STUDIES ON SEED-BORNE FUNGAL DISEASES OF TOMATO AND THEIR MANAGEMENT

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By

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CERTIFICATE

This is to certify that the thesis entitled " STUDIES ON SEED-BORNE FUNGAL DISEASES OF TOMATO AND THEIR MANAGEMENT" submitted by Ms. JAYASHREE RAJAPUT, for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this university under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop grown in the world, next to potato. It is used as a fresh vegetable as well as processed and canned, paste, juice, sauce, powder or as a whole (Barone and Frusciante, 2007). The ripe fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food. Recently, it started gaining more medicinal value because of the antioxidant property (Anon., 2000). Hence, tomatoes are called as poor man's apple (Rick, 1969).

Tomato offers significant nutritional advantages, as it contains significant source of dietary lycopene, β-carotene, carotenoids, vitamin C, potassium, fiber, color, flavor and antioxidant properties in a low energy dense food (Rani and Khetarpual, 2009 and Britt and Kristin, 2011). Several human studies indicated a relationship between a high intake of tomato products and a decreased risk of several types of cancer, atherosclerosis and cardiovascular diseases (Cecilia *et al*., 2010). Recently, this crop is recognized as a model for plant-pathogen interactions (Arie *et al*., 2007).

At present, the total tomato cultivation is 46.16 lakh hactares in the world with the production of 1,279.93 lakh tonnes (Anon., 2015). In India, area under cultivation is 7.77 lakh hactares with the production of 182.86 lakh tonnes (Anon., 2015). The major tomato growing states in India are Andhra Pradesh, Karnataka, Odisha, Maharashtra and West Bengal. In Karnataka, tomato production is 20.34 lakh tonnes (Anon., 2015).

Many factors operate in successful cultivation as well as marketing of quality tomatoes of which diseases play an important role. Several diseases affecting tomato are caused by fungi, bacteria, viruses and nematodes (Balanchard *et al.,* 1992) and many of them are seed-borne in nature. These seed-borne pathogens are known to cause economically important diseases like early blight, late blight, fusarium wilt, septoria leaf spot, damping off and fruit rot. Seed-borne fungi are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. The infected seeds may fail to germinate, or transmit disease from seed to seedling and/or from seedling to growing plant (Islam and Borthakur, 2012). Fungal pathogens may be externally or internally seed-borne, extra- or intra-embryal, or associated with the seeds as contaminants (Singh and Mathur, 2004). Other fungi, including saprophytes and very weak pathogens, may lower seed's quality causing discoloration, which reduces the commercial value of the seeds (Al-Askar *et al*., 2012).

Good seed is a basic input in agricultural production. Successful agriculture depends on quality of seed used for sowing. Thus the seed producer holds a greater responsibility in producing genetically pure but viable seed, besides preserving its quality from harvest to next one or two planting seasons. It is known fact that the choice of chemicals for seed treatment exerts a positive effect on the viability and vigour of seeds during storage.

Seed-borne infections or infected seed is very important discouraging factor, which posses a serious problem in seed certification. Although infected seeds which may otherwise be viable with prescribed germinability as per certification standards, may not be acceptable as seed because of poor physical appearance, high incidence of seed-borne fungi and mycotoxin such as aflatoxin. Many seed-borne fungi are known to infect tomato *viz*., *Alternaria solani, A. alternata, Colletotrichum gloeosporioides, Bipolaris maydis, Curvularia lunata, Fusarium moniliforme, Fusarium solani, Fusarium equiseti, Cladosporium* sp., *Aspergillus clavatus*, *A. flavus, A. niger, Penicillium digitatum*, *Pythium* sp., *Verticillium* sp., *Rhizoctonia* sp., *Rhizopus arrhizus*, *R. stolonifer* and *Sclerotinia* sp.

The disease caused by seed-borne fungi may lead to seed abortion or elimination of germination capacity. Thus, the disease control programme is important at each stage of growth. Seed treatment with bio-control agents along with priming agents may serve as an important means of managing many of the soil and seed-borne diseases, the process often known as 'bio-priming". The bio-priming seed treatment developed for control of *Pythium* seed rot of sweet corn involves coating seed with a bacterial biocontrol agent such as *Pseudomonas aureofaciens* Kluyver AB254 and hydrating for 20 h under warm (23 ^{0}C) conditions in moist vermiculite or on moist germination blotters in a self-sealing plastic bag. The seeds are removed before radical emergence. The bacterial bio-control agent may multiply substantially on seed during biopriming (Callan *et al*., 1990). Seed encapsulation with beneficial microorganisms is now becoming common.

The priming agents along with bioagents can protect the seeds from biotic as well as abiotic stresses like moisture stress and thereby they can protect the seeds and the seedlings for extended periods. Hence there is a need to screen the new and the available seed dressing molecules including fungicides, bio agents and bio agents along with priming agents for their efficacy in overcoming the seed-borne fungal infections in tomato. Accordingly, the present investigation is being carried out with the following objectives:

1. Collection of seed samples and standardization of different seed health testing methods.
2. To study the threshold limit of seed borne inoculum.
3. To develop novel management strategies for seed-borne fungal infections of tomato.

3. MATERIAL AND METHODS

The present investigations were carried out in the laboratory and field during 2015-16. The field experiment was conducted at the farmer's field in Bailhongal while the laboratory experiments were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. The details of materials used and the methodology followed in conducting the experiments are described in this chapter.

3.1 Seed health testing

Tomato seed samples were collected from the different tomato growing areas of Dharwad, Belagavi and Haveri districts. Following are the details of the seed samples collected:

District	Taluk	Cultivars
Belagavi	Belagavi	Navodaya, PKM-1, Megha
	Bailhongal	Abhinav, Local, Chenya
	Gokak	S-22, Pusa Ruby
Dharwad	Dharwad	Pusa Ruby, DMT- 1, DMT- 2, Local, PKM-1
	Hubblli	Megha, S-85
	Kundagol	Punjab Chhahura, H-24
	Kalaghatagi	Laxmi, Soubhagya
Haveri	Haveri	Ramya, Rakshita, PKM-1, Nutan
	Byadgi	Laxmi, Shivam, Soubhagya
	Ranebennur	Abhinav, Abhishek, Laxmi, Shivam

Collected seed samples were stored at room temperature (25 ± 2ºC) and further they were subjected to seed health testing by standard blotter method (Anon., 1999).

3.1.1 Standard blotter method

The standard blotter method was developed by Doyer in 1938 which was later included in the International Seed Testing Association Rules (Anon., 1999). Seeds of each variety were tested by employing standard blotter method in three replications. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri plates after draining excess water. Untreated seeds were placed at the rate of 25 seeds per petri plate at equal distance. The plates were incubated at room temperature (25 ± 2 ºC) under alternate cycles of 12 hours NUV light and darkness. After eight day of incubation, the seeds were examined under stereoscopic-binocular microscope for the associated fungi and they were identified based on colony characters (Anon., 1999). Observations were recorded on per cent seed infection.

3.1.2 Identification of fungi

The identification of fungi was done based on the spore morphology and colony character. The culture of *Alternaria solani, A. alternata* and *Fusarium* isolated from the infected seed and the culture was further purified by following single spore isolation technique (Tuite, 1969), thus obtained pure culture was maintained on potato dextrose agar slants. Such culture tubes were preserved in a refrigerator at 5 ºC and renewed once in a month for further studies.

3.2 Evaluation of seed health testing methods

To know the efficacy of different seed health testing methods in detecting seed borne fungi of tomato, following methods were employed as described below.

3.2.1. Standard blotter method

Seeds of moderately infected tomato variety PKM-1 were placed at the rate of 25 seeds per Petri plate on moistened blotters as described under standard blotter method. Such seeds were examined under stereoscopic-binocular microscope for the infection of seed-borne fungi.

3.2.2 Deep freezing blotter method

Seeds of moderately infected tomato variety PKM-1 were placed at the rate of 25 seeds per plate on moistened blotter in the way as described under standard blotter method. The Petri plates were incubated at 20 ± 2 ºC for 24 hours under alternate cycles of 24 h NUV light and darkness, for next 24 hours the plates are incubated at -20 ºC in dark and then kept back under normal/ambient condition for the next six days. After eight days of incubation the seeds were examined under stereoscopic-binocular microscope for the infection of seed-borne fungi.

3.2.3 2, 4-D Blotter method

Seeds of moderately infected tomato variety PKM-1 were placed at the rate of 25 seeds per Petri plates with moistened blotters dipped in 0.2 % sodium salt of 2, 4 – dichlorophenoxy acetic acid. The Petriplates were incubated in the way as described under standard blotter method. After eight days of incubation the fungal growth on seeds was examined using stereoscopic-binocular microscope (Khare, 1996).

3.2.4 Water agar method

Seeds of moderately infected tomato variety PKM-1 were placed at the rate of 25 seeds per Petriplate containing 20 ml of 2 per cent water agar. The Petriplates were incubated for seven days as described under standard blotter method. After seven days of incubation, the fungal growth was examined under stereoscopic-binocular microscope.

3.2.5 Agar plate method with potato dextrose agar

Seeds of moderately infected tomato variety PKM-1 were surface sterilized with one per cent sodium hypochlorite solution for 1-2 min and then placed at the rate of 25 seeds per Petriplate containing 20 ml of potato dextrose agar. The petriplates were incubated for seven days as described under standard blotter method. After seven days of incubation the fungal growth was examined under stereoscopic binocular microscope.

3.2.6 Seed washing method

The washing test is a seed health testing method, employed to test seeds for seed-borne pathogens, the inoculum of which is present loosely on the seed surface. Two grams of tomato seed samples were taken in a test tube with 10 ml of sterilized water and shaken for 10 minutes on a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 20 minutes. The supernatant was discarded and the spores are again suspended in two ml of lactophenol and the suspension was then examined under the microscope for the presence of fungal spore load.

3.2.7 Seedling symptom test

Tomato seed samples collected from various places were examined for seedling symptom test. Culture tubes (100 × 16 mm) were filled with 10ml of 2 per cent water agar and solidified to have slight slant. Seeds of variety PKM-1 were artificially inoculated with different conidial suspensions of *Alternaria solani, A. alternata* and *Fusarium* spp. separately from 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} concentration, and apparently healthy seed treated with only distilled water without conidial suspension served as control. Randomly selected seeds of each samples were placed individually in each tube and incubated at 20 ± 2ºC with alternate cycles of 12 h light and dark period for 15 days. The cotton plug was removed after seedling reached to rim portion of the tube and observations was taken on symptom expressed in the seedling (Khare, 1996).

3.3 Transmission studies

Seed to plant transmission studies were carried out both under *in vitro* and pot culture studies. Under *in vitro* condition, transmission test was carried out by employing seedling symptom test as described earlier.

3.3.1 Effect of different seed inoculum levels on disease incidence

This study was under taken by using apparently healthy seeds to know the effect of different seed inoculum densities and extent of disease incidence. *Alternaria solani, A. alternata* and *Fusarium* spp. isolated from naturally infected tomato seeds, was cultured on PDA at 20 ± 2 ºC for one week. The fungal propagules (mycelial bits) were isolated by taking a 0.5 cm disc using a cork borer. The discs were gently mixed with 5ml of sterile water by using a magnetic stirrer and propagules were adjusted to required concentration by using haemocytometer.

No of propagules in the diluted suspension/milliliter = Average no of propagules above one large square × 1ml / 0.004 mm3 (or)

No of propagules in the diluted suspension/milliliter = No of propagules counted × 2,50,000

This number has to be multiplied by the original dilution of the suspension to ascertain the density of original suspension in number of cells /ml (Karuna and Kolte, 2005).

Apparently healthy seeds of tomato cultivars were surface sterilized for 3 min with 0.5 % sodium hypochlorite solution, and washed in sterile distilled water. The sterilized seeds were soaked in different inoculum level under vacuum for 30 minutes, and dried at room temperature overnight. The seeds of control treatments were similarly treated except that they were soaked in sterile distilled water. Treatments were laid out in comlete randomized block design (CRD) with four replications. Ten seeds were sown in each pot and replicated 4 times in each treatment. The pots were incubated under glasshouse condition at 26 ± 2ºC. Observations were recorded on the rapidity of disease incidence in each treatment, every alternate days and the per cent disease incidence and per cent seed germination were calculated by using the formula,

$$\% \text{ Disease incidence} = \frac{\text{No. of diseased plants /pot}}{\text{Total no. of plants /pot}} \times 100$$

$$\% \text{ Seed germination} = \frac{\text{No. of seed germinated /pot}}{\text{Total no. of seed sown /pot}} \times 100$$

The same experiment was repeated under laboratory conditions by employing growing on test in test tubes.

3.3.2 Location of fungi in seed

The location of fungi in seed was studied by employing component plating technique (Maden *et al*, 1975). Naturally infected tomato seed samples, of variety PKM-1 was used for the study. Twenty five seeds were washed four times with tap water then surface sterilized in one per cent sodium hypochlorite solution for one minutes. These seeds were again washed with sterile water and soaked in water for 1 hr and then the seeds were dissected aseptically using sterile needle and forceps. The pericarp was separated and other seed parts were difficult to separate they were fused together (*i.e.*, embryo endosperm and hilum). The separated parts were plated immediately before drying on potato dextrose agar plates. The plates were incubated at 20 ± 2 ºC for seven days, the seed component were examined under stereoscopic binocular microscope for the presence of fungal structures in different seed parts.

3.4 Management strategies for overcoming seed-borne fungal infections of tomato

3.4.1 Evaluation of seed dressing fungicides

This study was carried out to know the efficacy of different seed dressing fungicides in eliminating the seed-borne fungal infections in the infected seed sample of variety PKM-1. The experiment included 8 treatments were allotted in factorial complete randomized block design (CRD) with 3 replications.

Seeds were treated with fungicides by following dry seed treatment. 0.2 and 0.3 per cent concentration of the fungicide was prepared and treated with seeds by sprinkling the water. Then the seeds were dried under shade. Three replications of 100 seeds per treatment were tested in moist paper towel (rolled towel) method as described earlier and then incubated in BOD incubators at 25 ± 2 ℃ under 12 h of light and 12 h of darkness. The untreated sample served as control. The per cent germination and per cent infection were recorded after seven days of incubation. Vigour index was calculated by the following formula, given by Abdul and Anderson (1973) *i.e.*

Vigour Index = Seed germination (%) × Seedling length (Shoot + Root length (cm))

$$\% \text{ Seed germination} = \frac{\text{No. of seed germinated}}{\text{Total no. of seed used}} \times 100$$

$$\% \text{ Seed infection} = \frac{\text{No. of seed infected}}{\text{Total no. of seed used}} \times 100$$

The trade name, common name and chemical names of fungicides used in the experiment are given below.

Sl. No.	Common name	Trade name	Chemical name	Dosage	
1	Carbendazim	Bavistin 50 WP	2-(methoxy carbomyl)-benzimidazole	0.2 %	0.3 %
2	Carbendazim + Mancozeb	Sprint	Manganese ethylene bis (dithiocarbamate) polymeric complex + methyl-2-benzimidazole carbamate	0.2 %	0.3 %
3	Carboxin + Thiram	Vitavax Power [Carboxin (37.5 WP) +	3-(3-5-dichlorophenyl)-N-(1-methyl ethyl)- 2-4-dioxo-1-lemadazolidine	0.2 %	0.3 %

		thiram (37.5 WP)]	carboximide		
4	Captan (Standard check)	Captaf 50 WP	Captaf 50 WP N-trichloromethyl mercapta 4-cyclolexene-1-2-dicarboximide-Ntrichlormethyl thiotetrahydropthalamide	0.2 %	0.3 %
5	Captan + Hexaconazole	Taquat	N-trichloromethyl mercapta 4-cyclolexene-1-2-dicarboximide-Ntrichlormethyl thiotetrahydropthalamide + 2- (2,4-dichlorophenyl)-(1-14-1,2,4-triazole-yl) hexan-2	0.2 %	0.3 %
6	Metalaxyl	Apron	Methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate	0.2 %	0.3 %
7	Tebuconazole	Raxil 2DS	(RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4- triazol-1-yl methyl) pentan-3-ol	0.2 %	0.3 %
8	Control				

3.4.2 *In vitro* evaluation of bioagents by rolled towel method

The powder formulations of antagonists' *viz., Trichoderma harzianum* Rifai, *Pseudomonas fluorescens* Migula, *Bacillus subtilis* Cohn and VAM fungi were taken for seed treatment to test their efficacy in providing protection against seed borne infection under *in vitro* conditions by rolled towel method. These bioagents were pracured from IOF ((Institute of Organic Farming), UAS, Dharwad. Seeds of moderately infected tomato variety PKM-1 were treated with different bioagents at the rate of 0.4 and 0.8 per cent concentrations. The seeds were shaken along with bioagents for 20 minutes in mechanical shaker for uniform application and then stored in separate boxes for 24 hours. The treated seeds were tested in 3 replications of 100 seeds by employing rolled paper towel method. The treatments were laid out in factorial CRD design. Seeds without treatment served as control. These paper towels were incubated at 25 ± 2 ℃ for seven days under 12 h light and 12 h darkness. After seven days of incubation, per cent germination, per cent infection was recorded and seedling vigour was calculated as described earlier.

3.4.3 Evaluation of different biopriming agents

3.4.3.1 *In vitro* evaluation of bioagents with priming agents by rolled towel methods

In bio-priming, two grams of tomato seeds (variety - PKM 1) were treated with bacterial bio-control agent *Pseudomonas fluorescens* with priming agents like vermiculite, coir pith, jelly and coco peat. Same treatment was followed for fungal biocontrol agent *Trichoderma harzianum.* After pre-soaking of seeds in sterile distilled water, seeds were coated with powder formulations of *P. fluorescens and T. harzianum.* at 0.8 per cent concentration along with moist vermiculite in the proportion of 2:1 (2 parts of vermiculite and 1 part of seed) and mixed thoroughly to give uniform coating. These seeds were dried in shade and stored at 25 ± 2 °C for 24 h in a self– sealing plastic bags. The treated seeds were tested three replications of 100 seeds by employing paper towel method in complete randomized block design (CRD). These paper towels were incubated at 25 ± 2 °C for twelve days under 12 h light and 12 h darkness. After twelve days of incubation, per cent germination and per cent infection were recorded and seedling vigour was calculated as stated earlier. The same procedure was employed for coco peat, coir pith also.

For bio-priming in jelly, (a commercial preparation containing water absorbent organic polymer which absorbs, stores and then keeps releasing the moisture slowly). 10 g of jelly was poured into 500 ml of cold sterilized water and kept in water 5 to 6 hours till the small pieces turned into sparkling jelly cubes. After pre-soaking of seeds in sterilized water, seeds were coated with powder formulations of *P. fluorescens* and *T. harzianum* at 0.8 per cent concentration along with the jelly cubes in 2:1 proportion as stated earlier. Such treated seeds were tested in three replications of 100 seeds by employing paper towel method. These paper towels were incubated at 25 ± 2 °C for twelve days under 12 h light and 12 h darkness. After twelve days of incubation per cent germination, per cent infection was recorded and seedling vigour was calculated as stated earlier.

3.4.4 Effects of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery

An experiment was conducted during *rabi* 2015-16 at Bailhongal in the farmer's field. Two best seed dressing fungicides, bio agent and bio priming agents were selected for the management of seed-borne fungal diseases of tomato. Tomato variety PKM-1 was used for the study. All other packages of practices were followed and kept common to all the treatments. Following treatments were used for the studies.

T_1 – Seed treatment with Carbendazim + Mancozeb @ 0.3 %

T_2 - Seed treatment with Carboxin + Thiram @ @ 0.3 %

T_3 – Seed treatment with *Trichoderma harzianum* @ 0.8 %

T_4 - Seed treatment with *Pseudomonas fluorescens* @ 0.8 %

T_5 – Seed treatment with Jelly + *Pseudomonas fluorescens* @ 0.8 %

T_6 – Seed treatment with Coco peat + *Trichoderma harzianum* @ 0.8 %

T_7 – Control

Observations were taken after 15 and 25 days on per cent seed germination, per cent seedling mortality and seedling vigour were calculated as stated earlier.

3.4.4.1 Nursery bed preparation

Tomato seeds were sown on nursery beds to raise seedlings for transplanting in the field. The beds were prepared at the size 4 m length x 0.75 m width and 10 cm in height. Sowing was done thinly in lines spaced at 10-15 cm distance at a depth of 2-3 cm and covered with a fine layer of soil followed by light watering. The beds were covered with dry straw to maintain required temperature and moisture. The cover was removed immediately after completion of germination. Observation was taken at 15 and 25 days after sowing.

3.4.5 Field evaluation of different seed treatments for the management of seed- borne fungal diseases of tomato

Seedlings from respcted treatments were transplanted after 25 DAS to the main field and common foliar spray of hexaconazole @ 0.1 % at 45 DAS was given to all the treatments except control.

Design and layout

The experiment was laid out in randomized block design (RBD) with three replications. The treatments were randomly allotted to the plots.

Plot size : 3.6 m × 6 m

Treatments : 8

Spacing : 60 cm × 45 cm

Variety : PKM-1

Treatments details:

T_1 – ST with Carbendazim + Mancozeb @ 0.3 % + Hexaconazole @ 0.1 % spray

T_2 – ST with Carboxin + Thiram @ @ 0.3 % + Hexaconazole @ 0.1 % spray

T_3 – ST with *Trichoderma harzianum* @ 0.8 % + Hexaconazole @ 0.1 % spray

T_4 - ST with *Pseudomonas fluorescens* @ 0.8 % + Hexaconazole @ 0.1 % spray

T_5 – ST with Jelly + *Pseudomonas fluorescens* @ 0.8 % + Hexaconazole @ 0.1 % spray

T_6 – ST with coco peat + *Trichoderma harzianum* @ 0.8 % + Hexaconazole @ 0.1 % spray

T_7 – Hexaconazole @ 0.1 % spray alone

T_8 – Control

(ST = Seed treatment)

Observations were recorded after 45 DAS, 60 DAS and 90 DAS on per cent disease index of early blight and wilt incidence

a) Alternaria leaf spot (early blight): 0-5 disease rating scale (Mayee and Datar, 1986)

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	21-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem, and fruit.

Further, the PDI was calculated with the above scales using the formula given by Wheeler (1969).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

$$\text{Wilt incidence} = \frac{\text{No of wilted plants in a microplot}}{\text{Total no of plants in a microplot}} \times 100$$

b) Yield:

Crop was harvested at fruit ripening stage from randomly selected 5 plants from each replication and after harvest fruit weight of each plant in kilograms were recorded and yield per each treatment from each replication of 4 pickings in kilograms were recorded. Yield per hectare was computed by using net plot yield data and it was then converted to tonnes per hectare.

c) Benefit cost ratio (B:C ratio):

Total cost (Rs. ha^{-1}) incurred for the application of each treatment including cost of cultivation, cost of chemicals and labour was worked out. Gross return (Rs.

ha^{-1}) was calculated on the basis of market price of the produce during harvest period. Net returns (Rs. ha^{-1}) was calculated by deducting the total cost (Rs. ha^{-1}) from the gross return (Rs. ha^{-1}). Benefit cost ratio was calculated by using the formula.

$$\text{B:C ratio} = \frac{\text{Net returns (Rs. ha}^{-1}\text{)}}{\text{Total cost (Rs. ha}^{-1}\text{)}}$$

3.5 Statistical analysis

Statistical analysis was carried out by following the standard procedures (Panse and Sukhatme, 1967). Data in percentage were transformed to angular values (Arc sine transformation) before analyzing.

2. REVIEW OF LITERATURE

Seeds are the efficient medium for survival and dissemination of plant pathogens. In addition, the plants resulting from the infected seeds will not only be diseased but they also serve as infection foci for secondary spread of the disease. The reports are available in literature on different aspects of seed-borne fungi of tomto. However reviews relevant to the aspects investigated have been included here.

2.1 Seed mycoflora of tomato

Orlava *et al*. (1982) isolated 23 fungal species from tomato seeds grown in the Moscow area. Most predominate were *Penicillium, Aspergillus, Alternaria, Chaetomium, Ulocladium* and *Fusarium* spp.

Naseema *et al*. (1983) reported the *Aspergillus niger* and *Rhizopus stolonifer* from the tomato seeds as an internally seed-borne fungi.

Mridha *et al.* (1986) collected seeds of four tomato varieties for seed health test from Noakhali and Chittagong districts following blotter method. They revealed the association of *Alternaria solani, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* f. sp. *lycopersici, Penicillium* spp. and *Rhizopus* spp. with the tomato seeds.

Surface-borne saprophytes, *Alternaria alternata, Aureobasidium pullulans, Cladosporium* spp., *Penicillium* spp., *Rhizopus stolonifer, Stemphylium* spp., *Vlocladium atrum* and several pathogens like *Ascochyta pisi, Botrytis cinerea*, *Fusarium oxysporum* and *Phoma medicoginis* isolated from chickpea seed by employing standard blotter method (Bretag and Mebalds, 1987).

