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by Muhammad Noman

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Detection of different fungal pathogens in vegetables from market

Yasir Iftikhar[1], Muhammad Noman[1], Muhammad Ahmad Zeshan*[1], Ashara Sajid[1], Qaiser Shakeel[2]

1. Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha. Pakistan. 40100.
2. Department of Plant Pathology, Faculty of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Pakistan. 63100.

*Corresponding author's email: ahmd_1566@yahoo.com

092-3016804364

ABSTRACT

Different fungal species attack on vegetables and cause common post-harvest diseases like rotting and irregular black spots in vegetables. The objective of this research was to find out the cause of post-harvest diseases and their *in-vitro* management by using different fungicides at various concentrations. Surveys of different markets of Sargodha were conducted for the collection of infected samples of available summer vegetables (Potato, tomato, pumpkin, cucumber, eggplant). PDA media was used for the isolation of pathogen. The fungal culture was identified on the basis of macroscopic characteristics like colony color, morphology, shape and appearances as well as microscopic characteristics of vegetative and reproductive structure by using microscope. Food poisoning technique was used for the evaluation of five fungicides against *Aspergillus* and *Fusarium* spp. For each fungicide three different concentrations 100, 200 and 300 ppm were used. Data showed that all five fungicides significantly inhibited mycelial growth of *Aspergillus* and *Fusarium* spp. Highest percent inhibition on *Aspergillus* sp. was given bytubocanazole at all concentrations 100-300 ppm while, least percent inhibition was given by metalaxyal+mancozeb at all concentrations. The same trend was found in mycelial growth of *Fusarium*sp. that was significantly inhibited by all five fungicides. It could be concluded that Bytubocanazole is the most effective fungicide against post harvest fungi at 300 ppm concentration.

Keywords: Postharvest, Fungi, In-vitro, Management

Introduction

Vegetables and fruits deliver diverse benefits and in human nourishment they play an important role, particularly as a source of vitamin, mineral, and dietetic content (Wargovich, 2000). Vegetables and fruits are accompanying strongly in diet of daily and they reduce threat of cancer, disease of heart, heat stroke, and other prolonged diseases (Wargovich, 2000; Tomas-Barberan). Vegetables and fruits have some constituents that are strongly

antioxidants and they activate metabolic and vary the poisons detoxification, and processes of stimulus which change the path of cells which cause tumor (Wargovich, 2000).Mostly, variation of traditional agricultural merchandises into high value horticultural production and exports has been specified as a sector that can provide real prospects for improving export performance (Keno, 2011). However, high worth horticulture is challenging and the practical risks involved at every stage are so high that the chances of essentially getting such achievements are relatively low, and extremely dependent on management performance (Joosten et al., 2011)

Fungus are generally present in almost all type of environment and as a decomposer they are the most important component of an ecosystem. Fruits and vegetables plays an important role in human nourishment and in human daily diet they provide essential growth factors like vitamins and minerals, these factor helps to keep a normal and good health. In nature fruits and vegetables are widely distributed, pathogens attacks on fruits and vegetables and they reduce the shelf life, this is one of the most limiting factors which affect the fruits and vegetables commercial worth. During the handling of harvested fruits and vegetables, it is estimated that 20–25% fruits and vegetables may decay by the attack of pathogens and this loss even occur in developing countries (Droby et al., 2006).

Due to post-harvest handling, storage and marketing the quality of fruits and vegetables greatly affected. This can result in rotting and development of microorganisms. As fruits and vegetables change their physiological states, due to this microorganisms activate. (Wilson et al., 1991). From one lac species of fungus less than 10% are plant pathogenic and more than 100 species of fungus are accountable for post-harvest diseases. World food resources are monitor by international agencies and they acknowledge that to meet the future need of food is to reduce the post-harvest losses (Kelman, 1984). Vegetables are at risk to damage from mechanical damage because of their structure and quite soft surface related with their high moisture content. In postharvest handling injury can occur at any point. Losses from diseases and disorders also caused high cash loss for vegetable crops. Damage by insect relatively minor importance in comparison to decay caused by micro organism. One of the cause for losses of Royal project of postharvest losses of vegetables was estimated to be about 20-30% (The Royal Project, 1989).

