INVESTIGATION ON POST HARVEST FRUIT ROT [*Fusarium solani* (Mart.) Sacc.] OF TOMATO [*Lycopersicon esculentum* Mill.] UNDER SOUTH GUJARAT CONDITIONS

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ABSTRACT

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is extensively grown vegetable fruit crop in south Gujarat. During the survey, occurrence of fruit rot disease on tomato was noticed in serious proportion inflicting heavy losses in vegetable markets of Navsari and neighbouring cities in popular varieties Namdhari and Pusa Ruby. Considering the seriousness of the problem, the present investigation was carried out to point out exact cause and to find out suitable control measures for the disease.

The repeated isolations from infected fruits revealed the association of *Fusarium* sp. which was identified as *Fusarium solani* (Mart) Sacc. (I.T.C.C.No.6123-05). The pathogenicity was proved by artificial inoculation methods *viz.*, knife, cork borer and pin-prick injury methods. All of them gave cent per cent infection.

Market survey revealed maximum per cent disease incidence (PDI) in market of Surat (15.52%) followed by Navsari and Billimora. Out of three seasons, maximum fruit rot was recorded during *kharif* 2005. Variety Namdhari followed by Pusa

Ruby, Abhinav and Avinash were found susceptible to the disease. Local variety (Desi) was found less prone to the disease.

Inoculation through various avenues revealed that the fungus cannot enter the fruits through stem end region with and without calyx and also through other area of fruit when artificial injury was not provided. Less and moderate infection was found when inoculated through pin and pressed injury and severe through insect bored holes and knife injury.

Out of eight media tested, Richards', tomato fruit extract, wheat meal and potato dextrose proved better for growth and sporulation of *F. solani*.

Out of ten varieties screened, all the varieties were found susceptible, but minimum infection was recorded in PKM-1 followed by Junagadh Ruby. The fruit rotting was initiated within two days in all varieties screened. Among them, the earliest infection was observed in Pusa Ruby followed by GT-1, Pusa Early Dwarf, DT-11, and NS-2535.

Studies on hot water dip treatment (HWT) against the disease revealed that HWT treatment at 52°C for 5 minutes recorded low PDI (11.67%). All the treatments proved significantly better in delaying the rot over control. Among them, HWT at 52°C for 5 minutes found superior over the others.

The phytoextracts of eleven plant species evaluated *in vitro* against *F. solani* revealed that the leaf extracts of gandobaval exhibited maximum growth inhibition of the pathogen (51.76%) and it was significantly superior over the rest. Next best

in order of merit was the extracts of black tulsi leaves followed by neem leaves, garlic bulbs, nilgiri leaves and karanj leaves.

All the botanicals tested at 10 and 5 per cent concentrations proved significantly superior in checking the rot over control. Gandobaval (10%) proved significantly superior over the rest (88.79%). Next best in order of effectiveness was neem (10 and 5%) followed by gandobaval (5%) and black tulsi (10 and 5%). All botanicals significantly delayed the symptoms over the control. However, significantly more duration was taken in gandobaval (10%).

Out of seven oils tested, all the oils significantly checked the rot over control. Palm kernel oil and neem oil recorded minimum fruit rot (3.33%) and found significantly superior over the rest. Next best was groundnut oil in effectiveness. All the oils showed significantly longer duration over control to start the initiation of symptoms. However, palm kernel oil followed by neem oil gave protection for longer duration.

Interaction studies effect of known antagonists carried out by three methods *viz.*, dual culture, pathogen at periphery and pathogen at centre, showed strong antagonistic effect of *T. viride*, *B. subtilis* and *A. niger* against *F. solani in vitro*.

Pre inoculated tomato fruits dipped in cell suspension of *B. subtilis* and *T. viride* recorded minimum PDI. *B. subtilis* recorded highest PDC over check. All the antagonists took significantly long duration to start the initiation of symptoms over control.

Eleven fungicides at three different concentrations screened *in vitro* against *F. solani*, showed that carbendazim, thiophanate methyl and flusilazole were highly fungitoxic followed by MEMC, propiconazole, carboxin + thiram, copper oxy chloride and mancozeb at all the concentrations they had tested.

Out of six fungicides tested for the control of fruit rot, all showed significantly lower fruit rot as compared to the control. Among them, carbendazim was found superior (3.33%) over the rest but found statistically at par with thiram (4.16%). The next best in order of merit was flusilazole. All fungicides gave protection significantly for long duration over control. Out of them, carbendazim gave long time protection (5.12 days) over the rest.

Evaluation of physical, chemical and biological treatments at pre and post inoculation conditions revealed that all treatments except pre inoculation fruit dip in hot water at 52°C temperature recorded significantly lower per cent disease index over control. Among these, pre and post inoculation fruit dip in carbendazim and palm kernel oil gave significantly lower per cent disease index (3.33%). Except pre inoculation HWT, all the treatments gave more than 88 per cent disease control over check. Overall, the treatments given before or after inoculation, the efficacy more or less was found similar except in case of HWT.

Tomato fruits took significantly a long duration in pre inoculation fruit dip in carbendazim (6 days) as compared to the rest.

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CERTIFICATE

This is to certify that the thesis entitled **"INVESTIGATION ON POST HARVEST FRUIT ROT [*Fusarium solani* (Mart) Sacc.] OF TOMATO [*Lycopersicon esculentum* Mill.] UNDER SOUTH GUJARAT CONDITIONS"** submitted by Mr. **RAVISHANKAR H. B.** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** of the **NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafied research work carried out by him under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

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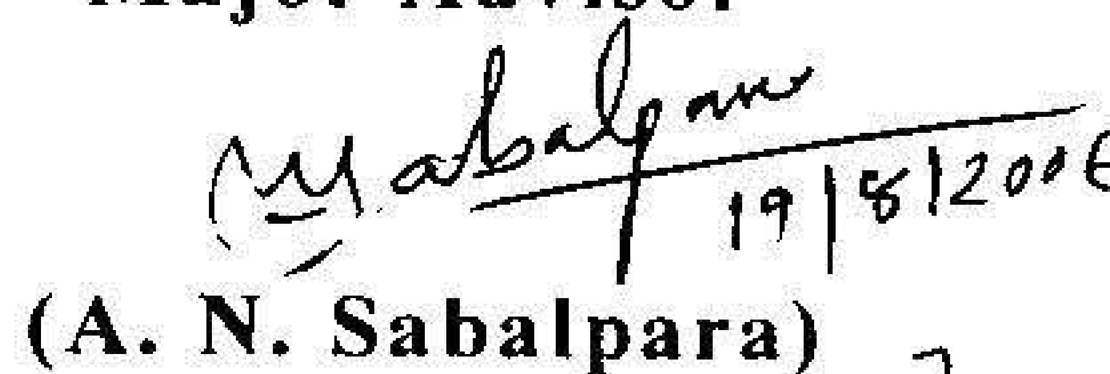

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DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment 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 direct guidance and supervision of **Dr. A. N. SABALPARA**, Professor, Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

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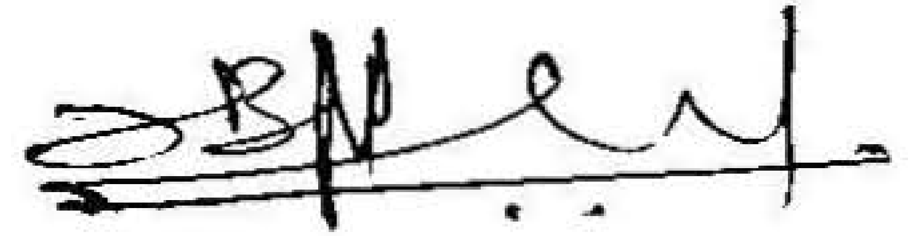

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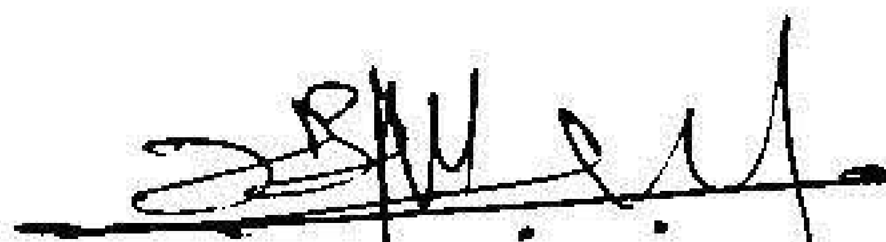

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INTRODUCTION

I INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a solanaceous fruit vegetable believed to have its origin in Tropical America (Thompson and Kelly, 1957). Tomato is known as Love apple, Tomate, Tomat, Tomatar, Rangam and Tomati in different parts of the world. It is also popularly called as 'Poorman's orange'. It is grown extensively and marketed throughout the world. It ranks third largest vegetable crop after potato and sweet potato. In India, it occupies an area of 5 lakh hectares with a production of 85 lakh tones (Anon., 2003). In Gujarat, the area under tomato crop is 22,912 hectares with total production of 4,20,689 metric tones. Ahmadabad, Banaskantha, Bhavnagar, Mehsana, Rajkot, Baroda, Valsad and Navsari are the major tomato growing districts of Gujarat (Anon., 2005).

Tomato is an attractive fruit vegetable in market. Attractive red colour of fruit is due to the presence of lycopene and pigment carotene imparts yellow colour to fruits (Kalloo, 1985). It is highly nutritious and delicious fruit vegetable, rich in vitamin A, B and an excellent source of vitamin C. The 100g edible fruit of tomato contains 94.7 g water, 1.0 g protein, 0.1 g fat, 1.6 g fiber, 1.9 g carbohydrates, 0.51 g organic acids, 19.1 mg vitamins and 224.5 mg minerals (Mc Glasson, 1993). Reducing sugars are the main carbohydrates present in tomato fruit and free sugars such as fructose, glucose and α-ketoheptose present in fruit represent more than 60 per cent of the solid.

Organic acids such as citric, malic, formic and acetic acids known as health acids are present in fresh tomato fruits (Kalloo, 1985).

Tomato has got importance in human diet due to its nutritive value and low price. It can be consumed as fresh ripe fruit and is one of the most popular salad vegetables. It is taken after cooking or raw or is made into soups, pickles, ketchups, sauces and many other products. Hence, there is a great demand for tomato in the market as a fresh fruit. It has many other uses also. The tomato seeds contain 24 per cent oil and the semi-drying oil is used as salad oil and in the manufacture of margarine. It has medicinal value too, as its pulp and juice are easily digestible, a promoter of gastric secretion and blood purifier. It is also considered to be an intestinal antiseptic. It stimulates the torpid liver and useful in chronic dyspepsia. It is a good appetizer and removes the constipation and has a pleasing taste (Tiwari and Choudhary, 1973).

Despite its wide spread cultivation in our country, the availability of good quality tomato fruits are inadequate in the market because a large numbers of fungal, bacterial and viral pathogens affect the crop in field as well in market conditions. A great damage is caused to the tomato fruits in the field, during transit, storage and marketing by the fungal rots followed by the bacterial rots, which are responsible for decaying the tomato fruits. Tomatoes are highly perishable and deteriorate in their quality after harvest. Sharma (1994) recorded 0.5 to 81.3 per cent damage in tomato fruits annually due to post-harvest fungal rots,

while Garg and Gupta (1979) reported severe post harvest rot of tomato fruits due to *Fusarium solani* (Mart.) Sacc. alone. In field and market conditions, 23 to 35 per cent tomato fruit rots have been reported due to *Rhizopus stolonifer* (Fr.) Lind., *Alternaria solani* (Ell. and Mart.) Jones and Grout and *Penicillium notatum* Westling (Ramgiry *et al.*, 1997).

Tomato fruits are attacked by many fungi in the field as well as in transit, storage and marketing. *Colletotrichum pomoides* (Sacc.) Chester, *Curvularia geniculata* (Tray and Eale.) Boedjn, *Gliocladium* sp., *Helminthosporium* sp., *Rhizopus arrhizus* Fisher. and *Monilia* sp. (Ratnam and Nema, 1967), *R. stolonifer* (Barkai *et al.*, 1993), *R. arrhizus* (Bilgrami *et al.*, 1979), *Aspergillus niger* Van Tieghem (Mandal and Dasgupta, 1981), *Myrothecium roridum* (Fr.) Tode (Kalra and Sohi, 1985), *Penicillium oxalicum* Durrie and Thom (Mandal and Dasgupta, 1981), *Mucor circinelloides* Van Tieghem, *M. hiemalis* Wehmer (Dasgupta and Mandal, 1989), *Penicillium expansum*, *R. oryzae* Went and Prinsen Geerlings, *Trichoderma koningii* Oudemans, *Pestalotiopsis* sp. (Badyal, 1994), *Alterniaria* sp. (Sood and Sharma, 2003), *A. tenuis* Auct. (Vir., *et al.*, 1967), *Botrytis cinerea* Pers. Ex Fr. (Fallik, 2002), *Rhizoctonia solani* Khin. (Strashnov *et al.*, 1985), *Thanatephorus cucumis* (Frank) Donk (Waraitch and Munshi, 1975), *Oospora* sp., (Grover and Aulakh, 1968), *Aspergillus* sp., *Gliocladium* sp. and *Sclerotium* sp. (Sumbali and Mehrotra, 1980), *Cladosporium fulvum* Cooke., *Curvularia lunata* (Wakker) Boedijin., *Myrothecium roridum*

Tode Ex. Fr., *Oospora lactis* (Fres.) Sacc. f. sp. *parasitica*, *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterh., *A. tenuis*, *Phoma destructiva* Plowr. and *Septoria lycopersici* Speg. Have (Kalra and Sohi, 1985) have been reported to cause tomato fruit rots during transit, storage and marketing.

Tomato fruits are also attacked by many species of *Fusarium*. *Fusarium* sp. (Kalra and Sohi, 1985), *F. nivale* (Fr.) Ces. (Singh *et al.*, 1980), *F. roseum* (Schwe) Snyd. (Khanna and Chandra, 1976), *F. solani* (Amadoha and Uchendu, 2003), *F. equiseti* (Corda) Saccardo (Sharma, 1994), *F. parasitica, F. moniliforme, F. nivale* (Patel and Patel, 1991), *F. pallidoroseum* Cook. Sacc. (Sharma, 1994) have been reported from rotted tomato fruits.

Fusarial rot of tomato fruits has been reported from vegetable markets of Agra, Haryana, Ahmednagar and other places (Garg and Gupta, 1979; Thakur and Yadav, 1971; Patel and Patel, 1991), but tomato fruit rot caused by *F. solani* observed for the first time from Gujarat state. The tomato fruit rot caused by *F. solani* is observed in the markets of south Gujarat throughout the year causing considerable losses and becoming very important post harvest problem. Considering this fact, the present investigation was selected. A very little work has been done for the management of fruit rot of tomato caused by *F. solani*, therefore, present studies were aimed for effective management with following objectives.

1 Collection of samples and isolation of the pathogen

2 Pathogenicity test and symptomatology

3 Identification and morphology of the pathogen

4 Survey and loss assessment on natural incidence of the disease in different markets and seasons in south Gujarat area

5 Detection of mode of entry of the pathogen

6 Search for superior media for better growth and sporulation of *F. solani*

7 Control measures

7.1 Varietal screening

7.2 Physical control: Effect of hot water dip treatment on fruit rot of tomato

7.3 Biological control

7.3.1 Bio-efficacy of botanicals against *F. solani in vitro.*

7.3.2 Control of Fusarial fruit rot of tomato by fruit dip treatment in different botanicals

7.3.3 Control of Fusarial fruit rot of tomato by fruit dip treatment in different oils

7.3.4 Testing antagonistic effect of different microorganisms to *F. solani*

7.3.5 Control of Fusarial fruit rot of tomato by fruit dip treatment in different antagonist suspensions

7.4 Chemical control

7.4.1 *In vitro* screening of different fungicides against *F. solani*

7.4.2 Control of Fusarial fruit rot of tomato by fruit dip treatment in different fungicidal solution

7.5 To test the efficacy of physical, chemical and biological treatments against the disease

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Several constraints are responsible for heavy post harvest loss of tomato, the diseases are one among them, causing serious losses in terms of quality and/or quantity. Like other fruits and vegetables, tomato is also attacked by many diseases right from harvest till it reaches to final consumers. Fruit rot caused by *Fusarium solani* (Mart.) Sacc. is one among important post harvest disease of tomato.

During the survey, tomato fruits were found severely rotted at various vegetable markets of Navsari, Billimora and Surat during *kharif* and *rabi* seasons of 2005 and summer season of 2006. The information on fruit rot caused by *F. solani* is reviewed and presented here.

2.1 Isolation of the pathogen

Many fungal pathogens have been reported from rotted tomato fruits as, as pre or post harvest disease. Among them, various species of *Fusarium* are very important.

Garg and Gupta (1979) isolated *F. solani* (Mart.) Sacc. from the diseased tomato fruits collected from local vegetable markets of Agra. Singh *et al.* (1980) also mentioned *F. solani* as an important cause of tomato fruit rot. Amadioha and Uchendu (2003) also isolated *F. solani* and considered as an important tomato fruit rot causing organism in storage.

William and Batson (1973) isolated *Fusarium* spp., *Rhizoctonia solani* Khin, *Sclerotium rolfsii* Sacc., *Erwinia* sp., *R. stolonifer* (Fr.) Lind., *Pythium aphanidermatum* (Edwon) Fitz., *Colletotrichum dematium* (Pers.) Grove, *P. parasitica* Dastur., *Alternaria tenuis* Auct., *Phomopsis* sp., *Xanthomonas vesicatoria* (Doidge) Dowson, *Curvularia* sp. and *Verticillium* sp. from rotted fruits of tomato collected from Mississippi state in USA. Thakur and Yadav (1971) isolated *F. nivale* from diseased tomatoes collected from different vegetable markets of Haryana.

Adisa (1985) mentioned tomato fruit rot as global occurrence and the major cause he found was *F. equiseti* in Nigeria. Thapa and Sharma (1976) and Kalra and Sohi (1985) found association of *Fusarium* sp. on rotted tomato fruits collected from Solan and Chandigarh vegetable markets, respectively.

Patel and Patel (1991) isolated *F. parasitica*, *F. moniliforme*, *F. nivale* and other fruit rot causing fungi from diseased tomato fruits. Tausson and Snyder (1961) also isolated one of the well known fruit rot fungus, *F. solani* from infected fruits of cucurbits.

2.2 Symptomatology

Garg and Gupta (1979) reported that *F. solani* caused small brownish water soaked lesion on tomato fruits at initial stages but later gradually increased in size and infected tissue get macerated. They also observed that the rot covered the entire fruit

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within a week; finally the fruit became pulpy and emitted an unpleasant odour. Thakur and Yadav (1971) noticed initially water-soaked lesions on tomato fruits affected by *F. nivale*. Later, they observed gradual increase in size of lesion, the tissue beneath the lesion became depressed and sunken and finally the lesion became full of whitish to pinkish fungal growth.

Sharma *et al.* (1979) observed association of *F. solani* in soft fruit rot disease of *Aegle marmelos* (Lin) Correa. They also recorded that the pathogen developed brownish soft rot with three distinct zonations of colour on infected fruits.

Roy (1983) from Agra isolated *F. solani* from infected fruits of bottle gourd. He observed white brownish hallow cavity on fruits which later embraced the whole fruit. He also noticed growth of sporulated mycelia on outer and inner surfaces and tissue became pulpy and slimy and emitted foul odour.

2.3 Identification and morphology of the pathogen

F. solani was first described and illustrated by Martius (1842) as *Fusisporium solani*, which was later on transformed to *Fusarium solani* by saccardo (1881).

According to Booth (1977), *F. solani* produced three types of spores, i.e., microconidia, macroconidia and chlamydospores. Microconidia were cylindrical to oval, one septate produced from long phialides and measured 8-16 X 2-4 µm in size; macroconidia were inequilaterally fusoid with widest point about the centre, 5-9 septate measured 35-55 X 4.5-6.0 µm

to 45-100 X 5-8 µm, while chlamydospores were globose, smooth to rough walled, produced singly or in pairs on short lateral branches or intercalary and measured 9-12 X 8-10 µm.

According to Kore and Mashalkar (1987) *F. solani* produced one celled oval to oblong and hyaline microconidia measuring 9.98-13.64 x 2.52-3.54 µm to 11.59 x 14.9 µm in size. Macroconidia were curved and narrow towards the end, pedicelate at the tip and measured 33.43-51.3 x 3.52-4.2 µm to 71.4-88.97 x 6.56-6.94 µm, while chlamydospores were produced seldom, intercalary, 1-2 celled in cluster on hyphae and measured 9.2-21.4 x 10.5-25.6 µm.

Gupta and Mathew (1999) reported that *F. solani* produced sparse to grayish white mycelium. Macroconidia were hyaline and fusiform with a pointed, slightly-beaked apical cell, measuring 44-55 x 5.1-5.3 µm and number of septa ranged from 3 to 5 depending upon culture medium used and incubation conditions. Microconidia were rare and developed from sparsely-branched conidiophores. They were broad, oval and one septate. Chlamydospores were measuring 6-16 µm in diameter and formed terminally or short lateral branch or intercalary. They formed singly, in pairs or occasionally in short chains and round to subglobular to pear shaped.

According to Pandav (2002) mycelium of *F. solani* was white to dirty white, sparse, highly branched, septate, measuring 2.41 µm in thickness and producing vinacious pigmentation on PDA. Microconidia he noticed were hyaline,

elliptical, unicellular, measuring 9.64-16.67 x 3.62-4.82 µm. While macroconidia varied from fusiform to nearly straight with rounded or pointed ends, hyaline, measuring 24.10-45.97 x 3.62-4.82 µm, having 1 to 4 septa. He also noticed abundant chlamydospores in aged culture which were found spherical to globose rough walled, light brownish, terminal and intercalary, ranging from 9.64-12.05 µm in diameter formed singly or in chain.

2.4 Market survey and loss assessment

Garg and Gupta (1979) reported severe post harvest fruit rot of tomato due to *F. solani* in Agra. While Thakur and Yadav (1971) reported large number of rotted tomato fruits due to *F. nivale* in different vegetable markets of Haryana.

Patel and Patel (1991) reported 0.80, 0.30 and 0.20 per cent losses due to *F. parasitica*, *F. moniliforme* and *F. nivale* in vegetable markets of Ahmednagar. Kalra and Sohi (1985) conducted regular market surveys (1980-83) of vegetable market at Chandigarh. They noticed *A. tenuis*, *P. nicotianae* var. *parasitica*, *C. fulvum*, *C. lunata*, *M. roridum*, *O. lactis* f.sp. *parasitica*, *P. destructiva*, *S. lycopersici* and *Fusarium* sp. on rotted tomato fruits.

Ratnam and Nema (1967) surveyed local vegetable markets and stores at Jabalpur during July, 1964 to March, 1965 and recorded various fungi *viz. A. tenuis*, *C. pomoides*, *C. geniculata*, *R. arrhizus*, *Gliocladium* sp. *Helminthosporium* sp.

and *Monilia* sp. associated with decayed fruits of tomato. Sharma (1994) also reported fungal rots in tomato fruits collected from vegetable markets of Himachal Pradesh and found the incidence of 0.5 to 81.3 per cent due to *F. equiseti*, *F. Pallidoroseum*, *G. candidum*, *Didumella lycopersici* Kleb., *A. alternata* and *P. nicotianae* var. *parasitica*.

Singh *et al.* (1980) conducted the survey of vegetable markets of Haryana during 1975-76 and 1976-77 and reported that large quantity of tomatoes were destroyed due to the attack of *A. tenuis*, *A. niger*, *F. nivale*, *F. solani*, and *R. stolonifer*. The losses ranged from 5.2 to 12.4 per cent. Losses were more in summer than winter season. A survey was conducted in Delhi vegetable market to asses month-wise loss due to *R. stolonifer* and *A. tenuis* in tomato fruits. Maximum loss of 19.0 per cent was observed during June followed by 18.5 per cent during July (Dasgupta and Mandal, 1989).

Thanatephorus cucumis caused 2 to 3 per cent loss of harvested tomato fruits in Punjab state (Waraitch and Munshi, 1975).

2.5 Pathogenicity test and Mode of entry

The fungal pathogens may get entry in tomato fruits through injuries which may occur by mechanical means, growth cracks, sunscald, insect injury or the injuries caused by any other means. Some fungal pathogens may also get entry through stem-end. Infection of some fungal pathogens may occur on tomato

fruits in the field, which manifest during transit, storage and marketing. The avenues for natural infection of fruits may be exposed surface of the attached pedicel or the stem-end (scar) after pedicel removed (Pathak and Srivastava, 1967, 1969).

Garg and Gupta (1979) successfully proved the pathogenicity of tomato fruit rot caused by *F. solani* by artificial inoculation on healthy fruits through knife injury method. Vir *et al.* (1967) reported that tomato fruits were vulnerable to injury and consequent infection of *Alternaria solani* (Ellis and Mart.) during transportation.

Thakur and Yadav (1971) noticed cent per cent infection of tomato fruits inoculated with *F. nivale* by syringe inoculation or by Granger and Horne's method of inoculation or by knife injury. They also showed that the fungus was a wound parasite because that it failed to initiate any type of symptoms on uninjured inoculated fruits.

Mehta *et al.* (1975) reported that *Alternaria* spp. caused fruit rot of tomato when inoculum of the test fungi placed on intact tomatoes or at petiolar region of fruits. Khanna and Chandra (1976) conducted pathogenicity tests of *F. roseum* by inoculating spore suspension on healthy tomato fruits bruised with sterilized needles. They noticed typical symptoms after 8 days of incubation at 28°C (± 2°C) temperature.

Reddy and Reddy (1988) were able to produce rotting of cucurbitaceous vegetables when inoculated with Fusarial isolates by scalpel injury and pin prick injury methods.

2.6 Search for superior media for better growth and sporulation of *F. solani*

Booth (1977) suggested potato dextrose agar and oat meal agar as good media for the growth of *F. solani*, Bilay's medium modified by Joffe for sporulation and Armstrong *Fusarium* medium to increase inoculum potential of *Fusarium* spp. Garg and Gupta (1979) mentioned that *F. solani* causing tomato fruit rot grew best on potato dextrose and host extract media.

Among the different media tested, maximum growth of *F. solani* was observed on Richards' medium and sporulation on PDA in case of solid media while in case of liquid media, it was on Sabouraund's medium (Gaur and Agnihotri, 1980). Out of six synthetic and semi-synthetic solid media tested, PDA and Richards' agar proved best followed by wheat meal agar. Among the liquid media, Richards' medium proved to be the best for growth and sporulation of *F. solani* (Chauhan, 1997).

Tripathi *et al.* (1999, a) cultured 5 different *Fusarium* spp. including *F. solani* on 10 different media to study growth and development of the fungi and found that modified Asthana and Howker's medium provided fair growth and excellent sporulation, while Akhtar *et al.* (1999) reported potato dextrose agar as superior medium for mycelial growth and sporulation of *Fusarium* sp. Better growth and sporulation by *F. solani* in wheat meal broth was reported by Pandav (2002). He also noticed Richards' solution and potato dextrose broth as best liquid media

for sporulation of the test fungus. Rawal and Thakore (2003) also noticed better growth and sporulation of *F. solani* on PDA medium.

