

**Molecular characterization of *Fusarium solani* (Mart.)
Sacc. causing dry rot of Potato tubers (*Solanum
tuberosum* L.) and its management**

**A
THESIS
SUBMITTED TO THE
ANAND AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE**

OF

Master of Science

(AGRICULTURE)

**IN
PLANT PATHOLOGY**

BY

PANDYA JANKI PINAKINBHAI

B. Sc. (Agri.)

**DEPARTMENT OF PLANT PATHOLOGY
B. A. COLLEGE OF AGRICULTURE
ANAND AGRICULTURAL UNIVERSITY
ANAND – 388 110 (GUJARAT)**

JUNE-2015

(Reg. No. 04-2152-2013)

Dr. R. K. Patil
Professor
Department of Plant Pathology
B. A. College of Agriculture
Anand Agricultural University
Anand – 388 110
Gujarat (India)

CERTIFICATE

This is to certify that the thesis entitled “**Molecular characterization of *Fusarium solani* (Mart.) Sacc. causing dry rot of Potato tubers (*Solanum tuberosum* L.) and its management**” submitted by **PANDYA JANKI P. (Reg. No. 04-2152-2013)** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** of the Anand Agricultural University, Anand is a record of bonafide research work carried out by her under my personal guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place : Anand
Date : 5th June, 2015

(R.K. Patil)
Major Guide

Molecular characterization of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers (*Solanum tuberosum* L.) and its management

Name of the Student

Pandya Janki P.

Major Guide

Dr. R. K. Patil

**B. A. COLLEGE OF AGRICULTURE
ANAND AGRICULTURAL UNIVERSITY
ANAND – 388 110**

ABSTRACT

Potato is known as “The king of vegetables”, and has emerged as fourth most important food crop in India after rice, wheat and maize. Potato tubers lose their market value due to damage caused by many fungi during storage. Among all, the dry rot caused by *Fusarium solani* adversely affects the tuber quality, quantity and ultimately reduces the market value. As the Fusarium dry rot of potato causes enormous yield losses, the detailed investigations on various aspects were carried out in the present study.

The potato tubers (Cultivar- Khufri Badshah) showing typical symptoms of Fusarium dry rot were collected from Sardar Patel vegetable market, Anand. The infected tubers exhibited brown to darker wrinkles on skin. These wrinkles were often arranged in irregular concentric circles. Sometimes a hole may be produced in the centre of the concentric ring with whitish or pinkish growth of fungal mycelia. The pathogen inciting dry rot was isolated from the infected tubers on PDA and the pathogenicity was proved by following Koch’s Postulates. The pure

culture obtained was sent for identification to Indian Type Culture Collection (I.T.C.C.), I.A.R.I., New Delhi – 110 012 and was identified as *Fusarium solani* (Mart.) Sacc. (ID.No. 9255.13).

The results of morphological studies revealed that the fungus (*F. solani*) initially produced whitish to light pink mycelial growth rapidly becoming colourless to dark purple. The hyphae was septate and hyaline. Chlamydospores were globose to oval, terminal or intercalary with smooth or rough wall either formed in singly or in chain were observed. Microconidia produced in chains, remain connected loosely, one celled (6-11 x 2-4 μm) or two celled (10-18 x 2-4 μm), round to oval shaped. Macroconidia (31-58 x 3.1-4.7 μm) were spares, very slightly sickle-shaped to nearly straight with 3-4 septa.

The weekly survey carried out from 1st week of June to 4th week of February during 2014-2015, revealed that the *Fusarium* dry rot incidence was predominant among all rots and recorded to the tune of 14.05 and 12.31 per cent at Sardar Patel vegetable market, station road and Goya Talav market, Anand, respectively.

Among the fungicides screened *in vitro*, carbendazim (100 ppm) and carbendazim (12 %) + mancozeb (63 %) at both the concentrations (500 & 1000 ppm) completely inhibited the mycelial growth of *F. solani*. The result of *in vivo* study revealed that the dry rot severity was not observed in tubers treated with carbendazim (50 & 100 ppm) on 4th and 8th day in pre- and post- inoculation treatments.

Complete mycelial growth inhibition of *Fusarium solani* was recorded in aluminum acetate (0.2 M) and aluminum chloride (0.2 M) salts. Further no dry rot severity was recorded in tubers treated with aluminum acetate both in pre-and post-inoculation treatments.

Red Kaner found significantly superior in inhibiting the mycelial growth (69.17%) of *F.solani*. Significantly lowest Fusarium dry rot severity was noticed in tubers treated with Red Kaner (0.20 & 0.60%).

Trichoderma asperellum found most efficient antagonist in inhibiting the mycelial growth of *F. solani* on 4th (89.95%) and 8th day (93.99%) in *in vitro* and also found most effective in reducing the Fusarium dry rot severity both in pre- (0.70 & 3.40%) and post-inoculation (0.73 & 5.60%) treatments.

Among the different temperatures studied in *in vivo*, *F. solani* did not produced dry rot symptoms on tubers preserved at 5, 10 and 40°C.

The results of cultural variability revealed that the isolates FS-14, FS-15 and FS-24 produced maximum luxuriant mycelial growth (90.00 mm). The isolates produced different pigmentations *viz.*, yellow, pale yellow, dark yellow, pink, orange, red, while isolate FS-7 and FS-24 did not produce pigmentation.

The molecular characterization study was carried out by using six RAPD primers. The primer OPA-6, OPA-7, OPA-11and OPA-13 showed the highest percentage (100%) of polymorphism. Highest similarity was observed between FS-10 and FS-13 (0.86). The dendrogram clearly exhibited one big cluster of twenty four isolates, while only one isolate FS-6 was separately situated with similarity matrix of 0.349 with FS-1.The results of PCR amplification with fuminosin mycotoxin specific primer FUM1 showed that all isolates have a potential to produce the toxin fuminosin except FS-6, FS-13 and FS-14 isolates.

Potato tubers when inoculated with *Fusarium solani* showed progressive decrease in total soluble sugar (2.90%), total reducing sugar (1.02%), non reducing sugar (1.88%) and starch content (3.41%), while the total phenol (3.74%) content was increased as the incubation period is increased as compared to uninoculated tubers.

DECLARATION

This is to declare that the whole of the research work reported here in the thesis is for the partial fulfilment of the requirements for the Degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** by the undersigned is the result of investigations done by me under the guidance and supervision of **Dr. R. K. Patil**, Professor, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand and no part of the work has been submitted for any other degree so far.

Place: Anand

Date: 05th June, 2015

(Pandya Janki P.)

Countersigned by

(R. K. Patil)

Professor
Department of Plant Pathology
B. A. College of Agriculture
A. A. U., Anand- 388 110.

ACKNOWLEDGEMENT

*The great accomplishments of man have resulted from the transmission of
Ideas and enthusiasm.*

This thesis is the end of my journey in obtaining my M.Sc. I have not traveled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people. At the end of my thesis, it is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me.

*At this moment of accomplishment, first of all I pay homage to my guide, **Dr. R. K. Patil**, Professor, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, for his constructive criticism, unflagging enthusiasm, valuable guidance, constant encouragement, most co-operative and painstaking attitude and everlasting inspiration during whole tenure of the investigation. Under his guidance I successfully overcame many difficulties and learned a lot. His unflinching courage and conviction will always inspire me to become an independent researcher and helped me realize the power of critical reasoning. He also demonstrated what a brilliant and hard-working scientist can accomplish.*

*My special words of thanks should also go to my co-guide, **Dr. T. M. Bharpoda**, Associate Professor, Department of Agril. Entomology, B. A. College of Agriculture, Anand Agricultural University, Anand, for always being so kind, helpful and motivating.*

*I greatly acknowledge the technical guidance, encouragement and moral support received from my advisory committee **Dr. A. B. Brahmhatt**, Associate Professor, Department of Nematology, **Dr. P. R. Vaishnav**, Professor & Head, Department of Agricultural Statistics, and **Dr. N.J. Patel**, Assistant professor, Department of Biochemistry, B. A. College of Agriculture, Anand Agricultural University, Anand for every arena of difficulty.*

*I vest to place on record my heartfelt thanks to **Dr. R. N. Pandey**, Professor & Head, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand for his kind co-operation during my research work and persuade me to complete my research work expeditiously.*

*I have also treasured immense assistance and incomparable affection from **Dr. V. P. Gohil**, **Dr. R. G. Parmar**, **Dr. N. M. Gohel**, and other staff members of Department of Plant Pathology, B. A. College of Agriculture, AAU, Anand for their valuable suggestions, co-operation and help during the course of study.*

I am highly thankful to Dr. N. C. Patel, Vice-Chancellor of Anand Agricultural University and Dr. K.P. Patel, Principal and Dean, B. A. College of Agriculture, Anand Agricultural University, Anand for providing me with all the required facilities to complete my research.

My heart feels indebted for the unstinted cooperation, guidance and help rendered by seniors Hemant and Jignesh. I also take the opportunity to acknowledge my friends Neha, Kinjal, Riddhi, Ravi, Kuldeep, Rishi and Kiran for supporting me. I would be remiss, if I do not articulate my cordial feeling of thanks into words to my juniors Alka, Mahima, Chaitra, Kirtana, Lipi, Ramapriya, Dhanya, Gunjan, Hardy, Bindesh, Vishvajeet and Harshit.

*I would like to acknowledge my career navigators **Dr. D. K. Patel** and **Shri. B.A. Pathak** who helped me to took First step in Agricultural science.*

*I would like to acknowledge my friends **Yash Patel** and **Bhoomi Mehta** for their moral support and motivation, which drives me to give my best. My special gratitude to **Prashant Arade** for always there cheering me up and stood by me through the good times and bad. I find myself lucky to have friends like them in my life.*

*Finally, I would like to acknowledge the people who mean world to me. My angel mother **Mrs. Shilpa Pandya** and my adorable father **Mr. Pinakin Pandya**. Thank you mom & dad, Words cannot begin to describe my appreciation and gratitude for sacrificing all that you have to guarantee my future, for showing faith in me and giving me liberty to choose what I desired. I could not have become the person I am today without you both in my life.*

*I hertly thankful to my yonger brother **Shivang** for always makes me smile in all my ups and downs. I find myself bleesed to have such a sweet brother.*

From the phone calls to the road trips up to see me dance, I'm lucky to have such a caring and supportive family, standing behind me with their love and support. Thank you for your endless support and encouragement.

*Love and adurance that had always been showered by my lovely grandparents dadu **Aniruddha Pandya** and dadi **Mrudula Pandya** and nanu **Lt. Himmatlal Mehta** and Nani **Hansaben** and all my family members have energized to mount the climax of this exploration. I don't imagine a life without their love and blessings.*

*Above all, I lay down my head before the almighty "**My Guruji**" and "**Lord Shiva and Krishna**" whose blessing gave me the strength to make this a successful venture and without which this piece of work would not have been crafted into the present shape.*

Place : Anand

Date : 05th June, 2015

(Pandya Janki P.)

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Abbreviations and Acronyms

| | |
|--------------------|--|
| % | Per cent |
| @ | At the rate of |
| μ | Micron |
| μg | Microgram |
| μl | Microliter |
| μm | Micrometer |
| °C | Degree Celsius |
| AAU | Anand Agricultural University |
| Anon. | Anonymous/ Unacknowledged |
| B.A. | Bansilal Amritlal |
| BOD | Biological Oxygen Demand |
| C.D. | Critical difference |
| C.V. | Coefficient of variation |
| Cfu | Colony forming unit |
| e.g | For example |
| EC | Emulsifiable concentrate |
| <i>et al.</i> | <i>et alii</i> ; and coworkers |
| etc. | Et cetera., pretty much the alike |
| Fig. | Figure |
| G/g | Gram |
| Ha/ha | Hectare |
| Hrs/hrs. | Hours |
| I.A.R.I. | Indian Agricultural Research Institute |
| i.e. | That is |
| I.T.C.C. | Indian Type Culture Collection |
| <i>In vitro</i> | Laboratory condition |
| <i>In vivo</i> | In the host |
| Kg/cm ² | Kilogram per centimeter square |
| L | Litre |
| M/m | Meter |

| | |
|---------|--|
| M | Molar |
| M ha | Million hectare |
| Mg | Milligram |
| Min | Minutes |
| ml | Milliliter |
| mM | milli Molar |
| mm | Millimeter |
| MT | Metric tone |
| N | Normal |
| O.D. | Optical density |
| PDA | Potato Dextrose Agar |
| PGI | Per cent Growth Inhibition |
| pH | Potential of hydrogen ion |
| ppm | Parts per million |
| RH | Relative humidity |
| rpm | Revolution per minute/ rotation per minute |
| S.Em | Standard Error of mean |
| spp. | Species |
| Sr. No. | Serial Number |
| Tr. No. | Treatment Number |
| TSS | Total Soluble Sugar |
| v/v | Volume by volume |
| viz., | Namely |
| w/v | Weight by volume |
| WP | Wettable powder |

I. INTRODUCTION

The potato (*Solanum tuberosum* L.) is herbaceous annual that grows up to 100 cm tall. It belongs to the Solanaceae or “nightshade” family of flowering plants.

Potato is also known as “The king of vegetables”, and has emerged as fourth most important food crop in India after rice, wheat and maize. Potato was first domesticated in the region of modern-day Southern Peru and extreme North Western Bolivia between 8,000 and 5,000 BCE (Spooner *et al.*, 2005). It was well established as a crop in India by the late 18th century and in Africa by the mid-20th century. Later it spread around the world and became a staple food crop of many countries.

About 365 million metric tons of potato is produced in the world over an area of about 19.13 million hectare (Anon.2014). India contributes about 15 per cent of total potato production of the world. India is the second largest producer of potato (45 million MT) in the world after China (85 million MT) and both the countries put together contribute nearly one third of the global potato production today (Scott and Suarez, 2011).

Indian vegetable basket is incomplete without Potato. The potato is rich in starch, vitamins, minerals and trace amounts of thiamin,

riboflavin, folate, niacin, magnesium, phosphorus, iron and zinc, as well as an assortment of phytochemicals such as carotenoids and natural phenols. The annual diet of an average global citizen in the first decade of the 21st century included about 33 kg of potato (Anon. 2011).

Potato is grown almost in all states of India. However, the major potato growing states of India are Himachal Pradesh, Punjab, Uttar Pradesh, Madhya Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Bihar and Assam. The annual production of potato in India for the year 2013-14 was 41,555 MT (Anon. 2014). Potato is one of the most important *Rabi* vegetable cash crop, which has got an unlimited potentiality of its cultivation in Gujarat. The total area under potato cultivation in Gujarat was about 81,000 hectares with the total production of 25 lacks MT with an average yield of 30.10 MT/ha during the year 2013-2014.

The fungal diseases like powdery scab (*Spongospora subterranea*), wart (*Synchytrium endobioticum*), late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), black rot (*Rosellinia* sp.), stem canker / black scurf (*Rhizoctonia solani*) and fusarium dry rot (*Fusarium* spp.) etc. cause substantial yield losses to potato crop every year. Fusarium dry rot of potato (*Solanum tuberosum* L.) can be a devastating postharvest disease worldwide and is incited by several species of *Fusarium* (Boyd, 1972). Dry rot affects both tubers in storage and seed tuber pieces in the

field (Choiseul *et al.*, 2006). It is mainly incited by *Fusarium* spp. particularly in warm and sandy soils. The skin of the dry rot infected tubers first becomes brown then turns darker and develops wrinkles. These wrinkles are often arranged in irregular concentric circles. In the later stage of infection, a hole may be observed in the centre of the concentric ring with whitish or pinkish growth of fungal mycelium. On cutting these affected tubers, whitish or brownish tissues are seen with one or more cavities. Eventually the infected tubers loose water and become dry, hard and shriveled.

Investigations carried on dry rot in the UK showed 70 per cent edible tubers and 100 per cent seed tubers were found infected with *Fusarium solani* (Mart.) Sacc. and more than 1 per cent of the tubers showed the symptoms of dry rot (Bradshaw *et al.*, 2001). Bhardwaj (2012) reported 25 to 60 per cent yield loss in India due to dry rot caused by *Fusarium solani* (Mart.) Sacc. Crop losses attributed to dry rot have been estimated to the tune of 6 to 25 per cent (Chelkowski, 1989). *Fusarium* spp. cause tuber rot, with severe reductions in crop yield, upto 25 per cent annually (Lui and Kushalappa, 2002).

Very meagre research work has been carried out on post harvest rots of potato tubers and their management in India, therefore to extend the storage life of potato and to reduce the losses caused by post harvest diseases; it is felt worthwhile to carry out the investigations on dry rot of

potato tubers incited by *Fusarium solani* (Mart.) Sacc. and its management under Middle Gujarat condition with following research aspects.

RESEARCH ASPECTS:

1. Collection, isolation, purification, pathogenicity and identification of pathogen causing dry rot of potato tubers.
2. Survey of post-harvest diseases of potato tuber at Sardar Patel vegetable market, Station road and Goya Talav market at Anand.
3. Evaluation of newer fungicides, some organic and inorganic salts, natural products and antagonists against *F. solani* (Mart.) Sacc. causing dry rot of potato tubers *in vitro* and *in vivo*.
4. Effect of different temperatures on development of Fusarium dry rot of potato tubers *in vivo*.
5. Cultural and morphological variability among the isolates of *F. solani* (Mart.) Sacc. causing dry rot of potato tubers.
6. Molecular variability among the isolates of *F. solani* (Mart.) Sacc. causing dry rot of potato tubers using RAPD markers.
7. Biochemical factors responsible for disease resistance: phenol, sugar and starch.

II. REVIEW OF LITERATURE

2.1 Collection, isolation, purification, pathogenicity and identification of pathogen causing dry rot of potato tubers

Moghadam and Hosseinzadeh (2013) reported that inoculation with conidial suspension (1×10^4 conidia/ml) of all the species of *Fusarium* into the potato tubers showed that *F. solani* had the highest pathogenic severity among all other species, while *F. lateritium* had the lowest one. *Fusarium oxysporum*, *F. solani*, *F. equiseti* and *F. sambusinum* were identified as main pathogens causing dry rot disease in potato from the Ardabil province.

El-Hassan *et al.* (2007) proved the pathogenicity of 33 isolates of *Fusarium* spp. isolated from potato tubers. Among all isolates eight isolates were found highly pathogenic (FSA7, FSA9, FSO15, FSO17, FSO21, FSO27, FSO30 and FOX31).

Leszek Lenc (2011) studied pathogenicity and potential capacity for producing mycotoxins by *Fusarium sambucinum* and *Fusarium solani* isolates isolated from potato tubers.

Gashgari and Gherbawy (2013) proved pathogenicity of some *Fusarium* spp. associated with superficial blemishes of potato tubers.

Chehri *et al.* (2011) studied occurrence and pathogenicity of *Fusarium* spp. on the potato tubers in Malaysia.

2.2 Survey of post-harvest diseases of potato tuber at Sardar Patel vegetable market, Station road and Goya Talav market at Anand

Eighteen per cent of potato tubers arrived at New York markets between 1972-1980 showed symptoms of *Fusarium* dry rot (Cappellini *et al.*, 1984), while as high as 60 per cent of graded tubers in Scotland were found affected by dry rot (Carnegie *et al.*, 1990).

Masum *et al.* (2011) reported that potato tubers were found infected with soft rot (3.58%), dry rot (1.06%) and scab (0.96%) in Dhaka district of Bangladesh.

Hossain *et al.* (2010) conducted a survey during October 2006 to June 2008 and observed higher leaf infection due to early blight of potato and tuber infection by soft rot (*Erwinia carotovora*) of potato to 37 and 39 per cent, respectively.

Bayona *et al.* (2011) reported that potato tubers exhibited symptoms of extensive dry rot (*Fusarium oxysporum*) at a supermarket in Bogota, Colombia in August, 2008.

Gachango *et al.* (2012) carried out a survey of seed potato tubers in Michigan seed production storage during 2009 and 2010 and reported 228 isolates of different 11 *Fusarium* spp. infecting seed potato tubers isolated from 370 samples collected from a total of 51 seed lots. Among all 11 species, *Fusarium oxysporum* was predominant (30.3%), followed by *F. equiseti* (19.3%). *F. sambucinum* and *F. avenaceum* were

third most prevalent (each at 13.6%). Less prevalent species (each at 4 to 10%) included *F. cerealis*, *F. solani*, and *F. acuminatum*.

Khan *et al.* (1973) reported 9.5 to 22 per cent tuber losses due to soft rot (*Erwinia carotovora*) in cold storage in Bangladesh in which 3 to 11 per cent tuber loss were from only soft rot.

2.3 Evaluation of newer fungicides, some organic and inorganic salts, natural products and antagonists against *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers *in vitro*

2.3.1 Fungicide:

Rawal and Thakore (2003) reported that carbendazim, triadimefon and kitazin at 10, 25, 50 and 75 ppm concentrations were found most effective in inhibiting the mycelial growth as well as fruit infection by *Fusarium solani* in sponge gourd.

Onwuzulu *et al.* (1995) observed complete inhibition of *Fusarium oxysporum* in imazalil at 50 and 100 µg/ml concentrations by poisoned food technique. The decay reduction index for imazalil was 86.7 and 73.3 at 1000 µg/ml for *F. oxysporum* and *Alternaria alternate*, respectively in artificially inoculated ripe tomato fruits.

Terao *et al.* (2006) showed that thiabendazole (0.03%) and imazalil (0.4%) completely inhibited the mycelial growth and sporulation of *F. proliferatum* causing melon rot, whereas azoxystrobin (0.01%) treatment resulted in 87.09 per cent mycelial inhibition *in vitro*.

Thiabendazole and imazalil provide better control in refrigeration up to 20 days of storage.

Benato *et al.* (2002) studied the effect of different fungicides on passion fruit rot caused by *Colletotrichum gloeosporioides*, *Cladosporium* spp., *Rhizopus stolonifer*, *Lasidiplodia theobromae*, *Phoma* spp., *Alternaria* spp., *Fusarium* spp. and *Penicillium* spp. Prochloraz and imazalil found most effective in controlling the fruit rot by fruit dip treatment and then stored at $25 \pm 1^{\circ}\text{C}$ for 10 days at 70-80 per cent RH.

Ge Yong Hong *et al.* (2005) reported that pre- and post-harvest dip treatment with harpin (60 mg/l) found most effective in controlling the Fusarium rot (*F. semitectum*) and Trichothecium rot (*T. roseum*) of muskmelon.

Wagan *et al.* (2004) observed that benomyl at lower concentration gave significant reduction in colony growth of *Fusarium solani*, *Alternaria alternata*, *Aspergills niger*, *Penicillium* spp., *Stemphyllium* sp. and *Phomopsis* sp. infecting pawpaw fruit.

Saha and Kaiser (2005) found that bavistin at 400 ppm concentration gave complete inhibition of *Fusarium semitectum* infecting litchi fruits followed by benlate (450 ppm) and topsin M (600 ppm) *in vitro*.

Singh (2011) reported complete mycelial growth inhibition of *F. moniliforme* in benomyl, carbendazim (12%) + mancozeb (63%),

thiophanate methyl, carbendazim and propiconazole at both the concentrations (500 & 1000 ppm). She reported that significantly lowest Fusarium rot severity was recorded in banana fruits treated with benomyl (5.82 & 4.16 %) and propiconazole (6.66 & 4.99%) in pre and post inoculation treatments, respectively.

Gleosporium musarum and *Fusarium roseum* infecting banana were effectively controlled by thiabendazole (200 ppm) and benomyl (300 ppm) when used as post harvest dips (Shillingford, 1970).

Pre- and post-inoculation dips with benomyl (100 ppm) was found most effective against Fusarium rot of banana (Khanna and Chandra, 1975).