Losses due to decay and rotting are difficult to determine, these losses are differs broadly with goods, area of production and season, these losses and damages are known to be important (Pathak, 1997). The losses in under developing and tropical countries on perishable commodities are 50% or more than 50% (Coursey and Booth, 1972).Vegetables also plays an

important role in urban and rural surroundings of Pakistan in food security of underprivileged (Burney and Akmal, 1991). These vegetables are the source of primary and secondary foods and other traditional vegetables used to prepared dishes. In diet vegetables are the valued mean of energy and micronutrients (Belitz andGrosch, 1999).

Pakistan is lacking in using of common and traditional vegetables. Vegetables are very important to prevent the diseases and they also maintain the health (Belitz and Grosch, 1999). These vegetables are esteemed for their high vitamin and mineral contents. They might be edible stems, roots, fruits and seeds. Each group of vegetableplay important role to the human diet. To control postharvest diseases the primary mean is the use of fungicides. European and Asian plant protection markets use 26% fungicides and United States market used 6% fungicides (Jutsum, 1988). Annual application of fungicides on fruits and vegetables is 23 million kilograms, and this use is greatly accepted because their use is important to provide perishable products to markets and consumers (Ragsdaleand Sisler, 1994).

To reduce postharvest diseases the vegetables and fruits are treated with fungicides, human are exposed to fungicides due to their direct use. As chemicals have high residual toxicity, they pollute the environment and also affect food the use of chemicals to manage postharvest losses is limited (Unnikrishnan and Nath, 2002).Due to insufficient storage and transportation problems postharvest losses in developing countries are more severe. Postharvest diseases of fruits and vegetables are controlled by fungicides as a primarily control (Zhu, 2006). To identify the fungal species different techniques are used for purification and identification and to confirm the fungal culture. But single spore isolation technique is best method to obtain pure culture, for pure identification and for the subculture of fungal isolates. (Noman, 2016).

Fungal spp. causes most serious and economically important diseases of vegetables. Crop production losses occur from nursery to fruit production. Disease caused by fungal spp. are grey mold, fusarium rot, alternaria rot, charcoal rot, cottony leak, rhizopus rot, dry rot, black scurf. Most important species involve in these diseases are *Aspergillus* spp, *Pythium* spp, *Rhizopus* spp, *Sclerotium* spp, *Sclerotinia* spp, *Fusarium* spp, *Botrytis* spp. Management of these diseases is necessary to reduce yield losses as well as to maintain fruit quality. Keeping in view the importance current study was carried out with following objectives. (1) Isolation and identification of different fungal spp. related with post-harvest diseases of vegetables. (2) Evaluation of different fungicides against different fungal spp. related with post-harvest diseases of vegetables.

Material and Methods

Present research was carried out under laboratory conditions, Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, during 2018-2020.

Collection of diseased samples

From different markets of Sargodha diseased samples of five vegetables were collected. For this purpose, a survey was conducted in vegetable markets of district Sargodha during 2018-2019. Samples were collected on the basis of characteristic symptoms of disease were observed specifically on five vegetables including cucumber, pumpkin, eggplant, potato and tomato in the vegetable market. Infected vegetables were collected in polythene bags and label them with necessary information as area, name of vegetable, name of disease, market name and date. Samples brought into laboratory in an ice box to maintain the temperature for pathogen. Stored these samples in refrigerator at 4°C until use.

Incidence of disease

Five different markets of Sargodha were visited (Sabz mandi, Choti mandi, Qenchi mor, 49 tail and Gol chowk) to measure the disease incidence on five vegetables. In each survey of markets whole markets were visited to calculate the disease incidence. The incidence of disease was calculated as follows:

$$\text{Disease incidence} = \frac{\text{Number of infected samples}}{\text{Total number of samples}} \times 100$$

On the basis of symptoms of rotting to record the data for disease incidence the samples were collected in polythene bag. Data was recorded by visiting the different stalls in the markets. Data was recorded by visual examination of symptoms on different vegetables.

Isolation of fungal pathogens

Culture medium (PDA)

Different fungal pathogenswere isolated and multiplied on potato dextrose agar (Appendix I) evaluated against fungicides. Different fungal species were isolated by using tissue planting isolation technique.

Tissue planting method

Infected tissues were used for the isolation of pathogens in tissue planting method. The diseased samples cut into small pieces of 3 mm length with prominent diseased symptoms. These samples were washed under tap water followed by washing with 70% ethanol and again washed away under tap water. Samples were placed on tissue paper for drying. Potato dextrose agar (PDA) was suspended in 1000 ml media bottle and autoclaved at 121°C for 20 minutes at 15 Pascal pressure with other materials like petri plates, inoculating needle etc. After autoclaving, the PDA was brought to laminar flow chamber and was allowed to cool

and 50μL/ml amount of streptomycin was added to PDA to inhibit the growth of bacteria. Then onto each petri plate, 15 ml PDA was poured and allowed to solidify. Small pieces of diseased samples were placed onto each petri plate, wrapped with Para film tape and incubated at 25±2°C temperature. The colony was observed between 3 to 7 days. (Ghosh & Shamsi, 2014).