2.7 Control measures

2.7.1 Varietal screening

Thakur and Yadav (1971) noticed cent per cent infection on two commercial varieties of tomato *viz.*, S-12 and Pusa Ruby when inoculated on uninjured fruits with *F. nivale*.

Waraitch and Munshi (1975) tested 36 tomato varieties against *T. cucumis* fruit rot. Varieties, T-1 and Kalyanpur Kuber showed highest resistance against the patnogen and showed no infection even when injury caused before inoculation.

Thapa and Sharma (1976) reported Local gola variety as susceptible to post harvest tomato fruit rot caused by *Fusarium* spp. as it showed severe damage.

2.7.2 Physical control: Effect of hot water dip treatment on fruit rot of tomato

The control of diseases of fruits and vegetables by post-harvest treatment in most other parts of world has always involved the physical and chemical treatments (Eckert and Sommer, 1967; Eckert, 1977). However, the problems of fungicidal toxicity and residue on such treated fruits and related produce are of common occurrence.

Following the success of post-harvest hot water treatment of papaya by Akamine and Arisumi (1953), and mango by Pennock and Maldonaldo (1962) in reducing fungal infection, a large number of studies have been undertaken. Adisa (1985) suggested storage of tomato fruits at 15°C temperature to avoid fungal storage loss caused by *F. equiseti*. Roy (1981) got 70 per cent control of *R. stolonifer* infection on tomato fruits by hot water dip at 55°C temperature for 6 minutes. A hot water dip treatment at 50°C for 2 min was also more effective in reducing *Botrytis cinerea* Pers. ex Fr. and *R. stolonifer* decay in inoculated light-red tomatoes (Barkai *et al.*, 1993).

Sood and Sharma (2003) reported that *Alternaria* sp. was unable to cause infection in 0°C, marginal infection was recorded at 5°C, whereas storage of tomatoes between 10 to 15°C temperature was quite satisfactory as the growth of the *Alternaria* sp. was checked fairly well. They also reported that hot water treatment at 52°C for 5 minutes gave better control of *Alternaria* sp. from another trial.

Fallik (2002) noticed that hot water rinse of freshly harvested tomato fruits at 52°C temperature for 15 seconds extended storability and enhanced resistance against *B. cinerea* during storage. Madhukar and Reddy (1990) found that hot water treatment at 50°C temperature for 30 minutes markedly reduced the post-harvest decay of guava caused by *Pestalotia* sp. and *Rhizoctonia* sp.

Majumdar and Pathak (1991) reported a significant decrease in the severity of infections caused by *B. theobromae*, *Colletotrichum gloeosporioides* (Penzing) Penzing & Sacc., *Pestalotia versicolor* Spegazzini, *Phomopsis psidi* Nagaraj and Ponnappa and *R. arrhizus* when the guava fruits after harvest were treated with hot water at 50 °C temperature for 5 minutes.

Pathak *et al.* (1976) reported that hot water dip treatment of papaya fruits at 49±1°C temperature for 3 minutes gave best control against post harvest fruit rot caused by *R. stolonifer* (Her. Ex Fr.) Vuill and *F. solani*.

2.7.3 Biological control

2.7.3.1 Bio-efficacy of botanicals against *F. solani in vitro*

Plants are the richest source of organic chemicals on earth and they are claimed to produce a wide variety of secondary metabolites which are used as defensive weapons. The importance of researches on several products other than plant protection chemicals have been realized in recent years due to the hazards of toxic chemicals to human beings and animals. Botanicals possess the great potentialities, being used as botanical fungicides without any adverse effect on the environment for the management of plant diseases.

Dey and Chaudhuri (1984) noticed eugenol, caryophylline and methyleugenol in tulsi responsible for strong inhibition of growth of *F. solani in vitro*. Shivpuri *et al.* (1997) tested ethanol leaf extract of 10 plant species and reported that

Azadirachta indica Juss., *Datura stramonium* L., *Ocimum sanctum* L. and *Polyalthia longifolia* (Sooner) Thw. were found more toxic to *F. oxysporum*, *Alternaria brassicola* (Berk.) Sacc. *Colletotrichum capsici* (Syd.) Butler and Bisby, *R. solani* and *S. sclerotiorum* at 100 ppm concentration. Thribhuvanamala and Narasimhan (1998) reported leaf extracts of *Delonix regia* (Bojer ex Hook) Raf., *Pongamia pinnata* L., and *Acacia nilotica* L. inhibiting the spore germination, mycelial growth and spore production of *F. solani*, significantly.

Patel and Vala (2004) noticed maximum growth inhibition of *F. solani in vitro* by un-sterilized extracts of garlic bulb (*Allium sativum* L.). Leaf extracts of *Lantana cammara* Linn. followed by *A. indica*, *Acalypha indica* L. and *Bacopa monnieri* (L.) Pennell. were found effective in inhibiting the growth of *F. solani in vitro* (Mamata and Rai, 2004).

2.7.3.2 Control of Fusarial fruit rot of tomato by fruit dip treatment in different botanicals

Pre inoculation spray with bark extracts of neem (*A. indica*) controlled tomato fruit rot caused by *F. solani* in storage (Amadioha and Uchendu, 2003).

Ark and Thompson (1959) demonstrated that aqueous and organic solvent extracts of garlic contained potent fungicidal and bactericidal activity against several plant pathogens. They controlled post harvest brown rot (*Monilinia fructicola* (Wint.)

Honey) of peach by dipping fruits in 20 per cent odourless garlic extract for 5 minutes.

Various workers have discovered effectiveness of tulsi (*Ocimum* sp.) extract against various fruit rots (Saini and Pathak, 1991; Patil, 1992; Vyas, 1993; Godara, 1994). Prakasam *et al.* (2001) noticed that cold water extract of *Vitex negundo* Linn. var. *purpurescens* was more effective at 10 per cent concentration in reducing the post harvest rot (*F. solani*) of carrots up to four days.

2.7.3.3 Control of Fusarial fruit rot of tomato by fruit dip treatment in different oils

Fruit dip in coconut, groundnut, mustard, castor and cotton seed oil emulsions (75%) for five minutes prevented *Aspergillus*, *Gliocladium* and *Sclerotium* rot of tomato fruits (Sumbali and Mehrotra, 1980). Adisa (1985) reported that 75 per cent palm kernel oil in 1 per cent soap solution was more effective against *Rhizopus*, *Curvularia*, *Phoma* and *Fusarium* rots of tomato fruits.

Aulakh and Grover (1968) reported that fruit rot of tomato could be inhibited by castor, cotton seed and paraffin oils. Mustard oil effectively controlled the decay of mango fruits caused by *A. niger* followed by vegetable oil, groundnut oil, coconut oil and linseed oil (Raoof and Prakash, 1983).

Saini and Pathak (1991) reported that post-treatment of mustard oil was effective in controlling black rot of mango fruits

caused by *A. niger*. Pathak (1980) found many vegetables and other oils effective against post harvest rot of fruits.

2.7.3.4 Antagonistic effect of different microorganisms to *F. solani in vitro*

Antagonism among the microorganisms is now well established, known and exploited in biological control of plant pathogens. Because of withdrawal of key fungicides used to control rot fungi causing most of the post-harvest losses, biological control offers an attractive alternate option (Wilson and Wisniewiski, 1992).

Zakhi (1980) reported significant growth inhibition of *F. solani*, *F. moniliforme*, and *F. oxysporum* by the species of *Aspergillus*, *Penicillium* and *Trichoderma*. Neweigy *et al.* (1982) observed strong antagonistic effect of species of *Aspergillus*, *Trichoderma* and *Bacillus* sp. against *F. solani*, *R. solani* and *S. rolfsii*.

Selvarajan and Jeyarajan (1996) reported that production of macro and microconidia and growth of *F. solani* was inhibited by antagonists *viz. Trichoderma* spp., *Laetisaria arvalis* Burds, *Bacillus subtilis* (natto.) and *Pseudomonas fluorescens* (Migula). Ram *et al.* (2000) showed inhibition of *F. solani* and *Pythium myritylum* Drech. by the *Trichoderma harzianum* Rifai, *T. auroviride* Rifai, *Gliocladium virens* (Miller) and *T. viride* Pers. Fr.

Pandav (2002) found strong antagonism of *A. niger* and *Trichoderma* spp. against *F. solani. B. subtilis*, *Chaetomium globosum* Krunze ex Fr. and *G. virens* also appeared as potential antagonists. Gurjar *et al.* (2004) observed the antagonistic effect of *T. harzianum*, *T. viride*, *G. virens*, *P. fluorescens* against pathogenic fungi of okra *viz.*, *C. lunata*, *Macrophomina phaseolina* (Tussi.) Goid, *F. oxysporum*, *F. moniliforme*, and *F. pallidoroseum*.

2.7.3.5 Control of Fusarial fruit rot of tomato by fruit dip treatment in different antagonist suspensions

Biological control of post harvest diseases by microbial antagonists was emerged as effective technology (Janisiewicz, 1990; Pusey *et al.*, 1993). This was illustrated by registration of six micro organisms for biocontrol of plant pathogens in U. S. (Cook, 1993).

Strashnov *et al.* (1985) suggested *T. harzianum* for the management of *Rhizoctonia* rot of tomato fruits. Singh and Deveral (1984) found that dipping of citrus fruits in the suspension of cells of *B. subtilis* very effectively controlled the decay of fruits caused by *A. citri* Pierce, *G. candidum* and *P. digitatum*.

Pande (1985) reported that culture filtrates of three species of *Aspergillus* and *T. viride* retarded the growth of *A. alternata*, *F. oxysporum* and *S. rolfsii*. Utkhede and Sholberg

(1986) found that 11 isolates of *B. subtilis* provided effective control of *Alternaria* rot of cherry fruits.

2.7.4 Chemical control

2.7.4.1 *In vitro* screening of different fungicides against *F. solani*

Shukla and Bhargava (1977) observed that the growth of *F. solani* isolated from various pulses and oil seed crops was inhibited by Agrosan, Benlate, Blitox-50, Ceresan, Kirticopper, Plantavax, Thiram and Vitavax *in vitro* condition. Bavistin (Carbendazim), Topsin-M (Thiophanate-methyl) and Tecto (Thiabendazole) were also found more effective against *F. solani in vitro* (Neweigy *et al.*, 1985).

Mishra and Rath (1986) found Bavistin as most effective in reducing mycelial growth and conidial germination of *F. solani*, the cause of post harvest decay of colocasia. Patel (1987) observed that Derosal, Bavistin, Agrozim and Pausin-M proved highly fungitoxic to *F. solani*.

Singh and Saxena (1990) tested twelve fungicides *in vitro*, out of them seven *viz.*, Aureofungin, Bavistin, Captan, Emisan-6, Hexaferb, Vitavax and Ziram were variably effective, while Blitox, Captafol, Dithane M-45, Streptocyclin, Sulfex were ineffective against cauliflower wilt pathogen, *F. solani*. Monga and Grover (1991) evaluated 16 fungitoxicants against *F. solani* under *in vitro* condition and observed Captafol as the most superior to inhibit the spore germination and mycelium growth

followed by Thiram, Mancozeb and M.E.M.C. at less than 10 μg ml^{-1} concentration.

Pandav (2002) also tested ten fungicides *in vitro*. Out of those, he found M.E.M.C. was more effective against *F. solani* causing wilt of cowpea. Mamata and Rai (2004) noticed higher fungitoxic effect of Captan and Dithane M-45 against *F. solani in vitro*.

2.7.4.2 Control of Fusarial fruit rot of tomato by fruit dip treatment in different fungicidal solutions

Vir *et al.* (1967) reported that post harvest dip treatment with Aureofungin prolonged the life of mango and tomato fruits by 18 to 20 and 11 to 20 days, respectively due to effective control of Fusarial fruit rots.

Pre inoculation fruit dip of tomato fruits in Dithane M-45, Difolatan (both at 0.3 %) and Thiram (0.2%) for five minutes completely checked *Alternaria* fruit rot of tomato (Kalra and Sohi, 1985). Daradhiyal (1984) found that Difolatan, Sodium2, -phenyl phenolate and Aureofungin were effective in controlling fruit rot of tomato caused by *A. alternata* when applied as post-treatment dips at 200 ppm concentration or more.

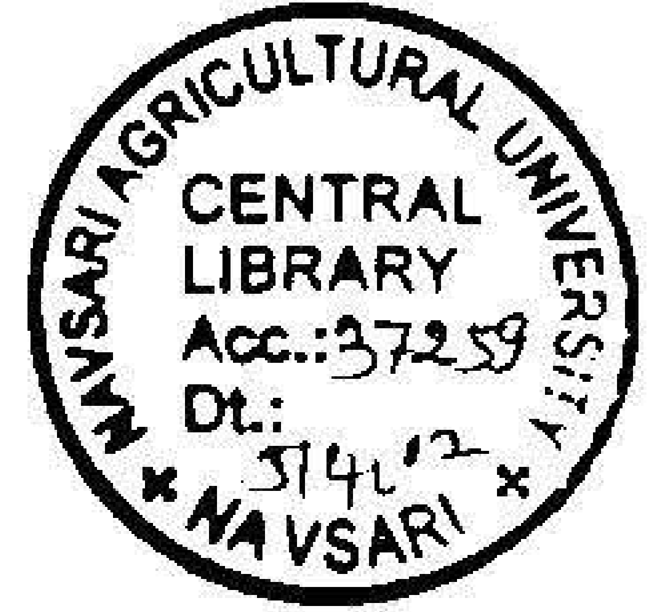
Fungicidal dip treatments in 0.2 per cent Antracol, Bavistin, Benlate, Cupravit and Dithane M-45 were found effective in reducing *Alternaria* rot of tomato fruits (Kassim, 1986). Patel and Patel (1991) found that the use of Boric acid (500 ppm), Copper sulphate (2500 ppm) and Potassium

permanganate (20 ppm) extended the keeping quality of tomato fruits by one day at 30°C temperature over the control.

De *et al.* (1994) successfully controlled post harvest rot of banana (*Fusarium* sp.) by fruit dip and shower-spraying of triazole fungicides (propiconazole, myclobutanil, flusilazole and bitertanol) at low conccentration (50 ppm) and avoided accumulation of residues on fruits. Khan (1995) found propiconazole as the most effective while thiophanate methyl as least effective at 10, 20 and 30 ppm concentration for the control of tomato fruit rot caused by *A. alternata*.

Fageria *et al.* (2002) noticed pre harvest spray of ber fruits with 0.1 per cent Bavistin effectively checking post harvest rottage (*F. solani*) up to two days after storage. Patel *et al.* (2004) also noticed good control over *F. solani* infection in okra seeds when treated externally with Bavistin.

MATERIAL AND METHODS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III MATERIALS AND METHODS

The details of the materials used and the methods adopted in the present investigation are described here as under.

3.1 Pathological investigation

3.1.1 Collection of samples

The diseased tomato fruits showing typical brownish water-soaked lesions and white mycelial growth over the surface were collected in *kharif* season of 2005 from the Dudhiya talav vegetable market, Navsari. The infected samples were brought into the laboratory in separate polythene bags to avoid contamination and were subjected to microscopic examination. The symptoms and signs observed in nature were critically examined and recorded.

3.1.2 Isolation of the pathogen

Fresh infected tomato fruits showing typical symptoms were used to isolate the pathogen. The infected area was subjected to tissue isolation. The infected portion of the fruit was cut into small bits in such a way that each bit consist of infected as well as healthy tissues.

The bits were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds followed by three washings with sterilized distilled water and then transferred aseptically under laminar air flow system (cabinet manufactured by klenzoid contamination control Ltd.) on sterilized petriplates

containing 20 ml Potato Dextrose Agar (PDA) medium. These petriplates were incubated at room temperature. The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA slants. The pure culture thus obtained was microscopically examined for identification and was further purified by using single hyphal tip isolation technique. The single hyphal tip culture obtained was maintained on PDA slants at room temperature for further future investigations.

3.2 Identification and morphology of the pathogen

Identification of the pathogen causing post harvest rot of tomato fruits was carried out by studying the cultural and morphological characters. The cultural characters were recorded right from initiation of mycelial growth till the period of 15 days.

The morphological characters *viz.*, mycelial growth and sporulation were studied under low power magnification (10 X) from 10 days old culture of *Fusarium* sp. and were compared with those described in literature. The microphotographs of mycelia and conidia were also taken. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 for identification and confirmation.

3.3 Pathogenicity test

To know the pathogenic nature of *Fusarium* sp. isolated from rotted tomato fruit samples, it was tested on semi ripened tomato fruits of cv. Pusa Ruby. Approximately equal

sized healthy fruits harvested from field were placed in paper bags and brought to the laboratory. The fruits were surface sterilized for 30 seconds with 0.1 per cent mercuric chloride solution and washed thoroughly with distilled sterilized water to remove traces of the mercuric chloride. The mycelial suspension (1×10^6 cfu/ml) was prepared from the 8 days old pure culture of *Fusarium* sp. by homogenizing culture in distilled sterilized water in homogenizer. The inoculation was carried out on surface sterilized fruits by following three methods.

1) Knife injury

2) Cork borer injury (Granger and Horne's (1924) method)

3) Pin prick injury

In all the above three methods, the pathogen was inoculated on fruits by dipping fruits in mycelial suspension of *Fusarium* sp. ($1\text{x}10^6$ cfu/ml) isolated from rotted tomato fruits. Suitable controls by fruit dip in only distilled water were maintained. All the fruits were then kept in separate clean polyethylene bags. Observations with regard to infection and symptoms development were recorded. The fungus was re-isolated from the inoculated diseased fruits and the morphological and cultural characters were compared with those of *Fusarium* sp., which was originally isolated form diseased tomato fruits and thus Koch's postulate was proved.

3.4 Market survey

Survey was conducted in *kharif* and *rabi* season of 2005 and summer season of 2006. To study the incidence of the disease, vegetable market at Dudhiya talav, Morarji Desai market yard and Vegetable shops and vendors at char rasta in Navsari, Sardar Market and Kala market in Billimora and Sardar market in Surat were selected. At each market, data were collected from five randomly selected vegetable shops. The observations were taken 4 times at an interval of 15 days in each case. Total number of fruits and diseased fruits from each lot were counted with the help of typical symptoms and per cent incidence/loss was estimated by using following formula suggested by Ratnam and Nema (1967).

$$A = \frac{Y}{X} \times 100$$

Where,

A= Per cent incidence/loss due to fusarial decay.

X= Total number of fruits

Y= Number of decayed fruits.

The average per cent incidence of fusarial fruit rot for each visit was calculated. Infected fruits represented typical fusarial fruit rot were collected in fresh polythene bags from all the places at each visit and brought to the laboratory for further isolation and symptomalogical studies.

3.5 Mode of entry

To know the mode of entry of *Fusarium* sp., equally ripened tomato fruits of equal size of cultivar Pusa Ruby were selected and the inoculum of test fungus which was prepared as mentioned earlier was inoculated through the following avenues.

1. Stem end with calyx.
2. Stem end without calyx
3. Uninjured fruit except at stem end
4. Insect bored holes
5. Pressed injury
6. Pin injury
7. Lateral injury with knife

Before inoculation, fruits were washed and surface sterilized by swabbing non absorbent cotton dipped in 0.1 per cent mercuric chloride ($HgCl_2$) solution followed by subsequent three washes in sterilized distilled water to ensure the fruit surface free from the residual traces.

In first four above mentioned methods, no artificial injury was provided. Sterilized knife, pins and other materials used to provide injury on tomato fruits in rest of the treatments.

After inoculation, the fruits were kept on surface sterilized laboratory table under bell jar at room temperature and observations on appearance of the fruit rot were recorded as (-) = no infection (+) = mild infection, (++) = moderate infection and

(++++) = severe infection at regular interval as suggested by Tandon and Mishra (1969).

3.6 Media test

With a view to find out the superior medium for the growth and sporulation of the fungus, three semi synthetic and five synthetic media in solid and liquid states were compared. The compositions of various media were given in Appendix-I

3.6.1 Solid media test

These agar agar based sterilized media were poured aseptically into 90 mm diameter previously sterilized corning petriplates @ 20 ml $plate^{-1}$. The petriplates were inoculated aseptically after solidification by placing 5 mm diameter culture block at the centre, cut aseptically with cork borer from 5 days old pure culture of *F. solani* grown on PDA medium. Three replications were kept for recording observations on colony diameter and colony character of the fungus. The petriplates were incubated at room temperature. The linear growth of the fungal colony was daily measured up to the complete coverage of plates with fungus mycelium.

3.6.2 Broth media test

All the solid media used earlier in section 3.6.1 of this chapter were used as broth media with the same ingredients omitting agar agar.

Fifty ml of the liquid medium was filled in each 150 ml corning conical flasks which were plugged with nonabsorbent

cotton. These were sterilized at 1.2 kg cm^{-2} pressure for 20 minutes in autoclave. The flasks were inoculated aseptically by placing 5 mm diametered culture block, cut aseptically with the help of cork borer from 5 days old pure culture. Three repetitions for recording growth and fourth repetition for recording conidial count were maintained in each case. The flasks were incubated at room temperature. After 15 days of inoculation mycelial mats were harvested on previously weighed oven dried Whatman's filter paper No. 42, giving sufficient washings with warm distilled water. The filter paper with mycelial mats were dried in an oven at 60° C temperature till constant weight was obtained.

The observations were recorded to compare the dry mycelial weight. The conidial count was recorded from fourth repetition. At the end of incubation period, the whole mycelial substrate was homogenized in 50 ml distilled water with the help of Sumeet homogenizer. The homogenate was filtered through muslin cloth. A drop of suspension was examined under low power magnification (10 X) microscope. The number of conidia per microscopic field were recorded from four randomly selected microscopic fields in each case. The data were statistically analyzed and presented.

3.7 Management measures

3.7.1 Varietal screening in laboratory condition.

With a view to determine comparative resistance, 10 tomato varieties *viz.*, GT-1, GT-2, Pusa Ruby, Pusa early dwarf,

DT-11, S-22, PKM-1, NS-2535, Durgesh Amar and Junagadh Ruby were screened under laboratory conditions against the fruit rot during 2005 in PG research laboratory, N. M. College of Agriculture, N.A.U., Navsari. Healthy fruits of uniform size and maturity of all the 10 varieties were freshly harvested from vegetable research station, Navsari Agricultural University, Navsari and brought to laboratory in separate labeled polyethylene bags to avoid the mix up between the varieties. Further, fruits were washed, surface sterilized, inoculated by Granger and Horne's (1924) method and incubated as mentioned in section 3.5 of this chapter. Three replications with each of five fruits were maintained for observation. Observations with regard to infection and symptoms development were recorded on the basis of following graded scale of 0-6 suggested by Mckinney (1923) with required modifications.

Rating scale

Scale		Per cent fruit area infection
0	=	No visible symptoms
1	=	1 to 5 per cent fruit area infected
2	=	6 to 10 per cent fruit area infected
3	=	11 to 25 per cent fruit area infected
4	=	26 to 50 per cent fruit area infected
5	=	51 to 75per cent fruit area infected
6	=	76 to 100 per cent fruit area infected

Per cent Disease Index (PDI)

In order to reduce the disease intensity complex to a single expression the following formula as suggested by Mc Kinney (1923) was used with required modifications.

$$PDI = \frac{\text{Sum of the rating scales of the fruits in the treatment}}{\text{Total number of fruits examined} \times \text{maximum rating scale}} \times 100$$

Grading of varieties

PDI		Reaction
Nil	=	Free (F)
0.1 to 10.0	=	Resistant (R)
10.1 to 25.0	=	Moderately resistant (MR)
25.1 to 40.0	=	Moderately susceptible (MS)
40.1 to 50.0	=	Susceptible (S)
>50	=	Highly susceptible (HS)

3.7.2 Physical control: Effect of hot water dip treatment on tomato fruit rot

In order to find out the efficacy of fruit dip treatment in hot water against the pathogen at different temperatures, fruits of variety Pusa Ruby were selected, washed, surface sterilized, inoculated by pin prick injury method and incubated as mentioned as mentioned in section 3.5 of this chapter.

After 24 hours of incubation, the fruits were dipped in hot water at 40, 45, 48, 50 and 52°C temperature for 5 minutes in

thermostatically controlled sensitive water bath containing sterilized distilled water. Fruits were dipped in distilled sterile water at room temperature for control treatment. Treatments were replicated four times with each of five fruits and treated fruits were kept for incubation as mentioned in section 3.5 of this chapter. Observations were recorded and PDI was calculated as mentioned in section 3.7.1 of this chapter.

3.7.3 Biological control

3.7.3.1 Bio-efficacy of botanicals against *F. solani in vitro*

The effect of phyto extracts of various plant species as listed in Table-3.1 were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *F. solani*.

Healthy fresh plant parts i.e., leaves, bulbs or rhizomes were taken, washed thoroughly with fresh water and finally rinsed with sterilized distilled water. Fifty grams of plant parts was cut into small pieces and minced with the help of a grinder by adding 50 ml sterilized distilled water. The phytoextracts were filtered through double-layer muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 1.2 kg. cm^{-2} pressure for 20 minutes. Autoclaved extract was individually added into previously sterilized PDA @ 10 per cent (i.e., 2 ml extract +18 ml PDA $plate^{-1}$) and mixed thoroughly at the time of pouring in the previously sterilized petriplates. The petriplates were inoculated aseptically after

Table- 3.1: List of plants used for preparing phytoextracts for testing efficacy against *F. solani in vitro*

Sr. No.	Common or local name	Botanical name	Family	Plant part used for preparing extract
1	Baugainvillea	*Baugainvillea spectobilis* L.	Nyctaginaceae	Leaves
2	Dhatura (black)	*Datura metel* L.	Solanaceae	Leaves
3	Gando baval	*Prosopis juliflora* L.	Mimosaceae	Leaves
4	Garlic	*Allium sativum* L.	Liliaceae	Bulbs
5	Ginger	*Zingiber officinalis* Roxb.	Zingiberaceae	Rhizomes
6	Karanj	*Pongamia glabra* L.	Papilionaceae	Leaves
7	Neem	*Azadirachta indica* Juss.	Meliaceae	Leaves
8	Nilgiri	*Eucalyptus citridora* Hook.	Myrtaceae	Leaves
9	Onion	*Allium cepa* L.	Liliaceae	Bulbs
10	Ratan jyot	*Jatropha curcas* L.	Euphorbiaceae	Leaves
11	Tulsi	*Ocimum sanctum* L.	Labiatae	Leaves

solidification of the medium by placing 5 mm diameter mycelial disc at the centre, cut aseptically with the cork borer from 5 days old pure culture of *F. solani*. Three replications of each treatment were maintained. The plate without phyto extract served as control. The petriplates were incubated at room temperature for 5 days.

The observation on radial mycelial growth was recorded by averaging two diameters of colony at right angles to one another and subtracting 5 mm of the mycelial discs. The amount of growth inhibition was calculated by using the formula given by Vincent (1927).

$$PGI = \frac{100\,(DC-DT)}{DC}$$

Where,

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony of control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

3.7.3.2 Testing of efficacy of botanicals against the tomato fruit rot

For determining the efficacy of botanicals against fruit rot disease, three superior botanicals *viz.*, gandobaval, tulsi and neem found *in vitro* trial were tested at 5 and 10 per cent concentrations. One litre of each phytoextract solution of both concentrations were prepared by adding 50 and 100 ml of

prepared phyto extract as explained earlier, into 950 and 900 ml of sterilized distilled water, respectively.