Seed mycoflora of 31 seed samples of brinjal collected from different location of Chittagong following the blotter method. Eight different fungi were encountered namely, *Alternaria solani, Aspergillus flavus, Aspergillus niger, Botrytis* spp., *Curvularia* spp., *Fusarium moniliforme, Penicillium* spp. and *Rhizopus* spp. (Basak *et al*., 1989)

Malik *et al*. (1991) reported the presence of *Alternaria solani, A. alternata, A. tenussima, Aspergillus niger, Curvularia lunata, Fusarium oxysporum* and *Rhizoctonia solani* on tomato seeds.

Oladiran and Iwu (1993) noticed that the association of *Fusarium equiseti*, *F. chlamydosporum, Alternaria solani*, *Geotrichum candidum*, *Acremonium recifei*, *Aspergillus flavus* and *A. niger* with fruit rot of tomato.

Abdel *et al.* (1995) isolated 39 species of fungi belonging to 16 genera from healthy tomatoes, among them *Aspergillus niger* being the most prevalent.

Bankole (1996) isolated 18 fungal species as internal seed-borne from two tomato varieties. Among them *Aspergillus flavus, A. niger, Alternaria longissima, Fusarium* spp. and *Phoma destructive* were most abundant.

Perveen and Ghaffar (1995) isolated 37 species of fungi belonging to 20 genera, 22 species of fungi belonging to 15 genera from 24 samples of tomato seeds collected from different parts of Pakistan. *Fusarium solani, F. moniliforme, Aspergillus flavus, Alternaria alternata and Drechslera australiensis* were predominant.

Conducted an investigation to find out the prevalence of seed borne fungi in tomato seeds, totally 12 fungi were detected namely, *Colletotrichum capsici, Fusarium moniliforme, Fusarium oxysporum, Alternaria alternata, Bipolaris sorokinicma. Curvularia lunata, Curvularia geniculata, Aspergillus flavus, Aspergillus niger, Chaetomium dolichotrichum, Chaetomium ereclum and Chaetomium homopilatum* were asociated with tomato seeds. Of the 10 fungi, *Fusarium* sp. (*Fusarium moniliforme* and *Fusarium oxysporum)* was most predominant followed by *Aspergillus* sp. (Khan and Ahmed, 1998).

Krishna *et al.* (1998) recorded 12 different fungal species from a local variety of tomato in India by using different seed health testing methods. *Alternaria solani* was detected by them in 7.5 per cent of seed samples by using standard blotter method.

Alam (2001) reported that most of the pathogens from different vegetables seeds by using standard blotter method. He recorded *Altrnaria, Aspergillus, Penicillium, Curvularia* and *Rhizopus* from tomato seeds. *Aspergillus, Penicillium, Curvularia, Fusarium, Phomopsis* and *Rhizopus* from brinjal seeds. *Aspergillus, Penicillium* and *Rhizopus* from onion seeds and *Aspergillus, Penicillium, Fusarium, Rhizopus, Colletotricum, Alternaria* and *Macrophomina* from okra seeds.

The six seed-borne mycoflora namely, *Alternaria solani, Aspergillus flavus, Penicillium* spp., *A. fumigatus, Fusarium oxysporum* and *Phytophthora infestans* were reported on tomato seeds (Fakir, 2001).

Alam (2002) studied the health of some vegetable seeds collected from different sources. He found six fungi, *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Curvularia* spp., *Fusarium* spp. and *Rhizopus* spp. in tomato and brinjal seeds.

Shome (2002) studied quality of brinjal, tomato and onion seeds. He recorded *Aspergillus* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. where maximum prevalence of *Fusarium* spp. was (12 %) in farmer's seeds.

Singh *et al*. (2005) isolated nine fungal species belonging to eight genera namely; *Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Helminthosporium sativum, Mucor* sp., *Penicillium notatum* and *Rhizopus nigricans* were observed on seven seed samples of tomato.

The effect of seed-borne fungal pathogen on the planting value of brinjal (*Solanum melongena*) seeds collected from different sources *i.e*. Bangladesh Agriculture Development Corporation (BADC), Bangladesh Rural Advancement Committee (BRAC), Local Seed Company (LSC), Local Seed Trader (LST) and Farmer (F) of Mymensingh, Bangladesh during March 2002 and 2003. *Apergillus flavus, Alternaria alternata, Colletotrichum dematium, Phomopsis* spp. *Curvularia lunatu [Cochliobolus lunatus], Fusarium moniliforme [Gibberella moniliformis],* *F. oxysporum, Penicillium* spp*. and Rhizopus* spp. were commonly found prevalent in all the seeds (Sarkar *et al*., 2006).

Seed mycoflora were isolated from 25 seed samples of different varieties of eggplant collected from local seed market. Collected seed lots yielded various fungi including *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum* and *Fusarium solani* ranging from 6.75-13 per cent. Some saprophytic and non-pathogenic species of *Epicoccum, Mucor* and *Penicillium* were also isolated, which ranged from 1.29 - 37.8 per cent (Habib *et al*., 2007).

Dabbas *et al*. (2008) observed association of *Alternaria solani, A. tenuis, A. alternata, Fusarium oxysporum, Rhizopus infestans, Aspergillus niger, Sclerotinia sclertivorum, Rhizopus* spp. *Mucar* and *Botrytis* spp. with tomato seeds by standard blotter method.

Sultana (2009) found eight fungi *viz*., *Aspergillus* sp., *Fusarium* sp., *Botrytis* sp., *Curvularia* sp., *Colletotrichum* sp., *Rhizopus* sp. and *Phomopsis* sp. on tomato seeds. Among these pathogens *Fusarium* sp. was highly prevalent in all the crop seeds ranging from 1.60 to 30 %.

Dabbas *et al*. (2010) noticed *Aspergillus niger, Fusarium solani, Alternaria alternata, Alternaria solani, Aspergillus flavus, Rhizopus* sp. with discolored and abnormal seeds of tomato variety Azad T- 6 in standard blotter method.

The association of *Aspergillus clavatus, A. flavus, A. niger*, *Cladosporium* sp., *Penecillium digitatum, Pythium* sp., *Rhizoctonia* sp., *Rhizopus arrhizus*, *Rhizopus stolonifer* and *Sclerotinia* sp. with solanaceous seeds, the highest per cent of repetition of *A. flavus* and *Pythium* sp. were 0.42 and 0.4 per cent respectively in tomato seeds (Ismael, 2010).

The fungi associated with seeds of seven cultivars of sunflower were detected by using agar plate and blotter paper methods. A total of 13 phytopathogenic fungal species including *Alternaria alternata, A. helianthi, Aspergillus flavus, A. fumigates, A. niger, Curvularia lunata, Drechslera tetramera*, *Fusarium solani, F. moniliforme*, *Macrophomina phaseolina*, *Mucor mucedo, Penicillium* and *Rhizopus* spp. were identified and isolated fungi reduced germination (Rukhsana *et al.,* 2010).

Chamling *et al*. (2011) isolated eight fungal species on four cultivars of tomato seeds in Gujarat by standard blotter method. The fungi isolated were *Aspergillus niger, A. flavus, Alternaria alternata, Fusarium moniliforme, Fusarium* sp., *Cladosporium* sp., *Penicillium* sp. and *Cunnninghamella echinulata*. Among them the dominance of *Alternaria alternata* (20.64 %) followed by *Aspergillus niger* (13.44 %) and *Fusarium* sp. (20.33 %).

Thippeswamy *et al*. (2011) observed the dominance of *Alternaria solani* (12.0 %), *Alternaria alternata* (3.0 %) and *Fusarium oxysporum* (18.0 %) on tomato seeds in Karnataka.

Abdulaziz *et al*. (2014) reported that among the one hundred samples of tomato seeds were collected from tomato-cultivated fields in Saudi Arabia and screened for their seed-borne mycoflora, a total of 30 genera and 57 species of fungi were recovered from the collected seed samples using agar plate and deep-freezing blotter methods.

Meraj and Nandkar (2015) who observed 29 fungi associated with tomato seed samples collected from different localities of Vidarbha region. Fungi like *Alternaria solani, Aspergillus flavus, A. niger, Botrytis cinera, Cladosporium fulvum, Colletotrichum capsici, Curvularia lunata, Fusarium solani, F. lycopersici, Penicillium* sp. were found to be most dominant fungi on tomato seeds.

2.2 Seed health testing methods

Several methods have been developed to detect the seed-borne fungal microflora and these have been reviewed by Neergaard (1977). The emphasis has been given to those methods which are simple, easy, economic, sensitive, reproducible and efficient.

Neergaard (1956) suggested the dipping of blotters in 0.2 per cent 2-4 dichlorophenoxy acetic acid for detecting fungal infection in cabbage seeds since it avoids germination of seeds.

Deep freezing blotter method was advocated for evaluation and identification of fungus as an alternative method to 2, 4-D blotter method since it is also known to suppress germination of seed (Limonard, 1968).

Seed-borne mycoflora associated with soybean seeds were noticed and examined 19 species of fungi and a bacterium. The blotter method proved superior over agar plate method. Pre-treatment of seeds in the agar plate method reduced a number of fungi to a great extent (Singh *et al.*, 1973).

Kumar *et al*. (1981) reported the superiority of standard blotter method over agar plate method for the detection of 32 spp. of fungi from brinjal seeds. Among them *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Rhizopus, Stachybotrys, Penicillium* and *Phoma* were predominant.

Paul (1989) reported the association of *Aspergillus* (12 %), *Cladosporium* (56 %), *Fusarium* (75.25 %), *Penicillium* (7.25 %), *Phoma* (25.25 %) and *Rhizopus* (12 %) with soybean seeds when assayed under blotter method.

Dwivedi and Shukla (1990) reported the predominance of *Aspergillus, Fusarium* and *Penicillium* with tomato seeds when assayed under blotter method compared to agar plate method.

Perveen (1996) reported the efficacy of standard blotter method over agar plate and deep freezing blotter method for the detection of *Alternaria* spp., *Fusarium* spp. and *Dreschleria* spp. on tomato seeds.

Krishna *et al.* (1998) reported that standard blotter method is an efficient one for detection *of Alternaria solani* causing early blight in tomato.

Advocated that dry seed examination itself is not an adequate method and is a supplement to the incubation method with some modifications, which are imperative to provide full information regarding the identification of the pathogen. Besides, dry seed examination does not provide information on viability of the seeds (Anon., 1999).

Singh *et al*. (2005) reported that the standard blotter method was best method in terms of number of fungal species isolated from chickpea seed followed by agar plate method.

Vinaya and Laxminarayanarao (2008) reported that among the different seed health testing methods tested, standard blotter method was found to be most efficient for the quick and accurate diagnosis of *Colletotrichum capsici* causing fruit rot in chilli.

Ishrat and Shahnaz (2009) reported that deep freezing method was the best method for the detection of *Drechslera* spp., *Fusarium* spp. and *Penicillium* spp., while agar plate method was suitable for the detection of *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp. and *Rhizopus* spp. from maize seeds.

Kumara *et al.* (2012) reported that standard blotter method to be superior over agar plate method for detection of *Aspergillus niger, A. flavus, Alternaria alternata, Fusarium moniliforme* and *Penicillium* sp. in chilli seeds of varieties GVC101 and GVC111.

Standard blotter method was found to be good for the detection of seed-borne fungal infections in tomato over agar plate method (Sowley and Kodua, 2012)

Meraj and Nandkar (2015) suggested that standard blotter method was the best method for detection of *Cercospora capsici, Chaetomium globosum, Drechslera* spp. *Phytophthora infestans* and *Trichothecium roseum*, while agar plate method was suitable for the detection of *Aspergillus ochraceus, A. sulphureus, Curvularia* sp. *Fusarium solani* and *Penicillium spp*. from tomato, chilli and brinjal seeds.

2.3 Effect of inoculum levels, transmission studies and proving pathogenicity

Elliott and Crawford (1922) reported the seed borne nature of the fungus by recovering it from seed, from infected plants and proving that isolates were pathogenic on tomato. Approximately 3.5 per cent of the seeds from fruits produced on heavily infected plants contained the pathogen.

Westerlund *et al*. (1973) reported 37 isolates of *Fusarium solani* and five isolates of *F. oxysporum* were found to be pathogenic to tomato in green house inoculation trials.

Orlava *et al*. (1982) studied the pathogenecity of 3 sp. of *Fusarium,* isolated from tomato seeds and observed that pathogenecity was higher when tomato seeds were dipped in spore suspension for 1 hour before sowing. Perveen (1996) reported that *Fusarium* and *Alternaria* sp. inoculated on tomato seed reduced per cent seed germination.

Thippeswamy *et al*. (2006) reported that inoculum sprayed on brinjal plants showed the symptoms of *Phomopsis vexans* and *Alternaria solani* in 2 to 3 days. They observed 1-10 per cent spots in one and two month old seedlings and 20-30 per cent in three months old plants while no spots observed in control plants.

The pathogenic effect of six *Fusarium* spp. *viz*., *F. equiseti*, *F. longipes, F. scirpi, F. oxysporum, F. pallidoroseum* and *F. solani* in sunflower plant by artificial inoculation. Symptom produced by these *Fusarium* spp. includes root rot, collar rot, seedling rot, damping-off, stunting, wilting, tip burning and reduction in growth, wilting and seedling rot were found to be the most prominent symptom produced by all *Fusarium* spp. high wilt incidence was observed in plants inoculated by *F equiseti*, *F. scripi* and *F. solani* (Sharfun and Muhammad, 2007).

Shovan *et al.* (2008) studied the pathogenicity test with 33 isolates of *Fusarium solani* under pot culture, all the tested isolates were found to be pathogenic on tomato. Ismael (2010) observed harmful influence on germination rate of pepper and tomato cultivars by some fungal exudates. Fungal exudates significantly decreased germination rate of all the tested solanaceous seeds.

Ignjatov *et al.* (2012) reported *Fusarium oxysporum* was the main source of tomato wilt and fruit rot, the symptoms appears on older plants with typical signs of leaf chlorosis.

Abdulaziz *et al*. (2014) studied pathogenicity test under pot culture of seven fungal species isolated from tomato seeds, were *Alternaria solani* caused the highest percentage of rotted seeds (56.7 %) followed by *Fusarium solani* (38.6 %), *Alternaria alternata* (38.3 %), *F. equiseti* (30 %), *Phoma lycopersici* (28.3 %), *F. oxysporum* (26.7 %) and *F. verticillioides* (23.3 %) as compared with the check (2 %). After 60 days, plants showed 28.4 per cent seedling mortality due to infection by *A. solani* while *A. alternata* caused 25 per cent infection. Among *Fusarium* species tested, *F. solani* caused 18.4 per cent wilting on seedlings followed by *F. oxysporum* and *F. equiseti* (13.3 % and 10 % respectively).

The pathogenicity test of seed-borne fungi, *Alternaria alternata, Fusarium solani* and *Fusarium oxysporum* on 10 tomato cultivars. All the germinated seeds were infected by these fungi with varying degree of variability or aggressiveness and each cultivar significantly reduced germination and produced more abnormal seedlings compared to control. They also reported the transmission of *Alternaria alternata, F. solani* and *F. oxysporum* from seeds to seedlings. The highest transmission was observed during germination stage followed by seedling stage on leaves and on stem (Hayat *et al*., 2014).

2.3.1 Histopathology

Neergaard (1979) found many seed borne fungi infected the seed coat causing conspicuous black brown to black necrotic discoloration, germination reduction and germination failure. The best known samples were the effects of *Aspergillus* spp. in tomato, okra and radish; *Fusarium* spp. in onion and *Colletotrichum gloeosporioides* in okra.

Saad *et al.* (1988) observed that *Aspergillus flavus* and *Fusarium solani* were associated with damage to plumule, radical and hypocotyl of germinating tomato seedlings.

Thippeswamy *et al*. (2006) studied the location and transmission of *Fusarium oxysporum* and *Alternaria solani* in naturally infected tomato seeds. The results revealed that both pathogens were located in seed coat, cotyledons and in embryonic axis of tomato seedlings at various concentrations.

2.4 Management of seed-borne fungal diseases of tomato through novel seed dressing fungicides, bioagents and bioagents along with priming agents (bio-priming)

2.4.1 Evaluation of fungicides

The advantage of fungicides goes beyond increasing yield. Seed-borne fungi not only reduce seed quality and yield but also serve as efficient medium for transport of such pathogens to longer destinations, as well as source of primary inoculum in the field, which can be controlled with the use of fungicides.

Among 11 fungicides used as seed treatment assayed under *in vitro* condition against *Fusarium solani* inciting piper betel decline, carbendazim (0.1 %) was most effective followed by duter, captan and blitox (Hiremath *et al*., 1981). *Fusarium solani* the incitant of wilt disease in Kagzi lime was completely inhibited under *in vitro* condition by carbendazim (0.1 %) (Dhrub, 1988).

Kapoor and Kumar (1991) reported that seed treatment with captan was least effective against *F. oxysporum* and *F. solani* infecting tomato compared to carbendazim. Growth and sporulation of *F. solani* causing root rot of Garden Rue was completely inhibited by methoxyethyl mercury chloride (100 ppm), thiram (1000 ppm) and carbendazim (50 ppm) (Rathnamma, 1994).

Singh *et al*. (2000) reported the efficacy of seed treatment of tomato variety pusa ruby with seed dressing fungicides *viz*., carbendazim, vitavax, thiram and carbendazim + mancozeb at 0.2 per cent concentration significantly reduced the incidence of early blight caused by *Alternaria solani* and increased the fruit yield.

Rahman and Bhattiprolu (2005) an experimental results revealed that seed treatment with thiride @ 2 g/ kg seed alone or along with soil application of thiride or Ridomil MZ-72 @ 0.2 % twice, first immediately after germination and second at 20 days after germination resulted in the effective control of damping-off disease in the solanaceous crop nurseries.

Thippeswamy *et al*. (2006) suggested that among all the fungicides, seed treatment with mancozeb, carbendazim and captaf at 0.20 per cent concentration effective against *phomopsis* blight and leaf spot in brinjal caused by *Phomopsis vexans* and *Alternaria solani* respectively.

Tomato seed treated with carbendazim (0.2 %) was more effective than captan (0.2 %) by improving germination at 86.00 per cent while the seeds treated with captan (0.2 %) the germination percentage was 77.33 per cent (Dabbas *et al*., 2008).

Srivastava *et al*. (2009) reported that seed treatment with carbendazim 0.1 per cent was more effective against *Fusarium oxysporum* f. sp. *lycopersici* by improving per cent germination (83.1 %) compared to seed treatment with thiram.

Seed treatment with carbendazim + mancozeb @ 2 g/ kg seed gave highest seed germination (94.02 %) and maximum control of the seed mycoflora of tomato (Dabbas *et al*., 2010).

Taskeen *et al*. (2011) evaluated the efficacy of systemic and non-systemic fungicides *viz.,* carbendazim, myclobutanil, bitertanol, hexaconozole, mancozeb, captan and zineb on mycelial growth and spore germination of *Fusarium oxysporum* under *in vitro.* Maximum inhibition in mycelial growth was observed in the hexaconozole at 1000 ppm followed by other fungicides at the same concentration.

Tewari and Vishunavat (2012) reported the efficacy of seed treatment with thiram, carbendazim + mancozeb and carbendazim against early blight of tomato caused by *Alternaria solani*, they observed that significantly increased seed germination and reduction in seedling infection in all the fungicides treated seeds as compared to check.

Suman and Pardeep (2013) reported that systemic fungicides, carbendazim and captan as seed treatment and seedling dip, followed by spray of non- systemic fungicides mancozeb, ridomil MZ and polyram were effective against fruit rot of brinjal caused by fungal complex involving species of *Alternaria, Fusarium, Colletotrichum, Phytophthora* and *Phomopsis.*

Roopa *et al*. (2014) evaluated the efficacy of twelve fungicides against *Alternaria solani* casing early blight of tomato under *in vitro*. The result revealed that contact fungicide, mancozeb @ 0.2 per cent, systemic fungicide, hexaconazole @ 0.1 per cent and the combi fungicide hexazconazole 4 % + zineb 68 % @ 0.2 per cent were recorded the maximum inhibition (87.21 % 88.88 % and 88.88 %) of mycelial growth respectively.

2.4.2 Evaluation of bio agents

Ramamoorthy *et al*. (2002) evaluated twenty isolates of fluorescent pseudomonads for their ability to control damping-off in tomato (*Lycopersicon esculentum*) and hot pepper (*Capsicum annuum*). These isolates were characterized as *Pseudomonas fluorescens* and *Pseudomonas putida*. Two isolates, PFATR and KKM 1 belonged to *P. putida* and the remaining 18 isolates belonged to *P. fluorescens*. Among these isolates, *P. fluorescens* isolate Pf1 showed the maximum inhibition of mycelial growth of *Pythium aphanidermatum* and increased plant growth promotion in tomato and hot pepper in greenhouse and field conditions.

Panchal and Patil (2009) reported the superiority of *Trichoderma viride and T. harzianum* in inhibiting the mycelia growth of the *Alternaria alternata* followed by *T. virens.*

Seed treatment with *Trichoderma harzianum*, *T. Viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were effectively control of *Alternaria solani, Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Pythium* sp. in tomato seeds (El-Mougy *et al*., 2012).

Mumtaz *et al*. (2012) reported the efficacy of seed treatment with bio control agents (*Trichoderma viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis)* in controlling seed borne fungi like, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani* and *Alternaria alternata* (Fr.) Keissler in tomato seeds.

Bioefficacy of seed treatment with three bioagents *viz*., *Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* under *in vitro* against *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato. Among them *Pseudomonas fluorescens* showed significantly higher seed germination (71.85 %) followed by *T. harzianum* (65.93 %) and *T. viride* (58.65 %) (Ganie *et al*., 2013).

Sundaramoorthy and Balabaskar (2013) studied the effect of fifteen native isolates of *Trichoderma* species against *Fusarium oxysporum* f. sp. *lycopersici* which causes Fusarium wilt of tomato under *in vitro* and *in vivo* conditions. The results revealed that *Trichoderma harzianum* (ANR-1) isolate effectively inhibit the radial mycelial growth of the pathogen (53 %) compared to all other isolates.

Vandna and Priya (2014) studied the effeicancy of *Trichoderma harzianum* as seed treatment against wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* showed maximum seed germination (78.33 %) and disease control (66.53 %).

Seed treatment with *T. harzianum* + soil application of neem cake powder + foliar spray of carbendazim, significantly reduce the severity of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) over control (Synder and Hansen, 2015).

2.4.3 Evaluating the effect of bio-priming in increasing the efficacy of biocontrol agents

Harman *et al.* (1989) reported the increased plant stand in soils infested with *F. graminarum* and *Pythium ultimum* by priming of wheat seeds with *Trichoderma harzianum*. They further reported that, in field trial stands of peas were not significantly enhanced by seed treatment with *T. harzianum* strains in the absence of priming but were improved by *T. harzianum* + vermiculite.

Seed treatment with bio-control agents along with priming agents may serve as an important means of managing many of the soil and seed-borne diseases, the process often known as ‘bio-priming”. The bio-priming seed treatment developed for control of *Pythium* seed rot of sweet corn, involves coating seed with a bacterial biocontrol agent such as *Pseudomonas aureofaciens* Kluyver AB254 and hydrating for 20 h under warm (23 °C) conditions in moist vermiculite or on moist germination blotters in a self-sealing plastic bag. The seeds are removed before radical emergence. The bacterial bio-control agent may multiply substantially on seed during biopriming (Callan *et al*., 1990).

Vidhyasekaran and Muthamilan (1995) reported that biopriming of seeds increased rhizosphere population. Seed treatment with the antagonist formulation effectively controlled chickpea wilt disease in the two field trials and increased the yield.

Biological seed treatments for control of seed and seedling diseases offer the grower an alternative to chemical fungicides. While biological seed treatments can be highly effective, it must be recognized that they differ from chemical seed treatments by their utilization of living microorganisms. Storage and application conditions are more critical than with chemical seed protectants and differential reaction to hosts and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than chemicals. Conversely, some biocontrol agents applied as seed dressers are capable of colonizing the rhizosphere, potentially providing benefits to the plant beyond the seedling emergence stage (Nancy *et al*., 1997).

El-Mohamedy and Abd El-Baky (2008) investigated / evaluated the efficacy of different types of seed treatments *i.e*., bio-priming, seed coating with bio-control agents (*T. harzianum, Bacillus subtilis* and *P. fluorescens*) seed priming and seed dressing with these antagonistic micro-organisms in control of root rot disease incidence compared to other treatments.