Post- harvest dips of banana fruits in thiabendazole (500 & 1000 ppm) and benomyl (250 & 500 ppm) immediately after harvest gave good control against crown rot of banana (Ramma *et al.*, 1999 and Peerthum, 1998).

Sharma (2006) reported that bavistin and ziram-500 at 750, 1000 and 1250 ppm concentrations were found most effective in inhibiting fruit rot incited by *F. moniliforme* in Ivy gourd (*Coccinia indica*).

Datar and Ghule (1998) reported that pathogens associated with fruit rot of banana (*Cylindrocarpon tokinensis*, *Penicillium funiculosum*, *Fusarium solani*, *Fusarium* sp. and *Colletotrichum* sp.) can be effectively controlled by dipping the fruits in carbendazim (1000 ppm) for 10 min.

Lapeyre and De Nolin (1994) showed that triazole fungicides viz. propiconazole, myclobutanil, flusilazole and bitertanol when applied at low concentration (50 ppm) successfully controlled post-harvest diseases of banana incited by *Colletotrichum musae* and *Fusarium* sp. Fruit dipping and shower spraying were more efficient than low or high volume sprays.

Jones (1991) found prochloraz and flusilazole most effective in controlling crown rot of banana incited by *Fusarium pallidroseum* and *Verticillium* sp.

2.3.2 Antifungal Activity of Some Salts

Ghadiri *et al.* (2013) reported that ammonium phosphate gave highest inhibition of the mycelial growth of *F. solani* (92.29%) on PDA media followed by potassium carbonate (54.92%), potassium bicarbonate (46.14%), sodium carbonate (42.60%) and sodium bicarbonate (42.33%).

Mecteau *et al.* (2008) noted that exposure of *F. solani* var. *coeruleum* conidia to aluminium acetate, potassium sorbate, sodium benzoate, sodium metabisulfite or trisodium phosphate at 0.2 M resulted in 100 per cent mortality after 1h, while aluminium chloride and aluminium lactate caused 100 per cent mortality of conidia when exposed for 24 h.

Hervieux *et al.* (2002) reported that aluminium chloride (0.2M) significantly reduced silver scurf (*H. solani*) severity when

applied either at 2, 4 or 7 days after inoculation, while aluminium lactate, potassium sorbate, sodium carbonate, sodium metabisulfite, and trisodium phosphate markedly reduced silver scurf severity in potato when applied at 2 or 4 days after inoculation.

Mills *et al.* (2004) found that mycelium growth and spore germination of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium alboatrum*, and *V. dahliae* were consistently inhibited by sodium metabisulfite followed by the aluminium compounds like aluminium chloride and aluminium acetate.

Das (1991) reported that decreasing in the concentration of K⁺ ions below 3mM in agar medium which contained starch, casein hydrolysate, MgSO₄, and K₂HPO₄ changed the growth pattern of *Fusarium solani* illuminated in diurnal 12h light/12h dark cycles from zonation to a feathery growth mode.

Shekhar *et al.* (2009) reported that ammonium carbonate, potassium carbonate and sodium carbonate were found very effective in reducing the radial growth of *A. flavus* at 20 mM and found fungitoxic at 30 mM.

2.3.3 Natural products:

Pandya *et al.* (2009) reported that the mycelial growth of *Fusarium solani* was inhibited by tulsi leaf extract (15%) using poisoned

food technique followed by acalypha (10%), garlic (5%), lantana (10%), naffatia (15%), barmasi (10%) and neem (15%).

Joshi (1985) studied the effect of different plant extracts on pathogens infecting banana fruits and concluded that application of turmeric rhizome extract (10%) found most effective in inhibiting the growth of several pathogens (*Fusarium* spp., *Botryodiplodia theobromae*, *Colletotrichum musae*, *Penicillium* spp. *Rhizopus* spp. and *Aspergillus* spp.).

Singh and Chand (2004) reported that neem leaf extract completely inhibited the spore germination of *Fusarium* sp. causing chickpea wilt.

Win *et al.* (2007) studied the antifungal activities of cinnamon, piper and garlic extract at 0, 0.1, 0.5, 10.0 and 0.75g/l concentrations on banana crown rot fungi (*Colletotrichum musae*, *Fusarium* sp. and *Lasiodiplodia theobromae*) *in vitro*. Cinnamon extract (0.5g/l) completely inhibited conidial germination and mycelial growth of all fungi.

Rawal and Thakore (2003) reported that extracts of medicinal plants like *Datura stramonium*, *Azadirachta indica* and *Ocimum sanctum* each at 5, 10, 15 and 20 per cent concentrations were found most effective in inhibiting the growth of *Fusarium solani* and effectively controlled the sponge gourd rot when applied before and after inoculation.

Joseph *et al.* (2008) studied *in vitro* efficacy of different plant extracts viz., *Azardiachta indica*, *Artemessia annua*, *Eucalyptus globulus*; *Ocimum sanctum* and *Rheum emodi* each at 5, 10, 15 and 20 per cent to control brinjal wilt pathogen (*Fusarium solani* f. sp. *melongenae*). All the plant extracts showed significant reduction in the mycelial growth of the pathogens. Among the different extracts, *Azardiachta indica* (1.45 cm) found most effective at 20 per cent concentration followed by *Rheum emodi* (1.52 cm), *Eucalyptus globules* (1.81 cm), *Artemessia annua* (1.89 cm) and *Ocimum sanctum* (1.91 cm).

Singh (2011) reported that complete mycelial growth inhibition of *F. moniliforme* was recorded in garlic, cinnamon and arduisi extracts each at 10 per cent concentration. However, lowest severity of fruit rot of banana was found in cinnamon leaf extract (14.16 & 13.00%) in pre- and post-inoculation treatments, respectively.

Bhardwaj (2012) showed that the mycelial growth of *Fusarium solani* was inhibited by 15 per cent mixed leaf extracts of *Lawsonia alba* and stem extracts of *Acacia catechu* (78.64%).

Mathan *et al.* (2008) revealed that neem leaf extract (60%), buffalo urine (20%) and poultry litter (40%) completely inhibited the mycelial growth of *Fusarium moniliforme*.

The alcohol extract from different parts of neem, especially the bark, gave the highest growth inhibition of the *Fusarium solani* in culture

and rot development of the tomato fruits in pre inoculation (Amadioha and Uchendu, 2003).

2.3.4 Biological control

Abeysinghe (2007) reported that *T. harzianum* (RU01) found highly inhibitory in dual Petri plate assays against *F. solani* f. sp. *phaseoli* followed by *B. subtilis* (CA32).

Schisler *et al.* (2000) found that *P. fluorescens* (P22:Y:05) and *Enterobacter cloacae* (S11:T:07) reduced the potato dry rot severity incited by *Gibberella pulicaris* (Anamorph: *Fusarium sambucinum*) to 25 and 17 per cent, respectively.

Trichoderma harzianum was found most effective in reducing the *B. theobromae* rot by 53.5 to 84.5 per cent. The rot reduction caused by *F. solani* ranged from 59.6 to 87.1 per cent followed by *Pseudomonas syringae* and *P. chlororaphis* in water yam (Okigbo and Emeka, 2010).

Choudhari *et al.* (2012) reported that *T. viride* was found best in inhibiting the mycelial growth of pathogen causing root rot (*Fusarium solani*) disease in mulberry by 73.6 per cent followed by *T. harzianum* (64.0%) and *Pseudomonas fluorescence* (51.1%).

Rajendiran *et al.* (2010) found that *T. viride* inhibited the radial growth of post harvest pathogens of fruits and vegetables namely *A. niger* (55.0%), *A. flavus* (51.0%), *A. fumigatus* (52.0%), *Fusarium* spp. (64.0%) and *Penicillium* spp. (54.0%) in dual culture method. Maximum growth

inhibition of *A. niger* (64.0%), *A. fumigatus* (49.0%) and *A. flavus* (48.0%) was recorded in 50 per cent culture filtrate.

Singh (2011) reported that *Trichoderma harzianum* found most efficient antagonist in inhibiting the mycelial growth of *F. moniliforme* (53.06 %) *in vitro*.

Padmodaya and Reddy (1996) found *Trichoderma viride* as highly inhibitory to *Fusarium* sp. causing wilt in tomato followed by *T. harzianum*.

Xiao *et al.* (2007) showed that *T. harzianum* antagonized *Rhizoctonia solani*, *Fusarium moniliforme*, *Colletotrichum capsici* and *Sclerotium rolfsii*. The colonies of pathogens were either overgrown or invaded by *Trichoderma* spp., leading to inhibition of growth along with debasement and reduction in spore concentration.

Lassois *et al.* (2008) reported two yeast strains (*Pichia anomola* strain K and *Candida oleophila* strain O) showed antagonism against *Fusarium moniliforme* and *Colletotrichum musae* causing banana crown rot.

Costa and Subasinghe (1998) observed that *Pseudomonas* isolates associated with the fruit skin of banana cv. Seenikehel inhibited the growth of *Fusarium* spp., *Colletotrichum musae*, *Botryodiplodia* spp. and *Ceratocystis paradoxa* (30-42%) causing crown rot of banana in *in vitro*.

Adebesin *et al.* (2009) studied the fruit treatment with conidia and culture filtrate of *Trichoderma asperellum* (NG-T161) for 30 min prior to inoculation with pathogen in banana fruits, provided a better control. At 50 per cent (v/v) concentrations, the filterates inhibited the mycelial growth of *Fusarium oxysporum* and *Colletotrichum musae* by 49.7 and 60.3 per cent respectively.

Pratella and Mari (1993) found that *T. viride*, *T. harzianum*, *Gliocladium roseum* and *Paecilomyces varioti* when applied as spray treatment to fruits partially controlled *Botrytis cinerea* in strawberry and kiwi fruit, *Fusarium oxysporum* in potatoes and *Alternaria citri* in lemon.

Ozbay and Newman (2004) reported that *Trichoderma* sp. are well documented as effective biological control agents of plant diseases caused by both soil borne fungi, leaf and fruit infecting plant pathogenic fungi. They are fast growing and rapidly colonize *Fusarium* spp.

Wang *et al.* (2008) reported that one isolate of *Bacillus amyloliquefaciens* (EXWB3) and three isolates of *Bacillus subtilis* (EXWB1, EXWB2 & EXWB4) were effective against seven post-harvest pathogens including *B. cinerea*, *A. alternata*, *F. oxysporum*, *A. niger*, *T. roseum*, *Penicillium* sp. and *Cladosporium* sp. of melon fruits. Among the *Bacillus subtilis*, strain EXWB1 was found to be the most effective in controlling the rots.

Candida oleophila strain O was tested against *Colletotrichum musae*, *Fusarium moniliforme* and *Cephalosporium* sp. inciting crown rot of banana at three concentrations 10^6 , 10^7 , 10^8 cfu/ml. Strain O (10^8 cfu/ml) showed the highest protective level (56%) when applied 24 hrs before the complex (Jijakli *et al.* 2004).

Zhan *et al.* (2005) isolated *Trichoderma* spp. from the soil, leaves, and fruits of sugar apple and they found strong antagonism against *Phytophthora palmivora*, *P. parasitica* and *Fusarium* sp. by pairing culture on PDA.

Calistru *et al.* (1997) tested nine isolates of *Trichoderma* against *Aspergillus flavus* and *Fusarium moniliforme* in dual culture and it was revealed that out of nine, four isolates of *Trichoderma* sp. (*T. harzianum* & *T. viride*) inhibited the fungal growth of the pathogens.

2.4 Effect of different temperatures on development of *Fusarium* dry rot of potato tubers *in vivo*

Kausar *et al.* (2009) recorded 2.5 and 3.81 mm mycelial growth of *L. theobromae* and *F. solani* at 40 °C temperature, respectively.

Llamas *et al.* (2013) recorded minimum mycelial growth of *Fusarium proliferatum* after 9 days of incubation at 5 (0.03 ± 0.04 mm) and 40°C temperature (0.008 ± 0.02 mm).

Cherian and Varghese (2007) reported that *F.semitectum* was unable to produce disease symptoms at 10 °C, and spore germination did not observed at 10°C even after 24h of incubation.

Khilare and Ahmed (2012) found that temperature ranging from 10 to 20⁰C did not found suitable for the growth of *F. oxysporum* f. sp. *ciceri* isolates.

Remadi *et al.* (2006) studied the effect of temperature on aggressivity of Tunisian Fusarium species inciting potato dry rot *in vivo* and found two thermal pics of aggressivity first one at low temperatures (10-15°C) and a second at higher temperatures (30-35°C). Nevertheless, at temperatures less than 25°C, *F. sambucinum* and *F. graminearum* were found most aggressive, while *F. solani* was the most aggressive at temperatures superior to 30°C.

Seppanen (1982) observed variation in thermal requirements of tuber infection of *Fusarium* species which varied from 24 to 30°C. The most conducive temperature rang for *F. coeruleum* and *F. avenaceum* was 18 to 24°C and for *F. sambucinum* temperature 12 to 30°C.

Tivoli and Jouan (1981) classified French isolates of *F. graminearum* and *F. culmorum* within a group of higher thermal optimum situated between 25 and 30°C, while *F. solani* seems to have

its thermal optimum of *in vivo* development situated between 30 and 35°C.

2.5 Cultural and morphological variability among the isolates of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers

2.5.1 Cultural variability:

Out of eight different solid media (PDA, MA, CDA, RBA, SDA, OMA, YMA & NA) and six liquid broth media (PDB, SDB, PGB, MB, YEB & NB) studied, *Fusarium moniliforme* (KUMBF1201) gave good mycelial growth, pigment production and sporulation on Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media (Pradeep *et al.*, 2013).

Gupta *et al.* (2010) studied cultural and physiological (temperature and pH) characters of *Fusarium oxysporum* and *F. solani*, causal agents of wilt in guava. The results revealed that maximum mycelial growth was obtained in PDA as semi-solid media (78.00 mm) for *F. oxysporum* and 73.83 mm for *F. solani*. While malt extract broth as liquid broth media *i.e.* 1385 mg mycelium for *F. oxysporum* and 1491 mg for *F. solani*. Maximum sporulation was recorded in oatmeal agar and mycological broth. The optimum temperature and pH for growth for both *Fusarium* spp. was 28°C and 5.5, respectively.

Mwang'ombe *et al.* (2008) studied cultural and morphological characters of *Fusarium solani* (Mart) f.sp. *phaseoli* (Burk) Synd. and

Hans, causal agent of root rot of common bean. They found that all 52 isolates showed high variability in aerial mycelial growth, mycelial texture, pigmentation (mycelia colour) when cultured on potato dextrose agar medium. They grouped colonies into luxuriant, moderately luxuriant and scanty on aerial mycelial growth; fluffy and fibrous based on mycelial texture; purple, pink and white based on mycelial colour; and long, medium and short macro conidial length.

Sharma *et al.* (1996) studied difference among the five isolates of *Fusarium solani* (Mart.) Sacc. causing root rot of mulberry and found that all isolates differed from each other in morphological, cultural and other colony characters as well as in virulence.

Bolton and Donaldson (1972) observed variation in pathogenicity and in cultural characteristics such as color, growth rate, ability to produce pionnotes, topography, macrospore shape and size and tendency to sector in isolates of *Fusarium solani* f. *pisi* and *F. oxysporum* f. *pisi* isolated from diseased peas.

Barreto *et al.* (2003) reported that *Fusarium solani* (Mart.) Sacc. developed colonies after 2-3 days of incubation which were characterized by typical dirty-white or white blue pigmentation, long and branched phialides, hyaline to milky-white microconidium droplets, and pionnotes with macroconidia.

2.5.2 Morphological variability:

Chandran and Reddi (2012) reported that the isolates of *F. solani* showed significant variation in radial growth and the size of macro conidia (13-15 x 3-4 μm to 27-29 x 4-5 μm) and the micro conidia (3-4 x 1-2 μm to 9-10 x 1-3 μm) on PDA medium. The number of septa in macro and micro conidia were 3-5 and 0-1, respectively and conidia were hyaline. The macro conidia were sickle shaped with blunt end and micro conidia were round to oval shaped.

Sang-Do Cha *et al.* (2007) recorded the microscopic observation of *Fusarium oxysporum* isolates isolated from paprika and showed that the conidiophores were unbranched and monophialides, the microconidia have oval-ellipsoidal shape with no septa (3.0~11 \times 1.5~3.5 μm) and macro conidia (15~20 \times 2.0~3.5 μm) have slightly curved or slender shape with 2~3 septa.

Balali and Iranpoor (2006) reported considerable intraspecific variation *i.e.* colony growth, shape of macro and micro-conidia and rare to dense chlamydospores formation among the *Fusarium* spp. However *Fusarium solani* isolates on PDA were cream or white and in rare cases the lower surface was light violet.

Yong Sung Jung *et al.* (1999) reported that microconidia of *Fusarium solani* f. sp. *pisi* formed on the long conidiophore with ovoid or oblong shape with 5~14 \times 2.5~5.0 μm size. Macroconidia of *Fusarium*

solani f. sp. *pisi* were formed on the multibranched conidiophores on carnation leaf agar media with 4.8~ 5.3× 32.0~40.7 μm size.

2.6 Molecular variability among the isolates of *Fusarium solani* causing dry rot of potato tubers using RAPD markers

Gupta *et al.* (2009) studied the genetic polymorphism of the isolates of *Fusarium solani* and found that 10 randomly amplified polymorphic DNA markers (OPA1-OPA10) tested in the genome of *Fusarium solani* and grouped on the basis of allelic data. This pattern of genetic variability in the isolate was also supported by the analysis of the similarity indices and UPGMA dendrogram.

Younes *et al.* (2013) studied the genetic variation of *Fusarium solani* isolates based on RAPD-PCR analysis and results indicated high levels of variations in banding patterns of all isolates tested. Unique banding profiles were obtained with two isolates of *F. solani* (I₄ & I₆).

El-Fadly *et al.* (2008) studied identification of some *Fusarium* spp. using RAPD-PCR technique and found genetic variability among such species and formae specialis under study.

Sutthisa *et al.* (2010) studied genetic variation of 30 isolates of *Fusarium* spp. using random amplified polymorphic DNA (RAPD) markers. Four primers A02, A03, A07 and A09 produced fingerprint profiles, which clearly distinguished between the different *Fusarium* spp.

Sankar *et al.* (2014) studied molecular characterization of *Fusarium solani* isolates inciting dry root rot of sweet orange using random amplified polymorphic DNA (RAPD) markers. Twelve representative isolates were characterized using 20 random primers of OPM series, out of which 17 primers gave a total of 174 amplified products, showing 90.22% polymorphism.

2.7 Biochemical factors responsible for disease resistance: Phenol, sugars and starch

2.7.1 Phenol:

Majumdar and Raychaudhari (1977) showed that total phenol content get decreased in the pea plants when infected with *Fusarium* spp.

Bagwan and Meshram (2003) observed that total sugar content of banana fruit found decreased when infected with *Trichotehcium roseum*, *Rhizopus arrhizus* and *Penicillium expansum* by 5.02, 6.03 and 5.00 per cent respectively as compared to control (13.12%), whereas total phenol content increased by 2.31, 2.20 and 2.40 per cent respectively as compared to control (2.17%).

Tang *et al.* (2006) revealed that total phenol and ferulic acids in banana increased when inoculated with *F. oxysporum* f. sp. *cubense*. Total phenol and ferulic acid content in infected cv. Fenjiao was 546 and 74.8 micro g/ml at 28 days after inoculation, while control plants showed 184 and 68.6 micro g/ml phenol and ferulic acid content, respectively.

Singh (2011) estimated the total phenol content in banana fruits inoculated with *Fusarium moniliforme* over control fruits and noted appreciable increase in total phenol content when banana fruits were inoculated with *F. moniliforme*. Highest amount of total phenol content 2.71 per cent was recorded on 6th day after inoculation followed by 2.69, 2.68 and 2.64 per cent on 5th, 4th and 3rd day of inoculation, respectively.

Srivastava and Kumar (2013) studied the phenol content in onion bulbs inoculated with *Botrytis* sp. and capsicum inoculated with *Colletotrichum* sp. The results revealed that phenol level was high in infected onion in comparison to fresh onion. However, negative trend of results noticed in capsicum, phenolic compounds get decreased due to higher activity of phenolic metabolic enzymes or change in auxin metabolism leading to less synthesis and/or more oxidation of phenol.

2.7.2 Sugars:

Srivastava and Kumar (2013) studied the sugar content in onion bulbs inoculated with *Botrytis* sp. and capsicum inoculated with *Colletotrichum* sp. The results showed that reducing and non reducing sugar decreased in pathogen inoculated vegetables as compared to uninoculated control.

Seager and Haslemore (1993) reported that the phenol-sulphuric acid assay found to be a most suitable method for estimating the TSS from tissue extracts of Kiwi fruits.

Singh *et al.* (1991) reported that total sugar, reducing and non reducing sugar and ascorbic acid content get decreased faster in banana fruits inoculated with *Botryodiplodia theobromae*, *Fusarium oxysporum* and *Aspergillus niger* than in healthy fruits.

Singh (2011) estimated the total soluble sugar content in banana fruits inoculated with *Fusarium moniliforme* and control fruits and found that TSS in inoculated (*F. moniliforme*) fruits get decreased progressively with an increase in incubation period *i.e.* 14.24, 13.20, 12.73, 11.52, 11.26 and 11.19 per cent at 1st, 2nd, 3rd, 4th, 5th and 6th day after inoculation, respectively, over control (18.05%).

2.7.3 Starch

Choudhari and Kareppa (2013) studied biochemical changes in healthy and infected rhizomes of ginger due to infection by *Fusarium* spp. and found that starch content get increased by 49.6 per cent in the infected rhizomes over healthy rhizomes (47.0%).

Markson *et al.* (2014) studied starch grain depletion in Irish potato infected with *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Penicillium expansum* and reported that after one week of incubation period, about 10 per cent of starch grains were lost due to infection by *B. theobromae* as against 20 per cent reduction obtained with *P. expansum* and *R. stolonifer*.

III. MATERIALS AND METHODS

Present investigation on “Molecular characterization of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers (*Solanum tuberosum* L.) and its management” was carried out in the Department of Plant Pathology at Bansilal Amritlal College of Agriculture, Anand Agricultural University, Anand during the year 2014-2015. The materials used and methods adopted during the studies are described here in this chapter.

3.1 General

3.1.1 Location

Anand Agricultural University, Anand, where the present investigation was carried out is situated on 22⁰-35’ North latitude and 72⁰-55’ East longitude and has an elevation of 45 meter above the mean sea level.

3.1.2 Climate

Monsoon is warm and moderately humid. It commences by the middle of June and ends in middle of September. An average rainfall of the tract is about 1015.88 mm. Monsoon in this area is often erratic and uncertain, both in respect of total rainfall and its distribution. Winter is fairly cool and dry, while summer is quite hot and dry.

3.1.3 Laboratory procedures

3.1.3.1 Glassware cleaning

Standard glasswares were used during all the laboratory experimentation. The glasswares were dipped in cleaning solution—chromic acid (60 g of potassium dichromate dissolved in 300 ml of distilled cooled water, then after 400 ml of concentrated sulphuric acid was added in it with constant stirring) for 24 hours. Afterwards the glasswares were cleaned with detergent powder, followed by washing in running tap water and finally rinsed with distilled sterile water and dried before use.

3.1.3.2 Sterilization

The cleaned glasswares were sterilized at 180⁰C for 2 hours in hot air oven. Media and water were sterilized in an autoclave at 121⁰C at 1.1kg/cm² pressure for 20 min.

