STUDIES ON ONION BASAL ROT CAUSED BY *Fusarium oxysporum* Schlecht Fr f. sp. *cepae* (Hans.) Snyd. and Hans.

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1. INTRODUCTION

Onion (*Allium cepa*) is one of the major bulb crop of the world, which is grown in India. Onion has been considered as rich source of carbohydrates and minerals like phosphorus, calcium. It also contains protein and vitamin C. It is used for its flavor and pungency in daily food.

In India, onion is grown in an area of 1.02 million ha with a production of 14.82 m tonnes and productivity of 14.61t ha^{-1} (Anon., 2011). The prominent growing states are Maharashtra, Gujarat, Uttar Pradesh, Orissa, Karnataka, Tamil Nadu and Andra Pradesh. In Karnataka, it occupies an area of 0.15 m ha with the production of 2.38 m tones and productivity of 16.05 t ha^{-1} (Anon., 2011). Dharwad, Chitradurga, Bijapur, Bellary and Gulbarga are major districts of onion cultivation. Karnataka is the second leading producer of onion in India. It contributes 18.6 per cent to the total onion production in the country. In Karnataka, Dharwad occupied the highest area of 26,978 hectares and the production is 4,35,276 metric tonnes with the productivity of 16.13 tonnes per hectare. Dharwad alone contributes 20 per cent of the total state production. Chitradurga is the second leading producer in the state with area, production and productivity is 16,784 hectares, 3.33 lakh tonnes and 19.89 tonnes per hectare respectively. The four major districts *viz*., Dharwad, Chitradurga, Gadag and Bijapur contribute 59 per cent of the total production in the state.

Onion is a seasonal crop and has comparatively low storage ability and bulbs are usually stored until the harvest of next season crop or for longer period due to seasonal glut in the market. Significant losses in quality and quantity of onion occur during storage. Storage of onion bulbs has, therefore, become a serious problem in the tropical countries like India.

The major constraints in onion production are lack of varieties capable of producing high yields of uniform sized bulbs, imbalanced use of fertilizer, poor management of disease and pest. Defects which are commonly seen are premature bolting, doubling or splitting of bulb at initial stages of crop growth as well as after bulb development and also after harvesting.

Among diseases the soil borne diseases are becoming widespread and are serious enough to limit production and it includes damping off, pink rot, fusarium rot or plate rot, and smut.

Fusarium basal rot occurs in the most onion growing areas of the world; it is more prevalent where onion is grown under high temperature condition. Basal rot was first reported in United States in 1910. Basal rot of onion caused by *Fusarium oxysporum* f.sp *cepae* is an economically important and widespread disease, causing rot of basal plate of the bulb, further infection of bulb scales occurs and most severe loss are found in storage period. *F. oxysporum* f. sp. *cepae* causing fusarium basal rot in a number of *Allium* species in addition to onion, such as chive, garlic and shallot (Schwartz and Mohan, 1995). *F. oxysporum* f. sp. *cepae* produces mycelium as well as three types of asexual spores: microconidia, macroconidia and chlamydospores. Chlamydospores are produced in or on older mycelium, have one or two round cells and have thick cell walls, which defend the cells against degradation and antagonists. This type of spores helps *F. oxysporum* f. sp. *cepae* survive in the soil, in the absence of its host, for a very long time, usually indefinitely (Cramer, 2000).

As there is little work with respect to pathogen and management of the disease, the present investigation gives a clear picture of morphological, cultural and physiological characters of fungal pathogen and it also helps to develop integrated management practices by using biocontrol agents, botanicals and fungicides. Thus with the help of these strategies we can reduce the losses caused in onion due to basal rot disease and grow onion successfully. Hence, the present investigation includes the following objectives.

1. Survey and surveillance of basal rot of onion.
2. Isolation, morphology and physiological characters of the pathogen.
3. Screening of botanical, bioagents, fungicides and nutrients.
4. Management of basal rot of onion.

2. REVIEW OF LITERATURE

Onion (*Allium cepa* L.) is one of the economically important vegetable crop grown in india.The basal rot disease of onion caused by *Fusarium oxysporum* Schlechtend Fr f .sp. *cepae* (Hans) Snyd. and Hans is the most destructive disease of onion and causes yield losses in all growing areas of the world. Since there is not much research work is being carried out, a detailed study was undertaken on the disease and pathogen and the review pertaining to *Fusarium oxysporum* f.sp. *cepae* on onion are presented here under.

Disease

The genus *Fusarium* was coined by Link in 1809 for the species with fusiform, nonseptate spores borne on a stroma Booth (1971). Ozer and Koycu (1987) reported that contaminated seeds and soil have been determined as the principle source of inocula .Christopher (2000) reported the Fusarium basal rot is a root and bulb fungal disease of onion grown in temperate and sub tropical regions.

Ozer *et al*. (2004) reported that basal rot disease caused by *Fusarium oxysporum* f. sp. *cepae* Synder and Hanes is the most destructive disease of onion and causes significant yield losses in all the growing parts of the world.

Pathogen

Pryal (1909) reported for the first time the fungal pathogen *Fusarium oxysporum* Schlech Fr f .sp. *gladioli* (Massey) Snyd. & Hans. causing *Fusarium* wilt on Gladiolus from California. Massey (1926) opined that *Fusarium oxysporum* var *gladioli* was primarily a storage disease. Reported its existence for the first time from India.

Kistler (1997) reported that the formae specialis *cepae* is one of the host specific groups within *F. oxysporum*, a complex and diverse species with large diversity in specific host ranges as well as non-pathogenic forms.

Cramer (2000) reported *F. oxysporum* f. sp. *cepae* occurs worldwide, causing fusarium basal rot in a number of *Allium* species in addition to onion, such as chive, garlic and shallot.The pathogen is a deuteromycete and has no known teleomorphic (sexual) stage (Brayford, 1996). *F. oxysporum* f. sp. *cepae* produces mycelium as well as three types of asexual spores (microconidia, macroconidia and chlamydospores). Microconidia are the most commonly produced spores and are 5-12 µm in length. They are typically without septate and their shape varies from oval to kidney shaped. Macroconidia have a characteristic falcate shape making them easily identifiable. In addition, they typically have three or four septa (Cramer, 2000). Chlamydospores are produced in or on older mycelium, have one or two round cells and have thick cell walls, which defend the cells against degradation and antagonists. This type of spores helps *F. oxysporum* f. sp. *cepae* survive in the soil, in the absence of its host, for a very long time.

Symptomology

August *et al*. (1962) reported that diseased plants were stunted and yellowing and dieback of leaf-tips occurred. Pink discolorations of the roots were observed, and root development was poor.

Abawi and Lorbeer (1972) reported that, the fungus attacks seedlings, causing pre- and post emergence damping-off. In addition, root rot of older plants, stem plate discoloration, and basal rot of bulbs can be observed in the field and storage.

Entwistle (1990) reported that the fungus infects the roots or the basal plate of the bulbs. Further infection of bulb scales occurs later in the season, and most severe losses were found in post-harvest storage. The fungus is spread worldwide, and also infects other cultivated *Allium* species such as garlic, onion and shallot.

Sumner (1995) reported that Fusarium basal rot, caused by *Fusarium oxysporum* f. sp. *cepae*, is a devastating disease in onion-growing areas worldwide . Beginning from the leaf tips, progressive wilting, yellowing, curving, and eventually dying back symptoms appear on infected plants. The pathogen causes a brownish, watery rot on infected bulbs. The roots eventually rot and become covered with a whitish mycelium.

Brayford (1996) reported that the affected tissue appears brown or reddish-brown and watery when the onion was cut in half. The stem plate was often the first part of the onion to show symptoms, usually as brown discolouration or occasionally white mycelium. When the entire stem plate was fully decayed it can easily be separated from the rest of the bulb. The roots typically rot, causing the plant to die. Some bulbs that were infected in the field may appear healthy and later develop rot in storage

Sunitha (1999) explained the characteristic symptoms of the wilt disease of gladiolus caused by *F. oxysporum.* f .sp. *gladioli*. The symptoms included intervenial leaf tip yellowing which extended down the leaf and whole leaf gradually turned brown and became narrow. As infection, advanced the plant suddenly wilted or turned yellow and premature death was observed. The centre of the bulb turned black and rotted completely. Corms were depressed, leading to mummified corms.

Cramer (2000) reported that the initial symptoms of fusarium basal rot on the leaves of seedlings can be difficult to observe and plants can be killed before any other symptoms can be visually recognized. Symptoms on seedlings include delayed emergence, seedling damping-off and stunted growth. The symptoms above ground of mature bulbs are chlorosis and the curving of all leaves. The chlorosis progresses to necrosis from the tip of the leaves and downwards, eventually killing the plant.

The fungus infects the roots or the basal plate of the bulbs and infection of bulbs scales occurred later in the season and most losses occurred are found at post harvest storage period (Rabiei-Motlag *et al.* 2010).

2.1 Survey and surveillance of basal rot of onion

Bacher *et al*. (1989) reported that, onion grown on soils naturally infested with *Fusarium oxysporum* f. sp. *cepae* can have 23% field and storage losses.

Sudarshan Rao (1975) stated that, survey and surveillance form the basis for any successful plant protection strategy. Successful plant protection depends upon early detection of the disease incidence followed by timely adoption and application of preventive measures.

Padghan and Gade (2006) reported that, combination of *Fusarium oxysporum* Schelchtend. Fr. f. sp. *gerberae* Gordon and *Pythium sp*. were major causes of root rot complex of gerbera. *Fusarium oxysporum* f. sp. *gerberae* was predominant in wilted samples followed by *Pythium* sp. whereas, some samples yielded the association of both the pathogens.

Garibaldi and Minuto (2007), reported wilt disease of gerbera (*Gerbera jamesonii* cv. Excellence) plants, grown for cut flowers, in a soilless cultivation system (coconut fiber substrate) in one farm in the Cadiz area (southwestern Spain) caused by *Fusarium oxysporum.* They reported that currently, the wilt of gerbera in Spain is limited to a few farms and a very limited per cent (2 to 3%) of plants.

Sudhasha *et al*. (2008) conducted survey in important onion growing pockets in two districts of Tamil Nadu *viz*., Coimbatore and Dindigul. At each place, five points were selected. In all places, incidence of basal rot was recorded during different stages of the crop growth. Highest disease incidence was observed in Pollachi.

Rabiei –Motlag *et al*. (2010) conducted survey to estimate the frequency and occurrence of fungi associated with onion roots, samples obtained over a two year period (2007-2008). Out of 109 isolates 52 isolates were identified as *Fusarium oxysporum* Synder f. sp.*cepae.*

2.2 Isolation

Sunita and Monica (2007) isolated pathogen from infested roots, corms and collar region of the gladiolus by hyphal tip method and was identefied as *F. oxysporum* f. sp. *gladioli.*

Rabiei –Motlag *et al*. (2010) from their result showed that all the *Fusaria* that isolated from infected roots, bulbs, seeds causes basal rot in onion.

Mishra *et al*. (2010) isolated five isolates *of F. oxysporum* f. sp. *psidii* from N. States of India and they isolated these on PDA at 28±1℃ by incubating for six days.

2.2.1 Morphology

Smith *et al.* (1988) reported that, *F. oxysporum* exhibits varying cultural morphology on Potato Dextrose Agar (PDA). The aerial mycelium first appears white and then it may change to a variety of colours, ranging from violet to dark purple according to the strain of *F. oxysporum*.

Fusarium oxysporum produces three types of asexual spores *viz.,* microconidia, macroconidia and chlamydospores (Nelson *et al.,* 1983).

Burgess *et al.* (1994) stated that the pathogen *F. oxysporum* f. sp. *cepae* is one the hundreds of formae species of *Fusarium oxysporum* and produces chlamydospores, macrospores, and less often that the microspores (Havey, 1995) chlamydospores are the primary source of inocula.

McCulloch (1944) observed that, in cultures, the pathogen *F. oxysporum* f .sp. *gladioli* produced white to peach pale salmon or purple mycelium. Microconidia were abundant, hyaline, ovoid to ovate. Macroconidia were scarce, often lacking and variable, three septate. The chlamydospores were hyaline, usually vacuolated and spherical.

Sowmya (1993) studied four isolates of banana panama wilt pathogen on different nutrient media and observed maximum growth and sporulation of the pathogen on Potato sucrose agar and Richards's agar respectively.

Susan Groenewald (2005) divided the different *F. oxysporum* f. sp. *cubense* isolates, according to their morphology, into three morphological types, namely sporodochial, cottony, and slimy pinnotal, out of these sporodochial type was the most dominant morphological type.

Among ten solid media tested, maximum radial growth of *F. oxysporum* f .sp. *gladioli* was observed on potato dextrose agar followed by Richards's agar. The least mycelial growth was observed on potato carrot agar and malt extract. Mycelium was pink in potato dextrose agar and Sabouraud's agar (Kulkarni, 2006).

Sharma *et al*. (2012) isolated twenty four different isolates of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and isolated from fourteen different states including different agroclimatic condition of India from the plant showing typical wilting symptom. They studied the influences of different media on the growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* under laboratory condition in Petri plates. Different media namely Corn meal agar (CMA), V-8 juice agar medium (V-8 JA), Oat meal agar (OMA) and Potato dextrose agar (PDA) were used to find out the mycelial growth and sporulation of FOL *in vitro*. OMA was observed to be the best medium suited for the growth and sporulation of the FOL isolates.

2.2.3 Physiology

2.2.3.1 Effect of temperature

August *et al.* (1962) studied pathogenicity of four isolates of *F. oxysporum* f. sp. *cepae* at temperature range of 20 to 38℃, temp of 26℃ and higher induce the pathogenic effect. Sumner (1995) reported that the disease development was optimum when soil temperature ranged from 25 to 28℃. Sushma Gupta (1996) studied chlamydospore production at different temperature range of 15, 20, 30, 35, 40 and 45℃, maximum were observed at 35℃, and least at 20℃.

Abawi and Lorbeer (1972) studied the growth of *Fusarium oxysporum* f sp *cepae* in culture at different temperatures by making monocultural transfer to PDA plates which were incubated in the dark for several days. Effect of temperature were tested at 3℃ interval from 0 to 36℃. The optimum growth of *Fusarium oxysporum* was observed at temperature range 24 to 27℃ and no growth was observed at 0, 3, 6, 9 and 36℃.

Muthukumar (2009) reported that maximum growth of *F. oxysporum* f. sp. *polyanthi* at temperature of 28℃ on PDA.

Gupta *et al*. (2010) studied colony growth of *F. oxysporum* f. sp. *psi*dii at different temperature viz., 10, 16, 22, 28, 34, and 40℃. They observed maximum growth at 28^0C followed by 34℃. Optimum sporulation was recorded at 34℃ followed by 40℃.

Mishra *et al.* (2010) studied colony growth of 5 isolates of each *F. oxysporum* f. sp. *psidii* and *F. solani* at six different temperature viz. 10°, 16°, 22°, 28°, 34°, and 40℃. The data indicated radial growth at 28℃ (72.50 mm for both *F. oxysporum* f. sp. *psidii* and *F. solani*) followed by 34℃ (66.5 mm for *F. oxysporum* f. sp. *psidii*; 69.5 mm for *F. solani*). Optimum sporulation was recorded at of 34℃ (3.6x105 per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.9x105 per ml for macro-conidia for *F. solani* and 2.6x105 per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; 2.8x105 per ml for micro-conidia for *F. solani*) followed by 40℃ (3.2x105 per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; 3.3x105 per ml for macro-conidia for *F. solani* and 2.2x105 per ml for micro-conidia for both *F. oxysporum* f. sp. *psidii* and *F. solani*).

Sharma *et al*. (2012) isolated twenty four (24) different isolates of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) from fourteen different states having different agroclimatic conditions. They studied the influences of temperature on the growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* under laboratory condition at different temperature conditions i.e. 15-30 ℃, maximum growth and sporulation of the pathogen was recorded in temperature range of 25℃ to 30℃.

2.2.4 Hydrogen ion concentration

Jadhav *et al*. (2000) reported profuse growth and sporulation of *F. oxysporum* f. sp *sesamum* at pH 6.6 to 7.5 and for *F. chlamydosporum* at pH 6.5 which was followed by pH 6.0 and 5.5. Pokhar Rawal *et al*. (2003) reported maximum growth and sporulation of *Fusarium* sp.at pH 6.5.

Gupta *et al.* (2010) studied the mycelial growth of five isolates of *F. oxysporum* f. sp. *psidi* at 8 pH level, maximum growth was observed at pH 5.5 followed by 6.0. Maximum sporulation was recorded at pH 6.5 followed by pH 6.0.

Mishra *et al*. (2010) studied mycelial growth of the ten isolates of *Fusarium* sp. (5 isolates each of *F. oxysporum* f. sp. *psidii* and *F. solani*) at 8 pH levels 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.The maximum growth was recorded at the pH 5.5 (1208 mg for *F. oxysporum* f. sp. *psidii*; 1275 mg for *F. solani*) followed by a pH of 5.0 (956 mg for *F. oxysporum* f. sp. *psidii*; 1083 mg for *F. solani*). Maximum sporulation was recorded at a pH of 6.5 for ($4.4x10^5$ per ml for macro-conidia for *F. oxysporum* f. sp. *psidii*; $4.8x10^5$ per ml for macro-conidia for *F. solani* and $2.8x10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.9x10^5$ per ml for micro-conidia for *F. solani*) followed by a pH of 6.0 ($3.8x10^5$ per ml for macro-conidia for both of *F. oxysporum* f. sp. *psidii* and *F. solani*; $2.4x10^5$ per ml for micro-conidia for *F. oxysporum* f. sp. *psidii*; $2.5x10^5$ per ml for micro-conidia for *F. solani*).

Sushma Gupta (1996) reported maximum yield of chlamydospore when pH below 6.0 and above 7.0.

Sharma *et al*. (2012) studied the influences of pH on the growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* under laboratory condition, and reported that, slightly acidic medium with a pH of 5.5 and 6.5 supported profuse growth and conidia production of all the FOL isolates.

