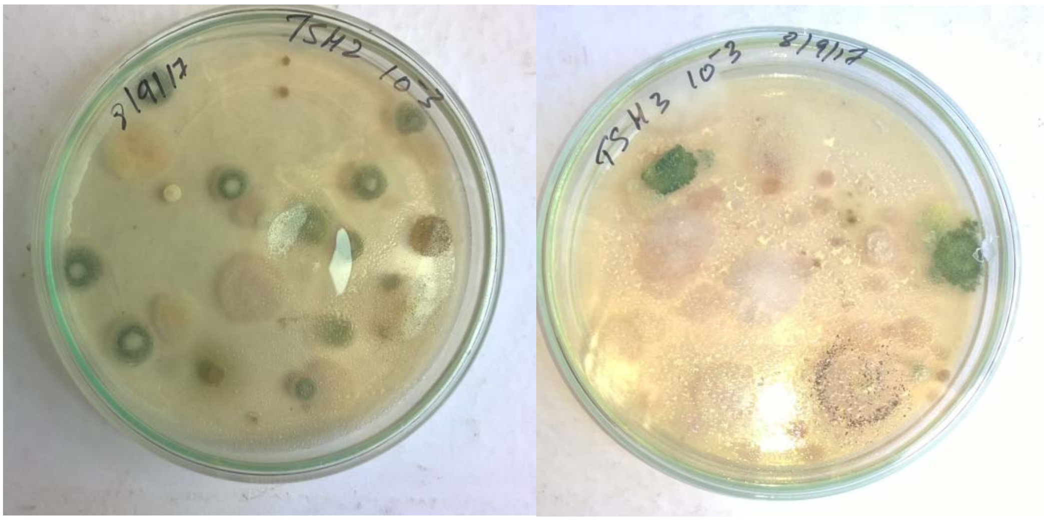
T_1 : Propiconazole 61.3 % + Iprovalicarb 5.5 %
T_2 : Pyraclostrobin 5% + Metiram 55 % WG
T_3 : Carbendazim 12% + Mancozeb 63 WP
T_4 : Tebuconazole + Trifloxystrobin
T_5 : Hexaconazole + Captan

T_6 : Carboxin 37.5 % + Thiram 37.5 WP
T_7 : Thiophante methyl 70 % WP
T_8 : Difenocozole
T_9 : Propiconazole
T_{10} :Carbendazim

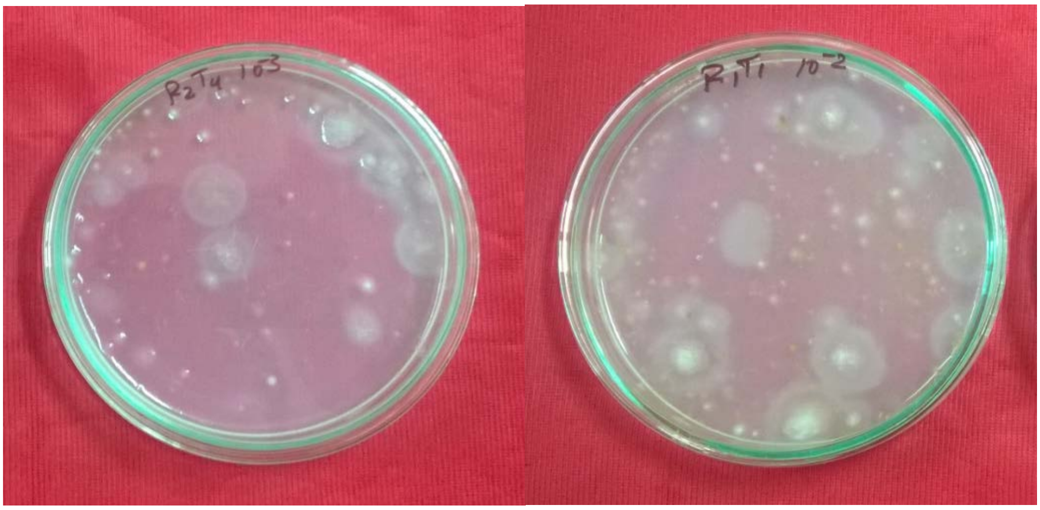
Plate 13. Effect of fungicides on castor wilt in cultivar GCH-4 under glass house conditions



White coloured colonies of Fusarium on FSM.