Sub culturing

When growth of fungi initiated on PDA media a bit of mycelia was transferred to new petri plates having PDA. Fungi, that colonized was observed under microscope and relevant fungus was transferred to new petri plates having PDA media for purification and multiplication. Plates were incubated at 25±2°C and growth will be observing after every 24 hours.

Identification

Identification was done on the bases of macroscopic and microscopic observations. Macroscopic like colony pattern and color were observed visually. Glass slides of isolates were prepared and observed under compound microscope at 10x, 40x and 100x. A drop of water was placed on the slide and then single spore was placed on the drop from isolates. Cover slip was placed on the drop gently to avoid the bubbles and slide was placed under microscope to observe the spores.

Pathogenicity test

Pathogenicity test was conducted to prove Koch postulate. The surface of healthy vegetables was sterilized in 10% sodium hypochlorite solution. Then these samples were washed 3 times under running tap water and allowed to dry. 2mm diameter circle was made on each sample with the help of ruler and fungal streak was inoculated on the marked portions with the help of sterilized needle. The samples that were placed as a control were injected with distilled water. Samples were placed in boxes and these boxes placed on the laboratory bench at room temperature. On the 4th day the diseased portions were removed with the help of Sterilized forceps and placed on freshly prepared PDA plates. These plates were incubated at 25 ±2°C for 3 days. After 3rd day the fungal growth was observed.

Evaluation of fungicides

Preparation of stock solution

For each fungicide stock solution was separately prepared by dissolving 1g of fungicide in 999ml of distilled water. This stock solution was use for preparation of different concentration.

Preparation of fungicidal concentration

Three different concentrations were prepared for each fungicide by using stocksolution. 100ppm concentration was prepared by adding 100ml of stock solution in 900ml distilled water. For 200ppm concentration 200ml of stock solution will be taken and dissolved in 800ml of distilled water. 300ml of stock solution will be dissolved into 700ml of distilled water for preparation of 300ml concentration. Fungicides that were used are given in Appendix II.

Food poisoning technique

Food poisoning technique was used to check the efficacy of five different fungicides (Fitsum et al., 2014). In vitro experiment was conducted by preparing three concentrations 100 ppm, 200 ppm and 300 ppm of each fungicide was used. For each treatment three replicate were used. In each plate 10 ml media amended with 5 ml fungicide was poured. 5 mm mycelia plug was placed in the center of each petri plate after solidification. (Gautam et al., 2017). After wrapping petri plates were incubated at 25±2 ºC and data was taken after 3 days, 5 days and 7 days. Percentage inhibition was measured by using the formula:

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

C= Colony diameter in control

T= Colony diameter in treatment

Results

Disease incidence in different markets of Sargodha

Different markets were visited to calculate the disease incidence of different vegetables. In Sabz mandi the disease incidence was 05% on potato. The disease incidence was 20% on tomato while, disease incidence was 05% on cucumber. The disease incidence was 6.25% on brinjal while, the disease incidence was 05% on pumpkin in Sabz mandi Sargodha (Table).In Choti mandi disease incidence was 05% on potato. The disease incidence was 18.33% on tomato while, disease incidence was 05% on cucumber. The disease incidence was 5.6% on brinjal while, the disease incidence was 4.4% on pumpkin in Choti mandi Sargodha (Table).

In Qenchi morthe disease incidence was 4.16% on potato. The disease incidence was 16.67% on tomato while, the disease incidence was 05% on cucumber.The disease incidence was 05% on brinjalwhile, the disease incidence was 4.4% on pumpkin in Qenchi mor Sargodha (Table).

In Gol chowk thedisease incidence was 4.16% on potato. The disease incidence was 20% on tomato while, the disease incidence was 2.5% on cucumber. The disease incidence was

6.25% on brinjal while, the disease incidence was 05% on pumpkin in Gol chowk Sargodha (Table).

In 49 tail the disease incidence was 4.58% on potato.The disease incidence was 20% on tomato while, the disease incidence was 2.5% on cucumber.The disease incidence was 6.25% on brinjal while the disease incidence was 05% on pumpkin in 49 tail Sargodha (Table).