2.3 Management through fungicides

Sintayehu *et al*. (2011) evaluated five fungicides, Prochloraz 50 WP, Tebuconazole 25 EC, Penncozeb 80 WP, Seed plus 30 WS and Matalaxyl-m+ mancozeb 68 WG as seed bulb dressing and bulb dip treatments against basal rot in the field and storage. Bulb dressing with prochloraz, by 40%, respectively over control. These fungicides also resulted in a significant reduction in severity, basal rot affected cull bulbs on shallot. Bulb rot during three months of storage on concrete ground floor and on wire mesh shelves was also reduced by seed bulb treatment over control. The highest increase in yield was obtained from bulb dressing with prochloraz (42%) and from bulb dip treatment in seed plus (44%) over control. Fusarium basal rot caused 45% loss in yield and 12-30% of bulb loss in the storage. The study showed that basal rot of shallot can be managed effectively by seed bulb dressing or dip treatment in prochloraz or Seed plus.

Taskeen-Un-Nisa *et al.* (2011) found that all systemic fungicides at different concentrations significantly inhibited the mycelial growth of *F. oxysporum.* However, the hexaconozole at highest concentration (1000 ppm) caused highest reduction of mycelail growth (8.80 mm) followed by carbendazim (9.40 mm), bitertanol (18.60 mm) and myclobutanil (20 mm) at the same concentration. It was also observed from the study that amongst the non-systemic fungicides, mancozeb was found most effective (14.20 mm) in reducing mycelia growth of the fungi followed by captan (20.00 mm) and zineb (22.00 mm).

Naik and Burden (2009) evaluated fungicide treatments in two field trials. The mean establishment was 47 per cent. In the first trial involving dusting of the sets before planting Granosan 200 (benomyl 15% + mancozeb 60%) increased establishment by 28%, reduced basal rot of harvested bulbs by 77 per cent and increased yield by 106 per cent. Benomyl decreased basal rot and increased yields but captan and thiram treatments were ineffective. When bulbs from this trial were stored for six weeks under ambient conditions losses were 94% in controls and 45% in the benomyl + mancozeb treatment, with losses from other treatments intermediate. In the second trial, using pre-planting dips of benomyl the optimum concentration/time of 100 µ/ml for 15 min, reduced basal rot by 65 per cent and increased yield by 54 per cent.

Musmade *et al.* (2009) evaluated fungicides (Carbendazim and Thiram) both *in vitro* and *in vivo* conditions. *In vitro* evaluation, of Carbendazim (0.1%) completely inhibited the growth of tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* and was found significantly superior over the rest of fungicides. Under field condition, seedling dip treatment of Carbendazim (1 gl^{-1} water) was found most significant followed by Carbendazim+*T. viride* (1+100 gl^{-1} water) and *T. viride* (100 gl^{-1} water) significantly reduced wilt incidence by 73.91, 69.56 and 68.11 per cent respectively as against 71.88 per cent wilting in control (under epiphytotic condition i.e. wilt sick soil).

Gullino *et al.* (2002) tested strobilurins, azoxystrobin, kresoxym-methyl and trifloxystrobin, in experimental trials in the growth chamber or glasshouse against Fusarium wilts of carnation (*F. oxysporum* f. sp. *dianthi)*, cyclamen (*F. oxysporum* f. sp. *cyclaminis*) and Paris daisy (*F. oxysporum* f. sp. *chrysanthemi)*, in comparison with benomyl and in some experiments prochloraz. The three strobilurins controlled Fusarium wilt on carnation when applied at transplant at 1–2 g/m^2 as soil drenching. Azoxystrobin, at 250 mg/l of medium controlled also Fusarium wilt on cyclamen and on Paris daisy. Kresoxim methyl at 250 mg/l controlled Fusarium wilt on cyclamen but it was phytotoxic; at the same dosage it was not effective on Paris daisy. Trifloxystrobin was only partially or not effective in controlling Fusarium wilt on cyclamen and Paris daisy. Particularly kresoxim-methyl caused chlorosis and plant stunting. This study shows the high efficacy of azoxystrobin against Fusarium wilts of three important ornamental crops. Azoxystrobin provided control similar or better to those shown by benomyl, applied at higher dosages in all trials.

3.3.1 Management through bioagents

Coskuntuna and Ozer (2007) reported that *Trichoderma harzianum* inhibit the growth of FOC16 isolate at rate of 73.3% in dual culture.

Malathi and Mohan (2011) evaluated the efficacy of biocontrol agents for the potential to manage the basal rot of onion caused by *F. oxysporum*.f. sp. *cepae*. *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas* sp. were collected from different onion growing areas of Tamil Nadu and were tested for their antagonistic activity against the pathogen by dual culture technique. Among the tested isolates of *Trichoderma* sp., *T. harzianum* (TH3) recorded the maximum (82.77%) inhibition followed by *T. viride* (TV5) which recorded 78.52 per cent inhibition on the mycelial growth. Among the sixty two isolates of *Pseudomonas* sp., Pf 12 significantly exerted the highest (74.68) per cent reduction of mycelia growth of pathogen.

Shanmugam *et al.*(2011) reported that Talc-based formulations of plant growth promoting rhizobacterial strain S2BC-2 (*Bacillus atrophaeus*) and strain mixture, S2BC-2 þ TEPF-Sungal (*Burkholderia cepacia*), inhibitory to the growth of *Fusarium oxysporum* f. sp. *gladioli* (FOG), were developed for corm dressing and soil application in gladiolus. In comparison to the individual strain, the strain mixture recorded maximum spike and corm production of 100 and 150%, respectively with less vascular wilt and corm rot incidences of 73.6 and 54.8% reduction over the pathogen control in greenhouse when inoculated with FOG.

Mishra and Prasad (2004) tested the biogens like *Aspergillus niger, Trichoderma* sp. *Penicillum citrinum*,and stated that *Aspergillus niger* was fast growing and most effective.

Mishra *et al.* (2010) reported that *Trichoderma virens* and *T. viride* were superior in inhibiting the growth of *Fusarium sp*.

Mishra *et al*. (2005) reported integrated and biological control of gladiolus corm rot and wilt caused by *Fusarium oxysporum* f. sp. *gladioli*. Isolate of *T. virens* Miller and carboxin, individually and in combined form, significantly reduced disease incidence in both glasshouse and field conditions. Mishra *et al.* (2010) reported that *Trichoderma* and *Aspergillus* were effective in complete suppression of wilt incidence.

Edirisinghe and Deshapriya (2011) reported that under *in vitro* condition dual culture of the pathogen with the *Trichoderma* isolate on Potato Dextrose Agar (PDA) indicated that the *Trichoderma* isolate was capable of controlling the growth of the onion basal rot pathogen *i.e. F. oxysporum.* The isolated *Trichoderma* species produced loops and clamps around the pathogen hyphae restricting the growth of the pathogenic fungus. In addition, the *Trichoderma* isolate was able to utilize chitin and glucan as the sole C source when grown on modified Czapek – Dox Agar medium indicating the involvement of extracellular enzymes.

Kamala and Indira Devi (2012) reported that out of the 114 isolates of *Trichoderma*, 80 per cent shows high degree of antagonism against *Fusarium oxysporum* while 68 per cent *Trichoderma* isolates gives strong activity against *Rhizoctonia solani*. Based on their antifungal activity in dual plate assay, 25 isolates were selected for further analysis. The interaction between the *Trichoderma* and fungal pathogens were examined microscopically. Several biocontrol mechanisms were studied and analysis data showed that the clearing zone diameter of protease activity of these indigenous *Trichoderma* isolates ranges from 10 to 60 mm. Among them, 84% gave high chitinase activity and their activity ranges between 10 to 85 mm. whereas, ß-1,3-glucanases activity showed a clearing zone diameter ranging from 10 to 70 mm. Based on their relative biocontrol potency, three indigenous *Trichoderma* isolates (T10, T17 and T83) were selected for pot culture experiment for testing their biocontrol efficacy against wilting and damping off diseases of common beans. Among all the treatments, T83 showed better biocontrol efficacy against the two test fungus as compared to the exotic *Trichoderma harzianum* (ITCC No. 6276) strain.

2.3.2 Management through botanicals

Bowers and James (2000) investigated the effect of several commercial formulations of botanical extracts and oils for control of Fusarium wilt diseases. Pepper/mustard, Cassia and clove extracts added as 10 per cent aqueous emulsions reduced the population density of *F. oxysporum* f. sp*. chrysanthemi* Littrell, Armstr & Armstr by 99.9, 96.1, and 97.55 per cent respectively. In a green house experiment, Pepper/Mustard, Cassia, and clove extracts added to soil as 1, 5, and 10 per cent aqueous emulsions suppressed disease development.

Bhatnagar *et al.* (2004) reported that the highest percentage of inhibition for *Fusarium oxysporum* f.sp *psidi* was achieved by extacts from *Achyranthes rosea*, *Curcuma longa* L.

Shrivastava and Yadav (2008) reported that the flower extrect of desmostachya bipinnata linn was found to be maximum fungitoxic and inhibited 96%mycelial growth,fallowed by *Callistemon lanceolatus* DC (92%),*Rumex dentus* Linn (83%).

Kulkarni (2006) reported that among the ten botanicals tested *in vitro* against *F. oxysporum* f .sp. *gladioli*, neem seed kernal extract at 10 per cent (54.49%) was found superior followed by custard apple leaf extract at 10 per cent (52.50%).

Kumar *et al*. (2007) reported that the oil of *Chenopodium ambrosioides* can completely inhibit the mycelia growth of *Aspergillus flavus* Link and *Fusarium oxysporum* at 100 ppm

Uzma sitara *et al*.(2008) Essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) were evaluated for their antifungal activity @ 0.5, 0.1 and 0.15% against eight seed borne fungi viz., *Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, F. nivale, F. semitectum, Drechslera hawiinesis* and *Alternaria alternata*. Ridomyl gold (MZ 68%WP) was used for comparison. All the oils extracted except mustard, showed fungicidal activity of varying degree against test species. Of these oils, Asafoetida oil @ 0.1% and 0.15% significantly inhibited the growth of all test fungi except *A. flavus* and *Nigella sativa* oil @ 0.15 was also effective but showed little fungicidal activity against *A. niger* followed by neem, Ridomyl gold and mustard oils.

2.3.3 Management through nutrients

Prabhu *et al.* (2007) observed that application of K either before or after planting has effective in reducing the incidence of fusarium wilt and root rot caused by *Fusarium oxysporum* in case of cotton and other crops.

Sanjeev and Eswaran (2008) conducted experiment to know the effect of micronutrients on the growth of the *Fusarum oxysporum* f.sp *cubense,* among the eight micronutrients they tested borax recorded 100% control fallowed by zinc sulphate.

Sohaibani (2011) observed Variation among four organic acids (tannic acid, oxalic acid salicylic acid and ascorbic acid), four salts (Potassium chloride, dipotassium phosphate, sodium chloride and disodium phosphate) and two growth regulators (Indole acetic acid and indole butyric acid) on the percentages of root rot disease caused by *Macrophomina Phaseolina, Rhizoctonia solani* and *Fusarium oxysporum* f. sp*. basilici.* Salicylic acid was the most effective treatment at 8 mM for decreasing pre-emergence damping off in case of *M. phaseolina* and *F. oxysporum* f. sp. *basilici* while, oxalic acid was the most effective acids as inducer in case of *R. solani.* Potassium chloride at 4% was the most effective salt as inducer for decreasing emergence of damping off.

Kolaeia (2012) reported that, in the search for alternatives to synthetic fungicides to control postharvest disease, they were evaluated sulfur-containing salts for their effects on the mycelial growth of various fungal or fungus-like pathogens and their ability to control carrot cavity spot *(Pythium sulcatum*) and potato dry rot (*Fusarium sambucinum*). Results showed that metabisulfite-containing salts provided strong inhibition of all the tested fungi. Furthermore, some sulfate-containing salts were also directly inhibitory to *P. sulcatum* (calcium sulfate and ammonium sulfate) and to *F. sambucinum* (sodium sulfate). The metabisulfite salts also provided 100% inhibition of cavity spot and dry rot at concentrations of 50 and 200 mM, respectively. Calcium sulfate and sodium sulfate also significantly reduced carrot cavity spot lesions at 50 mM and ammonium sulfate, magnesium sulfate, potassium sulfate and sodium sulfate reduced potato dry rot lesions at 200 mM. These results indicate that various sulfate and metabisulfite salts could be used to control these post-harvest microorganisms.

3. MATERIAL AND METHODS

Present investigations were carried out during 2011-12. Laboratory experiments were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka. Survey for basal rot of onion was undertaken in Bijapur, Dharwad, Gadag, and Uttar Kannada districts of Karnataka.

3.1 General procedure

3.1.1 Cleaning of glassware

For all laboratory experimental studies, Corning and Borosil glassware were used. They were kept for a day in the cleaning solution containing 60.0 g potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water. Then they were cleaned by washing with detergent solution followed by rinsing several times in tap water and finally in distilled water.

3.1.2 Sterilization

All the glassware used in the studies were sterilized in an autoclave at 1.1 kg/cm^2 pressure for 20 min and kept in hot air oven at 60°C for one hour. Both solid and liquid media were sterilized at 1.1 kg/cm^2 pressure for 15 minutes.

3.2 Survey and surveillance for the incidence of the disease

Rapid roving survey was conducted during 20011-12, to know the incidence of basal rot disease, in onion growing districts viz., Bijapur, Dharwad, Gadag, and Kumta area of Uttar Kannada districts. After the harvest of the bulbs, farmers adopt sun drying of the bulbs, during that time, 100 bulbs were collected randomly and from that bulbs showing characteristic symptoms of basal rot were collected and per cent incidence was calculated by following formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of bulbs showing basal rot symptom}}{\text{Total No. of bulbs observed}} \times 100$$

3.2.1 Isolation of the pathogen

The infected bulbs showing typical symptoms of basal rot disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 60 seconds and washed separately in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized petriplates containing potato dextrose agar (PDA). The petriplates were incubated at room temperature (27±1°C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27±1°C for 15 days. Then such slants were used to study characters.

3.2.2 Hyphal tip isolation

This method was followed for maintaining pure culture, since this fungus is known to be heterokaryotic in nature. Hyphal tip isolation was done on water agar plates. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Single spore was then marked under the microscopic field with ink on the glass surface of the plate and it was allowed to germinate. Such plates were incubated at 27±1^{0}C and periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 27±1°C for 10 days. Later, mycelial bits of the fungus were placed in the center of Petriplates containing potato dextrose agar medium and incubated at 27±1°C for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such culture was used for further studies.

3.2.3 Maintenance of the culture

The fungal pathogen was subcultured on PDA slants and allowed to grow at 27±1^{0}C for ten days and such slants were preserved in a refrigerator at 5^{0}C and revived once in 30 days.

Identification of the pathogen

The morphological characters of the fungus such as mycelial culture obtained was compared with the original description of the fungus.

Mass Multiplication of *F. oxysporum* f .sp. *cepae*

Sand- maize medium was prepared in the proportion 95:5 in order to get maximum inoculum of the fungus. About 400g of Sand- corn meal medium was taken in 1000ml flask and watered to 20 percent of its weight and sterilized at 1.33 kg/sq.cm for one hour. The pure culture of *F. oxysporum.* f .sp. *cepae* was inoculated separately to the flask under aseptic condition and incubated at 27±1ºC for 15 days. The flasks were shaken on alternate days to get uniform growth. The giant culture so obtained was used for preparing sick soil for further studies.

Proving the pathogenicity

Onion was planted in a steam sterilized potting media consisting of soil: sand: farm yard manure in 3:1:1 ratio. Further sick soil was made by inoculating the giant culture of *F. oxysporum.* f. sp. *cepae* to the sterile soil. A control treatment was maintained without adding the inoculum. Observations were made regularly for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected bulbs. The isolate obtained was compared with the original culture for confirmation.

3.3 Cultural studies

3.3.1 Growth characters on solid media

The growth characters of *Fusarium oxysporum* f .sp. *cepae* were studied on six solid media *viz.,* Potato Dextrose Agar, Oat Meal Agar, Richards's agar, Czapek's agar, Sabouraud's agar and Rose Bengal agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1°C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete petriplate in any one of the media. The colony diameter and sporulation was recorded. The data on radial growth was analyzed statistically. The composition of each medium used is furnished below.

1. Potato dextrose agar (Tuite, 1969)

Peeled and sliced potatoes	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

The potatoes were boiled in 400 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was added to the mixture. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

2. Oat meal agar (Tuite, 1969)

Oat flakes	30 g
Agar-agar	20 g
Distilled water	1000 ml

Oat flakes were boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made up to 1000 ml with distilled water and sterilised at 1.1 kg/cm^2 pressure for 15 min.

3. Sabouraud's agar

Dextrose ($C_6H_{12}O_6$)	20 g
Peptone	10 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

Agar-agar was melted in 400 ml of distilled water. All other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. This was sterilized at 1.1 kg/cm^2 pressure for 15 min.

4. Richards's agar (Ainsworth, 1971)

Sucrose ($C_{12}H_{22}O_{11}$)	50 g
Potassium dihydrogen phosphate (KH_2PO_4)	5 g
Potassium nitrate (KNO_3)	10 g
Magnesium sulphate ($MgSO_4.7H_2O$)	2.5 g
Ferric chloride ($FeCl_3.6H_2O$)	0.02 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made up to 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at 1.1 kg/cm^2 pressure for 15 min and subsequently mixed together.