Healthy tomato fruits were selected, washed, surface sterilized, inoculated with test fungus by pin prick injury and incubated as mentioned in section 3.5 of this chapter for 24 hours. After incubation, the fruits were dipped in respective phyto extract solutions for 5 minutes and treated fruits were incubated again as mentioned in section 3.5 of this chapter. Tomato fruits dipped in distilled sterile water for same duration served as control. Treatments were replicated four times with each of five fruits. Observations were recorded at regular intervals on number of days taken for initiation of symptoms and per cent disease index (PDI) was calculated as mentioned in section 3.7.1 of this chapter.

3.7.3.3 Testing of efficacy of oils against the tomato fruit rot

To know the efficacy of oils in controlling tomato fruit rot caused by *F. solani*, pre inoculated and incubated tomato fruits as mentioned in section 3.7.2 of this chapter were dipped for 5 minutes in 75 per cent groundnut, castor, mustard, karanj, eucalyptus, neem and palm kernel oils emulsions made in 1 per cent soap solution. Treated fruits were incubated as mentioned as mentioned in section 3.5 of this chapter. Inoculated fruits dipped in sterile distilled water served as control.

Treatments were replicated thrice with each of five fruits and observations were recorded as mentioned in section 3.7.1 of this chapter.

3.7.3.4 Effect of antagonists on *F. solani in vitro*.

Nine known antagonists *viz*., *T. viride*, *T. longibrachiatum* Rifai, *T. harzianum*, *A. niger*, *A. flavus* Link Fries, *G. virens*, *C. globosum*, *B. subtilis* and *P. fluorescens* were tested *in vitro* for their antagonistic effect to *F. solani*. Three different methods were employed in the study are as under.

[A] Dual culture method (Dennis and Webster, 1971)

The Petri plates containing 20 ml Richards' agar medium were inoculated aseptically with the pathogen, *F. solani* and the test organism (antagonist) by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three repetitions of each treatment were kept and the petriplates with only pathogen at centre served as control. All the plates were incubated at room temperature. Observations on colony diameter were recorded up to the complete coverage of control plates with pathogen. The amount of growth inhibition was calculated by using the formula given by Vincent (1927) as mentioned in section 3.7.3.1 of this chapter.

[B] Pathogen at centre (Asalmol *et al.*, 1990)

Each petriplate containing 20 ml Richards' agar medium was inoculated aseptically in the centre by transferring a 5 mm diameter mycelial disc of the pathogen and four discs of the

test organism (antagonist) were placed 35 mm away radially from the centre in the same petriplate simultaneously. Each treatment was repeated three times. The plates inoculated in the centre with only pathogen served as control. The plates were incubated at room temperature. Observations on radial growth of the pathogen and the test organism were recorded after the complete coverage of plates with fungal growth in plate, which was kept at ambient temperature. The per cent growth inhibition (PGI) was worked out by using the formula given by Vincent (1927) as mentioned earlier in section 3.7.3.1 of this chapter.

[C] Pathogen at periphery (Asalmol *et al.*, 1990)

Mycelial discs of 5 mm diameter of the pathogen and the test organism (antagonist) were cut uniformly with the help of a cork borer and inoculated aseptically by placing these blocks in petriplates containing 20 ml Richards' agar medium keeping four mycelial discs of the pathogen 35 mm away radially from the centre of the petriplates and one culture disc of the test organism was placed in the centre of the same petriplates simultaneously. Three repetitions of each treatment were kept and the petriplates with only pathogen at centre served as control. Inoculated plates were incubated at room temperature and radial growth of the test organism and pathogen was recorded after the complete coverage of the plates with fungal growth in control plate inoculated only with the pathogen. The per cent growth inhibition (PGI) was worked out by using the formula given by Vincent (1927) as mentioned in section 3.7.3.1 of this chapter.

3.7.3.5 Efficacy of antagonists against tomato fruit rot

Three superior antagonists *viz.*, *T. viride*, *B. subtilis* and *T. longibrachiatum* found *in vitro* test were selected for this study. Tomato fruits were inoculated with the test pathogen as mentioned earlier in section 3.7.2 and then dipped in antagonist suspension at two different concentrations *viz.*, 10^6 and 10^8 cfu/ml for 5 minutes.

Each treatment was replicated four times with each of three fruits. Separate sets of the fruits which were dipped only in sterile distilled water at room temperature for 5 minutes served as control. Treated fruits were incubated on a clean surface sterilized laboratory table under separate transparent bell jars at room temperature. Observations were recorded on number of days taken for initiation of symptoms and per cent disease index was worked out as mentioned in section 3.7.1 of this chapter.

3.7.4 Chemical control

3.7.4.1 *In vitro* screening of different fungicides against *F. solani*

Eleven fungicides belonging to different chemical groups at three different concentrations as listed in Table-3.2 were tested for their efficacy *in vitro* against *F. solani* using poisoned food technique.

The required quantities of each test fungicides were incorporated in a conical flask containing 100 ml molten Richards' agar medium so as to get required concentration in

Table 3.2: Details of fungicides used against *F. solani in vitro*

Sr. No	Technical name	Trade name	Chemical name	Concentrations (ppm)			Source (Manufacturer)
				1st	2nd	3rd	
1	Thiram	TMTD 75 WP	Bis (dimethylthiocarbamoyl) disulhide	1000	1500	2000	Cement Chemicals Ltd., (India) Ahmedabad
2	Captan	Captaf 50 WP	N-trichloromethylthio 4-cyclohexane-1, 2- dicarboximide	1000	1500	2000	Rallis India Pvt.Ltd. Mumbai
3	Mancozeb	Dithane M-45 75 WP	Zinc ion and manganese ethylene bisdithiocarbamate	1000	1500	2000	Indofil Chemicals Ltd., Mumbai
4	Copper oxychloride	Blitox 50 WP	Basic cupric chloride	1000	1500	2000	Bharat Rasayan Ltd., New Delhi
5	MEMC	Emisan 6 WP	Methoxy ethyl mercuric chloride	1000	1500	2000	Excel industries Ltd., Mumbai
6	Copper Hydroxide	Kocide101 75 WP	Cupric hydroxide	1000	1500	2000	Griffin corporation.Yeldosta (USA)
7	Carbendazim	Bavistin 50 W.P.	2(Methoxy carbamyl)-benzimadazole	250	500	1000	BASF India Ltd., Bombay
8	Propiconazole	Tilt 25 EC	1-[2-(2, 4-dichlorophenyl)-4- propyl-1, 3-dioxolan-2, 1,methyl] 1, 2, 4-triazole	250	500	1000	Novartis India Ltd., Mumbai
9	Thiophanate-methyl	Topsin-M 70 WP	1,2 bis (3- Methloxy carbamyl 2-thioruie dobenzene	250	500	1000	Motilal Pesticides (India) Ltd., New Delhi.
10	Carboxin (37.5%) + Thiram (37.5%)	Cosko 75 DS	5,6 dihydro-2-methyl-1,4-oxathiin-3-carboxanilide and bis (dimethylthiocarbomoyl)disulphide	250	500	1000	P.I. Industries Ltd. Pesticides (India) Udaipur
11	Flusilazole	Nustar 40 EC	1-[Bis (4-fluorophenyl) (methylisilyl methyl)]-1H-1,2,4-triazole	250	500	1000	Du Pont (India) Pvt. Ltd., Haryana

parts per million (ppm). The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized petriplate. On solidification of the medium, the plates were inoculated in the centre by placing 5 mm diameter mycelial culture block cut aseptically with the help of a cork borer from 5 days old actively growing pure culture of *F. solani* grown on Richards' agar medium. Three repetitions were kept for each concentration of the respective fungicide. The inoculated plates were incubated at room temperature. The observations on linear growth of fungus were recorded at 24 hours. interval till the entire plate in control was completely covered with mycelium. The per cent growth inhibition (PGI) of the pathogen over control was worked out as mentioned in section 3.7.1 of this chapter.

3.7.4.2 Evaluation of different fungicides against the fruit rot in laboratory

Considering the importance of disease and variation in recommendations of different fungicides for the control of the fruit rot disease, a laboratory experiment was conducted with six chemicals, *viz*., carbendazim, flusilazole, propiconazole, copper hydroxide, mancozeb and thiram which were found effective under *in vitro* screening.

Pre inoculated and incubated tomato fruits for 24 hours as mentioned in section 3.7.2 were dipped in fungicidal solutions of respective concentration for 5 minutes. The fruits

then, were air dried and incubated at room temperature as mentioned in section 3.5 of this chapter.

Each treatment was replicated four times with each of five fruits. Separate set of inoculated fruits which were dipped only in sterile distilled water for 5 minutes served as control. Treated fruits were incubated as mentioned earlier. The observations were recorded on number of days taken for initiation of the symptom and per cent disease index was worked out as mentioned in section 3.7.1 of this chapter.

3.7.5 Evaluation of physical, chemical and biological treatments for the management of fruit rot of tomato

Superior treatments from previous trials which found effective in controlling tomato fruit rot were selected and re-evaluated.

Five treatments *viz*., fruit dip in hot water (52°C), leaf extract of gandobaval (10%), cell suspension of *B. subtilis* (10^8 cfu/ml), palm kernel oil emulsion (75%) and bavistin suspension (500 ppm) for 5 minutes were tested at both pre and post inoculation conditions, where fruits were inoculated with the test fungus 12 hours before and after giving the treatments, respectively. Each treatment was replicated three times with each of five fruits. Separate sets of inoculated fruits which were dipped only in sterile distilled water for 5 minutes served as control. Treated fruits were incubated as mentioned in section

5. The observations were recorded on number of days taken for
itiation of the symptom and per cent disease index was worked
t as mentioned under 3.7.1 of this chapter.

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

Tomato (*Lycopersicon esculentum* Mill.) is extensively grown vegetable fruit crop in south Gujarat. Post harvest diseases are the major constraints in transit, storage and market conditions as they inflict heavy losses. In tomato, post harvest diseases are of complex nature jointly caused by various pathogens. Among the various post harvest diseases infecting tomatoes, fruit rot caused by *Fusarium solani* (Mart.) Sacc. has become a severe problem in recent years with a severe threat to successful handling, storage and marketing of tomato fruits in south Gujarat. The popular variety Pusa Ruby was found susceptible inflicting severe losses. The fruit rot was observed throughout the year in the market but found more during *kharif* season. Considering the severeness of the problem and lack of systematic information on this disease, the present investigation was carried out on various aspects to generate scientific information and also to find out suitable management strategies for preventing the losses.

4.1 Pathological investigation

4.1.1 Collection of specimen and isolation of the pathogen

The samples of infected tomato fruits cv. Pusa Ruby were collected showing typical fruit rot symptoms from Dudhia Talav vegetable market, Navsari and brought to the laboratory. The symptoms and signs were observed visually and the presence of the pathogen was confirmed by microscopic observation. Isolation was done by tissue isolation technique on potato

dextrose agar (PDA) medium which yielded the pure culture of *Fusarium* sp. This was further purified by the single hyphal tip isolation technique and was maintained on PDA for further investigations.

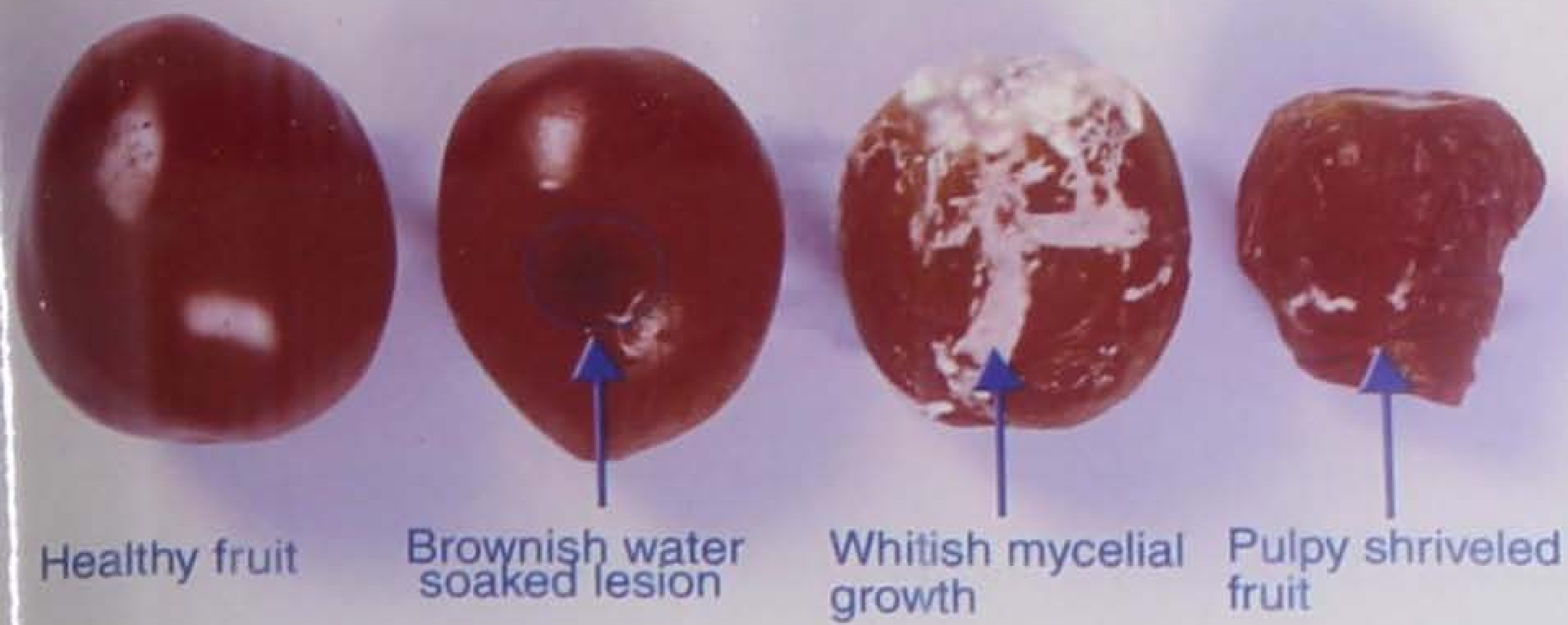
4.1.2 Symptoms

Under natural market conditions, the symptoms and signs observed on the infected fruits during the survey were as under.

The symptoms initiated as small circular to irregular, brownish water soaked lesions on the fruit surface. The lesions gradually spreaded into circular to irregular large necrotic area. In severe infection, tissue got macerated and a fluffy white cottony growth of fungal mycelium was observed over the infected area on fruit surface (Plate-I, a). Finally, fruit became pulpy, fruit juice got drained from the infected fruits and emitted an unpleasant odour (Plate-I, b). The symptoms studied here is in accordance with Garg and Gupta (1979).

4.2 Identification and morphology of the pathogen

After purification of the fungus as described under materials and methods, morphological and cultural characters of the fungus grown on potato dextrose agar were studied for the purpose of identification and taxonomy. It was also compared with those described in the literature. The pure culture was also sent for identification and confirmation to Indian Type Culture Collection (I.T.C.C.), Division of plant pathology, IARI., New



(b)

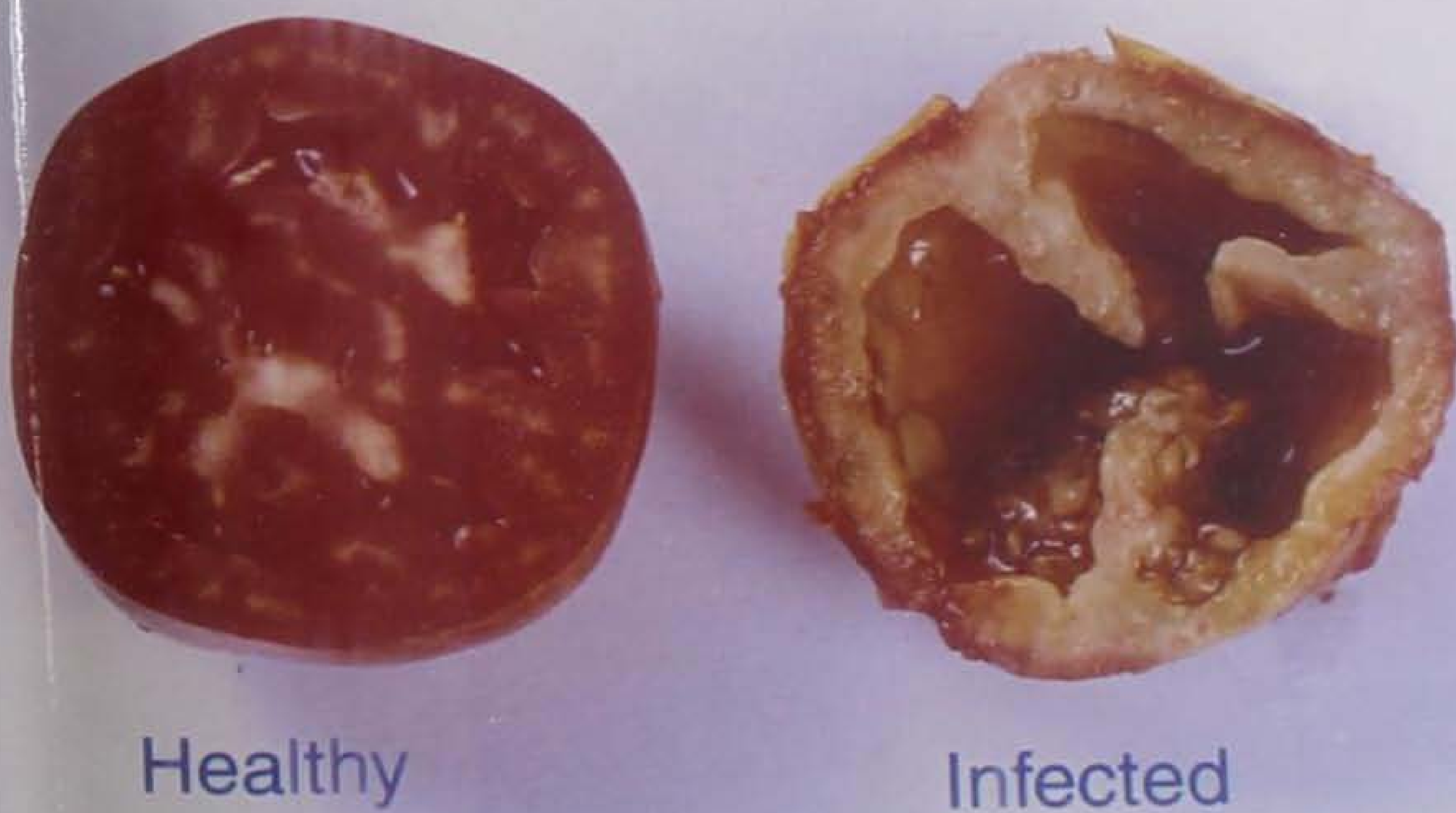


Plate II : (a) Symptoms of fruit rot of tomato &
(b) Transverse section of infected fruit

Delhi 110012 and was identified as *Fusarium solani* (Mart.) Sacc. (I.T.C.C. No. 6123-05). This confirmed that the fruit rot infection of tomato in south Gujarat vegetable markets is caused by *Fusarium solani*.

4.2.1 Cultural characters

The colonies of *F. solani* grew fast on potato dextrose agar (PDA) and attained diameter of 90 mm within 7 days at room temperature. Initially colony was flat with sparse mycelial growth, but later it showed profuse growth with fluffy, cottony to dull white mycelium (Plate-II).

4.2.2 Morphological characters

The microscopic observation of the test fungus revealed that the hyphae was septate and hyaline (Plate-III, a) measuring 2.53 to 3.87 µm in thickness. The mycelium was branched producing round to almost round colony and vinacious pigmentation on PDA.

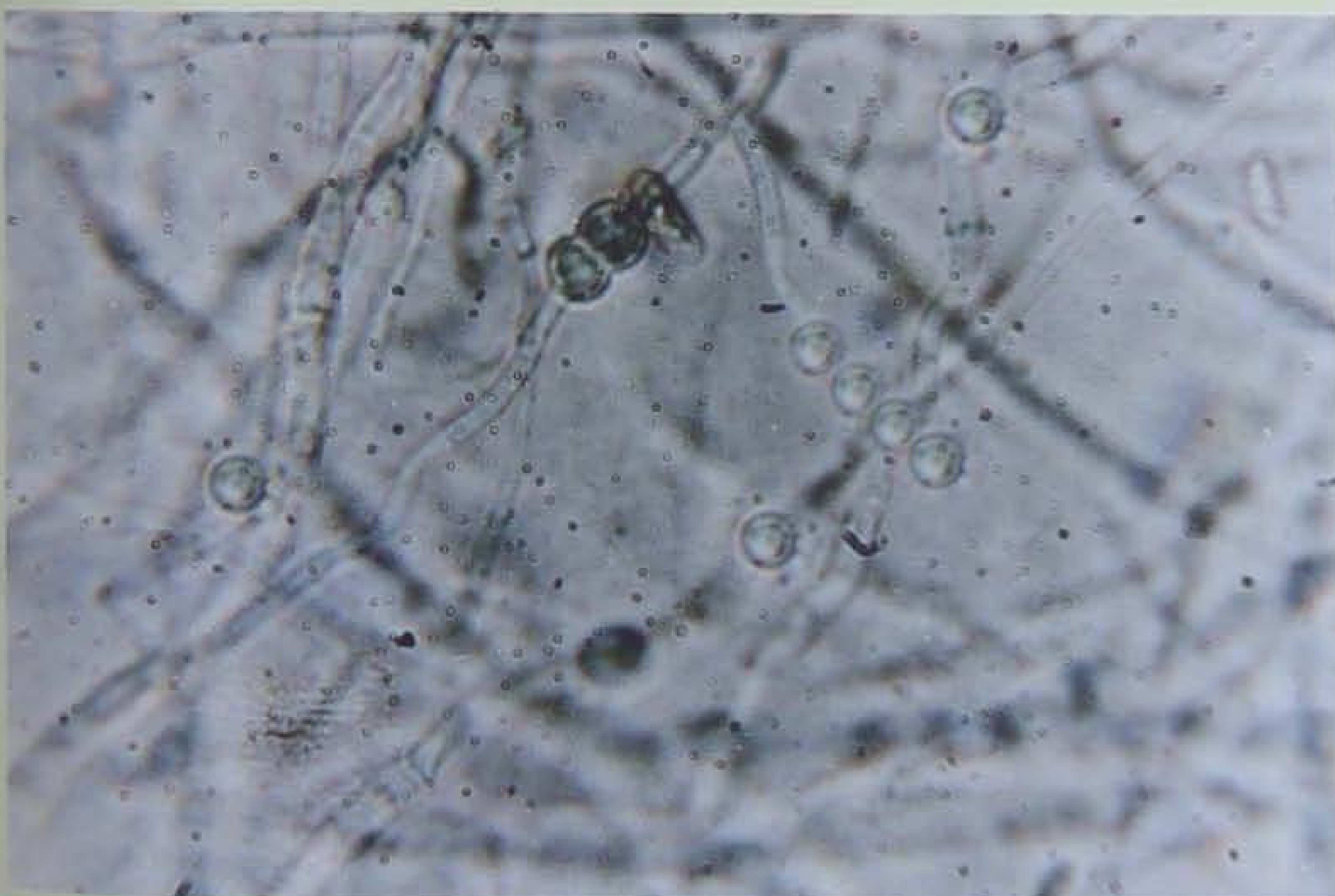
Microconidia were found hyaline, elliptical, unicellular without septation, measuring 11.3-15.56 x 2.97-3.88 µm. Macroconidia formed were varied from fusiform to nearly straight with nearly pointed ends, hyaline, measuring 24.44-42.76 x 4.21-5.38 µm, and had 1 to 4 septa (Plate-III, b). Mycelium also exhibited abundant chlamydospores in aged culture. Chlamydospores were spherical to globose, rough walled, light brown, terminal and intercalary, ranged from 8.97 to 11.78 µm in

Plate-II : Pure culture of *F. solani*

Plat[e]-III : Microphotographs of *F. solani* (High power magnification 450 x)

: Thin branched and septate mycelium

(b) : Hyaline, micro and macro conidia



(C) : Globose rough walled chlamydospores produced intercalary and chain in aged culture

diameter and were produced singly or in chains (Plate-III, c). The results are in accordance with Pandav (2002).

4.3 Pathogenicity test

To fulfill Koch's postulate, the pathogenic nature of the fungus (*Fusarium* sp.) isolated from diseased fruits was established by employing three different inoculation techniques *viz.*, making injury by cork borer (Granger and Horne's (1924) method), pin-prick and knife. The results pertaining to these aspects are presented in Table-4.1. From the table, it is clearly revealed that cent per cent infection was observed in all the methods of inoculation. Dipping of uninjured fruits only in distilled sterilized water (control) produced no diseased symptoms on tomato fruits (Plate-IV).

Typical symptoms became evident after 24 hours of inoculation. Initially small, brownish, water soaked lesions appeared on the surface of inoculated fruits. Later, white cottony mycelial growth at inoculated site was produced and gradually covered entire fruit within a week. Infected tissue got macerated finally, fruit became pulpy and emitted an unpleasant odour. Typical symptoms of the disease developed were similar to those described by Garg and Gupta (1979). Re-isolation from artificially inoculated diseased fruits yielded *Fusarium* sp. which was identical with original one.

Thus, the pathogenicity was successfully proved by all the inoculation techniques employed. The present results are in conformity with the findings of Garg and Gupta (1979) who

Table-4.1: **Pathogenicity of *Fusarium* sp. on tomato fruits under laboratory conditions**

Sr. No.	Inoculation Technique	No. of fruits		Per cent diseased fruits
		Inoculated	Infected	
1	Knife injury	4	4	100
2	Granger and Horne's (1924) method	4	4	100
3	Pin prick injury	4	4	100
4	Control i.e. no injury (dipping of fruits in sterilized water)	4	0	00

proved the pathogenicity of *F. solani* successfully on tomato fruits by making knife injury. Rawal and Thakore (2003) noticed highest infection of sponge gourd (*Luffa cylindrica* (L.) Roem.) when the test fungus (*F. solani*) was inoculated through cork borer injuries.

4.4 Survey and loss assessment

To find out the loss caused by the pathogen (*F. solani*) market survey was conducted in three different seasons *viz.*, *kharif* and *rabi* seasons of 2005 and summer season of 2006 in vegetable markets at Navsari and neighbouring cities *viz.*, Billimora and Surat. Data were collected from five vegetable shops in each market and averaged out the per cent incidence obtained and are presented in Table-4.2, Fig.-1 and Plate-V.

From the table, it is clearly revealed that the disease was recorded from all the markets surveyed throughout the year. Maximum Per cent Disease Incidence (PDI) was observed in the market of Surat (15.25%) followed by Navsari (12.26 to 14.31%) and Billimora (11.18 to 12.52%) during *kharif* season of 2005. Among the three different seasons, maximum fruit rot (Av. 13.05%) was recorded during *kharif* 2005 and minimum (Av. 2.06%) during *rabi* 2005. However, it was moderate (Av. 5.04%) during summer season of 2006. Majority of tomato fruits came from Nasik and Sangam Districts of Maharashtra. Tomato variety Namdhari was observed to be more affected as compared to Pusa Ruby, Abhinav and Avinash. While Local (Desi) varieties found relatively less damaged to the disease.