El-Mougy and Abdel-Kader (2008) evaluated the effect of bio-priming of faba bean seeds against root rot pathogens (*Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii*). They noticed that bio-primed faba bean seeds showed a highly significant effect causing complete reduction of root rot incidence at both pre and post emergence stages of plant growth compared with the control treatment.

Begum *et al.* (2010) reported that bio-priming with *P. aeruginosa* was the most effective treatment for controlling pre and post-emergence damping-off caused by *Colletotrichum truncatum,* with reductions in disease incidence with increase in germination ranging from 48.6 to 51.9 per cent and 65.0 to 97.2 per cent, respectively. Moreover, *P. aeruginosa* resulted in enhancement of seed germination and healthy seedling stand ranging from 32.4 to 60.0 per cent and 56.0 to 73.9 per cent, respectively. Bio-priming with *T. harzianum* reduced pre- and post-emergence damping-off by 42.8-46.8 and 35.0-85.1 per cent, respectively. Bio-priming with *P. aeruginosa* or *T. harzianum* offered an effective biological seed treatment system and an alternative to the fungicide Benlate for the control of damping-off of soybean caused by *C. truncatum* of soybean.

Ananthi *et al*. (2014) evaluated the efficacy of bio-priming with the bio-control agents *Trichoderma viride* or *Pseudomonas fluorescens* in order to improve seed germination and seedling vigour using PKM 1 chilli seed. To optimise the concentration and duration of bio-priming, seeds were bio-primed with *T. viride* or *P. fluorescens* at 40, 60 or 80 per cent (w/v) in water for 3, 6, 9 or 12 h. Seeds were also hydro-primed for 3, 6, 9 or 12 h and non-primed seeds were used as controls. Bio-priming with *T. viride* at 60 per cent (w/v) for 3 h gave the highest values for all parameters measured including the germination percentage (93 %), biomass production and seedling vigour index (1,330) and *P. fluorescens* treatment at 60 per cent (w/v) for 12 h also increased the germination percentage (95 %), biomass production and vigour index (1,406) compared to non-primed seed. They concluded that seed bio-priming with 60 % (w/v) preparations of *T. viride* or *P. fluorescens* for 3 h or 12 h respectively, can improve the seed germination and seedling vigour in chilli.

Ravindra *et al.* (2014) evaluate the effect of bio priming with bacterial strains of *Enterobacter* spp. on seed germination and seedling growth of tomato cultivars (Arka Meghali and Pusa Ruby) under osmotic stress conditions. Seed treatment with bacterial strains influenced the germination and seedling vigour index of both cultivars as compared to the untreated seeds.

4. EXPERIMENTAL RESULTS

The results of the present investigation including *in vitro* studies conducted at Department of Plant Pathology, College of Agriculture, UAS, Dharwad and field experiment conducted under farmer's field at Bailhongal during 2015-16 are presented here.

4.1 Seed health testing of different seed samples of tomato

The seed samples of tomato collected from different parts of northern Karnataka were tested initially by employing standard blotter method as described in 'Material and Methods' and the results are presented in Table 1 and Plate 1. Totally six fungi including both saprophytic as well as pathogenic were encountered. The results of this study indicated the dominance of *Fusarium* spp. (34.87 %), *Alternaria solani* (30.78 %), *Alternaria alternata* (26.77 %), *Phoma* spp. (2.02 %) and *Curvularia* spp. (2.29 %). Other saprophytic fungi included species of *Aspergillus* (3.49 %). Of the 30 tomato seed samples tested, PKM-1 from Haveri area showed maximum seed borne infection (93.2 %) followed by Soubhagya (83.8 %) from Kalaghatagi and Laxmi (75.8 %) from Ranebennur.

Among seed samples, PKM-1 cultivar from Haveri area showed maximum infection of *A. solani* (40 %), Soubhagya from Kalaghatagi area showed maximum infection of *Fusarium* spp. (37.3 %), Abhinav from Bailhongal area showed maximum infection of *A. alternata* (30.6 %). In general seed samples from Dharwad and Haveri districts exhibited maximum infection of *Fusarium* spp. and *A. solani* and the infection of *A. alternata* was maximum at Belagavi and Haveri seed samples. Among the 30 seed samples tested, per cent infection of *Fusarium* spp. varied from 8 to 37.3 per cent, *A. solani* varied from 4 to 40 per cent and *A. alternata* varied from 4 to 30.6 per cent.

4.1.1 Identification of fungi

The identification of above fungi was done based on the morphological and colony characters (Ellis, 1971 and Barnett and Hunter., 1972). Six fungi were noticed in tomato seed samples collected from different tomato growing areas of northern Karnataka. Among them, *Fusarium* spp., *Alternaria solani* and *A. alternata* often appeared in many samples along with species of *Phoma, Curvularia* and *Aspergillus*. Spore morphology and colony characters are given below.

1. *Fusarium* spp.

The fungus produced abundant loose, aerial, white mycelium on incubated seed. In this mycelium, several shiny, hyaline and transparent to milky white spherical droplets can be seen hanging at the tips of long thin stalks. These stalks were primary conidiophores, which arise laterally from the hyphae in the aerial mycelium. The hanging droplets were moist false heads in which conidia were produced. Mucoid and moist pionnotes and snow white to dull white sporodochia were also produced on the seed surface. Micro-conidiophores (monophialides) that bear the microconidia were very long and slender and measures 15-40 x 2-3 µm, whereas those bearing macroconidia (macro-conidiophores) were short and measures 10-25 x 3-4.5 µm. Microconidia were hyaline, 1-2 septate, oval, ellipsoid to sub-cylindrical and measure 5-20 x 2.8-7 µm. Macroconidia were hyaline, stout, measure 22-75 x 35-7 µm, subcylindrical (or) slightly curved, with short blunt and rounded apical cells and indistinctly pedicillate basal cells. The walls of the conidia were thick, with dorsal and ventral surfaces parallel for most of their length. They were mostly 3-septate but 4-7 septate conidia were not uncommon. Chlamydospores were formed singly and in pairs or in clusters in sporodochia. They were globose to subglobose, smooth (or) rough-walled and 6-11 µm in diameter (Kraft, 1969; Ramnath *et al.*, 1970; Booth, 1971) (Plate 2).

2. *Alternaria solani* (Ellis and Martin) Jones and Grout

The fungus produced conidiophores in singly or in groups, straight or flexuous brown to olivaceous brown. On the incubated seed fungus produces conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown. The length of conidia 150-300 µm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks were flexuous, pale and sometimes branched (Ellis, 1971) (Plate 2).

3. *Alternaria alternata (*Fr.) Keissler

The fungus produced wooly or powdery chains of dark brown conidia of variable lengths and shapes. The colour of the colony was usually extremely variable between olive green to dark brown. On incubated seed, the fungus produced woolly or powdery chains of dark brown conidia of variable lengths and shapes. The mycelium may be either sparse or abundant and variable in colour, usually light olive green to dark brown. Hyphae were dark brown, thick, septate and branched. Conidiophores were simple, erect, 40-50 µm long, 2-6 µm thick and often clustered. They produce darkly pigmented conidia in an acropetal succession of simple or branched chains. The chains normally branch at the beak of a spore, or sometimes from the short lateral projection of the beak. Conidia have 4-5 transverse and 5-6 longitudinal septa; measuring 23.5 to 30.00 x8.5-16µm and were ovoid to obovoid, obclavate, pyriform, ellipsoidal, muriform, with an elongated terminal cell. Conidia have a short conical or cylindrical beak, which is about one third the length of the conidium and measure 2-5 X 10-20 µm (Plate 2).

4. *Phoma* spp.

The fungus *Phoma* produced dark brown to black, shiny or dull pycnidia scattered on the surface of incubated seed. The mycelium was dark gray to black. Hyphae were thick, branched and have rough surfaces Pycnidia arise singly or in aggregate masses with extremely variable shape, often globose to subglobose, and 60-150 µm in diameter. The beak was very small or inconspicuous and cirrhus was not usually produced. Conidia were hyaline, single celled, variable in shape, usually globose to ovoid or shortly cylindrical, they measure 1.4- 4.4 x 3.5-8.8 µm in diameter and they were straight and guttulate. Chlamydospores were small, thick, opaque, dark brown to black, spherical to irregular and have smooth to rough surfaces, they may be terminal or intercalary of formed in chains (Plate 2).

Table 1: Seed health testing of different tomato cultivars collected from different parts of northern Karnataka by standard blotter method during *kharif* 2015

District	Taluk	Cultivar	Per cent seed infection of the pathogen						Total
			Alternaria solani	***A. alternata***	***Fusarium* spp.**	***Phoma* spp.**	***Curvularia* spp.**	***AsperglIus* spp.**	
Belagavi	Belagavi	Megha	18.6	13.3	26.6	0	0	1.3	59.8
		PKM-1	22.6	21.3	29.3	1.3	0	0	74.5
		Navodaya*	5.3	10.6	10.6	1.3	1.3	5.3	34.4
	Bailhongal	Chenya*	10.6	10.6	12	0	2.6	2.6	38.4
		Local	26.6	20	22.6	1.3	1.3	2.6	74.4
		Abhinav	12.0	30.6	13.3	1.3	4.0	5.3	66.5
	Gokak	S-22	8.0	9.3	8	0	1.3	0	26.6
		Pusa Ruby	18.6	28.0	14.6	2.6	1.3	2.6	67.7
Dharwad	Dharwad	DMT-1	26.6	12.0	21.3	0	2.6	0	62.5
		DMT-2	4.0	4.0	12	0	0	1.3	21.3
		PKM-1	22.6	12	21.3	1.3	1.3	2.6	61.1
		Pusa Ruby	29.3	9.3	12	1.3	2.6	4	58.5
		Local	16.0	13.0	22.3	2.6	0	0	54.4
	Hubblli	Megha	14.6	12.0	22.3	1.3	0	0	50.5
		S-85*	12.0	5.3	10.3	1.3	0	1.3	30.5
	Kundgol	Punjab chhahura	30.6	9.3	12.0	1.3	1.3	2.6	54.5
		H-24	6.6	9.3	16.0	0	1.3	0	33.2
	Kalaghatagi	Laxmi	12.0	13.3	18.6	2.6	0	2.6	49.1
		Soubhagya	22.6	21.3	37.3	1.3	1.3	0	83.8

Contd…

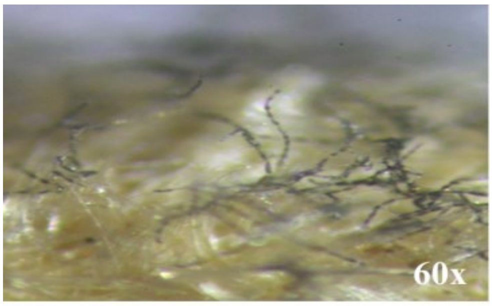
Haveri	Haveri	Ramya*	4.0	9.0	10.6	0	0	1.3	25.2
		Rakshita*	4.0	5.0	9.3	0	0	1.3	19.9
		Nutan*	3.0	12	12	0	0	0	28
		PKM-1	40	21.3	26.6	1.3	0	4	93.2
	Byadgi	Laxmi	16	18.6	24	2.6	1.3	0	66.5
		Shivam	18.6	13	30.6	0	0	1.3	63.8
		Soubhagya	22.6	14.6	22	1.3	0	2.6	63.7
	Ranebennur	Abhinav	13.6	9	14.6	2.6	2.6	0	45.1
		Abhishek	4	5.3	5.3	0	0	2.6	17.2
		Laxmi	14.6	28	22	1.3	1.3	4	75.8
		Shivam	13	6.6	16	1.3	0	2.6	33.1
		Total	473.5	412.4	537.2	31.2	35.4	53.8	1540.5
			30.73 %	26.77 %	34.87 %	2.02 %	2.29 %	3.49 %	

*Seed samples pre-treated with Captan

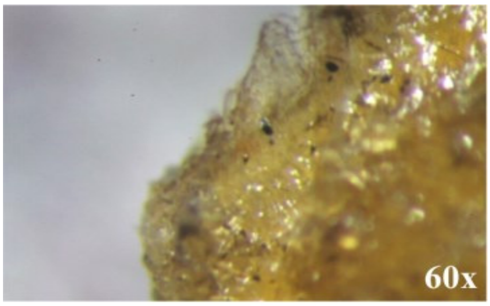
	A. solani	*A. alternata*	*Fusarium* spp.	*Phoma* spp.	*Curvularia* spp.	*Aspergillus* spp.
(orange)	122.3	143.7	137.0	7.8	11.8	19.7
(green)	196.9	120.8	205.4	13.0	10.4	14.4
(cyan)	153.4	142.4	193.0	22.5	5.2	19.7

Plate 1: Map showing seed-borne fungal infection of tomato in northern Karnataka

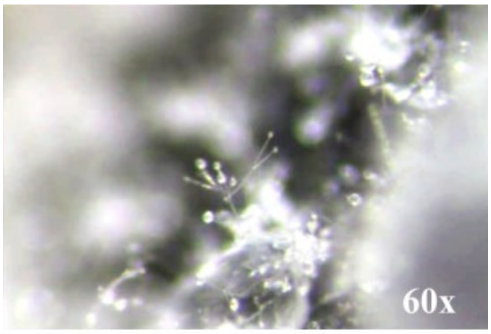
Standard blotter method



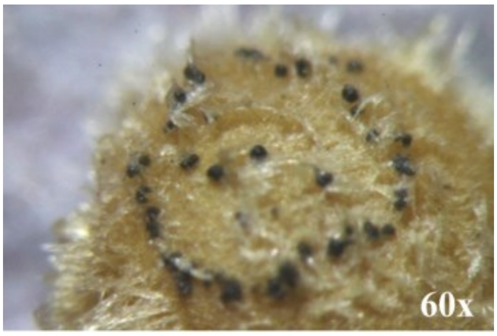
Alternaria alternata



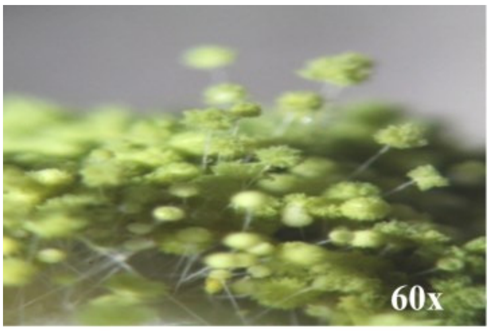
Alternaria solani



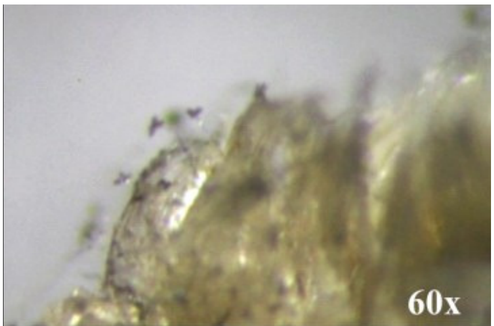
Fusarium spp.



Phoma spp.



Aspergillus flavus



Curvularia spp.

Plate 2: Microphotographs of different seed-borne fungi of tomato

5. *Curvularia* spp.

The fungus *Curvularia* produced dark brown colonies on incubated seed. The mycelium was mostly immersed in the seed coat and was composed of branched, septate, pale brown, thick hyphae. The hyphae were swollen at the point of origin of the conidiophore. Conidiophores arise singly or in groups and erect, simple, straight or flexuous, sometimes geniculate, mid to dark reddish-brown and paler towards the apex, 68.4-200 µm long, 6-8 µm wide with a swollen base of 10-16 µm and 3-5 µm wide at the apex. Conidia were produced acro-pleurogenously.The 3-septate conidia were clavate and almost always slightly curved; septa were thick and visible at higher magnifications under the stereobinocular microscope.The third cell from the base of the conidium was larger than the others and darker in color. Cells at either ends were subhyaline or pale and measure 29-42x13-20 µm (Plate 2).

6. *Aspergillus* spp.

Colonies effuse, variously coloured, often green or yellowish, sometimes brown or black. Mycelium partly immersed or partly superficial. Conidiophores macronematous, often with a foot cell, straight or flexuous, colorless or with the upper part mid to dark brown, usually smooth, swollen at the apex into a spherical or clavate vesicle the surface of which covered by short branches or in some species by phialides. Conidiogenous cells discrete, several arising together at the end of terminal branches or over the surface of vesicle. Conidia catenate, semi-endogenous or acrogenous, spherical, variously coloured, smooth, rugose, echinulate, sometimes with spines arranged spirally (Plate 2).

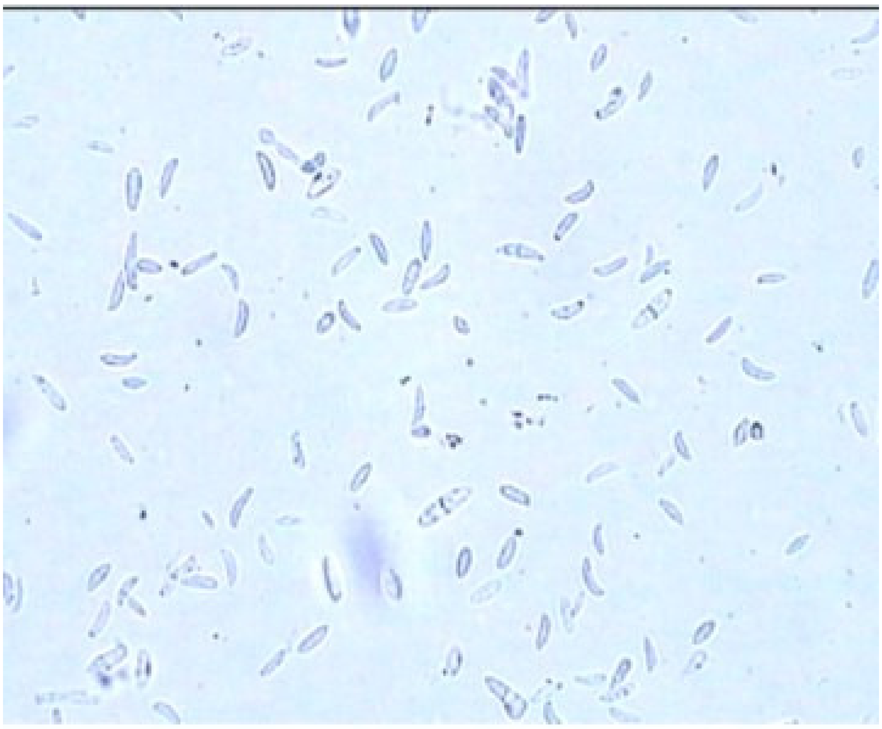
4.1.2 Seed health testing of tomato seed samples by seed washing technique

Seed health testing of tomato seed samples was carried out by seed washing technique as described in 'Material and Methods'. The examination of seed washing revealed only the presence of spores of saprophytic fungus like *Aspergillus* sp. Seed washing technique was found unsuitable for the detection of *A. solani, A. alternata, Fusarium* spp., *Phoma* spp. and *Curvularia* spp. as no fungal structures or spores of these fungi were detected.

4.2 Evaluation of seed health testing methods

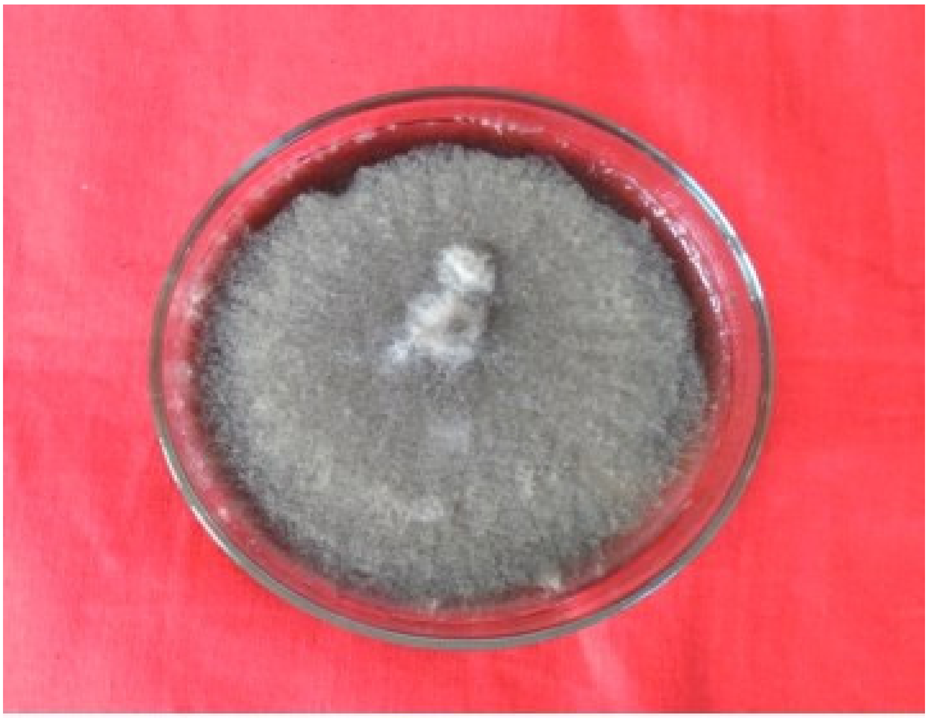
Seed samples of tomato variety PKM-1 was used for the evaluation of seed health testing methods. The results of the experiment are presented in Table 2, Fig. 1 and Plate 5. Among the five different methods employed for the detection of seed-borne fungal pathogen of tomato, standard blotter method was found to be good for the detection of *A. solani*, *A. alternata* and *Fusarium* spp. Significantly, higher counts of *Fusarium* spp. were recorded in standard blotter method (36.00 %) followed by 2, 4-D blotter method (33.33 %). For *A. solani,* standard blotter method (34.67 %) followed by water agar method (24.33 %) and for *A. alternata,* standard blotter method (31.33 %) followed by water agar method (26.00 %) were found to be suitable. Deep freezing blotter method was found to be ineffective for the detection of seed-borne fungi in tomato as it recorded least seed infection.

Pure culture of *Fusarium* sp. Isolated from infected seeds



Microconidia and macroconidia (45x)

Plate 3: Pure culture and microconidia and macroconidia of *Fusarium* spp.