5. Czapek's agar (Tuite, 1969)

Sucrose ($C_{12}H_{22}O_{11}$)	30 g
Sodium nitrate ($NaNO_3$)	2 g
Potassium dihydrogen phosphate (K_2HPO_4)	1 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5g
Potassium chloride (KCl)	0.5g
Ferrous sulphate ($FeSO_4.7H_2O$)	0.01g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

Agar-agar was melted in 500 ml of distilled water. All the other ingredients were mixed in 400 ml of distilled water. The two solutions were mixed thoroughly. The volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

3.3.2 Growth phase

The growth phase study was conducted on potato dextrose broth. Thirty ml of broth was added in each of the 100 ml conical flasks and sterilized at 1.1 kg/cm^2 pressure for 15 min. These flasks were allowed to cool and 5 mm disc of *F. oxysporum* f. sp. *cepae* was inoculated to each of the conical flasks. They were incubated at 27±1°C. Each treatment was replicated thrice. Culture was filtered through Whatman No. 42 filter paper disc of 12.15 cm diameter, which was dried to a constant weight at 60°C in an electrical oven, prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any

salts likely to be associated with it. One set of flasks was harvested on second day. Subsequent harvesting was done at an interval of two days upto 20th day. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60°C, cooled in a dessicator and weighed immediately in an analytical electric balance & dry mycelial weight was calculated. Results were analyzed statistically.

3.3.3 Growth in liquid media

The liquid media used were same as that of solid media. The composition and preparations of different liquid media used were the same as that of solid media except that the agar-agar was not added. Thirty ml of the medium was added to each of 100 ml flask. All the flasks were sterilized at 1.1 kg/cm^2 pressure for 15 min. Inoculum disc of five mm size was inoculated to all flasks and incubated at 27±1°C for 12 days. Each treatment was replicated thrice. The mycelial mat was harvested, dried and weighed as described above. The best synthetic medium was found out and used as a basal medium for further studies.

3.4 Physiological studies

3.4.1 Temperature requirement

Potato dextrose liquid medium was used in this experiment. Conical flasks of 100 ml capacity and each containing 30 ml of liquid medium were inoculated with 5 mm mycelial disc and incubated at different temperature levels *viz.,* 20, 25, 30, 35 and 40°C. In each case, three replications were maintained. The dry mycelial weight at each temperature level was recorded after incubating for ten days and the results were analyzed statistically.

3.4.2 Hydrogen ion concentration

pH of the liquid media was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). Potato dextrose liquid medium was used as a basal medium. The reaction of the medium was adjusted to the desired pH by using di-hydrogen phophate citric acid buffer according to schedule of Vogel (1951). The pH of the medium was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9 .0. After sterilization there was slight change in pH, which was negligible. The culture was inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 27±1°C for ten days. Three replications were maintained in each treatment. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

3.5 Management studies

3.5.1 *In vitro* evaluation of biocontrol agents

Six biocontrol agents such as, *Trichoderma harzianum*, *Trichoderma viride* Persex. Fr., *Trichoderma koningii* Oudern*, Trichoderma virens* Miller, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn Emend Pras were tested against *Fusarium oxysporum.*f.sp. *cepae.* The biocontrol agents and test fungus were cultured on potato dextrose agar by using dual culture technique.

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus was inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at ends of the petriplates and mycelial discs of the fungus was placed at the centre. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The percent inhibition of the growth of the pathogen was calculated by using the formula (Vincent, 1947).

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

Where,

I = Per cent inhibition.

C = Radial growth in control.

T = Radial growth in treatment.

3.5.2 *In vitro* evaluation of botanicals

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some plant extracts. The following plant leaf extracts were selected.

List of botanicals

Common name	Botanicals	Plant parts used	Concentration (%)
Garlic	*Allium sativum*	Clove	5, 7.5, 10
Onion	*Allium cepa*	Bulb	
Neem	*Azaridicta indica*	Seed	
Datura	*Datura stromenium L.*	Leaf	
Lantana	*Lantana camera*	Leaf	
Tulsi	*Ocimum sanctum*	Leaf	
Prickly chaff flower	*Achyrantus rosea.*	Leaf	
Turmeric	*Curcuma longa*	Rhizome	

3.5.2.1 Preparation of cold aqueous extract

Fresh plant leaf materials and seeds were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1982). Five, seven point five and ten ml of stock solution was mixed with 95, 92.5 and 90 ml of sterilized molten PDA media, respectively so as to get 5, 7.5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile petriplates, mycelium of five mm size discs form periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1°C temperature for ten days and radial growth was taken when maximum growth occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the formula suggested by Vincent (1947).

3.5.3 *In vitro* evaluation of fungicides

The efficacy of seven systemic fungicides (at the concentration of 0.025, 0.05 and 0.1 per cent) and seven non-systemic fungicides (at the concentrations of 0.1, 0.2 and 0.3 per cent) were assayed. The fungicides used are given here under.

List of systemic fungicides

Common name	Formulation	Trade name	Concentration (%)		
Thiophenate methyl	70% WP	Topsin	0.025	0.05	0.1
Carbendazium	50% WP	Bavistin			
Benomyl	50% WP	Benlate			
Tebuconazole	25% EC	Folicur			
Propiconazole	25% EC	Tilt			
Diffenconazole	25% EC	Score			
Hexaconazole	5% EC	Contaf			

List of Non systemic chemicals and combi products

Common Name	Formulation	Trade name	Concentration (%)		
Captan	50% WP	Captaf	0.1	0.2	0.3
Mancozeb	75% WP	Dithane M-45			
Mancozeb 63% + Carbendazib 12%	72 % WP	Saaf			
Carboxin 37.5% + Thiram 37%	75% WP	Vitavax power			
Zineb 64% + Hexaconazole 4%	68% WP	Avatar			
Trifloxystrobin 25% + Tebuconazole 50%	75% WP	Natio			
Copper oxychloride	50% WP	Blue copper			

Required quantity of individual fungicide was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of fungicides. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated thrice. Then such plates were incubated at room temperature for ten days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947).

3.5.4 *In vitro* evaluation of nutrients

Potato dextrose agar medium was amended with different nutrients *viz*., zinc sulphate, potassium chloride, magnesium sulphate at different concentrations *viz*., 100, 250, 500ppm. Mycelial growth was tested by using poison food technique.

3.6 *In vivo* studies

A pot culture experiment was conducted in the glasshouse of University of Agricultural Sciences, Dharwad to find out the best treatment for control of basal rot caused by *Fusarium oxysporum* f.sp *cepae.* The fungus was multiplied by inoculating in sand maiz medium (Muthusamy 1972). Sand and ground maize seed were mixed at the ratio of 95:5, moistened and autoclaved at 1.33kg/cm^2 for an hour about 2-3 times consecutively. In this sterilized medium, one week old culture of *F oxysporum* f.*sp cepae* was inoculated and incubated at 27±1℃ for 14 days. Sieved garden soil was autoclaved for 2hour about 2days consecutively. About six kg of sterile soil was filled in each earthern pots of 12" size. Inoculum multiplied on sand maize medium was incorporated at 10g/pot. The onion seeds were surface sterilized by immersing them in 1% solution of sodium hypochlorite for 15min, rinsing in sterile distilled water and air dried. Later the seeds were treated with *T. harzianum* in 50ml conical flasks. The flasks gently rotated for 10 min to distribute the powder homogenously. Then the seeds were sown in earthen pots containing sick soil (Sudhasha 2008 and Coskuntuna, 2008). Each treatment was replicated thrice. After proper establishment of the seedlings, the effective fungicides, botanicals, and bioagents evaluated in *in vitro* studies were tested in pot culture, thereby percent disease incidence and post harvest loss over control were calculated.

The post harvest loss over control was calculated by the formula.

$$\text{The post harvest loss over control} = \frac{\text{Yield in treatment - Yield in control.}}{\text{Yield in treatment}} \times 100$$

Treatments

1. Seed treatment with *T. harzianum* + Soil application of *T. harzianum* 0.4%.
2. Seed treatment with *T. harzianum* + Drenching with tebuconazole 0.1%.
3. Seed treatment with *T. harzianum* + Drenching with mancozeb 0.3%.
4. Seed treatment with *T. harzianum* + Drenching with NSKE 10%.
5. Seed treatment with *T. harzianum* + Drenching with mancozeb 63%+ carbendazim 12% 0.3%
6. Seed treatment with *T. harzianum* + control.
7. Untreated Control

Observations were recorded for per cent disease incidence, bulb yield, post harvest losses.

4. EXPERIMENTAL RESULTS

The results of investigation on basal rot of onion caused by *Fusarium oxysporum* f .sp. *cepae* Snyd. & Hans. during the period 2011 to 2012 with reference to survey and surveillance of disease; cultural, morphological, physiological aspects of pathogen; management of disease by fungicides, bioagents, botanicals and results so obtained are presented here under.

4.1 Survey for the incidence of basal rot disease of onion

An intensive roving survey was conducted during 2011–12 in different villages of Bijapur, Dharwad, Gadag and Uttar Kannada districts, to know the incidence of the disease. The data pertaining to this study are given in Table 1.

The results indicated that, the per cent disease incidence was noticed in all the locations surveyed with a range of 11.71 to 42.77 per cent. The maximum disease incidence was noticed in Dharwad district (36.18%) followed by Gadag district (35.95%) and Uttar Kannada (28.09%). Least disease incidence was recorded in Bijapur district (15.30%). Among the villages surveyed highest per cent incidence 42.77 was recorded in Annigere village of Dharwad district followed by 41.56 per cent in Asundi and 38.32 per cent in Anturu bantur (Gadag). The least per cent incidence 11.71 observed in Managoli village (Bijapur). The disease was observed in all the varieties *viz.*, Telgi red, Nasik red, N-53, Bellary red and Local varieties.

4.2 Symptomatology

F. oxysporum f .sp.*cepae* was one of the most important fungal pathogens of onion, which showed characteristic symptoms on leaves and bulbs. The first above symptoms is a yellowing of leaf blades at the tip. This yellowing progresses downward to entire leaf blade. Later such leaves shrivel and decay. Infected plants can be pulled easily because they have a retarded root system. Affected roots are dark brown, flattened, hollow, and transparent.

The discoloration starts at the outermost layer of the stem plate and extends upward. At later stage, the stem plate tissue become pitted and exhibited a dry rot.

4.2.1 Isolation of Pathogen.

Standard tissue isolation technique was followed to get culture of causal organism from diseased bulbs. Hyphal tip method was used to obtain pure culture as detailed in material and methods.

4.2.2 Proving pathogenicity.

Artificial inoculation of fusarium pathogen were carried out as explained in material and methods and symptoms developed were recorded. The inoculated onion plants started showing symptoms like yellowing of leaves from 21 days after inoculation and the plants started wilting (Plate 1) and died within 45 days after inoculation. *Fusarium* was re-isolated from the artificially inoculated onion roots.

4.2.3 Identification of Pathogen

The culture isolated from rotted bulbs of onion and causal organism was identified as *F. oxysporum* f. sp.*cepae* based on the morphological and cultural characters as per Hanson (1971) and by its pathogenicity on onion. The fungus produced white, cottony mycelium with abundant microconidia, hyaline, continuous or 1-septate, ovoid to ovate and measured 3.5 - 8.0 x 2.5 – 3.5 µm(average 6x2.8µm). Macroconidia were sparse and variable, 3 septate or rarely 4–5 septate measured 19.5 – 29.5 x 3 – 5 µm(average 25x3.8µm). Chlamydospores were hyaline, usually vacuolated and spherical, measured 4.5 – 9.5 µm(average 6.5 µm) in diameter.

Table 1: Survey for basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae* during 2011-12

District	Taluk	Village	Variety	Stage of crop	Type of soil	PDI
Bijapur	Bijapur	Hitnali	Telgi Red	After harvest	Black	17.54
		Jumnal	Telgi Red	After harvest	Black	13.05
	Basavana bagewadi	Telgi	Telgi Red	After harvest	Black	14.19
		Muttagi	Telgi Red	After harvest	Black	20.82
		Managoli	Bijapur local	After harvest	Black	11.71
District mean						**15.30**
Dharwad	Dharwad	Shivalli	Nasik Red	After Harvest	Black	35.76
		Somapur	Nasik Red	Harvesting	Black	36.19
	Hubli	Annigere	Nasik Red	After Harvest	Black	42.77
		Hebsur	N-53	Harvesting	Black	30.00
District mean						**36.18**
Gadag	Gadag	Asundi	Bellary local	After Harvest	Black	41.56
		Anturu bantur	Bellary local	Harvesting	Black	38.32
		Hulkoti	Bellary local	Harvesting	Black	37.30
		Lakkundi	N-53	Harvesting	Black	26.68
District mean						**35.95**
Uttar Kannada	Kumta	Alvikodi	Local variety	After Harvest	Sandy	26.54
		Handigona	Local variety	After Harvest	Sandy	29.65
District mean						**28.09**

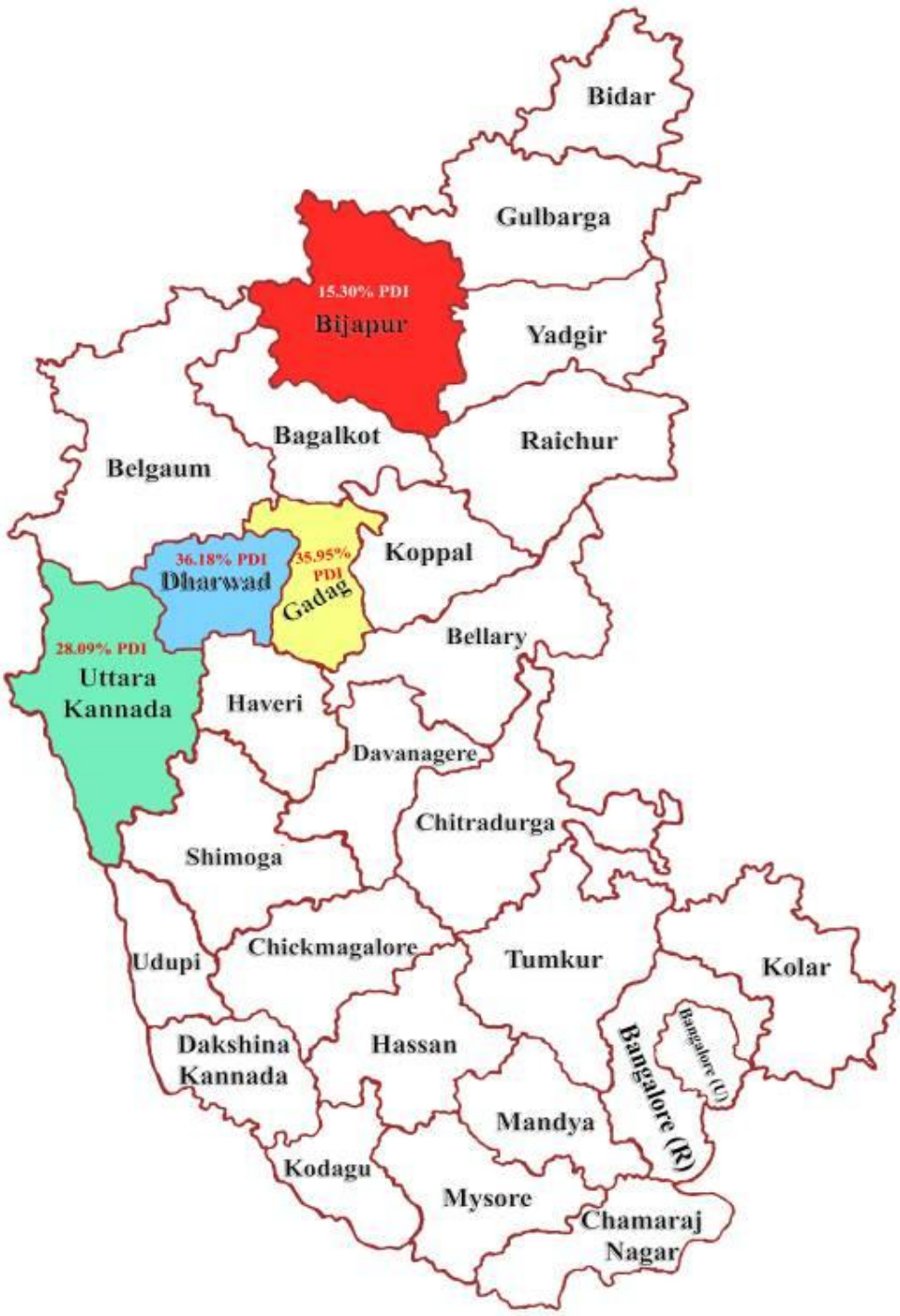


Fig. 1: Map of Karnataka showing the districts surveyed for basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae* during 2011-12

Pathogenicity test

Pathogenicity test

Retarded roots

Retarded roots

Symptoms on bulb

Symptoms on bulb

Plate 1: Symptoms of basal rot of onion

4.3 Morphological and cultural studies of pathogens

4.3.1 Morphological studies of *F. oxysporum.* f. sp. *cepae.*

Microconidia : Microconidia were abundant, hyaline, continuous, or single septate, ovoid and Ovate and measured 3.5-8.0 x 2.5-3.5 µm (Average 6x2.8µm)

Macroconidia : Macroconidia were sparse, fusoid, and variable, usually 3 septate, rarely 4-5septate, measuring 19.5-29.5 x 3.0-5.0 µm (Average 25 x 3.8 µm)..

Chlamydospores : Chlamydospores were hyaline, usually vacuolated, spherical and with no septa, produced either singly or in chain and 4.5 – 9.5 µm in diameter (Average 6.5 µm diameter)..

4.3.2 Cultural studies

4.3.2a Growth phase

The experiment was conducted as detailed in material and methods to ascertain the period for the maximum growth of the fungus by dry mycelial weight method, starting from the 2^{nd} day to 20^{th} day. The results obtained are presented in the Table 2.

The data from table 2 indicated that, there were significant differences in the different incubation periods. Dry mycelial weight of *F. oxysporum* f. sp. *cepae* recorded gradual increase starting from second day (43.33mg) and reached peak growth on 10^{th}day (360.00 mg), and remained significantly superior over remaining treatments. Later the dry mycelia weight declined to reach 140.00 mg on 20^{th} day of inocubation.

As highest growth of the fungus was recorded at 10^{th} day it was taken for further studies.

4.3.2b Growth characters on different solid media

Diversity in cultural and morphological characters of *F. oxysporum* f. sp*. cepae* were studied in different media at room temperature 27 ± 1^{o} C as described in "Material and Methods" and the results obtained are presented in Table 3.