Table – 4.2 : Market survey

Sr. No	Name of the vegetable market	City	Average per cent disease incidence		
			Kharif 2005	*Rabi* 2005	Summer 2006
1	Dudhia talav market	Navsari	12.26	1.96	3.35
2	Morarji Desai market yard	Navsari	14.31	0.88	4.01
3	Vegetable shops and vendors	Navsari	12.83	0.50	3.12
4	Kala market	Billimora	11.18	3.06	6.88
5	Sardar market	Billimora	12.52	3.48	7.31
6	Sardar market	Surat	15.25	2.50	5.58
	Mean value		13.05	2.06	5.04

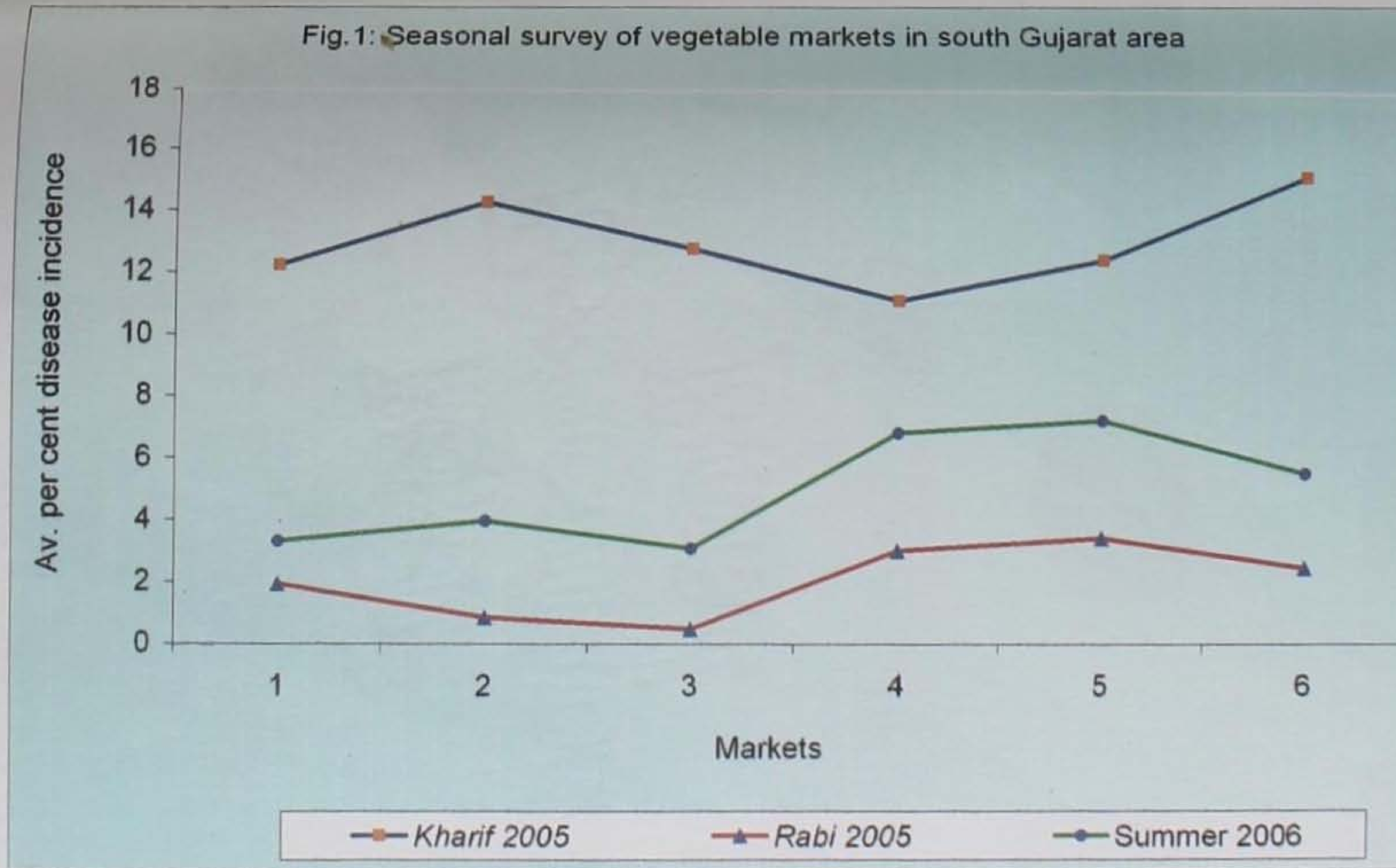
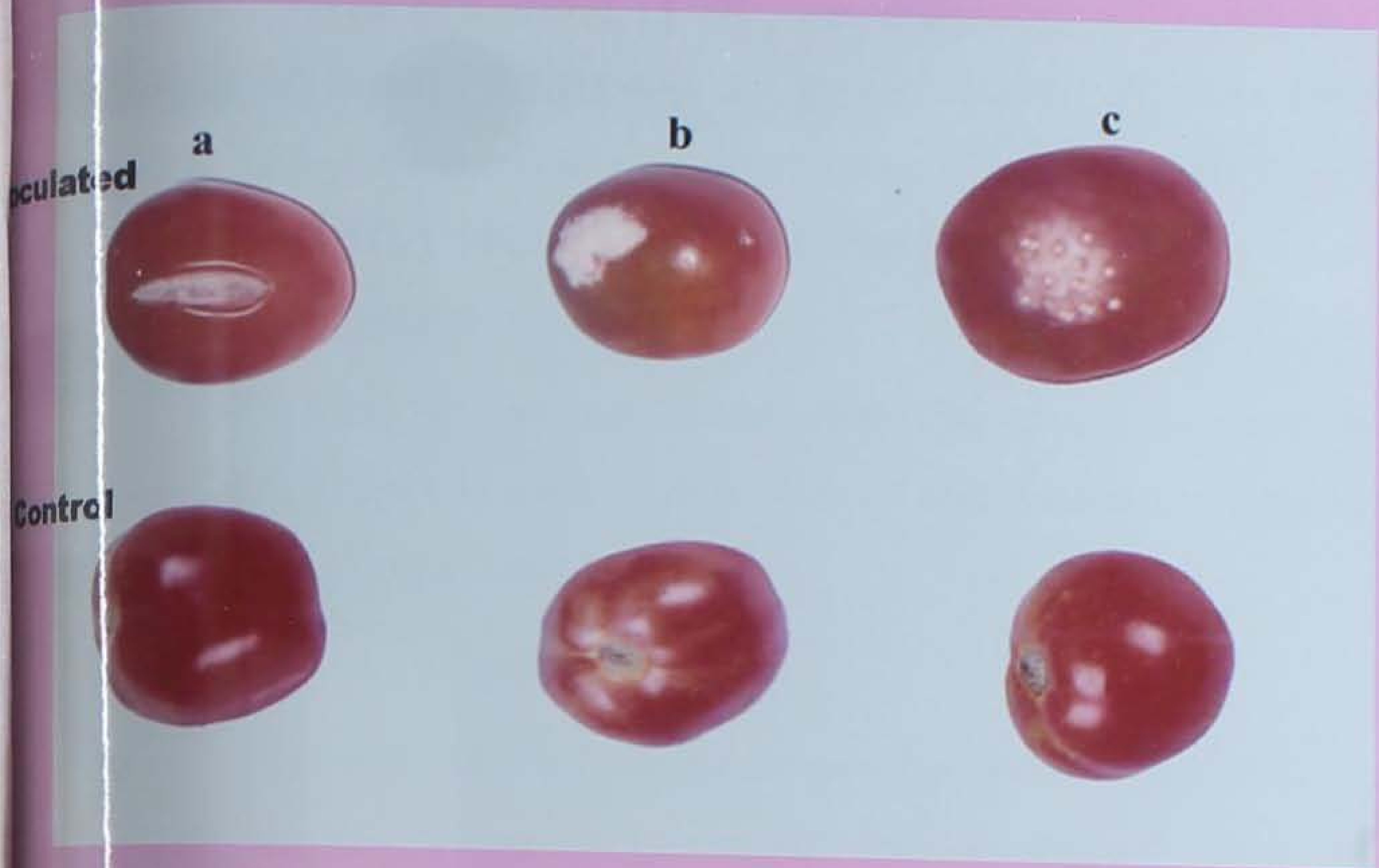


Fig. 1: Seasonal survey of vegetable markets in south Gujarat area

1	Dudhia talav market	(Navsari)	4 Kala market	(Billimora)
2	Morarji Desai market yard	(Navsari)	5 Sardar market	(Billimora)
3	Vegetable shops and vendors	(Navsari)	6 Sardar market	(Surat)

Plate-IV : Pathogenicity of Fusarium sp., showing symptoms of fruti rot of toamto



(a) Injury by knife (b) Injury by cork borer (c) Injury by pin prick

Plate-V : Market view of fruit loss

Patel and Patel (1991) recorded 0.8, 0.3 and 0.2 per cent loss of tomato fruits due to *F. parasitica*, *F. moniliformae* and *F. nivale*, respectively in Ahmednagar markets. Earlier, Garg and Gupta (1979) also reported severe post harvest fruit rot of tomato (*F. solani*) from Agra.

Thus, it is concluded that the post harvest rot of tomato (*F. solani*) is distributed widely and found throughout the year causing greater loss.

4.5 Mode of entry

Inoculation of the pathogen on tomato fruits was done through various avenues to detect the mode of entry of the pathogen and the data recorded are presented in the Table-4.3 and depicted in Plate-VI, a & b.

It is evident from the above table that there was no infection when the pathogen was inoculated through stem end of the fruit with and without calyx and also when inoculated through other region of the fruit. In all the above three cases, artificial injury was not provided (Plate-VI a, 1, 2 & 3). Severe infection was observed when the tomato fruits were inoculated with the test fungus through natural insect bored holes and knife injury provided artificially at lateral position of the tomato fruits (Plate-IV b, 2 & 3). However mild and moderate infection was observed when the fruits were artificially inoculated after making pinprick (Plate-IV a, 4) and pressed injuries (Plate-IV b, 1), respectively. It is evident from this result that, the fungus needed

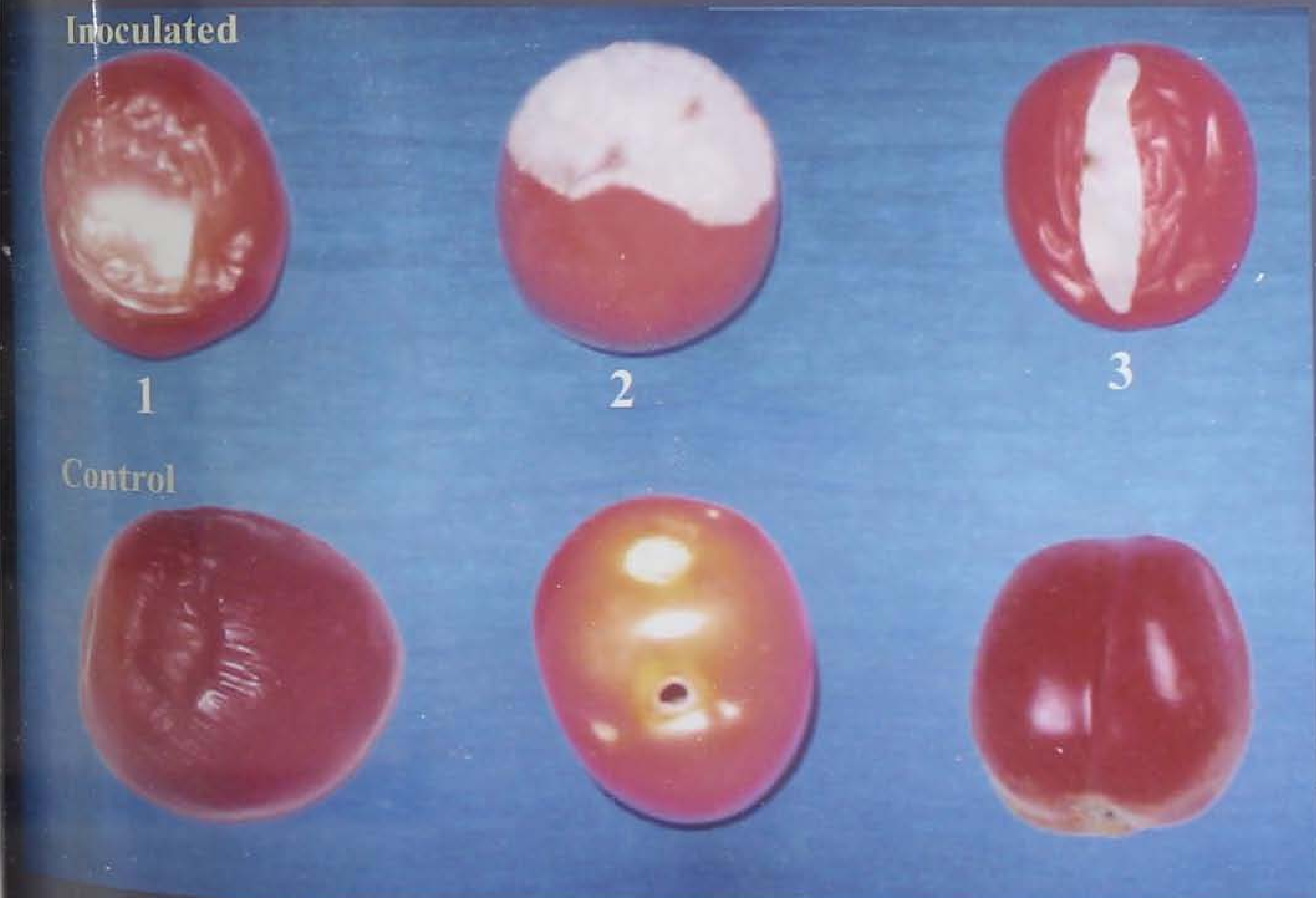
Table- 4. 3 : **Mode of entry of *F. solani* into tomato fruits.**

Sl. No.	Avenues for entry	Natural (N) / Artificial (A)	Reaction
1	Stem end region with calyx	N	-
2	Stem end region without calyx	N	-
3	Uninjured area except at stem end region	N	-
4	Pressed injury	A	++
5	Pin injury	A	+
6	Insect bored holes	N	++++
7	Lateral injury with knife	A	++++

"-" Indicates no infection
"+" Indicates mild infection
"++" Indicates moderate infection
"++++" Indicates severe infection

Plate-VI : Mode of entry of *F. solani* into tomato fruits

(a) : Inoculation through
1. Stem end with calyx.
2. Stem end without calyx
3. Uninjured fruit except at stem end
4. Insect bored holes



(b) : Inoculation through
1. Pressed injury
2. Pin injury
3. Lateral injury with knife

wounds either natural or artificial to get entry into the tomato fruits and to cause infection.

Above results are also in confirmatory with the findings of Tandon and Mishra (1969) who have noticed no infection when *R. stolonifer* was inoculated through general surface area and stalk region of mature and young banana fruits. Thakur and Yadav (1971) recognized *F. nivale* as a wound parasite, since the fungus was able to initiate symptoms on knife injured tomato fruits but not on fruits without injury. Vir *et al.* (1967) suspected transportation injuries as the main mode of entry of *A. solani* on tomato fruits.

From these results it is very clear that, the fruit rot (*F. solani*) is occurring only when there are injuries. It is suggested that, the injuries may be avoided by controlling insects, proper harvesting, transporting, handling and storage.

4.6 Search for superior media for the growth and sporulation of *F. solani*

4.6.1 Solid media

Eight different synthetic and semi-synthetic media were tested for their suitability to the growth and sporulation of the pathogen. The data pertaining to media test are presented in Table-4.4 and depicted in Fig.2 and Plate VII.

It is evident from the results that, among the eight solid media tested, significantly higher mycelial growth of *F. solani* was recorded on Richards' agar (90.00 mm) as compared to

1	Potato dextrose agar	9.12* (82.83)**	Dense growth, mycelium cottony to dull white, round colony, no zonations, moderate sporulation,
2	Wheat meal agar	8.52 (72.16)	Dense growth with dull white projected aerial mycelium, round colony, no zonations, abundant sporulation, macro conidia more than micro conidia with 1-4 septation
3	Richards' agar	9.51 (90.00)	Fluffy cottony growth, mycelium pure white, all most round colony without zonations, abundant sporulation
4	Czapek's (Dox) agar	8.15 (66.00)	Fluffy growth, white mycelium, slightly variegated colony boundary, no zonations, moderate sporulation
5	Elliot's agar	7.53 (56.33)	Fluffy growth, dull white mycelium, variegated colony boundary, no zonations, moderate sporulation
6	Asthana and Hawker's agar	8.04 (64.16)	Flat growth, sparse, dull white mycelium, all most round colony without zonations, moderate sporulation
7	Brown's agar	6.76 (45.33)	Dense growth, pure white round colony, no zonations and less sporulation
8	Tomato fruit extract agar	9.18 (83.83)	Dense growth, dull white and round colony without zonations, abundant sporulation
	S. Em. ±	0.11	-
	C.D. at 5%	0.32	-
	C.V. %	2.22	-

* Figures indicate SQR + 0.5 transformed values

** Figures in parentheses indicate retransformed values

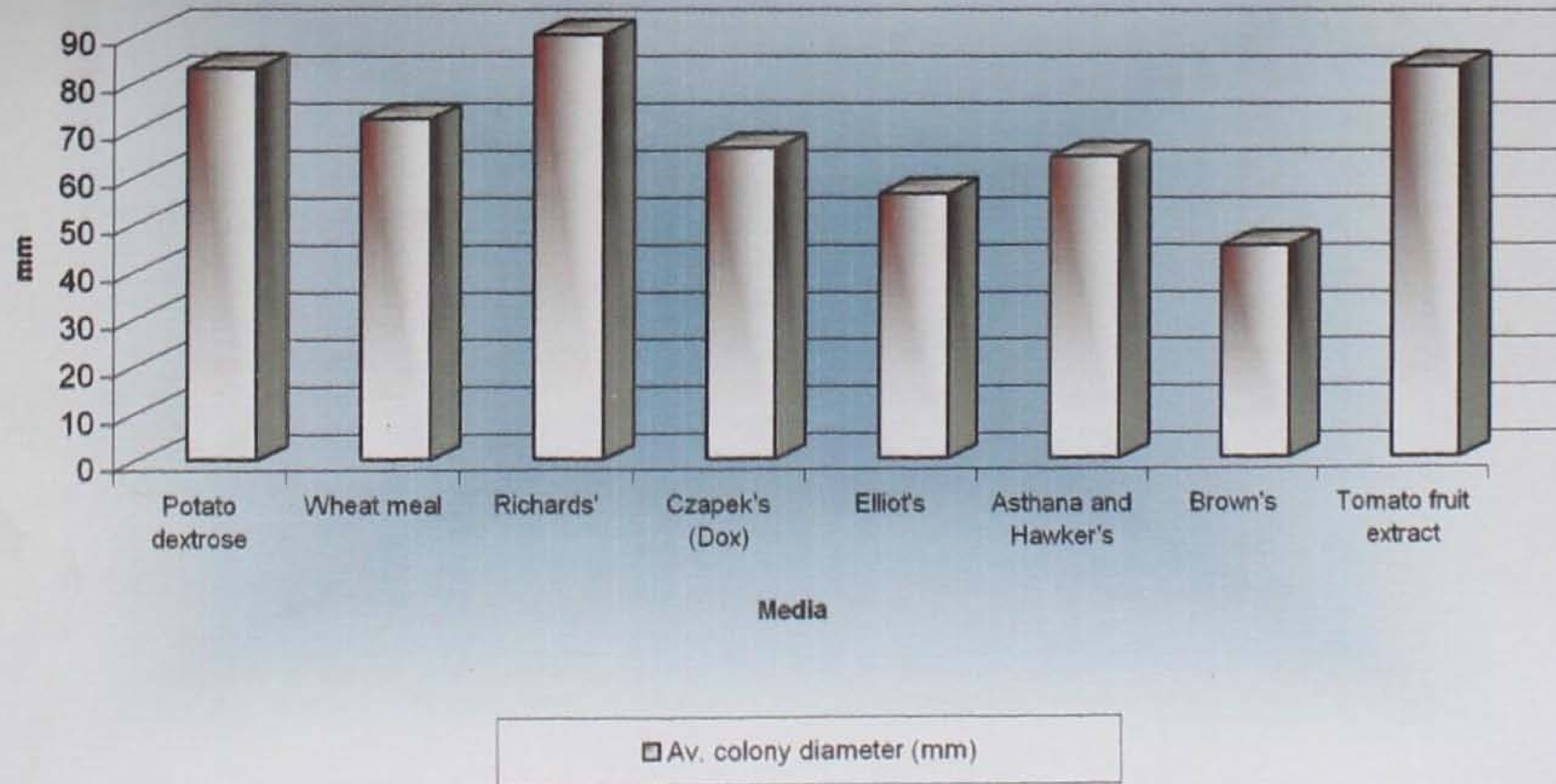


Fig.-2 : Effect of different solid media on growth of F. solani in vitro
90
80
70
60
50
40
30
20
10
0
mm
Potato dextrose
Wheat meal
Richards'
Czapek's (Dox)
Elliot's
Asthana and Hawker's
Brown's
Tomato fruit extract
Media
Av. colony diameter (mm)

Plate-VII : Growth of *F. solani* on different solid media

1. Richards' agar
2. Tomato fruit extract agar
3. Potato dextrose agar
4. Wheat meal agar
5. Czapek's (Dox) agar
6. Asthana and Hawker's agar
7. Elliot's agar
8. Brown's agar

the rest (Plate-VII, 1). Next best in order of merit was tomato fruit extract agar (83.83 mm) which was statistically at par with potato dextrose agar (82.83 mm). The rest of the media *viz.*, wheat meal, czapek's Dox, Asthana and Hawker's and Elliot's agar media were moderate in supporting the growth of the pathogen.

Among the semi-synthetic media, tomato fruit extract agar and potato dextrose agar and among the synthetic media, Richards' agar medium supported better growth of the pathogen.

Richards' agar (Gaur and Agnihotri, 1980) and potato dextrose agar (Garg and Gupta, 1979; Chauhan, 1997 and Akhtar *et al.*, 1999) have also been reported as better media for the growth and sporulation of *F. solani*. Rawal and Thakore (2003) also noticed better growth of *F. solani* on PDA medium. The result of present investigation is in the line of these.

4.6.2 Broth media

The results pertaining to liquid media are presented in Table-4.5 and depicted in Fig.3. The results revealed that, significantly higher dry mycelial weight was recorded in wheat meal broth (753.48 mg) as compared to the rest of the liquid media. The next best medium in order of merit was Richards' solution (512.79 mg) followed by Brown's solution (346.36 mg), tomato fruit extract broth (233.03 mg), potato dextrose broth (208.00 mg) and Asthana and Hawker's broth (132.54 mg).

Czapex (Dox) broth (115.96 mg) and Elliot's broth (111.99 mg) were poorer in supporting the growth.

The conidial formation was of high level in wheat meal broth (536.66 spores/low power microfield) which was at par with Richards' solution (515.72 spores/LPM). Next best in order of merit was tomato fruit extract broth (416.66 spores/LPM) and potato dextrose broth (334 spores/LPM) but were statistically differing from each other. Brown's solution (214.66 spores/LPM) and Czapeck's (Dox) broth (210 spores/LPM) were also supported sporulation to some extent and at par from each other. Elliot's broth (83 spores/LPM) followed by Asthana and Hawker's broth (75.33 spores/LPM) yielded less sporulation.

Looking to the dry mycelial weight and sporulation, wheat meal and tomato fruit extract broths, among semi-synthetic media while Richards' and Brown's solutions among the synthetic media proved better for *F. solani*.

Garg and Gupta (1979) recorded host extract media as the best media next to potato dextrose media for better growth of *F. solani* causing tomato fruit rot, which is also confirmatory with the above findings. Chauhan (1997) also recognized Richards' solution as the best medium for growth and sporulation of *F. solani*. Pandav (2002) recorded better growth of *F. solani* in wheat meal broth, Richards' solution and potato dextrose broth media.

Table-4.5: **Growth and sporulation of *F. solani* in liquid media**

Sr. No.	Name of media	Av. Dry mycelial weight (mg)	Av. No. of conidia / Low power microscopic field (10X)
1	Potato dextrose broth	2.31* (208.00)**	2.52* (334)**
2	Wheat meal broth	2.82 (753.48)	2.72 (536.66)
3	Richards' broth	2.70 (512.79)	2.71 (515.72)
4	Czapek's (Dox) broth	2.06 (115.96)	2.32 (210.00)
5	Elliot's broth	2.04 (111.99)	1.91 (83.00)
6	Asthana and Hawker's broth	2.12 (132.54)	1.87 (75.33)
7	Brown's broth	2.53 (346.36)	2.33 (214.66)
8	Tomato fruit extract broth	2.36 (233.03)	2.61 (416.66)
	S. Em. ±	0.02	0.01
	C.D. at 5%	0.05	0.03
	C.V. %	1.18	0.82

* Figures indicate logarithmic transformed values

** Figures in parentheses indicate retransformed values

4.7 Management measures

4.7.1 Varietal screening in laboratory condition

Use of resistant varieties is an ideal, simplest and cheapest method for the control of plant disease. Moreover, it does not disturb natural eco-system and avoid hazards of environmental pollution. The identification of the source of resistance is a basic need in breeding for disease resistance. Considering these facts, ten tomato varieties were screened under laboratory condition at PG research laboratory, N. M. College of Agriculture, NAU, Navsari. The observations on fruit rot infection were recorded. These varieties were grouped under different degrees of resistance on the basis of 0-6 scale (Plate-VIII). The per cent disease index and reaction of different varieties are presented in Table-4.6 and depicted in Fig-4 and Plate-IX.

All the varieties tested, showed susceptible to highly susceptible reaction. Among them, minimum infection was recorded in PKM-1 (41.11%) followed by Junagadh Ruby (46.66%). The rest of the varieties had higher infection (54.44 to 93.33%).