Pure culture of *Alternaria solani* isolated from infected seeds

Conidia of *A. solani* (400x)

Plate 4: Pure culture and conidia of *A.* solani

Table 2: Evaluation of seed health testing methods in detecting the seed-borne fungal infections in tomato

Sl. No.	Treatment	Per cent seed infection by		
		Alternaria solani	*A. alternata*	*Fusarium* spp.
1	Standard blotter method	34.67 (36.06)*	31.33 (34.04)	36.00 (36.87)
2	Deep freezing blotter method	12.00 (20.27)	8.67 (17.10)	8.00 (16.43)
3	2,4-D blotter method	16.00 (23.58)	14.00 (21.97)	33.33 (35.26)
4	Water agar method	24.33 (29.56)	26.00 (30.66)	11.33 (19.66)
5	Potato dextrose agar method	20.00 (26.57)	21.33 (27.50)	17.33 (24.60)
	S. Em. ±	**0.37**	**0.41**	**0.40**
	C. D. at 1 %	**1.68**	**1.83**	**1.79**

*Figures in parentheses indicates arcsine transformed value

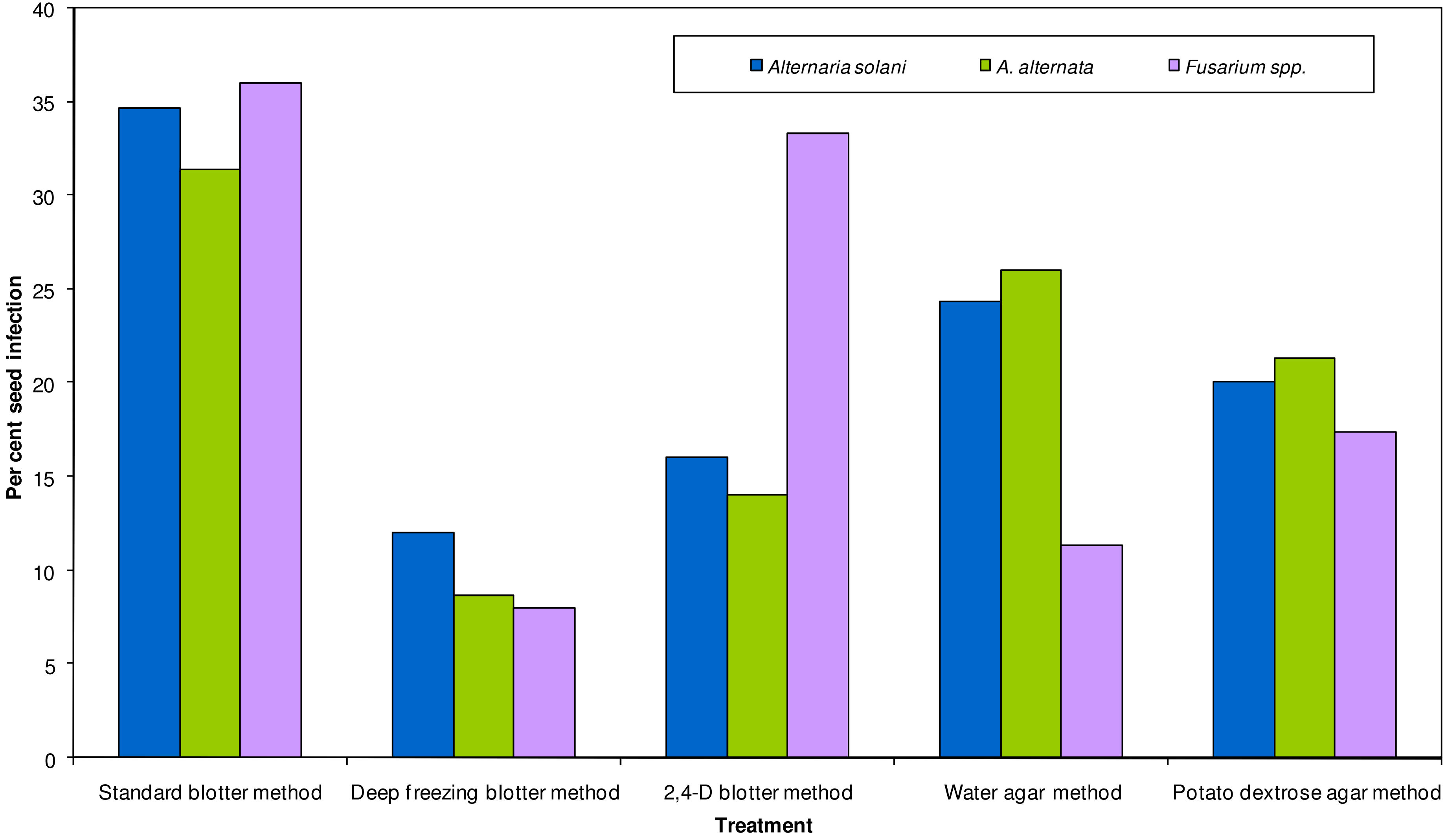
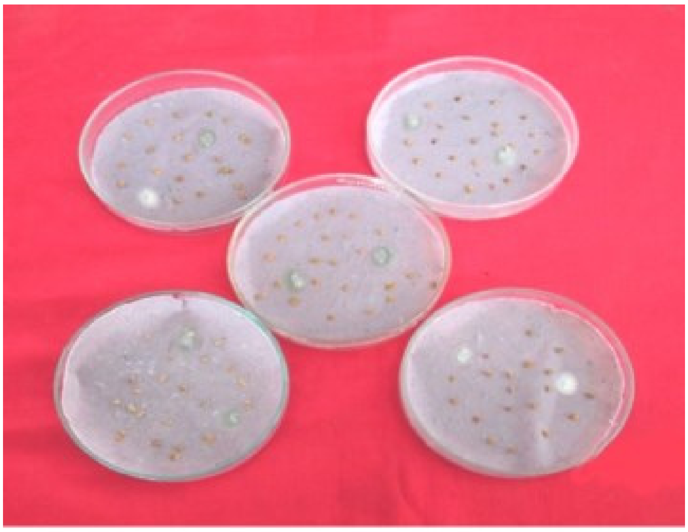
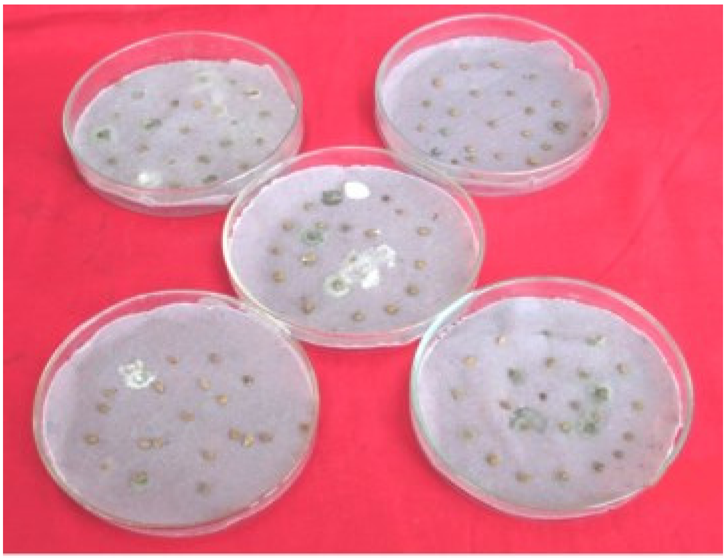


Fig. 1: Evaluation of different seed health testing methods in detecting the seed-borne fungal infections in tomato

Standard blotter method



Deep freezing blotter method



2, 4-D blotter method

Plate 5: Evaluation of seed health testing methods

Water agar method

Potato dextrose agar

Plate 5: Contd…..

4.3 Transmission studies

Transmission studies were carried out by seedling symptom test under *in vitro* conditions and also in pot culture as described in 'Material and Methods'.

4.3.1 Seedling symptom test

Selected seed samples of variety PKM-1 were subjected to water agar seedling symptom test to diagnose the seed-borne infection and to see the seed to plant transmission. The germinated seedling from the moderately infected seed sample exhibited the respective symptoms such as cotyledonary infection, leaf necrosis after 12 days of incubation. Severely infected seed samples aborted and failed to germinate and exhibited seed rot.

Seeds of variety PKM-1 were artificially inoculated with different conidial suspensions of *Alternaria solani, A. alternata* and *Fusarium* spp. separately from 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} concentration, and apparently healthy seed treated with only distilled water without conidial suspension served as control.

In case of *Alternaria solani*, seeds soaked in 2.4 x 10^6 conidia/1ml showed seed abortion and at 2.4 x 10^5 conidia/1ml concentration showed cotyledonary infection. The results of the experiment are presented in (Plate 6). In case of *Alternaria alternata* seeds soaked in 2.12 × 10^6 and 2.12 × 10^5 conidia/1 ml showed cotyledonary infection and leaf necrosis. The results of the experiment are presented in (Plate 7). Seeds soaked in 3.25 x 10^6 and 3.25 x 10^5 conidia/1 ml concentration of *Fusarium* spp. got aborted and they exhibited seed rotting (Plate 8).

4.3.2 Pot culture studies

Apparently healthy and artificially inoculated seed samples of variety PKM-1 were used to demonstrate the effect of different seed inoculum levels on per cent disease incidence and to prove seed to plant transmission of *A. solani, A. alternata* and *Fusarium* spp. This study was conducted under pot culture in controlled conditions in glasshouse. The results of the experiment (Table 3a to 4) indicated that, apparently healthy seed and seeds soaked in 10^3 concentration showed maximum seed germination in case of all the fungi.

In case of *A. solani*, seeds soaked in 2.4 × 10^6 and 2.4 × 10^5 conidia/ 1 ml showed 82.75 and 78.25 per cent seedling infection. Whereas lower per cent seedling infection was recorded in treatments T_3 and T_4 (*i.e.*, T_3: seeds soaked in 2.4 x 10^4 conidia/1ml and T_4: seeds soaked in 2.4 x 10^3 conidia /1 ml respectively). The results of the experiment are presented in Table 3a, Fig.2 and Plate 6. The characteristic symptom *i.e*. brown to dark brown concentric ring (target board effect) was observed on older leaves at 30 DAS onwards. The maximum infection was observed in treatments T_1 and T_2 (82.75 and 78.25 per cent) whereas lower infection was observed in T_3 and T_4 (62.50 and 42.50 per cent) the seeds soaked in 2.4×10^4 and 2.4 ×10^3 conidia/1ml respectively.

Table 3a: Effect of seed inoculum levels of *Alternaria solani* on per cent germination and seedling infection under pot culture

Treatments	Per cent	
	Seedling infection	**Seed germination**
T_1- Seeds soaked in 2.4×10^6 conidia/1 ml	82.75 (65.47)*	37.50 (37.73)
T_2-Seeds soaked in 2.4×10^5 conidia /1 ml	78.25 (62.21)	47.50 (43.56)
T_3-Seeds soaked in 2.4×10^4 conidia /1 ml	62.50 (52.27)	57.50 (49.33)
T_4- Seeds soaked in 2.4×10^3 conidia /1 ml	42.50 (40.67)	82.00 (65.01)
T_5-Un-inoculated (Apparently healthy seeds)	22.50 (28.23)	97.00 (80.67)
S. Em. ±	**1.22**	**1.62**
C. D. at 1 %	**5.08**	**6.77**

*Figures in parentheses indicates arcsine transformed values

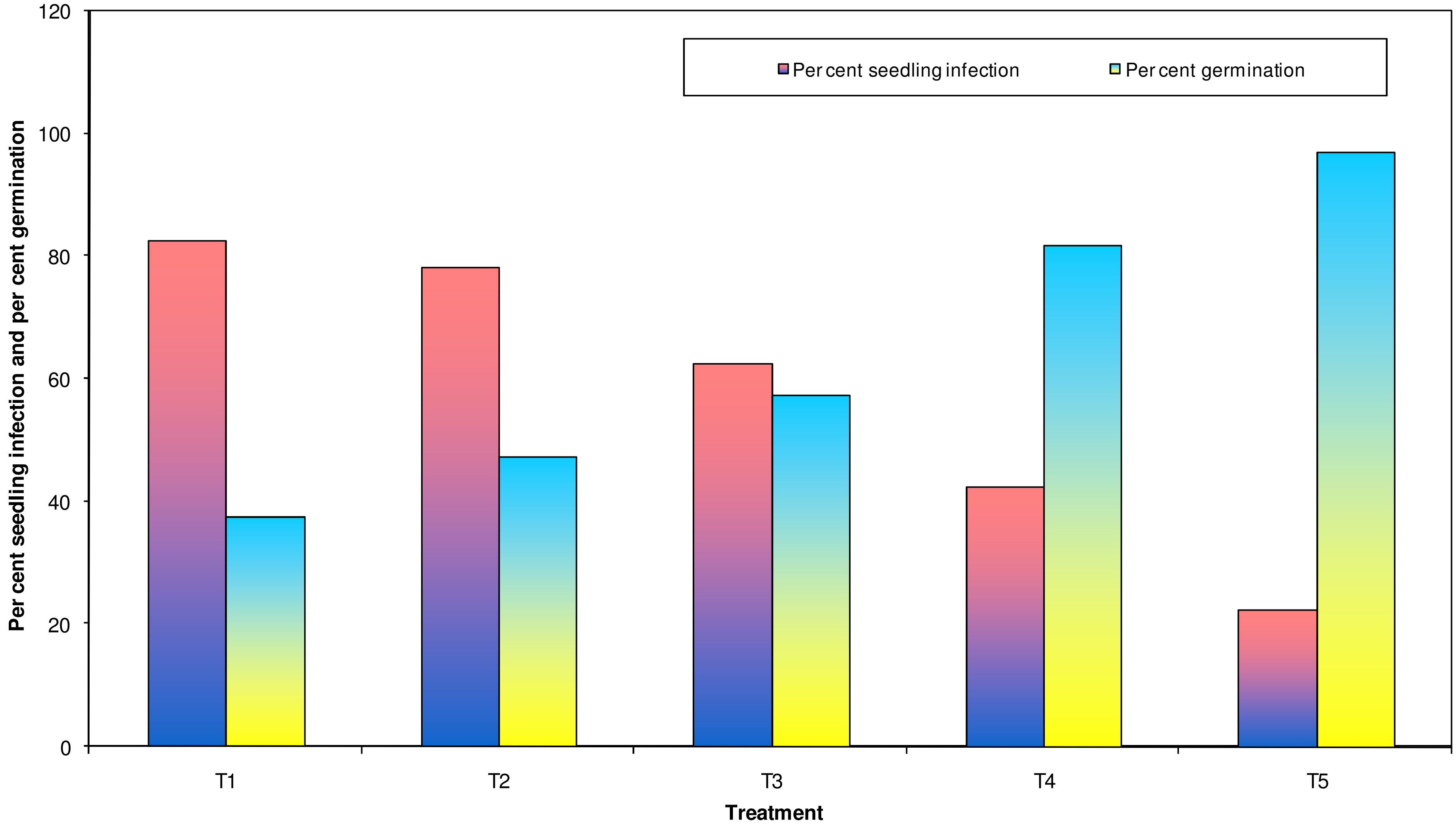


Fig. 2: Effect of seed inoculum levels of *Alternaria solani* on per cent germination and seedling infection under pot culture

Seedling symptom test

Pot culture studies

30 days after inoculation

40 days after inoculation

Plate 6: Transmission studies (*Alternaria solani*)

Table 3b: Effect of seed inoculum levels of *A. alternata* on per cent germination and seedling infection under pot culture

Treatments	Per cent	
	Seedling infection	**Seed germination**
T_1 -Seeds soaked in 2.12×10^6 conidia /1 ml	66.67 (54.37) *	37.50 (37.72)
T_2-Seeds soaked in 2.12×10^5 conidia /1 ml	62.49 (52.33)	48.75 (44.30)
T_3-Seeds soaked in 2.12×10^4 conidia /1 ml	45.83 (42.59)	56.66 (53.77)
T_4 -Seeds soaked in 2.12×10^3 conidia /1 ml	41.58 (40.10)	83.08 (65.75)
T_5- Un-inoculated (Apparently healthy seeds)	15.25 (22.99)	80.25 (63.65)
S. Em. ±	**2.36**	**2.16**
C. D. at 1 %	**9.84**	**9.00**

*Figures in parentheses indicates arcsine transformed values

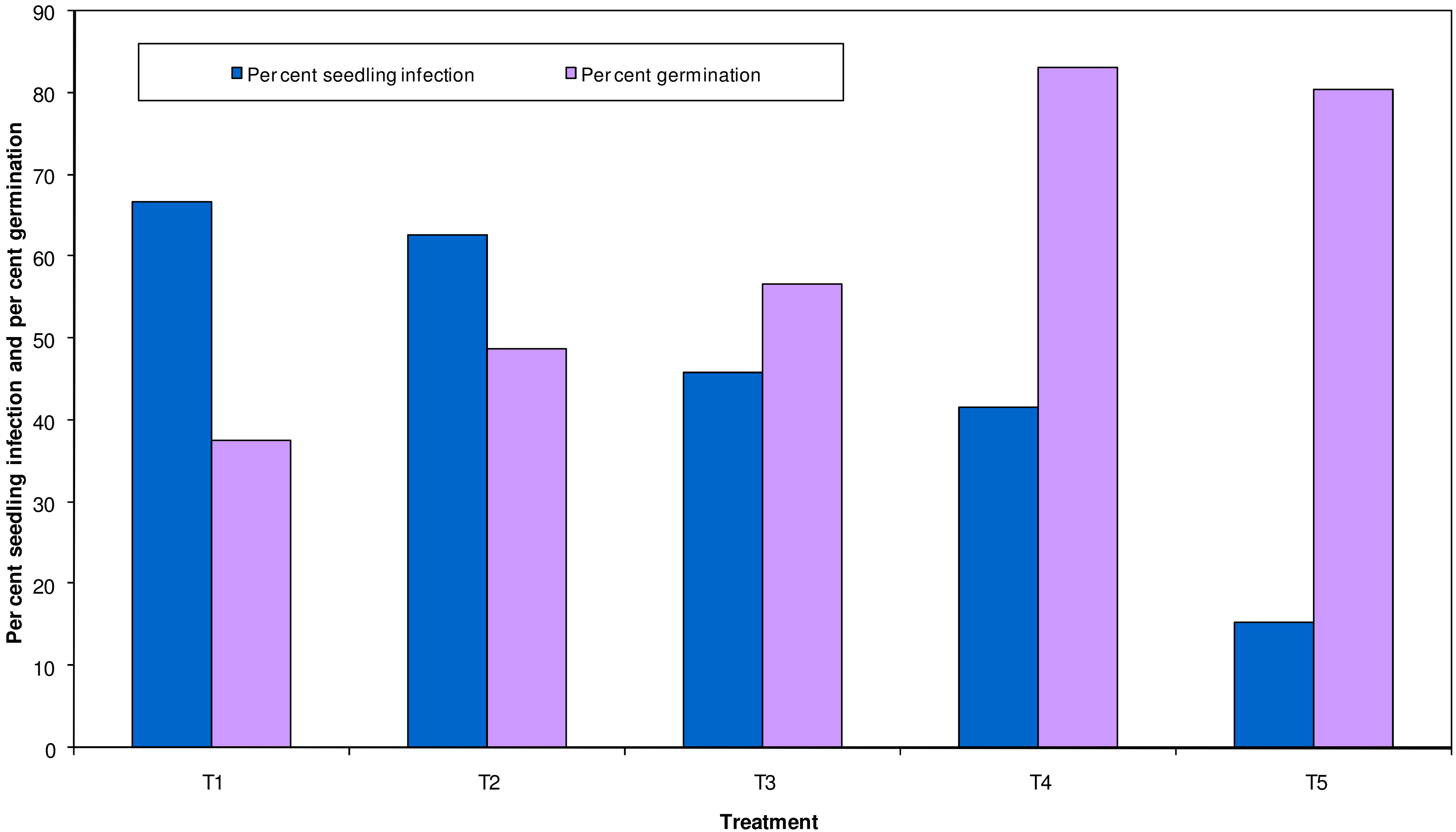
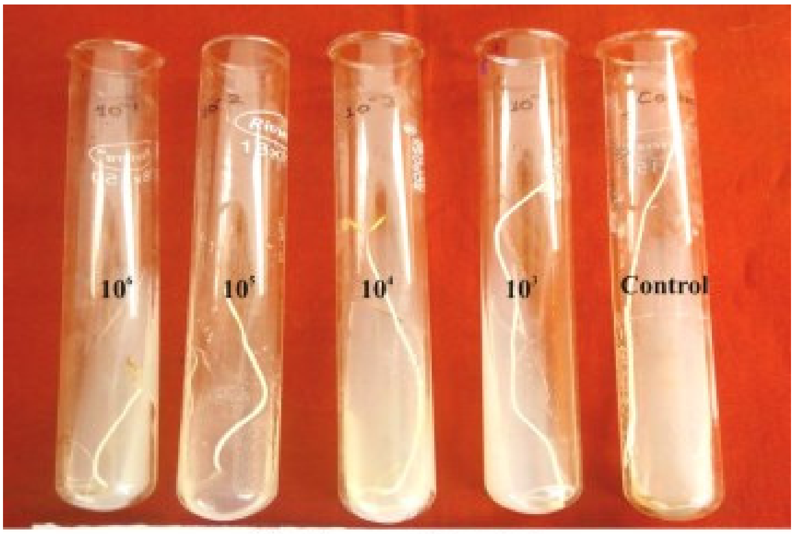


Fig. 3: Effect of seed inoculum levels of *A. alternata* on per cent germination and seedling infection under pot culture



Seedling symptom test

Pot culture studies

Plate 7: Transmission studies (A. Alternata)

Table 4: Effect of seed inoculum levels of *Fusarium* spp. on per cent germination and wilt incidence under pot culture

Treatments	Per cent	
	Wilt incidence	**Seed germination**
T_1- Seeds soaked in 3.25×10^6 conidia /1 ml	99.25 (87.51)*	2.50 (9.05)
T_2- Seeds soaked in 3.25×10^5 conidia /1 ml	97.00 (80.17)	32.50 (34.72)
T_3- Seeds soaked in 3.25×10^4 conidia /1 ml	87.50 (69.53)	67.50 (55.28)
T_4- Seeds soaked in 3.25×10^3 conidia /1 ml	62.50 (52.27)	85.00 (67.50)
T_5- Un-inoculated (Apparently healthy seeds)	18.50 (25.42)	100.00 (90.00)
S. Em. ±	**1.73**	**1.44**
C. D. at 1 %	**7.19**	**5.99**

*Figures in parentheses indicates arcsine transformed values

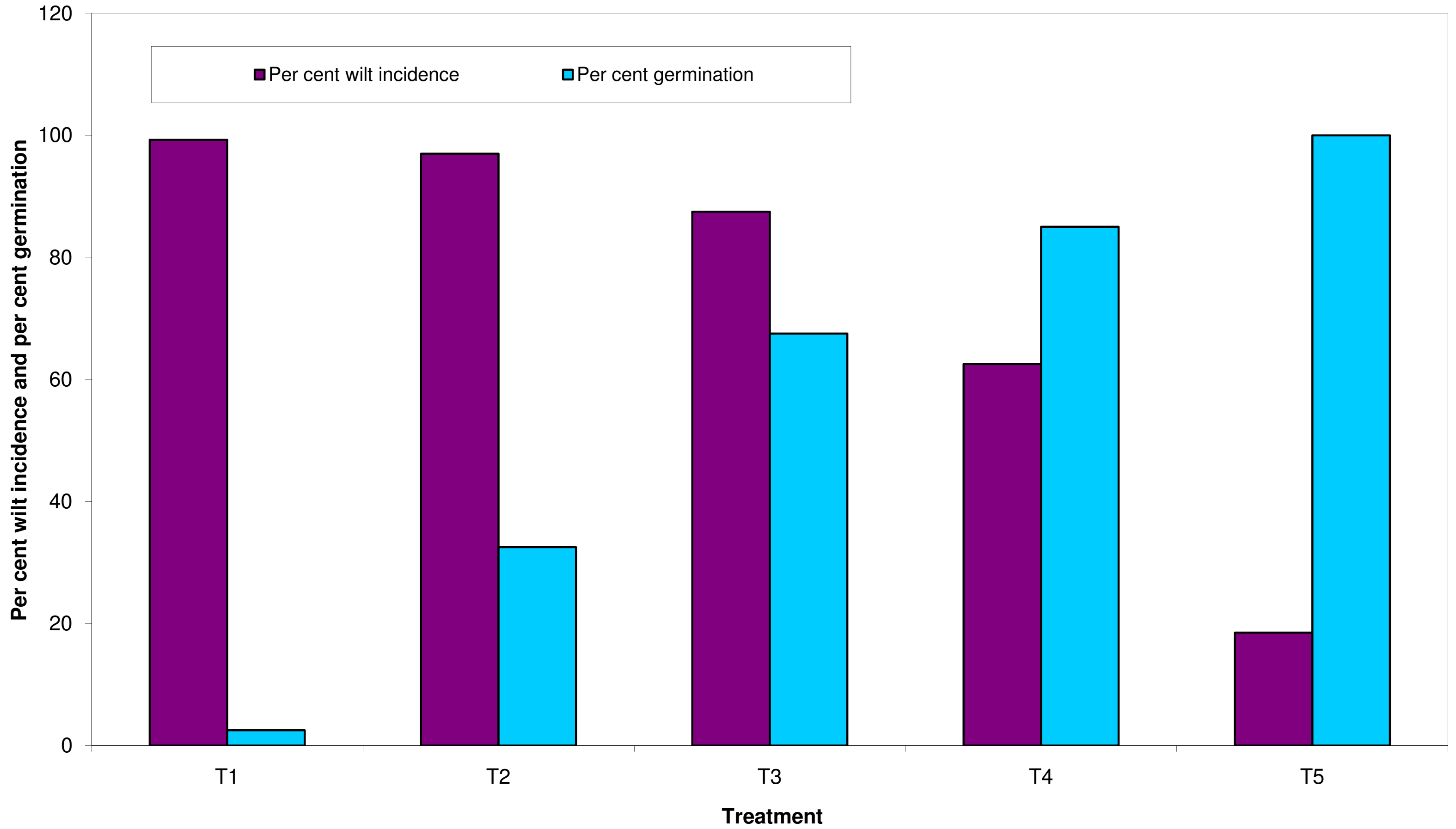


Fig. 4: Effect of seed inoculum levels of *Fusarium* spp. on per cent germination and wilt incidence under pot culture

Seedling symptom test

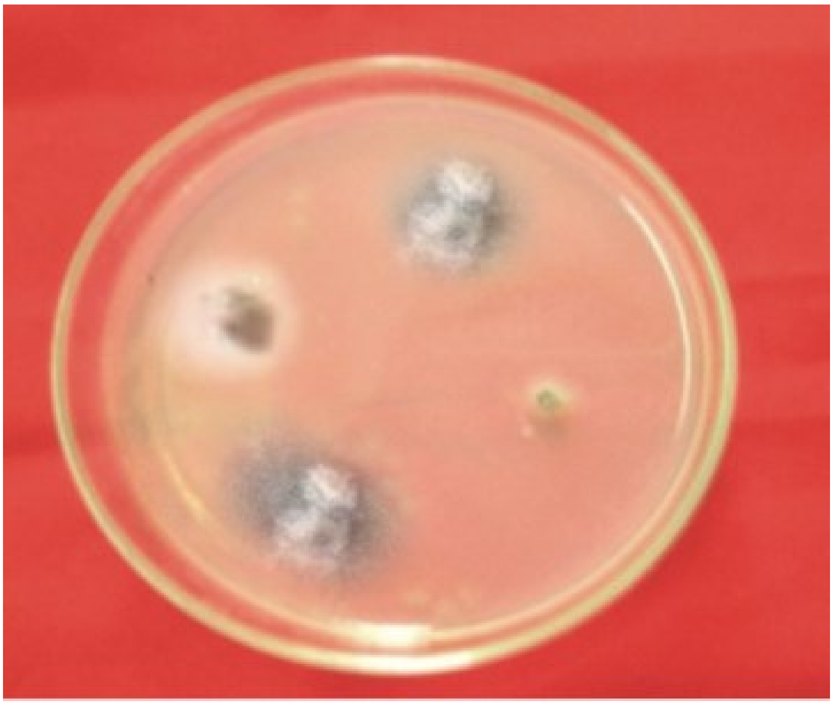
Pot culture studies

Plate 8: Transmission studies (Fusarium spp.)