3.1.4 Experimental materials

Healthy potato tubers of uniform size were collected from vegetable market, station road, Anand. Whereas, dry rot infected potato tubers were collected from different vegetable markets of Gujarat as well as markets of different states of India for collection of different isolates of *Fusarium solani*.

3.1.5 Selection of variety

The most popular potato variety ‘Kufri Badshah’ was selected for studies as it is better in terms of yield, size, easy to cook, floury texture,

mild flavor, free from discoloration after cooking and preferred by the growers and consumers.

3.2 Collection, isolation, purification, pathogenicity and identification of pathogen causing Fusarium dry rot of potato tubers

3.2.1 Collection of samples

The diseased potato tuber samples exhibiting typical dry rot symptoms were collected from different 25 markets of India. The infected samples were brought to the laboratory and were subjected to tissue isolation for further studies.

Table 3.1: Sources of *Fusarium solani* isolates of potato

| Sr. No. | Name of Isolate | Market | District | State |
|----------------|------------------------|-------------------------------|-----------------|--------------|
| 1. | FS-1 | Sardar Patel vegetable market | Anand | Gujarat |
| 2. | FS -2 | Goya Talav market | Anand | ----”---- |
| 3. | FS -3 | Jamalpur market | Ahmedabad | ----”---- |
| 4. | FS -4 | APMC market | Navsari | ----”---- |
| 5. | FS -5 | Talav gate vegetable market | Junagadh | ----”---- |
| 6. | FS -6 | Idar vegetable market | Sabarkantha | ----”---- |
| 7. | FS -7 | Sardar market | Surat | ----”---- |
| 8. | FS -8 | Halol local vegetable market | Panchmahals | ----”---- |
| 9. | FS-9 | Local vegetable market | Bhavnagar | ----”---- |
| 10. | FS-10 | W.T.P.S | Kheda | ----”---- |
| 11. | FS-11 | Khanderao market | Vadodara | ----”---- |

| | | | | |
|-----|-------|-----------------------------|--------------------------|------------------|
| 12. | FS-12 | Local vegetable market | Rajkot | ---”--- |
| 13. | FS-13 | APMC market | Banaskantha | ---”--- |
| 14. | FS-14 | Azadpur mandi | North West | New Delhi |
| 15. | FS-15 | Shahu market yard | Kolhapur | Maharashtra |
| 16. | FS-16 | Choithram market | Indore | Madhya Pradesh |
| 17. | FS-17 | Naini sabji mandi | Varanasi | Uttar Pradesh |
| 18. | FS-18 | Sarai road vegetable market | Ludhiana | Punjab |
| 19. | FS-19 | Palayam market | Thiruvanantha - puram | Kerala |
| 20. | FS-20 | Mukharjee chowk mandi | Udaipur | Rajasthan |
| 21. | FS-21 | Bazar samiti | Nalanda | Bihar |
| 22. | FS-22 | Ek number market | Khurda | Orissa |
| 23. | FS-23 | APMC market | Kullu | Himachal Pradesh |
| 24. | FS-24 | LBS market | Bijapur | Karnataka |
| 25. | FS-25 | Sheoraphuli bazar | Hoogly | West Bengal |

3.2.2 Isolation of pathogen:

Small pieces from dry rot diseased tissues along with adjoining healthy tissues were cut and surface sterilized by dipping in 1.0 per cent NaOCl solution for one minute followed by three successive washings with distilled sterile water and these pieces were placed on Potato Dextrose Agar (PDA) medium (20 ml) poured in Petri plates (90 mm) under aseptic condition. The inoculated plates were incubated for growth of the pathogen at $28\pm 1^{\circ}\text{C}$ in BOD incubator for seven days. The pure mycelial growth was subcultured and maintained on PDA slants. From seven days old pure culture, spore suspension was made following serial dilution in distilled sterile water so as to get two to three spores per drop of suspension. One ml

of such diluted suspension was poured aseptically on the solidified sterilized molten water agar medium. Petri plates were tilted in different direction to get uniform spread of spore suspension. Petri plates were examined under the stereoscopic binocular research microscope. Well separated single spore of pathogen was marked and then transferred on PDA slants separately under aseptic conditions and incubated at $28\pm 1^{\circ}\text{C}$ in BOD incubator for seven days. The pure culture of each isolate thus obtained was maintained by periodical transfer on PDA slants throughout the investigations.

3.2.3 Pathogenicity

Healthy potato tubers of 'Kufri Badshah' variety were collected from Sardar Patel vegetable market, Anand. The tubers were thoroughly washed in tap water, then surface sterilized by dipping in 1.0 per cent NaOCl solution for one minute followed by three successive washings with distilled sterile water and then inoculated with isolated fungus. The spores, harvested from seven days old culture of dry rot isolate (*Fusarium solani*), was mixed in 200 ml of distilled sterile water. The tubers were pricked at epicarp with sterilized pins fixed on cork. Spore suspension (10^6 spores/ml) of the isolate was inoculated with the help of cotton swab on healthy tubers following smearing method. The inoculated and uninoculated (dipped in distilled sterile water) tubers were placed separately in sterilized polythene bags. One tuber was kept in one bag. A piece of sterilized moist absorbent

cotton swab was placed inside the bag to create humidity and mouth of the bag was loosely tied with rubber band. The bagged tubers were kept at $28\pm 1^{\circ}\text{C}$ in BOD incubator for seven days. The tubers were examined daily for symptom expression. The tubers showing typical dry rot symptoms were used for re-isolation of the organism. The cultural and morphological characters of re-isolated pathogen were compared with previously isolated pathogen from naturally infected diseased potato tubers.

3.2.4 Identification

Identification of the pathogen was carried out by studying the cultural and morphological characters. The photomicrographs of mycelium and spore structure were taken with the help of digital camera. The pure culture was sent for identification to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi – 110 012.

3.3 Survey of post-harvest diseases of potato tubers at Sardar Patel vegetable market, station road and Goya Talav market at Anand

Weekly survey of post-harvest diseases of potato tubers was carried out at Sardar Patel vegetable market, station road and Goya Talav market at Anand to study the incidence of post-harvest diseases, from first week of June to fourth week of February during 2014-2015. Five samples were selected randomly each containing 100 tubers from each locations and

were examined for the incidence of post-harvest diseases caused due to different pathogens. The per cent disease incidence was calculated by following standard formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tubers}}{\text{Total number of tubers examined}} \times 100$$

3.4 Evaluation of newer fungicides, some organic and inorganic salts, natural products and antagonists against *F. solani* (Mart.) Sacc. causing dry rot of potato tubers *in vitro*.

3.4.1 Fungicides:

3.4.1.1 *In vitro*:

Bio-efficacy of different fungicides were studied *in vitro* by following Poisoned Food Technique method (Nene and Thapliyal, 1979) against Fusarium dry rot pathogen with different concentrations. Hexaconazole, carbendazim and tebuconazole were tested at 50 and 100 ppm; azoxystrobin; cymoxanil 8% + mancozeb 64% and carbendazim 12% + mancozeb 63% were tested at 500 and 1000 ppm. While copper oxychloride, metiram and mancozeb were tested at 1000 and 2000 ppm concentrations. The list of fungicides screened is given in Table 3.2 along with their common name, trade name and chemical name. Required quantity of each fungicide under study was mixed thoroughly in sterilized 100 ml PDA media filled in 250 ml flask separately under aseptic

condition. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The poisoned medium was then poured in sterilized Petri plates (20 ml) and allowed it to solidify. The plates were then inoculated with five mm diameter disc of seven day old culture of test pathogen by placing in the centre of the plate. Control was maintained for each set where fungal disc were placed on PDA medium without fungicide. Each treatment was replicated three times. The inoculated plates were then incubated at $28 \pm 1^{\circ} \text{C}$ in BOD incubator.

Table 3.2: Statement showing the common name, trade name, chemical name and concentration of fungicides

| Sr. No. | Common name | Trade name | Chemical name | Concentration (ppm) | |
|---------|--------------------------------|----------------|--|---------------------|------|
| | | | | | |
| 1. | Hexaconazole | Contaf 5 EC | 2-(2,4-Dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol | 50 | 100 |
| 2. | Carbendazim | Bavistin 50 WP | 1 H – Benzimidazol – 2 – ylcabamic acid methyl ester | 50 | 100 |
| 3. | Tebuconazole | Elite 250 EW | 1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan-3-ol | 50 | 100 |
| 4. | Azoxystrobin | Amistar 25 SC | methyl(αE)2-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene) benzeneacetate | 500 | 1000 |
| 5. | Cymoxanil 8% + Mancozeb 64% | CurzateM8 72WP | 2- cyno- N- (Ethylamino carbonyl) - 2- (Methoxyimino acetamide) | 500 | 1000 |
| 6. | Carbendazim 12% + Mancozeb 63% | Sixer 75 WP | 1 H – Benzimidazol – 2 – ylcabamic acid methyl ester + (Ethylenebis (dithiocarbamato)) manganese mixture with (ethylenebis (dithiocarbamato)) zinc | 500 | 1000 |

| | | | | | |
|----|--------------------|------------------------|---|------|------|
| 7. | Copper Oxychloride | Blitox 50 WP | Dicopper chloride trihydroxide | 1000 | 2000 |
| 8. | Mancozeb | Dithane M- 45 75 WP | (Ethylenebis dithiocarbamate)) manganese mixture with (ethylenebis (dithiocarbamate)) zinc | 1000 | 2000 |
| 9. | Metiram 80 WP | Polyram 80 WP | Tris[ammine[ethylenebis(dithiocarbamate)]zinc(2+)] [tetrahydro 1,2,4,7-dithiadiazocine-3,8- dithione],polymer | 1000 | 2000 |

Observations recorded:

Observations on the radial growth (mm) were recorded on 4th and 8th day of incubation at 28±1°C till the complete growth of test pathogen appeared in control plates. Per cent growth inhibition (PGI) over control was calculated by using following formula (Asalmol *et al.*, 1990).

$$I = \frac{C-T}{C} \times 100$$

Where, C = Colony diameter in control plates (mm)

T = Colony diameter in treated plates (mm)

3.4.1.2 *In vivo*:

The most effective five fungicides were screened further to test their efficacy in controlling Fusarium dry rot of potato tubers *in vivo*.

3.4.1.2.1 Pre-inoculation

In case of pre-inoculation treatment, healthy uniform tubers of ‘Kufri Badshah’ were surface sterilized by dipping in 1.0 per cent NaOCl solution for one minute followed by three successive washings with

distilled sterile water and inoculated separately with the pathogen by the pin pricking method. The tubers were first dipped in fungicidal solution for five minutes; air dried and then inoculated with dry rot pathogens (10^6 spores/ml). The interval between fungicidal treatment and inoculation was kept twelve hours. The severity of tuber rot was recorded on 4th and 8th day after inoculation with the help of assessment key (Plate 3.1).

3.4.1.2.2 Post- inoculation

The procedure detailed in 3.4.1.2.1 was followed except that the tubers were first inoculated with test pathogen and then treated with fungicides.

3.4.2 Salts

3.4.2.1 *In vitro*:

Bio-efficacy of different salts were studied *in vitro* by following Poisoned Food Technique method (Nene and Thapliyal, 1979) against Fusarium dry rot pathogen at 0.2 M concentration. The list of salts screened is given in the Table 3.3 along with their chemical formula. Required quantity of each salt under study was mixed thoroughly in sterilized 100 ml PDA media filled in 250 ml flask separately under aseptic condition. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The poisoned medium was then poured in sterilized Petri plates (20 ml) separately and allowed it to solidify. The plates were then inoculated with five mm diameter disc of seven days old

culture of test pathogen by placing in the centre of the plate. Control was maintained for each set where fungal disc were placed on PDA medium without salt. Each treatment was replicated three times. The inoculated plates were then incubated at $28\pm 1^{\circ}\text{C}$ in BOD incubator for 8 days.

Table 3.3: Statement showing the name of salts, chemical formula and concentration

| Sr. No | Salts | Chemical formula | Concentration |
|--------|-----------------------|---|---------------|
| 1. | Aluminum acetate | $\text{C}_2\text{H}_5\text{O}_4\text{Al}$ | 0.2 M |
| 2. | Aluminum chloride | $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ | 0.2 M |
| 3. | Ammonium chloride | NH_4Cl | 0.2 M |
| 4. | Calcium chloride | $\text{CaCl}_2, \text{H}_2\text{O}$ | 0.2 M |
| 5. | Potassium chloride | KCl | 0.2 M |
| 6. | Potassium sorbate | $\text{C}_6\text{H}_7\text{O}_2\text{K}$ | 0.2 M |
| 7. | Sodium benzoate | $\text{C}_7\text{H}_5\text{O}_2\text{Na}$ | 0.2 M |
| 8. | Sodium bicarbonate | NaHCO_3 | 0.2 M |
| 9. | Sodium carbonate | Na_2CO_3 | 0.2 M |
| 10. | Sodium chloride | NaCl | 0.2 M |
| 11. | Ammonium phosphate | $\text{H}_{12}\text{N}_3\text{O}_4\text{P}$ | 0.2 M |
| 12. | Potassium carbonate | K_2CO_3 | 0.2 M |
| 13. | Potassium bicarbonate | KHCO_3 | 0.2 M |

Observations recorded:

The procedure mentioned earlier in 3.4.1.1 was followed.

3.4.2.2 *In vivo*

The most effective five salts were screened further to test their efficacy in controlling Fusarium dry rot of potato tubers at 0.2 M concentration by following both pre- and post-inoculation methods *in vivo*.

3.4.2.2.1 Pre- inoculation

In pre-inoculation treatment, the tubers were first dipped in salts separately and then inoculated with the pathogen by keeping 12 hours interval. Further procedure was followed as mentioned earlier in 3.4.1.2.1.

3.4.2.2.2 Post- inoculation

In the post inoculation method, the tubers were first inoculated with pathogen and then treated with the salts. The procedure described earlier in 3.4.1.2.2 was followed.

3.4.3 Natural products:

3.4.3.1 *In vitro*

Efficacy of different phytoextracts of plant species having medicinal value were tested at 10 per cent concentration *in vitro* by Poisoned Food Technique against potato dry rot pathogen (Nene and Thapliyal, 1979). The list of plant species used for phytoextracts study is given in Table 3.4 indicating their scientific name, common name, family and plant part used.

Fresh and healthy 100 g plant parts of each species were thoroughly washed with tap water and then with distilled sterile water. They were macerated separately in grinder mixture by adding 100 ml ethanol.

The mixture was filtered through two fold sterilized muslin cloth and the filtrate was centrifuged at 5,000 rpm for 10 min. and the clear supernatant extract was collected in sterilized conical flasks. After

evaporating the ethanol from extract, the clear extract was collected and diluted with 100 ml distilled sterile water to make volume 1:1 (W/V). This was considered as 100 per cent concentration for the study to test the efficacy of plant extracts (Sinha and Saxena, 1989). Each phytoextracts (10 %) were mixed thoroughly in sterilized 100 ml PDA medium filled in 250 ml flask under aseptic condition. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. Further procedure was same as followed earlier in 3.4.1.1.

Table 3.4: Statement showing the list of natural products, local name, family and plant part used

| Sr. No. | Botanical name | Local name | Family | Plant part used |
|---------|------------------------------------|---------------|-----------------------|-----------------|
| 1. | <i>Adhatoda vasica</i> Nees. | Ardusi | <i>Acanthaceae</i> | Leaf |
| 2. | <i>Aegle marmelos</i> L. | Bael Patra | <i>Rutaceae</i> | Leaf |
| 3. | <i>Azadirachta indica</i> A. Juss. | Neem | <i>Meliaceae</i> | Leaf |
| 4. | <i>Allium sativum</i> L. | Garlic | <i>Amaryllidaceae</i> | Cloves |
| 5. | <i>Lantana camera</i> L. | Ghaneri | <i>Verbenaceae</i> | Petal |
| 6. | <i>Melia azadirach</i> L. | Maha neem | <i>Meliaceae</i> | Leaf |
| 7. | <i>Curcuma longa</i> L. | Turmeric | <i>Zingiberaceae</i> | Rhizome |
| 8. | <i>Nerium indicum</i> Mill. | Red Kaner | <i>Apocynaceae</i> | Leaf |
| 9. | <i>Nicotiana tabacum</i> L. | Tobacco | <i>Solanaceae</i> | Leaf |
| 10. | <i>Annona reticulate</i> L. | Custard apple | <i>Annonaceae</i> | Leaf |
| 11. | <i>Ocimum sanctum</i> L. | Tulsi | <i>Labiatae</i> | Leaf |

3.4.3.2 *In vivo*

The procedure described in 3.4.1.2 was followed. The phytoextracts studied *in vitro* were tested at 10 per cent concentration by following both pre- and post-inoculation methods.

3.4.3.2.1 Pre- inoculation

In pre-inoculation treatment, the tubers were first dipped in phytoextracts separately and then inoculated with the pathogen by keeping 12 hours interval. Further procedure was followed as mentioned earlier in 3.4.1.2.1.

3.4.3.2.2 Post- inoculation

In the post inoculation method, the tubers were first inoculated with pathogen and then treated with the phytoextracts. The procedure described earlier in 3.4.1.2.2 was followed.

3.4.4 Antagonists

3.4.4.1 *In vitro*

Antagonistic effect of different bioagents *i.e.* *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested by dual culture technique for their antagonism against potato dry rot pathogen (Dennis and Webster, 1971).

Seven days old culture of the bioagents and the pathogen were employed by following dual culture method. Mycelial disc of 5 mm diameter cut from the periphery of fungal antagonist and test pathogen and were placed at 50 mm apart from each other in Petri plates and in case of bacterial bioagents half portion of plates streaked with bacterial bioagents and 5 mm diameter mycelial disc placed at centre of Petri plates. In control, only test pathogen was kept in the centre of Petri plate. Each

treatment was replicated four times. The Petri plates were incubated at $28\pm 1^{\circ}\text{C}$ in BOD incubator. Observations on per cent growth inhibition were recorded after 8 days of incubation by following the procedure mentioned earlier in 3.4.1.1.

3.4.4.2 *In vivo*

Antagonists studied *in vitro* were used for further investigation to test their antagonism in controlling Fusarium dry rot of potato following both pre- and post-inoculation methods.

3.4.4.2.1 Pre- inoculation

The healthy uniform size tubers were pricked with pin pricking and inoculated with spore suspension (10^6 spores/ml) of seven days old culture of different antagonists separately and after 12 hrs, the tubers were inoculated at the same site with spore suspension (10^6 spores/ml) of seven days old culture of test pathogens. Control was maintained separately with pathogen. Each treatment was replicated three times. Further procedure mentioned earlier in 3.4.1.2.1 was followed.

3.4.4.2.2 Post-inoculation

The procedure described in 3.4.1.2.2 was followed except that the tubers were first inoculated with pathogen and then with antagonists.

3.5 Effect of different temperatures on development of *Fusarium* dry rot of potato tubers *in vivo*

Influence of different temperatures (5, 10, 15, 20, 25, 30, 35 and 40°C) on dry rot development of potato tubers incited by *Fusarium solani* (Mart.) Sacc. was studied *in vivo*.

The healthy uniform tubers of 'Kufri Badshah' variety were collected from Sardar Patel vegetable market, Anand. The tubers were thoroughly washed in tap water followed by distilled water and then dipped in 1% NaOCl solution for 1 min. and then subsequently washed with sterile distilled water. The tubers were then pricked on epicarp with sterilised pins fixed on cork. Then the tubers were inoculated with dry rot pathogen (10^6 spores/ml) and ware bagged in sterilised polythene bags containing moist absorbent cotton swab. The mouth of the bag was loosely tide with the rubber band and inoculated tubers were incubated at 5, 10, 15, 20, 25, 30, 35 and 40°C temperature separately for eight days.

Observations on dry rot severity were recorded on 4th and 8th day after inoculation by following the procedure mentioned earlier in 3.4.1.2.1.

3.6 Cultural and morphological variability among the isolates of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers

3.6.1 Cultural and morphological variability:

Twenty five isolates were separately cultured on potato dextrose agar (PDA) media and incubated at $28\pm 1^{\circ}\text{C}$ for seven days. After seven days of incubation cultural characteristics like colony colour, type and growth (mm) of colony were recorded.

3.6.1.1 Size and shape of micro and macroconidia.

The morphological characters like size (length and width) and shape of the conidia (micro & macro) and chlamydospores were studied.

The observations were recorded in three repetitions of each isolate. The study was carried out using ocular and stage micrometer after mounting them on the slides containing sterile distilled water at 400X magnification. Data obtained were analyzed statistically using complete randomized design.

3.6.1.2 Sporulation:

The spores, harvested from seven days old culture of each isolate, were mixed in 200 ml of distilled sterile water and made a uniform spore suspension (10^6 spores/ml). The numbers of spores were counted with the help of haemocytometer.

3.7 Molecular variability among the isolates of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers using RAPD markers

3.7.1 Genomic DNA isolation and purification

DNAs of twenty five purified isolates of *F. solani* were extracted using a modified CTAB method (Leisova *et al.*, 2005) and were purified by ethanol precipitation.

Isolates were grown on potato dextrose agar plates at $28 \pm 1^\circ\text{C}$ for 7 days. About one gram of young growing mycelia were ground in a pre-chilled mortar with liquid nitrogen. The powder was transferred to an Eppendorf tube and resuspended in 500 μl of CTAB extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-HCl, pH 8.0). The tubes were incubated at 65°C for 30 min with occasional gentle swirling. Two third volumes of chloroform: isoamylalcohol (24:1, v / v) was added to this sample and mixed for 15–25 min and then centrifuged at 10,000 rpm for 10 min. For purification, 10 μl RNase (10 mg/ml) was added to the supernatant and incubated for 30 min at 37°C . The incubated sample was again extracted with chloroform : isoamyl alcohol solution and centrifuged at 10,000 rpm for 10 min. Chilled isopropanol (0.57 volume) was added to the upper phase, mixed by inverting and centrifuged at 10,000 rpm for 10 min at 4°C . The DNA pellet was rinsed twice with 70% ethanol for 10–15 min. and then dried at room

temperature. The dried pellet was dissolved in 250 µl TE buffer (pH 8.0). The purified DNA was checked by running 2 µl of the sample on a 0.8% agarose gel. The quality and quantity of DNA were measured at 260/280 nm and 260 nm respectively using nanospectrophotometer.

3.7.2 RAPD primer amplification and analysis

For the molecular characterization of *F. solani* isolates, the 20 different 10-mer RAPD primers (Eurofins Genomics India Pvt. Ltd.) were screened for the amplification of template DNA of *F. solani* (Table 3.5). The primers that gave clear and polymorphic amplification patterns were used for further analysis of all the 25 isolates of *Fusarium solani*.

The amplified DNA fragments for each accession were scored as present (1) or absent (0). Data generated by 20 RAPD primers were used to compile a binary matrix for cluster analysis. Genetics similarity among accessions was calculated according to Jaccard's coefficient (Jaccard, 1908).