Infection percent of *Aspergillus* and *Fusarium* sp. in Sabz mandi

From Sabz mandi 15 diseased samples of potato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on potato was 60%, while the attack of *Fusarium* sp. was 40% on potato. While 15 diseased samples of tomato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on tomato was 53.33%, while the attack of *Fusarium* sp. was 46.67% on tomato. Whil 15 diseased samples of cucumber were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on cucumber was 73.33%, while the attack of *Fusarium* sp. was 26.67% on cucumber. While 15 diseased samples of brinjal were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on brinjal was 60%, while the attack of *Fusarium* sp. was 40% on brinjal. While 15 diseased samples of pumpkin were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on pumpkin was 66.76%, while the attack of *Fusarium* sp. was 33.33% on pumpkin (Table).

Interaction percent of *Aspergillus* and *Fusarium* sp. in Choti mandi

From Choti mandi 12 diseased samples of potato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on potato was 58.33%, while the attack of *Fusarium* sp. was 41.37% on potato. While 12 diseased samples of tomato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on tomato was 50%, while the attack of *Fusarium* sp. was 50% on tomato. While 12 diseased samples of cucumber were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on cucumber was 75%, while the attack of *Fusarium* sp. was 25% on cucumber. While 12 diseased samples of brinjal were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on brinjal was 58.33%, while the attack of *Fusarium* sp. was 41.67% on brinjal. While 12 diseased samples of pumpkin were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on pumpkin was 66.67%, while the attack of *Fusarium* sp. was 33.33% on pumpkin (Table).

Infection percent of *Aspergillus* and *Fusarium* sp. in Qenchi mor

From Qenchi mor 10 diseased samples of potato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on potato was 70%, while the

attack of *Fusarium* sp. was 30% on potato. While 10 diseased samples of tomato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on tomato was 50%, while the attack of *Fusarium* sp. was 50% on tomato. While 10 diseased samples of cucumber were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on cucumber was 80%, while the attack of *Fusarium* sp. was 20% on cucumber. While 10 diseased samples of brinjal were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on brinjal was 50%, while the attack of *Fusarium* sp. was 50% on brinjal. While 10 diseased samples of pumpkin were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on pumpkin was 70%, while the attack of *Fusarium* sp. was 30% on pumpkin (Table).

Infection percent of *Aspergillus* and *Fusarium* sp. in Gol chowk

From Gol chowk 10 diseased samples of potato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on potato was 60%, while the attack of *Fusarium* sp. was 40% on potato. While 10 diseased samples of tomato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on tomato was 50%, while the attack of *Fusarium* sp. was 50% on tomato. While 10 diseased samples of cucumber were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on cucumber was 80%, while the attack of *Fusarium* sp. was 20% on cucumber. While 10 diseased samples of brinjal were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on brinjal was 60%, while the attack of *Fusarium* sp. was 40% on brinjal. While 10 diseased samples of pumpkin were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on pumpkin was 70%, while the attack of *Fusarium* sp. was 30% on pumpkin (Table 4.5).

Infection percent of *Aspergillus* and *Fusarium* sp. in 49 tail

From 49 tail 12 diseased samples of potato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on potato was 66.67%, while the attack of *Fusarium* sp. was 33.33% on potato. While 12 diseased samples of tomato were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on tomato was 58.33%, while the attack of *Fusarium* sp. was 41.37% on tomato. While 12 diseased samples of cucumber were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on cucumber was 83.33%, while the attack of *Fusarium* sp. was 16.67% on cucumber. While 12 diseased samples of brinjal were collected for the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on brinjal was 50%, while the attack of *Fusarium* sp. was 50% on brinjal. While 12 diseased samples of pumpkin were collected for

the isolation of pathogen. It was reported that the attack of *Aspergillus* sp. on pumpkin was 66.67%, while the attack of *Fusarium* sp. was 33.33% on pumpkin (Table 4.6).

Pathogenicity test results

Pathogenicity test showed different symptoms on different vegetables after 3rd day of inoculation. These symptoms were observed to 7th day and following symptoms were observed (Table 4.7).

Evaluation of fungicides against *Aspergillus*and *Fusarium* sp.