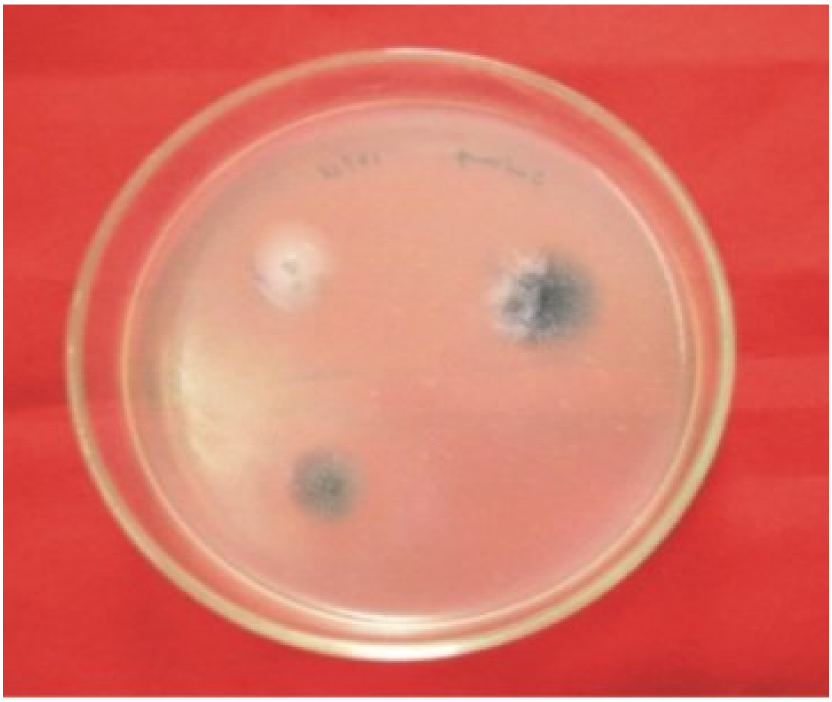
Table 5: Location of seed borne fungi in different seed parts of infected tomato seed by component plating technique

Sl. No.	Fungi observed	Seed parts tested	
		Pericarp	Other than pericarp
1	*Alternaria solani*	+	+
2	*Alternaria alternata*	+	-
3	*Fusarium* spp.	+	+
4	*Phoma* spp.	+	-
5	*Aspergillus* spp.	+	-

+ = Presence of pathogen
- = Absence of pathogen



Pericarp



Other than pericarp

Plate 9: Component plating technique

In case of *A. alternata* seeds soaked in 2.12×10^6 and 2.12×10^5 conidia/ 1 ml showed 66.67 and 62.49 per cent seedling infection. Whereas lower per cent seedling infection was recorded in treatments T_3 and T_4 (i.e., T_3: seeds soaked in 2.12 x 10^4 conidia/1ml and T_4: seeds soaked in 2.12 x 10^3 conidia/1ml respectively). The characteristic symptom *i.e.* minute water soaked specks with yellow halo on leaves was recorded after 30 days of sowing. The maximum infection was in treatments T_1 and T_2 (66.67 and 62.49 per cent) seeds soaked in 2.12×10^6 and 2.12×10^5 conidia/ 1 ml concentration, whereas lower infection was recorded in seeds soaked in 2.12 x 10^4 and 2.12 x 10^3 conidia /1ml treatments at per cent infection of 45.83 and 41.58 per cent (Table 3b, Fig. 3 and Plate 7).

In case of *Fusarium* spp. seeds soaked in 3.25×10^6 and 3.25×10^5 conidia/ 1ml showed least germination per cent ((*i.e.* 2.50 % and 32.50 %). But germination per cent increased with the corresponding decrease in concentration of fungal propagules. The wilting symptom was observed 20 DAS. The maximum wilt incidence was recorded in treatments T_1, T_2 and T_3 (99.25 %, 97.00 % and 87.50 % respectively) whereas lower wilt incidence was recorded in treatment (T_5) (Apparently healthy seed, 18.50 %) and T_4 (seeds soaked in 3.25 x 10^3 conidia/1ml, 62.50 %) This experiment reveals that T_4 (seeds soaked in 3.25 x 10^3 conidia/1ml) had suppose to be the minimum threshold seed inoculum level for wilt disease to occur (Table 4, Fig. 4 and Plate 8).

4.3.3 Location of fungi in seed

The location of fungi in the seed was studied by employing, component plating technique, as described in 'Material and Methods' and results are presented in Table 5 and Plate 9. Naturally infected tomato seed sample of variety PKM-1 was used for the study. The presence of seed borne pathogen in the separated seed parts *viz.,* pericarp and other than pericarp (embryo endosperm and hilum) were recorded as indicated in Table 5. *Alternaria alternata, Phoma* spp. and *Aspergillus* spp*.,* were confined to pericarp only, where as *Fusarium* spp. and *Alternaria solani* were noticed other than pericarp also *viz*., endosperm, embryo and hilum.

4.4 Management strategies for overcoming seed-borne infections of tomato

4.4.1 Evaluation of fungicides

4.4.1.1 *In vitro* evaluation of seed dressing fungicides by rolled towel method

Efficacy of seven seed dressing fungicides @ 0.2 and 0.3 per cent were tested against seed borne fungal infections of tomato (variety-PKM-1) using rolled towel method, as explained in 'Material and Methods' and results are presented in Table 6, Fig. 5a & 5b and Plate 10. Among the seven seed dressing fungicides tested, seed treatment with carbendazim + mancozeb (Sprint) exhibited higher per cent seed germination (96.50 %) followed by seed treatment with carboxin + thiram (Vitavax Power) (95.17 %) but they were found on par with respect to per cent seed infection (10.84 and 12.17 per cent) and vigour index (2319.22 and 2239.45). Treatment with carbendazim + mancozeb and

Table 6: Efficacy of fungicides as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

Sl. No.	Treatments	Per cent seed infection			Per cent seed germination			Vigour index		
		0.2 %	0.3 %	Mean	0.2 %	0.3 %	Mean	0.2 %	0.3 %	Mean
1	Carbendazim	15.33 (23.05)*	13.33 (21.41)	14.33 (22.23)	92.33 (73.93)	94.33 (76.24)	93.33 (75.09)	2032.37	2011.60	2021.98
2	Carbendazim + Mancozeb	11.00 (19.37)	10.67 (19.06)	10.84 (18.88)	95.67 (78.00)	97.33 (80.64)	96.50 (79.32)	2299.23	2339.20	2319.22
3	Carboxin + Thiram	12.33 (20.56)	12.00 (20.27)	12.17 (19.94)	94.67 (76.66)	95.67 (78.00)	95.17 (77.33)	2237.27	2241.63	2239.45
4	Captan	16.67 (24.09)	15.00 (22.78)	15.84 (23.44)	90.33 (71.89)	91.33 (72.92)	90.83 (72.41)	1990.13	2064.13	2027.13
5	Captan + Hexaconazole	15.67 (23.31)	13.67 (21.69)	14.67 (22.50)	92.67 (74.30)	93.67 (75.43)	93.17 (75.06)	2089.63	2143.90	2116.76
6	Metalaxyl	24.33 (29.56)	21.67 (27.74)	23.00 (28.65)	74.33 (59.56)	76.33 (60.89)	75.33 (60.23)	1417.40	1521.63	1469.52
7	Tebuconazole	21.33 (27.51)	19.33 (26.08)	20.33 (26.79)	76.67 (61.12)	80.67 (63.93)	78.67 (62.52)	1663.47	1675.73	1669.60
8	Control	30.00 (33.21)	30.00 (33.21)	30.00 (33.21)	68.00 (55.55)	68.00 (55.55)	68.00 (55.55)	919.20	919.20	919.20
	Mean	18.62 (25.08)	17.13 (24.02)	17.87 (24.55)	85.58 (68.88)	87.17 (70.45)	86.38 (69.66)	1831.09	1863.50	1847.30
		Fungicide (F)	**Conc (C)**	**FXC**	**Fungicide (F)**	**Conc (C)**	**FXC**	**Fungicide (F)**	**Conc (C)**	**FXC**
	S. Em. ±	0.27	0.14	0.38	0.30	0.15	0.42	18.16	9.08	25.68
	C. D. at 1 %	1.05	0.52	1.48	1.14	0.57	1.62	70.33	35.16	99.46

*Figures in parentheses indicate arcsine transformed values

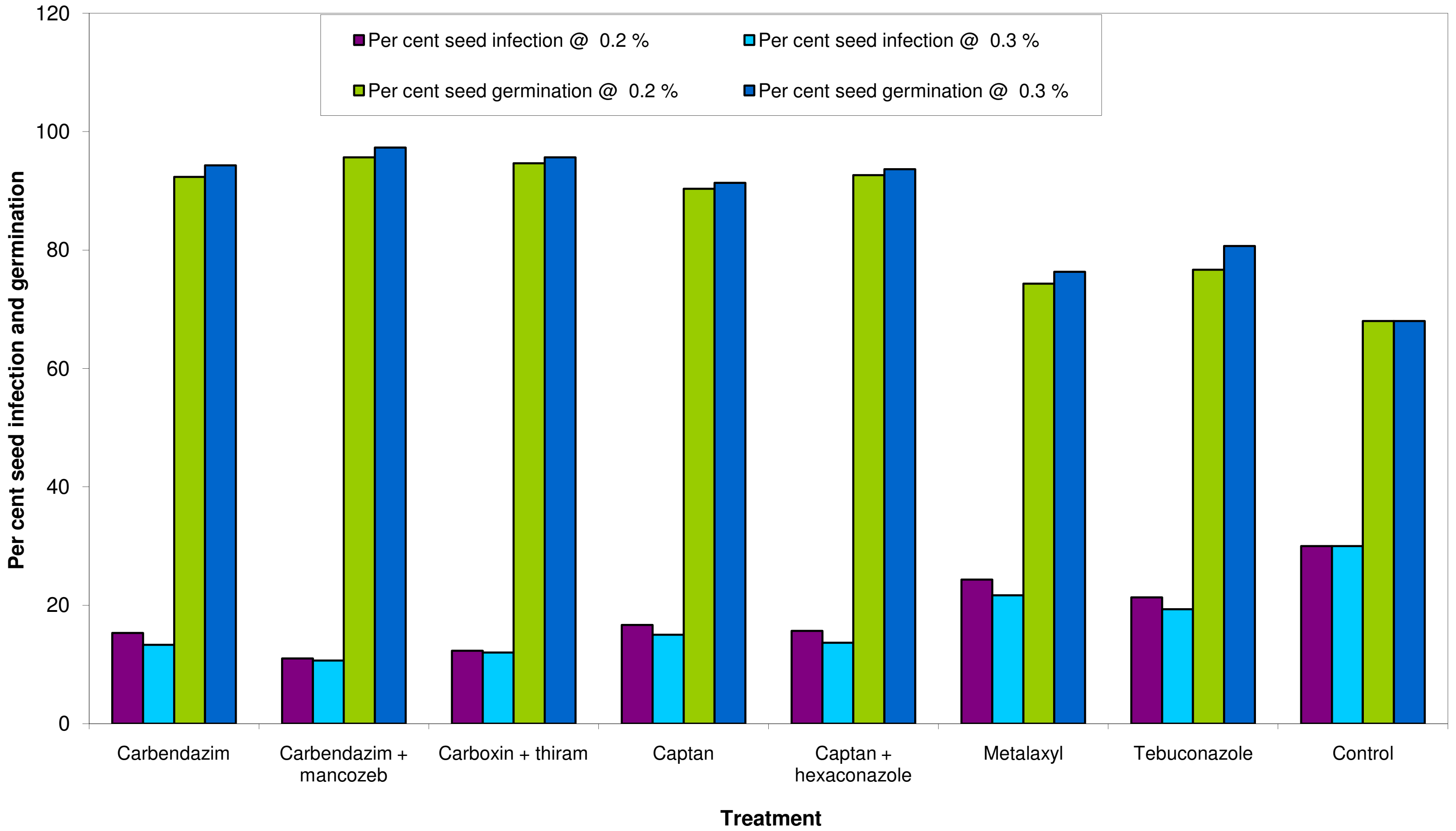


Fig. 5a: Efficacy of fungicides as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

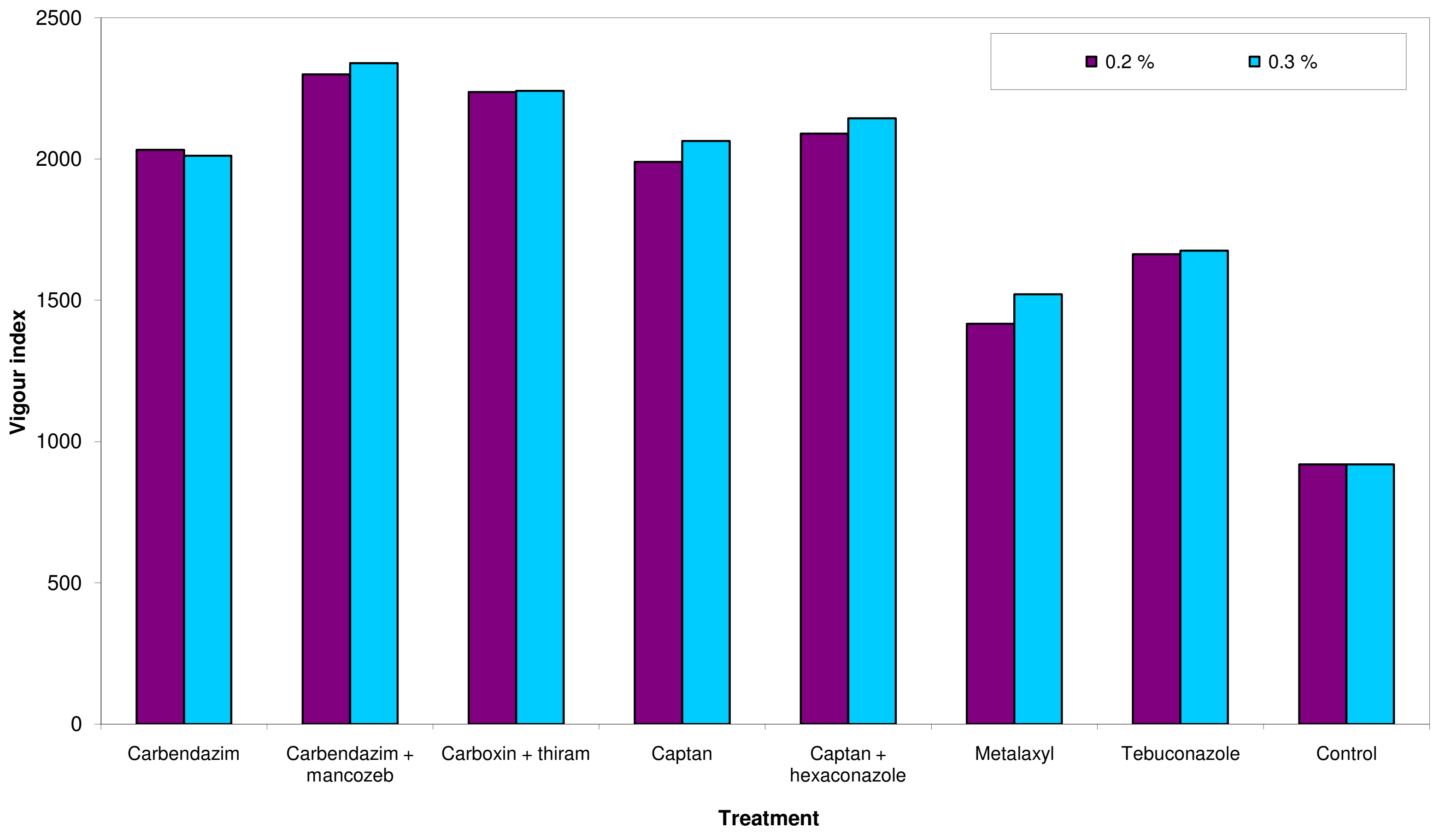


Fig. 5b: Efficacy of fungicides as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

carboxin + thiram showed significant difference over treatment with Metalaxyl and Tebuconazole with respect to per cent seed infection, per cent seed germination and vigour index.

Seed treatment carbendazim, captan and captan + hexaconazole were found to be on par with respect to per cent seed infection (14.33 %, 15.84 % and 14.67 %), per cent germination (93.33 % and 93.17 %) and vigour index (2021.98, 2027.13 and 2112.27) but differed with respect to per cent germination in case of captan (90.83 %).

Seed treatment with carbendazim + mancozeb and carboxin + thiram at 0.2 and 0.3 per cent were found to be on par with each other with respect to per cent seed infection and vigour index in case of carboxin + thiram, but they significantly differed with respect to per cent germination and vigour index in case of carbendazim + mancozeb. Among the seven seed dressing fungicides, carbendazim + mancozeb @ 0.3 % and carboxin + thiram @ 0.3 % were selected for further study (for field evaluation) as they were found to be superior over other treatments with respect to the quality parameters.

4.4.2 Evaluation of bioagents

4.4.2.1 *In vitro* evaluation of bioagents by Rolled towel method

Four bioagents were tested for their efficacy in the management of seed borne fungal infections of tomato (variety-PKM-1) by using rolled towel method, as described in 'Material and Methods' and results are presented in Table 7, Fig. 6a & 6b and Plate 10. The four bioagents tested in two concentrations, i.e., 0.4 and 0.8 per cent for their efficacy in the management of seed-borne fungal infection. Seed treatment with *Pseudomonas fluorescens* showed least seed infection of 13.00 per cent followed by *Trichoderma harzianum* with per cent seed infection of 15.50 per cent but they were found to be on par with respect to per cent germination (94.00 and 92.67 per cent) and vigour index (2031.17and 2006.78) respectively.

Seed treatment with *Pseudomonas fluorescens* at 0.8 per cent differed significantly over seed treatment with *Trichoderma harzianum* @ 0.8 per cent with respect to per cent seed infection (12.67 % and 14.67 %) per cent germination (95.33 % and 94.00 %). Seed treatment with *Pseudomonas fluorescens* at 0.4 per cent differed significantly with seed treatment of *P. fluorescens* at 0.8 per cent with respect to per cent seed infection (13.33 % and 12.67 %), per cent germination (92.67 and 95.33 %) and vigour index (1955.47 and 2106.87). Similarly in case of *T. harzianum* @ 0.4 % which differed significantly with seed treatment of *T. harzianum* @ 0.8 % with respect to per cent seed infection, per cent germination and vigour index. Seed treatment with VAM fungi was found ineffective as it resulted in seed infection of 23.00 per cent, with a germination of 77.67 per cent and vigour index of 1424.78, but was found significantly superior over control, which exhibited a seed infection of 32.00 per cent, with germination and vigour index of 65.00 per cent and 1027.83 respectively.

Table 7: Efficacy of bioagents as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

Treatments	Per cent seed infection			Per cent seed germination			Vigour index		
	0.4 %	**0.8 %**	**Mean**	**0.4 %**	**0.8 %**	**Mean**	**0.4 %**	**0.8 %**	**Mean**
Pseudomonas fluorescens	13.33 (21.41)*	12.67 (20.85)	13.00 (21.13)	92.67 (74.43)	95.33 (77.58)	94.00 (76.01)	1955.47	2106.87	2031.17
Trichoderma harzianum	16.33 (23.83)	14.67 (22.52)	15.50 (23.17)	91.33 (72.92)	94.00 (75.85)	92.67 (74.39)	1914.70	2098.87	2006.78
Bacillus subtilis	19.67 (26.31)	17.33 (24.60)	18.50 (25.46)	86.67 (68.64)	90.00 (71.57)	88.33 (70.10)	1684.47	1713.00	1698.73
VAM fungi	24.67 (29.78)	21.33 (27.50)	23.00 (28.64)	73.33 (58.91)	82.00 (64.90)	77.67 (61.90)	1398.17	1451.40	1424.78
Control	32.00 (34.45)	32.00 (34.45)	32.00 (34.45)	65.00 (53.73)	65.00 (53.73)	65.00 (53.73)	1029.00	1026.67	1027.83
Mean	**21.20 (27.42)**	**19.60 (26.28)**	**20.40 (26.85)**	**82.80 (65.50)**	**86.27 (68.25)**	**84.79 (66.84)**	**1596.36**	**1679.36**	**1637.86**
	T	**C**	**T x C**	**T**	**C**	**T x C**	**T**	**C**	**T x C**
S. Em. ±	**0.27**	**0.17**	**0.37**	**0.55**	**0.35**	**0.78**	**24.74**	**15.65**	**34.99**
C. D. at 1 %	**0.78**	**0.49**	**1.11**	**1.63**	**1.03**	**2.31**	**72.98**	**46.16**	**103.21**

*Figures in parentheses indicate arcsine transformed values

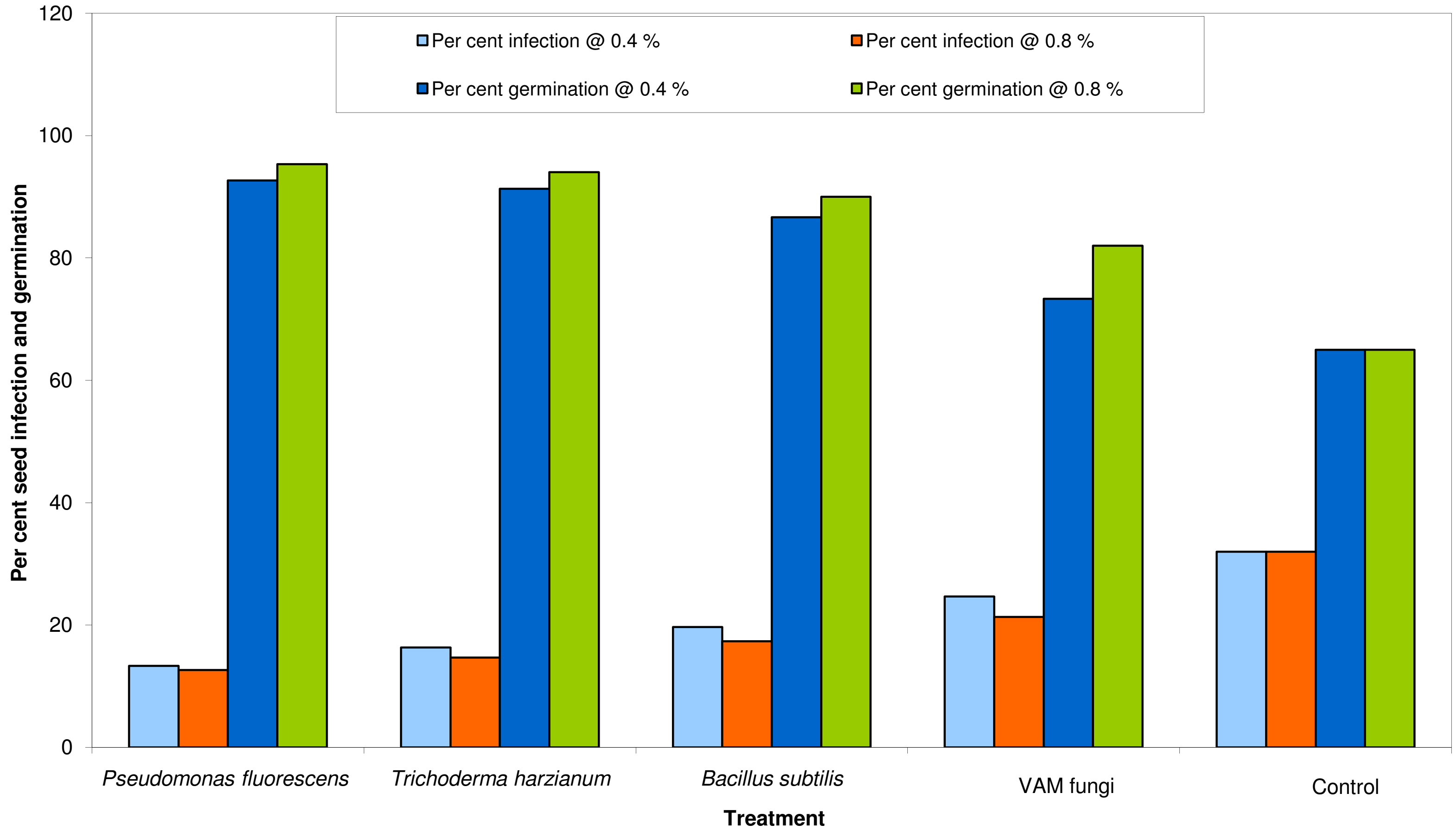


Fig. 6a : Efficacy of bioagents as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