The radial growth and sporulation of the fungus were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungi differed significantly. The maximum radial growth was observed on Oatmeal agar (89.62 mm) which is on par with Potato dextrose agar (87.40 mm) followed by Richard's agar (86.05 mm). The minimum radial growth was obtained on Sabouraud's agar (72.22 mm) followed by Rose Bengal agar (76.60 mm),Czapek's dox agar (78.57 mm).

Sporulation was also showed greater variation in different media, ranging from excellent to poor sporulation. Excellent sporulation was recorded on Potato dextrose agar, Richards's agar and Czapek's Dox. It was moderate in Oatmeal agar and Sabouraud's agar. Poor sporulation was observed in Rose Bengal agar (Table 3).

4.3.2c Growth in different liquid media:

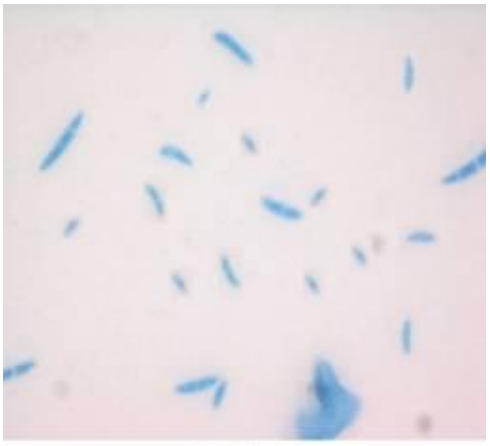
The experiment was conducted as explained in material and methods to find out the best liquid medium for mycelial growth of *F. oxysporum* f .sp. *cepae* by average dry mycelial weight of fungus after 10 days of incubation is given in Table 4.

The results indicated that, there were significant differences among the different liquid media on growth of *F. oxysporum* f. sp. *cepae*. Maximum dry mycelial weight of fungus was obtained in potato dextrose broth (363.66 mg) which was found significantly superior over all the liquid media tested followed by oatmeal broth (343.33 mg) and Richard's broth (323.33 mg).Least mycelial weight was obtained in Rose Bengal broth (126.33 mg) followed by Sabouraud's broth (184.33 mg).

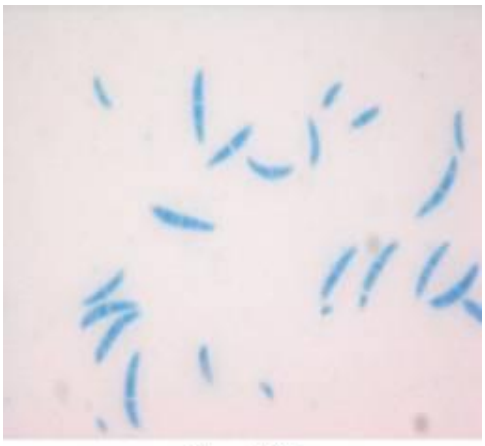
As the maximum growth was recorded in Potato dextrose broth, it was selected as a basal liquid medium for further studies.

Culture

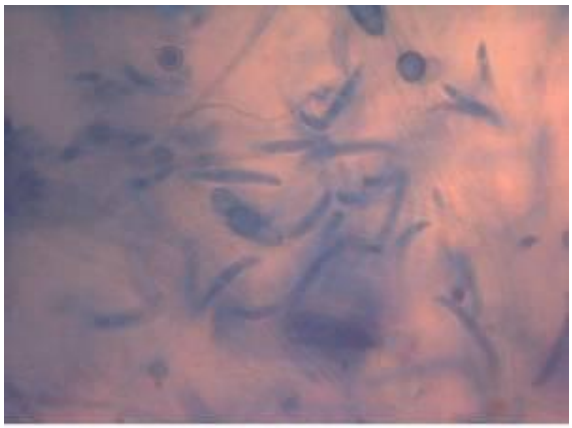
Conidia (100X)



Micro conidia (400X)



Macro conidia (400X)



Chlamydospores (400X)

Plate 2: Morphological characters of Fusarium oxysporum f. sp. Cepae

Table 2: Effect of incubation period on dry mycelial weight of *F. oxysporum* f. sp. *cepae* in Potato dextrose broth

No of days after inoculation	Dry myclelial weight (mg)
2	43.33
4	90.00
6	150.00
8	246.67
10	360.00
12	313.33
14	270.00
16	210.00
18	186.67
20	140.00
S.Em ±	2.10
CD at (1%)	8.48

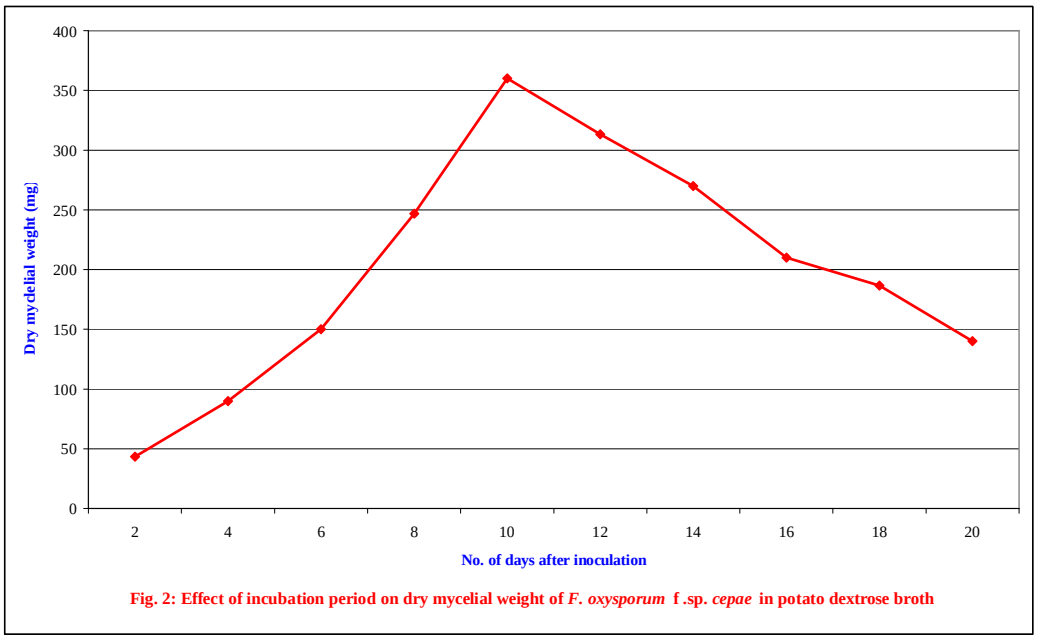


Fig. 2: Effect of incubation period on dry mycelial weight of F. oxysporum f .sp. cepae in potato dextrose broth

Table 3: Effect of solid media on growth of *Fusarium oxysporum* f. sp*. cepae*

Media	Colony diameter (mm)	Sporulation
Czapek's dox agar	78.57	+ + +
Oat meal agar	89.62	+ +
Potato dextrose agar	87.40	+ + +
Richard's agar	86.05	+ + +
Rose Bengal agar	76.60	+
Sabouraud's agar	72.22	+ +
S.Em±	0.67	
CD at 1%	2.89	

+ + + : Good sporulation >50 macroconidia/microscopic field

+ + : Moderate sporulation 30-50 macroconidia/microscopic field

+ : Scanty sporulation <30 macroconidia/microscopic field

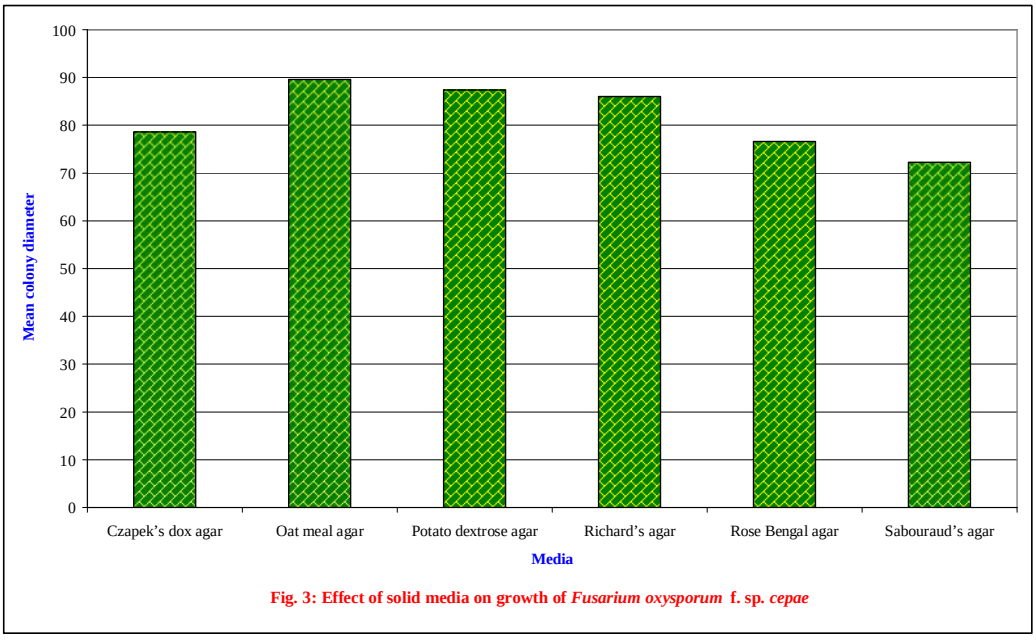


Fig. 3: Effect of solid media on growth of Fusarium oxysporum f. sp. Cepae

1) Rose Bengal broth 2) Oat meal broth 3) Sabouraud's broth
4) Richard's broth 5) Czapek's dox broth 6) Potato dextrose broth

Effect of solid media on growth of *Fusarium oxysporum* f. sp. *cepae*

1) Richard's broth 2) Sabouraud's broth 3) Czapek's dox broth
4) Rose Bengal broth 5) Potato dextrose broth 6) Oat meal broth

Effect of liquid media on growth of *Fusarium oxysporum* f. sp. *cepae*

Plate 3: Cultural characters

4.3.3 Physiological studies.

4.3.3a Effect of temperature

The effect of temperature on the growth of *F. oxysporum* f .sp. *cepae* was studied as explained in Material and methods and results are presented in Table 5.

The effect of different temperatures on the growth of the fungus was significant. Growth of *F oxysporum* f.sp.*cepae* on PDA showed gradual increase as temperature increased from 20 to 30^{o}C and later declined with further increase in temperature.The maximum dry mycelial weight of fungus was observed at a temperature of 30°C (350.00 mg) which was significantly superior over all other temperature levels tested. This was followed by 25°C (330.00 mg), 35°C (220.00 mg) and 20°C (150.00 mg) which were in decreasing order and differed significantly. However, no mycelial growth was observed at 40°C.

4.3.3b Effect of hydrogen ion concentration

The experiment was carried out to know the effect of pH on the growth of *F. oxysporum* f .sp.*cepae*. The growth of the fungus was studied at various pH levels as detailed in Material and methods and the results are presented in Table 6.

The fungus growth increased with the increase in pH from 4.0 to 7 and then onwards there was decline in the growth. The maximum dry mycelial weight of the fungus was noticed at pH level of 6.0 (367.77 mg) which was significantly superior over rest of the pH levels tested. This was followed by the 7.0 (250.00 mg) and 8.0 (176.66 mg). The least growth was observed at pH 4.0 (53.44 mg)

4.4 Management studies

4.4.1 *In vitro* evaluation of bio control agents.

Efficacy of bacterial and fungal bioagents *viz*., *Bacillus subtilis, Pseudomonas fluorescens*, *Trichoderma koningii, T. harzianum*, *T. viride*, and *T. virens* were evaluated against *F. oxysporum* f .sp.*cepae* under *in vitro* condition by following dual culture method as described in “Material and Methods” the results are presented in Table (7)

The results revealed that, the antagonists significantly reduced the growth of *F. oxysporum* f. sp*. cepae* either by over growing or by exhibiting inhibition zones. Maximum reduction in colony growth was observed in *T. harzianum* (75.92%) which was significantly superior over all other bioagents tested. Next best was T*. virens* (74.81%) followed by *T. koningii* (73.7%) and *T. viride* (72.59%). However, *B. subtilis* (57.4%) and *Pseudomonas fluorescens* (52.96%) were least effective in inhibiting mycelia growth of the pathogen.

4.4.2 *In vitro* evaluation of botanicals

The antifungal activity of ten leaf extracts was assayed, at three concentrations in the laboratory for their efficacy against the *F. oxysporum* f. sp*. cepae* using poisoned food technique as described in Material and Methods. The data are presented in Table 8.

The effect of plant extracts on the per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *cepae* at three concentrations differed significantly.

The results revealed that, among the eight plant extracts, Neem seed kernel extract (43.95%) was found effective in inhibiting mycelial growth which was followed by prickly chaff flower (34.81%), turmeric (32.53%) and datura (31.95%), which were on par with lantana (31.85), tulsi (25.92%). The least inhibition was observed in garlic bulb extract (17.90%) followed by onion bulb extract (19.50%).

The plant extract at 10 per cent was significantly superior over 7.5 and 5 per cent. Neem seed kernel extract (54.1%) at 10 per cent was the best and significantly superior over all other plant extracts. Next best was prickly chaff flower (41.9%) and lantana (39.03%). Least inhibition was observed in onion bulb extract (23.0%) and garlic bulb extract (23.3%). Similar trend was observed at 5 and 7.5 percent concentrations.

Table 4: Effect of liquid media on growth of *Fusarium oxysporum* f. sp. *cepae*

Name of media	Dry mycelial weight(mg)
Czapek's dox broth	244.33
Oat meal broth	343.33
Potato dextrose broth	363.33
Richard's broth	323.33
Rose Bengal broth	126.67
Sabouraud's broth	186.67
S.Em ±	3.33
CD (1%)	14.40

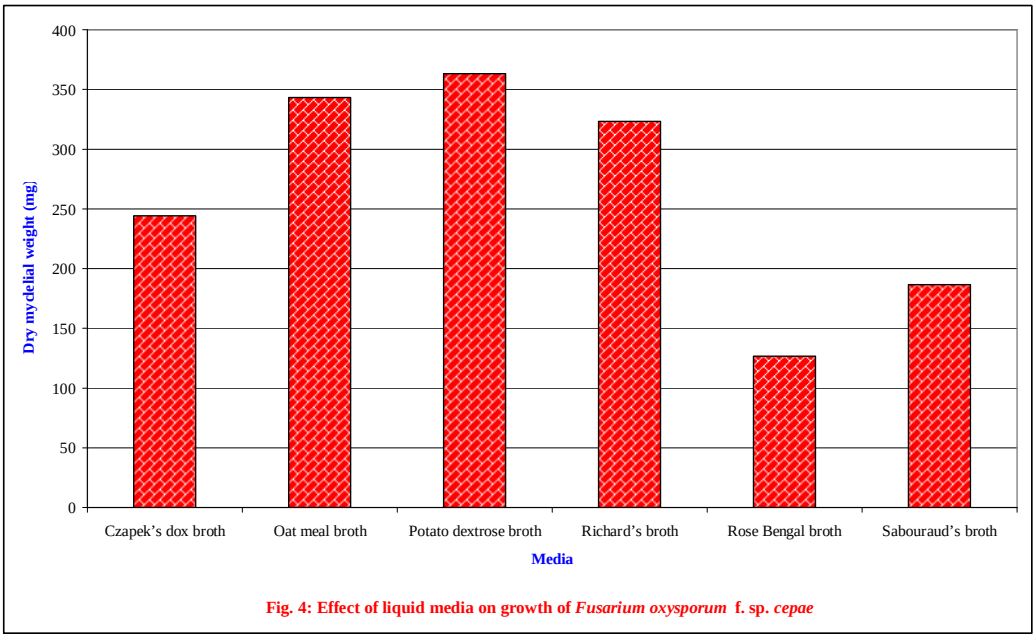


Fig. 4: Effect of liquid media on growth of Fusarium oxysporum f. sp. cepae

Table 5: Effect of temperature on dry mycelial weight of *F. oxysporum* f. sp. *cepae* a causal agent of basal rot

Temperature	Dry mycelial weight (mg)
20	150.00
25	330.00
30	350.00
35	220.00
40	0.00
S.Em±	2.58
CD at 1%	11.57

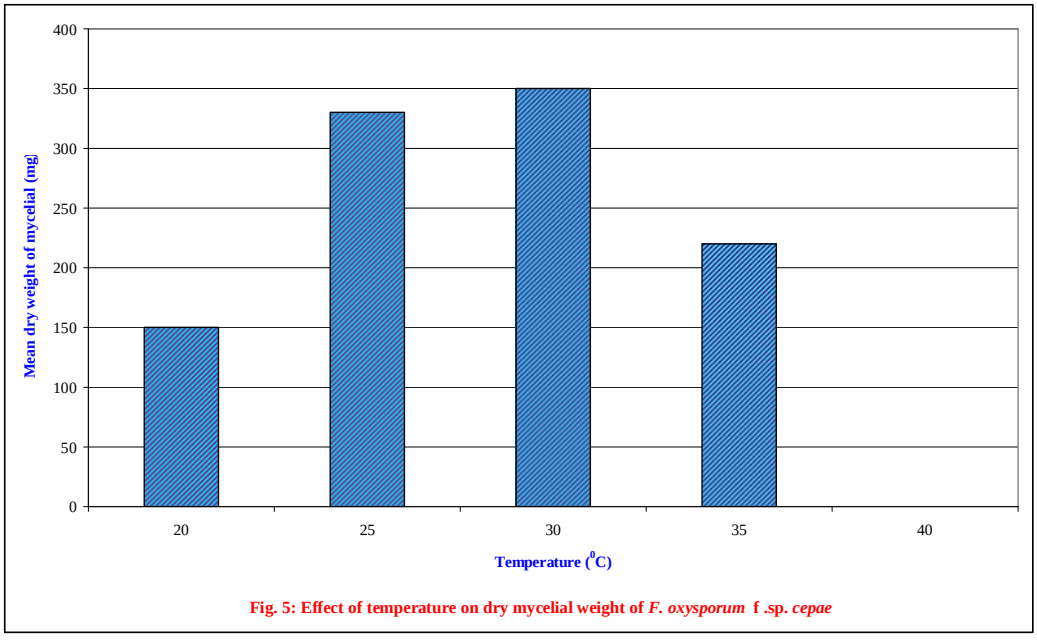


Fig. 5: Effect of temperature on dry mycelial weight of F. oxysporum f .sp. cepae