Table 3.5: Statement showing the list of primers used for RAPD analysis and toxin detection of *Fusarium solani*

| Primer | Sequence (5'-3') | Temp. Value (°C) | GC Content (%) |
|---------------|-----------------------------|---------------------------------|-------------------------------|
| OPA1 | CAGGCCCTTC | 34 | 70 |
| OPA2 | TGCCGAGCTG | 34 | 70 |
| OPA3 | AGTCAGCCAC | 32 | 60 |
| OPA4 | AATCGGGCTG | 32 | 60 |
| OPA5 | AGGGGTCTTG | 32 | 60 |
| OPA6 | GGTCCCTGAC | 34 | 70 |
| OPA7 | GAAACGGGTG | 32 | 60 |
| OPA8 | GTGACGTAGG | 32 | 60 |
| OPA9 | GGGTAACGCC | 34 | 70 |
| OPA10 | GTGATCGCAG | 32 | 60 |
| OPA11 | CAATCGCCGT | 32 | 60 |
| OPA12 | TCGGCGATAG | 32 | 60 |
| OPA13 | CAGCACCCAC | 34 | 70 |
| OPA14 | TCTGTGCTGGC | 32 | 60 |
| OPA15 | TTCCGAACCC | 32 | 60 |
| OPA16 | AGCCAGCGAA | 32 | 60 |
| OPA17 | GACCGCTTGT | 32 | 60 |
| OPA18 | AGGTGACCGT | 32 | 60 |
| OPA19 | CAAACGTCGG | 32 | 60 |
| OPA20 | GTTGCGATCC | 32 | 60 |
| FUM1 For. | CCATCACAGTGGGACACAGT | 53.8 | 55 |
| FUM1 Rev. | CGTATCGTCAGCATGATTAGC | 52.4 | 47.6 |
| FUM2 For. | GAGGCCCGAGCGAGCACTGG | 62 | 75 |
| FUM2 Rev. | CCAGCCGCGGAAATTAGGGATGTG | 60.8 | 58.3 |

3.8 Studies on biochemical factors responsible for disease resistance:

Phenol, sugars and starch

3.8.1 Total phenol content

Total phenol content from the potato pulp of both inoculated and uninoculated was estimated by Folin Ciocalteu method as described by Bhatnagar *et al.* (2005).

Fifty mg of pulp from inoculated and healthy tubers was separately mixed with 25 ml of 0.3 N HCl and was kept for shaking for about one hour. After shaking, crude extract was centrifuged at 8,000 rpm for about 10 min. One ml supernatant obtained was evaporated to dryness on waterbath. Hot water was added to the residue and final volume was adjusted to 250 ml with water in volumetric flask. One ml of the above aliquot was taken in the test tube. To this, one ml each of Folin Ciocalteu reagent diluted 1: 2 and 1 ml of 35 per cent sodium carbonate was added. After one hour, 2 ml water was added to adjust the final volume (5 ml). Intensity of the colour was recorded at 650 nm in spectrophotometer. Blank was prepared by taking one ml distilled water and the rest of the procedure was followed as described for sample estimation as above.

Standard curve of the pyrocatechol was prepared in the range of 2-10 mg pyrocatechol. Hundred mg of pyrocatechol was dissolved in 10 ml water. From this stock solution 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and

1.0 ml solution was pipetted in duplicate into series of test tubes. Blank was prepared by taking 1.0 ml of water. Volume was adjusted to 1.0 ml with water in those tubes where it was necessary 1.0 ml of each reagent *i.e.* Folin reagent (1:2) and sodium carbonate (35%) is added one after another. Final volume was adjusted to 5 ml with water after 1 hr. and intensity of the colour was measured at 650 nm in spectrophotometer. Standard graph was plotted to calculate the amount of phenol present in samples.

Total Phenol (g/100 g) = Sample O.D x Graph factor (mg) x Dilution factor

3.8.2 Sugars

3.8.2.1 Total soluble sugar content

Total soluble sugar content from the potato pulp of both inoculated and uninoculated was estimated by phenol sulphuric acid method as described by Dubois (1956).

Hundred mg. of pulp sample was macerated in 5 ml 80% alcohol and taken in 30 ml sugar test tubes and total volume was made to 10 ml with 80 % alcohol. The test tubes were kept for overnight. Next day take one ml supernatant from each test tube and was evaporated to dryness in water bath. After evaporation make the volume to 25 ml with distilled water in beaker. From this 25 ml, one ml test solution was used for assay in which freshly prepared 1 ml 5 per cent phenol solution was added followed by immediate direct addition of 5 ml concentrated

sulphuric acid solution. The tubes were kept for 10 min. at room temperature for colour development. After mixing the solution it was kept for further 15 min. in cold water bath. The intensity of stable yellow colour developed was recorded at O.D. 490 nm in spectrophotometer.

In a similar way take 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard glucose solution having 10 to 50 µg glucose, was pipetted out into a series of test tubes. The volume of each test tube was made up to 1ml with distilled water. For blank, one ml of distilled water was taken. Sugar content was determined by using the following formula:

$$\text{TSS (g/100 g)} = \text{Sample O.D} \times \text{Graph factor (mg sugar)} \times \frac{250}{1000}$$

3.8.2.2 Estimation of Reducing Sugars

An aliquot from the extract prepared for the estimation of total soluble sugar was used for the estimation of total reducing sugars according to the Nelson's modification of Somogyi's method (Nelson, 1944; Somogyi, 1952).

Preparation of Somogyi's Copper Reagent

This reagent was prepared by dissolving 24 g. of anhydrous sodium carbonate and 12 g. of sodium potassium tartrate (Rochelle salt) in about 250 ml of distilled water. To this 4 g. of copper sulphate as a 10% (w/v) solution was added and mixed followed by the addition of 16 g. of sodium bicarbonate. Then 180 g. of sodium sulphate was dissolved in about 500 ml of distilled water and boiled to expel air. After cooling, the

two solutions were mixed and the final volume was made up to 1,000 ml (Somogyi, 1952).

Preparation of Nelson's Arsenomolybdate Reagent

Nelson's arsenomolybdate reagent was prepared by dissolving 25 g. of ammonium heptamolybdate in 450 ml of water. Then 21 ml of sulphuric acid was added and mixed well. To the mixture 3.0 g. of disodium hydrogen arsenate dissolved in 25 ml of distilled water was added. The solution was mixed well and incubated for 24 hours at 37°C (Nelson, 1944).

Calculation of reducing sugars

From the sample, a known volume of aliquot was pipetted out and was made up to 1.0 ml using distilled water. To this 1.0 ml of Somogyi's copper reagent was added. The mixture was then placed in a bath of boiling water and heated for 20 minutes. After cooling under tap water 1.0 ml of Nelson's arsenomolybdate reagent was added with immediate mixing till the effervescence ceased. The intensity of colour was measured after proper dilution at 620 nm using a spectrophotometer. Glucose was used as the standard.

3.8.3 Starch

Extraction

From healthy and infected tubers 200 mg pulp was weighed and homogenized in diethyl ether to remove the lipids that may interfere with the processes of extraction and purification of starch. Then the diethyl ether was decanted off and the residue was ground in 30% (v/v) perchloric acid for the extraction of starch. The homogenate was centrifuged at 4,000 rpm for 5 min and the supernatant was collected. The residue was again homogenised in 30% (v/v) perchloric acid and centrifuged. The processes of homogenisation, centrifugation and extraction were repeated till it was ensured that the entire starch content of the pulp was extracted. Volume of the combined supernatant was noted. A known volume of the aliquot was taken from the combined supernatant and an equal volume of freshly prepared iodine–potassium iodide reagent was added to the tube and mixed well using a vortex shaker. The mixture was then kept undisturbed for 10-20 minutes and centrifuged for 10 minutes. The supernatant was decanted off. The excess iodine reagent present in the residue was removed by washing with alcoholic sodium chloride followed by centrifugation. After centrifugation, the coloured residue was treated with alcoholic sodium hydroxide till the blue colour was found disappeared. The residue was again washed with alcoholic sodium chloride. It was then dissolved in a

known volume of 10% (v/v) sulphuric acid by heating in a hot water bath. After cooling, the supernatant was collected and used for the estimation of starch.

Starch content

Starch content was estimated using the method described by McCready *et al.* (1950).

Two hundred mg of sample from healthy and infected tubers were taken and separately mixed with 5 ml of distilled water and 25 ml of 80% ethyl alcohol was taken in 50 ml centrifuge tube. This was centrifuged at 8,000 rpm for 6 mins supernatant was discarded, to the pellet 30 ml of 80% ethyl alcohol was added and centrifuged again, supernatant was discarded, to the residue 20 ml of distilled water plus 6.5 ml of perchloric acid was added and centrifuged. Repeated it twice and then transferred the aqueous phase to 100 ml volumetric flask and final volume made up to 100 ml. One ml of filtrate was taken and diluted to 100 ml with distilled water. Again 5 ml from this was taken and 10 ml of freshly prepared 10 % anthrone reagent was added and boiled them for 7.5 min in boiling water bath. The tubes were allowed to cool down at room temperature and read at 630 nm in spectrophotometer.

Starch content was calculated as per the following formula:

$$\% \text{ Glucose} = \text{O.D.} \times \text{Graph factor}$$

$$\% \text{ Starch} = \% \text{ Glucose} \times 0.9$$

3.9 Statistical analysis

Data obtained from various investigations were subjected to statistical analysis by making use of analysis of variance technique (Steel and Torrie, 1980). The standard methods of analysis of variance for complete randomized design and transformations were used in the experiments. The test of significance among the treatments was worked out by 'F' test. The appropriate standard error (S.Em. \pm) was computed in each case. For the treatments effects, which were found to be significant, the critical difference (CD) at 5 per cent level of probability was worked out to compare two treatment means.

IV. RESULTS AND DISCUSSION

The present investigations on “Molecular characterization of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers (*Solanum tuberosum* L.) and its management” was carried out in the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Sardar Patel vegetable market and Goya Talav market, Anand. The results obtained on various aspects are presented here under.

4.1 Collection, isolation, purification, pathogenicity and identification of pathogen causing dry rot of potato tubers

4.1.1 Collection of samples

Diseased potato tubers (variety- Kufri Badshah) exhibiting typical symptoms of *Fusarium* dry rot were collected from Sardar Patel vegetable market, Anand. The infected tubers exhibit brown to darker wrinkles on skin. These wrinkles are often arranged in irregular concentric circles. Sometimes a hole may be produced in the centre of the concentric ring with whitish or pinkish growth of fungal mycelia. On cutting these affected tubers, whitish or brownish tissues are seen with one or more cavities (Plate 4.1).

4.1.2 Isolation and purification

The freshly dry rot infected potato tubers brought to the laboratory and were subjected to isolation by tissue isolation technique to obtain the culture of pathogen inciting Fusarium dry rot of potato tubers. The culture thus obtained was further purified by single spore isolation. The pure culture of each isolate was maintained separately by periodical transfer on PDA slants throughout the investigations.

4.1.3 Pathogenicity

To prove the Koch's postulates, the isolated pathogen was artificially inoculated on healthy potato tubers following pin-prick method as per the procedure described earlier in 3.2.3. The tubers inoculated with the test pathogen exhibited typical Fusarium dry rot symptoms on 8th day after inoculation. The artificially inoculated tubers exhibited brown to darker wrinkles on skin. These wrinkles were often arranged in irregular concentric circles with whitish or pinkish growth of fungal mycelia. With the advancement of infection the tubers become pulpy and oozed out a juice emitting foul odour. Later on, white cottony growth of the fungus consisting of profusely branched hyphae appeared over the rotted tissues of the tuber. The control tubers without inoculation of pathogen did not produced any symptoms (Plate 4.2). The Fusarium dry rot symptoms produced on artificially inoculated tubers were similar to those observed on

naturally infected tubers. Further, the reisolations were made from artificially inoculated tubers which produced whitish mycelial fungal growth and was identical to the one which was used for inoculation of the tubers. Results similar to the present investigation showing typical dry rot symptoms produced by *Fusarium* spp. on artificially inoculated potato tubers was reported by Gachango *et al.* (2012) and Nasr-Esfahani (1998). They isolated and proved the pathogenicity of the fungi associated with dry rot of potato (*Fusarium sulphureum*, *F. solani*, *F. oxysporum*, *F. equiseti* and *F. sambucinum*) from Michigan and Iran, respectively. The inoculated tubers developed typical potato dry rot symptoms showing brown and dry decay regardless of isolate of *Fusarium* spp. which was inoculated.

4.1.4 Identification

After purification of the fungus, the cultural and morphological characters were studied for identification of the pathogen. The pure culture obtained was sent for identification to Indian Type Culture Collection (I.T.C.C.), Division of Mycology and Plant Pathology, I.A.R.I., New Delhi and was identified as *Fusarium solani* (Mart.) Sacc. (ID.No. 9255.13). The cultural and morphological characters of the fungus were found similar to that of *Fusarium solani* causing Fusarium dry rot of potato tubers (Chehri *et al.*, 2011; Gachango *et al.*, 2012 and Nasr-Esfahani, 1998). Thus, the

causal agent of Fusarium dry rot of potato in middle Gujarat region was confirmed as *Fusarium solani* (Mart.) Sacc.

4.1.4.1 Cultural characters

The fungus (*Fusarium solani*) produced white to light pink mycelial growth with abundant sporulation on PDA covering the entire Petri plate (90 mm) within 8 days of incubation at room temperature ($28 \pm 1^\circ \text{C}$). In initial stage, the fungal growth was white to light pink, rapidly becoming tinged with lavender to dark pink or colourless to dark purple or dark brown with the production of micro and macro conidia.

4.1.4.2 Morphological characters

The fungus produced whitish to light pink septate mycelial growth on PDA, later on turned lavender to pink and finally colourless to dark purple or dark brown. The hyphae is septate, hyaline, 3-5 μ in diameter, septa at a distance of 20-40 μ . Chlamyospores were present. Microconidia produced in chains, remain connected loosely, one celled or two celled, round to oval shaped, one celled conidia measured 6-11 x 2-4 μm and two celled conidia 10-18 x 2-4 μm in size. Macroconidia were spores, very slightly sickle-shaped to nearly straight with 3-4 septa, measuring 31-58 x 3.1-4.7 μm in size (Plate 4.3).

4.2 Survey

The weekly survey carried out from first week of June to fourth week of February during 2014-2015, revealed the presence of bacterial soft rot, Fusarium dry rot (*Fusarium* spp.), common scab (*Streptomyces scabies*), sclerotium rot (*Sclerotium rolfsii*) and brown rot (*Ralstonia solanacearum*) at Sardar Patel vegetable market, station road and Goya Talav market, Anand.

4.2.1 Sardar Patel vegetable market, Anand

The data presented in Table 4.1 and Fig. 4.1 revealed that at Sardar Patel vegetable market, station road, Anand, incidence of dry rot was predominant (14.05%) among all rots, followed by bacterial soft rot (11.28%) and common scab (6.39%). The incidence of sclerotium rot and brown rot were 3.72 and 2.01 per cent, respectively. The highest incidence of dry rot (18.10%) and bacterial rot (17.00%) was recorded in 1st week of December and 4th week of October, respectively. The incidence of common scab (9.80%) was severe in 3rd week of October followed by sclerotium rot (6.60%) in 1st week of September and brown rot (4.40%) in 3rd week of August.

Table 4.1: Incidence of potato diseases at Sardar Patel vegetable market, station road, Anand(2014-2015)

| Sr. No. | Name of diseases | Per cent Disease Incidence | | | | | | | | | | | | | | | | | | | |
|---------|--------------------|----------------------------|------|------|------|------|------|------|-------|--------|-------|-------|-------|-----------|-------|-------|-------|---------|-------|-------|-------|
| | | Months | | | | | | | | | | | | | | | | | | | |
| | | June | | | | July | | | | August | | | | September | | | | October | | | |
| | | Weeks | | | | | | | | | | | | | | | | | | | |
| | | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| 1 | Bacterial soft rot | 7.20 | 7.10 | 8.40 | 8.80 | 7.80 | 9.00 | 9.70 | 10.10 | 10.20 | 11.00 | 11.60 | 12.50 | 12.90 | 13.70 | 14.50 | 14.90 | 15.60 | 16.40 | 16.90 | 17.00 |
| 2 | Dry rot | 5.70 | 6.20 | 7.40 | 7.90 | 8.10 | 8.90 | 9.30 | 9.90 | 11.20 | 11.90 | 12.60 | 13.20 | 14.00 | 14.30 | 15.20 | 15.80 | 16.00 | 16.50 | 16.70 | 17.00 |
| 3 | Common Scab | 2.60 | 2.90 | 3.50 | 3.80 | 4.10 | 4.60 | 4.90 | 5.10 | 5.60 | 6.10 | 6.50 | 6.90 | 7.20 | 7.80 | 8.30 | 8.70 | 8.90 | 9.30 | 9.80 | 9.70 |
| 4 | Sclerotium rot | 1.90 | 2.20 | 2.60 | 3.50 | 4.20 | 4.80 | 5.30 | 5.80 | 5.60 | 5.90 | 6.20 | 5.60 | 6.60 | 5.70 | 5.30 | 5.90 | 5.40 | 4.00 | 4.30 | 4.60 |
| 5 | Brown rot | 1.30 | 1.60 | 1.70 | 1.40 | 3.20 | 2.80 | 2.00 | 2.60 | 2.40 | 4.00 | 4.40 | 4.20 | 3.80 | 4.30 | 2.80 | 3.60 | 1.80 | 2.40 | 2.40 | 2.00 |

| Sr. No. | Name of diseases | Months | | | | | | | | | | | | | | | | Mean Per cent Disease Incidence |
|---------|--------------------|----------|-------|-------|-------|----------|-------|-------|-------|---------|-------|-------|-------|----------|-------|-------|-------|---------------------------------|
| | | November | | | | December | | | | January | | | | February | | | | |
| | | Weeks | | | | | | | | | | | | | | | | |
| | | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | |
| 1 | Bacterial soft rot | 14.60 | 13.80 | 13.10 | 12.50 | 12.00 | 11.80 | 11.30 | 10.50 | 10.20 | 9.80 | 9.50 | 9.10 | 8.80 | 8.60 | 8.30 | 7.00 | 11.28 |
| 2 | Dry rot | 17.30 | 17.80 | 18.00 | 18.00 | 18.10 | 17.60 | 17.80 | 17.50 | 16.80 | 16.50 | 16.10 | 15.90 | 15.70 | 15.60 | 14.80 | 14.50 | 14.05 |
| 3 | Common Scab | 9.70 | 9.00 | 8.70 | 8.40 | 8.20 | 7.50 | 6.90 | 6.40 | 6.20 | 5.80 | 5.30 | 4.80 | 4.70 | 4.40 | 4.10 | 3.60 | 6.39 |
| 4 | Sclerotium rot | 4.70 | 4.10 | 3.80 | 3.20 | 3.40 | 2.90 | 2.10 | 2.40 | 2.40 | 2.10 | 2.00 | 1.60 | 1.50 | 0.00 | 1.00 | 1.20 | 3.72 |
| 5 | Brown rot | 1.70 | 2.20 | 2.40 | 2.60 | 2.50 | 1.60 | 1.30 | 0.90 | 0.70 | 0.60 | 0.00 | 0.50 | 0.70 | 0.00 | 0.00 | 0.00 | 2.01 |

Table 4.2: Incidence of potato diseases at Goya Talav market, Anand (2014-2015)

| Sr. No. | Name of diseases | Per cent Disease Incidence | | | | | | | | | | | | | | | | | | | |
|---------|--------------------|----------------------------|------|------|------|------|------|------|------|--------|-------|-------|-------|-----------|-------|-------|-------|---------|-------|-------|-------|
| | | Months | | | | | | | | | | | | | | | | | | | |
| | | June | | | | July | | | | August | | | | September | | | | October | | | |
| | | Weeks | | | | | | | | | | | | | | | | | | | |
| | | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| 1 | Bacterial soft rot | | 6.20 | 7.60 | 7.80 | 6.70 | 8.00 | 8.50 | 9.50 | 9.80 | 10.00 | 10.50 | 10.90 | 11.40 | 11.90 | 12.30 | 13.20 | 13.60 | 14.30 | 14.80 | 15.70 |
| 2 | Dry rot | 4.80 | 5.20 | 5.80 | 6.70 | 6.90 | 7.60 | 8.10 | 8.50 | 8.90 | 9.50 | 10.10 | 11.60 | 11.90 | 12.70 | 13.00 | 13.50 | 13.90 | 14.60 | 15.10 | 15.30 |
| 3 | Common Scab | 1.40 | 1.80 | 2.10 | 2.60 | 2.90 | 3.50 | 3.90 | 4.60 | 4.90 | 5.00 | 5.40 | 5.60 | 6.00 | 6.30 | 6.80 | 7.30 | 7.60 | 7.90 | 8.30 | 8.40 |
| 4 | Sclerotium rot | 1.10 | 1.30 | 1.70 | 2.10 | 2.60 | 2.90 | 3.40 | 3.60 | 3.90 | 4.20 | 4.80 | 5.30 | 5.90 | 6.40 | 6.80 | 7.00 | 7.20 | 7.20 | 7.00 | 7.10 |
| 5 | Brown rot | 1.00 | 1.20 | 0.80 | 1.40 | 2.40 | 2.60 | 1.50 | 2.00 | 1.60 | 3.10 | 3.50 | 3.20 | 3.60 | 3.80 | 4.30 | 4.30 | 2.80 | 2.60 | 1.60 | 2.30 |

| Sr. No. | Name of diseases | Months | | | | | | | | | | | | | | | | Mean Per cent Disease Incidence |
|---------|--------------------|----------|-------|-------|-------|----------|-------|-------|-------|---------|-------|-------|-------|----------|-------|-------|-------|---------------------------------|
| | | November | | | | December | | | | January | | | | February | | | | |
| | | Weeks | | | | | | | | | | | | | | | | |
| | | I | II | III | IV | I | II | III | IV | I | II | III | IV | I | II | III | IV | |
| 1 | Bacterial soft rot | 15.70 | 15.40 | 14.30 | 13.60 | 13.00 | 12.30 | 11.80 | 11.00 | 11.00 | 10.10 | 9.70 | 9.30 | 8.10 | 7.20 | 6.00 | 5.10 | 10.63 |
| 2 | Dry rot | 15.80 | 16.00 | 16.40 | 16.30 | 16.50 | 15.40 | 15.30 | 15.30 | 15.40 | 15.00 | 14.80 | 14.00 | 13.90 | 13.60 | 13.20 | 12.60 | 12.31 |
| 3 | Common Scab | 8.70 | 8.60 | 8.60 | 8.40 | 8.00 | 7.90 | 7.70 | 7.10 | 6.70 | 6.40 | 6.00 | 5.70 | 5.00 | 4.80 | 4.30 | 4.00 | 5.84 |
| 4 | Sclerotium rot | 6.80 | 6.40 | 6.00 | 5.40 | 5.30 | 5.00 | 4.60 | 4.10 | 3.80 | 3.40 | 3.10 | 2.60 | 2.40 | 2.30 | 1.90 | 1.80 | 4.34 |
| 5 | Brown rot | 1.30 | 1.50 | 2.10 | 1.90 | 2.20 | 2.00 | 1.80 | 1.60 | 1.60 | 1.40 | 1.50 | 1.30 | 1.00 | 0.80 | 0.00 | 0.00 | 1.99 |

4.2.2 Goya Talav market, Anand

The data presented in Table 4.2 and Fig. 4.2 revealed that during 2014-2015 at Goya Talav market, Anand, incidence of dry rot (12.31%) was predominant, followed by bacterial soft rot (10.63%) and common scab (5.84%). The incidence of sclerotium rot and brown rot were 4.34 and 1.99 per cent, respectively.

The highest incidence of dry rot (16.50%) recorded in 1st week of December, while bacterial soft rot (15.70%) was recorded in 4th week of October and 1st week of November. Highest incidence of common scab (8.70%) recorded in 1st week of November, while sclerotium rot (7.20%) in 1st and 2nd week of October. The highest incidence of brown rot (4.30%) was recorded in 3rd and 4th week of September. Disease incidence of various rots were found more at Sardar Patel vegetable market, Station Road, Anand rather than Goya Talav vegetable market, Anand. Thus, at both the locations dry rot was predominant over all other rots followed by bacterial soft rot. Very low incidence of brown rot was recorded at both the markets.