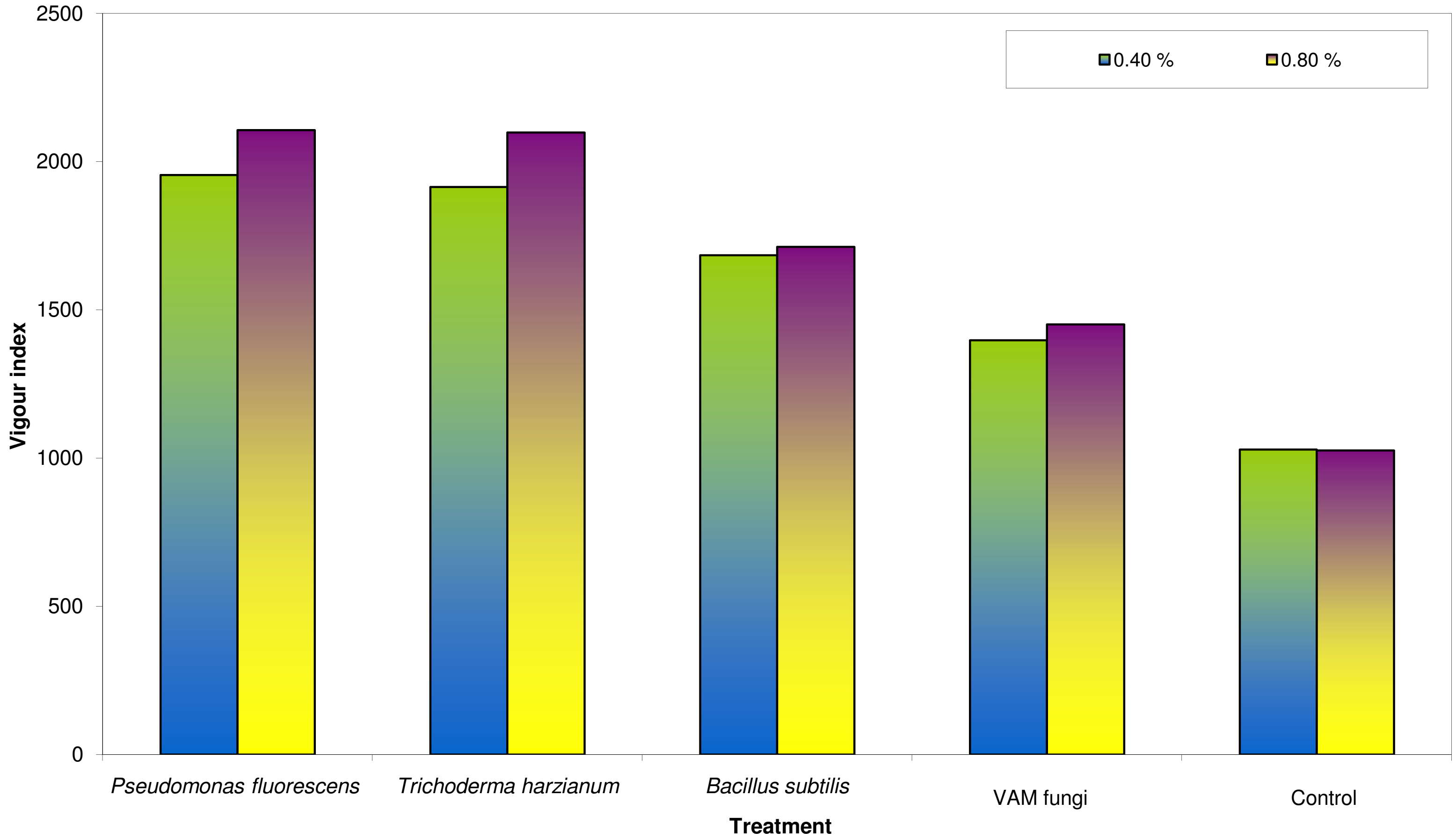


Fig. 6b: Efficacy of bioagents as seed dressers against seed-borne fungal infections and seed quality parameters of tomato

ST with
Crabendazim + mancozeb @ 0.3%

ST with
Carboxin + thiram @ 0.3%

ST with
***Pseudomonas fluorescens* @ 0.8%**

ST with
***P. fluorescens* @ 0.8% +jelly**

Control

Plate 10: *In vitro* evaluation of seed dressing fungicides, bioagents and biopriming agents by rolled towel method

4.4.3 Evaluation of Biopriming

4.4.3.1 Efficacy of biopriming against seed-borne fungal infections and seed quality parameters of tomato

Four priming agents with the selected two bioagents were tested for their efficacy in overcoming seed borne fungal infections of tomato (variety-PKM-1) by using rolled towel method as explained under 'Material and Methods' and results are presented in (Table 8, Fig. 7a & 7b and Plate 10). Among the four priming agents treated with respect to *Pseudomonas fluorescens,* seed treatment with *Pseudomonas fluorescens +* jelly recorded significantly higher per cent seed germination (98.33 %) and vigour index (1235.57) with least per cent seed infection (10.00 %) compared to seed treatment with *Pseudomonas fluorescens* @ 0.8 % alone. Seed treatment with *Pseudomonas fluorescens* + vermiculite and *Pseudomonas fluorescens* + coco peat were found to be on par with each other with respect to per cent seed infection (12.33 and 12.00 per cent) and per cent germination (95.33 and 96.33 per cent) respectively.

With respect to *Trichoderma,* seed treatment with *Trichoderma harzianum* + coco peat recorded significantly higher per cent seed germination (97.00 %) and vigour index (2091.07) with least per cent seed infection (11.33 %) compared to seed treatment with *Trichoderma harzianum* alone. However seed treatment with *Pseudomonas fluorescens +* jelly was found to be on par with *Trichoderma harzianum* + coco peat with respect to per cent germination, per cent infection and vigour index. These two biopriming agents were found to be superior over rest of the treatments with respect to per cent germination and vigour index. Hence these two bioprimings were selected for further field evaluation.

4.4.4 Effect of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery.

An experiment was conducted in tomato nursery during *rabi* 2015-16 at Bailhongal to compare different seed treatment procedures in tomato variety PKM-1. The best two seed dressing fungicides, bioagents and biopriming options *viz*., carbendazim + mancozeb @ 0.3 % and carboxin + thiram @ 0.3 %, *Pseudomonas fluorescens* @ 0.8% and *T. harzianum* @ 0.8 %, jelly + *Pseudomonas fluorescens* @ 0.8 % and coco peat + *T. harzianum* @ 0.8 % respectively were evaluated for their efficacy in the management of seed-borne fungal diseases of tomato in nursery and the results are presented in Table 9, Fig. 8a & 8b and Plate 11.

Among the seven treatments, seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 % and carboxin + thiram (Vitavax Power) @ 0.3 % were found on par with respect to per cent seedling mortality (12.73 and 13.79per cent) and per cent germination (92.00 and 90.33 per cent). Among the bioagents, seed treatment with jelly + *Pseudomonas fluorescens* @ 0.8 % and coco peat + *Trichoderma harzianum* @ 0.8% were found to be on par with respect to per cent germination (86.67 and 84.33), per cent seedling mortality (17.22 and 18.41 %) and vigou index (1759.27 and 1703.50), but found to be inferior over chemicals.

Table 8: Efficacy of biopriming agent as seed dressers against seed-borne fungal infection and seed quality parameters of tomato

Sl. No.	Treatments	Per cent seed infection	Per cent seed germination	Vigour index
1	*P. fluorescens* alone @ 0.8 %	14.00 (21.96)*	92.00 (73.57)	1291.07
2	*P. fluorescens* @ 0.8 % + Jelly	10.00 (18.43)	98.33 (82.67)	2058.23
3	*P. fluorescens* @ 0.8 % + Vermicullite	12.33 (20.56)	95.33 (77.87)	1738.33
4	*P. fluorescens* @ 0.8 % + Coco peat	12.00 (20.27)	96.33 (78.98)	1916.53
5	*P. fluorescens* @ 0.8 % + Coir pith	13.33 (21.40)	94.00 (75.82)	1316.87
6	*T. harzianum* alone @ 0.8 %	14.67 (22.52)*	91.67 (73.23)	1235.57
7	*T. harzianum* @ 0.8 % + Jelly	12.67 (20.85)	95.00 (77.08)	1811.33
8	*T.harzianum* @ 0.8 % + Vermicullite	13.67 (21.69)	92.67 (74.30)	1640.77
9	*T. harzianum* @ 0.8 % + Coir pith	15.33 (23.04)	91.33 (72.90)	1212.60
10	*T. harzianum* @ 0.8 % + Coco peat	11.33 (19.67)	97.00 (80.03)	2091.97
11	Control	29.67 (33.00)	68.67 (55.96)	1007.07
	Mean	**14.45 (22.13)**	**92.03 (74.76)**	**1574.58**
	S. Em. ±	**0.39**	**0.75**	**20.27**
	C. D. at 1 %	**1.57**	**2.98**	**80.81**

*Figures in parentheses indicate arcsine transformed values

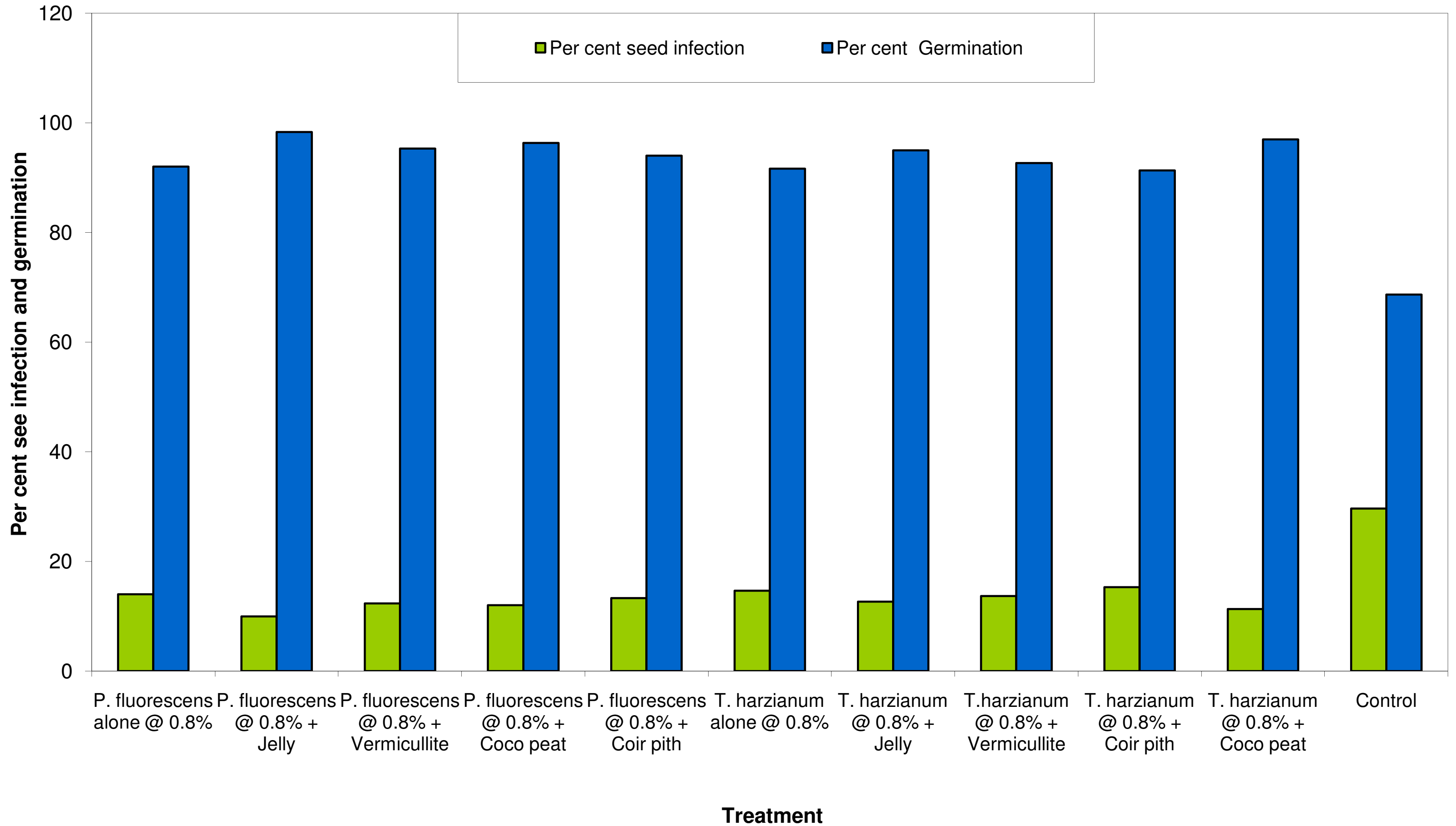


Fig. 7a: Efficacy of biopriming agent as seed dressers against seed-borne fungal infection and seed quality parameters of tomato

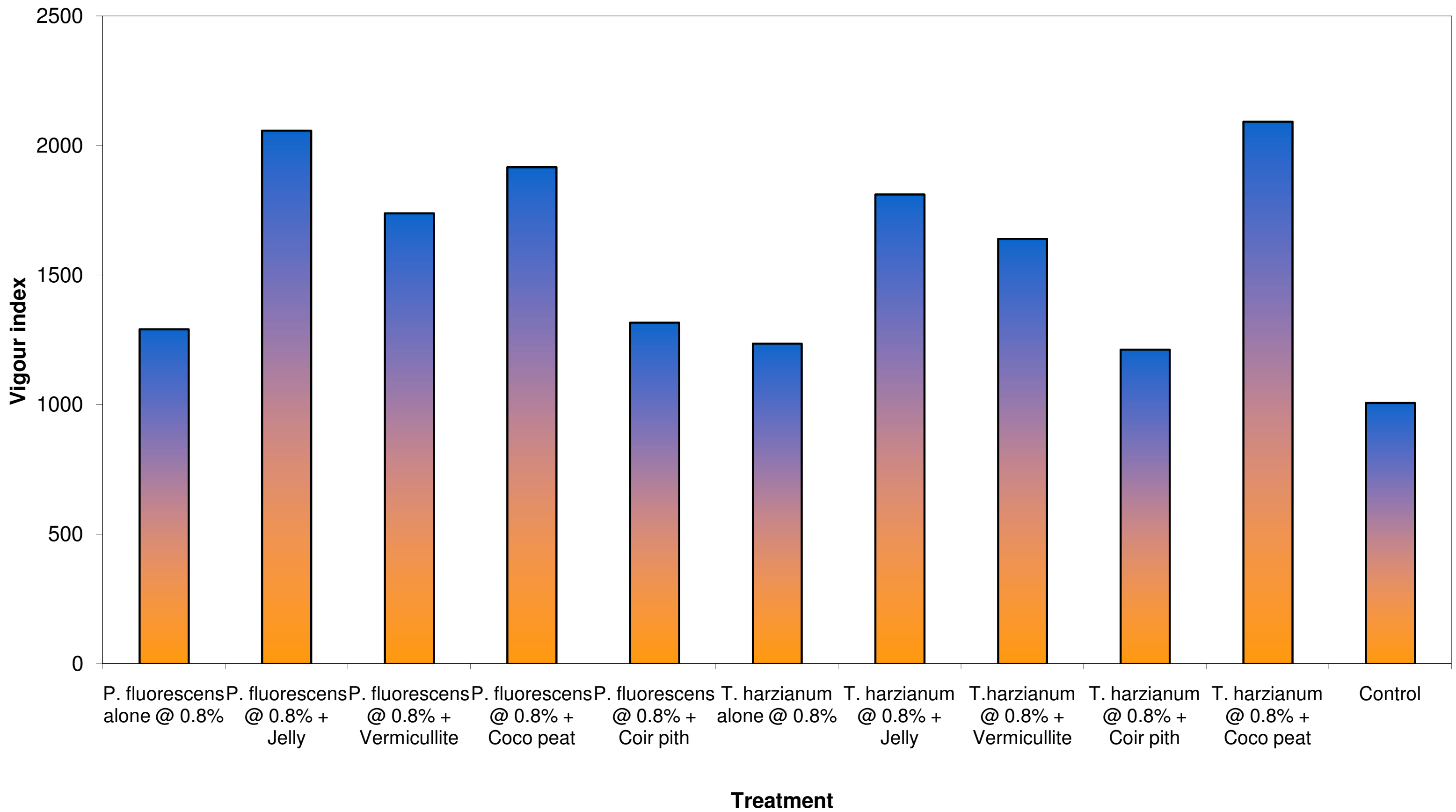


Fig. 7b: Efficacy of biopriming agent as seed dressers against seed-borne fungal infection and seed quality parameters of tomato

4.4.5 Field evaluation of different seed treatments for the management of seed-borne fungal diseases of tomato

A field experiment was conducted during *rabi* season 2015-16 at farmer's field in Bailhongal to assess the efficacy of fungicides, bioagents and bioagent with priming agent as seed dresser against seed-borne fungal diseases of tomato. Variety PKM-1 is used for the study. Treatments were allocated under Randomized Complete Block Design as described in 'Material and Methods' and observations were recorded on per cent disease index of Alternaria blight, per cent disease incidence of wilt and yield. Results are presented in Table 10, Fig. 9a & 9b Plate 11.

In case of Fusarium wilt seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 % + foliar spray with hexaconazole @ 0.1 % was found to be on par with seed treatment with carboxin + thiram (Vitavax Power) @ 0.3 % + foliar spray with hexaconazole (0.1 %) with respect to per cent disease incidence at 45 days (8.24 % and 9.17 %), 60days (10.18 % and 11.23 %), 90 days (15.11 % and 15,74 %) and fruit yield (20.74 and 19.26t/ha). Among the bioagents, seed treatment with *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) and *Trichoderma harzianum* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) was found to be on par with respect to per cent disease incidence at 45 days (13.60 and 14.96 per cent) and 90 dasy (22.06 and 24.24 per cent). Seed treatment with Jelly + *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) and coco peat + *Trichoderma harzianum* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) was found to be on par with each other with respect to per cent disease incidence at 45 days, 60 days, 90 days and fruit yield.

In case of Alternaria blight seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 % + foliar spray with hexaconazole @ 0.1 % was found to be on par with seed treatment with carboxin + thiram (Vitavax Power) @ 0.3 % + foliar spray with hexaconazole (0.1 %) with respect to per cent disease index at 45 days (9.97 % and 10.73 %), 60 days (12.73 % and 13.07 %), 90 days (16.13 % and 17.23 %) and fruit yield (20.74 and 19.26 t/ha). Among the bioagents, seed treatment with *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) and *Trichoderma harzianum* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) was found to be on par with respect to per cent disease incidence at 45 days, 60 days, 90 dasy and fruit yield. Seed treatment with Jelly + *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) and coco peat + *Trichoderma harzianum* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) were found to be on par with respect to per cent disease index at 45 days, 60 days, 90 days and fruit yield.

With respect to benefit cost ratio seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 % + foliar spray with hexaconazole (0.1 %) exhibited maximum benefit cost ratio (3.39) with respect to per cent wilt incidence and per cent disease index. Hence this treatment is considered as effective for the management of seed-borne fungal diseases of tomato. Among the bioagents, seed treatment with *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) exhibited higher benefit cost ratio (2.46) with respect to per cent wilt incidence and PDI of Alternaria blight. Seed treatment with Jelly + *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) were recorded maximum benefit cost ratio with respect to per cent wilt incidence and PDI of Alternaria blight. Hence seed treatment with Jelly + *Pseudomonas fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) were considered as effective for the management of seed-borne fungal diseases of tomato.

Table 9: Effect of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery

Sl. No.	Treatments	Per cent germination	vigour index	Per cent seedling mortality
1	Seed treatment (ST) with Carbendazim + Mancozeb @ 0.3 %	92.00 (73.92)*	1910.53	12.73 (20.90)
2	ST with Carboxin + Thiram @ 0.3 %	90.33 (71.89)	1891.10	13.79 (21.80)
3	ST with *Trichoderma harzianum* @ 0.8 %	73.00 (58.70))	1462.17	22.02 (27.98)
4	ST with *Pseudomonas fluorescens* @ 0.8 %	75.67 (60.44)	1483.13	20.59 (26.97)
5	ST with Jelly + *P. fluorescens* @ 0.8 %	86.67 (68.59)	1759.27	17.22 (24.51)
6	ST with Coco peat + *T. harzianum* @ 0.8 %	84.33 (66.69)	1703.50	18.41 (25.41)
7	Control	62.33 (52.14)	880.90	26.11 (30.71)
	S. Em. ±	**0.96**	**34.00**	**0.48**
	C. D. at 5 %	**2.95**	**104.78**	**1.48**