Table 6: Effect of pH on dry mycelial weight of *F. oxysporum* f. sp. *cepae* a causal agent of basal rot

pH	Dry mycelial weight (mg)
4	53.33
5	230.00
6	367.77
7	250.00
8	176.66
S.Em±	2.72
CD at 1%	11.75

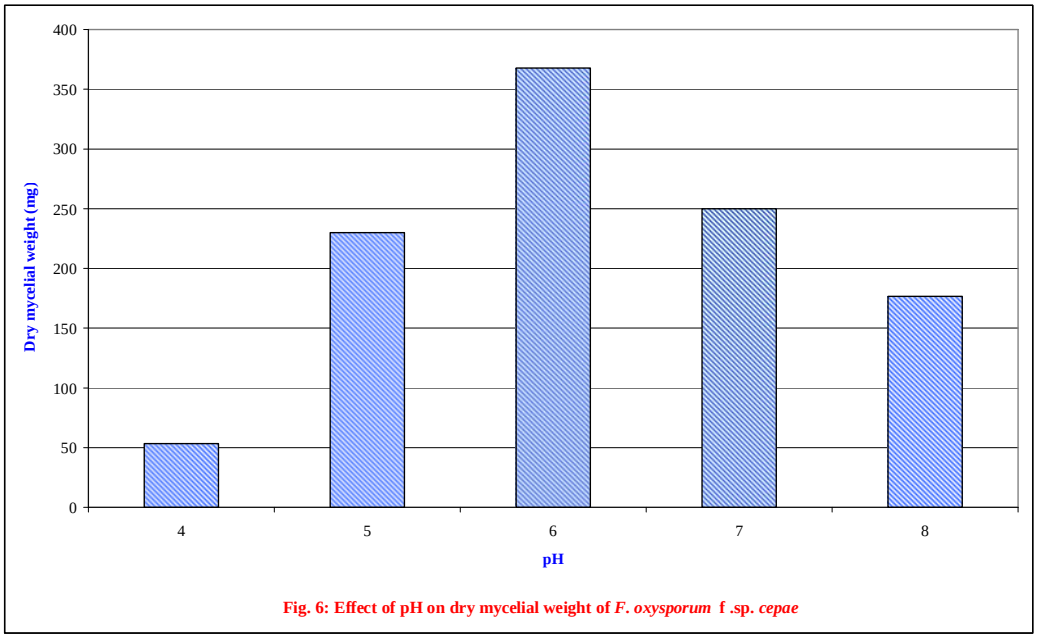
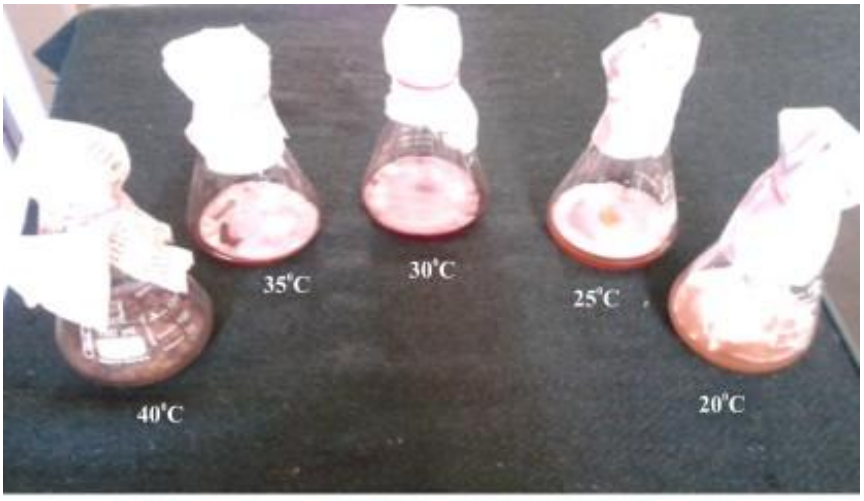


Fig. 6: Effect of pH on dry mycelial weight of F. oxysporum f .sp. cepae



Effect of temperature on *Fusarium oxysporum* f. sp. *cepae*

Effect of pH on *Fusarium oxysporum* f. sp. *cepae*

Plate 4: Physiological characters

Table 7: Evaluation of biocontrol agents against *F. oxysporum* f. sp. *cepae* a causal agent of basal rot

Biocontrol agents	Per cent inhibition of mycelial growth
Bacillus subtilis	57.40 (49.28)*
Pseudomonas fluorescens	52.96 (46.72)
Trichoderma harzianum	75.92 (60.64)
Trichoderma koningii	73.70 (59.18)
Trichoderma virens	74.81 (59.90)
Trichoderma viridae	72.59 (58.46)
S.Em±	0.37
CD at 1%	1.59

* Figures in parenthesis indicate arc sin transformed values

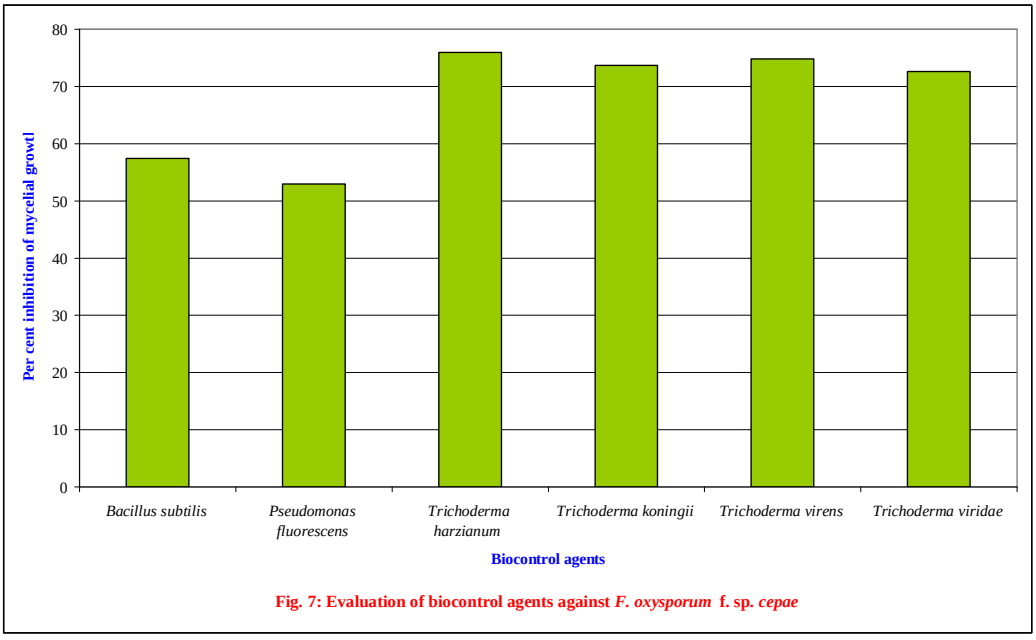


Fig. 7: Evaluation of biocontrol agents against F. oxysporum f. sp. Cepae

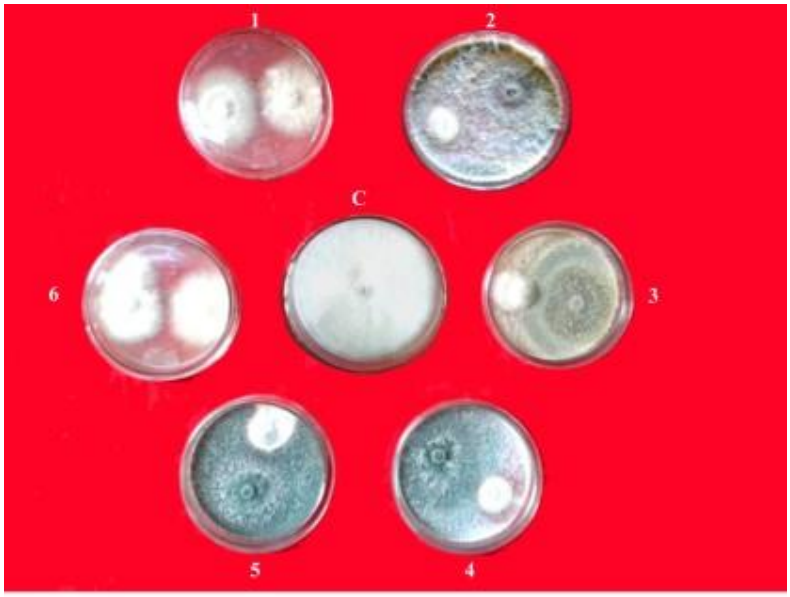


Plate 5: Evaluation of biocontrol agents against F. oxysporum f. sp. Cepae

4.4.3 *In vitro* evaluation of fungicides

Screening of fungicides was done against *F oxysporum* f.sp.*cepae* under laboratory condition by following poisoned food technique as described in “Material and Methods”.

Seven systemic and three non systemic and four combi product were screened at three concentrations in the laboratory for their efficacy against *F. oxysporum* f. sp*. cepae.* Poisoned food technique was followed as detailed in material and methods. Data with respect to inhibition of mycelial growth of *F oxysporum* f.sp.*cepae* at three concentrations of seven systemic fungicides was recorded and presented in Table 9.

Data from table 9 revealed that, the efficacy of different systemic fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *cepae* differed significantly.

Among the seven systemic fungicides evaluated, tebuconazole (100%) was significantly superior over other treatments, followed by carbendazim (95.84%), thiophenate methyl (87.68%) which was on par with benomyl (87.03%). Least inhibition was observed in diffenconazole (74.26%).

Among the tested three concentrations, 0.1 per cent concentration of all fungicides was significantly found superior to 0.025 and 0.05 per cent. Maximum per cent inhibition of mycelial growth (100%) of the fungus was recorded in tebuconazole followed by carbendazim (95.84) which remained on par with thiophenate methyl (92.03%) followed by hexaconazole (90.74%), benomyl (89.07%) and propiconazole (86.11%).Least inhibition was observed in diffenconazole (78.51%).

At 0.05 percent concentration, maximum per cent inhibition of mycelial growth (100%) of the fungus was recorded in tebuconazole. The least per cent inhibition of mycelial growth was recorded in diffenconazole (74.81%). Further thiophanate methyl at 0.025% remain on par with benomyl at 0.05% and propiconazole at 0.1%.

Table 8: *In vitro* evaluation of plant extracts against *Fusarium oxysporum* f. sp. *cepae*

Botanicals	Per cent inhibition of mycelial growth			Mean
	Concentration (%)			
	5	7.5	10	
Garlic bulb extract	12.20 (20.47)*	18.50 (25.50)	23.00 (28.65)	17.90
Onion bulb extract	15.2 (22.94)	20.00 (26.58)	23.30 (28.90)	19.50
NSKE	34.80 (36.18)	43.00 (40.97)	54.10 (47.36)	43.95
Datura leaf extract	25.60 (30.38)	32.20 (34.60)	38.10 (38.16)	31.97
Lantana leaf extract	22.60 (28.39)	33.30 (35.28)	39.60 (39.03)	31.85
Tulsi leaf extract	20.40 (26.84)	25.90 (30.62)	31.50 (34.15)	25.92
Prickly chaff flower leaf extract	27.40 (31.58)	35.20 (36.40)	41.90 (40.33)	34.81
Turmeric rhizome extract	26.80 (31.22)	31.50 (34.15)	39.30 (38.81)	32.53
	Botanicals (B)	**Concentration (C)**	**B × C**	
S.Em±	0.22	0.13	0.39	
CD at 1%	0.84	0.53	1.50	

* Figures in parenthesis indicate arc sin transformed values

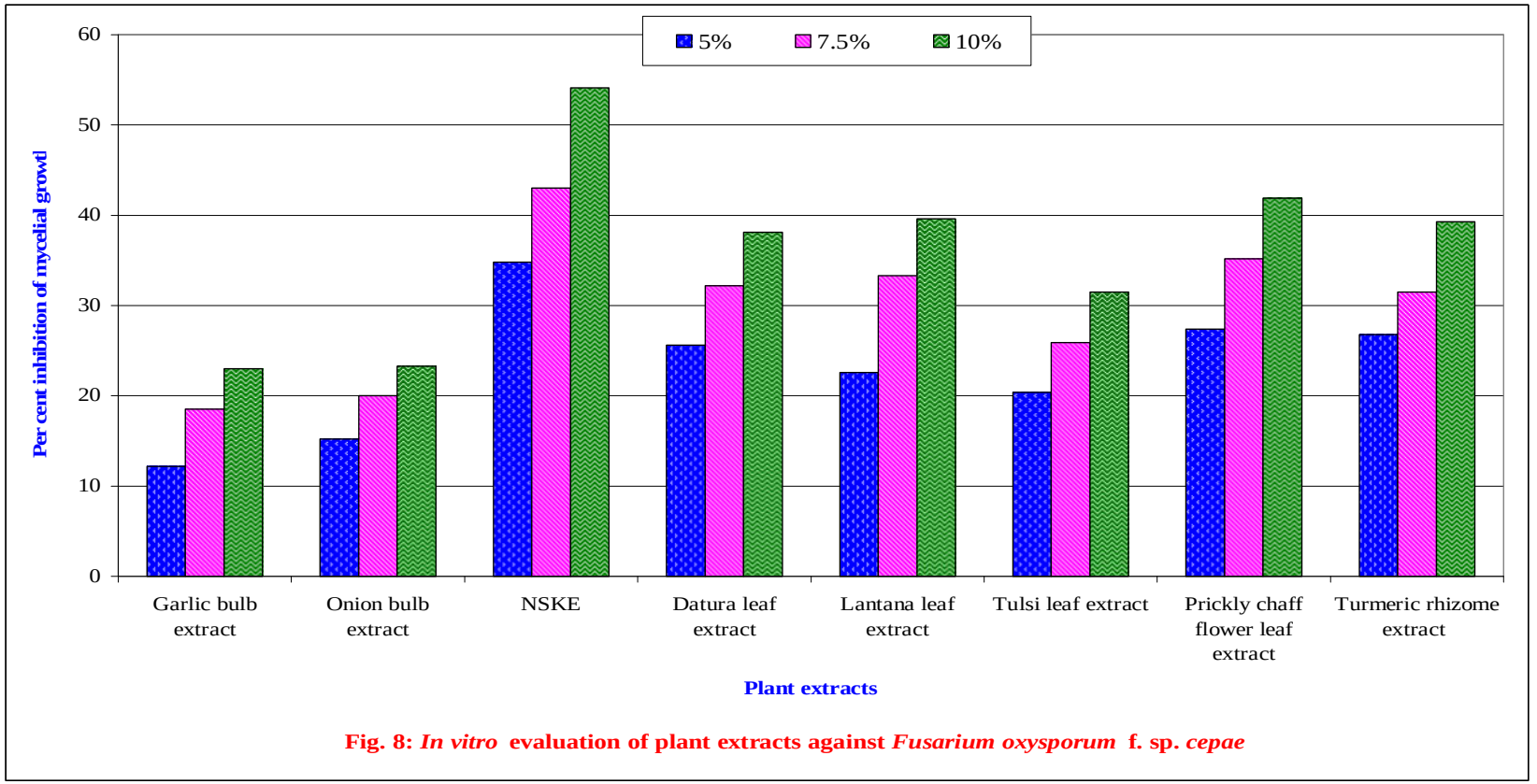


Fig. 8: *In vitro* evaluation of plant extracts against *Fusarium oxysporum* f. sp. *cepae*

Fig. 8: In vitro evaluation of plant extracts against Fusarium oxysporum f. sp. Cepae

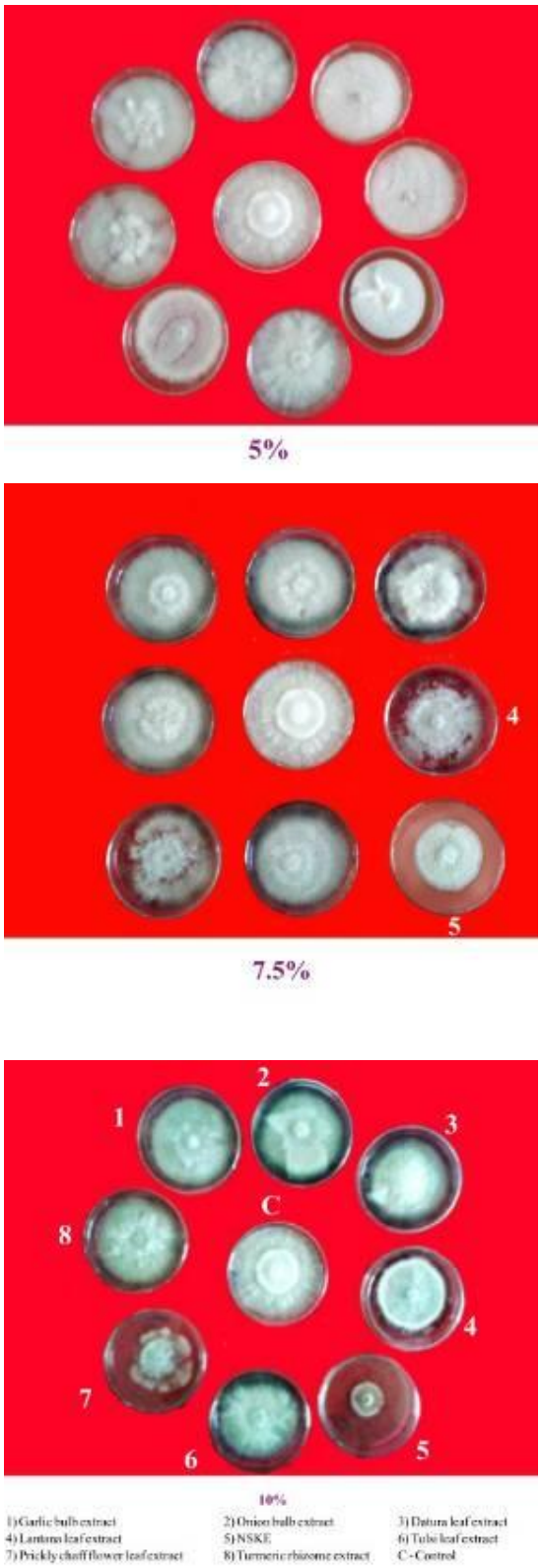


Plate 6: *In vitro* evaluation of plant extracts against *Fusarium oxysporum* f. sp. *Cepae*

Data with respect to inhibition of mycelial growth of *F. oxysporum* f .sp*. cepae* at three concentrations of three non-systemic fungicides and four combi product was recorded and presented in Table 10.