Green coloured colonies of Trichoderma on TSM



White depressed colonies of Actinomycetes

Plate 15. Enumeration of soil microflora from wilt sick plot of castor

Table 4.14 Effect of main and sub treatments on per cent disease incidence of castor wilt in field

Treatments	30 DAS					60 DAS					90 DAS					120 DAS				
	Sbt1	**Sbt2**	**Sbt3**	**Sbt4**	**Mean**	**Sbt1**	**Sbt2**	**Sbt3**	**Sbt4**	**Mean**	**Sbt1**	**Sbt2**	**Sbt3**	**Sbt4**	**Mean**	**Sbt1**	**Sbt2**	**Sbt3**	**Sbt4**	**Mean**
Mt1	0.8* (3.0)	0.0 (0.2)	0.8 (3.0)	1.5 (4.2)	**0.8**	7.8 (16.1)	6.4 (14.6)	9.2 (17.6)	20.3 (26.7)	**10.9**	14.4 (20.0)	11.7 (19.6)	25.4 (30.2)	30.5 (33.4)	**21.0**	22.4 (28.2)	21.9 (27.9)	33.7 (36.3)	36.7 (37.2)	**28.7**
Mt2	1.8 (4.6)	2.0 (6.7)	3.3 (8.7)	3.4 (8.4)	**2.6**	24.8 (29.8)	24.9 (29.9)	40.2 (39.3)	50.6 (45.3)	**35.1**	39.7 (39.0)	34 (35.4)	64.7 (53.5)	68.7 (55.9)	**51.7**	51.1 (45.6)	46.8 (43.1)	78.8 (62.6)	92.2 (73.9)	**67.2**
Mt3	0.8 (3.0)	0.8 (3.0)	2.2 (7.0)	2.3 (7.1)	**1.5**	16.7 (24.0)	17.7 (24.8)	23.9 (29.2)	32.4 (34.6)	**22.7**	20.9 (27.1)	23.7 (29.0)	38.1 (38.0)	42.6 (40.7)	**31.3**	33.6 (35.3)	34.4 (35.8)	50.3 (45.1)	54.4 (47.5)	**43.2**
Mt4	0.8 (3.0)	2.3 (5.2)	3.3 (8.6)	5.1 (10.5)	**2.9**	47.1 (43.3)	45.4 (42.3)	49.5 (44.6)	51.9 (46.0)	**48.5**	62.7 (52.3)	58.3 (49.6)	65.8 (54.2)	74.7 (59.9)	**65.3**	68.5 (55.8)	60.9 (51.3)	86.9 (68.8)	96.4 (79.5)	**78.2**
Mean	**1.1**	**1.3**	**2.4**	**3.1**		**24.1**	**23.6**	**30.7**	**38.8**		**34.4**	**32.5**	**48.5**	**54.1**		**43.9**	**41.0**	**62.4**	**69.9**	
	Mt	**Sbt**	**Mt at Sbt**	**Sbt at Mt**		**Mt**	**Sbt**	**Mt at Sbt**	**Sbt at Mt**		**Mt**	**Sbt**	**Mt at Sbt**	**Sbt at Mt**		**Mt**	**Sbt**	**Mt at Sbt**	**Sbt at Mt**	
±SEm						1.11	0.71	1.56	1.20		1.35	0.84	2.11	1.82		0.80	0.47	1.23	1.05	
CD(p=0.05)	NS	NS	NS	NS		3.83	1.73	4.62	3.57		4.67	2.88	6.26	5.42		2.76	1.65	3.65	3.10	

*Mean of three replications

Figures in parenthesis are arc sin transformed values

Mt_1: Soil solarisation

Mt_2: Application of dazomet (30-40 g/m^2)

Mt_3: Application of neem cake (1.78 kg/m^2)

Mt_4 : Control.

Sbt_1: Seed treatment with *Trichoderma harzianum*, Th 4d WP 10g/kgand soil application of *T.harzianum*

Sbt_2: Seed treatment with carbendazim 2g/kg seed

Sbt_3: Soil application of carbofuron 2kg a.i./ha

Sbt_4 : Control

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