*Figures in parentheses indicate arcsine transformed values

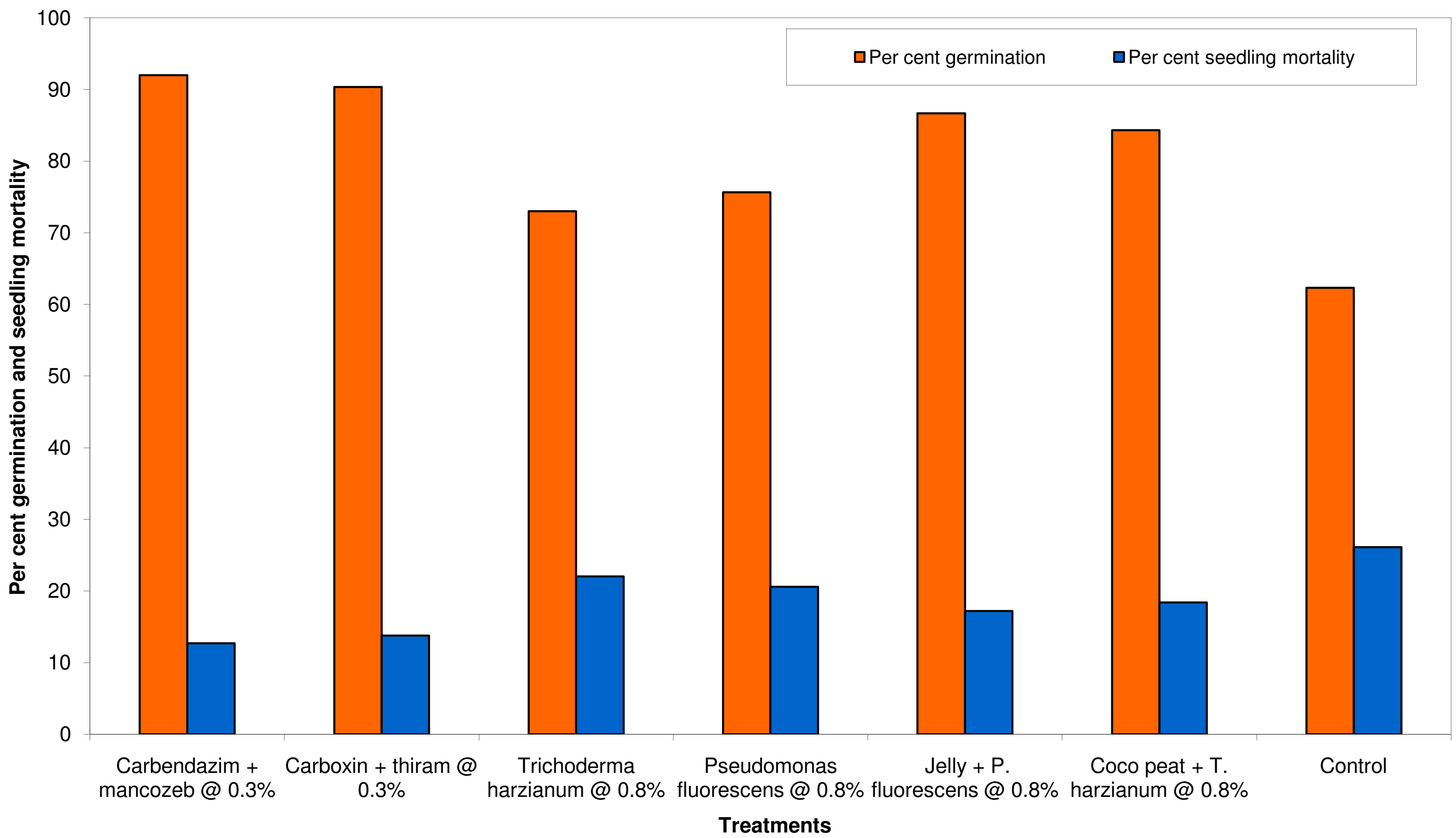


Fig. 8: Effect of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery

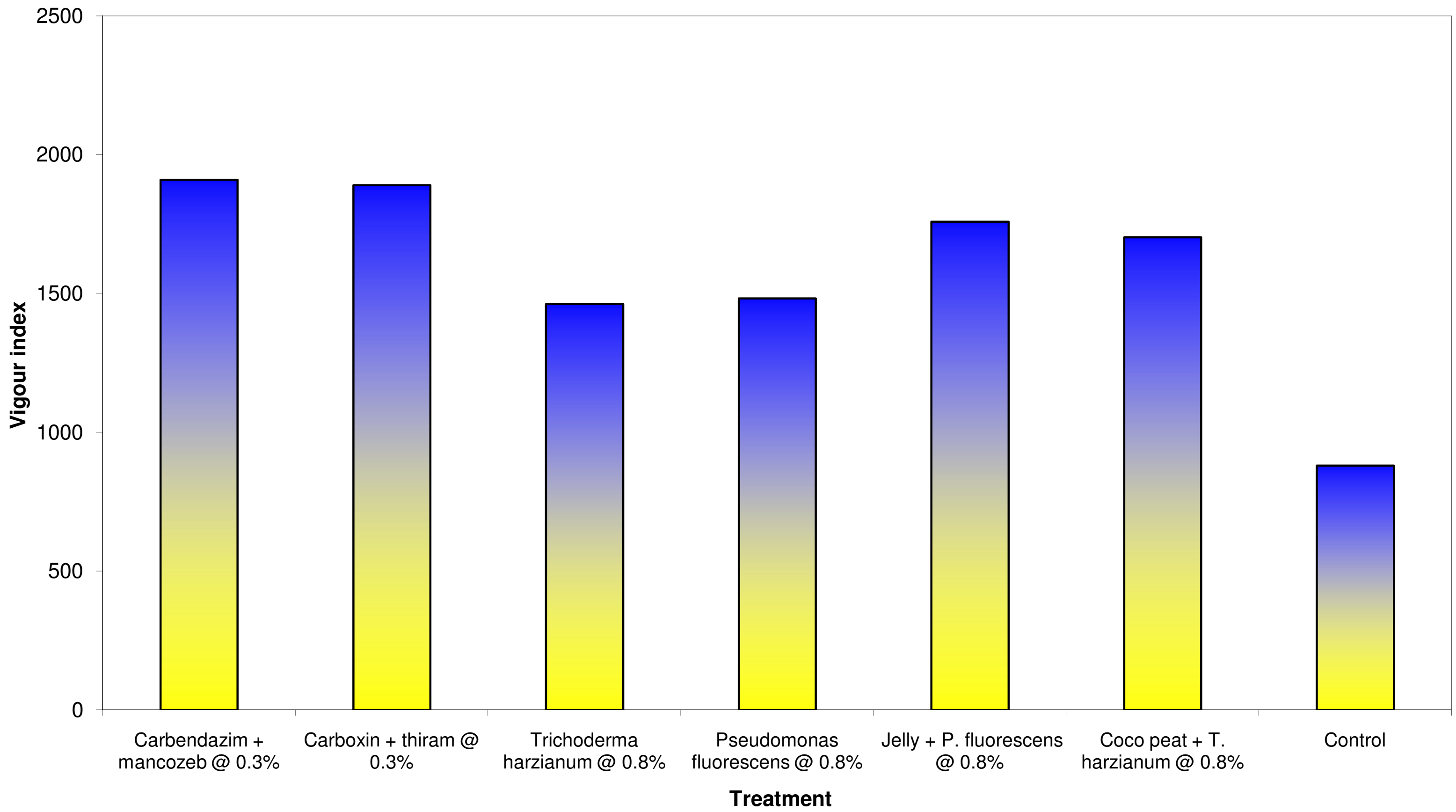


Fig. 8b: Effect of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery

ST with carbendazim + mancozeb @ 0.3%

ST with carboxin + thiram @ 0.3%

ST with *P. fluorescens* @ 0.8% + jelly

Control

Affected plant from control block
showing symptom after transplant

Plate 11: Effect of different seed treatments for the management of seed-borne fungal diseases of tomato under nursery

Table 10: Field evaluation of different seed treatments for the management of seed-borne fungal diseases of tomato

Sl. No.	Treatments	Fusarium wilt incidence (%)			Alternaria blight (PDI)			Fruit yield (t/ha)	B:C ratio
		45 DAS	60 DAS	90 DAS	45 DAS	60 DAS	90 DAS		
1	Seed treatment with Carbendazim + Mancozeb @ 0.3 % + Hexaconazole @ 0.1 % spray	8.24 (16.65)*	10.18 (18.60)	15.11 (22.87)	9.97 (18.34)	12.73 (20.85)	16.13 (23.64)	20.74	3.39
2	ST with Carboxin + Thiram @ 0.3 % + Hexaconazole @ 0.1 % spray	9.17 (17.62)	11.23 (19.57)	15.74 (23.36)	10.73 (19.04)	13.07 (21.15)	17.23 (24.47)	19.26	3.15
3	ST with *Trichoderma harzianum* @ 0.8 % + Hexaconazole @ 0.1 % spray	14.96 (22.75)	19.65 (26.31)	24.24 (29.49)	18.07 (25.15)	20.03 (26.59)	26.53 (31.00)	14.59	2.39
4	ST with *Pseudomonas fluorescens* @ 0.8 % + Hexaconazole @ 0.1 % spray	13.60 (21.63)	17.19 (24.49)	22.06 (28.00)	17.13 (24.45)	18.43 (25.43)	24.17 (29.44)	15.04	2.46
5	ST with Jelly + *P.fluorescens*@ 0.8 % + Hexaconazole @ 0.1 % spray	11.11 (19.47)	13.33 (21.41)	18.26 (25.30)	14.90 (22.71)	15.03 (22.78)	20.07 (26.61)	17.78	2.91
6	ST with Coco peat + *T.harzianum* @ 0.8 % + Hexaconazole @ 0.1 % spray	11.38 (19.71)	14.04 (22.00)	19.44 (26.17)	13.27 (21.36)	15.23 (22.97)	19.17 (25.90)	17.04	2.79
7	Hexaconazole @ 0.1 % alone	18.04 (25.13)	21.05 (27.30)	28.33 (32.13)	19.57 (26.25)	21.10 (27.34)	29.13 (32.67)	12.00	1.96
8	Control	18.98 (25.83)	21.75 (27.77)	28.44 (32.23)	22.20 (28.11)	24.20 (29.45)	34.57 (36.01)	10.37	1.70
S. Em. ±		**0.42**	**0.50**	**0.51**	**0.73**	**0.64**	**0.80**	**0.86**	
C. D. at 5 %		**1.29**	**1.53**	**1.56**	**2.20**	**1.94**	**2.41**	**2.61**	

*Figures in parentheses indicate arcsine transformed values DAS - days after sowing PDI – Per cent Disease Index

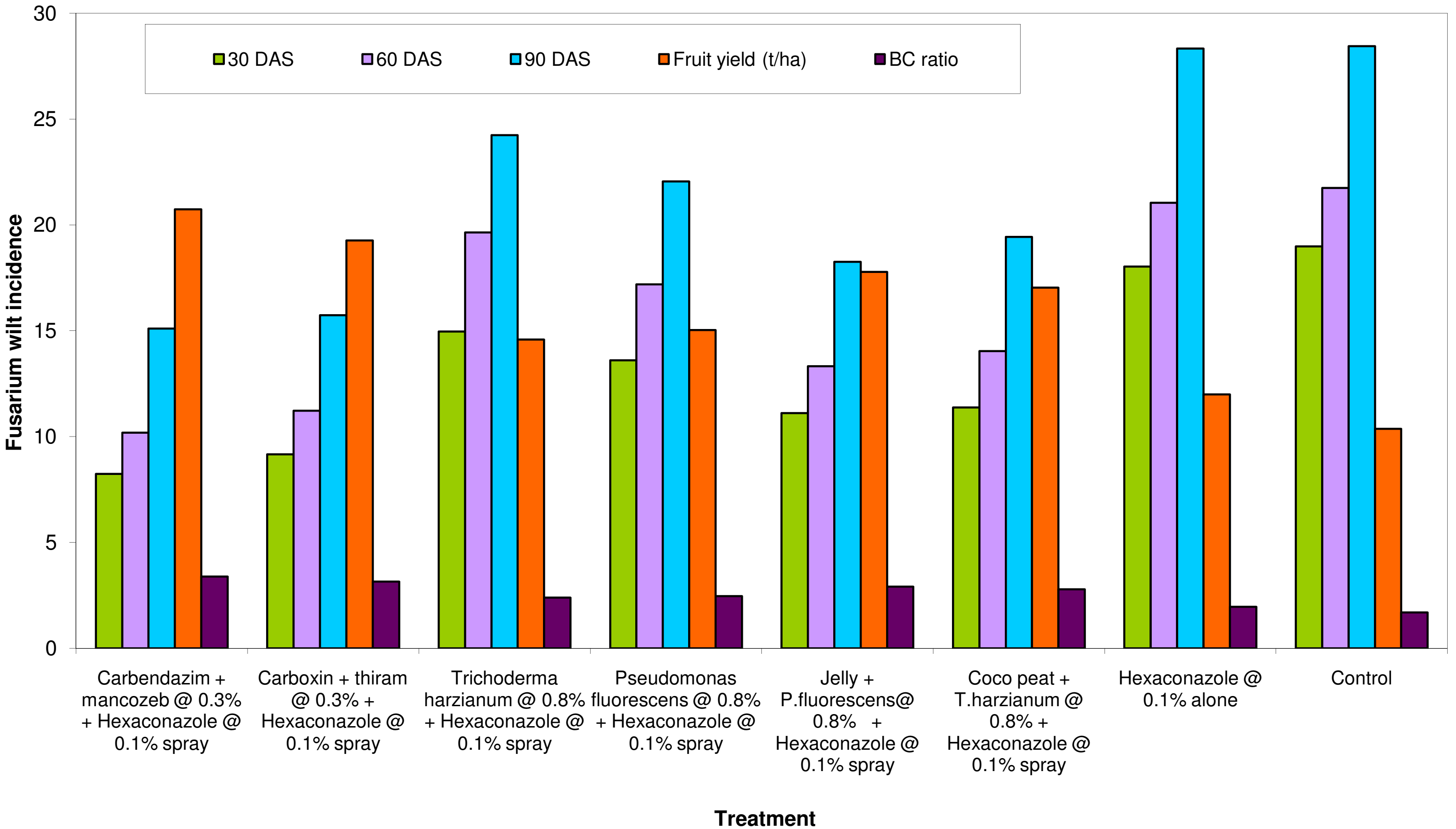


Fig. 9a: Field evaluation of different seed treatments for the management of seed-borne fungal diseases of tomato

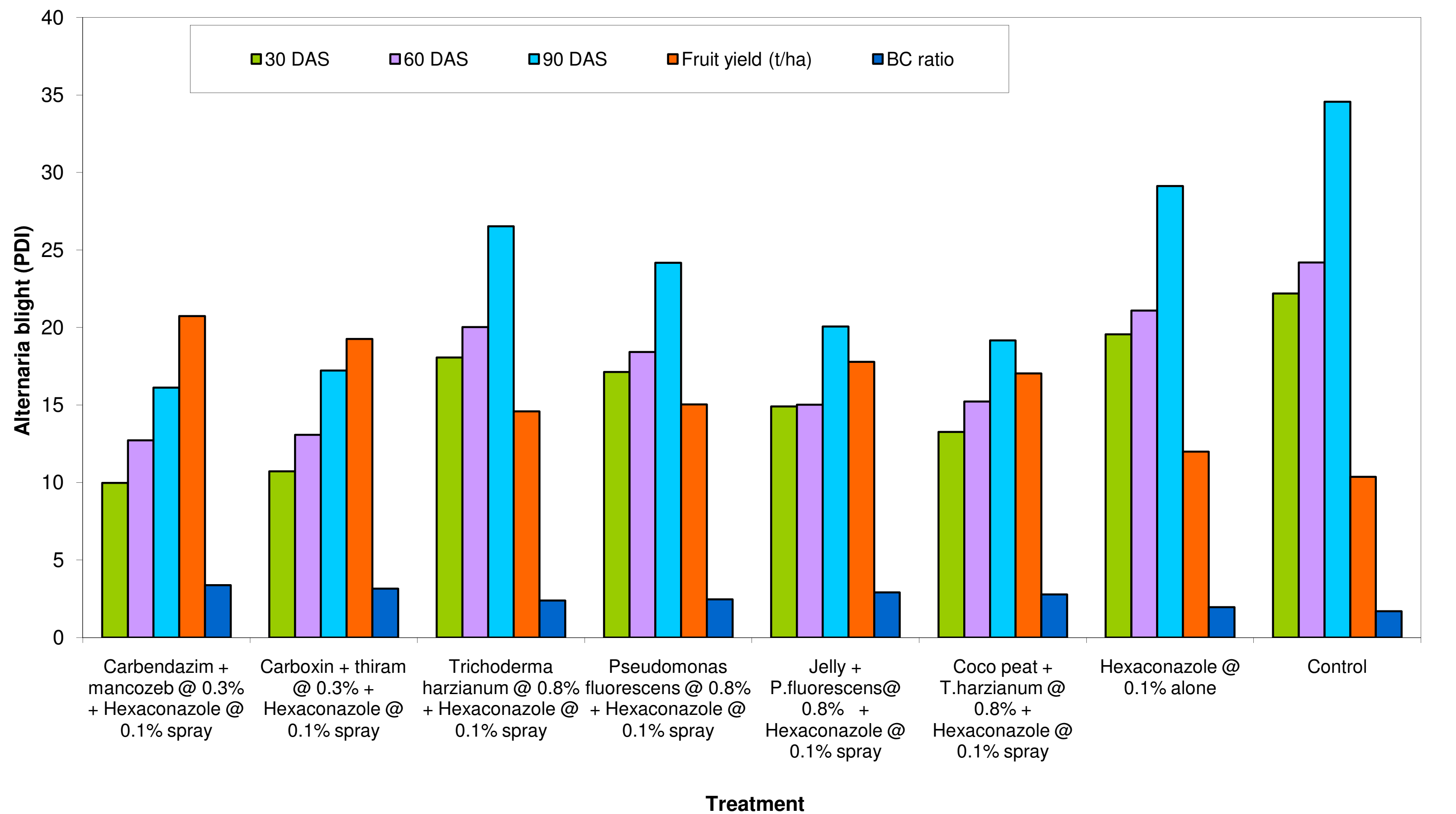


Fig. 9b: Field evaluation of different seed treatments for the management of seed-borne fungal diseases of tomato

5. DISCUSSION

The present investigation, "Studies on seed-borne fungal diseases of tomato and their management" included seed health testing of different tomato cultivars, evaluation of seed health testing methods, location of the pathogen in the seed, seed to plant transmission studies, integrated disease management through use of seed dressing fungicides, bioagents and bioagents along with priming agents. The results of these investigations are discussed below.

5.1 Seed health testing of different seed samples of tomato

The seed samples of tomato collected from different parts of northern Karnataka were tested initially by employing standard blotter method. The results of this study indicated the dominance of *Fusarium* spp., *Alternaria solani* and *Alternaria alternata* and species of *Phoma*, *Curvularia* and *Aspergillus* were appeared in traces hence further studies were restricted to *Fusarium* spp., *Alternaria solani* and *Alternaria alternata.* Similar trend was noticed by Thippeswamy *et al*. (2011) who observed the dominance of *Alternaria solani* (12.0 %), *Alternaria alternata* (3.0 %) and *Fusarium oxysporum* (18.0 %) on tomato seeds in Karnataka.

Malik *et al*. (1991) reported the presence of *Alternaria solani, A. alternata, A. tenussima, Aspergillus niger, Curvularia lunata, Fusarium oxysporum* and *Rhizoctonia solani* on tomato seeds. Chamling *et al*. (2011) isolated eight fungal species on four cultivars of tomato seeds in Gujarat by standard blotter method. The fungi isolated were *Aspergillus niger, A. flavus, Alternaria alternata, Fusarium moniliforme, Fusarium* sp., *Cladosporium* sp., *Penicillium* sp. and *Cunnninghamella echinulat*. Among them they noticed the dominance of *Alternaria alternata* (20.64 %) followed by *Aspergillus niger* (20.33 %) and *Fusarium* sp. (13.44 %).

In the present investigation, among the seed samples of different cultivars tested from different places, PKM-1 cultivar from Haveri area exhibited maximum seed-borne infections compared to other cultivars. In general seed samples from Dharwad and Haveri districts exhibited maximum infection of *Fusarium* spp. and *A. solani* and the *A. alternata* infections were maximum at Belagavi and Haveri seed samples. This might be because of provenance effect and microclimatic conditions existing in these areas for which the seeds were exposed during and after harvest. Even the pre treated seeds like Navodaya, Chenya, Ramya etc., exhibited seed-borne infections. This might be due to improper seed treatment or the chemical treated may not be having broad spectrum of activity. Among the 30 seed samples tested, per cent infection of *Fusarium* spp. varied from 8 to 37.3 per cent, *A. solani* varied from 4 to 40 per cent and *A. alternata* varied from 4 to 30.6 per cent. Similar results were obtained by Meraj and Nandkar (2015) who observed 29 fungi associated with tomato seed samples collected from different localities of Vidarbha region. Fungi like *Alternaria solani, Aspergillus flavus, A. niger, Botrytis cinera, Cladosporium fulvum, Colletotrichum capsici, Curvularia lunata, Fusarium solani, F. lycopersici* and *Penicillium* sp. were found to be most dominant fungi on tomato seeds.

Among the different seed-borne fungi observed in the current investigations, fungi like *Fusarium* spp., *Alternaria solani and A. alternata* were found to be dominant. From the seed health point of view, these fungi are known to cause economically important diseases like wilt, early blight and leaf spot respectively. These seed-borne diseases are known to affect overall yield and seed quality parameters like per cent germination and vigour index. Hence further studies were carried out like evaluation of seed heath testing, transmission studies and management aspects etc.

5.1.2 Seed health testing of tomato seed samples by seed washing technique

Examination of seed washing revealed only the presence of spores of saprophytic fungi like *Aspergillus* spp. The method was found unsuitable for the detection of *A. solani, A. alternata, Fusarium* spp., *Phoma* spp*.* and *Curvularia* spp. as no fungal structures or spores of these fungi were detected.

5.2 Evaluation of seed health testing methods

Several criteria are involved in selecting a suitable method for the detection of seed borne fungi. The primary criterion is that the method should be sensitive, simple and reproducible. (Anon., 1999) advocated that dry seed examination itself is not an adequate method and is a supplement to the incubation method with some modifications, which are imperative to provide full information regarding the identification of the pathogen. Besides, dry seed examination does not provide information on viability of the seeds. Keeping this in view, a study to compare the efficacy of five routine seed health testing methods in detecting the seed borne fungal infection in tomato was undertaken.

Among the five different methods employed for the detection of seed-borne fungal pathogens of tomato, standard blotter method was found to be good for the detection of *A. solani*, *A. alternata* and *Fusarium* spp. Similar results were obtained by Krishna *et al.* (1998) who reported that standard blotter method is an efficient one for detection *of Alternaria solani* causing early blight in tomato. Perveen (1996) reported the efficacy of standard blotter method over agar plate and deep freezing blotter method for the detection of *Alternaria* spp., *Fusarium* spp. and *Dreschleria* spp. on tomato seeds. Standard blotter method being very simple method is most suitable for the routine seed health diagnosis of *Alternaria* and *Fusarium*. Significantly, higher counts of *Fusarium* spp. were recorded in standard blotter method followed by 2, 4-D blotter method, and higher count of *Alternaria* spp*.* were recorded in standard blotter method followed by water agar method. These finding are in conformity with the findings of Sowley and Kodua (2012) who reported the efficacy of standard blotter method for the detection of seed-borne fungal infections in tomato over agar plate method. Deep freezing blotter method was found to be ineffective for the detection of seed-borne fungi in tomato as it recorded least seed infection.

Hence, it can be concluded from the present study that Standard blotter method can be recommended for routine seed health diagnosis of seed-borne fungal infection in tomato as the method is simple, sensitive and reliable.

5.3 Effect of inoculum levels, transmission studies and proving pathogenicity

Seed transmission studies helps to confirm the seed to plant transmission to prove the pathogenicity and also to design the suitable management strategy. Hence an attempt was made to know the mode of transmission and type of damage caused by *viz*., *A. solani, A. alternata and Fusarium* spp. under existing microclimatic conditions. Transmission studies were carried out by water agar seedling symptom test *in vitro* and also under pot culture. Transmission studies with different inoculum densities of the pathogen helps in fixing the certification limits for the management of these seed-borne pathogens.

5.3.1 Seedling symptom test

Selected seed samples of variety PKM-1 were subjected to water agar seedling symptom test to diagnose the seed borne infection and to see the seed to plant transmission. Seeds of variety PKM-1 were artificially inoculated with conidial suspension of *Alternaria solani, A. alternata* and *Fusarium* spp. separately from 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions and apparently healthy seeds treated with only distilled water without conidial suspension which served as control. In *Alternaria solani* inoculated seeds with higher concentration of conidia (with 10^{-1} and 10^{-2} dilution) seed got aborted. In case of *A. alternata* seeds after germination exhibited cotyledonary leaf necrosis upto 10^{-2} dilution. This might be because of higher concentration of toxin produced in lower dilution, where as *Fusarium* inoculated seeds with higher concentration of conidia (with 10^{-1} and 10^{-2} dilution) seeds got aborted and they exhibited seed rotting, further dilutions *i.e.*,10^{-3} and 10^{-4} failed to induce any visible symptoms. These findings are in accordance with the findings of Elliott and Crawford (1922) who reported the seed borne nature of the fungus by recovering it from seed, from infected plants and proving that isolates were pathogenic on tomato. Approximately 3.5 per cent of the seeds from fruits produced on heavily infected plants contained the pathogen. Perveen (1996) reported the reduced per cent seed germination in *Fusarium* and *Alternaria* sp. inoculated tomato seeds.

5.3.2 Pot culture studies

The results of pot culture studies conducted to study the seed to plant transmission of *Alternaria solani, A. alternata and Fusarium* spp. revealed that apparently healthy and artificially inoculated seeds exhibited severe reduction in per cent germination and disease incidence was noticed after 20 DAS. Per cent seedling infection increased with increase in conidial concentration.

In case of *A. solani*, seeds soaked in 2.4×10^6 and 2.4×10^5 conidia/ 1ml recorded maximum per cent seedling infection. The characteristic symptom *i.e.* brown to dark brown concentric rings (target board effect) were observed on older leaves at 30 DAS onwards. The maximum infection was observed in treatments T_1 and T_2 whereas lower infection was observed in T_3 and T_4 (seeds soaked in 2.4×10^4 and 2.4×10^3 conidia/ 1 ml respectively). In case of *A. alternata* seeds soaked in 2.12×10^6 and 2.12×10^5 conidia/ 1ml showed maximum per cent seedling infection. The characteristic symptom *i.e.* minute water soaked specks with yellow halo on leaves was recorded after 45 days of sowing.

The maximum infection was in treatments T_1 and T_2 (seeds soaked in 2.12 × 10^6 and 2.12 × 10^5 conidia/ 1 ml concentration), whereas lower infection was recorded in T_3 and T_4 seeds soaked in 2.12 x 10^4 and 2.12 x 10^3 conidia /1ml treatments. Higher seedling infection was noticed with lower dilutions, might be due to the increased level of toxin produced by the fungi.

This study reveals the significance of seed-borne fungal infection. Seeds with high dose of inoculum yielded more of disease incidence and the incidence was noticed upto10^{-3} and at 10^{-4} there was no significant visible symptoms, indicating that 10^{-3} *i.e.,* conidial concentration of (2.4 x 10^4) is the minimum threshold inoculum required for causing the disease under Dharwad conditions. Similar result was obtained by Hayat *et al.* (2014) who reported the pathogenicity of seed-borne fungi like *Alternaria alternata, Fusarium solani* and *Fusarium moniliforme* on ten tomato cultivars. All the germinated seeds were infected by these fungi with varying degree of variability or aggressiveness and each cultivar significantly reduced germination and produced more abnormal seedlings compared to control. They also reported the transmission of *Aspergillus niger, Alternaria alternata, F. solani* and *F. oxysporum* from seeds to seedlings. The highest transmission was observed during germination stage followed by seedling stage on leaves and stem.