All the fungicides significantly inhibited fungal growth. Among tested fungicides tubocanazole was most effective followed by chlorothalonil+metalaxyl, thiophanate methyl, sulphur and metalaxyl+mancozeb. These fungicides gave significant results at 3rd, 5th and 7th day against mycelial growth of *Aspergillus* and *Fusarium* sp.the casual organism of different fungal diseases in vegetables.

Percent inhibition of day 3rd at 100ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 100ppm, tubocanazole was highest at 3rd day with 96%, chlorothalonil+metalaxyl gave 82.22% inhibition rate, thiophanate methyl gave 75.55% inhibition, and sulphur gave 28.88 inhibition rate while metalaxyl+mancozeb gave 13.11% inhibition rate at 3rd day. While percentage inhibition of mycelium of *Fusarium* sp. at 100ppm, tubocanazole was highest at 3rd day with 97.27%, chlorothalonil+metalaxyl gave 87.87% inhibition rate, thiophanate methyl gave 83.33% inhibition, and sulphur gave 51.51 inhibition rate while metalaxyl+mancozeb gave 40.75% inhibition rate at 3rd day (Table 4.8).

Percent inhibition of day 3rd at 200 ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 200 ppm, tubocanazole was highest at 3rd day with 97.55%, chlorothalonil+metalaxyl gave 85.55% inhibition rate, thiophanate methyl gave 82.22% inhibition, and sulphur gave 62.22% inhibition rate while metalaxyl+mancozeb gave 19.77% inhibition rate at 3rd day. While percentage inhibition of mycelium of *Fusarium* sp. at 200 ppm, tubocanazole was highest at 3rd day with 97.27%, chlorothalonil+metalaxyl gave 89.39% inhibition rate, thiophanate methyl gave 84.84% inhibition, and sulphur gave 56.06% inhibition rate while metalaxyl+mancozeb gave 42.42% inhibition rate at 3rd day (Table 4.9).

Percent inhibition of day 3rd at 300ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 300ppm, tubocanazole was highest at 3rd day with 98.88%, chlorothalonil+metalaxyl gave 92.22% inhibition rate, thiophanate methyl gave 91.11% inhibition, and sulphur gave 88.88% inhibition rate while

metalaxyl+mancozeb gave 26.44% inhibition rate at 3rd day. While percentage inhibition of mycelium of *Fusarium* sp. at 300ppm, tubocanazole was highest at 3rd day with 97.87%, chlorothalonil+metalaxyl gave 89.39% inhibition rate, thiophanate methyl gave 86.21% inhibition, and sulphur gave 54.54% inhibition rate while metalaxyl+mancozeb gave 43.78% inhibition rate at 3rd day (Table 4.10).

Percent inhibition of day 5th at 100 ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 100 ppm, tubocanazole was highest at 5th day with 97.27%, chlorothalonil+metalaxyl gave 81.81% inhibition rate, thiophanate methyl gave 80.30% inhibition, and sulphur gave 40.60% inhibition rate while metalaxyl+mancozeb gave 15.15% inhibition rate at 5th day. While percentage inhibition of mycelium of *Fusarium* sp. at 100 ppm, tubocanazole was highest at 5th day with 97.85%, chlorothalonil+metalaxyl gave 85.71% inhibition rate, thiophanate methyl gave 84.52% inhibition, and sulphur gave 53.33% inhibition rate while metalaxyl+mancozeb gave 33.33% inhibition rate at 5th day (Table 4.11).

Percent inhibition of day 5th at 200ppm

Percentage inhibitionof mycelium of *Aspergillus* sp. at 200ppm, tubocanazole was highest at 5th day with 98.33%, chlorothalonil+metalaxyl gave 86.51% inhibition rate, thiophanate methyl gave 84.84% inhibition, and sulphur gave 65.15% inhibition rate while metalaxyl+mancozeb gave 24.24% inhibition rate at 5th day. While percentage inhibition of mycelium of *Fusarium* sp. at 200ppm, tubocanazole was highest at 5th day with 98.09%, chlorothalonil+metalaxyl gave 85.83% inhibition rate, thiophanate methyl gave 88.09% inhibition, and sulphur gave 54.76% inhibition rate while metalaxyl+mancozeb gave 34.52% inhibition rate at 5th day (Table 4.12).