The fruit rotting was initiated within two days in all the varieties screened. Among them, the earliest infection was observed in Pusa Ruby (0.5 DAI) followed by GT-1, Pusa Early Dwarf, DT-11, NS-2535 (1 DAI) while in rest of the varieties infection started slightly later (1.20 to 1.93 DAI).

able-4.6: Reaction of different tomato varieties to *F. solani* infection under laboratory conditions

Sr. No.	Variety	Per cent disease index	Reaction	First symptoms developed (DAI)
1	GT-1	69.99	HS	1.00
2	GT-2	57.77	HS	1.26
3	Pusa Ruby	93.33	HS	0.50
4	Pusa Early Dwarf	76.66	HS	1.00
5	DT-11	61.11	HS	1.00
6	S-22	54.44	HS	1.67
7	PKM-1	41.11	S	1.93
8	NS-2535	77.77	HS	1.00
9	Durgesh Amar	61.11	HS	1.20
10	Junagadh Ruby	46.66	S	1.73

DAI Days after inoculation

PDI		Reaction
Nil	=	Free (F)
0.1 to 10.0	=	Resistant (R)
10.1 to 25.0	=	Moderately resistant (MR)
25.1 to 40.0	=	Moderately susceptible (MS)
40.1 to 50.0	=	Susceptible (S)
>50	=	Highly susceptible (HS)

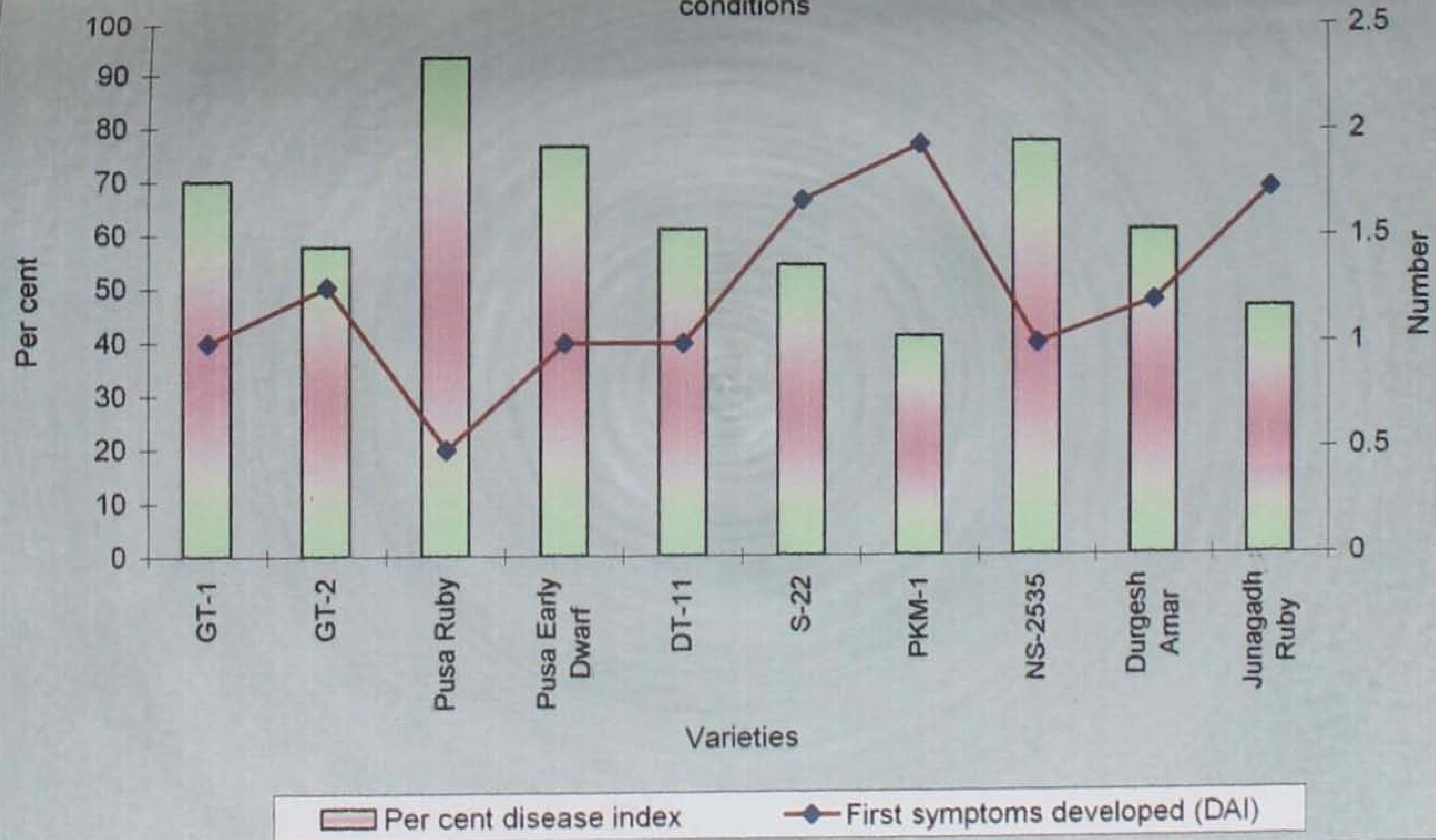


Fig.-4 : Reaction of different tomato varieties to *F. solani* infection under laboratory conditions

Plate-VIII : Standardized 0-6 rating scale of the fruit rot used in this study

Scale	Per cent fruit area infection
0 =	No visible symptoms
1 =	1 to 5 per cent fruit area infected
2 =	6 to 10 per cent fruit area infected
3 =	11 to 25 per cent fruit area infected
4 =	26 to 50 per cent fruit area infected
5 =	51 to 75per cent fruit area infected
6 =	76 to 100 per cent fruit area infected

Plate-IX : Varietal screening

1. PKM-1
2. Junagadh Ruby
3. S-22
4. GT-2
5. DT-11
6. Durgesh Amar
7. GT-1
8. Pusa Early Dwarf
9. NS-2535
10. Pusa Ruby

From above findings, none of the varieties found resistant or moderately resistant against the disease. However, less per cent disease incidence was recorded in PKM-1 and also comparatively delayed the symptoms.

Cent per cent infection on tomato fruits of commercial variety Pusa Ruby was observed by Thakur and Yadav (1971) when inoculated with the test fungus *F. nivale*. The same variety was also found highly susceptible in above findings.

The varietal screening done here is the first report and is very useful information. It is suggested to discourage varieties incurring huge losses.

4.7.2 Physical control: Effect of hot water dip treatment for the control of fruit rot of tomato

The control of diseases of fruits and vegetables by post harvest treatment in most other parts of the world always involved physical and chemical treatments. But the problems of fungicidal toxicity and residue on such treated and related produce are of common occurrence. Considering these facts, six different temperatures were tested as hot water dip treatment against the disease and results are presented in Table-4.7 and depicted in Fig.5 and Plate-X.

The results of investigation revealed that, all the treatments proved significantly superior in controlling the disease as compared to control. Among them, hot water dip treatment (HWT) at 52°C for five minutes recorded significantly

Table – 4.7: Effect of hot water dip treatment on fruit rot of tomato

Sr. No.	Temperature (°C)	Per cent disease index	PDC over check	First symptoms developed (DAI)
1	40	46.89* (53.34)**	44.81	0.85
2	45	33.17 (29.99)	68.97	1.22
3	48	32.13 (28.33)	70.69	1.65
4	50	25.31 (18.33)	81.03	1.77
5	52	19.91 (11.67)	87.92	2.17
6	Control	89.44 (96.66)	-	0.5
	S.Em ±	0.70		0.04
	C.D. 5%	2.08	-	0.11
	C.V. %	3.41		5.54

* Figures indicate arc sine transformed values

** Figures in the parentheses indicate retransformed values

DAI Days after inoculation

PDC Per cent disease control

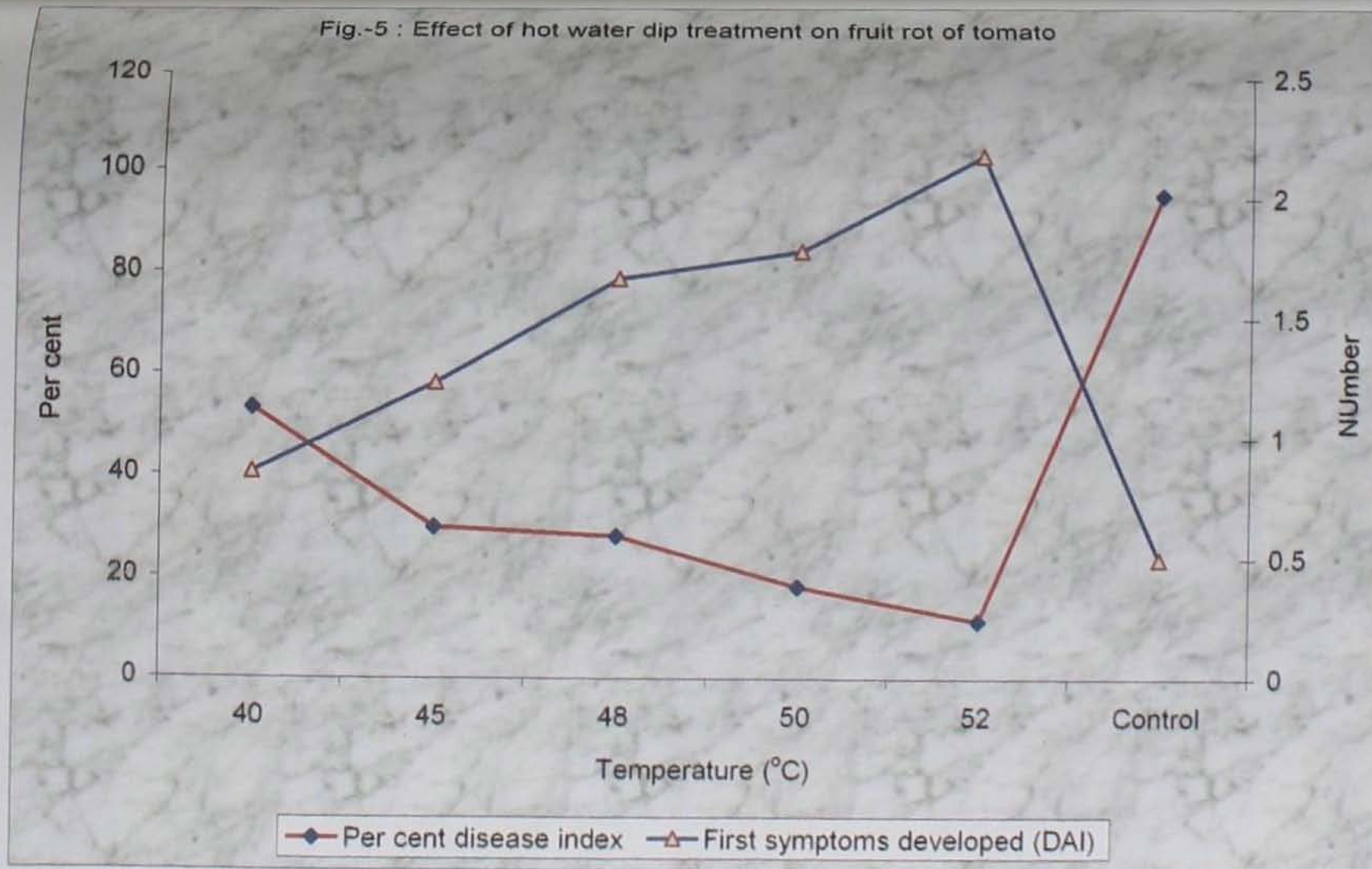


Fig.-5 : Effect of hot water dip treatment on fruit rot of tomato
120
100
80
60
40
20
0
Per cent
2.5
2
1.5
1
0.5
0
NUmber
40
45
48
50
52
Control
Temperature (°C)
Per cent disease index
First symptoms developed (DAI)

Plate-X : **Effect of hot water dip treatment for control of fruit rot of tomato**

1. 52°C
2. 50°C
3. 48°C
4. 45°C
5. 40°C
6. Control

lower per cent disease index (11.67%) as compared to the rest (Plate-X, 1). Next best treatment in order of merit was 50°C (18.33%) followed by 48°C (28.33%), 45°C (29.99%) and 40°C (53.34%) temperature.

All the treatments proved significantly better in delaying the rot as compared to the control. Among them, HWT at 52°C for 5 minutes (2.17 days) proved significantly superior over the others. The rest of the treatments were less effective. Maximum disease control was recorded in the treatment of 52°C/5 minutes (87.92%).

Hot water dip treatment tested here is the first report. However, the results are confirmatory with the findings of Sood and Sharma (2003), had also got good control of post harvest rot of tomato fruits caused by *Alternaria* spp. when fruits were dipped in hot water at 52°C temperature for 5 minutes. Similar findings were also noticed by Majumdar and Pathak (1991) in guava fruits.

Best control of the disease by HWT may be due to thermal sensitivity of the pathogen. However, further studies are needed to confirm under large scale experiment. Confirmation of thermal sensitivity of fruits is also needed.

4.7.3 Biological control

The hazardous effect of chemicals used in plant disease management has diverted plant pathologists to find out the alternative methods with little or no adverse effect on

environment and non targeted living things. Noble success of post harvest disease control through the use of botanicals and antagonistic microorganisms at pre-harvest and post-harvest conditions have been achieved during past several years on various fruits and vegetables. Nowadays the commercial formulation of various botanicals and bio-control agents are available in the market. However, inadequate information on the performance of these under varying conditions is a major constraint in the large scale adoption of this technology.

4.7.3.1 Evaluation of various botanicals against *F. solani in vitro*

The aqueous extracts of commonly available plant species belonging to eleven families were evaluated *in vitro* for their inhibitory effect on the mycelial growth of *F. solani*.

The results presented in Tabnle-4.8 and depicted in Fig.6 and Plate-XI revealed that all the plant extracts inhibited the growth of the fungus significantly more as compared to control except ginger and datura.

The leaf extract of gandobaval exhibited maximum growth inhibition (51.76%) of the pathogen and it was significantly superior over the rest (Plate-XI, 1). Next best in order of merit was the extract of black tulsi leaves (34.50 %) followed by neem leaves (29.80%), garlic bulbs (27.45%), nilgiri leaves (23.13%) and karanj leaves (12.94%), while extracts of ratanjyot leaves, bougainvillea leaves and onion bulb were found least effective.

Table-4.8: **Effect of various botanicals on the growth of *F. solani in vitro***

Sr. No.	Local name of phytoextract	Botanical name of phytoextract	Average colony diameter (mm)	Growth inhibition over control (%)
1	Onion	*Allium cepa* L.	9.03* (81.00)**	4.70
2	Neem	*Azadirachta indica* Juss.	7.75 (59.67)	29.80
3	Tulsi	*Ocimum sanctum* L.	7.49 (55.67)	34.50
4	Garlic	*Allium sativum* L.	7.88 (61.67)	27.45
5	Ginger	*Zingiber officinalis* L.	9.22 (84.67)	0.39
6	Nilgiri	*Eucalyptus citridora* Hook	8.11 (65.33)	23.13
7	Ratan jyot	*Jatropha curcas* Sant. and Farn.	8.85 (78.00)	8.23
8	Datura	*Datura metel* L.	9.17 (83.67)	1.56
9	Bougainvillea	*Bougainvillea spectabilis* L	8.97 (80.00)	5.88
10	Gandobaval	*Prosopis juliflora* L.	6.43 (41.00)	51.76
11	Karanj	*Pongamia glabra* L.	8.63 (74.00)	12.94
12	Control (Richards' agar only)	-	9.24 (85.00)	-
	S.Em. ±	-	0.06	-
	C.D. at 5 %		0.18	
	C.V. %		1.30	

* Figures indicate SQR + 0.5 transformed values

** Figures in parentheses indicate retransformed values

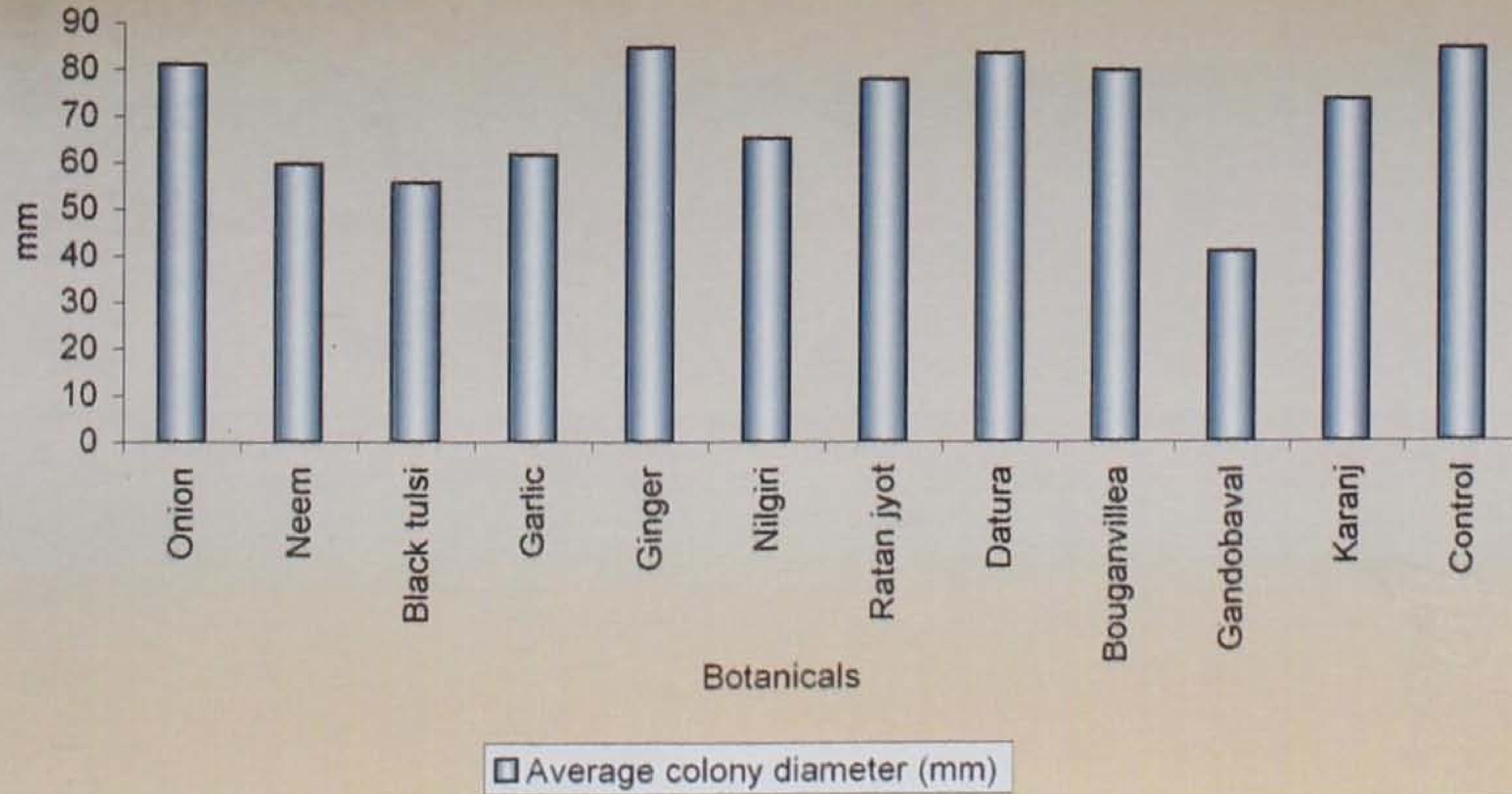


Fig.6 : Effect of various botanicals on growth of F. solani in vitro
90
80
70
60
50
40
30
20
10
0
mm
Onion
Neem
Black tulsi
Garlic
Ginger
Nilgiri
Ratan jyot
Datura
Bouganvillea
Gandobaval
Karanj
Control
Botanicals
Average colony diameter (mm)

Plate-XI : Effect of various botanicals on growth of *F. solani in vitro*

1. Gandobaval
2. Tulsi
3. Neem
4. Garlic
5. Nilgiri
6. Karanj
7. Ratan jyot
8. Bougainvillea
9. Onion
10. Datura
11. Ginger

Dey and Chaudhuri (1984) noticed euginol, caryophyllene and methyleugenol in tulsi responcible for strong inhibition of growth of *F. solani in vitro*. Mamata and Rai (2004) recognized leaf extract of *A. indica* effective against *F. solani in vitro*. Similar findings were also noticed by Prakasam *et al.* (2001) and Rawal and Thakore (2003).

From this experiment, it is very clear that extracts of gandobaval, black tulsi, neem, garlic, nilgiri and karanj may have some strong toxic principles which directly affect on growth of *F. solani*. The fungitoxic effect of gandobaval tested here is the new information.

4.7.3.2 Evaluation of botanicals for the control of fruit rot of tomato

Three botanicals *viz*., gandobaval, tulsi and neem performed better *in vitro* trial were tested at two different concentrations *viz*., 5 and 10 per cent by fruit dip technique for their efficacy against the disease and results were depicted in Table 4.9, Fig.7 and Plate-XII.

It is evident from the results that all the treatments proved significantly superior in checking the rot development over control. The leaf extracts of gandobaval at 10 per cent concentration proved significantly more effective (10.83%) in checking the fruit rot over the rest (Plate-XII, 1). Next best in order of effectiveness were neem extracts used at 10 and 5 per cent concentrations followed by gandobaval at 5 per cent and black tulsi at 10 and 5 per cent concentrations respectively.

Gandobaval at 10 per cent concentration recorded maximum PDC (88.79%) and proved as potential botanical for the management of the disease.

All the botanicals at both the concentration tested significantly delayed the symptoms over the control. However, significantly more duration was taken in treatment of gandobaval (2.97 days) to start the initiation of symptoms at it's 10 per cent concentration. Neem (2.05 days) and gandobaval (1.95 days) at 10 and 5 per cent concentration, respectively were next best in delaying the symptoms but found at par with each other. Neem (1.87 days) at 5 per cent concentration and black tulsi at 10 (1.45 days) and 5 per cent concentration (1.2 days) were also found effective.

Results obtained in present investigation is the new information. However, Dey and Chaudhary (1984) noticed that, essential oil contents of leaf, inflorescence and stem of black tulsi (*O. sanctum*) were responsible for antifungal activity against *F. solani*. Complete inhibition of spore germination of *F. solani* by aqueous extract of *A. indica* was recognized by Tripathi *et al.*, (1999, b). Amadioha and Uchendu (2003) controlled tomato fruit rot (*F. solani*) by pre inoculation spray with bark extracts of *A. indica*. Rawal and Thakore (2003) also recognized the inhibitory effect of leaf extracts of medicinal plants like datura, neem and tulsi against *F. solani in vitro*.

The results obtained in present study are very useful and new information. The fruit rot can be effectively controlled

Sr. No.	Local name of phytoextract	Botanical name phytoextract	Concen tration (%)	Per cent disease index	PDC over check	First symptoms developed (DAI)
1	Gandobaval	*Prosopis juliflora* L.	5	27.71* (21.66)**	77.59	1.95
2	Gandobaval	*Prosopis juliflora* L.	10	19.17 (10.83)	88.79	2.97
3	Neem	*Azadirachta indica* Juss.	5	27.13 (20.83)	78.45	1.87
4	Neem	*Azadirachta indica* Juss.	10	23.36 (15.83)	83.62	2.05
5	Tulsi	*Ocimum sanctum* L	5	34.73 (32.49)	66.38	1.2
6	Tulsi	*Ocimum sanctum* L.	10	28.29 (22.49)	76.73	1.45
7	Control	-	-	89.44 (96.66)	-	0.5
	S. Em. ±			0.70		0.04
	C.D. at 5%	-	-	2.06	-	0.11
	C.V. %			3.94		4.5

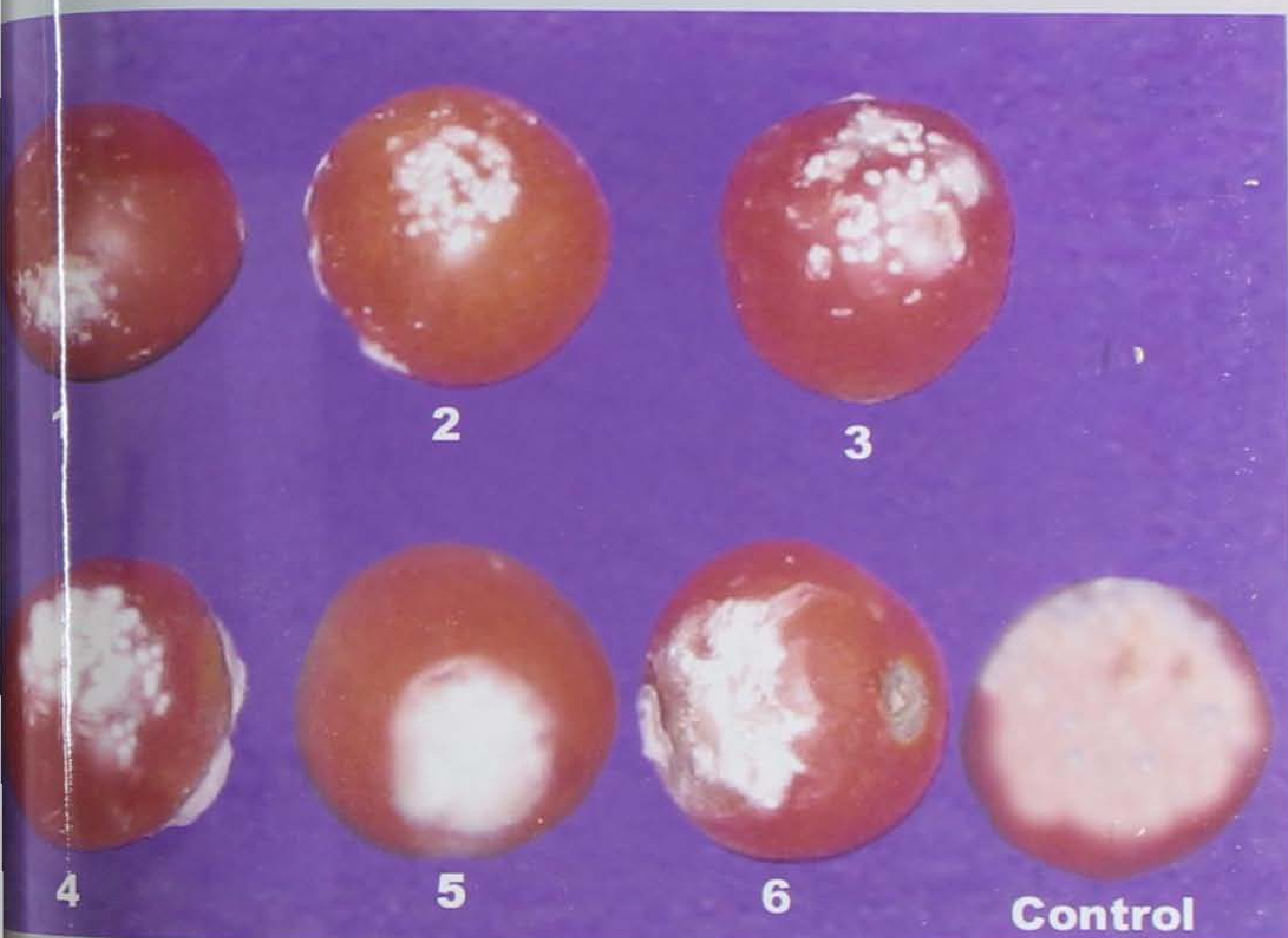
* Figures indicate arc sine transformed values

** Figures in the parentheses indicate retransformed values

DAI Days after inoculation

PDC Per cent disease control

te-XII : Evaluation of botanicals on fruit rot of tomato



1. Gandobaval (10%)
2. Neem (10%)
3. Neem (5%)
4. Gandobaval (5%)
5. Tulsi (10%)
6. Tulsi (5%)

by using botanicals instead of chemicals especially gandobaval. However, further confirmation in large scale experiment is suggested.