Among the three non systemic fungicides and four combi-products evaluated against *F. oxysporum* f .sp*. cepae,* Mancozeb 63% + carbendazim 12% gave maximum inhibition of mycelial growth (98.51%), which was followed by trifloxystrobin 25%+ tebuconazole 50% (95.67), carboxin 37.5%+ thiram 37.5% (93.45%), mancozeb (87.53%), zineb 64%+ hexaconazole (81.72%). Least inhibition of mycelial growth was observed in Captan (60.24%) followed by copper oxychloride (53.95%) (Table 10).

Among the different concentrations tested, 0.3 per cent was significantly superior to 0.1 and 0.2. Mancozeb 63% + carbendazim 12%, trifloxystrobin 25%+ tebuconazole 50% and carboxin 37.5%+ thiram 37.5% at 0.3 per cent (100%) concentration gave complete inhibition of the mycelial growth of pathogen. This was followed by mancozeb at 0.3 per cent (91.48%), zineb 64%+ hexaconazole 4% at 0.3 per cent (86.29%).Least effective fungicide was copper oxychloride at 0.1 per cent (45.55%), followed by captan at 0.1 per cent (45.92%). mancozeb at 0.3 per cent remain on par with carboxin 37.5%+ thiram 37.5% at 0.2 per cent concentration.

4.4.4 *In vitro* evaluation of nutrients

Evaluation of nutrients was done against *F oxysporum* f.sp.*cepae* under laboratory condition by following poisoned food technique as described in “Material and Methods”.

Data from table 11 revealed that, the efficacy of three nutrients, their concentrations and interaction on per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *cepae* differed significantly.

Among the tested nutrients Potassium chloride gave the maximum inhibitation of mycelia growth (21.82%), followed by Magnesium sulphate (15.55%). Least inhibition of mycelial growth was observed in Zinc sulphate (10.18%).

Among the different concentration tested, 500ppm was significantly superior over 100 and 250ppm. Potassium chloride at 500ppm gave the maximum inhibition mycelial growth (27.77%), followed by zinc sulphate (23.70%). Potassium chloride at 100ppm was on par with zinc sulphate at 250ppm. Least inhibitation of mycelial growth (6.11%) was noticed in zinc sulphate at 100ppm.

4.5 *In vivo* evaluation

The experiment was conducted with six treatments and one untreated control as described in “Material and Methods”.

Among the treatments tested per cent disease incidence was low in seed treatment with *T. harzianum* + drenching with Mancozeb 63% + Carbendazib 12% (15.55%) followed by seed treatment *T. harzianum* + soil application of *T. harzianum* (18.38%), seed treatment with with *T harzianum* + drenching with tebuconazole (21.75%) which is on par with seed treatment with *T. harzianum* + drenching with mancozeb (21.43%), highest disease incidence was noticed in seeds treatments with *T. harzianum* + no drenching(68.88%) and in control 100 per cent disease was noticed.

Among the treatments tested highest bulb yield was noticed in seed treatment with *T. harzianum* + soil application of *T. harzianum* (55.68g) followed by seed treatment with *T. harzianum* + drenching with mancozeb 63%+ carbendim 12% (54.29g) which is on par with seed treatment with *T. harzianum* + drenching with tebuconazole (54.23g) followed by seed treatment with *T. harzianum* + drenching with mancozeb (51.11g). Least bulb yield was noticed in seed treatment with *T. harzianum* + drenching with NSKE (37.53) followed by seed treatment with *T. harzianum* + control (42.49g) and control (17.15g).

Post harvest loss over control was minimum in seed treatment with *T. harzianum* + soil application of *T. harzianum* (31.37%),which is on par with seed treatment with *T. harzianum* + drenching with mancozeb 63% + carbendazim 12% (31.59%), seed treatment with *T.harzianum* + drenching with tebuconazole (31.56%) followed by seed treatment with *T. harzianum* + drenching with mancozeb (33.56%), seed treatment with *T. harzianum* + drenching with NSKE (36.09%) and least was in seed treatment with *T. harzianum* + control (40.37%).

Table 9: ***In vitro*** **evaluation of systemic fungicides against** ***F oxysporum*** **f. sp.** ***cepae***

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.025	**0.05**	**0.1**	
Thiophenate Methyl	86.29 (68.31)*	89.07 (70.73)	92.03 (73.64)	87.68 (69.49)
Carbendazim	92.18 (73.80)	95.35 (77.59)	100 (89.96)	95.84 (78.27)
Benomyl	85.18 (67.39)	86.85 (68.77)	89.07 (70.73)	87.03 (68.93)
Tebuconazole	100.00 (89.96)	100.00 (89.96)	100 (89.96)	100.00 (89.96)
Propiconazole	81.48 (64.54)	84.26 (66.66)	86.11 (68.15)	82.87 (65.58)
Difenconazole	73.70 (59.18)	74.81 (59.90)	78.51 (62.41)	74.26 (59.54)
Hexaconazole	80.59 (63.89)	84.44 (66.80)	90.74 (72.32)	82.51 (65.31)
Mean	**85.63 (67.76)**	**87.82 (69.61)**	**90.92 (72.50)**	
	Fungicides (F)	**Concentration (C)**	**F × C**	
S.Em±	0.21	0.14	0.35	
CD at 1%	0.82	0.54	1.36	

* Figures in parenthesis indicate arc sin transformed values

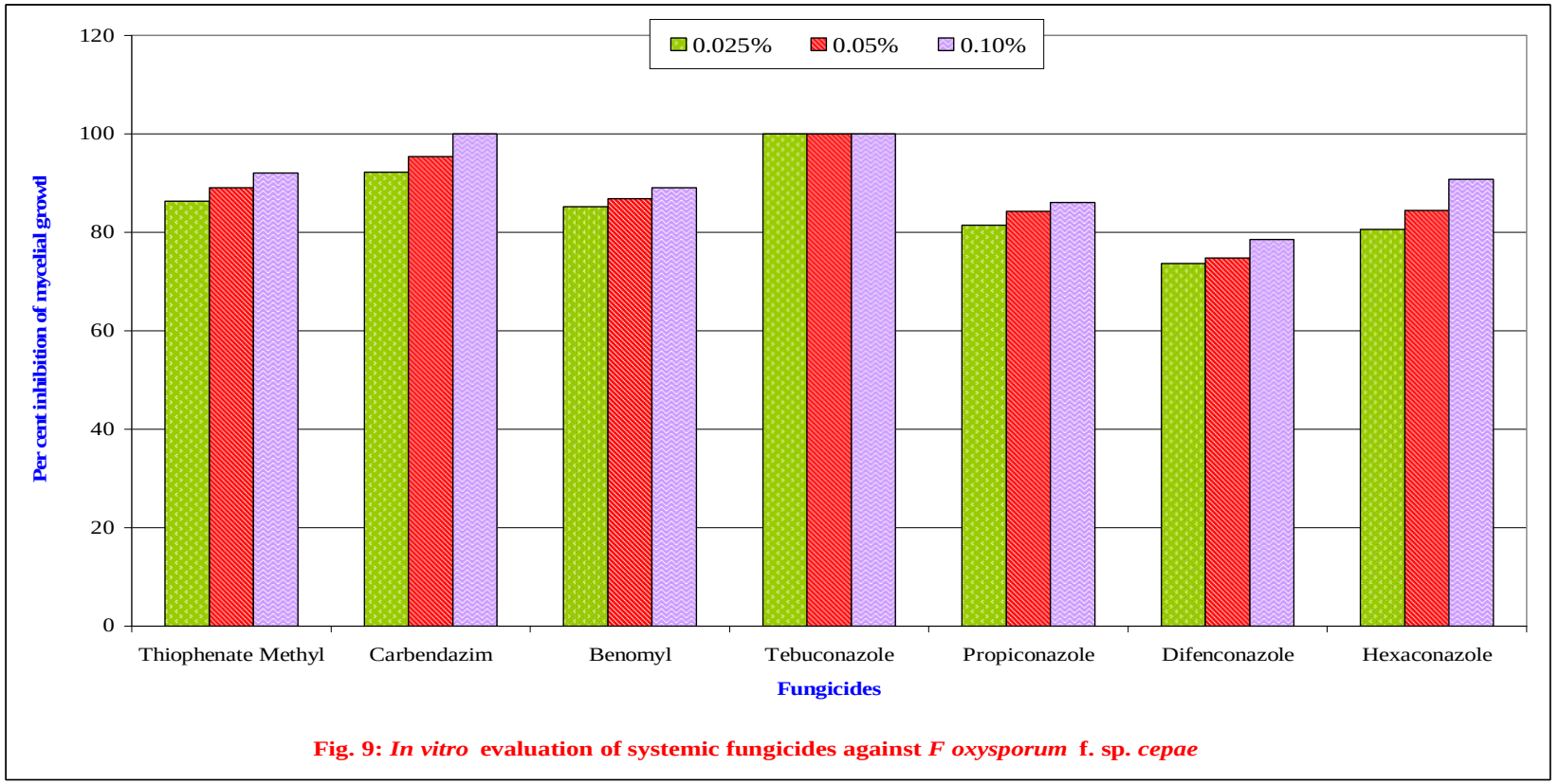


Fig. 9: *In vitro* evaluation of systemic fungicides against *F oxysporum* f. sp. *cepae*

Fig. 9: In vitro evaluation of systemic fungicides against F oxysporum f. sp. Cepae

Table 10: *In vitro* evaluation of non systemic fungicides and combi products against *F. oxysporum* f. sp. *cepae*

Fungicides	Per cent inhibition of mycelial growth			Mean
	Concentration (%)			
	0.1	**0.2**	**0.3**	
Captan	45.55 (42.47)*	63.70 (52.98)	71.48 (57.75)	60.24 (51.07)
Mancozeb	82.59 (65.37)	88.51 (70.22)	91.48 (73.07)	87.53 (69.55)
Mancozeb 63% + Carbendazim 12%	95.53 (77.83)	100 (86.96)	100 (86.96)	98.51 (83.03)
Carboxin 37.5% + Thairam 37.5%	88.51 (57.99)	91.85 (73.45)	100 (89.96)	93.45 (77.91)
Zineb 64% + Hexaconazole 4%	75.92 (60.64)	82.96 (68.30)	86.29 (68.30)	81.72 (64.87)
Trifloxystrobin 25% + Tebuconazole 59%	91.48 (73.07)	95.55 (77.86)	100.00 (89.96)	95.67 (77.86)
Copper oxychloride	48.14 (43.96)	54.07 (47.07)	59.62 (50.51)	53.95 (49.29)
Mean	**75.38 (60.28)**	**82.37 (65.21)**	**86.98 (68.88)**	
	Fungicides (F)	**Concentration (C)**	**F × C**	
S.Em±	0.17	0.11	0.29	
CD at 1%	0.65	0.42	1.13	

* Figures in parenthesis indicate arc sin transformed values

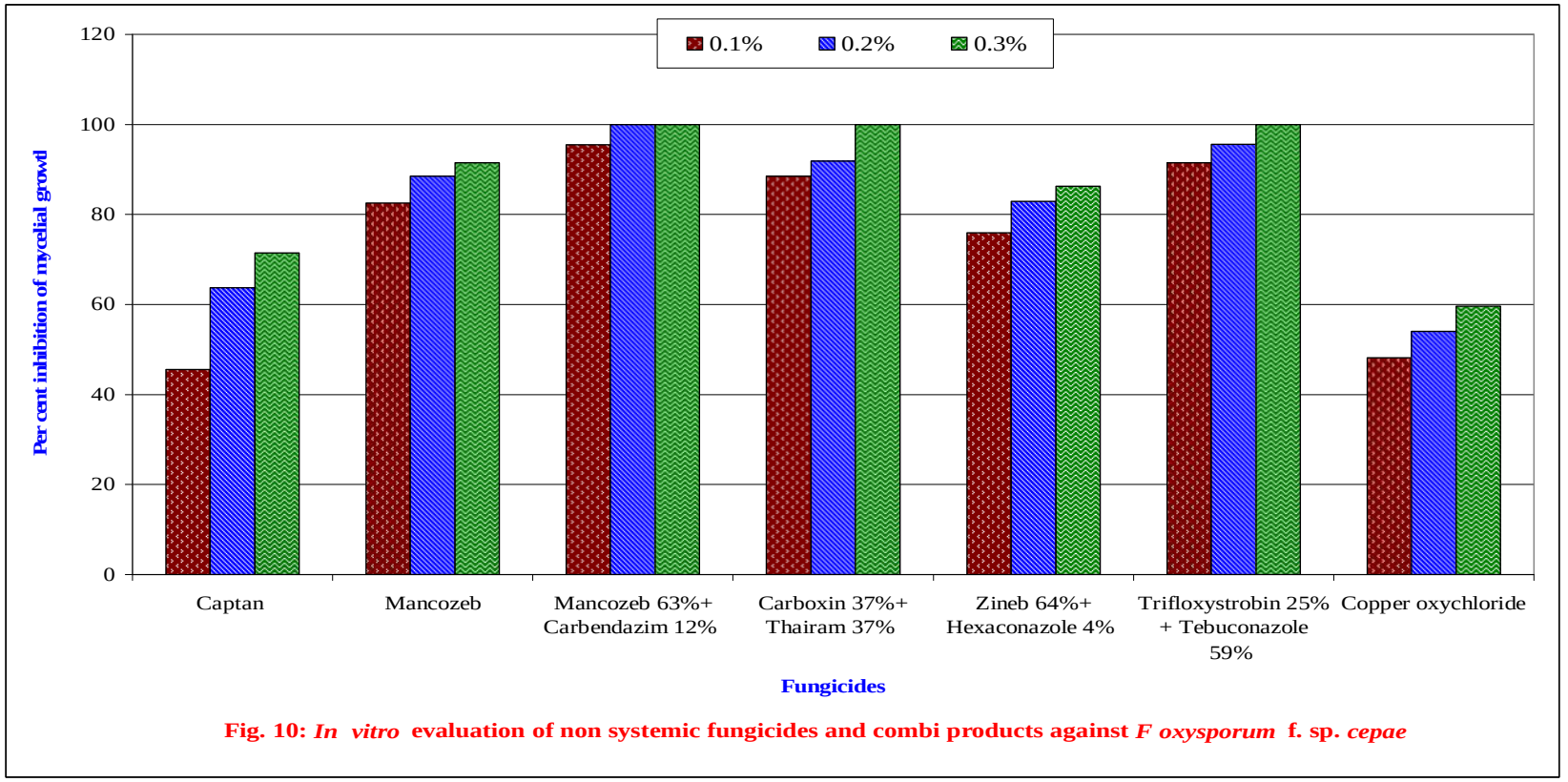
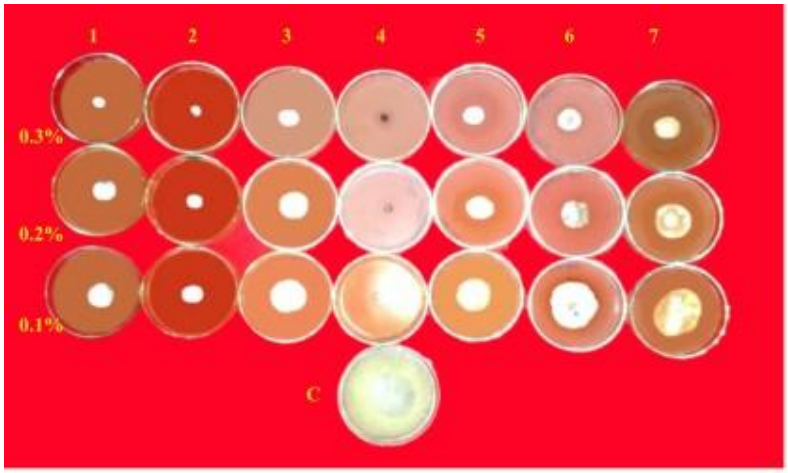


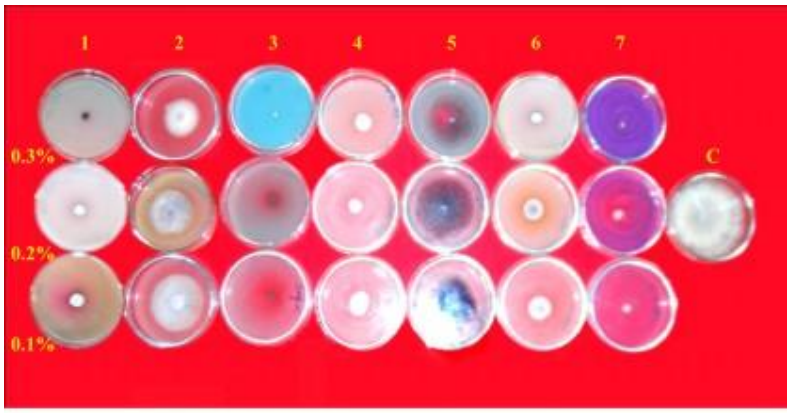
Fig. 10: *In vitro* evaluation of non systemic fungicides and combi products against *F oxysporum* f. sp. *cepae*

Fig. 10: In vitro evaluation of non systemic fungicides and combi products against F oxysporum f. sp. Cepae