Percent inhibition of day 5th at 300 ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 300 ppm, tubocanazole was highest at 5th day with 99.24%, chlorothalonil+metalaxyl gave 92.72% inhibition rate, thiophanate methyl gave 93.93% inhibition, and sulphur gave 74.24% inhibition rate while metalaxyl+mancozeb gave 37.87% inhibition rate at 5th day. While percentage inhibition of mycelium of *Fusarium* at 300 ppm, tubocanazole was highest at 5th day with 97.61%, chlorothalonil+metalaxyl gave 86.66% inhibition rate, thiophanate methyl gave 85.35% inhibition, and sulphur gave 55.95% inhibition rate while metalaxyl+mancozeb gave 35.59% inhibition rate at 5th day (Table 4.13).

Percent inhibition of day 7that 100ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 100ppm, tubocanazole was highest at 7th day with 98%, chlorothalonil+metalaxyl gave 56.55% inhibition rate, thiophanate methyl gave 81.11% inhibition, and sulphur gave 27.77% inhibition rate while metalaxyl+mancozeb gave 17.77% inhibition rate at 7th day. While percentage inhibition of mycelium of *Fusarium* sp. at 100ppm, tubocanazole was highest at 7th day with 98.28%, chlorothalonil+metalaxyl gave 62.76% inhibition rate, thiophanate methyl gave 83.80% inhibition, and sulphur gave 38.09% inhibition rate while metalaxyl+mancozeb gave 29.52% inhibition rate at 7th day (Table 4.14).

Percent inhibition of day 7th at 200ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 200 ppm, tubocanazole was highest at 7th day with 98.77%, chlorothalonil+metalaxyl gave 59.66% inhibition rate, thiophanate methyl gave 85.55% inhibition, and sulphur gave 32.22% inhibition rate while metalaxyl+mancozeb gave 27.77% inhibition rate at 7th day. While percentage of mycelium of *Fusarium* sp. inhibition at 200 ppm, tubocanazole was highest at 7th day with 98.47%, chlorothalonil+metalaxyl gave 63.80% inhibition rate, thiophanate methyl gave 84.76% inhibition, and sulphur gave 41.90% inhibition rate while metalaxyl+mancozeb gave 30.47% inhibition rate at 7th day (Table 4.15).

Percent inhibition of day 7th at 300ppm

Percentage inhibition of mycelium of *Aspergillus* sp. at 300ppm, tubocanazole was highest at 7th day with 99.44%, chlorothalonil+metalaxyl gave 73.55% inhibition rate, thiophanate methyl gave 92.22% inhibition, and sulphur gave 37.77% inhibition rate while metalaxyl+mancozeb gave 41.11% inhibition rate at 7th day. While percentage inhibition of mycelium of *Fusarium* sp. at 300ppm, tubocanazole was highest at 7th day with 98%, chlorothalonil+metalaxyl gave 64.76% inhibition rate, thiophanate methyl gave 84.76% inhibition, and sulphur gave 41.90% inhibition rate while metalaxyl+mancozeb gave 31.04% inhibition rate at 7th day (Table 4.16).

Growth inhibition (mean±SE) of mycelial of *Aspergillus* sp. at 100 ppm at 3rd, 5th and 7th day after treatment. Mean sharing letter are not significantly different at P<0.05 (Table 4.17).

Growth inhibition (mean±SE) of mycelial of *Aspergillus* sp. at 200 ppm at 3rd, 5th and 7th day after treatment. Mean sharing letter are not significantly different at P<0.05 (Table 4.18).

Discussions

Post-harvest diseases are severe threat to vegetables. *Aspergillus* and *Fusarium* spp.are two most important pathogens of vegetable diseases. Irregular brown spots, chilling injury symptoms, softening of fruits blackish watery decaying spots, small whitish rings, light

brown watery lesions, broken skin are the symptoms that caused by these pathogens. These diseases present in almost all vegetables producing areas of the world and produce losses to 20-30% every year (The Royal Project, 1989). During the handling of harvested fruits and vegetables, it is estimated that 20–25% fruits and vegetables may decay by the attack of pathogens and this loss even occur in developing countries (Droby et al., 2006). These numbers shows the diseases caused by these pathogens are really serious. There are many characters of these spp. which makes them worst vegetable enemy. These characters include the ability of pathogens to spread through water and air quick production of inoculum and production of chlamydospores and oospores for their survival outside plant tissues. Without control of these pathogens it is difficult to serve maximum healthy vegetables to the consumers.

Chemical control is not eco-friendly but still there is no other way to replace chemical controlof plant diseases with superior and effective option. So, we have to depend on chemical use due to unavailability of eco-friendly products. However, if we successfully find an appropriate fungicide that inhibit our target pathogen with minimum dose then it would be a great success of our research. This may also help to reduce excess use of fungicides on farmer's field which may leads to environmental pollution and also minimize the chances of resistance. In this study, we evaluated different fungicide products against different isolates.