The tubers are injured during harvesting, grading, packaging and transportation, which make major avenues for infection by various pathogens. Further more number of rainy days and high humidity provide conducive conditions for infection by the pathogens and this leads to deterioration of the tubers in storages.

Masum *et al.* (2011) surveyed the Dhaka district of Bangladesh and reported that the tubers were found infected with soft rot (3.58%), dry rot (1.06%) and scab (0.96%).

Eighteen per cent of potato tubers arrived at New York markets between 1972-1980 showed symptoms of *Fusarium* dry rot (Cappellini *et al.*, 1984), while as high as 60 per cent of graded tubers in Scotland were found affected by dry rot (Carnegie *et al.*, 1990).

4.3 Evaluation of newer fungicides, some organic and inorganic salts, natural products and antagonists against *F. solani* (Mart.) Sacc. causing dry rot of potato tubers *in vitro*.

4.3.1 Fungicides:

4.3.1.1 *In vitro*:

Three fungicides (mancozeb, copper oxychloride and metiram) with two concentrations (1000 & 2000 ppm) and three fungicides *i.e.* azoxystrobin, cymoxanil (8 %) + mancozeb (64 %) and carbendazim (12 %) + mancozeb (63 %) at 500 and 1000 ppm concentrations where as hexaconazol, carbendazim and tebuconazol at 50 and 100 ppm concentrations along with control were screened to study their efficacy on mycelial growth of *F. solani in vitro* following standard poison food technique (Nene and Thapliyal, 1979). The observations on mycelial growth and per cent growth inhibition (PGI) recorded after 4th and 8th

days of incubation and the results obtained are presented in Table 4.3, Fig. 4.3 and Plate 4.4.

The perusal of results revealed that all the fungicides were found significantly superior in inhibiting the mycelial growth of *F. solani* showing more than 50 per cent growth inhibition of test pathogen at lower concentrations as compared to control except copper oxychloride and azoxystrobin which gave 16.60 and 13.81 per cent inhibition, respectively after 4 days of incubation.

Carbendazim (100 ppm) and carbendazim (12 %) + mancozeb (63 %) at both the concentrations (500 & 1000 ppm) completely inhibited the mycelial growth (100 %) of *F. solani* over control and found significantly superior over rest of the treatments. The next best treatment in order of merit were carbendazim (50 ppm) (93.98%) followed by cymoxanil (8 %) + mancozeb (64 %) (75.93%) at higher concentration (1000 ppm). While hexaconazole (100 ppm) (75.64%), tebuconazole (50 & 100 ppm) (75.59 & 70.76%), cymoxanil (8 %) + mancozeb (64 %) (1000 ppm) (75.93%) and mancozeb at higher concentration (2000 ppm) (73.89%) were found at par. Copper oxychloride and azoxystrobin at both the concentrations were less effective in inhibiting the mycelial growth of *Fusarium solani*.

On 8th day of incubation carbendazim and carbendazim (12 %) + mancozeb (63 %) at both concentrations showed complete mycelial

growth inhibition and found significantly superior over all other treatments. Azoxystrobin (62.12%) and copper oxychloride (52.28%) at lower concentrations found least effective in inhibiting the mycelial growth of *F. solani*.

The results of present investigation corroborate with the results reported by Singh (2011). She noted complete mycelial growth inhibition of *F. moniliforme* in benomyl, carbendazim (12%) + mancozeb (63%), thiophanate methyl, carbendazim and propiconazole at both the concentrations (500 & 1000 ppm).

Sultana and Ghaffar (2010) observed complete inhibition of colony growth of *F. solani* in aliette, benlate and carbendazim at 100 ppm concentration.

Nisa *et al.* (2011) reported maximum inhibition in mycelial growth of *Fusarium oxysporum* (8.80 mm) with hexaconazole at 1000 ppm concentration followed by carbendazim (9.40 mm), bitertanol (18.60 mm) and myclobutanil (20 mm) at the same concentration.

Kapadiya *et al.* (2013) reported that tebuconazole at 50, 100, 250 and 500 ppm concentration (25.9% EC) showed complete mycelial growth inhibition of *Fusarium solani* followed by epoxyconazole (250 & 500 ppm) 5% WP (92.69%).

Choudhari *et al.* (2012) reported that carbendazim at 100,250 and 500 ppm concentration completely inhibited the mycelial growth of *Fusarium solani*.

Table 4.3: Bio-efficacy of fungicides against *Fusarium solani* in vitro

| Sr. No. | Treatments | Concentration (ppm) | Radial growth (mm) | | Percent growth inhibition (PGI) | |
|---------|-----------------------------------|---------------------|---------------------|---------------------|---------------------------------|---------------------|
| | | | 4 th day | 8 th day | 4 th day | 8 th day |
| 1. | Hexaconazole | 50 | 6.33 | 10.00 | 54.09 | 79.29 |
| | | 100 | 1.67 | 7.67 | 75.64 | 89.81 |
| 2. | Carbendazim | 50 | 0.00 | 0.67 | 100.00 | 100.00 |
| | | 100 | 0.00 | 0.00 | 100.00 | 100.00 |
| 3. | Tebuconazole | 50 | 3.33 | 12.00 | 75.59 | 82.00 |
| | | 100 | 1.00 | 6.67 | 70.76 | 93.98 |
| 4. | Azoxystrobin | 500 | 12.00 | 29.67 | 13.81 | 62.12 |
| | | 1000 | 4.33 | 14.00 | 27.57 | 73.84 |
| 5. | Cymoxanil 8%+ Mancozeb 64% | 500 | 3.33 | 19.33 | 52.81 | 70.21 |
| | | 1000 | 1.67 | 11.00 | 75.93 | 90.05 |
| 6. | Carbendazim 12% + Mancozeb 63% | 500 | 0.00 | 0.00 | 100.00 | 100.00 |
| | | 1000 | 0.00 | 0.00 | 100.00 | 100.00 |
| 7. | Copper oxychloride | 1000 | 10.33 | 34.17 | 16.60 | 52.28 |
| | | 2000 | 3.67 | 17.67 | 25.95 | 77.78 |
| 8. | Mancozeb | 1000 | 3.67 | 15.33 | 62.59 | 80.19 |
| | | 2000 | 1.33 | 7.33 | 73.89 | 92.13 |
| 9. | Metiram | 1000 | 5.00 | 18.33 | 55.37 | 72.98 |
| | | 2000 | 1.67 | 10.00 | 63.98 | 90.05 |
| 10. | Control | - | 14.00 | 41.00 | 0.00 | 0.00 |
| | S.Em.± | | 0.45 | 0.62 | 3.60 | 1.50 |
| | C.D. at 5% | | 1.28 | 1.77 | 10.29 | 4.29 |
| | C.V. (%) | | 17.21 | 7.36 | 10.92 | 3.45 |

4.3.1.2 In vivo:

Five fungicides which were found most effective *in vitro* were further screened to test their efficacy in controlling *Fusarium* dry rot of potato tubers *in vivo*.

Table 4.4: Bio-efficacy of fungicides on the severity of Fusarium dry rot of potato *in vivo*

| Sr. No. | Fungicides | Dry rot severity (%) | | | | | | | |
|---------|---|----------------------|---------------------|---------------------|---------------------|----------------------|---------------------|----------------------|---------------------|
| | | Pre-inoculation | | | | Post-inoculation | | | |
| | | 50 ppm | | 100 ppm | | 50 ppm | | 100 ppm | |
| | | 4 th day | 8 th day | 4 th day | 8 th day | 4 th day* | 8 th day | 4 th day* | 8 th day |
| 1 | Carbendazim | 0.00 | 0.00 | 0.00 | 0.00 | 0.0 (0.0) | 0.00 | 0.70 (0.00) | 0.66 |
| 2 | Tebuconazole | 3.28 | 4.61 | 2.46 | 4.08 | 2.27 (4.65) | 15.93 | 2.20 (4.34) | 5.01 |
| 3 | Hexaconazole | 6.66 | 13.80 | 5.73 | 12.73 | 3.23 (9.93) | 22.88 | 3.06 (8.86) | 13.81 |
| | | 500 ppm | | 1000 ppm | | 500 ppm | | 1000 ppm | |
| 4 | Carbendazim 12% + Mancozeb 63% | 0.83 | 2.66 | 0.66 | 1.56 | 1.09 (0.68) | 1.90 | 1.02 (0.54) | 3.15 |
| | | 1000 ppm | | 2000 ppm | | 1000 ppm | | 2000 ppm | |
| 5 | Mancozeb | 5.58 | 12.80 | 4.36 | 11.03 | 2.55 (6.00) | 19.90 | 2.31 (4.83) | 12.86 |
| 6 | Control | 18.76 | 52.16 | 19.10 | 52.63 | 4.40 (18.86) | 57.30 | 4.38 (18.68) | 52.60 |
| | S.Em. ± | 0.39 | 1.06 | 0.33 | 1.09 | 0.19 | 0.64 | 0.17 | 1.02 |
| | C.D. at 5 % | 1.21 | 3.29 | 1.04 | 3.36 | 0.60 | 1.99 | 0.54 | 3.16 |
| | C.V. % | 11.69 | 12.90 | 10.86 | 13.82 | 14.19 | 5.70 | 13.52 | 12.11 |

* Figures in the parenthesis are retransformed values of square root transformation.

4.3.1.2.1 Pre-inoculation:

The results presented in Table 4.4 and Fig. 4.4 revealed that the dry rot disease was not observed in tubers treated with carbendazim (50 & 100 ppm) on 4th day after inoculation and it was at par with carbendazim (12 %) + mancozeb (63 %) (0.83 & 0.66%). The next best treatment in order of merit was tebuconazole (3.28 & 2.46%) at 50 and 100 ppm concentrations, respectively on 4th day after inoculation.

Hexaconazole (6.66 & 5.73%) found least effective in controlling the dry rot at both the concentrations (50 & 100 ppm).

Trend similar to that observed on 4th day was recorded on 8th day after inoculation. No dry rot severity was observed in tubers treated with carbendazim and it was at par with carbendazim (12%) + mancozeb (63%) (2.66 & 1.56 %) at 500 and 1000 ppm concentrations. The next best treatment in order of merit was tebuconazole (4.61 & 4.08%) at 50 and 100 ppm concentrations, respectively. Hexaconazole (13.80 & 12.73%) found least effective in controlling the dry rot over control (52.16 & 52.63%) (Plate 4.5).

4.3.1.2.2 Post-inoculation:

The results presented in Table 4.4 and Fig. 4.4 revealed that the dry rot disease was not observed in tubers treated with carbendazim (50 ppm) on 4th day after inoculation followed by carbendazim (12%) + mancozeb (63%) (1.09 & 1.02%) at 500 and 1000 ppm concentrations, respectively. The next best treatment in order of merit was tebuconazole (2.27 & 2.20%) at 50 and 1000 ppm concentrations, respectively on 4th day after inoculation. Hexaconazole (3.23 & 3.06%) found least effective in controlling the rot at both the concentrations over control (4.40 & 4.38%).

More or less similar trend of results which was observed on 4th day was recorded on 8th day after inoculation. No dry rot severity was observed in tubers treated with carbendazim at 50 ppm concentration; while mild dry rot severity was recorded at 100 ppm concentration (0.66%) and it was found at par with carbendazim (12%) + mancozeb (63%) (1.90 & 3.15%) at 500 and 1000 ppm concentrations, respectively. The next best treatment in order of merit was tebuconazole (15.93 & 5.01%) at 50 and 100 ppm concentrations, respectively. Both the concentrations (50 & 100 ppm) of hexaconazole (22.88 & 13.81%) found least effective in controlling the dry rot over control (57.30 & 52.60) (Plate 4.5).

The results of present investigation corroborate with the results reported by Singh (2011). She observed lowest Fusarium rot severity in banana fruits treated with benomyl (5.82 & 4.16 %) at 500 and 1000 ppm concentrations followed by propiconazole (6.66 & 4.99 %) and carbendazim (12 %) + mancozeb (63 %) (9.16 & 8.33 %) at 500 and 1000 ppm concentrations, respectively.

Datar and Ghule (1998) reported that pathogens associated with fruit rot of banana viz. *Cylindrocarpon tokinensis*, *Penicillium funiculosum*, *Fusarium solani*, *Fusarium* sp. and *Colletotrichum* sp. can

be effectively controlled by dipping the fruits in carbendazim (1000 ppm) for 10 min.

Yadav *et al.* (2012) reported lowest *Penicillium* rot severity in aonla fruits treated with carbendazim at 1000 ppm both in pre (12.49 %) and post-inoculation (12.83 %) followed by benomyl @ 1000 ppm both in pre (13.49 %) and post inoculation (15.03 %) treatments at 7 days after inoculation.

Post-harvest application of thiabendazole (0.5%), benomyl (0.05%) and carbendazim (0.05%) were found most effective against the various fungal rots caused by *Aspergillus niger*, *Penicillium digitatum* and *Penicillium italicum* in citrus fruits (Verma and Tikoo, 2003).

Carbendazim at 1000 ppm concentration gave 100 per cent control of *Penicillium italicum* in kinnow fruits up to 60 days in storage (Singh and Thakur, 2005)

Rathod and Patel (2005) found carbendazim (500 and 1000 µg/ml) and mancozeb (2000 and 4000 µg/ml) most effective against the *Colletotrichum*, *Penicillium* and *Alternaria* rots in aonla fruits both in pre and post inoculation treatments.

Verma (2008) reported that post inoculation treatments with thiabendazole, benomyl and carbendazim each at 0.05% were found most

effective against the green mould (*Penicillium digitatum*) and blue mould (*Penicillium italicum*) rots of mandarin oranges.

Meena (2006) observed that application of bavistin (0.05 %) and kavach (0.2 %) found most effective in minimizing the *Penicillium* rot (*P. fellutanum*) severity in aonla fruits.

4.3.2 Salts

4.3.2.1 *In vitro*:

Effect of thirteen salts (0.2M) were tested against the mycelial growth inhibition of *F. solani in vitro* following standard poison food technique (Nene and Thapliyal, 1979). The observations on the mycelial growth was recorded after 4th and 8th days of incubation and the results obtained are presented in Table 4.5, Fig. 4.5 and Plate 4.6.

All the salts screened, were found significantly superior in inhibiting the mycelial growth of *F. solani* over control. Aluminum acetate and aluminum chloride completely inhibited the mycelial growth (100 %) over control on 4th day after inoculation. The next best treatment in order of merit were potassium sorbate (0.33 mm), sodium carbonate (0.67 mm) and ammonium chloride (1.67 mm) showing 96.19, 92.27 and 80.74 per cent mycelial growth inhibition, respectively over control (8.67 mm) on 4th day after inoculation. Calcium chloride (3.00 mm) and

potassium carbonate (3.00 mm) found least effective in restricting the mycelial growth of the *F. solani* (65.40 %) (Fig 4.5).

Similar trend of results was noted on 8th day of incubation. Aluminum acetate and aluminum chloride completely inhibited the mycelial growth over control (33.33 mm). The next best treatment in order of merit were potassium sorbate (2.67 mm), sodium carbonate (3.00 mm) and ammonium chloride (8.33 mm) showing 91.99, 91.00 and 75.01 per cent mycelial growth inhibition, respectively over control (33.33 mm) on 8th day after inoculation.

The results of present investigation corroborate with the results reported by Mecteau *et al.* (2008). They reported complete mycelial growth inhibition of *Fusarium solani* var. *coeruleum*, a causal agent of potato dry rot through aluminium acetate, aluminium chloride, potassium sorbate, sodium benzoate, sodium metabisulfite and trisodium phosphate each at 0.2 M concentration.

Aluminium acetate, aluminium chloride, potassium sorbate, sodium benzoate, sodium metabisulfite, trisodium phosphate each at 0.2 M concentration completely inhibited the conidial germination and mycelial growth of various potato pathogens *i.e.* *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora*

erythroseptica, *P. infestans*, *Verticillium albo-atrum* and *V. dahlia* (Mills *et al.*, 2004).

Abdel-Kader *et al.* (2012) reported that calcium chloride at 1 and 2 per cent concentrations gradually reduced mycelial growth of *F. solani* and *F. oxysporum* by 22.2 and 33.3 per cent, respectively. As well as the highest reduction in mycelial growth of *Alternaria solani* was observed in calcium chloride at 1, 2 and 4 per cent concentrations showing 33.3, 34.4 and 38.8 per cent reduction in mycelial growth, respectively.

Karabulut *et al.* (2005) reported 84 and 68 per cent reduction in spore germination of *Botrytis cinerea* on potato dextrose agar after 30 seconds immersion in 0.5 and 1.0 per cent potassium sorbate, respectively.

Nadia *et al.* (2014) recorded complete mycelial growth inhibition of both test fungi (*Alternaria alternata* & *Rhizopus stolonifer*) at 1.0 and 1.05 per cent concentrations of potassium sorbate. However highest reduction in mycelial growth inhibition of *A. alternata* (63.3 & 49.5%), and *R. stolonifer* (61.1 & 63.0%) was recorded at 1.0 per cent concentration of sodium benzoate and calcium chloride, respectively.

Table 4.5: Bio-efficacy of salts against *Fusarium solani* in vitro

| Sr. No. | Treatments | 4 th day | | 8 th day | |
|---------|-----------------------|---------------------|---------------------------------|---------------------|---------------------------------|
| | | Radial growth (mm) | Percent growth inhibition (PGI) | Radial growth (mm) | Percent growth inhibition (PGI) |
| 1. | Aluminum acetate | 0.00 | 100.00 | 0.00 | 100.00 |
| 2. | Aluminum chloride | 0.00 | 100.00 | 0.00 | 100.00 |
| 3. | Ammonium chloride | 1.67 | 80.74 | 8.33 | 75.01 |
| 4. | Calcium chloride | 3.00 | 65.40 | 9.33 | 72.01 |
| 5. | Potassium chloride | 2.67 | 69.20 | 12.67 | 61.99 |
| 6. | Potassium sorbate | 0.33 | 96.19 | 2.67 | 91.99 |
| 7. | Sodium benzoate | 2.33 | 73.13 | 9.67 | 70.99 |
| 8. | Sodium bicarbonate | 2.33 | 73.13 | 10.00 | 70.00 |
| 9. | Sodium carbonate | 0.67 | 92.27 | 3.00 | 91.00 |
| 10. | Sodium chloride | 2.00 | 76.93 | 9.33 | 72.01 |
| 11. | Ammonium phosphate | 1.67 | 80.74 | 10.00 | 70.00 |
| 12. | Potassium carbonate | 3.00 | 65.40 | 12.00 | 64.00 |
| 13. | Potassium bicarbonate | 2.00 | 76.93 | 11.67 | 64.99 |
| 14. | Control | 8.67 | --- | 33.33 | --- |
| | S.Em. ± | 0.03 | 3.76 | 0.07 | 1.44 |
| | C.D. at 5% | 0.09 | 10.86 | 0.20 | 4.18 |
| | C.V. (%) | 26.64 | 8.69 | 12.88 | 3.49 |

4.3.2.2 *In vivo*

Five salts which were found most effective *in vitro* were further tested to study their efficacy in controlling *Fusarium* dry rot of potato tubers at 0.2 M concentration by following both pre- and post-inoculation methods.

4.3.2.2.1 Pre-inoculation

The results presented in Table 4.6 and Fig. 4.6 revealed that the dry rot disease was not observed in tubers treated with aluminum acetate and aluminum chloride on 4th and 8th day after inoculation followed by

potassium sorbate (9.87 & 12.73%) and sodium carbonate (10.12 & 19.63%), respectively. Ammonium chloride (14.03 & 22.26%) proved least effective in managing the Fusarium dry rot (Plate 4.7).

Table 4.6: Bio-efficacy of salts on the severity of Fusarium dry rot of potato *in vivo*

| Sr. No. | Salts (0.2 M) | Dry rot severity (%) | | | |
|---------|-------------------|----------------------|---------------------|---------------------|---------------------|
| | | Pre-Inoculation | | Post-Inoculation | |
| | | 4 th day | 8 th day | 4 th day | 8 th day |
| 1 | Aluminum acetate | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | Aluminum chloride | 0.00 | 0.00 | 0.00 | 1.90 |
| 3 | Potassium sorbate | 9.87 | 12.73 | 10.30 | 15.93 |
| 4 | Sodium carbonate | 10.12 | 19.63 | 10.43 | 19.90 |
| 5 | Ammonium chloride | 14.03 | 22.26 | 14.53 | 22.88 |
| 6 | Control | 20.77 | 57.30 | 20.76 | 57.30 |
| | S.Em.± | 0.55 | 0.86 | 0.55 | 0.64 |
| | C.D. at 5% | 1.69 | 2.67 | 1.72 | 1.99 |
| | C.V. (%) | 10.42 | 8.05 | 10.35 | 5.70 |

4.3.2.2.2 Post-inoculation

Similar trend of results was noted in post-inoculation method. No dry rot severity was recorded in tubers treated with aluminum acetate and aluminum chloride on 4th day after inoculation. The next best treatment in order of merit was potassium sorbate (10.30%) followed by sodium carbonate (10.43%). Application of ammonium chloride (14.53%) found least effective in controlling the dry rot over control (20.76 %).

On 8th day after inoculation, no dry rot severity was recorded in potato tubers treated with aluminum acetate and it was found at par with aluminum chloride (1.90%). Potassium sorbate (15.93%), sodium

carbonate (19.90%) and ammonium chloride (22.88 %) found least effective in managing the dry rot over control (57.30 %).

The results of present study are in agreement with the results obtained by Mecteau *et al.* (2008). They showed that application of aluminium chloride gave 40 per cent decrease in dry rot severity of potato infected by *Fusarium solani* var. *coeruleum* compared with the control.

Hervieux *et al.* (2002) reported that sodium metabisulfite, aluminum chloride, aluminum lactate, potassium sorbate, trisodium phosphate and sodium carbonate reduced silver scurf severity by 85, 80, 70, 60, 60, and 50 per cent, respectively when applied 4 days after inoculation. However, aluminum chloride was the only salt that strongly reduced silver scurf severity when applied 7 days after the inoculation of *H. solani*.

Ghadiri *et al.* (2013) reported that application of ammonium phosphate showed average lesion area of 0.05 cm² and found most effective in inhibiting the dry rot (*F. solani*) development in potato tubers and placed in group D, while potassium carbonate (1.31 cm²) and potassium bicarbonate (1.40 cm²) placed in group C. Sodium carbonate (2.55 cm²) and sodium bicarbonate salts (2.44 cm²) placed in group B and control treatment with an average lesion area of 4 cm² placed in group A.

4.3.3 Natural products:

4.3.3.1 *In vitro*

Bio-efficacy of eleven phytoextracts (10%) were tested against the mycelial growth inhibition of *F. solani in vitro* following standard poison food technique (Nene and Thapliyal, 1979). The observations on the mycelial growth were recorded after four and eight days of incubation and the results obtained are presented in Table 4.7, Fig. 4.7 and Plate 4.8.

All the phytoextracts screened, were found significantly superior in inhibiting the mycelial growth of *F. solani* over control. Red Kaner (1.00 mm) and garlic extract (1.00 mm) found significantly superior in inhibiting the mycelial growth giving 69.17 per cent growth inhibition followed by bael patra (2.33 mm) with 57.45 per cent growth inhibition over control on 4th day after inoculation. Tobacco (3.00 mm & 51.85%), tulsi leaf extract (3.33 mm & 49.27%) and turmeric rhizome extract (3.67 mm & 46.85%) showed mediocre effect in inhibiting the mycelial growth of *F. solani*. Ardusi (39.69%), ghaneri (39.40%) and custard apple (39.40%) found least effective in inhibiting the mycelial growth of *F. solani*.