4.7.3.3 Testing of oils for the control of fruit rot of tomato

There is no exact correlation between the treatment of fruits and vegetables with fungicides and the residues which will be found on the food finally sold to the consumer markets. There had been reports of rot pathogens which produced resistant strains to some fungicides. Considering this, present investigation was carried out to find effectiveness of seven oils against the disease and results are depicted in the Table-4.10, Fig.8 and Plate-XIII.

It is evident from the results that, all the oils significantly checked the rot over control. Palm kernel oil and neem oil recorded minimum fruit rot (3.33%) and found significantly superior over the rest (Plate-XIII, 1). Next best effective oil was groundnut oil (6.66%) (Plate-XIII, 2). The rest of the oils were found moderately effective. Maximum disease control was obtained in the treatment of neem oil and palm oil (96.51%) followed by groundnut oil (93.02%).

All the treatments showed significantly longer duration over control to start the initiation of symptoms. However palm kernel oil followed by neem oil gave protection up to 4.06 and 3.90 days, respectively but found at par with each other. Groundnut oil (3.34 days) was found next best in order of merit followed by mustard oil (2.36 days), castor oil (2 days)

ble – 4.10: Testing of oils for the control of fruit rot of tomato

r. No.	Name of oil	Per cent disease index	PDC over check	First symptoms developed (DAI)
1	Groundnut oil	14.94* (6.66)**	93.02	3.34
2	Castor oil	26.55 (20.00)	79.06	2.00
3	Mustard oil	29.60 (24.44)	74.42	2.36
4	Karanj oil	50.12 (58.88)	38.37	0.76
5	Eucalyptus oil	39.86 (41.11)	56.97	1.00
6	Neem oil	10.51 (3.33)	96.51	3.90
7	Palm kernel oil	10.51 (3.33)	96.51	4.06
8	Control (Distilled water)	79.43 (95.55)	-	0.5
	S. Em. ±	0.41		0.07
	C.D. at 5%	1.24		0.22
	C.V. %	2.20		5.76

* Figures indicate arc sine transformed values

** Figures in the parentheses indicate retransformed values

DAI Days after inoculation

PDC Per cent disease control

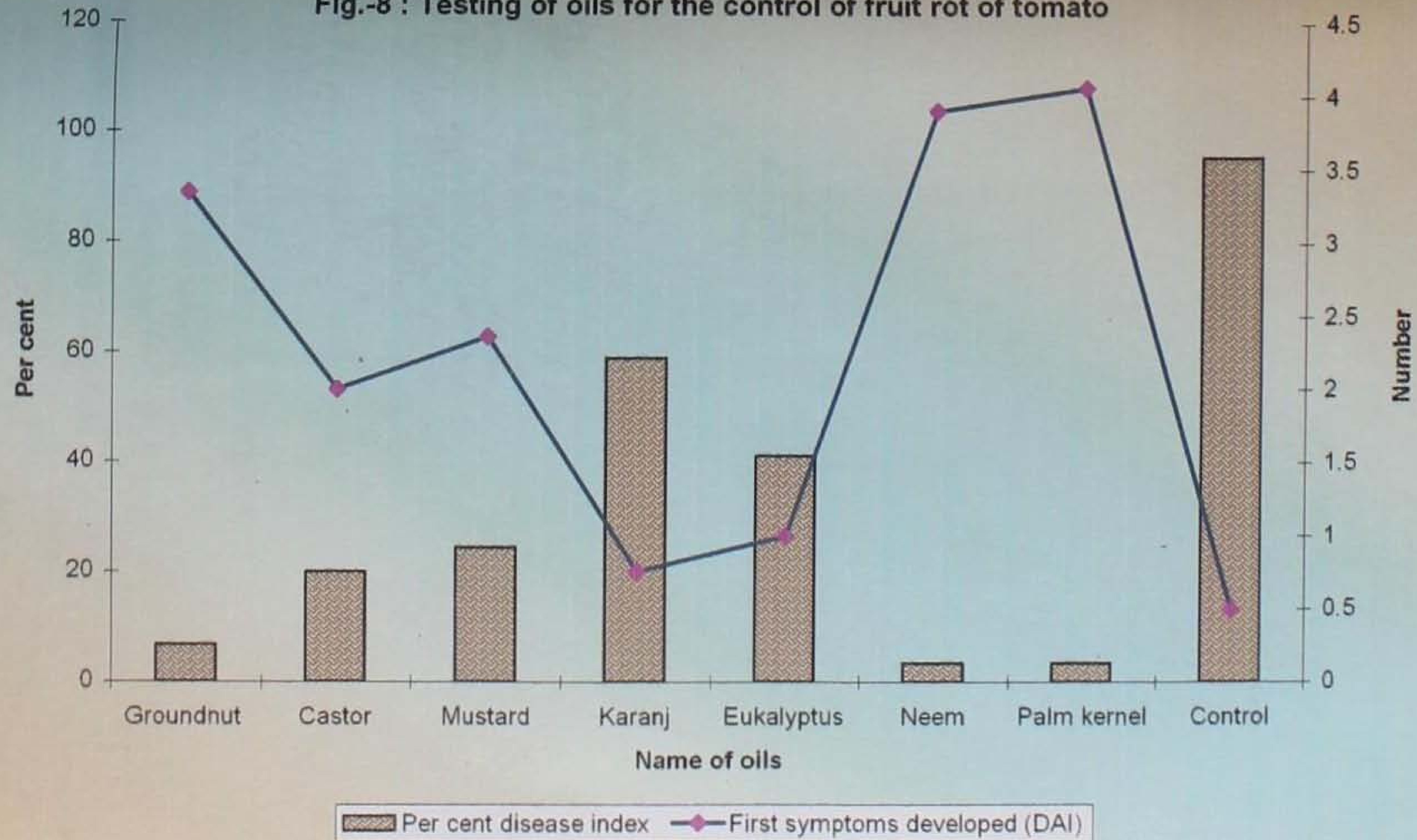
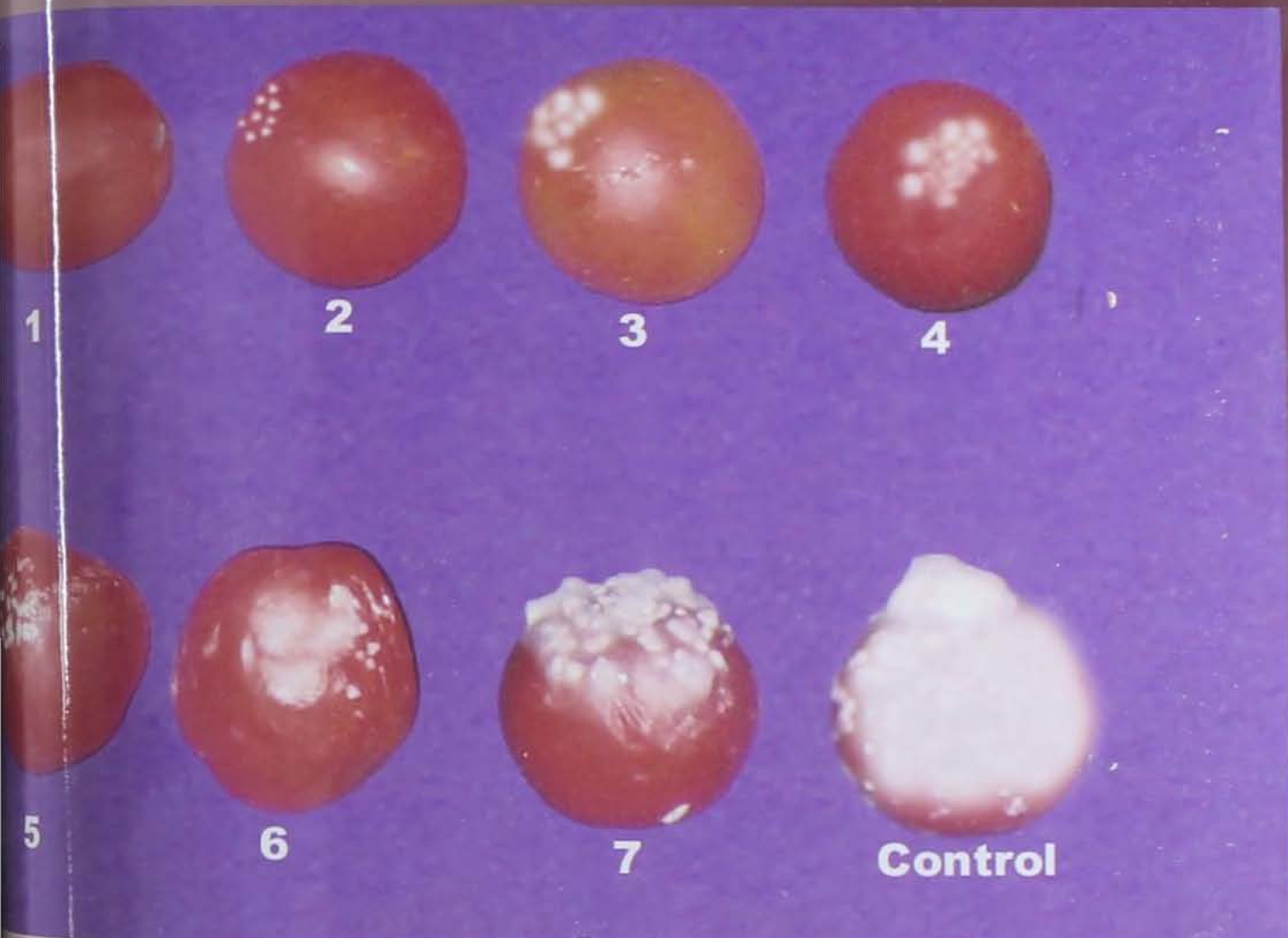


Fig.-8 : Testing of oils for the control of fruit rot of tomato
Per cent
120
100
80
60
40
20
0
Number
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Groundnut
Castor
Mustard
Karanj
Eukalyptus
Neem
Palm kernel
Control
Name of oils
Per cent disease index
First symptoms developed (DAI)

Plate-XIII : Testing of oils on fruit rot of tomato



1. Palm kernel oil
2. Neem oil
3. Groundnut oil
4. Castor oil
5. Mustard oil
6. Eukalyptus oil
7. Karanj oil

eucalyptus oil (1 day) and karanj oil (0.76 day) against the disease.

Variation in the effect of different oils against the disease may be due to differential toxicity of oils on the test fungus. Adisa (1985) reported inhibitory effect of palm kernel oil on spore germination of *F. equiseti* causing storage rot of tomato fruits. He also noticed that, eventhough spores germinated but germ tubes were distorted and malformed. Superiority of all oils over the control may also due to moisture or water repellent action and stable nature on the treated surfaces.

Encouraging results from oils were also observed in controlling *R. arrhizus* rot of pomegranate fruits (Kanwar and Thakur, 1974), *A. tenuis* rot, *C. fulvum* rot, *S. sclerotiorum* rot and *F. roseum* rot of ripe tomato fruits (Aulakh and Grover., 1968) in storage.

4.7.3.4 Antagonistic effect of different microorganisms to *F. solani*

In the present study, attempts were made to identify antagonistic microorganisms of *F. solani in vitro*. The effect of nine known antagonists was tested by three different methods viz., dual culture, by placing pathogen at center and pathogen at periphery.

4.7.3.4.A Dual culture technique

The results presented in Table-4.11 (Plate-XIV, a) revealed that, out of nine, six antagonists were found

Table-4.11: Antagonistic effect of different microorganisms to *F. solani* (Dual culture method)

Sr. No.	Test organism	Av. radius of pathogen (mm)	Growth inhibition over control (%)
1	*Trichoderma viride*	3.67* (13.00)**	62.85
2	*Trichoderma longibrachiatum*	4.45 (19.33)	44.76
3	*Trichoderma harzianum*	5.52 (30.00)	14.28
4	*Aspergillus niger*	5.84 (33.66)	3.80
5	*Aspergillus flavus*	4.73 (22.00)	37.14
6	*Gliocladium virens*	5.08 (25.33)	27.61
7	*Chaetomium globosum*	5.81 (33.33)	4.76
8	*Bacillus subtilis*	4.14 (16.66)	52.38
9	*Pseudomonas fluorescens*	5.86 (34.00)	2.85
10	Control	5.95 (35.00)	-
	S.Em. ±	0.09	-
	C.D. at 5 %	0.28	
	C.V. %	3.17	

* Figures indicate SQR + 0.5 transformed values

** Figures in parentheses indicate retransformed values

significantly effective in checking the growth of the pathogen over the control. Out of them, *T. viride* produced maximum inhibition (62.85%) and was significantly superior over the rest (Plate-XIV b, 1). Next best in order of merit was *B. subtilis* (52.38%) followed by *T. longibrachiatum* (44.76%), *A. flavus* (37.14%), *G. virens* (27.61%) and *T. harzianum* (14.28%). Rest of the antagonists *viz.*, *C. globosum* (4.76%), *A. niger* (3.80%) and *P. fluorescens* (2.85%) were found less effective.

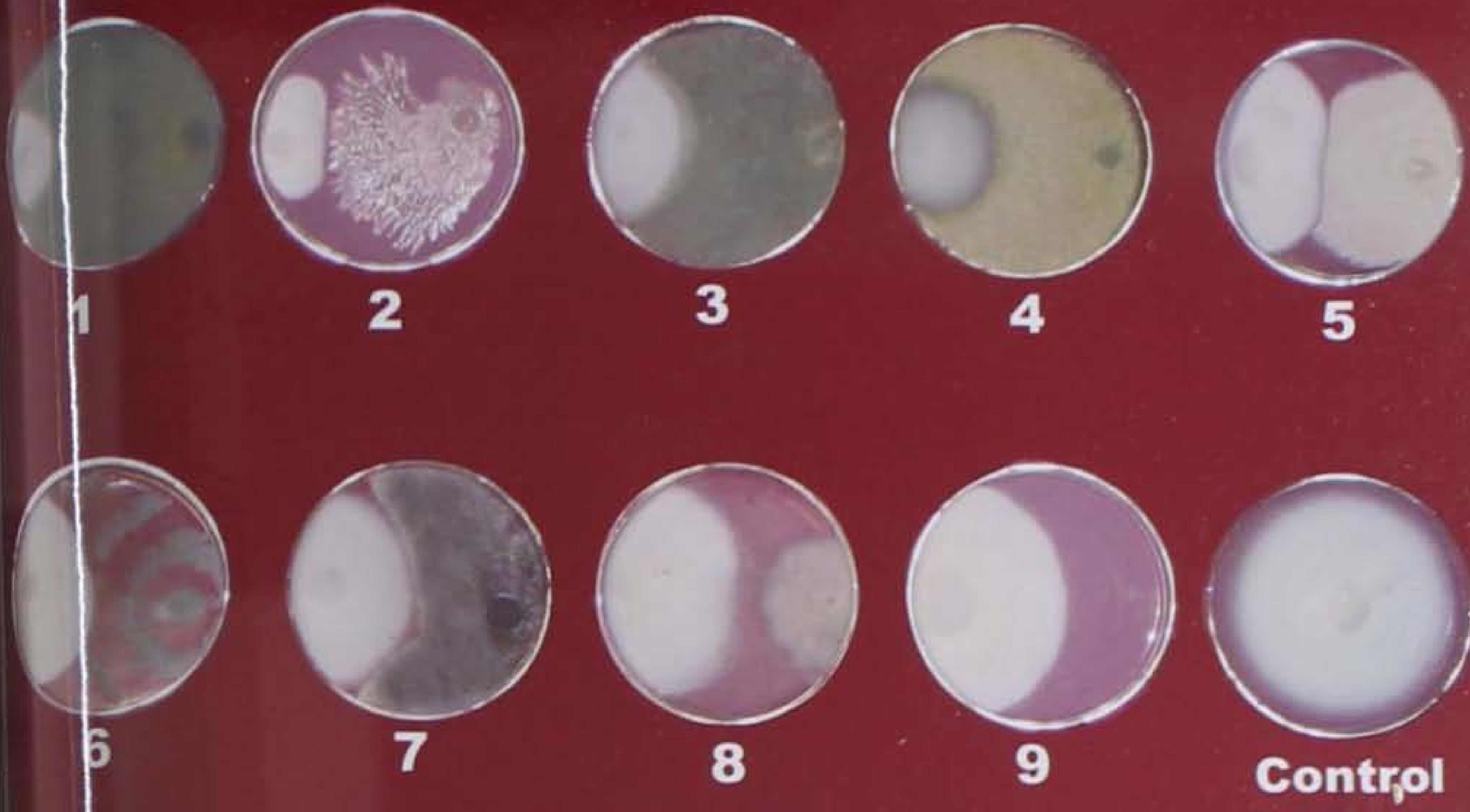
4.7.3.4.B Pathogen at centre

In this method, the pathogen was placed at centre surrounded by test organisms providing upper hand to the test organisms. The results presented in Table-4.12 (Plate-XIV, b) showed that all the antagonists significantly inhibited the growth of the pathogen except *P. fluorescens* as compared to control. Among them, maximum growth inhibition of the pathogen was recorded by *B. subtilis* (77.14%) which was at par with *T. viride* (76.19%) (Plate-XIV b, 1&2). *T. longibrachiatum* (57.38%) was found next best in order of merit and was at par with *A. flavus* (55.95%) followed by *C. globosum* (53.33%). *A. niger* (46.42%), *G. virens* (43.33%) and *T. harzianum* (39.76%).

4.7.3.4.C Pathogen at periphery

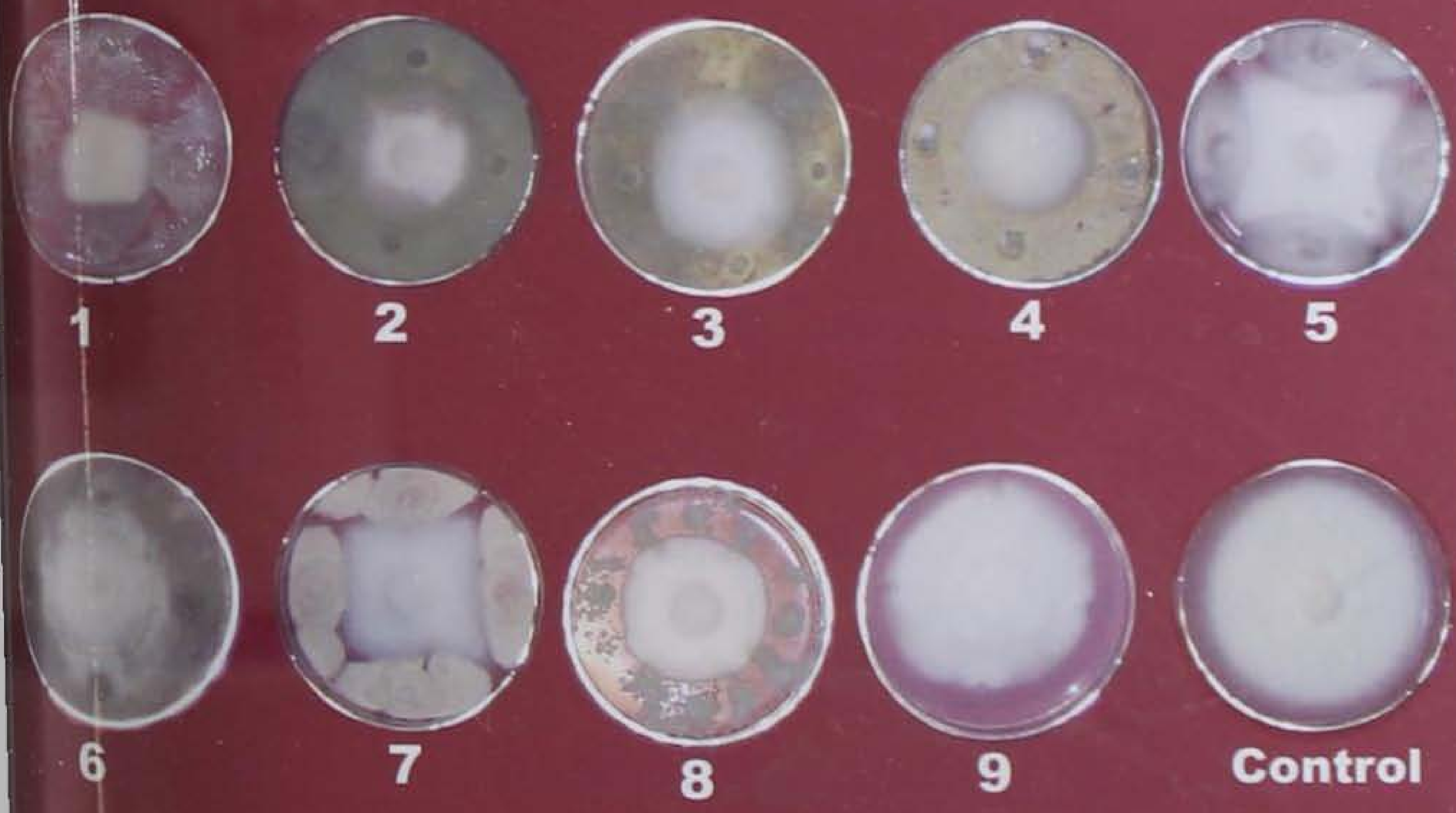
In this method, test organisms (antagonists) were placed in centre surrounded by the pathogen providing upper hand to the pathogen and real antagonistic properties of the test organism was exhibited. The results presented in Table-4.13

Plate-XIV : Efficacy of antagonists against *F. solani*



(a) : Dual culture

1. *Trichoderma viride*
2. *Bacillus subtilis*
3. *Trichoderma longibrachiatum*
4. *Aspergillus flavus*
5. *Gliocladium virens*
6. *Trichoderma harzianum*
7. *Aspergillus niger*
8. *Chaetomium globosum*
9. *Pseudomonas fluorescens*



(b) : Pathogen at centre

1. *Bacillus subtilis*
2. *Trichoderma viride*
3. *Trichoderma longibrachiatum*
4. *Aspergillus flavus*
5. *Chaetomium globosum*
6. *Aspergillus niger*
7. *Gliocladium virens*
8. *Trichoderma harzianum*
9. *Pseudomonas fluorescens*

ble-4.13: Antagonistic effect of different microorganisms to *F. solani* (Pathogen at periphery)

Test organism	Av. radius of pathogen (mm)	Growth inhibition over control (%)
Trichoderma viride	3.72* (13.5)**	61.42
Trichoderma longibrachiatum	3.93 (15.00)	57.14
Trichoderma harzianum	4.31 (18.16)	48.09
Aspergillus niger	3.05 (08.91)	74.52
Aspergillus flavus	4.20 (17.16)	50.95
Gliocladium virens	3.60 (12.50)	64.28
Chaetomium globosum	4.33 (18.33)	47.61
Bacillus subtilis	3.29 (10.33)	70.47
Pseudomonas fluorescens	5.95 (35.00)	00.00
Control	5.95 (35.00)	-
S.Em. ±	0.10	-
C.D. at 5 %	0.29	
C.V. %	4.02	

Figures indicate SQR + 0.5 transformed values

* Figures in parentheses indicate retransformed values

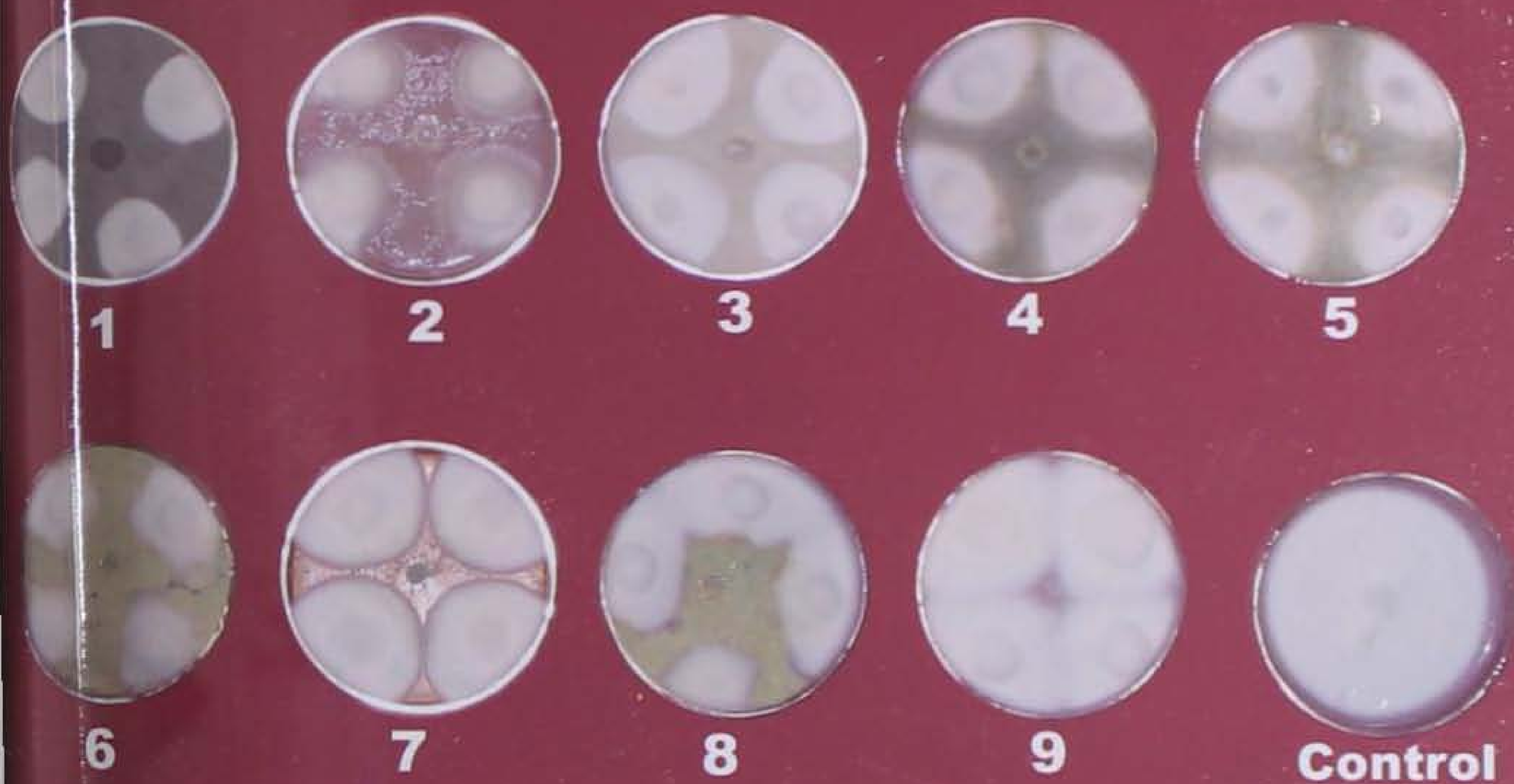


Plate-XIV (c) : Pathogen at periphery

1. *Aspergillus niger*
2. *Bacillus subtilis*
3. *Gliocladium virens*
4. *Trichoderma viride*
5. *Trichoderma longibrachiatum*
6. *Aspergillus flavus*
7. *Chaetomium globosum*
8. *Trichoderma harzianum*
9. *Pseudomonas fluorescens*

(Plate XIV, c) revealed that all the antagonists showed significantly maximum growth inhibition except *P. fluorescens* as compared to the control. Out of these, *A. niger* (74.52%) produced maximum inhibition but was at par with *B. subtilis* (70.47%). *G. virens* (64.28%) was found next best in order which was followed by *T. viride* (61.42%), *T. longibrachiatum* (57.14%), *A. flavus* (50.95%), *T. harzianum* (48.09%) and *C. globosum* (47.61%).