For in-vitro evaluation of five fungicides at three different concentrations food poisoning technique was used in this research. This technique is very simple and easy for in vitro evaluation (Sultana & Ghaffar., 2013). By using this technique under limited resources with short time best fungicide with its best concentration is tested against fungal pathogens (Hassan et al., 2013). Efficacy of any fungicides is tested in lab before field use. The use of food poisoning technique helps us to know about best fungicide at its best concentration against any fungal pathogen; in this technique we can save our time and resources. This technique was used by many by many researchers in recent years to check efficacy of different fungicides at different concentrations against fungal pathogens (Khanzada & Shah, 2012; Sahi et al., 2012; Rather et al., 2012; Dar et al., 2013; Elshahawy et al., 2016).

Present research was planned to evaluate different fungicides against *Aspergillus* and *Fusarium* spp. at different concentrations at different time intervals. In current research, efficacy of different fungicides was different at different concentrations and time intervals. Among these tested fungicides, tubocanazole gave higher percent inhibition against both species. Tubocanazole is broad spectrum systemic fungicide and has been found very effective against different fungi in previous studies (Sahi et al., 2012; Hassan et al., 2013).

Rather et al. (2012) evaluated ten different fungicides including and reported that tubocanazole gave significant inhibition by using food poisoning technique against different fungal pathogens. It was also noted that the tubocanazole efficacy increased by increasing concentration. Sahi et al. (2012) used food poisoning technique to check the efficacy of different fungicides at different concentrations at different day of intervals against fungal pathogens that cause diseases in fruits and vegetables. It was reported that among the tested fungicides, tubocanazole and thiophanate methyl was the most effective fungicide at different concentrations. They also reported that tubocanazole and thiophanate methyl efficacy was increased gradually by increasing concentration and efficacy was also increased by time. Sultana & Ghaffar (2013) conducted in vitro experiments to evaluate the efficacy of fungicides at different concentrations and at different day intervals, against *Fusarium oxysporum,* the causal organism of root and seed rot of cucumber and bottle gourd. It was reported that at 1000 ppm concentration of tubocanazole and thiophanate methyl completely inhibited the mycelial growth of fungus. Hassan et al. (2013) used food poisoning technique to check the efficacy of different fungicides against *Ilyonectria radicicola,* a soil borne fugnus. They reported that tubocanazole affected the mycelial growth of fungus and its efficacy was increased with increasing concentration at different day intervals.

In present study, metalaxyl+mancozeb was fifth best fungicide against *Aspergillus* and *Fusarium* spp. This fungicide protective and systemic and give significant control against these two spp.This fungicide significantly control the mycelial growth of both species at different concentrations and at different days interval. These results are similar to the studies conducted previously (Dar et al., 2013; Elshahawy et al., 2016; Turkolmez & Dervis, 2017). metalaxyl+mancozeb had been found effective against wilt disease of pepper (Rather et al., 2012). They concluded that metalaxyl+mancozeb significantly control the mycelial growth of different fungal spp. It had also been concluded that the efficacy was increased by increasing concentrations against different fungal pathogens. Dar et al. (2013) used food poisoning technique to check the efficacy of nine different fungicides and bio-control agents against root rot of fir. Results of metalaxyl+mancozeb showed the significantly inhibition of mycelial growth of different fungal pathogens at different concentrations at different day intervals. Elshahawy et al. (2016) conducted in vitro experiments by using food poisoning technique to control the fungal pathogens and to check the compatibility of metalaxyl+mancozeb and six other fungicides with *Trichoderma* spp. It was reported that metalaxyl+mancozeb was compatible with *Trichoderma* spp. and significantly inhibit the fungal pathogens at different concentrations. Turkolmez & Dervis (2017) noticed the activity of metalaxyl+mancozeb and

six other fungicides against *Phytophthora palmivora* that are responsible for root rot in cherry and apricot. It was concluded that this fungicide significantly inhibited *P. palmivora* and its effectiveness was more higher at higher concentrations.