1) Thiophenate Methyl 2) Carbendazim 3) Benomyl 4) Tebuconazole
5) Propiconazole 6) Difenconazole 7) Hexaconazole C - Control

a) Systemic fungicides



1) Trifloxystrobin 25% + Tebuconazole 59% 2) Captan
3) Mancozeb 63% + Carbendazim 12% 4) Mancozeb
5) Copper oxychloride 6) Zineb 64%+ Hexaconazole 4%
7) Carboxin 37% + Thairam 37% C - Control

b) Non-systemic and combi products

Plate 7: In vitro evaluation of fungicides

Table 11: *In vitro* evaluation of nutrients against *F oxysporum* f. sp. *cepae*

Nutrients	Per cent inhibition of mycelial growth			Mean
	Concentration(ppm)			
	100	**250**	**500**	
Potassium chloride	11.85 (20.13)	25.85 (30.57)*	27.77 (31.82)	21.82 (27.86)
Zinc sulphate	6.11 (14.31)	10.55 (18.97)	13.88 (21.89)	10.18 (18.62)
Magnesium sulphate	7.22 (15.59)	15.74 (23.38)	23.70 (29.15)	15.55 (23.24)
Mean	**8.39 (16.84)**	**17.38 (24.65)**	**21.78 (27.84)**	
	Nutrients (N)	**Concentration (C)**	**N × C**	
S.Em±	0.31	0.32	0.54	
CD at 1%	1.19	1.2	2.06	

* Figures in parenthesis indicate arc sin transformed values

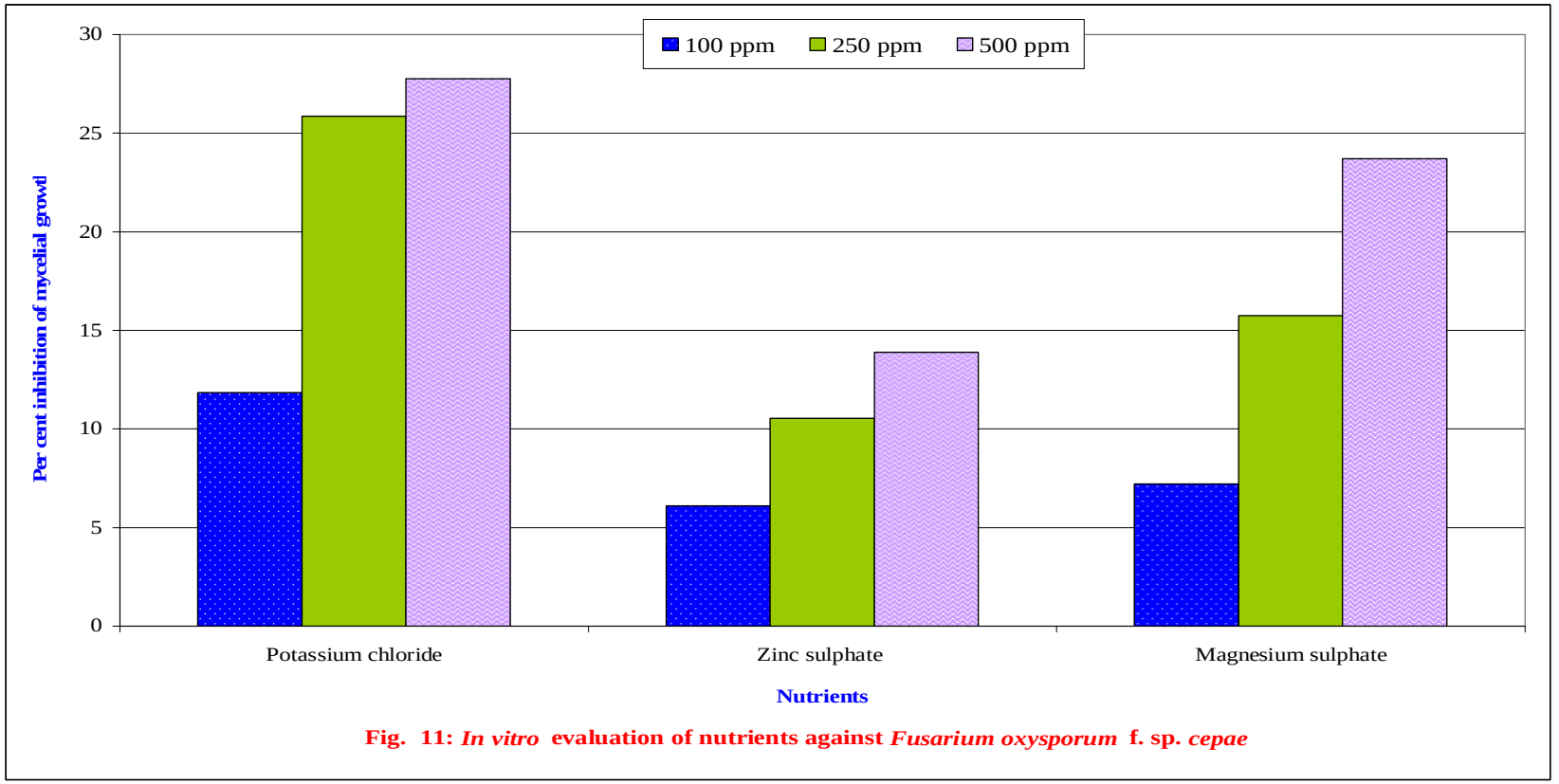


Fig. 11: *In vitro* evaluation of nutrients against *Fusarium oxysporum* f. sp. *cepae*

Fig. 11: In vitro evaluation of nutrients against Fusarium oxysporum f. sp. Cepae

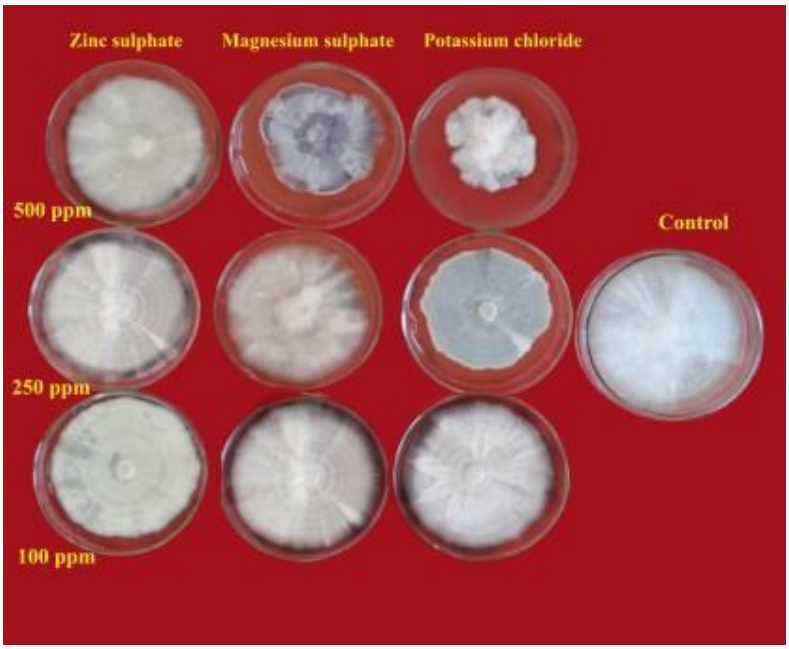


Plate 8: In vitro evaluation of nutrients

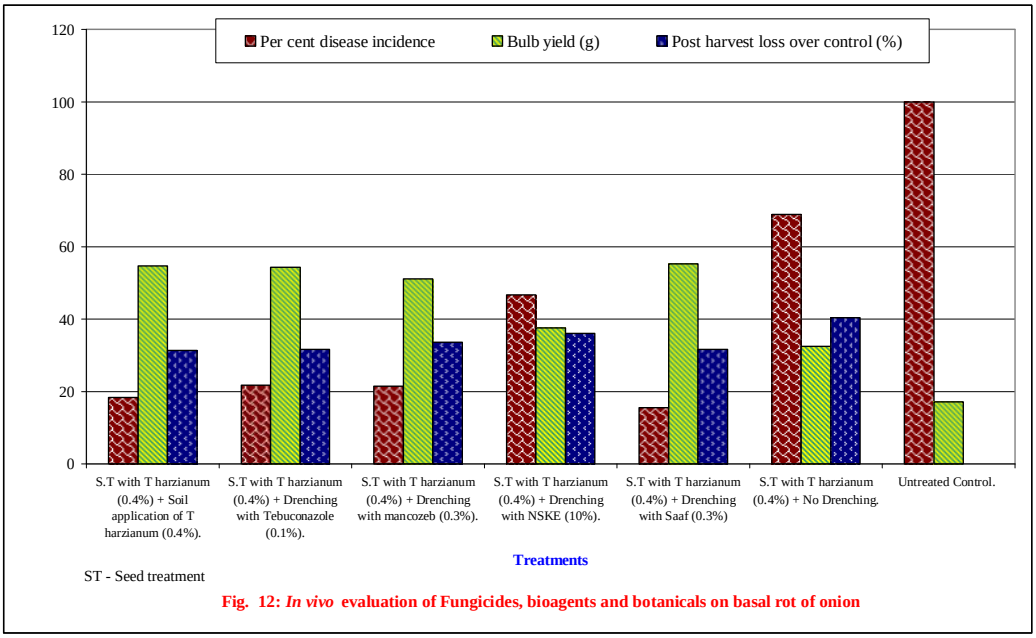


Fig. 12: In vivo evaluation of Fungicides, bioagents and botanicals on basal rot of onion

Table 12: *In vivo* evaluation of Fungicides, bioagents, and botanicals on basal rot of onion

Treatments	Per cent disease incidence	Bulb yield (g)	Post harvest loss over control (%)
S.T with *T harzianum* (0.4%) + Soil application of *T harzianum* (0.4%).	18.38 (21.33)*	54.68 (47.71)	31.37 (34.08)
S.T with *T harzianum* (0.4%) + Drenching with Tebuconazole (0.1%).	21.75 (26.56)	54.29 (47.47)	31.59 (34.22)
S.T with *T harzianum* (0.4%) + Drenching with mancozeb (0.3%).	21.43 (31.57)	51.11 (45.66)	33.56 (35.42)
S.T with *T harzianum* (0.4%) + Drenching with NSKE (10%).	46.66 (43.11)	37.53 (31.61)	36.09 (36.94)
S.T with *T harzianum* (0.4%) + Drenching with Saaf (0.3%)	15.55 (23.22)	55.29 (47.49)	31.59 (34.22)
S.T with *T harzianum* (0.4%) + No Drenching.	68.88 (56.13)	32.49 (30.70)	40.37 (39.47)
Untreated Control.	100.00 (89.96)	17.15 (24.48)	0.00 (0.00)
S. Em±	0.42	0.64	0.31
CD at 1%	1.60	1.94	1.34

S.T= seed treatment

*Figures in parenthesis indicate arc sin transformed values

1) *Tharzianum* + Soil application of *Tharzianum*
2) *Tharzianum* + Drenching with Tebuconazole
3) *Tharzianum* + Drenching with mancozeb
4) *Tharzianum* + Drenching with NSKE
5) *Tharzianum* + Drenching with Saaf
6) *Tharzianum* + No Drenching
C - Control.

Plate 9: In vivo Studies

5. DISCUSSION

Onion (*Allium cepa* L.) is one of the oldest bulb crops, known to mankind and consumed worldwide, rightly called as “queen of kitchen”. It is one of the most important commercial vegetable crop grown in India and believed to be originated in Central Asia. According to Vavilov (1951) the primary center of origin lies in central Asia. The near east and Mediterranean are the secondary centers of origin. The genus *Allium* is very large comprising of more than 500 spp.

Basal rot disease caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *cepae* (Hans.)Snyder and Hansen is the most destructive disease of onion and causes severe yield losses in all growing areas of the world. Contaminated seeds and soil have been determined as the principal source of inoculum, Which Causes severe loss in the productivity both in field and in storage condition (Coskuntuna and Ozer, 2008). The disease occurs at all stages of growth of the crop. Yield loss upto 50 per cent has been recorded in susceptible cultivars

There are few studies on the intensity and management aspects. Detail studies are essential to formulate management strategies of the disease. Investigations were carried out on various aspects of the pathogen with respect to symptomatology, incidence of the disease in different locations, morphological, cultural and physiological aspects of the pathogen, evaluation of fungicides, bioagents and botanicals against the pathogen under laboratory and glasshouse condition.

5.1 Survey and surveillance for the incidence of the disease

A detailed roving survey was undertaken during 2011-12 to gather information on the severity, distribution and spread of basal rot of onion from different localities. This information is highly useful to indentify the hot spots of this disease in Dharwad, Bijapur, Gadag and Uttar Kannada districts, where onion is extensively grown as commercial crop. From the survey it is revealed that the severity of this disease varied from locality to locality depending on the type of onion variety cultivated. The severity of disease was also dependent on inoculum load, environmental conditions prevailing in different localities. Among the districts surveyed, the severity of disease was more in Dharwad district (36.18) and less in Bijapur district (15.30), indicating that this disease was not consistent in all localities.

The basal rot of onion was severe in Dharwad district compared to Bijapur district. This could be because of favourable environmental conditions and initial inoculum prevailed. The variety used in Dharwad district was Nasik red which is susceptible. This might have helped in the development of the disease in *Kharif* when favourable environmental conditions prevailed. Working on survey of basal rot diseases of onion,in Tamil Nadu Sudhasha *et al*. (2008) concluded that the disease is the predominant in Coimbatore and Dindigul districts. Entwistle (1990) concluded that fungus infects the roots or the basal plate of the bulbs. Further infection of bulb scales occurs later in the season, and most severe losses are found in post-harvest storage. The fungus is prevalent worldwide, and also infects other cultivated *Allium* species such as garlic and shallot.

F. oxysporum f.sp*. cepae* was one of the most important fungal pathogens of onion, which showed characteristic symptoms on leaves and bulbs. The first above symptoms is a yellowing of leaf blades at the tip. This yellowing progresses downward until the whole blade is involved. Later such leaves shrivel and decay. Infected plants can be pulled easily because they have a retarded root system. Affected roots are dark brown, flattened, hollow, and transparent.

The discoloration starts at the outermost layer of the stem plate and extends upward. At later stage, the stem plate tissue become pitted and exhibited a dry rot. Sumner (1995) reported that Fusarium basal rot, caused by *Fusarium oxysporum* f. sp. *cepae* is a devastating disease in onion-growing areas worldwide. Beginning from the leaf tips, progressive wilting, yellowing, curving, and eventually dying back symptoms appear on infected plants. The pathogen causes a brownish, watery rot on infected bulbs. The roots eventually rot and become covered with a whitish mycelium. Similar symptoms were observed by August *et al*. (1962), Abawi and Lorbeer (1972) and Entwistle (1990).

5.2 Isolation, morphological and physiological characters of pathogen

5.2.1 Isolation of the pathogen.

The infected bulbs showing the typical disease symptoms were used for the isolation of the pathogen. The pathogen was isolated by following standard tissue isolation, and pure culture was obtained by following hyphal tip method and this culture of *F. oxysporum* f .sp.*cepae* was used in further studies.

5.2.2 Pathogenicity

The pathogen was identified as *F.oxysporum* f.sp.*cepae* based on their morphological characters as defined by Hansen (1929) and Booth (1971). Pathogenicity was proved by inoculating the giant culture of *F. oxysporum* f .sp.*cepae* to sterile soil and control was maintained without inoculum. Yellowing was observed at 30 DAS followed by wilting of the leaves at the end. Death of the plant was observed at 50th day similar observations were made by McCulloch (1944) and Sudhasha *et al*. (2008).

5.2.3 Identification

The culture isolated from rotted bulbs of onion and causal organism was identified as *F. oxysporum* f. sp.*cepae* based on the morphological and cultural characters as per Hansen (1929) and Booth (1971) and by its pathogenicity on onion. The fungus produced white, cottony mycelium with abundant microconidia, hyaline, continuous or 1-septate, ovoid to ovate and measured 3.5 -8.0 x 2.5 – 3.5 µm. Macroconidia were sparse and variable, 3 septate or rarely 4–5 septate measured 19.5 – 29.5 x 3 – 5 µm. Chlamydospores were hyaline, usually vacuolated and spherical, measured 4.5 – 9.5 µm in diameter. Similar observations were made by Hansen (1929), Burgess *et al.* (1994), Schwartz and Mohan (1995) and McCulloch (1944).

5.3 Cultural Studies

Maximum dry mycelial weight of *F oxysporum* f.sp.*cepae* was attained on 10^{th} day (360.00mg) of incubation in potato dextrose broth and was considered as optimum period for growth of fungus for future studies. The study indicated that further increase in the incubation period resulted in decrease in the dry mycelia weight of the fungus, which may have been due to autolysis of the mycelium, accumulation of toxins and exhaustion of nutrients in the medium after incubation for optimum number of days. Barnett (1951) have discussed the cellular enzymes begin to digest the various cell constituents. This was confirmed with the findings of Adiver (1996) in case of *Fusaium* sps, Sataraddi (1998) in *Fusarium udum* and Kulkarni (2006) in case of *F oxysporum* f.sp.*gladioli*.

Among six solid media evaluated maximum radial growth of *F. oxysporum* f.sp. *cepae* was observed on Oatmeal agar (89.62 mm) followed by Potato dextrose agar (87.40mm). Less growth of fungus was observed in Rose bengal agar and Sabouraud's agar. Sporulation of fungus was found to be abundant on Potato dextrose agar, Richards's agar and Czapeck's dox agar. The results are in confirmation with that of Mc Culloch (1944) in case of *F. oxysporum* f.sp. *gladioli,* Jhamaria (1972) in case of *F. oxysporum* f. sp. *niveum,* Sataraddi (1998) in case of *Fusarum udum* and Kulkarni (2006) in case of *F oxysporum* f.sp.*gladioli*.

However, in case of broth, maximum dry mycelial weight (363.33 mg) was recorded in potato dextrose broth and least (126.00 mg) in rose Bengal agar after ten days of incubation. Kulkarni (2006) who found that, maximum dry mycelial weight of fungus was obtained in potato dextrose broth which was followed by oat meal broth.