In case of *Fusarium* seeds soaked in 3.25 x 10^6 conidia/1ml and 3.25 x 10^5 conidia/1ml concentration showed least germination percentage but germination per cent increased with corresponding decrease in concentration of fungal propagules. Wilting of seedlings was noticed after 20 DAS. The maximum wilt incidence was recorded in treatments T_1, T_2 and T_3 with 3.25 x 10^6, 3.25 x 10^5 and 3.25 x 10^4 conidia/1ml. Wilt incidence was also recorded upto T_4 and also in T_5 (Apparently healthy seeds) this might be because of traces level infection of the pathogen in apparently healthy seeds and also there was no inoculum available for infection through sterilized soil. This is in contrast to the observation noticed in seedling symptom test where seed abortion was noticed at lower dilution this might be because of the direct impact of the pathogen under restricted area of the test tube. Similar observation was obtained by Orlava *et al*. (1982) who studied the pathogenecity of 3 species of *Fusarium,* isolated from tomato seeds and observed that pathogenecity was higher when tomato seeds were dipped in spore suspension for 1 hour before sowing. Shovan *et al.* (2008) they studied the pathogenicity test with 33 isolates of *Fusarium solani* under pot culture, all the tested isolates were found to be pathogenic on tomato. This shows that seed inoculums levels of T_1, T_2 and T_3 (3.25 x 10^6, 3.25 x 10^5 and 3.25 x 10^4 conidia/1ml) concentration can cause maximum wilting.

5.3.3 Location of fungi in seed

The location of seed-borne fungi was studied by inoculating seed parts *viz.,* pericarp and other than pericarp (embryo, endosperm and hilum) on PDA by component plating technique.

Results of the component plating technique to know the location of seed borne fungi in infected samples, revealed the presence of *A. alternata, Phoma* spp. and *Aspergillus* spp. in the pericarp only. Similar result was obtained by Neergaard (1979) found many seed borne fungi infected the seed coat causing conspicuous black brown to black necrotic discoloration, germination reduction and germination failure. The best known samples were effects of *Aspergillus* spp. in tomato, okra and radish; *Fusarium* spp. in onion and *Colletotrichum gloeosporioides* in okra. Whereas *Fusarium* spp. and *A. solani* were noticed on other than pericarp also *i.e.*, endosperm embryo and hilum. These results indicate the requirement of systemic seed dressing molecules (like combi-productes) for the effective control of the pathogen as the molecules can penetrate up to the embryo. Histopathological studies conducted by Thippeswamy *et al.* (2006) who studied the location and transmission of *Fusarium oxysporum* and *Alternaria solani* in naturally infected tomato seeds. The results revealed that both pathogens were located in seed coat, cotyledons and in embryonic axis of tomato seedlings at various concentrations.

5.4 Management strategies for overcoming seed-borne infections of tomato

5.4.1 Evaluation of fungicides

5.4.1.1 *In vitro* evaluation of seed dressing fungicides by rolled towel method

In the absence of resistant variety, use of fungicides is an alternate practice to manage the diseases. Seed treatment along with foliar spray of fungicide, gives maximum protection against seed-borne fungal diseases of tomato which can spread through seed and air as primary and secondary source of infection. *In vitro* evaluation of new seed dressing fungicides is very much necessary before they are taken to field. In the present study, seven seed dressing fungicides were tested for their efficacy in overcoming seed-borne fungal infections of tomato along with other seed contaminants by rolled towel method. The best two seed dressing fungicides were further tested under field conditions.

Results of the *in vitro* evaluation of fungicides by rolled towel method indicated that, the efficacy of carbandizim + mancozeb (Sprint) and carboxin + thiram (Vitavax Power) in eliminating seed borne fungal infection with higher percentage of germination and vigour index compared to untreated seeds (control). Seed treatment with carbandizim + mancozeb @ 0.3 % differed significantly with respect to per cent germination and vigour index over 0.2 % and similarly in case carboxin + thiram @ 0.3 % differed over 0.2 % significantly with respect to per cent germination though found to be on par with respect to per cent seed infection and vigour index. Hence 0.3 per cent concentration of carbandizim + mancozeb (Sprint) and carboxin + thiram (Vitavax Power) were selected for further field evaluation. Dabbas *et al.* (2010) reported the efficacy of seed treatment with carbendazim + macozeb @ 2 g/ kg for the maximum control of the seed mycoflora of tomato. In case of different seed dressing fungicides the efficacy of the fungicides depends upon tenacity, adhesiveness and other characters.

Result of the present study indicated the efficacy of combi-products than solo products as the combi-products consists of one systemic and one contact molecule which can take care of wide range of seed-borne inoculum.

5.4.2 Evaluation of bioagents

5.4.2.1 *In vitro* evaluation of bioagents by Rolled towel method

Biological control through the use of antagonistic microorganisms plays a key role in modern agriculture as it is cheap and eco-friendly. Such management options would help in preventing the pollution and also health hazards. Hence in the present investigations an attempt has been made to evaluate the effect of bioagents on seed borne fungal infections of tomato.

Among the bioagents, *Pseudomonas fluorescens* at 0.8 % differed significantly over *Trichoderma harzianum* with respect to per cent seed infection and per cent germination and further *P. fluorescens* at 0.8 % differed significantly over 0.4 % with respect to per cent seed infection, per cent germination and vigour index and similarly *T. harzianum* @ 0.8 % differed significantly over 0.4% with respect to per cent seed infection, per cent germination and vigour index. Hence these two bioagents *i.e.*, (*P. fluorescens* and *T. harzianum*) were selected for further field evaluation @ 0.8 per cent concentration. VAM fungi were found to be least effective in eliminating seed-borne infections. Similar results were obtained by El-Mougy *et al*. (2012) who reported that seed treatment with *Trichoderma harzianum*, *T. Viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were effectively control of *Alternaria solani, Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Pythium* sp. in tomato seeds. Ganie *et al*. (2013) studied the bio efficacy of seed treatment with three bioagents viz., *Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* under *in vitro* against *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato. Among them they noticed that *Pseudomonas fluorescens* showed significantly higher seed germination (71.85 %) followed by *T. harzianum* (65.93 %) and *T. viride* (58.65 %).

5.4.3 Evaluation of Biopriming

5.4.3.1 *In vitro* evaluation of biopriming agents against seed-borne infections of tomato

Seed priming (or) osmo-conditioning refers to a pre-sowing hydration treatment developed to improve seedling establishment. Primed seeds can be handled in a manner similar to conventional seeds and a seed company can perform this process. On-farm seed priming seems to be a robust, widely applicable technology and its effects are generally independent of the crop variety used. This is important, because priming can be used to add value to the benefits achieved by using improved modern varieties or by adoption of other improved technologies such as fertilizer or better crop protection.

Seed treatment with bio-control agents along with priming agents may serve as an important means of managing many of the soil and seed-borne diseases, the process often known as bio-priming. However, this may not give additional benefits under optimum conditions.

Results of the *in vitro* evaluation of bioagent along with priming agent against seed borne infection in tomato indicated the efficacy of jelly as priming agent in enhancing the efficacy of *P. fluorescens* in reducing seed borne fungal infection with increased germination and vigour index, where as *P. fluorescens @* 0.8 % + jelly was found to be on par with *Trichoderma harzianum @* 0.8 % + coco peat with respect to per cent seed infection, per cent germination and vigour index. Hence these two bioprimings *i.e.*, *P. fluorescens @* 0.8 % + jelly and *T. harzianum @* 0.8 % + coco peat were selected for further field evaluation. Seed treatment with *P. fluorescens @* 0.8 % + jelly differed significantly over seed treatment with *P. fluorescens @* 0.8% with respect to per cent seed infection, per cent germination and vigour index, indicating the role of jelly in enhancing efficacy of *P. fluorescens*, jelly being hydrophobic polymer, can imbibe water and provides suitable conditions for the multiplication of bioagent *P. fluorescens.* The present findings are in accordance with the findings of El-Mohamedy and Abd El-Baky (2008) who investigated / evaluated the efficacy of different types of seed treatments i.e., bio-priming, seed coating with bio-control agents (*T. harzianum, Bacillus subtilis* and *P. fluorescens*) seed priming and seed dressing with these antagonistic micro-organisms in control of root rot disease incidence compared to other treatments. Harman *et al.* (1989) reported increased plant stand in soils infested with *F. graminarum* and *Pythium ultimum* by priming of wheat seeds with *Trichoderma harzianum*. They further reported that in field trial, stands of peas were not significantly enhanced by seed treatment with *T. harzianum* strains in the absence of priming but were improved by *T. harzianum* + vermiculite.

5.4.4 Effect of different seed treatments for the management of seed-borne fungal diseases of tomato nuder nursery

To compare different seed treatment procedures on tomato variety PKM-1, the two seed dressing fungicides *viz*., carbendazim + mancozeb @ 0.3 % and carboxin + thiram @ 0.3 %, the two bioagents such as *Pseudomonas fluorescens* @ 0.8 % and *T. harzianum* @ 0.8 % and the two biopriming agents such as jelly + *Pseudomonas fluorescens* @ 0.8 % and coco peat + *T. harzianum* @ 0.8 % were evaluated for their efficacy in the management of seed-borne fungal diseases of tomato in nursery.

Results of this study revealed the efficacy of seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 per cent and carboxin + thiram (Vitavax Power) @ 0.3 per cent for the effective management of seed-borne fungal diseases of tomato in nursery. Both of these seed dressing fungicides being combi-products consists of both contact and systemic fungicides which can take care of broad range of seed-borne pathogens like, *Fusarium*, *Alternaria*, *Pythium*, *Septoria* etc. These findings are in the accordance with the findings of Tewari and Vishunavat (2012) who reported the efficacy of seed treatment with thiram, carbendazim + mancozeb and Carbendazim against early blight of tomato caused by *Alternaria solani*, they observed the significant increase seed germination and reduction in seedling infection in all the fungicides treated seeds as compared to check. Rahman and Bhattiprolu (2005) reported the effective control of damping-off disease in the solanaceous crop nurseries by seed treatment with thiram @ 2 g/ kg seed alone or along with soil application of Ridomil MZ-72 @ 0.2% twice, first immediately after germination and second at 20 days after germination.

Among the bioagents, seed treatment with *Pseudomonas fluorescens* @ 0.8 % + Jelly and *Trichoderma harzianum* @ 0.8 % + coco peat were found to be superior over seed treatment with bioagents alone (without priming agent) with respect to per cent seed germination, per cent seedling mortality and vigour index, indicating the efficacy of priming agents in providing protection to the seedlings over extended period. Similar results were obtained by Callan *et al*. (1990) who reported the increased efficacy of bacterial bio-control agent *Pseudomonas aureofaciens* Kluyver AB254 when bioprimed with moist vermiculite on moist germination blotter. They noticed rapid multiplication of bacterial bio-control agent on seed during biopriming. Vidhyasekaran and Muthamilan (1995) also reported the increased rhizosphere population of biocontrol agent with biopriming. Seed treatment with antagonist formulation effectively controlled chickpea wilt disease in the two field trials with increased yield. Begum *et al*. (2010) and Ananthi *et al*. (2014) reported increased efficacy of biocontrol agents along with priming agent.

5.4.5 Field evaluation of different seed treatments for the management of seed-borne fungal diseases of tomato

A field experiment was conducted during *Rabi* season 2015-16 at the farmer's field in Bailhongal (of Belagavi disticts) to assess the efficacy of fungicides, bioagents and bioagent with priming agent as seed dresser against seed-borne fungal diseases of tomato. Variety PKM-1 is used for the study. Results of the field evaluation of seed treatment procedures for the management of seed-borne fungal diseases of tomato, indicated the efficacy of seed treatment with carbendazim + mancozeb (Sprint) @ 0.3 % along with foliar spray of hexaconazole (0.1 %) at 45 DAS however this treatment was found on par with the seed treatment with carboxin + thiram (Vitavax Power) @ 0.3 per cent + hexaconazole (0.1 %) spray with respect to incidence of seed-borne diseases like Fusarium wilt and Alternaria blight and also fruit yield.

Seed treatment with carbendazim + mancozeb @ 0.3 % + hexaconazole (0.1 %) spray exhibited maximum B:C ratio compared to seed treatment with carboxin + thiram @ 0.3 per cent + hexaconazole (0.1 %) spray. Hence seed treatment with carbendazim + mancozeb @ 0.3% + hexaconazole (0.1 %) spray is preferred for the management of seed-borne fungal diseases of tomato. Similar results were obtained by Singh *et al*. (2000) they reported that the seed treatment of tomato variety Pusa Ruby with seed dressing fungicides, carbedazim, vitavax, thiram, and carbendazim + mancozeb at 0.2 per cent concentration significantly reduced the incidence of early blight caused by *Alternaria solani* and increased the fruit yield. Thippeswamy *et al*. (2006) suggested that among all the fungicides, seed treatment with Mancozeb, Carbendazim and Captaf at 0.20 per cent concentration was effective against *phomopsis* blight and leaf spot in brinjal caused by *Phomopsis vexans* and *Alternaria solani.*

Among the bioagents seed treatment with Jelly + *P. fluorescens* @ 0.8 per cent + foliar spray with hexaconazole (0.1 %) was found to be superior over seed treatment with coco peat + *T. harzianum* @ 0.8 % + foliar spray with hexaconazole (0.1 %) as the seed treatment with Jelly + *P. fluorescens* @ 0.8 % + hexaconazole (0.1 %) spray exhibited higher B:C ratio than coco peat + *T. harzianum* @ 0.8 % + hexaconazole (0.1 %) spray. Hence seed treatment with Jelly + *P. fluorescens* @ 0.8 per cent along with foliar spray of hexaconazole (0.1 %) can be recommended as an alternative to chemical seed treatment for the organic growers.

Seed treatment with bioagents alone (without priming agent) were found inferior to seed treatment with bioagents along with priming agent with reference to per cent wilt incidence, Alternaria blight index and fruit yield clearly indicate the significance of priming agent along with bioagent. The successe of biocontrol dependence on the survival of bioagents under varied climatic conditions. Similar observation were made by Callan *et al.* (1990) who reported the increased efficacy of bacterial bio-control agent *Pseudomonas aureofaciens* Kluyver AB254 when bioprimed with moist vermiculite on moist germination blotter. They noticed rapid multiplication of bacterial bio-control agent on seed during biopriming. Vidhyasekaran and Muthamilan (1995) also reported increased rhizosphere population of biocontrol agent with biopriming. Seed treatment with antagonist formulation effectively controlled chickpea wilt disease in the two field trials with increased yield.

Similar observations were recorded by Nancy *et al.* (1997) who recommended the biological seed treatment for control of seed and seedling diseases for the grower as an alternative to chemical fungicides. While biological seed treatments can be highly effective, it must be recognized that they differ from chemical seed treatments by their utilization of living microorganisms. Storage and application conditions are more critical than with chemical seed protectants and differential reaction to hosts and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than chemicals. Conversely, some biocontrol agents applied as seed dressers are capables of colonizing the rhizosphere, potentially providing benefits to the plant beyond the seedling emergence stage.

Results of this field trail clearly indicated the significance of seed treatment along with foliar spray for the management of diseases like early blight, Alternaria leaf spot and Fusarium wilt. Foliar spray with hexaconazole (0.1 %) alone was found inferior compared to seed treatment + foliar spray indicating the efficacy of seed treatment + foliar spray for the management of such diseases for which the primary source comes from seed and soil and secondary spread of inoculum takes place by air. Foliar spray with hexaconazole (0.1 %) cannot control fusarium wilt as the pathogen is soil and seed-borne in nature.

Future line of work

1. Results of the novel seed treatment options such as biopriming developed for the management of seed-borne fungal diseases of tomato can be confirmed by conducting large scale demonstrations and multi-location trails.
2. Seed treatment with biocontrol agents like *Pseudomonas fluorescens* with priming agents are known to induce systemic resistance in plants. Details of these mechanisms involved can be studied further.
3. Hydrogel encapsulations with biocontrol agents can be tried for effective delivery of biocontrol agents.

6. SUMMARY AND CONCLUSIONS

The present investigation was undertaken during 2015-16 at the Department of Plant Pathology, College of Agriculture, UAS, Dharwad which included seed heath testing of tomato seed samples for seed-borne mycoflora, evaluation of seed health testing methods, seed to plant transmission, management of seed-borne fungal infections of tomato through seed dressing fungicides, bioagents and biopriming procedures. The results obtained are summarized here under.

- Seed health testing of different tomato seed samples collected from different parts of northern Karnataka revealed the dominance of *Fusarium* spp. (34.87 %), *Alternaria solani* (30.73 %) and *Alternaria alternata* (26.77 %). Other saprophytic fungi included species of *Aspergillus*. In general seed samples from Dharwad and Haveri districts exhibited maximum infection of *Fusarium* spp. and *A. solani,* whereas *A. alternata* infection was maximum at Belagavi and Haveri seed samples. Variety PKM-1 exhibited maximum seed-borne infections compared to other seed samples.

- Seed health testing of tomato seed samples by seed washing technique revealed only the presence of saprophytic fungi like *Aspergillus* spp. and was found unsuitable for the detection of other pathogenic fungi *viz*., *A. solani, A. alternata, Fusarium* spp., *Phoma* spp.and *Curvularia* spp. as no fungal structures or spores of were detected.

- Among the five different seed health testing methods employed to assess their efficacy and reliability, standard blotter method was found to be good for the detection of seed borne infection of *A. solani*, *A. alternata* and *Fusarium* spp. in tomato. Hence, it can be concluded from the present study that standard blotter method can be recommended for routine seed health diagnosis of seed borne fungal infections in tomato as the method is simple, sensitive and reliable.

- The pathogenic ability of seed borne fungi *viz*., *Alternaria solani. A. alternata and Fusarium* spp. was proved in transmission studies carried out by seedling symptom test and in pot culture studies. Experiment on effect of seed inoculums levels on the incidence of seed-borne diseases indicated the increased incidence of the diseases at lower dilutions *i.e.*, 10^{-1} and 10^{-2} with inoculum levels varying from 3.25×10^{6} and 3.25×10^{5} (for *Fusarium*) and 2.4×10^{6} and 2.4×10^{5} (for *Alternaria solani*).

- Study on the location of seed borne fungi in the infected tomato seeds revealed that, *Alternaria alternata, Phoma* spp. and *Aspergillus* spp. were confined to pericarp only, where as *Fusarium* spp. and *Alternaria solani* were noticed on other than pericarp also.

- Among the seven seed dressing fungicides tested against seed-borne fungal infections of tomato by rolled towel method, carbendazim + mancozeb @ 0.3 % and carboxin + thiram @ 0.3 % were found on par with respect to per cent seed infection, and vigour index.

- Among the four bioagents tested for their efficacy in overcoming seed borne fungal infections of tomato by rolled towel method, seed treatment with *Pseudomonas fluorescens* @ 0.8 % showed least per cent seed infection with maximum per cent germination followed by *Trichoderma* *harzianum* @ 0.8 %. Seed treatment with *Pseudomonas fluorescens* at 0.8 % differed significantly over *Pseudomonas fluorescens* at 0.4 % with respect to per cent seed infection, per cent germination and vigour index.

- Among the four priming agents tested along with bioagents, *Pseudomonas fluorescens* @ 0.8 % + jelly recorded significantly higher per cent seed germination and vigour index with least per cent seed infection and found to be on par with *Trichoderma harzianum* @ 0.8 % + coco peat. Further, the results indicated the increased efficacy of bioagents along with priming agents.
- Nursery experiment conducted at Bailgonhal in farmer′s field to assess the efficacy of fungicides, bioagents, and bioagent with priming agent as seed dresser against, seed-borne fungal diseases of tomato, revealed that seed treatment with seed dressing fungicides, carbendazim + mancozeb (Sprint) @ 0.3 % and carboxin + thiram (Vitavax Power) @ 0.3 % were found on par with respect to per cent seedling mortality, vigour index and per cent germination.
- Field experiment conducted at farmer′s field in Bailhongal to assess the efficacy of fungicides, bioagents, and bioagent with priming agent as seed dresser against seed-borne fungal diseases of tomato, revealed the efficacy of seed treatment with carbenbazim + mancozeb @ 0.3 % in overcoming seed-borne fungal diseases of tomato along with foliar spray of hexaconazole (0.1 %) at 45 DAS. This was found on par with carboxin + thiram @ 0.3 % + hexaconazole (0.1 %) spray, but exhibited maximum benefit cost ratio hence this treatment is preffered for the effective management of seed-borne fungal diseases of tomato. Among the bioagents *Pseudomonas fluorescens @* 0.8 % + jelly + hexaconazole (0.1 %) spray was found to be superior over *Trichoderma harzianum* @ 0.8 % + coco peat + hexaconazole (0.1 %) spray as this treatment exhibited higher benefit cost ratio compared to *T. harzianum* @ 0.8 % + coco peat + hexaconazole (0.1 %) spray. Hence seed treatment with *P. fluorescens @* 0.8 % + jelly can be recommended as alternative to chemical seed treatment against seed-borne fungal diseases of tomao to organic growers.

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Appendix I: Weather parameters during crop growth period

Months	2014-15					2015-16				
	Temperature (^{0}C)		Rainfall (mm)	RH (%)	No. of rainy days	Temperature (^{0}C)		Rainfall (mm)	RH (%)	No. of rainy days
	Min	Max				Min	Max			
October	19.00	29.70	103.20	71.45	6	19.47	31.16	179.80	5	64.33
November	15.5	29.00	48.80	59.40	2	17.99	30.04	28.60	2	69.63
December	14.50	27.80	26.20	63.69	1	15.60	30.59	0.00	0	55.68
January	13.30	28.60	0.20	51.55	0	14.13	30.03	0.40	0	44.58
February	14.60	31.80	0.00	40.11	0	17.87	33.59	0.20	0	45.74
March	19.30	33.20	105.2	54.79	3	20.56	36.17	2.40	0	41.02
April	20.30	35.10	13.20	51.13	1	21.64	38.04	20.40	3	49.82

Media composition

Potato Dextrose Agar (PDA)

Peeled potato	200 g
Dextrose	20 g
Agar –agar	20 g
Distilled water	1000 ml

Two hundred grams of cleaned, washed and peeled potato tuber were chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose and agar-agar 20 g of each were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water.

2 per cent water agar

Agar	20 g
Water	1000 ml

Appendix II: Market prices of various components prevailing during 2015

Component	Price in rupees (□)
Carbendazim+ Mancozeb (Sprint)	□ 304 /250 g
Carboxin+Thiram (Vitavax power)	□ 176 /100 g
Trichoderma harzianum	□ 60 /500 g
Pseudomonas fluorescens	□ 60 /500 g
Jelly	□ 30 /10 g
Coco peat	□ 12 /500 g
Cost of cultivation	□ 18,300
Price of tomato fruit	□ 3000 /q

STUDIES ON SEED-BORNE FUNGAL DISEASES OF TOMATO AND THEIR MANAGEMENT

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ABSTRACT

The present investigation on seed-borne fungal diseases of tomato and their management was conducted during 2015-16, which included seed health testing of different tomato cultivars, evaluation of seed health testing methods, location of the pathogen in the seed, seed to plant transmission studies, integrated disease management through use of seed dressing fungicides, bioagents and biopriming agents.

Seed health testing of different tomato seed samples collected from different parts of northern Karnataka revealed the dominance of *Fusarium* spp., *Alternaria solani* and *Alternaria alternata.* In general, seed samples from Dharwad and Haveri districts exhibited maximum infection of *Fusarium* spp. and *A. solani,* whereas *A. alternata* infections were maximum at Belagavi and Haveri seed samples. Among the five different methods employed for the detection of seed-borne fungal pathogens of tomato, standard blotter method was found to be good for the detection of *A. solani*, *A. alternata* and *Fusarium* spp.

The pathogenic ability of seed borne fungi *viz*., *Alternaria solani, A. alternata and Fusarium* spp. was proved in seedling symptom test and transmission study. Component plating technique revealed that, *Alternaria alternata, Phoma* spp. and *Aspergillus* spp. were confined to pericarp only, where as *Fusarium* spp. and *Alternaria solani* were noticed on other seed parts also *viz*., endosperm, embryo and hilum. Seed dressing fungicide *viz*., carbendazim + mancozeb (0.3 %), bioagent *Pseudomonas fluorescens* (0.8 %) and biopriming with *Pseudomonas fluorescens* (0.8 %) + jelly were found most effective in eliminating seed-borne infections in tomato. Under field condition, seed treatment with carbendazim + mancozeb (0.3 %) along with foliar spray with hexaconazole (0.1 %) at 45 DAS exhibited higher benefit cost ratio (3.39).