It is evident from these studies that among all antagonists evaluated by three methods, *T. viride*, *B. subtilis* and *A. niger* showed strong antagonistic activity against *F. solani.* The present findings are in harmony with earlier findings of Ram *et al.*, (2000) and Mathur and Gurjar (2002). Gurjar *et al.*, (2004) reported that, *T. harzianum* and *T. viride* were effective antagonists against *Fusarium* sp. Pratibanda and Sen (2004) observed *A. niger* as a useful antagonist against *F. oxysporum* f. sp. *melonitis* causing wilt of musk melon.

4.7.3.5 Screening of antagonists for the control of tomato fruit rot

To find out the efficacy of antagonists against the disease, the antagonists proved better *in vitro* trial *viz.*, *B. subtilis, T. viride and T. longibrachiatum* were tested as fruit dip treatment at 10^6 and 10^8 cfu/ml concentrations. The results obtained are depicted in the Table-4.14, Fig.9 and Plate-XV.

From this experiment, it is very clear that, all the treatments recorded significantly low PDI over control. However *B. subtilis* at 10^8 cfu/ml recorded significantly lower PDI (7.5%) followed by *B. subtilis* at 10^6 cfu/ml (10.83%) and *T. viride* at 10^8 cfu/ml (11.6%) but were found at par with each other (Plate-XV, 1,2&3). Next best antagonists in order of merit was *T. viride* (16.66%) at 10^6 cfu/ml followed by *T. longibrachiatum* at 10^8 (22.49%) and 10^6 (25.82%) cfu/ml concentration. *B. subtilis* at 10^8 cfu/ml recorded highest PDC (92.24%) over check.

All the treatments took significantly maximum duration to start the initiation of symptoms after inoculation. However, *B. subtilis* at 10^8 cfu/ml (2.85 days) was found significantly superior over the rest. *B. subtilis* at 10^6 cfu/ml (2.1 days) and *T. viride* at 10^8 cfu/ml concentration found next best but were at par with each other. Rest of the treatments *viz.*, *T. viride* at 10^6 cfu/ml (1.87 days) and *T. longibrachiatum* at 10^6 (1.25 days) and 10^8 (1.05 days) also delayed the disease for considerable duration.

Strashnov *et al.* (1985) noticed up to 83 per cent fruit rot (*R. solani*) reduction when tomato fruits were coated with *T. harzianum*.

The mechanism of disease control in this case may be direct parasitism or production of antimetabolites which hinder the growth of the pathogen.

Table – 4.14: Screening of antagonists for the control of fruit rot of tomato

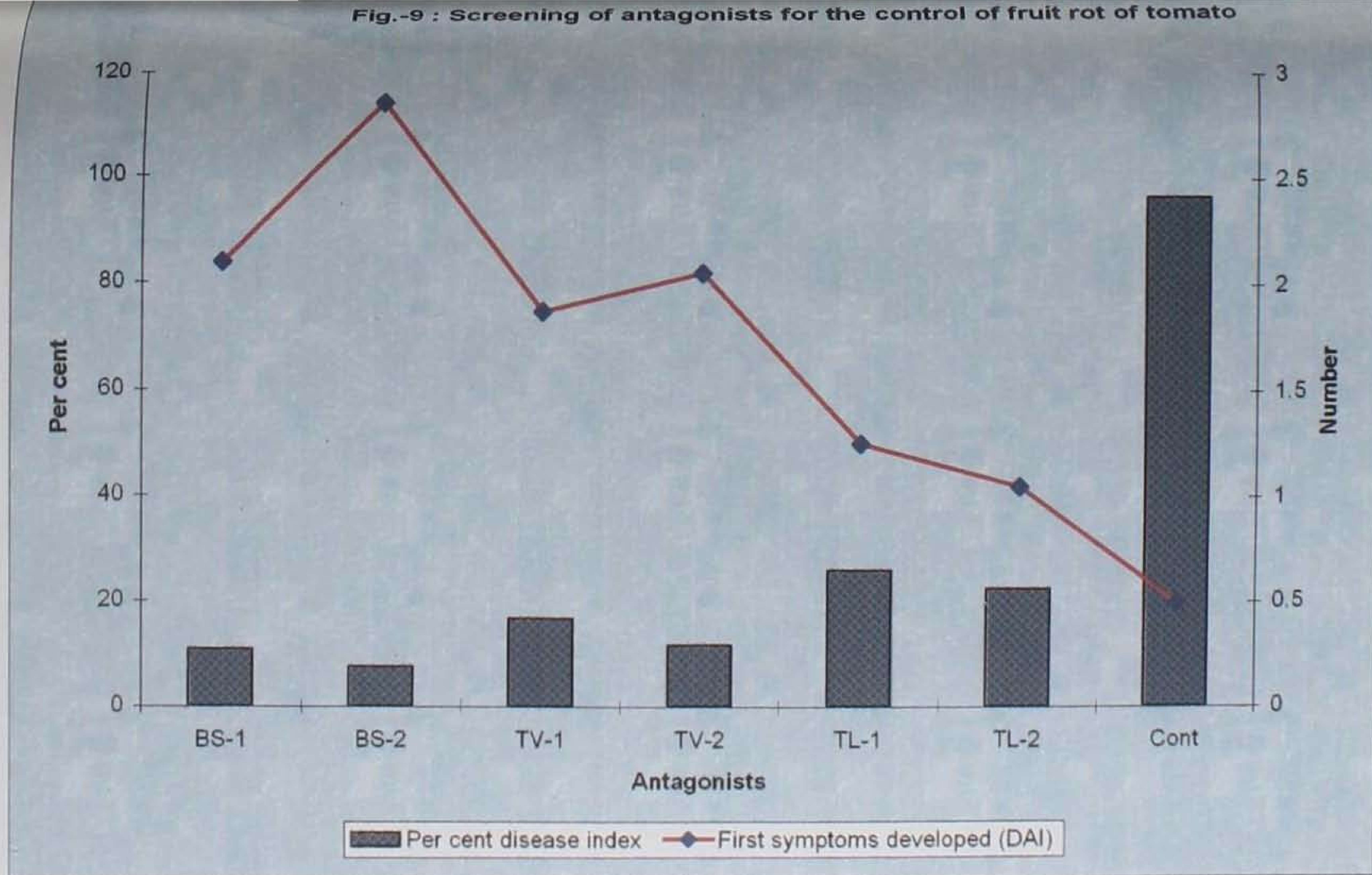
Sr. No	Test organism	Concentration (cfu/ml)	Per cent disease index	PDC over check	First symptoms developed (DAI)
1	*Bacillus subtilis*	10^6	19.17* (10.83)**	88.79	2.1
2	*Bacillus subtilis*	10^8	15.82 (7.50)	92.24	2.85
3	*Trichoderma viride*	10^6	24.02 (16.66)	82.76	1.87
4	*Trichoderma viride*	10^8	19.91 (11.66)	87.93	2.05
5	*Trichoderma longibrachiatum*	10^6	30.52 (25.82)	73.28	1.25
6	*Trichoderma longibrachiatum*	10^8	28.29 (22.49)	76.73	1.05
7	Control	-	89.44 (96.66)	-	0.5
	S. Em. ±		0.74		0.05
	C.D. at 5%	-	2.19	-	0.16
	C.V. %		4.53		6.51

* Figures indicate arc sine transformed values

** Figures in the parentheses indicate retransformed values

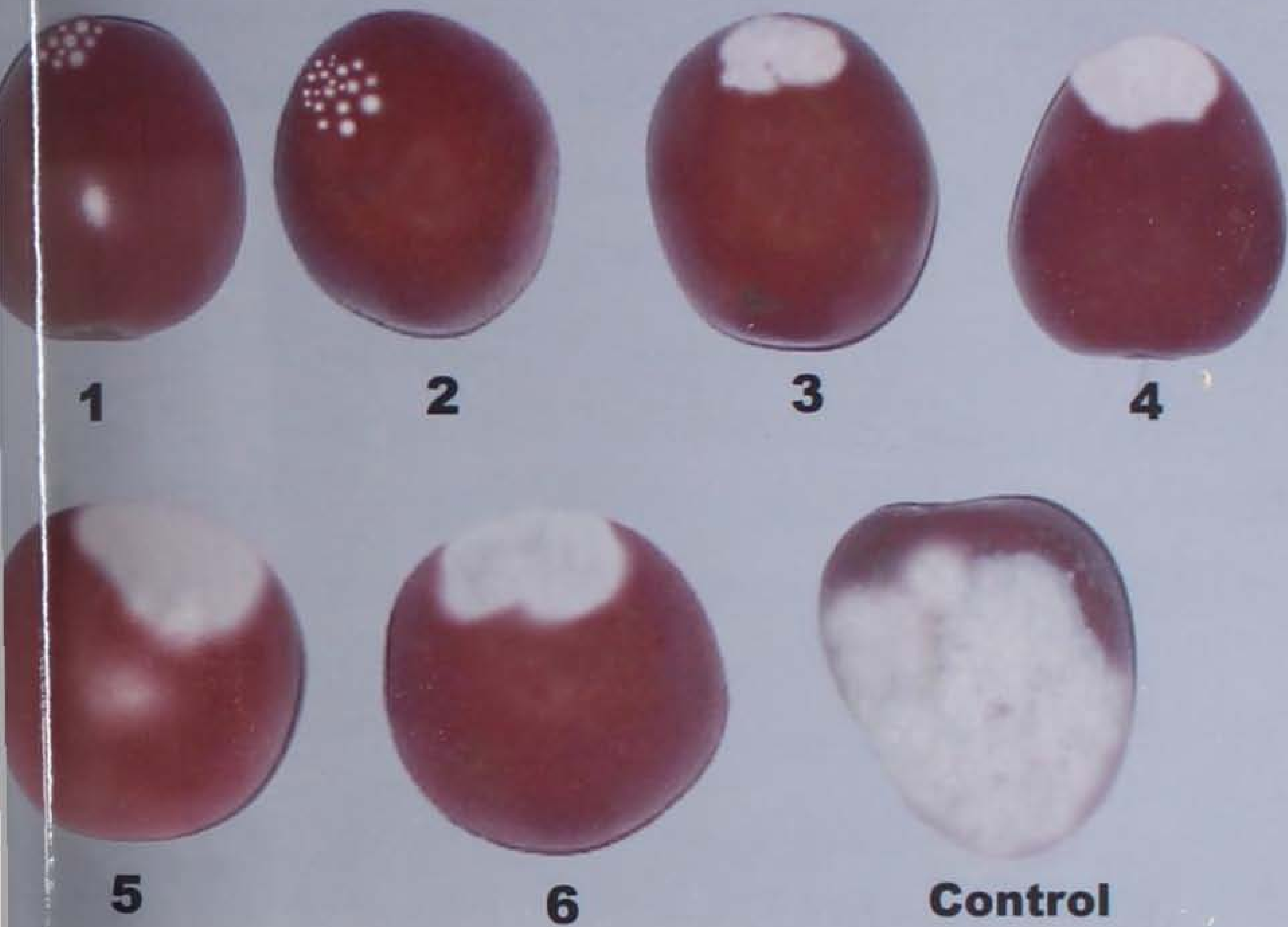
DAI Days after inoculation

PDC Per cent disease control



BS	*Bacillus subtilis*			
TV	*Trichoderma viride*	1	10^{6}	cfu/ml
TL	*Trichoderma longibrachiatum*	2	10^{8}	cfu/ml

te-XV : Screening of antagonists on fruit rot of tomato



1. *Bacillus subtilis* (10^8 cfu/ml)
2. *Bacillus subtilis* (10^6 cfu/ml)
3. *Trichoderma viride* (10^8 cfu/ml)
4. *Trichoderma viride* (10^6 cfu/ml)
5. *Trichoderma longibrachiatum* (10^8 cfu/ml)
6. *Trichoderma longibrachiatum* (10^6 cfu/ml)

4.7.4 Chemical control

4.7.4.1 *In vitro* screening of different fungicides against *F. solani*

Eleven fungicides from systemic and non-systemic groups were evaluated at three different concentrations by poisoned food technique for their efficacy against *F. solani*. The results presented in Table-4.15 and depicted in Fig.10 and Plate-XVI indicated that all fungicides have varied degree of efficacy against *F. solani*.

Out of these, carbendazim (Bavistin), thiophanate methyl (Topsin-M) and flusilazole (Nustar) recorded cent per cent inhibition of *F. solani* at all three concentrations (250,500 and 1000 ppm). Thiram (TMTD), mancozeb (Dithane M-45), MEMC (Emisan) and copper hydroxide (Kocide) at 2000 ppm, propiconazole (Tilt) and carboxin + thiram (Cosco) at 1000 ppm also gave cent per cent inhibition of the test fungus. Maximum inhibition was recorded in MEMC (99.60%) at 1500 ppm followed by propiconazole at 250 and 500 ppm (98.23%), copper oxychloride (Blitox) (98.86%) at 2000 ppm, MEMC (95.49%) and carboxin + thiram at 500 and 1000 ppm and carboxin + thiram (92.54%) at 250 ppm concentration. Copper oxychloride (89.60%) at 1500 ppm, mancozeb at 1500 (83.13%) and 1000 ppm (82.54%), copper oxychloride at 1000 ppm and captan (80.39%) at 200 ppm concentration have also proved very effective fungicides. Copper hydroxide at 1500 ppm (77.64%) and at 1000 ppm (66.49%), captan at 1500 ppm (64.90%) and at 1000 ppm

(61.76%), thiram at 1500 ppm (49.60%) and 1000 ppm concentration (46.27%) were moderately effective in checking the fungal growth.

It is evident from the results that the growth inhibition increased with an increase in the concentration of chemicals. Carbendazim, thiophanate methyl and flusilazole were proved most effective fungicides followed by MEMC, propiconazole, carboxin + thiram, copper oxychloride and mancozeb at all the concentrations tested.

As this pathogen is externally causing the rot of the tomato fruit and is wound parasite, the effective fungicides found here may be used for the protection and huge losses can be avoided. The further testing in field as preharvest treatment or postharvest treatment, their practical utility and feasibility is suggested.

Mishra and Rath (1984) reported complete inhibition of mycelial growth of *F. solani in vitro* by Bavistin. Same result was also noticed by Mishra and Rath (1986). Pandav (2002) recognized significantly lower mycelial growth of *F. solani in vitro* by carbendazim at 250, 500 and 1000 ppm concentration over the control. The results are also in confirmatory with the findings of Neweigy *et al.* (1985), Patel (1987), Singh and Saxena (1990), Bhat and Srivastava (2003) and Mamata and Rai (2004).

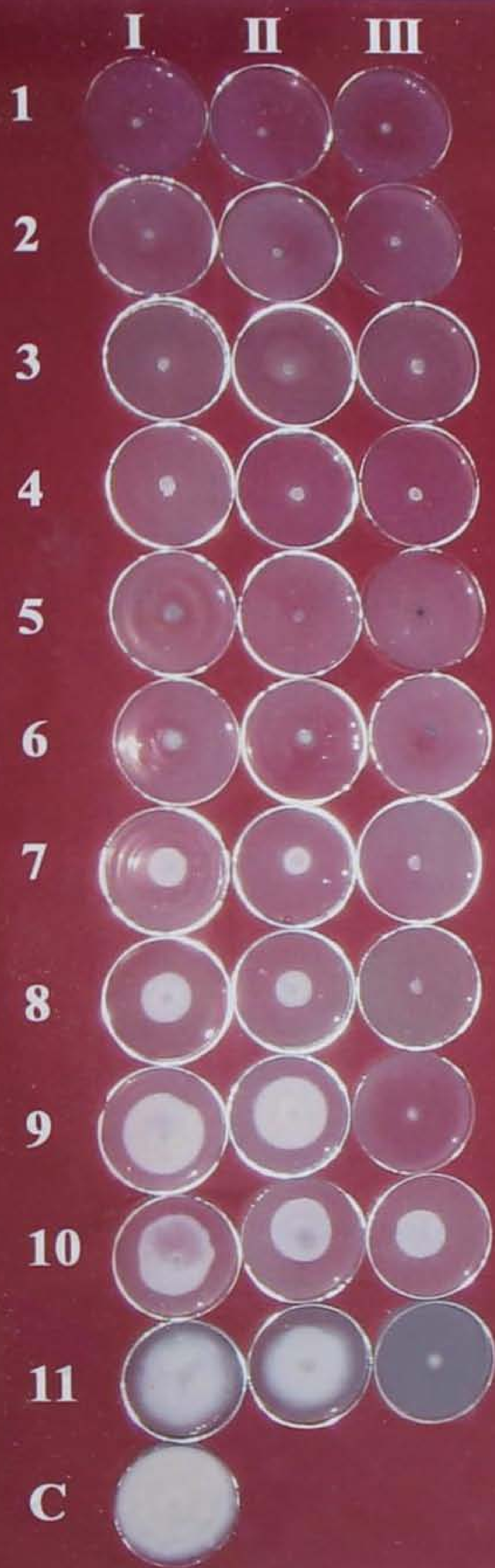
Table-4.15: **Evaluation of different fungicides against *F. solani in vitro***

S. No.	Fungicide	Concentration (ppm)	Average colony diameter	Per cent inhibition over control
1	Thiram (TMTD 75 WP)	1000	6.79* (45.66)**	46.27
		1500	6.58 (42.83)	49.60
		2000	0.70 (00.00)	100.00
2	Captan (Captaf 50 WP)	1000	5.74 (32.00)	61.76
		1500	5.50 (29.83)	64.90
		2000	4.14 (16.66)	80.39
3	Mancozeb (Dithane M-45, 75 WP)	1000	3.91 (14.83)	82.54
		1500	3.85 (14.33)	83.13
		2000	0.70 (00.00)	100.00
4	Copper oxychloride (Blitox 50 WP)	1000	4.14 (16.66)	80.39
		1500	3.05 (08.83)	89.60
		2000	1.77 (02.66)	96.86
5	MEMC (Emisan 6 WP)	1000	1.46 (01.66)	98.03
		1500	0.87 (00.33)	99.60
		2000	0.70 (00.00)	100.00
6	Copper hydroxide (Kocide101 75 WP)	1000	5.46 (29.33)	65.49
		1500	4.41 (19.00)	77.64
		2000	0.70 (00.00)	100.00
7	Carbendazim (Bavistin 50WP)	250	1.82 (02.83)	96.66
		500	0.70 (00.00)	100.00
		1000	0.70 (00.00)	96.86
8	Propiconazole (Tilt 25 EC)	250	1.41 (01.50)	98.23
		500	0.97 (00.50)	99.41
		1000	1.35 (01.33)	98.43
9	Thiophanate methyl (Topsin M 70 WP)	250	0.70 (00.00)	100.00
		500	0.70 (00.00)	100.00
		1000	0.70 (00.00)	100.00
10	Carboxin (37.5 DS) + Thiram (37.5 DS) (Cosco 75 WP)	250	2.97 (08.33)	90.19
		500	2.54 (06.00)	92.94
		1000	0.70 (00.00)	100.00
11	Flugilazole (Nustar 40 EC)	250	0.70 (00.00)	100.00
		500	0.70 (00.00)	100.00
		1000	0.70 (00.00)	100.00
12	Control	-	9.24 (85.00)	-
	S.Em. ±		0.05	
	C.D. at 5%		0.13	
	C.V. %		3.71	

* Figures indicate SQR + 0.5 transformed values

** Figures in parentheses indicate retransformed values

Plate- XVI : Effect of fungicides on growth of *F. solani in vitro*



1. Carbendazim
2. Thiophanate methyl
3. Flucilazole
4. MEMC
5. Propiconazole
6. Carboxin + Thiram
7. Copper oxychloride
8. Mancozeb
9. Copper hydroxide
10. Captan
11. Thiram
C. Control

.7.4.2 Testing of different fungicides against fruit rot of tomato

For controlling Fusarial fruit rot of tomato, the hemicals *viz.*, carbendazim, flusilazole, propiconazole, copper ydroxide, mancozeb and thiram which were found effective nder *in vitro* screening were further evaluated under laboratory ondition. The performance of each of these fungicides were ompared with control, where fruits were dipped only in sterilized listilled water. The per cent disease index (PDI) and number of lays taken to start the initiation of symptom were recorded and resented in Table-4.16, Fig.11and Plate-XVII.

The data presented in Table-4.16 revealed that, all the fungicidal treatments showed significantly lower fruit rot as compared to the control. Among them, fruit dip in carbendazim (3.33%) was found superior over the rest and found statistically at par with thiram (4.16%) (Plate-XVII, 1&2). The next best fungicide in order of merit was flusilazole (4.99%). Rest of the fungicides *viz.*, propiconazole (16.66%), copper hydroxide (24.99%) and mancozeb (26.66%) found moderately effective. Carbendazimm, thiram and flusilazole gave more than 94 per cent disease control over check.

All fungicides gave significantly long duration protection as compared to the control. However, carbendazim (5.12 days) gave significantly long time protection as compared to the rest. Next best fungicide in order of merit was flusilazole (4.85 days). Thiram (2.37 days), propiconazole (2.05 days),

ble – 4.16: Evaluation of fungicides against fruit rot of tomato

r. o.	Fungicides	Concen tration (ppm)	Per cent disease index	PDC over check	First symptoms developed (DAI)
1	Carbendazim (Bavistin 50 WP)	500	*10.51 **(3.33)	96.51	5.12
2	Flusilazole (Nustar 40 EC)	500	12.72 (4.99)	94.77	4.85
3	Propiconazole (Tilt 25 EC)	500	24.02 (16.66)	78.89	2.05
4	Copper hydroxide (Kocide101 75 WP)	2000	29.97 (24.99)	73.84	1.95
5	Mancozeb (Dithane M-45 75 WP)	2000	31.04 (26.66)	72.09	1.25
6	Thiram (TMTD 75 WP)	2000	11.62 (4.16)	95.64	2.37
7	Control	-	79.43 (95.55)	-	0.5
	S. Em. ±		0.69		0.07
	C.D. at 5%	-	2.01	-	0.19
	C.V. %		4.8		5.03

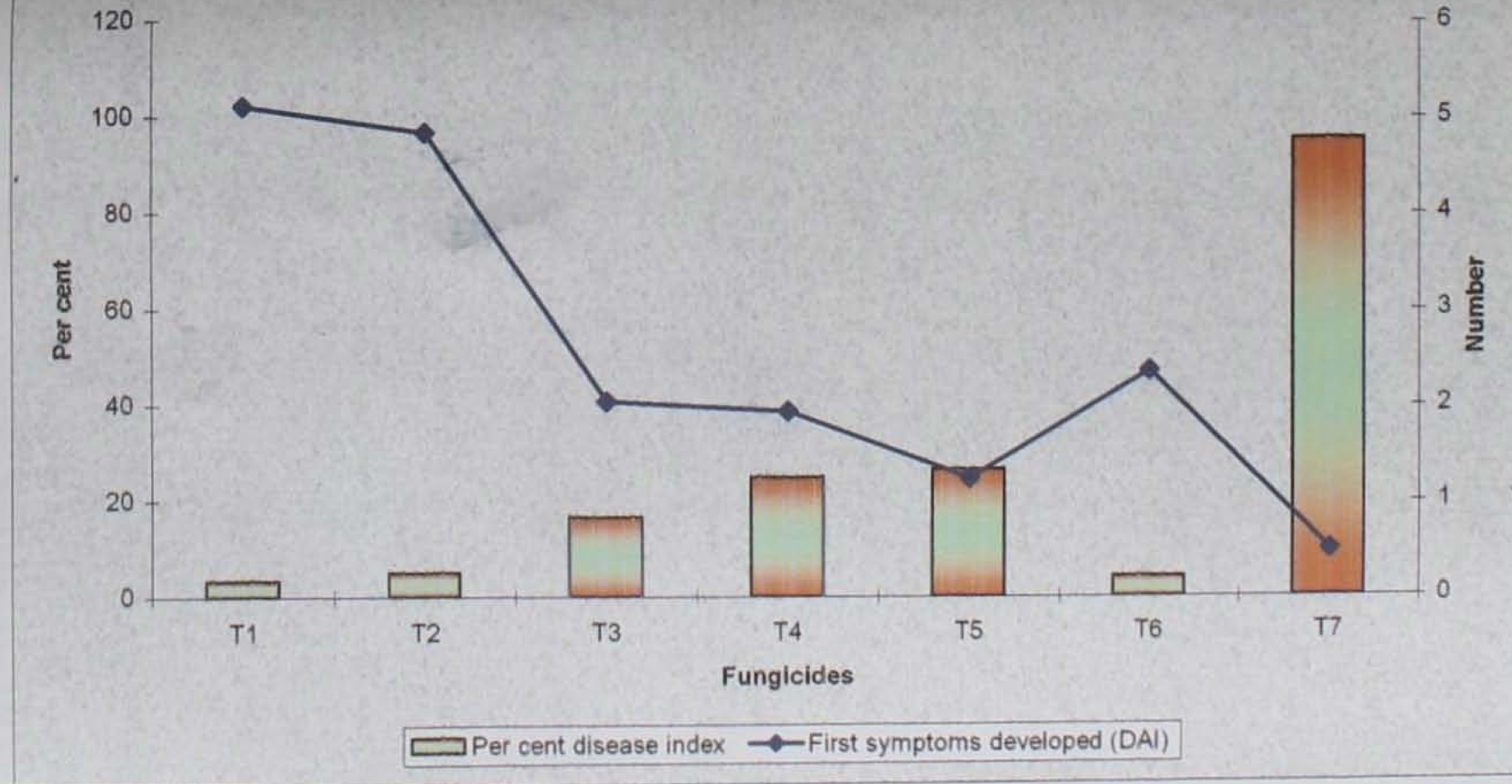
Figures indicate arc sine transformed values

Figures in the parentheses indicate retransformed values

Days after inoculation

Per cent disease control

Fig.-11 : Evaluation of fungicides against fruit rot of tomato



T1	Bavistin 50% WP (500 ppm)	T4	Kocide 101 75% WP (2000 ppm)
T2	Nustar 40% EC (500 ppm)	T5	Dithane M-45 75 WP (2000 ppm)
T3	Tilt 25% EC (500 ppm)	T6	TMTD 75 % WP (2000 ppm)
		T7	Control

ate-XVII : Testing of fungicides against fruit rot of tomato

1. Carbendazim (500 ppm)
2. Thiram (2000 ppm)
3. Flusilazole (500 ppm)
4. Propiconazole (500 ppm)
5. Copper hydroxide (2000 ppm)
6. Mancozeb (2000 ppm)

copper hydroxide (1.95 days) and mancozeb (1.25 days) protected tomato fruits only up to 1.25 days from fusarial infection.

Kalra and Sohi, (1985) got cent per cent control of Alternaria fruit rot of tomato by pre inoculation fruit dip for 5 minutes in Dithane M-45, Difolatan (both at 0.3 %) and Thiram (0.2%). While, Kassim (1986) reported that fungicidal dip treatments in 0.2 per cent Antracol, Bavistin, Benlate, Cupravit and Dithane M-45 were found effective in reducing Alternaria rot of tomato fruits.