5.4 Physiological characters of *Fusarium oxysporum* f.sp. *cepae*

Among the external factors which influence the growth of fungi, temperature plays an important role. All the fungi have minimum temperature, below which they cannot grow and above which they are inactivated or killed. Each fungus has its temperature range for the growth and sporulation. In the present study, maximum growth of *F. oxysporum* f .sp. *cepae* was obtained at 30°C, where as optimum temperature range was 20-30°C.

Similarly August *et al.* (1962) studied pathogenicity of four isolates of *F. oxysporum* f. sp. *cepae* at temperature range of 20 to 38℃, temp of 26℃ and higher induce the pathogenic effect. Sumner (1995) reported disease development is optimum when soil temperature ranges from 25 to 28℃. Abawi and Lorbeer (1972) also observed the optimum growth of *Fusarium oxysporum* f sp.*cepae* in 24 to 27℃ and no growth was observed at 0, 3, 6, 9 and 36℃in culture. Gupta *et al.* (2010) they observed maximum growth at 28℃ followed by 34℃.Since, *Fusarium* is mesophytic, perhaps, it requires higher temperature and low moisture for its better growth, spore formation, spore germination, mycelial growth and infection to plant. All these processes are favoured at a temperature of 25-30℃. Dhingra *et al*. (1974) also observed maximum growth of *F. oxysporum.* f. sp. *lintis* at 25℃. Sharma *et al*. (2012) observed same results in case of *F oxysporum* f.sp. *lycopersici*.

The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This brings importance of hydrogen ion concentration for the better fungal growth. The results indicated that maximum growth of fungus was observed at pH 6.0 (367.77mg). The present findings supports the reports of Sataraddi (1998) who observed that the optimum pH range for *Fusarium udum* was 6.0 to 7.0.Jadhav *et al*. (2000) observed profuse growth and sporulation of *F. oxysporum* f. sp *sesamum* at pH range of 6.6 to 7.5. Pokhar Rawal *et al*. (2003) noticed that the pH 6.5 favoured maximum growth and sporulation of *Fusarium* sp.

5.5 Disease management

5.5.1 *In vitro* evaluation of bioagents

Management of the disease through chemicals and use of resistant varieties is possible to some extent. But the hazardous impact of agrochemicals on the environment, development of resistant mutants, escalating cost of pesticides and frequent breakdown of resistantance strongly demand a sustainable and an alternative management approach to disease. Biological control assumes special significance as it is ecology conscious and cost-effective alternative strategy for disease management.

Chemical measures in management of soil borne pathogens, especially those infecting onion in late vegetative phase, are of limited. Therefore the presence of root colonizing antagonist seems acceptable for many reasons, including self propagation, longer persistence in soil environment, and ecological and toxicological benefit for workers and consumers. Hence, the present investigation was taken up to screen the bioagents for effective management of basal rot of onion.

In the present investigation four fungal and two bacterial biocontrol agents are tested against *F. oxysporum* f .sp.*cepae*. The results of dual culture technique on *F. oxysporum* f .sp.*cepae* revealed that all the four fungal antagonists significantly reduced the growth of *F. oxysporum* f.sp.*cepae* either by over growing or by exhibiting inhibition zones, except bacterial bioagents. Most of the antagonists inhibited colony growth of *F. oxysporum* f .sp. *cepae* by their fast and over growing nature as observed in antagonists. It was noticed that maximum reduction in colony growth was observed in *T. harzianum* (75.92%) which was significantly superior over all the bioagents tested. Next best was *T.virens* (74.81%) and *T.koningii* (73.7%).

Present studies recorded significant mycoparasitism of *Trichoderma harzianum* and *Trichoderma viride* on basal rot fungus that caused lysis of the hyphae and the spores *in vitro*. The results of the present study are in line with results of conditions Rajendran and Ranganathan (1996) who reported that fungal antagonists *T. viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* were effective against *Fusarium oxysporum* f.sp.*cepae* infecting onion under *in vitro*. Flori and Roberti (1993) reported that *T. harzianum* and *T.viride* reduced onion basal rot caused by *Fusarium oxysporum* f.sp.*cepae* to an extent of 88.7 and 77.3 per cent respectively and Coskuntuna and Ozer (2008) reported that seed treatment with *T. harzianum*, gave significant reduction in basal rot incidence on onion under pot and field conditions.

5.5.2 *In vitro* evaluation of botanicals

Extensive use of fungicides has led to various environmental problems, human health and their persistence in the bulbs. Contrary to the problems associated with the use synthetic chemicals, botanicals are environmentally non pollutive, renewable, largely non phytotoxic, readily biodegradable, relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the component in the integrated disease management, Khadar (1999).

In the present investigation, eight plant extracts were evaluated under *in vitro* condition against *F. oxysporum* f .sp.*cepae* to know the fungitoxic nature. Though complete inhibition of the pathogen was not observed in any of the leaf extract tested but considerable amount of inhibition was noticed in some of them.

Among eigtht plant extracts tested against *F. oxysporum* f .sp. *cepae,* Neem seed kernel extract at 10 per cent (54.1%) was significantly superior over all other plant extracts. Next best was prickly chaff flower leaf extract at 10 per cent (41.90%) followed by lantana leaf extract at 10 per cent (39.6%), which is on par with turmeric (39.3) and datura leaf extract (38.10%) and followed by tulsi at 10 per cent (39.1%). The least inhibition was noticed in garlic bulb extract (23.00%).In the present investigation, the mycelial growth of fungus was inhibited to a greater extent by neem seed kernel extract, which is said to have insecticidal property also. The fungicidal spectrum of *Azadirachta indica* has been attributed to azadirachtin which belongs to C_{25} terpenoides (Subramaniam, 1993). Similar results were observed by Ramaprasad shreeti (2005) and Kulkarni (2006) who reported that among the ten botanicals tested *in vitro* against *F. oxysporum* f .sp*. gladioli*, neem seed kernal extract at 10 per cent (54.49%) was found superior. Bhatnagar *et al.* (2004) reported that the highest percentage of inhibition for *Fusarium oxysporum* f.sp *psidi* was achieved by extacts from *Achyranthes rosea*, and *Curcuma longa* L.

5.5.3 *In vitro* evaluation of fungicides

In the absence of resistant cultivars, use of fungicides to manage the disease is an old-age practice. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know preliminary information regarding efficacy of fungicides against pathogens within a shortest period of time and therefore, serve as a guide for field testing.

Among systemic fungicides, Tebuconazole at 0.025, 0.05 and 0.1 per cent inhibited the growth of *F. oxysporum* f. sp*. cepae* completely. Next best systemic fungicide in inhibiting the mycelial growth is Carbendazim at 0.1 per cent inhibited complete growth of the fungus,followed by Thiophenate Methyl (87.68%), Propiconazole (82.87%) and Hexaconazole (82.51%). Tebuconazole being a of triazole, which inhibit sterol biosynthetic pathway in fungi. Carbendazim being a benzimidazole group of fungicides, they interfere with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1973). According to Davidse (1986) carbendazim induced nuclear instability by disturbing the mitosis and meiosis. Ozer and Koycu (1998) observed that Tebuconazole has also been reported to control Fusarium Basal Rot. Cramer (2000) and Ozer and Koycu (1998) reported that seed treatment with tebuconazole reduced the incidence of FBR in onion.

In the present investigation, among the non-systemic fungicides tested, mancozeb (91.48%) at 0.3 per cent was found very effective against all the non-systematic chemicals followed by captan (63.7%) at 0.3 per cent. Georgieva and Peikova (1976) observed that effective and excellent protection against *Fusarium* wilt disease of gladiolus was provided by captan and chlorothadonil. Taskeen-Un-Nisa *et al.* (2011) observed that amongst the non-systemic fungicides, mancozeb was found most effective (14.20 mm) in reducing mycelial growth of the fungi followed by captan (20.00 mm) and zineb (22.00 mm).

In the present investigation, among the combi-product tested mancozeb 63%+ carbendazim 12% and trifloxystrobin 25%+ tebuconazole 59% were found very effective, followed by carboxin 37.5% + thairam 37.5% and zineb 64%+ hexaconazole 4%. Kulkarni (2006) who reported that mancozeb at 0.3 per cent gave maximum inhibition of *F. oxysporum* f. sp. *gladioli.*

5.5.4 *In vitro* evaluation of nutrients.

The results showed that, among the three nutrients tested potassium chloride (21.82%) found superior, followed by Magnesium sulphate (15.55%). Prabhu *et al.* (2007) observed that application of K either before or after planting has effective in reducing the incidence of fusarium wilt and root rot caused by *Fusarium oxysporum* in case of cotton and other crops.

5.5.5 *In vivo* studies

The results showed that, among the various treatments applied, in case of onion, basal rot disease was least in seed treatment with *T. harzianum* + drenching with mancozeb 63%+ carbendazim12%, followed by seed treatment with *T. harzianum*+soil application of *T. harzianum*, and next best seed treatments were seed treatment with *T .harzianum* + drenching with tebuconazole and then in seed treatment with *T.harzianum* + drenching with mancozeb. Seed treatment with *T. harzianum* + drenching with NSKE (83.33 %) and seed treatment with *T. harzianum* + no drenching was found least effective and in control plot 100 per cent incidence of basal rot was recorded.

The results are comparable with Coskuntuna and Ozer (2007) reported that based on seed treatment with *T. harzianum*, resulted a significant reduction in basal rot incidence on onion sets under pot and field conditions. This formulation was also found superior to prochloraz by enhancing set diameter in both experiments. *T. harzianum* has the ability to stimulate a chemical response in onion. And they also showed that *T. harzianum* treatment of onion seeds induced the accumulation of antifungal compounds absorbing UV light in onion, which may be involved in the control of *F.oxysporum* f.sp.*cepae* during set development under pot and field conditions. Hunt *et al.* (1997) found that these compounds were involved in induced resistance. Rajendran and Ranganathan (1996) reported a combined seed treatment of *T.viride* was most effective for reducing disease incidence under pot and field conditions. Srivastava and Tiwari (2003) reported that seed treatment with *T. viride,* followed by its soil application, reduced damping-off disease in onion seedlings. *T. harzianum* has the ability to stimulate a chemical response in onion

Future line of work

There is no full stop to gain insight into scientific knowledge. Any amount of work does not satisfy the hunger of scientists as problems crept in, new ideas will continue to flow. The present investigations help to formulate new areas and given rise to new ideas on basal rot of onion caused by *F. oxysporum* f .sp.*cepae.* Hence, the following future lines of work are being suggested.

1. Identification of the hot spots for basal rot of onion and study of race pattern.
2. Environmental condition for the outbreak of the disease
3. Eco friendly management of basal rot onion as it is soil born pathogen.
4. Need to study resistance mechanism and screening of genotypes for resistance.
5. Develop an IDM practices to mitigate the disease.

6. SUMMARY AND CONCLUSIONS

An investigation on basal rot of onion caused by *F oxysporum* f.sp. *cepae* was carried out with reference to survey on the incidence and severity of basal rot, cultural, physiological and morphological characters of the *F oxysporum* f.sp. *cepae*, evaluation of fungicides, botanicals bioagents and nutrients against the disease both under laboratory and pot conditions. The results obtained are summarized here under,

An extensive roving survey was carried in Bijapur, Dharwad, Gadag, and Uttar Kannada districts of Karnataka to assess the severity of basal rot. Disease was severe in Dharwad and the lowest disease severity was recorded in Bijapur district.

The pathogen was isolated from infected bulbs of onion. The pure culture of pathogen was obtained by hyphal tip method. On the basis of morphological and cultural studies, the pathogen was identified as *Fusarium oxysporum* f .sp.*cepae.* Fungus produced three kinds of spores *viz.,* Microconidia, macroconidia and Chlamydospores. Among six solid media tested maximum radial growth of *F. oxysporum* f .sp.*cepae* was observed on potato dextrose agar (89.62 mm) followed by oatmeal agar (87.40mm). Less growth of fungus was observed in Czapeck's dox agar and Sabouraud's agar. Sporulation of fungus was found to be abundant on Potato dextrose agar, Richards's agar and Czapeck's dox agar media.

Among six liquid media tested maximum dry mycelial weight of fungus was obtained in potato dextrose broth (363.33 mg) which was found significantly superior over all the liquid media tested. The next best basal medium was oatmeal broth (343.33 mg) which was followed by Richard's broth (323.33 mg).The least mycelial weight was obtained in Rose Bengal broth (129.33 mg) and Sabouraud's broth (184.33 mg).

In temperature studies the maximum dry mycelial weight of fungus was observed at a temperature of 30°C (350.00 mg) which was significantly superior over all other temperature levels tested. This was followed by 25°C (330.00 mg), 35°C (220.00 mg) and 20°C (150.00 mg) which were in decreasing order and differed significantly. However, no mycelial growth was observed at 40°C.

F. oxysporum f .sp.*cepae* grew at different pH levels tested, however the maximum dry mycelial weight of the fungus was noticed at pH level of 6.0 (367.77 mg) which was significantly superior over rest of the pH levels tested. This was followed by the 7.0 (250.00 mg) and 8.0 (176.66 mg). The least growth was observed at pH 4.0 (53.44 mg) and 9.0 (76.66 mg)

The results of dual culture technique revealed that fungal bioagents were better than bacterial bio agents in inhibiting the growth of *F. oxysporum* f .sp.*cepae*. Maximum reduction in colony growth was observed in *T. harzianum* which was very effective when compared to all other bioagents tested.

The results revealed that, among the eight plant extracts, Neem seed kernel extract (54.1%)was found effective in inhibiting mycelial growth which is said to have insecticidal property. which was followed by *Achyrantus rosea* (41.90%)*, Lantana camera* at 10 per cent (39.6%), which is on par with *Curcuma longa* (39.3) *and Datura leaf extract* (38.10%) and followed by *Ocimum sanctum* at 10 per cent (39.1%), least inhibition was noticed in garlic bulb extract (23.00%).

Seven fungicides were tested *in vitro* against *F. oxysporum* f .sp. *cepae*. Among systemic fungicides, tebuconazole was highly effective in inhibiting the growth of *F. oxysporum* f .sp. *cepae,* followed by carbendazim. Among the non systemic fungicides mancozeb at 0.3 per cent was found significantly superior over other fungicides in inhibition of *F. oxysporum* f .sp. *cepae,* followed by captan at 0.3 per cent. Among the combi-product tested mancozeb 63%+ carbendazim 12% and trifloxystrobin 25%+ tebuconazole 59% was found very effective among the combi-product tested followed by carboxin 37.5 % + thiram 37.5 % and zineb 64%+ hexaconazole 4%.

The results showed that, among the three nutrients tested potassium chloride found superior among the nutrients tested, followed by zinc sulphate.

In vivo studies conducted in Department of Plant Pathology, Agricultural College, Dharwad. The results showed that, among the various treatments applied, in case of onion, basal rot disease was least in seed treatment with *T harzianum* + drenching with mancozeb 63%+ carbendazim12%, followed by seed treatment with *T. harzianum* + soil application of *T. harzianum*. The highest bulb yield was noticed in seed treatment with *T .harzianum* + drenching with mancozeb 63%+ carbendazim12%, followed by seed treatment with *T. harzianum* + soil application of *T. harzianum* which was on par with seed treatment with *T. harzianum* + drenching with tebuconazole. The least bulb yield was observed in control followed by seed treatment with *T. harzianum+* no drenching. And the post harvest loss was also calculated, among the tested treatments. The post harvest loss over control was least in seed treatment with *T .harzianum* + drenching with mancozeb 63%+ carbendazim 12%, followed by seed treatment with *T. harzianum* + soil application of *T. harzianum.*

Conclusions

- Survey: Disease was severe in Dharwad and the lowest disease severity was recorded in Bijapur district.
- Among six solid and liquid media tested maximum dry mycelial weight of fungus was obtained in potato dextrose broth.
- In temperature studies, the maximum dry mycelial weight of fungus was observed at a temperature of 30^0C.
- The maximum dry mycelial weight of the fungus was noticed at pH level of 6.0.
- Management studies : Effective bioagent was found *T. harzianum.*

  Effective botanical was found NSKE.

  Effective systemic chemical was found to be tebuconazole.

  Effective non systemic chemical was found to be mancozeb.

  Effective combi product was found to be mancozeb 63% + carbendazim 12%.

  In pot culture studies : seed treatment with *T. harzianum +* drenching with mancozeb 63%+ carbendazim12% was found effective.

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STUDIES ON ONION BASAL ROT CAUSED BY *Fusarium oxysporum* Schlencht Fr f.sp*. cepae* (Hans.) Snyd. and Hans

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ABSTRACT

Onion basal rot is a destructive disease, caused by *F. oxysporum* f .sp. *cepae*. The pathogen was isolated from infected bulb and on the basis of morphological and cultural studies was identified as *F. oxysporum* f. sp.*cepae.* The severity of the disease was more in Dharwad (42.77) district and least in Bijapur district (15.30%).The fungus produced microconidia, macroconidia and chlamydospores. Potato dextrose broth supported maximum dry mycelial weight on 10^{th} day of incubation and potato dextrose agar supported maximum sporulation and radial growth. The temperature of 30°C, pH 6 was found to be best for fungal growth.

Among the fourteen fungicides screened against pathogen tebuconazole 25 EC (0.1%) was found highly effective followed by combiproduct containing mancozeb 63%+ carbendazim 12%. Among eight botanicals screened against the pathogen NSKE was found better in inhibiting the pathogen followed by prickly chaff flower leaf extract at 5 per cent concentration. Among six bioagents evaluated, *T. harzianum, T. virens and T.koningii* were found effective in inhibiting the mycelia growth of the pathogen. Three nutrients viz., potassium chloride, zinc sulphate and magnesium sulphate were also screened against the pathogen, potassium chloride inhibited satisfactorily (21.82%) . The bioefficacy of fungicides, botanicals and bioagent which performed well *in vitro* condition were tested *in vivo* condition as well. Among them, seed treatment with *T. harzianum*+ drenching with mancozeb 63%+ carbendazim 12% at 0.3 per cent concentration were effective in reducing the per cent disease incidence of basal rot disease.