On 8th day after inoculation Red Kaner found significantly superior in inhibiting the mycelial growth (3.33 mm) showing 89.34 per cent mycelial growth inhibition followed by garlic (4.00 mm) with 87.21

per cent growth inhibition. Turmeric (8.33 mm & 73.38%), tulsi (10.00 mm & 67.97%) and tobacco (10.33 mm & 67.13%) leaf extract showed mediocre effect in inhibiting the mycelial growth, while bael patra leaf extract found least effective (16 mm & 48.76%) on 8th day after inoculation.

The results of present study are in agreement with the results obtained by Singh (2011). She reported complete mycelial growth inhibition of *F. moniliforme* causing fruit rot of banana in garlic, cinnamon and arduci extract each at 10 per cent concentration.

Shukla and Dwivedi (2012) observed 89.2 per cent mycelial growth inhibition of *Fusarium udum* in turmeric (15%) followed by garlic (88.26%) and black pepper (82.22%). In case of *Fusarium oxysporum* f. sp. *ciceri*, garlic, turmeric and black pepper each at 15 per cent concentration inhibited mycelial growth by 94.63, 87.96 and 77.74 per cent, respectively.

Al-Samarrai *et al.* (2013) reported that neem, pong-pong and chilli extract each at 3000 ppm concentration recorded the highest inhibition of *Penicillium digitatum* (90%) inciting green mold of orange followed by lemon grass and ginger (70%).

Adedokun and Ataga (2007) reported the extracts of *A. sativum* inhibited the mycelial growth of *F. solani* infecting the sweet potato tubers by 42.07 per cent as compared with the control.

Table 4.7: Bio-efficacy of natural products against *Fusarium solani* in vitro

| Sr. No. | Botanical name | Common name | 4 th day | | 8 th day | |
|---------|------------------------------------|---------------|---------------------|-----------------------------------|---------------------|----------------------------------|
| | | | Radial growth (mm) | Per cent growth inhibition (PGI)* | Radial growth (mm) | Per cent growth inhibition (PGI) |
| 1. | <i>Adhatoda vasica</i> Nees. | Ardusi | 4.67 | 39.69 (40.8) | 15.33 | 51.04 |
| 2. | <i>Aegle marmelos</i> L. | Bael Pattra | 2.33 | 57.45 (71.05) | 16.00 | 48.76 |
| 3. | <i>Azadirachta indica</i> A. Juss. | Neem | 4.00 | 45.00 (50.00) | 13.67 | 56.46 |
| 4. | <i>Allium sativum</i> L. | Garlic | 1.00 | 69.17 (87.35) | 4.00 | 87.21 |
| 5. | <i>Lantana camera</i> L. | Ghaneri | 4.67 | 39.40 (40.30) | 12.67 | 59.52 |
| 6. | <i>Melia azadirach</i> L. | Maha neem | 4.33 | 41.81 (44.45) | 12.00 | 61.68 |
| 7. | <i>Curcuma longa</i> L. | Turmeric | 3.67 | 46.85 (53.20) | 8.33 | 73.38 |
| 8. | <i>Nerium indicum</i> Mill. | Red Kaner | 1.00 | 69.17 (87.35) | 3.33 | 89.34 |
| 9. | <i>Nicotiana tabacum</i> L. | Tobacco | 3.00 | 51.85 (61.80) | 10.33 | 67.13 |
| 10. | <i>Annona reticulate</i> L. | Custard apple | 4.67 | 39.40 (40.30) | 11.00 | 64.94 |
| 11. | <i>Ocimum sanctum</i> L. | Tulsi | 3.33 | 49.27 (57.40) | 10.00 | 67.97 |
| 12. | Control | --- | 8.00 | 0.00 (0.00) | 31.33 | 0.00 |
| | S.Em.± | | 0.39 | 3.71 | 0.06 | 1.73 |
| | C.D. at 5% | | 1.12 | 10.84 | 0.18 | 5.04 |
| | C.V. (%) | | 17.91 | 14.06 | 8.55 | 4.93 |

* Figures in the parenthesis are retransformed values of arc sine transformation.

4.3.3.2 *In vivo*

Five phytoextracts which were found most effective *in vitro* were further tested to study their efficacy in controlling Fusarium dry rot of potato tubers at 10 per cent concentration by following both pre- and post-inoculation methods.

4.3.3.2.1 Pre- inoculation

The results presented in Table 4.8, Fig. 4.8 and Plate 4.9 revealed that significantly lowest Fusarium dry rot severity was noticed in tubers treated with Red Kaner (0.20 & 0.60%) and it was at par with garlic clove extract (0.70 & 1.13%) at 4th and 8th day after inoculation. Tumeric (3.20 & 3.96%) rhizome and tobacco (3.66 & 4.20%) leaf extract showed mediocre effect. Tulsi extract (4.51 & 4.86%) found least effective in reducing the dry rot severity at 4th and 8th day after inoculation, respectively over control (19.23 & 53.83%).

Table 4.8: Bio-efficacy of natural products on the severity of Fusarium dry rot of potato *in vivo*

| Sr. No. | Plant extracts (10%) | Dry rot severity (%) | | | |
|---------|----------------------|----------------------|-------------|------------------|-------------|
| | | Pre-Inoculation | | Post-Inoculation | |
| | | 4th day | 8th day | 4th day | 8th day |
| 1 | Red Kaner | 0.20 | 0.60 | 0.46 | 1.43 |
| 2 | Garlic | 0.70 | 1.13 | 1.33 | 2.06 |
| 3 | Turmeric | 3.20 | 3.96 | 3.76 | 4.43 |
| 4 | Tobacco | 3.66 | 4.20 | 4.46 | 5.70 |
| 5 | Tulsi | 4.51 | 4.86 | 5.56 | 6.40 |
| 6 | Control | 19.23 | 53.83 | 19.23 | 53.83 |
| | S.Em.± | 0.24 | 0.52 | 0.22 | 0.55 |
| | C.D. at 5% | 0.74 | 1.59 | 0.68 | 1.69 |
| | C.V. (%) | 7.92 | 7.83 | 6.60 | 7.73 |

4.3.3.2.2 Post- inoculation

Significantly lowest rot severity was recorded in Red Kaner (0.46%) followed by garlic cloves extract (1.33%) on 4th day after inoculation. The next best treatment in order of merit was turmeric (3.76%) followed by tobacco leaf extract (4.46%).

On 8th day after incubation Red Kaner (1.43%) proved best in reducing the dry rot severity and it was at par with garlic cloves extract (2.06%) followed by turmeric rhizome (4.43%) and tobacco leaf extract (5.70%) on 8th day after inoculation. Tulsi extract proved least effective in reducing the Fusarium dry rot severity (5.56 & 6.40%) at 4th and 8th day after inoculation (Plate 4.9).

The results of present study are in agreement with the results obtained by Amaeze *et al.* (2013). They observed highest rot (*Geotrichum candidum*) reduction in potato tubers with *Piper guineense* (91.70%) followed by *Allium sativum* (81.76%) (*F. solani*).

Singh (2011) reported lowest Fusarium fruit rot severity of banana in cinnamon leaf extract (14.16%) and it was at par with garlic (15.83%), piper (15.83%), ardusi (16.66%) and neem leaf extract (18.33 %) on 7th day after inoculation.

Adedokun and Ataga (2007) reported that the extracts of *A. sativum* reduced rot caused by *F. solani* on sweet potato tubers by 6.9 per cent as compared with the control.

The inhibition of mycelial growth of *Fusarium solani* and reduction in dry rot of tubers with the application of phytoextracts may be due to the fungitoxic compounds present in the phytoextracts. This may provide an alternative way of reducing and controlling the dry rot in potato tubers as it is less expensive, eco friendly and easy to prepare.

4.3.4 Antagonists

4.3.4.1 *In vitro*

Effect of six antagonists viz., *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were studied for their antagonism against *Fusarium solani* by dual culture method.

All the antagonists found significantly superior in inhibiting the mycelial growth of *F. solani* over control. Significantly highest mycelial growth inhibition (1.67 mm) was observed in *T. asperellum* (89.95 %) followed by *Pseudomonas fluorescens* (68.01%) and *T. harzianum* (61.89%) after 4 days of incubation. *Trichoderma virens* showed minimum mycelial growth inhibition (51.96%) (Table 4.9, Fig. 4.9 and Plate 4.10).

On 8th day after inoculation *T. asperellum* found significantly superior in inhibiting the mycelial growth (2.00 mm) showing 93.99 per cent growth inhibition followed by *P. fluorescens* (9.33 mm) (72.01%); *T. viride* and *T. harzianum* (10.67 mm & 68.00%) and *T. virens* (12.33 mm & 62.91%). *Bacillus subtilis* found least effective (12.67 mm & 61.99%).

Abeysinghe (2007) reported that *T. harzianum* (RU01) found highly inhibitory in dual Petri plate assays against *F. solani* f. sp. *phaseoli* followed by *B. subtilis* (CA32).

Singh (2011) reported that *T. harzianum* found most efficient antagonist in inhibiting the mycelial growth of *F. moniliforme* (53.06 %) *in vitro*.

Ozbay and Newman (2004) reported that *Trichoderma* sp. are well documented as effective biological control agents of plant diseases caused by both soil borne fungi, leaf and fruit infecting plant pathogenic fungi. They are fast growing and rapidly colonize *Fusarium* spp.

Awadalla *et al.* (2008) reported that *T. harzianum* was found most effective in inhibiting the mycelial growth of *F. solani* (64.44%).

Table 4.9: Effect of antagonists on per cent growth inhibition of *Fusarium solani* *in vitro*

| Sr. No. | Antagonist | 4 th day | | 8 th day | |
|---------|--------------------------------|----------------------|---------------------------------|----------------------|---------------------------------|
| | | Mycelial growth (mm) | Percent growth inhibition (PGI) | Mycelial growth (mm) | Percent growth inhibition (PGI) |
| 1 | <i>Trichoderma viride</i> | 6.67 | 59.93 | 10.67 | 68.00 |
| 2 | <i>Trichoderma harzianum</i> | 6.33 | 61.89 | 10.67 | 68.00 |
| 3 | <i>Trichoderma virens</i> | 8.00 | 51.96 | 12.33 | 62.91 |
| 4 | <i>Trichoderma asperellum</i> | 1.67 | 89.95 | 2.00 | 93.99 |
| 5 | <i>Pseudomonas fluorescens</i> | 5.33 | 68.01 | 9.33 | 72.01 |
| 6 | <i>Bacillus subtilis</i> | 7.00 | 58.09 | 12.67 | 61.99 |
| 7 | Control | 16.67 | ---- | 33.33 | ---- |
| | S.Em.± | 0.42 | 2.45 | 0.54 | 1.20 |
| | C.D. at 5% | 1.27 | 7.42 | 1.62 | 3.65 |
| | C.V. (%) | 9.81 | 7.61 | 7.12 | 3.74 |

4.3.4.2 *In vivo*

All the antagonists were found significantly superior in reducing the Fusarium dry rot severity on 4th and 8th day after inoculation in pre- and post-inoculation methods (Table 4.10, Fig. 4.10, Plate 4.11).

4.3.4.2.1 Pre-inoculation

Trichoderma asperellum was found significantly superior in reducing the Fusarium dry rot severity by 0.70 and 3.40 per cent on 4th and 8th day after inoculation, respectively followed by *T. viride* (2.23 & 7.30 %) and *T. harzianum* (1.34 & 5.60%) over control (4.63 & 57.20%). *Bacillus subtilis* found least effective in reducing the dry rot severity (4.63 & 16.43%).

4.3.4.2.2 Post- inoculation

The similar trend of results which was observed in pre-inoculation was obtained in post-inoculation treatment. *Trichoderma asperellum* found significantly superior in reducing the Fusarium dry rot severity (0.73 & 5.60%) followed by *T. viride* (5.68 & 11.10%) and *T. harzianum* (4.38 & 12.83%) over control (20.95 & 56.90%) on 4th and 8th day after inoculation, respectively. *Bacillus subtilis* found least effective in managing the dry rot severity (15.83 & 16.46 %).

The results of present investigation corroborate with the results obtained by Pratella and Mari (1993). They reported that when *T. viride*, *T. harzianum*, *Gliocladium roseum* and *Paecilomyces varioti* applied as spray

treatment to fruits partially controlled *Botrytis cinerea* in strawberry and kiwi fruit, *Fusarium oxysporum* in potato and *Alternaria citri* in lemon fruit.

Singh (2011) reported that *T. harzianum* found most effective in reducing the Fusarium rot severity in banana fruits (6.00 & 9.50 %) followed by *T. virens* (9.50 & 13.00%) over control (22.50 & 40.50%) on 4th and 7th day after inoculation, respectively.

Padmodaya and Reddy (1996) found *Trichoderma viride* as highly inhibitory to *Fusarium* sp. causing wilt in tomato followed by *T. harzianum*.

Schisler *et al.* (2000) showed that *P. fluorescens* (P22:Y:05) and *Enterobacter cloacae* (S11:T:07) reduced the potato dry rot severity incited by *Gibberella pulicaris* (Anamorph: *Fusarium sambucinum*) to 25 and 17 per cent, respectively.

Ram and Thakore (2009) reported that *T. harzianum* and *B. subtilis* completely suppressed the growth of *F. solani* and effectively minimized storage rot of ginger by dipping rhizomes in a combined suspension of *Pseudomonas fluorescens* and *T. harzianum* @ 0.5% for 30 min before storage.

Table 4.10: Effect of antagonists on the Fusarium dry rot severity of potato *in vivo*

| Sr. No. | Antagonist | Dry rot severity (%) | | | |
|---------|--------------------------------|----------------------|---------------------|---------------------|---------------------|
| | | Pre-Inoculation | | Post-Inoculation | |
| | | 4 th day* | 8 th day | 4 th day | 8 th day |
| 1 | <i>Trichoderma viride</i> | 2.23 (4.47) | 7.30 | 5.68 | 11.10 |
| 2 | <i>Trichoderma harzianum</i> | 1.34 (1.29) | 5.60 | 4.38 | 12.83 |
| 3 | <i>Trichoderma virens</i> | 2.89 (7.85) | 11.55 | 7.83 | 13.76 |
| 4 | <i>Trichoderma asperellum</i> | 0.70 (0.00) | 3.40 | 0.73 | 5.60 |
| 5 | <i>Pseudomonas fluorescens</i> | 3.31 (10.45) | 13.83 | 11.93 | 14.76 |
| 6 | <i>Bacillus subtilis</i> | 3.89 (14.63) | 16.43 | 15.83 | 16.46 |
| 7 | Control | 4.63 (20.93) | 57.20 | 20.95 | 56.90 |
| | S.Em.± | 0.27 | 0.58 | 0.64 | 0.25 |
| | C.D. at 5% | 0.82 | 1.74 | 1.93 | 0.75 |
| | C.V. (%) | 17.24 | 6.04 | 11.43 | 2.27 |

* Figures in the parenthesis are retransformed values of square root transformation.

4.4 Effect of different temperatures on development of Fusarium dry rot of potato tubers *in vivo*

Influence of different temperatures (5, 10, 15, 20, 25, 30, 35 & 40°C) on development of dry rot of potato tubers incited by *Fusarium solani* (Mart.) Sacc. was studied *in vivo*.

The results presented in Table 4.11, Fig. 4.11 and Plate 4.13 revealed that *F. solani* did not produced any symptoms on tubers incubated at 5, 10 and 40°C at 4th and 8th day after inoculation.

On 4th day after inoculation, it is observed that as the temperature increases, there is corresponding increase in dry rot severity was noted. The development of dry rot initiated at 15°C (5.38%) and found increased progressively upto 25°C temperature (32.77%). Further increase in temperature beyond 25°C, there is corresponding decline in dry rot was observed at 30°C (23.23%) and 35°C (17.90%). Higher temperature found (40°C) unfavourable for infection by the *F. solani* causing dry rot.

Similar trend of results which was observed on 4th day was noted on 8th day of incubation. The highest dry rot severity was recorded at 25°C (55.37%), while lowest dry rot severity was noted at 15°C (7.93%) temperature. It is concluded from the study that at lower temperature (5° & 10°C) pathogen (*F. solani*) unable to cause infection and further development, hence to increase the shelflife of tubers it is advisable to store the potato tubers in cold storages at 5°C and 10°C temperature.

The results of present investigation corroborate with the results obtained by Cherian and Varghese (2007). They reported that *F. semitectum* and *T. paradoxa* were unable to produce disease at 10°C, but *F. semitectum* caused rot at 15°C. Highest severity of the disease due to *T. paradoxa* and *F. semitectum* was found at 35° and 30°C temperature in the banana fruits.

Oliveira *et al.* (2014) found corky dry rot severity (*Fusarium semitectum*) in melons increased as the temperature increases from 15 to

25°C, whereas lesions were smaller when the temperature was higher than 25°C. The symptoms were not produced at lower temperature (10°C).

In banana fruits, higher crown rot severity caused by *F. semitectum* was observed at temperatures between 25° and 27°C, whereas the disease development was strongly inhibited below 15°C, and no symptoms were produced at 38°C (Perez and Vidal, 2002).

Dillard (1989) reported that no lesion development of *Colletotrichum coccodes* found at 7°C after 8 days of incubation of tomato fruits. The lesion diameter was found greater at 25° (24.4 mm) and 31°C (31.3 mm) than at 16°C (9.00 mm).

Colletti and Lourenco (2014) recorded highest disease incidence (100%) at 25° and 30°C after 6 hours of wetness duration for both *Colletotrichum gloeosporioides* and *C. acutatum* species at 10 days after inoculation in ‘Pedro Sato’ and at 12 days in ‘Kumagai’ guava varieties.

Granke and Hausbeck (2010) reported that no symptoms or signs of disease were observed on cucumber fruits incubated at 10° or 35°C, and the fruit rot pathogen (*Phytophthora capsici*) was not recovered from the fruits incubated at 35°C temperature.

Table 4.11: Effect of different temperatures on severity of *Fusarium* dry rot of potato *in vivo*

| Sr. No. | Temperature (°C) | Dry rot severity (%) | |
|---------|-------------------|----------------------|---------------------|
| | | 4 th day | 8 th day |
| 1 | 5 | 0.00 | 0.00 |
| 2 | 10 | 0.00 | 0.00 |
| 3 | 15 | 5.83 | 7.93 |
| 4 | 20 | 12.13 | 21.90 |
| 5 | 25 | 32.77 | 55.37 |
| 6 | 30 | 23.23 | 42.87 |
| 7 | 35 | 17.90 | 33.50 |
| 8 | 40 | 0.00 | 0.00 |
| | S.Em.± | 0.69 | 0.68 |
| | C.D. at 5% | 2.07 | 2.03 |
| | C.V. (%) | 12.01 | 5.83 |

4.5 Cultural and Morphological variability among the isolates of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers

4.5.1 Cultural and morphological variability:

The cultural and morphological variation in various isolates serves as tool in differentiation of isolates. All the isolates of *Fusarium solani* possessed characteristic features when cultured on PDA. The differences in colony characters: mycelial texture, aerial mycelia, growth, pigmentation, sporulation and colony diameter were observed.

The growth rate on PDA indicated that 3 isolates showed slow growth rate (<50 mm colony diameter), 6 showed medium growth rate (51-70 mm) and 16 were fast growing isolates (71-90 mm) after eight

days of incubation at $28\pm 1^{\circ}\text{C}$ temperature which ranged between 30.40 mm to 90.00 mm. after eight days of incubation at $28\pm 1^{\circ}\text{C}$ temperature (Table 4.12). The isolate FS-14, FS-15 and FS-23 showed maximum luxuriant mycelial growth (90.00 mm) followed by FS-12 and FS-8 with 88.00 mm and 86.00 mm radial growth, respectively. The isolate FS-13 showed minimum radial growth (30.40 mm).

Based on colony characters, the isolates were grouped into ten groups. Different colony characters *viz.*, sparse and fluffy, cottony, dense, sparse with smooth margin, sparse mycelium in concentric rings, sparse, fluffy and raised, dense and fluffy, sparse with irregular margin and fluffy growth were recorded. Two types of mycelial texture *viz.*, fluffy and fibrous were recorded; 20 isolates had fluffy growth and 5 isolates were showed fibrous type of growth.

Based on aerial mycelia, the isolates were grouped into seven groups. 7 isolates (FS-1, FS-9, FS-12, FS-20, FS-23, FS-24 & FS-25) had moderately luxuriant aerial mycelia, 6 isolates (FS-2, FS-7, FS-8, FS-15, FS-16 & FS-22) had luxuriant aerial mycelia, 4 isolates (FS-3, FS-4, FS-5 & FS-18) had luxuriant and uniform aerial mycelia, 3 isolates (FS-10, FS-11 & FS-21) had scanty and uniform aerial mycelia, 2 isolates (FS-6 & FS-14) had scanty and 2 isolates (FS-13 & FS-19) had suppressed aerial mycelia, while only FS- 17 had scanty and suppressed

aerial mycelia.

The isolates produced different colours ranging from white, pink, purple to orange yellow (Plate 4.13) on ventral surface whereas the reverse of the colonies were pink, brown, red, yellow, violet to colour less. Different pigmentations *viz.*, yellow, pale yellow, dark yellow, pink, orange, red and in some isolates no pigmentation were recorded.

Round to oval shaped microconidia ranging from 6.0 μm ~ 11.0 μm x 2.8 μm ~ 3.6 μm with 0-1 septation (Table 4.13). Macroconidia were sickle shaped to elongated with blunt end, size ranging from 38.0 μm ~ 47.4 μm x 4.5 μm ~ 4.7 μm with 3-4 septations. Sporulation of different isolates were ranged from 3.33 million spores/ml to 86.33 million spores/ml. Globose to oval, terminal or intercalary chlamydospores with smooth or rough wall either formed in singly or in chain were observed (Plate 4.14).

The results of present investigation corroborate with the results obtained by Mwang'ombe *et al.* (2008). They studied cultural and morphological variability of *Fusarium solani* (Mart) f.sp. *phaseoli* (Burk) Synd. and Hans, causal agent of root rot of common bean and reported that all 52 isolates showed high variability in aerial mycelial growth, mycelia texture, pigmentation (mycelia colour) when cultured on potato dextrose agar medium. They grouped colonies into luxuriant, moderately

luxuriant and scanty on aerial mycelial growth; fluffy and fibrous based on mycelial texture; purple, pink and white based on mycelia colour; and long, medium and short macroconidial length.

Gupta *et al.* (2010) studied cultural and physiological (temperature and pH) characters of *Fusarium oxysporum* and *F. solani*, causing wilt in guava. The results revealed that maximum mycelia growth was obtained in PDA as semi-solid media of *F. oxysporum* (78.00 mm) and *F. solani* (73.83 mm).

Sharma *et al.* (1996) studied variability among the five isolates of *Fusarium solani* (Mart.) Sacc. inciting root rot of mulberry and found that all isolates differed from each other in morphological, cultural and other colony characters as well as in virulence. Isolates showed differences in the growth pattern, colour of colony and in pigmentation. Pigmentation varied from yellow to slightly reddish.

Barreto *et al.* (2003) reported that *Fusarium solani* (Mart.) Sacc. developed colonies after 2-3 days of incubation, which were characterized by typical dirty-white or white blue pigmentation, long and branched phialides, hyaline to milky-white microconidium droplets, and pionnotes with macroconidia.