Datar and Ghule (1988) effectively controlled the fruit rot of banana (*F. solani*) by dipping the fruits in carbendazim (1000 ppm) for 10 minutes. De *et al.* (1994) successfully controlled post harvest rot of banana (*Fusarium* sp.) by fruit dip and shower-spraying of triazole fungicides (propiconazole, myclobutanil, flusilazole and bitertanol) at low conccentration (50 ppm) and avoided accumulation of residues on fruits.

Fageria *et al.* (2002) noticed that pre harvest spray of ber fruits with 0.1 per cent Bavistin effectively checked post harvest rottage (*F. solani*) up to two days after storage. Patel *et al.* (2004) also noticed good control of *F. solani* infection in okra seeds when treated externally with Bavistin.

4.7.5 Testing of physical, chemical and biological treatments for the control of fruit rot of tomato

For controlling fusarial fruit rot of tomato, physical, chemical and biological treatments *viz.*, fruit dip in hot water,

leaf extract of gandobaval, palm kernel oil, cell suspension of *B. subtilis* and carbendazim which found superior in previous trials were subjected for further evaluation and comparison in both pre and post inoculation conditions. The performance of each of these treatments were compared with control. The per cent disease index (PDI), per cent disease control (PDC) and number of days taken to start the initiation of symptoms were recorded and are presented in Table-4.17, Fig.12 and Plate-XVIII.

It is evident from the Table-4.17 that, all treatments except pre inoculation fruit dip in hot water at 52°C temperature recorded significantly lower per cent disease index over control. Among these, pre and post inoculation fruit dip in carbendazim and palm kernel oil gave significantly lower per cent disease index (3.33%). Next best treatment in order of merit was post inoculation fruit dip in *B. subtilis* at 10^8 cfu/ml (6.66%) but at par with pre inoculation dip treatment in gandobaval leaf extract (8.33%). Post inoculation fruit dip in gandobaval (10.83%) and hot water (11.67) were found moderately effective against the disease. Except pre inoculation HWT, all the treatments gave more than 88 per cent disease control over check.

Overall, the treatments given before or after inoculation, the efficacy more or less found similar except in case of HWT.

All the treatments except pre inoculation HWT significantly delayed the fruit rot initiation as compared to the control. Among these, pre inoculation fruit dip in carbendazim

leaf extract of gandobaval, palm kernel oil, cell suspension of *B. subtilis* and carbendazim which found superior in previous trials were subjected for further evaluation and comparison in both pre and post inoculation conditions. The performance of each of these treatments were compared with control. The per cent disease index (PDI), per cent disease control (PDC) and number of days taken to start the initiation of symptoms were recorded and are presented in Table-4.17, Fig.12 and Plate-XVIII.

It is evident from the Table-4.17 that, all treatments except pre inoculation fruit dip in hot water at 52°C temperature recorded significantly lower per cent disease index over control. Among these, pre and post inoculation fruit dip in carbendazim and palm kernel oil gave significantly lower per cent disease index (3.33%). Next best treatment in order of merit was post inoculation fruit dip in *B. subtilis* at 10^8 cfu/ml (6.66%) but at par with pre inoculation dip treatment in gandobaval leaf extract (8.33%). Post inoculation fruit dip in gandobaval (10.83%) and hot water (11.67) were found moderately effective against the disease. Except pre inoculation HWT, all the treatments gave more than 88 per cent disease control over check.

Overall, the treatments given before or after inoculation, the efficacy more or less found similar except in case of HWT.

All the treatments except pre inoculation HWT significantly delayed the fruit rot initiation as compared to the control. Among these, pre inoculation fruit dip in carbendazim

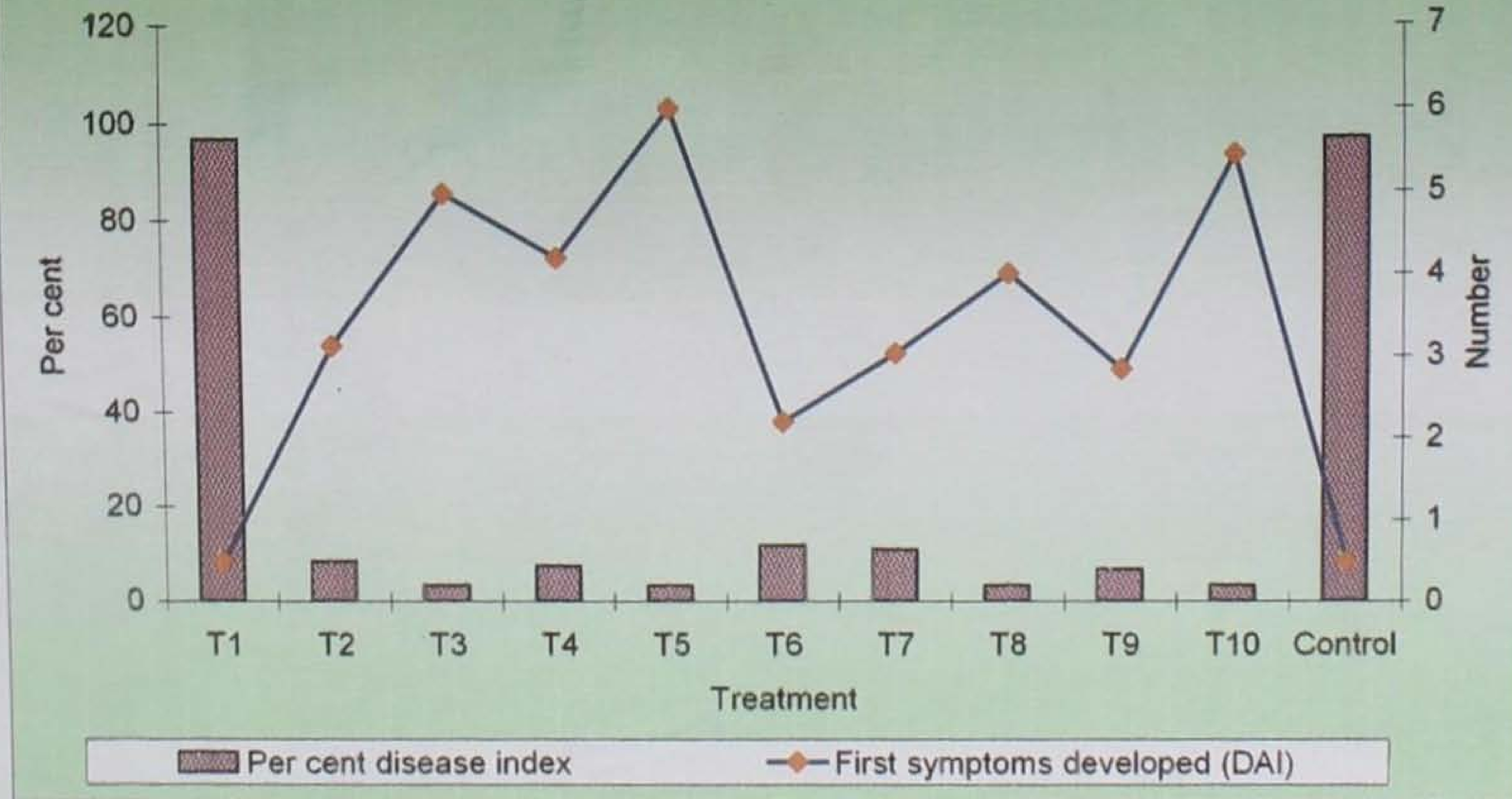
Sr. No.	Particulars	Temperature/ Concentration	Per cent disease index	PDC over check	First symptoms developed (DAI)
	<u>Pre inoculation fruit dip in</u>				
1	Hot water	52°C	89.44* (96.66)**	00.00	0.50
2	Gandobaval leaf extract	10 %	17.21 (08.33)	91.38	3.15
3	Palm kernel oil	75 %	10.51 (03.33)	96.55	5.00
4	*Bacillus subtilis* cell suspension	10^8 cfu/ml	16.66 (07.49)	92.25	4.22
5	Carbendazim (Bavistin 50 WP)	500 ppm	10.51 (3.33)	96.55	6.00
	<u>Post inoculation fruit dip in</u>				
6	Hot water	52°C	19.91 (11.67)	87.92	2.22
7	Gandobaval leaf extract	10 %	19.17 (10.83)	88.79	3.05
8	Palm kernel oil	75 %	10.51 (3.33)	96.55	4.02
9	*Bacillus subtilis* cell suspension	10^8 cfu/ml	14.94 (06.66)	93.10	2.85
10	Carbendazim (Bavistin 50 WP)	500 ppm	10.51 (3.33)	96.55	5.45
11	Control	-	89.44 (96.66)	-	0.5
	S. Em. ±		1.37		0.07
	C.D. at 5%		3.97		0.21
	C.V. %		13.51		4.28

* Figures indicate arc sine transformed values

** Figures in the parentheses indicate retransformed values

DAI Days after inoculation

Fig.-12 : Testing of physical, chemical and biological treatments for the control of fruit rot of tomato



Pre inoculation fruit dip in		Post inoculation fruit dip in	
T1	Hot water	T6	Hot water
T2	Gandobaval	T7	Gandobaval
T3	Palm kernel oil	T8	Palm kernel oil
T4	*B. subtilis*	T9	*B. subtilis*
T5	Bavistin	T10	Bavistin

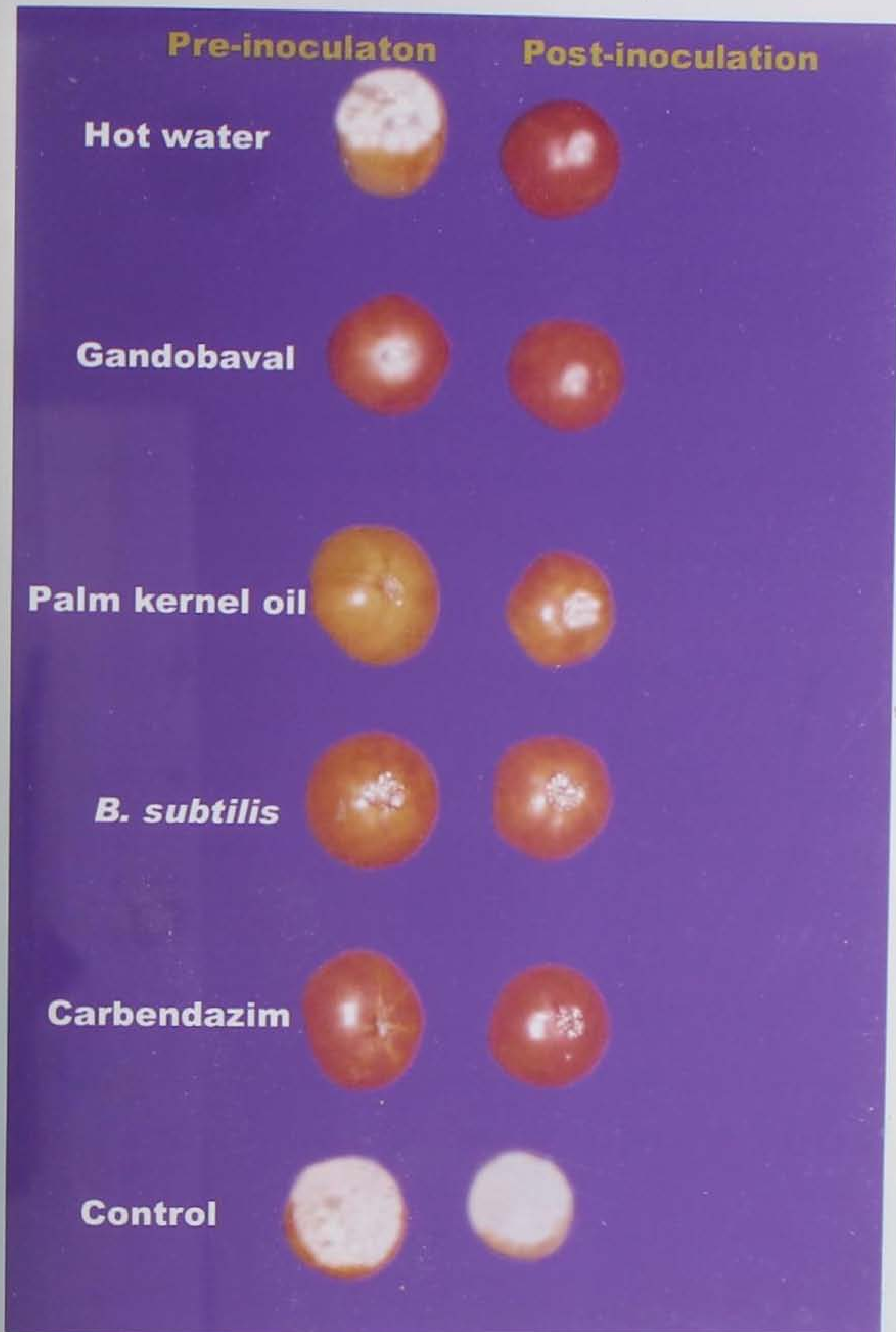


Plate-XVIII : Effect of physical, chemical and biological treatments on fruit rot of tomato

proved significantly better (6 days) in delaying the fruit rot as compared to the rest. Next best in order of merit was post inoculation fruit dip treatment in carbendazim (5.45 days) followed by, pre (4.22 days) and post inoculation dip treatment in palm kernel oil. Pre inoculation as well as post inoculation fruit dip in the extract of gandobaval and in the cell suspension of *B. subtilis* were also considerably effective in delaying the rot.

It is very clear from this study that the rotting can be avoided very effectively before or after infection by the pathogen, fruit dip in palm oil gave maximum disease control and proved as good as carbendazim. Thus instead of chemicals this oil can be used. Biological control by *B. subtilis* and phyto extracts (gandobaval) may also be very useful. The confirmation of these results in large scale experimentation, practical utility and feasibility is suggested. This can also be used as integrated fruit rot management after testing. The findings from the present investigation is the new and very useful information.

SUMMARY AND CONCLUSIONS

V SUMMARY AND CONCLUSION

Tomato is one of the important fruit vegetable of India. Fruit rot (*Fusarium solani* (Mart.) Sacc.) disease of tomato has become a major problem in recent past with a threat to successful and profitable marketing in south Gujarat. Considering this fact, the present investigation was carried out on various aspects to generate scientific information on this important pathological problem, which includes cause of the disease, identification of causal organism, symptomatology, market survey, detection of mode of entry, varietal screening, physical control, biological control, chemical control and further evaluation of physical, chemical and biological treatments for the control of the disease.

Microscopic examination and tissue isolation from the infected fruits consistently yielded the culture of *Fusarium* sp. The typical fruit rot symptoms observed in nature were small circular to irregular, brownish, water soaked lesion on the fruit surface. The lesions spread into circular to irregular large necrotic area. In severe infection, tissues got macerated and a fluffy white cottony growth of fungal mycelium was observed over the infected area on fruit surface. Finally fruit become pulpy. Fruit juice gets drained from the infected fruits and emitted an unpleasant odour.

The morphological and cultural characters of the isolated *Fusarium* sp. were studied, which exactly matched with

Fusarium solani and the identification was also confirmed by Indian Type Culture Collection, division of plant pathology, I.A.R.I., New Delhi-110 012 (I.T.C.C. No. 6123-05). The pathogenicity was carried out by three artificial inoculation methods *viz*., injury by pin prick, cork borer (Granger and Horne's (1924) method) and knife on fruits has confirmed the pathogenic nature of the fungus producing symptoms under artificial inoculation test. These were compared with those produced under natural conditions and found closely identical which have been described.

To find out the loss caused by the pathogen (*F. solani*), market survey was conducted in three different seasons *viz*., *kharif* and *rabi* seasons of 2005 and summer season of 2006 in vegetable markets at Navsari, Billimora and Surat. It revealed maximum per cent disease incidence (PDI) in market of Surat (15.25%) followed by Navsari (12.26 to 14.31 %) and Billimora (11.18 to 12.52 %). Out of three seasons, maximum fruit rot was recorded during *kharif* 2005 and minimum during *rabi* 2005. However, it was moderate during summer season of 2006. It was also revealed that majority of tomato fruits in south Gujarat markets came from Nasik and Sangam district of Maharashtra and varieties Namdhari followed by Pusa Ruby, Abhinav and Avinash were found susceptible to the disease. Local varieties (Desi) were found less prone to the disease.

Inoculation of the pathogen on tomato fruits was done through various avenues to detect the mode of entry of pathogen

into tomato fruits. Fungus failed to enter the fruits through stem end region with and without calyx and also through other areas of fruit when artificial injury was not provided. Less and moderate infection was observed when inoculated through artificially made pin and pressed injury and severe infection when inoculated through insect bored holes and knife injury at lateral region of the fruits.

Out of eight synthetic and semi synthetic solid and broth media tested, significantly higher mycelial growth of the fungus was recorded on Richards' agar (90 mm). Next best in order of merit was tomato fruit extract agar (83.83 mm) which was statistically at par with potato dextrose agar (82.83 mm). Among the liquid media, significantly higher dry mycelial weight was recorded in wheat meal broth (753.48 mg) as compared to the rest. The next best medium in order of merit was Richards' solution followed by Brown's solution, tomato fruit extract broth and potato dextrose broth. The conidial formation was of high level in wheat meal broth which was at par with Richards' solution.

Ten varieties were screened against fruit rot infection. All the varieties tested showed susceptible to highly susceptible reaction. Among them, minimum infection was recorded in PKM-1 (41.11%) followed by Junagadh Ruby (46.66%). The rest of the varieties had higher infection. The fruit rotting was initiated within two days in all the varieties screened. Among them, the earliest infection was observed in Pusa Ruby (0.5 DAI) followed

by GT-1, Pusa Early Dwarf, DT-11, NS-2535, and while in rest of the varieties infection started slightly later.

Six different temperatures were tested as hot water dip treatment against the disease. All the treatments proved significantly superior in controlling the disease as compared to control. HWT at 52°C for 5 minutes recorded significantly low PDI (11.67%) and also took long duration (2.17 days) to start initial symptoms. Maximum disease control (87.92%) was recorded in this treatment.

The phytoextracts of commonly available eleven plant species were evaluated under *in vitro* by poisoned food technique against *Fusarium solani*. The leaf extract of gandobaval exhibited maximum growth inhibition (51.76%) of the pathogen and it was significantly superior over the rest. Next best in order of merit was the extracts of black tulsi leaves followed by neem leaves, garlic bulbs, nilgiri leaves and karanj leaves.

Botanicals performed better *in vitro* trial were further tested each at 5 and 10 per cent concentrations by fruit dip technique for their efficacy against the disease. All the treatments proved significantly superior in checking the rot development over control. The leaf extracts of gandobaval (10%) was proved significantly more effective in checking the fruit rot over the rest (88.79%). Next best in order of effectiveness was neem (10 and 5%) followed by gandobaval (5%) and black tulsi (10 and 5%). Gandobaval (10%) recorded maximum PDC (88.79%) and proved as potential botanical for the management of the disease. All the

botanicals at both the concentrations tested significantly delayed the symptoms over the control. However, significantly more duration was taken in treatment of gandobaval to start the initiation of symptoms at it's 10 per cent concentration (2.97 days). Neem and gandobaval at 10 and 5 per cent concentration, respectively were next best in delaying the symptoms and found at par with each other.

Seven oils were tested to find their effectiveness against the disease. All the oils significantly checked the rot over control. Palm kernel oil and neem oil recorded minimum fruit rot and found significantly superior over the rest. Next best oil was groundnut oil. Maximum disease control was obtained in the treatment of neem oil and palm kernel oil (96.51%) followed by groundnut oil (93.02%). All the oils showed significantly longer duration over control to the initiation of symptoms. However, palm kernel oil (4.06 days) followed by neem oil (3.9 days) gave protection for longer duration but were found at par with each other.

Nine known antagonists were tested *in vitro* for their antagonism to *F. solani* by three methods *viz.*, dual culture, pathogen at centre and pathogen at periphery. Among all antagonists evaluated by three methods, *T. viride*, *B. subtilis* and *A. niger* showed strong antagonistic activity against *F. solani*.

Pre inoculated tomato fruits dipped in cell suspension of *B. subtilis* (10^8 cfu/ml) recorded significantly minimum PDI (7.50%) over the rest. Next best in order of merit was *B. subtilis*

(10^6 cfu/ml) (10.83%) followed by *T. viride* (10^8 cfu/ml) (11.66%). they were statistically at par with each other. *B. subtilis* at 10^8 cfu/ml recorded highest PDC (92.24%) over check. All the treatments took significantly maximum duration to start the initiation of symptoms after inoculation. Among them, *B. subtilis* (10^8 cfu/ml) found significantly superior over the rest (2.85 days). *B. subtilis* (10^6 cfu/ml) and *T. viride* (10^8 cfu/ml) found next best but at par with each other.

Eleven fungicides at three different concentrations were screened *in vitro* by poisoned food technique for evaluating their efficacy against *F. solani*. Carbendazim, thiophanate methyl and flusilazole were proved most effective fungicides followed by MEMC, propiconazole, carboxin + thiram, copper oxychloride and mancozeb at all the concentrations tested.

Six fungicides found superior *in vitro* trial were further tested against the disease. All the fungicidal treatments showed significantly lower fruit rot as compared to the control. Among them, fruit dip in carbendazim was found superior (3.33%) over the rest and found statistically at par with thiram (4.16%). The next best fungicide in order of merit was flusilazole. Rest of the fungicides *viz*., propiconazole, copper hydroxide and mancozeb were found moderately effective. Carbendazim, thiram and flusilazole gave more than 94 per cent disease control over check.

All fungicides gave significantly long duration protection to the fruits as compared to the control. However, carbendazim gave significantly longest time protection (5.12 days) as compared to the rest. Next best fungicide in order of merit was flusilazole. Thiram, propiconazole, copper hydroxide and mancozeb protected tomato fruits only up to 1.25 days from fusarial infection.

Further evaluation of physical, chemical and biological treatments at pre and post inoculation conditions revealed that all treatments except pre inoculation fruit dip in hot water at 52°C temperature recorded significantly lower per cent disease index over control. Among these, pre and post inoculation fruit dip in carbendazim and palm kernel oil gave significantly lower per cent disease index (3.33%). Next best treatment in order of merit was post inoculation fruit dip in *B. subtilis* at 10^8 cfu/ml which was at par with pre inoculation dip treatment in gandobaval. Post inoculation fruit dip in gandobaval (10.83%) and hot water (11.67) were found moderately effective against the disease. Except pre inoculation HWT, all the treatments gave more than 88 per cent disease control over check. Overall, the treatments given before or after inoculation, the efficacy more or less was found similar except in case of HWT.

All the treatments significantly delayed the fruit rot initiation as compared to the control. Pre inoculation dip in carbendazim proved significantly better in delaying the fruit rot by 6 days as compared to the rest. Next best in order of merit was

post harvest fruit dip in carbendazim followed by pre and post inoculation fruit dip treatment in palm kernel oil. Pre inoculation as well as post inoculation fruit dip in the extract of gandobaval and in the cell suspension of *B. subtilis* were also considerably effective in delaying the rot.

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*** Original not seen**

APPENDICES

Appendix-I: Composition of different cultural media under study

A] Semi-synthetic media

(1) Potato Dextrose Agar (PDA)

Peeled potatoes- 200.0 g

Dextrose ($C_6H_{12}O_6$) - 20.00 g

Agar agar - 20.00 g

Distilled water -1000.0ml

(2) Potato Carrot Sucrose Agar (PCSA)

Peeled potatoes- 100.0 g

Carrots- 100.0 g

Sucrose ($C_6H_{22}O_{11}$) - 20.00 g

Agar agar- 20.00 g

Distilled water- 1000.0 ml

(3) Oat meal agar

Oat meal agar- 100.00 g

Agar agar- 20.00 g

Distilled water- 1000.00 ml

(B) Synthetic media

(1) Czapek's (Dox) Agar (CzDA)

Sucrose ($C_6H_{22}O_{11}$) - 30.0 g

Sodium nitrate ($NaNO_3$) - 2.00 g

Dipotassiumhydrogen Orthophosphate(K_2HPO_4)- 1.00 g

Magnesium sulphate ($MgSO_4.7H_2O$) - 0.50 g

Potassium chloride (KCl) - 0.50 g

Ferrous sulphate ($FeSO_4$-$7H_2O$) - 0.01 g

Agar agar - 20.0 g

Distilled water- 1000.0 ml

(2) Richards' Agar (RA)

Potassium nitrate (KNO_3)-10.0 g

Potassium dihydrogen -5.00 g

Orthophosphate (KH_2PO_4)

Magnesium sulphate ($MgSO_4$-$7H_2O$)-2.50 g

Ferric chloride ($FeCl_3$-$6H_2O$)-0.02 g

Sucrose ($C_6H_{22}O_{11}$)-50.0 g

Agar agar -20.0 g

Distilled water -1000.0 ml

3) Asthana and Hawker's Agar (A & HA)

Glucose ($C_6H_{12}O_6$)-5.00 g

Potassium nitrate (KNO_3)-3.50 g

Potassium dihydrogen Orthophosphate(KH_2PO_4)-1.75g

Magnesium sulphate ($MgSO_4$-$7H_2O$)- 0.75 g

Agar agar -20.0 g

Distilled water -1000.0 ml

(4) Elliot's Agar (EA)

Sodium carbonate (Na_2CO_3) - 1.06 g

Magnesium sulphate ($MgSO_4.7H_2O$)-0.05 g

Asparagine -1.00 g

Dextrose ($C_6H_{12}O_6$)-5.00 g

Potassium dihydrogen Orthophosphate (KH_2PO_4)-1.36g

Agar agar -20.00 g

Distilled water -1000.0 ml

(5) Brown's medium (BA)

Dextrose ($C_6H_{12}O_6$)-2.00 g

Asparagine-2.00 g

Tribasic potassium phosphate (K_3PO_4)-1.25 g

Magnesium sulphate ($MgSo_4.7H_2O$)-0.75 g

Agar agar-20.00 g

Distilled water-1000.0 ml

CERTIFICATE

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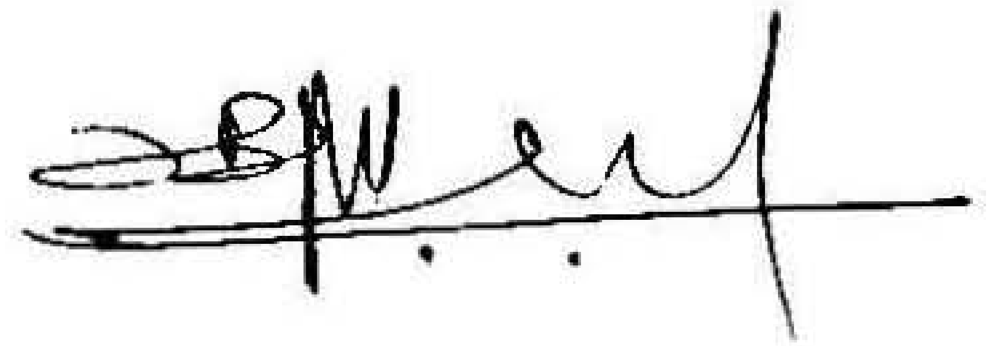

Place: Navsari.

Date : 19th August, 2006.

(Ravishankar H. B.)