Chandran and Reddi (2012) reported that the isolates of *F. solani* showed significant variation in radial growth and the size of

macro (13-15 x 3-4 μm to 27-29 x 4-5 μm) and the micro conidia (3-4 x 1-2 μm to 9-10 x 1-3 μm) on PDA medium. The numbers of septa in macro and micro conidia were 3-5 and 0-1, respectively and they were hyaline. The macro conidia were sickle shaped with blunt end and micro conidia were round to oval shaped.

Sang-Do Cha *et al.* (2007) recorded the microscopic observation of *Fusarium oxysporum* isolated from paprika and reported that the conidiophores are unbranched and monophialides, the micro conidia have oval-ellipsoidal shape with no septa (3.0~11 \times 1.5~3.5 μm) and macro conidia (15~20 \times 2.0~3.5 μm) have slightly curved or slender shape with 2~3 septa.

Yong Sung Jung *et al.* (1999) reported that microconidia of *Fusarium solani* f. sp. *pisi* formed on the long conidiophore with ovoid or oblong shape with 5~14 \times 2.5~5.0 μm size. Macro conidia of *Fusarium solani* f. sp. *pisi* were formed on the multibranched conidiophores on carnation leaf agar media with 4.8~ 5.3 \times 32.0~40.7 μm size.

Table 4.12: Cultural variability among the isolates of *Fusarium solani* on PDA medium

| Sr. No. | Isolate | Colony character* | Colony colour* | Growth (mm)* | Mycelial texture* | Aerial mycelia* | Pigmentation* |
|---------|---------|-------------------------------------|------------------------|--------------|-------------------|----------------------|--------------------------|
| 1 | FS-1 | Sparse and fluffy | White | 62.00 | Fluffy | Moderately luxuriant | Dark yellow |
| 2 | FS -2 | Sparse and fluffy | White | 84.00 | Fluffy | Luxuriant | Pale yellow |
| 3 | FS -3 | Cottony | White | 79.20 | Fluffy | Luxuriant uniform | Yellow |
| 4 | FS -4 | Dense | Pink | 64.10 | Fluffy | Luxuriant uniform | Pale pink |
| 5 | FS -5 | Dense | Purple | 80.00 | Fluffy | Luxuriant uniform | Blood red |
| 6 | FS -6 | Sparse and fluffy | Colour less to violate | 75.25 | Fluffy | Scanty | Orange |
| 7 | FS -7 | Cottony | White | 76.10 | Fluffy | Luxuriant | No pigmentation |
| 8 | FS -8 | Sparse with smooth margin | White | 86.00 | Fluffy | Luxuriant | Dark orange |
| 9 | FS-9 | Sparse mycelium in concentric rings | White to pink | 85.10 | Fluffy | Moderately luxuriant | Ruby red |
| 10 | FS-10 | Sparse mycelium in concentric rings | Pink | 78.20 | Fluffy | Scanty, uniform | Ruby red with brown dots |
| 11 | FS-11 | Sparse mycelium in concentric rings | Orange | 72.50 | Fluffy | Scanty, uniform | Orange |
| 12 | FS-12 | Cottony | White to yellow | 88.00 | Fibrous | Moderately luxuriant | Yellow |
| 13 | FS-13 | Cottony | Orane to yellow | 30.40 | Fluffy | Suppressed | Dusky red |
| 14 | FS-14 | Sparse with irregular margin | Dirty white | 90.00 | Fluffy | Scanty | Straw yellow |
| 15 | FS-15 | Dense, fluffy | Pink | 90.00 | Fluffy | Luxuriant | Dark yellow |
| 16 | FS-16 | Dense | White to orange | 82.10 | Fibrous | Luxuriant | Dark pink |
| 17 | FS-17 | Sparse | Pink to purple | 42.30 | Fluffy | Scanty, suppressed | Light pink |
| 18 | FS-18 | Cottony | White | 79.00 | Fibrous | Luxuriant uniform | Black to brown |
| 19 | FS-19 | Cottony | White | 66.40 | Fluffy | Suppressed | Yellow |
| 20 | FS-20 | Cottony | White | 58.20 | Fluffy | Moderately luxuriant | Pale orange |
| 21 | FS-21 | Fluffy and raised | Yellow | 54.40 | Fibrous | Scanty, uniform | Yellow |
| 22 | FS-22 | Fluffy | Pink to dark pink | 38.40 | Fluffy | Moderately luxuriant | Orange |
| 23 | FS-23 | Dense | Pink | 90.00 | Fluffy | Moderately luxuriant | Red |
| 24 | FS-24 | Dense | White | 85.00 | Fibrous | Moderately luxuriant | No pigmentation |
| 25 | FS-25 | Fluffy | Pink | 80.20 | Fluffy | Moderately luxuriant | Light pink |

* Average of three replications

Table 4.13: Size, shape, and septation of the macro and micro conidia of different isolates of *Fusarium solani*

| Sr. No. | Isolate | Micro conidia | | Macro conidia | | Septation | | Chlamyospores | Sporulation category |
|---------|---------|----------------|---------------|----------------|----------------------------------|-----------|-------|--|----------------------|
| | | Size* (µm) LxB | Shape | Size* (µm) LxB | Shape | Micro | Macro | | |
| 1 | FS-1 | 7x3.4 | Round to oval | 34 x4.5 | Sickle shaped with blunt ends | 0-1 | 3-4 | Globose and exist singly with smooth and rough walled | ++ |
| 2 | FS -2 | 8 x3.4 | Round to oval | 36 x4.2 | Elongated with blunt ends | 0-1 | 3-4 | Globose, smooth and formed singly and in chains intercalary and terminal | ++ |
| 3 | FS -3 | 6 x2.8 | Round to oval | 30 x4.2 | Sickle shaped | 0-1 | 3-4 | Oval and occurred singly and in chains with smooth and rough walled intercalary and terminal | ++ |
| 4 | FS -4 | 8 x3.0 | Round to oval | 37 x4.7 | Sickle shaped | 0-1 | 3-4 | Absent | ++ |
| 5 | FS -5 | 6 x3.2 | Round to oval | 48 x4.5 | Sickle shaped | 0-1 | 3-4 | Globose and exist singly with smooth and rough walled | ++ |
| 6 | FS -6 | 7 x3.4 | Round to oval | 34 x4.2 | Sickle shaped | 0-1 | 2-3 | Absent | + |
| 7 | FS -7 | 11 x3.6 | Round to oval | 39 x4.3 | Sickle shaped | 0-1 | 2-3 | Globose, smooth and formed singly and in chains intercalary and terminal | +++ |
| 8 | FS -8 | 9 x3.5 | Round to oval | 40 x4.1 | Sickle shaped | 0-1 | 2-3 | Globose and exist singly with smooth and rough walled | +++ |
| 9 | FS-9 | 8 x3.2 | Round to oval | 35 x3.5 | Sickle shaped | 0-1 | 2-4 | Globose, smooth and formed singly and in chains intercalary and terminal | ++ |
| 10 | FS-10 | 8 x3.5 | Round to oval | 36 x4.2 | Sickle shaped | 0-1 | 2-4 | Absent | +++ |
| 11 | FS-11 | 7 x2.9 | Round to oval | 28 x3.6 | Sickle shaped | 0-1 | 3-4 | Globose, smooth and formed singly and in chains intercalary and terminal | ++ |
| 12 | FS-12 | 9 x3.5 | Round to oval | 25 x3.5 | Sickle shaped with slight blunts | 0-1 | 3-4 | Globose and exist singly with smooth and rough walled | ++ |
| 13 | FS-13 | 10 x3.3 | Round to oval | 32 x3.6 | Sickle shaped | 0-1 | 2-4 | Globose and exist singly with smooth and | ++ |

| | | | | | | | | | |
|----|-------|---------|---------------|---------|----------------------------------|-----|-----|--|-----|
| | | | | | | | | rough walled | |
| 14 | FS-14 | 6 x3.3 | Round to oval | 36 x4.4 | Sickle shaped | 0-1 | 2-4 | Globose and exist singly with smooth and rough walled | +++ |
| 15 | FS-15 | 7 x3.6 | Round to oval | 34 x4.5 | Sickle shaped | 0-1 | 2-4 | Globose, smooth and formed singly and in chains intercalary and terminal | ++ |
| 16 | FS-16 | 8 x3.8 | Round to oval | 28 x3.8 | Sickle shaped | 0-1 | 2-3 | Absent | +++ |
| 17 | FS-17 | 7 x3.5 | Round to oval | 40 x4.5 | Sickle shaped | 0-1 | 2-3 | Absent | +++ |
| 18 | FS-18 | 6 x2.5 | Round to oval | 42 x4.2 | Sickle shaped | 0-1 | 2-3 | Absent | +++ |
| 19 | FS-19 | 7 x3.6 | Round to oval | 27 x3.5 | Sickle shaped with slight blunts | 0-1 | 2-4 | Globose, smooth and formed singly and in chains intercalary and terminal | + |
| 20 | FS-20 | 6 x2.5 | Round to oval | 24 x3.5 | Sickle shaped | 0-1 | 3-4 | Globose and exist singly with smooth and rough walled | +++ |
| 21 | FS-21 | 10 x3.8 | Round to oval | 26 x3.2 | Sickle shaped | 0-1 | 2-4 | Globose and exist singly with smooth and rough walled | ++ |
| 22 | FS-22 | 11 x3.9 | Round to oval | 28 x3.5 | Sickle shaped | 0-1 | 3-4 | Globose, smooth and formed singly and in chains intercalary and terminal | +++ |
| 23 | FS-23 | 8 x2.6 | Round to oval | 38 x3.6 | Sickle shaped with slight blunts | 0-1 | 2-4 | Absent | ++ |
| 24 | FS-24 | 9 x3.8 | Round to oval | 32 x3.2 | Sickle shaped | 0-1 | 3-4 | Absent | ++ |
| 25 | FS-25 | 7 x3.4 | Round to oval | 34 x3.1 | Sickle shaped | 0-1 | 3-4 | Absent | +++ |

*The average of three replications

+ Poor sporulation: 1-20 spores;

++ Medium sporulation: 21-60 spores;

+++ Good sporulation: More than 60 spores (**Ref.** Pradeep *et al.*,2013)

4.6 Molecular variability among the isolates of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers using RAPD markers

4.6.1 Assay of DNA from the isolates of *Fusarium solani* obtained through Nanodrop technique.

The results of spectrophotometric DNA analysis showed variability in DNA concentration in mycelia of twenty five isolates of *F. solani* (Table 4.14). DNA concentration was found ranging from 189.80 to 902.20 ng/ μ l, while the absorbance ratio of DNA at 260/280 wavelength was 1.80 to 2.10. Quality of DNA was further confirmed on agarose gel electrophoresis. DNA thus extracted was of good quality and utilized for molecular markers study through RAPD markers. Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at genetic level. This amplification technique (arbitrarily primed PCR on RAPD) can generate specific DNA fragments useful for genome mapping, identification of isolates and application in molecular biology (Hadrys *et. al.*, 1992).

Table 4.14: DNA assay of the *Fusarium solani* isolates obtained through “Nanodrop technique”

| Sr. No. | Isolate | (260/280) Wavelength Ratio | Concentration (ng/μl) |
|----------------|----------------|---|----------------------------------|
| 1 | FS-1 | 1.98 | 902.20 |
| 2 | FS -2 | 2.05 | 644.36 |
| 3 | FS -3 | 1.80 | 423.21 |
| 4 | FS -4 | 1.92 | 854.50 |
| 5 | FS -5 | 2.01 | 326.12 |
| 6 | FS -6 | 2.04 | 569.38 |
| 7 | FS -7 | 1.97 | 535.29 |
| 8 | FS -8 | 2.08 | 224.56 |
| 9 | FS-9 | 1.95 | 468.20 |
| 10 | FS-10 | 1.91 | 820.21 |
| 11 | FS-11 | 1.85 | 189.80 |
| 12 | FS-12 | 2.10 | 830.80 |
| 13 | FS-13 | 1.89 | 278.16 |
| 14 | FS-14 | 1.95 | 486.14 |
| 15 | FS-15 | 2.03 | 671.92 |
| 16 | FS-16 | 1.84 | 347.89 |
| 17 | FS-17 | 1.92 | 425.50 |
| 18 | FS-18 | 2.08 | 321.06 |
| 19 | FS-19 | 2.02 | 762.20 |
| 20 | FS-20 | 1.82 | 508.50 |
| 21 | FS-21 | 1.96 | 281.23 |
| 22 | FS-22 | 2.06 | 365.20 |
| 23 | FS-23 | 1.82 | 195.90 |
| 24 | FS-24 | 1.96 | 254.30 |
| 25 | FS-25 | 2.10 | 424.20 |

4.6.2 Random Amplification of Polymorphic DNA (RAPD) study:

In recent years, molecular markers have received arable attention and have been used for genetic diversity phylogenetic and evolutionary studies and mapping and tagging of agronomically important traits in different plant species. It can also be used in the study of molecular diversity in microorganism such as fungus, bacteria, viruses,

etc. In this study, the present investigation was carried out with the twenty five different isolates of *F. solani* to study the molecular variability in these isolates.

The present study showed a high level of genetic variability between twenty five isolates of *F. solani*. The highest polymorphism (100%) was recorded by the primer OPA-6, OPA-7, OPA-11 and OPA-13. Whereas, the lowest polymorphism was observed in the primer OPA-4(77.78%) (Table 4.15).

These six RAPD primers amplified a total of 55 scorable loci of which 51 loci were polymorphic, with a range of 6 to 13 alleles per primer. Overall, *Fusarium solani* isolates exhibited high level of genetic diversity. The maximum 13 alleles were generated by OPA-15; whereas, primer, OPA-4, OPA-6 OPA-7 and OPA-13 generated 9, 10, 8 and 9 alleles, respectively. The lowest numbers of alleles were generated by primer OPA-11 (6 alleles). This result showed the ability of RAPD to discriminate among isolates and suggested their application for species identification.

A total of 20 Primers consisted of di-nucleotide repeat motifs were used for initial screening with two isolates. Out of these, 14 primers gave no amplification at all, while only six primers were found to give clear banding patterns, and were subsequently used to analyze the entire

set of 25 isolates. The fragment size was detected by comparing the amplicons with a 100 bp Ladder.

1. Primer OPA-4 (AATCGGGCTG):

A maximum of 9 DNA alleles (Plate 4.15) were observed in OPA-4 primer. This primer showed 7 polymorphic loci out of 9 loci with 77.78 per cent polymorphism and the PIC value of 0.877 was observed (Table 4.15).

2. Primer OPA-6 (GGTCCCTGAC):

The RAPD profile of OPA-6 (Plate 4.15) produced 10 alleles which were found to be polymorphic showing 100 per cent polymorphism. The PIC value of 0.856 was observed (Table 4.15).

3. Primer OPA-7 (GAAACGGGTG):

Primer OPA-7 (Plate 4.15) amplified upto 8 alleles and all of them were polymorphic and hence showed 100 per cent polymorphism. The PIC value of 0.873 was obtained (Table 4.15).

4. Primer OPA-11 (CAATCGCCGT):

The PCR amplification with primer OPA-11 (Plate 4.15) generated 6 alleles out of which all were polymorphic with 100 per cent polymorphism. The PIC value for the primer was found to be 0.772.

5. Primer OPA-13 (CAGCACCCAC):

The RAPD profile of OPA-13 (Plate 4.15) produced 9 alleles which were found to be polymorphic showing 100 per cent polymorphism. The PIC value of 0.839 was observed (Table 4.15).

6. Primer OPA-15 (TTCCGAACCC):

The RAPD banding pattern generated through OPA-15 (Plate 4.15) showed maximum of 13 alleles out of which 11 were polymorphic and recorded 84.61 per cent polymorphism. The PIC value was 0.911 (Table 4.15).

Cophenetic Correlation Test

Cophenetic correlation value for RAPD was found to be 0.44 to 0.89. The R value obtained was 0.86. These cophenetic correlation values indicated that there was high level of diversity detected by RAPD despite the number of RAPD markers used in study was less.

Table 4.15: Details of amplification obtained with different RAPD primers

| Sr. No. | Name of primer | Sequence (5'-3') | Annealing temperature (°C) | No. of total alleles | No. of polymorphic loci | No. of monomorphic loci | Polymorphism percent (%) | Total No. of loci amplified in 25 isolates | PIC* value |
|----------------|-----------------------|-------------------------|-----------------------------------|-----------------------------|--------------------------------|--------------------------------|---------------------------------|---|-------------------|
| 1. | OPA-4 | AATCGGGCTG | 32 | 9 | 7 | 2 | 77.78 | 173 | 0.877 |
| 2. | OPA-6 | GGTCCCTGAC | 34 | 10 | 10 | 0 | 100.00 | 134 | 0.856 |
| 3. | OPA-7 | GAAACGGGTG | 32 | 8 | 8 | 0 | 100.00 | 163 | 0.873 |
| 4. | OPA-11 | CAATCGCCGT | 32 | 6 | 6 | 0 | 100.00 | 82 | 0.772 |
| 5. | OPA-13 | CAGCACCCAC | 34 | 9 | 9 | 0 | 100.00 | 126 | 0.839 |
| 6 | OPA-15 | TTCCGAACCC | 32 | 13 | 11 | 2 | 84.61 | 240 | 0.911 |
| Total | | | | 55 | 51 | 4 | 562.39 | 918 | 5.128 |
| Average | | | | 9.16 | 8.5 | 0.66 | 93.73 | 153 | 0.854 |

***PIC-Polymorphism Information Content**

Table 4.16: Jaccard's similarity coefficient between twenty five isolates of *F.solani* based on RAPD data

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| FS-1 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| FS -2 | 0.83 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| FS -3 | 0.71 | 0.67 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| FS -4 | 0.60 | 0.64 | 0.86 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| FS -5 | 0.67 | 0.64 | 0.89 | 0.82 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| FS -6 | 0.35 | 0.40 | 0.45 | 0.49 | 0.49 | 1.00 | | | | | | | | | | | | | | | | | | | |
| FS -7 | 0.65 | 0.65 | 0.73 | 0.74 | 0.73 | 0.44 | 1.00 | | | | | | | | | | | | | | | | | | |
| FS -8 | 0.60 | 0.57 | 0.67 | 0.68 | 0.67 | 0.45 | 0.77 | 1.00 | | | | | | | | | | | | | | | | | |
| FS-9 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.49 | 0.76 | 0.63 | 1.00 | | | | | | | | | | | | | | | | |
| FS-10 | 0.62 | 0.62 | 0.72 | 0.73 | 0.72 | 0.46 | 0.85 | 0.76 | 0.79 | 1.00 | | | | | | | | | | | | | | | |
| FS-11 | 0.71 | 0.71 | 0.79 | 0.76 | 0.79 | 0.47 | 0.72 | 0.71 | 0.67 | 0.79 | 1.00 | | | | | | | | | | | | | | |
| FS-12 | 0.70 | 0.73 | 0.73 | 0.70 | 0.73 | 0.49 | 0.78 | 0.77 | 0.72 | 0.81 | 0.77 | 1.00 | | | | | | | | | | | | | |
| FS-13 | 0.61 | 0.61 | 0.64 | 0.61 | 0.64 | 0.39 | 0.79 | 0.67 | 0.70 | 0.86 | 0.74 | 0.69 | 1.00 | | | | | | | | | | | | |
| FS-14 | 0.67 | 0.70 | 0.67 | 0.60 | 0.67 | 0.43 | 0.75 | 0.70 | 0.73 | 0.82 | 0.81 | 0.79 | 0.80 | 1.00 | | | | | | | | | | | |
| FS-15 | 0.59 | 0.63 | 0.51 | 0.55 | 0.51 | 0.46 | 0.47 | 0.51 | 0.48 | 0.51 | 0.63 | 0.54 | 0.50 | 0.55 | 1.00 | | | | | | | | | | |
| FS-16 | 0.60 | 0.71 | 0.57 | 0.64 | 0.60 | 0.41 | 0.62 | 0.64 | 0.53 | 0.69 | 0.67 | 0.70 | 0.64 | 0.70 | 0.67 | 1.00 | | | | | | | | | |
| FS-17 | 0.72 | 0.75 | 0.72 | 0.65 | 0.72 | 0.44 | 0.69 | 0.61 | 0.67 | 0.76 | 0.83 | 0.73 | 0.75 | 0.78 | 0.64 | 0.65 | 1.00 | | | | | | | | |
| FS-18 | 0.63 | 0.60 | 0.70 | 0.60 | 0.70 | 0.45 | 0.65 | 0.63 | 0.63 | 0.75 | 0.78 | 0.69 | 0.70 | 0.77 | 0.58 | 0.63 | 0.74 | 1.00 | | | | | | | |
| FS-19 | 0.79 | 0.78 | 0.71 | 0.61 | 0.67 | 0.34 | 0.69 | 0.57 | 0.67 | 0.72 | 0.74 | 0.66 | 0.71 | 0.77 | 0.52 | 0.64 | 0.71 | 0.78 | 1.00 | | | | | | |
| FS-20 | 0.69 | 0.69 | 0.65 | 0.62 | 0.69 | 0.48 | 0.70 | 0.69 | 0.61 | 0.77 | 0.80 | 0.78 | 0.72 | 0.79 | 0.53 | 0.73 | 0.80 | 0.76 | 0.72 | 1.00 | | | | | |
| FS-21 | 0.70 | 0.70 | 0.74 | 0.71 | 0.74 | 0.49 | 0.76 | 0.70 | 0.66 | 0.75 | 0.70 | 0.80 | 0.70 | 0.73 | 0.55 | 0.70 | 0.74 | 0.73 | 0.70 | 0.80 | 1.00 | | | | |
| FS-22 | 0.59 | 0.56 | 0.70 | 0.63 | 0.74 | 0.44 | 0.71 | 0.66 | 0.62 | 0.78 | 0.73 | 0.76 | 0.69 | 0.72 | 0.47 | 0.62 | 0.70 | 0.77 | 0.66 | 0.75 | 0.73 | 1.00 | | | |
| FS-23 | 0.62 | 0.62 | 0.65 | 0.59 | 0.65 | 0.44 | 0.63 | 0.62 | 0.68 | 0.73 | 0.80 | 0.74 | 0.72 | 0.75 | 0.57 | 0.58 | 0.80 | 0.76 | 0.69 | 0.74 | 0.65 | 0.71 | 1.00 | | |
| FS-24 | 0.61 | 0.61 | 0.72 | 0.65 | 0.72 | 0.46 | 0.77 | 0.68 | 0.67 | 0.77 | 0.80 | 0.78 | 0.75 | 0.78 | 0.56 | 0.64 | 0.80 | 0.75 | 0.65 | 0.73 | 0.75 | 0.74 | 0.77 | 1.00 | |
| FS-25 | 0.72 | 0.76 | 0.76 | 0.73 | 0.76 | 0.46 | 0.73 | 0.68 | 0.67 | 0.73 | 0.76 | 0.82 | 0.65 | 0.71 | 0.52 | 0.68 | 0.69 | 0.67 | 0.76 | 0.70 | 0.79 | 0.67 | 0.70 | 0.73 | 1.00 |

Pooled RAPD

Dendrogram was constructed according to Jaccard's coefficient (Jaccard, 1908) among the isolates of *F.solani* using the Unweighted Pair Group Method of Arithmetical averages (UPGMA) algorithm. The similarity coefficient ranged from 0.35 to 0.86 with 6 primers (Table 4.16). Highest similarity was observed between FS-10 and FS-13 (0.86) while lowest similarity was observed between FS-6 and FS-1 (0.35).

The dendrogram clearly revealed that one big cluster of twenty four isolates, while FS-6 isolate was separately situated in similarity matrix of 0.349 with FS-1. All isolates were sub divided in three clusters A, B and C. The cluster A contained twenty two isolates *i.e.* FS-1, FS-2, FS-19, FS-3, FS-5, FS-4, FS-7, FS-10, FS-13, FS-14, FS-11, FS-17, FS-23, FS-24 FS-12, FS-25,FS-20, FS-21, FS-18, FS-22, FS-8 and FS-9, while cluster B contain two isolates *i.e.* FS-15 and FS-16. Cluster C was containing only one isolate *i.e.* FS-6 which was diversified from all twenty four isolates. The sub-cluster A₁ was consisting isolates FS-1, FS-2 and FS-19, while sub-cluster A₂ was containing nineteen isolates *i.e.* FS-3, FS-5, FS-4, FS-7, FS-10, FS-13, FS-14, FS-11, FS-17, FS-23, FS-24 FS-12, FS-25,FS-20, FS-21, FS-18, FS-22, FS-8 and FS-9. Among all isolates FS-3 and FS-5 showed highly genetic similarity, while FS-7, FS-10 and FS-13 were found closely related.

In present study total 20 primers of OPA series were screened against isolates of *Fusarium solani*. Out of these 6 primers were found useful for amplification of DNA of *F. solani*. Among 6 primers the primer viz., OPA-6, OPA-7, OPA-11 and OPA-13 showed 100 per cent polymorphism, followed by OPA-15 (84.61%) and OPA-4 (77.78%) which helped to ascertain variability among the isolates. Therefore, these primers could be very useful for ascertaining variability among the population of other species of *Fusarium*.

Fumonisin are a new class of toxic secondary metabolites produced by *Fusarium* species. Two set of specific primer of FUM1 For./FUM1 Rev. and FUM2 For./FUM2 Rev. were used to determine the Fumonisin mycotoxin. The results of PCR amplification with primer FUM1 showed that all isolates have a potential to produce the toxin fumonisin except FS-6, FS-13 and FS-14, while results of PCR amplification with primer FUM2 showed that FS-6 and FS-13 were not able to produce toxin. An amplification product with this primer was detected yielding 186 bp amplicon with FUM1 primer, while 230 bp amplicon with FUM2 primer.

Results of the study revealed that PCR-based technique could be used not only to differentiate the *Fusarium* species from other genera of fungi but also to identify fumonisin-producing *Fusarium* spp.

Gupta *et al.* (2009) studied the genetic polymorphism of the isolates of *Fusarium solani* and found that 10 randomly amplified polymorphic DNA markers (OPA1-OPA10) tested in the genome of *Fusarium solani* and grouped on the basis of allelic data. This pattern of genetic variability in the isolate was also supported by the analysis of the similarity indices and UPGMA based dendrogram.

Younes *et al.* (2013) studied the genetic variation of *Fusarium solani* isolates based on RAPD-PCR analysis and results indicated that high levels of variation in banding patterns of all isolates. Unique banding profiles were obtained with two isolates of *F. solani* (I4 & I6).

Sutthisa *et al.* (2010) studied genetic variability among 30 isolates of *Fusarium* spp. using random amplified polymorphic DNA (RAPD) markers. Four primers A02, A03, A07 and A09 produced fingerprint profiles which clearly distinguished between the different species of *Fusarium* spp.

Sankar *et al.* (2014) studied molecular characterization of *Fusarium solani* isolates causing dry root rot of sweet orange using random amplified polymorphic DNA (RAPD) markers. Twelve representative isolates were characterized using 20 random primers of OPM series, out of which 17 primers gave a total of 174 amplified products, showing 90.22 per cent polymorphism.

Sreenivasa *et al.*, (2006) reported that out of 32 isolates of *Fusarium* species, 8 isolates showed a positive result with FUM1 gene set of primers at 183 bp, while no bands were seen in all other isolates of *Fusarium* and genera of *Aspergillus* and *Alternaria*. All the tested samples of *F. verticillioides*, *F. proliferatum*, and *F. anthophilum* exhibited the FUM1 gene of Fumonisin production and amplified fragments with the molecular size of 183 bp (Yazeed *et al.*, 2011).

4.7 Studies on biochemical factors responsible for disease resistance:

Phenol, sugars and starch

4.7.1 Total phenol content

Changes in Total phenol content in potato tubers inoculated with *F. solani* were compared with that of control (uninoculated) tubers at different periods. The data presented in Table 4.17 and Fig.4.13 revealed that there was appreciable increase in total phenol content in potato tubers when inoculated with *F. solani*. As the incubation period after inoculation of pathogen (*F. solani*) is increased, the corresponding increase in phenol content was observed. The highest amount of total phenol content (3.74%) was observed on 15th day after inoculation followed by 3.67, 3.60 and 3.54 per cent on 12th, 9th and 6rd day after inoculation, respectively. Thus, it was observed that total phenol content was increased in inoculated potato tubers as compared to control tubers (3.19 %).

Change in phenol metabolism is a ubiquitous response of host tissues to pathogenic invasion. Phenol content may get increased or decreased depending upon the kind of pathogen involved. The result revealed that, the possible reason might be due the breakdown of carbohydrate and intermediates of which it was utilized for the formation of phenolic compounds in infected tubers.

The content of phenolic compounds found increased after 7 days of incubation in apple fruit inoculated with *Gloeosporium* spp., *Penicillium expansum* and *Monilinia fructigena* (Schovankova and Opatova, 2011).

Srivastava and Kumar (2013) reported that the phenol level was high (52.6 mg/100 g) in neck rot (*Botrytis* sp.) infected onion as compared to fresh onion (32.00 mg/100 g).

Singh (2011) studied the total phenol content in banana fruits inoculated with *Fusarium moniliforme* over control fruits and noted appreciable increase in total phenol content when banana fruits were inoculated with *F. moniliforme*. The highest amount of total phenol content (2.71%) was recorded on 6th day after inoculation followed by 2.69, 2.68 and 2.64 per cent on 5th, 4th and 3rd day of inoculation, respectively.

Majumdar and Pathak (1989) also noted an increase in level of phenol in *Pestalotiopsis versicolor* and *Rhizopus arrhizus* infected guava fruits.

Table 4.17: Total phenol, sugars and starch content in potato tubers inoculated with *F. solani*

| Sr. No. | Treatments | Total Phenol (%) | TSS (%) | Reducing sugar (%) | Non reducing sugar (%) | Total starch (%) |
|---------|----------------------|------------------|-------------|--------------------|------------------------|------------------|
| 1 | 1 st day | 3.43 | 14.75 | 6.20 | 8.55 | 10.79 |
| 2 | 3 rd day | 3.48 | 10.66 | 5.82 | 4.84 | 8.20 |
| 3 | 6 th day | 3.54 | 8.09 | 4.65 | 3.44 | 7.40 |
| 4 | 9 th day | 3.60 | 6.37 | 3.70 | 2.67 | 5.93 |
| 5 | 12 th day | 3.67 | 4.33 | 2.25 | 2.08 | 4.37 |
| 6 | 15 th day | 3.74 | 2.90 | 1.02 | 1.88 | 3.41 |
| 7 | Uninoculated | 3.19 | 24.11 | 11.60 | 12.51 | 21.10 |
| | S.Em. ± | 0.02 | 0.03 | 0.02 | 0.02 | 0.03 |
| | C.D. at 5% | 0.07 | 0.10 | 0.06 | 0.06 | 0.09 |
| | C.V. (%) | 1.16 | 0.56 | 0.74 | 0.66 | 0.63 |

4.7.2 Total soluble sugar content

The result of total soluble sugar content in tubers inoculated with *Fusarium solani* and control tubers (without *F. solani*) at different periods are given in Table 4.17. The result showed that as the incubation period increases after inoculation of tubers with *F. solani*, there is a corresponding decrease is observed in total soluble sugar content, reducing and non reducing and starch content. The results revealed that TSS of inoculated potato tubers with *F. solani* decreased by 14.75, 10.66, 8.09, 6.37, 4.33 and 2.90 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers TSS was 24.11 per cent. Least TSS content (2.90 %) was found after 15th day of inoculation followed by 4.33 and 6.37 per cent on 12th and 9th day after inoculation, respectively. It was

observed that TSS of potato pulp decreased when inoculated with *F. solani* as compared to control tubers (Fig. 4.13).

4.7.2.1 Estimation of Reducing and Non reducing Sugars

Reducing sugar content in tubers inoculated with *Fusarium solani* and control tubers (without *F. solani*) at different periods are given in Table 4.17. The results revealed that reducing sugar of inoculated potato tubers with *F. solani* decreased by 6.20, 5.82, 4.65, 3.70, 2.25 and 1.02 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers reducing sugar was 11.60 per cent. Least reducing sugar content (1.02 %) was found after 15th day of inoculation followed by 2.25 and 3.70 per cent on 12th and 9th day after inoculation, respectively. It was observed that reducing sugar of potato pulp get decreased when inoculated with *F. solani* as compared to control tubers (11.60%) (Fig. 4.13).

The similar trend of results was obtained in non reducing sugar content. The results revealed that non reducing sugar of inoculated potato tubers decreased by 8.55, 4.84, 3.44, 2.67, 2.08 and 1.88 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers reducing sugar was 12.51 per cent.

In the present study tubers infected with *F. solani* showed reduction in total sugar, reducing and non-reducing sugar content. A possible reason for this may be the hydrolyses of sugars into units of lower

molecular weights or might be due to the utilization of storage starch and sugar as a carbon source by the pathogen during respiration and also a source of energy for microbial growth.

The results of present investigation corroborate with the results obtained by Singh (2011). She estimated the total soluble sugar content in banana fruits inoculated with *Fusarium moniliforme* and control fruits and found that TSS in inoculated fruits get decreased progressively with an increase in incubation period by 14.24, 13.20, 12.73, 11.52, 11.26 and 11.19 per cent at 1st, 2nd, 3rd, 4th, 5th and 6th day after inoculation, respectively, over control (18.05%).

Srivastava and Kumar (2013) estimated the sugar content in onion bulbs inoculated with *Botrytis* sp. and capsicum with *Colletotrichum* sp. The results showed that reducing sugar and non reducing sugar decreased in pathogen inoculated onion (5.08 & 9.39 g/100g) and capsicum (2.75 & 2.48 g/100g) as compared to uninoculated control onion bulb (5.73 & 11.87 g/100g) and capsicum (3.27 & 2.9 g/100g), respectively.

Bagwan (2003) observed similar trend of results in banana fruit infected with *Trichotehcium roseum*, *Rhizopus arrhizus* and *Penicillium expansum* with 5.02, 6.03 and 5.00 per cent decrease in total sugar, respectively as compared to control (13.12%).

Majumdar and Pathak (1989) reported that contents of ascorbic acid, sugars and proteins found declined in guava fruits infected by

Botryodiplodia theobromae, *Colletotrichum gloeosporioides*,
Pestalotiopsis versicolor, *Phomopsis psidii* and *Rhizopus arrhizus*.

4.7.3 Starch content

The results obtained on starch content in tubers inoculated with *Fusarium solani* and control (without *F. solani*) at different periods are given in Table 4.17. The results revealed that starch content of potato tubers inoculated with *F. solani* decreased by 10.79, 8.20, 7.40, 5.93, 4.37 and 3.41 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers starch was 21.10 per cent. Lowest starch content (3.41 %) was recorded at 15th day of inoculation followed by 4.37 and 5.93 per cent on 12th and 9th day after inoculation, respectively. It was observed that starch of potato pulp decreased when inoculated with *F. solani* as compared to control tubers (21.10%) (Fig. 4.13).

The starch content in inoculated potato tubers showed progressive decrease as the incubation period is increased. The probable reason for which may be due to utilization of nutritious compounds of the tubers by pathogen (*F. solani*) for their growth and metabolism which causes deterioration of the nutritious compounds of the tubers.

The results of present investigation corroborate with the results obtained by Chatage and Bhale (2012). They reported that starch

content of ivy gourd fruits infected with fruit rot (*Alternaria pluriseptata*) was decreased in sensitive (8.30 mg/100gm) and resistant (9.21 mg/100g) isolate of carbendazim over healthy isolate (15.00 mg/100g).

Tang *et al.* (2006) reported that amount of starch content of mulberry leaves was decreased due to infection of *Pyllactinia corylea* (3.94-4.44%) as compare to healthy leaves (6.38-7.22%).

Choudhari and Kareppa (2013) studied biochemical changes in healthy and infected rhizomes (*Fusarium* spp.) of ginger and found that starch content get increased in the infected rhizomes (49.6%) over healthy rhizomes (47.0%).

Markson *et al.* (2014) studied starch grain depletion in Irish potato infected with *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Penicillium expansum* and reported that after one week of incubation period, about 10 per cent of starch grains were lost due to infection by *B. theobromae* as against 20 per cent reduction with *P. expansum* and *R. stolonifer*.

V. SUMMARY AND CONCLUSION

The present investigations on “Molecular characterization of *Fusarium solani* (Mart.) Sacc. causing dry rot of potato tubers (*Solanum tuberosum* L.) and its management” was undertaken at Department of Plant Pathology, B. A. College of Agriculture, A.A.U, Anand during the year 2014-2015. The summary of the research work carried out and conclusions drawn are described hereunder.

Freshly infected diseased potato tubers (Variety-Kufri Badshah) showing typical symptoms of Fusarium dry rot were collected from Sardar Patel vegetable market, Anand. The infected tubers exhibit brown to darker wrinkles on skin. These wrinkles are often arranged in irregular concentric circles. Sometimes a hole may be produced in the centre of the concentric ring with whitish or pinkish fungal mycelia growth. When the infected tubers cut, a whitish or brownish tissues are seen with one or more cavities. Tissue isolations were made on PDA from freshly infected potato tubers. The cultural and morphological characters of the isolated pathogen were studied and they resembled similar to that of *Fusarium solani* (Mart.) Sacc.

The Koch’s postulates of isolated pathogen were proved by pin pricking method. The inoculated tubers exhibited typical Fusarium dry rot symptoms on 8th day after inoculation and which were identical to those

found under natural conditions. The artificially inoculated tubers exhibited brown to darker wrinkles on skin. These wrinkles are often arranged in irregular concentric circles with whitish or pinkish growth of fungal mycelia. With the advancement of infection the tubers become pulpy and oozed out a juice emitting foul odour. Later on, white cottony growth of the fungus consisting of profusely branched hyphae appeared over the rotted tissues of the tuber. The control potato tubers without inoculation did not produced any symptoms. Reisolations were made from artificially inoculated diseased tubers produced *Fusarium* sp. which was found identical to the original one. The reisolated culture of the pathogen was sent for identification to Indian Type Culture Collection (I.T.C.C.) Division of Mycology and Plant Pathology, I. A. R. I., New Delhi-110 012 and was identified as *Fusarium solani* (Mart.) Sacc. (ID.No. 9255.13).

On PDA, the fungus initially produced white to light pink septate mycelial growth with abundant sporulation covering the entire Petri plate (90 mm) within 8 days after incubation at room temperature ($28 \pm 1^\circ\text{C}$) and rapidly becoming tinged, lavender to dark pink, colourless to dark purple or dark brown coloured with the production of micro and macro conidia. The hyphae were septate, hyaline, 3-5 μ in diameter and septa at a distance of 20-40 μ . Chlamydospores were present. Microconidia produced in chains, remain loosely connected, one celled or two celled,

round to oval shaped, one celled conidia measured 6-11 x 2-4 μm and two celled conidia 10-18 x 2-4 μm in size. Macroconidia were sickle-shaped to elongated with blunt end with 3-4-septa, measuring 31-58 x 3.1-4.7 μm in size.

The weekly survey carried out from first week of June to fourth week of February during 2014-2015, revealed that the incidence of *Fusarium* dry rot (14.05 & 16.50%) was predominant among all rots followed by bacterial soft rot (11.28 & 15.70%) at Sardar Patel vegetable market, station road and Goya Talav market, Anand, respectively.

Among all fungicides screened, carbendazim (100 ppm) and carbendazim (12 %) + mancozeb (63 %) at both the concentrations (500 & 1000 ppm) completely inhibited the mycelial growth (100 %) of *F. solani* over control after 4th and 8th day of incubation.

The severity of *Fusarium* dry rot was not observed in tubers treated with carbendazim (50 & 100 ppm) on 4th and 8th day after inoculation in pre-inoculation treatment. While in case of post-inoculation treatment no dry rot severity was recorded in tubers treated with carbendazim at 50 ppm concentration and mild dry rot severity was recorded at 100 ppm concentration (0.66%) on 8th day after inoculation.

Total thirteen salts (0.2 M) were screened to know their efficacy in controlling the *Fusarium* dry rot of potato *in vitro* and *in vivo*. Aluminum

acetate and aluminum chloride salts completely inhibited the mycelial growth (100%) over control on 4th and 8th day after inoculation *in vitro*.

Severity of Fusarium dry rot was not observed in tubers treated with aluminum acetate and aluminum chloride on 4th and 8th day after inoculation in pre- and post-inoculation treatments.

The eleven phytoextracts were screened at 10 per cent concentration to know their efficacy in controlling the Fusarium dry rot of potato *in vitro* and *in vivo*. Among all phytoextracts screened *in vitro*, Red Kaner (69.17 & 89.34%) and garlic extract (69.17 & 87.21%) found significantly superior in inhibiting the mycelial growth over control on 4th and 8th day after inoculation.

Severity of Fusarium dry rot was significantly lowest in tubers treated with Red Kaner (0.20 & 0.60%) and it was at par with garlic clove extract (0.70 & 1.13%) at 4th and 8th day in pre-inoculation treatment, respectively. In case of post-inoculation treatment, the lowest rot severity was recorded in Red Kaner (0.46 & 1.43%) followed by garlic cloves extract (1.33 & 2.06%) on 4th and 8th day after inoculation.

Six antagonists *viz.*, *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were screened *in vitro* against *F. solani* by dual culture method. Highest mycelial growth inhibition was observed in *Trichoderma asperellum*

(89.95 & 93.99%) followed by *Pseudomonas fluorescens* (68.01 & 72.01%) on 4th and 8th day after incubation.

Trichoderma asperellum found significantly superior in reducing the Fusarium dry rot severity on 4th (0.70%) and 8th (3.40%) day after inoculation followed by *T. viride* (2.23 & 7.30%) in pre-inoculation treatment, respectively. In case of post-inoculation treatment *Trichoderma asperellum* found significantly superior in reducing the Fusarium rot severity (0.73 & 5.60%) followed by *T. viride* (5.68 & 11.10%) on 4th and 8th day after inoculation, respectively.

The potato tubers inoculated with *Fusarium solani* and incubated at 5, 10 and 40°C temperature did not produced symptoms on 4th and 8th day after inoculation. On 4th day after inoculation, it is observed that as the temperature increases, there is corresponding increase in dry rot severity. The initiation of dry rot started at 15°C temperature (5.38%) and found increased progressively upto 25°C temperature (32.77%) as the temperature is increased. On 8th day of inoculation highest dry rot severity was recorded at 25°C temperature (55.37%), while lowest rot severity was noted at 15°C (7.93%) temperature.

Studies on total sugar, total phenol and starch revealed that highest amount of total phenol content was observed on 15th day after inoculation (3.74%) followed by 12th (3.67%), 9th (3.60%) and 6th (3.54%) day after inoculation with *F.solani* as compared to control (3.19 %), while TSS of

inoculated potato tubers decreased by 14.75, 10.66, 8.09, 6.37, 4.33 and 2.90 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers it was 24.11 per cent.

In case of reducing and non reducing sugar, least reducing sugar content was recorded at 15th day of incubation (1.04%) followed by 12th (2.25%) and 9th (3.70%) day, respectively as compared to control (11.60%), while non reducing sugar content of inoculated potato tubers decreased by 8.55, 4.84, 3.44, 2.67, 2.08 and 1.88 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively as compared to control (12.51%). The starch content of inoculated potato tubers get decreased as the incubation period is increased and it was recorded 10.79, 8.20, 7.40, 5.93, 4.37 and 3.41 per cent at 1st, 3rd, 6th, 9th, 12th and 15th day after inoculation, respectively, while in control tubers it was 21.10 per cent.

The growth rate of various isolates on PDA revealed that 3 isolates (FS-13, FS-17 & FS-22) showed slow growth rate (<50 mm colony diameter), 6 (FS-1, FS-4, FS-19, FS-20, FS-21 & FS-23) showed medium growth rate (51-70 mm), while 16 isolates were found fast growing (71-90 mm) after eight days of incubation. The isolate FS-14, FS-15 and FS-23 showed maximum luxuriant mycelial growth (90.00 mm), while isolate FS-13 showed minimum radial growth (30.40 mm).

Two types of mycelial texture *viz.*, fluffy and fibrous were recorded. In 20 isolates the mycelial growth was fluffy, while in 5 isolates the mycelial growth was observed fibrous.

Based on aerial mycelia, the isolates were grouped into seven groups. Seven isolates (FS-1, FS-9, FS-12, FS-20, FS-23, FS-24 & FS-25) showed moderately luxuriant aerial mycelia growth, 6 isolates (FS-2, FS-7, FS-8, FS-15, FS-16 & FS-22) had luxuriant aerial mycelia growth, 4 isolates (FS-3, FS-4, FS-5 & FS-18) had uniform and luxuriant aerial mycelia growth, 3 isolates (FS-10, FS-11 & FS-21) had scanty and uniform aerial mycelia growth, 2 isolates (FS-6 & FS-14) had scanty and uniform aerial mycelia growth, 2 isolates (FS-13 & FS-19) had suppressed aerial mycelia growth, while only one isolate (FS- 17) had scanty and suppressed aerial mycelia growth.

The isolates produced different colours ranging from white, pink, purple to orange yellow on ventral surface, whereas, the reverse of the colonies were pink, brown, red, yellow, violet to colour less. Different pigmentations *viz.*, yellow, pale yellow, dark yellow, pink, orange, red and in some isolates no pigmentation were recorded.

Morphological studies of isolates also revealed the variation in shape and size of conidia, septation, sporulation and shape of chlamydospores. Round to oval shaped micro conidia ranging from 6.0

$\mu\text{m} \sim 11.0 \mu\text{m} \times 2.8 \mu\text{m} \sim 3.6 \mu\text{m}$ in size with 0-1 septation (Table 4.13). Macro conidia were sickle shaped to elongated with blunt end, measuring $38.0 \mu\text{m} \sim 47.4 \mu\text{m} \times 4.5 \mu\text{m} \sim 4.7 \mu\text{m}$ in size with 3-4 septations. Sporulation produced by different isolates ranged from 3.33 million spores/ml to 86.33 million spores/ml. The chlamydospores were globose to oval, terminal or intercalary with smooth or rough wall either formed singly or in chain.

The RAPD analysis was performed among twenty five isolates of *F. solani*. RAPD analysis with six primers showed amplification of a total of 55 alleles of which 51 loci were polymorphic, with a range of 6 to 13 alleles per primer. The maximum 13 alleles were generated by OPA-15; whereas, primer, OPA-4, OPA-6 OPA-7 and OPA-13 generated 9, 10, 8 and 9 alleles, respectively.

The highest polymorphism (100%) was revealed by the primer OPA-6, OPA-7, OPA-11 and OPA-13. Whereas, the lowest polymorphism (77.78%) was observed in the primer OPA-4.

The dendrogram clearly revealed that one big cluster of twenty four isolates, while FS-6 isolate was separately situated in similarity matrix of 0.349 with FS-1.

Two set of specific primer of FUM1 For./FUM1 Rev. and FUM2 For./FUM2 Rev. were used to determine the Fuminosin mycotoxin. The results of PCR amplification with primer FUM1 showed that all isolates

have a potential to produce the toxin fumonisin except FS-6, FS-13 and FS-14, while results of PCR amplification with primer FUM2 showed that FS-6 and FS-13 were not able to produce toxin.

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