Results of present research also indicated that chlorothalonil+metalaxylconsiderably inhibited the growth of *Aspergillus* and *Fusarium* spp. This fungicide gives two way systemic protection against fungal pathogen and it has multiple ways of action as it act to upward and downward in plant system. This fungicide control the spore formation as well as it stimulates the plant defence system. Data of this research is similar to the study conducted by (Shashidhara et al., 2008; Khanzada & Shah, 2012; Moradi et al., 2017). Shashidhara et al. (2008) conducted experiments to control the different fungal pathogens by using different fungicides at different concentrations. It was concluded that chlorothalonil+metalaxyl fungicide significantly suppress the growth of different fungus. (Khanzada & Shah, 2012) evaluated different fungicide against causal organism of rice blast disease. Results of their study showed that chlorothalonil+metalaxyl has the ability to control the mycelial growth of rice blast fungus. Moradi et al. (2017) conducted experiment to check the effectiveness of chlorothalonil+metalaxyl against causal organisms that cause rotting and gummosis. It was reported that the pathogens were significantly inhibited by this fungicide.

Conclusion

Results of present study showed that evaluated fungicides are effective against *Aspergillus* and *Fusarium* spp. at concentrations of 100, 200 and 300 ppm; however, their efficacy varies with days interval. Fungicide tubocanazole found most effective against both species while metalaxyl+mancozeb found least effective against both species.

Table: Disease incidence percent of different pathogens on host at different places

	Interaction percent of different pathogen on host (%)				
Area	Potato	Tomato	Cucumber	Brinjal	Pumpkin
Sabzmandi	5	20	5	6.25	5
Chotimandi	5	18.33	5	5.6	4.4
Qenchimor	4.16	16.67	5	5	4.4
Gol chowk	4.16	20	2.5	6.25	4.4
49 tail	4.58	20	2.5	6.25	5

Host	**Table:Infection percent of *Aspergillus* and *Fusarium* sp. in Sabzmandi**	
	Sabzmandi	
	Aspergillus sp. (%)	*Fusarium* sp. (%)
Potato	60	40
Tomato	53.33	46.67
Cucumber	73.33	26.67
Brinjal	60	40
Pumpkin	66.67	33.33

Host	**Table: Infection percent of *Aspergillus* and *Fusarium* sp. in Chotimandi**	
	Chotimandi	
	Aspergillus sp. (%)	*Fusarium* sp. (%)
Potato	58.33	41.37
Tomato	50	50
Cucumber	75	25
Brinjal	58.33	41.67
Pumpkin	66.67	33.33

Host	**Table: Infection percent of *Aspergillus* and *Fusarium* sp. in Qenchimor**	
	Qenchimor	
	Aspergillus sp. (%)	*Fusarium* sp. (%)
Potato	70	30
Tomato	50	50
Cucumber	80	20
Brinjal	50	50
Pumpkin	70	30

Host	**Table:Infection percent of *Aspergillus* and *Fusarium* sp. in Gol chowk**	
	Gol chowk	
	Aspergillus sp. (%)	*Fusarium* sp. (%)
Potato	60	40
Tomato	50	50
Cucumber	80	20
Brinjal	60	40
Pumpkin	70	30

Host	**Table:Infection percent of *Aspergillus* and *Fusarium* sp. in 49 tail**	
	49 tail	
	Aspergillus sp. (%)	*Fusarium* sp. (%)
Potato	66.67	33.33
Tomato	58.33	41.37
Cucumber	83.33	16.67
Brinjal	50	50
Pumpkin	66.67	33.33

Table: Symptoms observed

Host	Symptoms observed	
	By *Aspergillus*	By *Fusarium*
Potato	Soft rotted tissues	Watery soaked grey lesions
Tomato	Broken skin	Soft watery masses
Cucumber	Irregular in shape	Brown lesions
Brinjal	Irregular brown spots	Soft watery spots
Pumpkin	Blackish watery decaying symptoms	Soft rotted tissues

Fugicides	**Table: Mean percent inhibition of day 3rd at 100ppm**	
	Mean percent inhibition of day 3rd at 100ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	96%	97.27%
Chlorothalonil+Metalaxyl	82.22%	87.87%
Thiophanate methyl	75.55%	83.33%
Sulphur	26.88%	51.51%
Metalaxyl+Mancozeb	13.11%	40.75%

Fugicides	**Table: Mean percent inhibition of day 3rd at 200ppm**	
	Mean percent inhibition of day 3rd at 200ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	97.55%	97.27%
Chlorothalonil+Metalaxyl	85.55%	89.39%
Thiophanate methyl	82.22%	84.84%
Sulphur	62.22%	56.06%
Metalaxyl+Mancozeb	19.77%	42.42%

Fugicides	**Table: Mean percent inhibition of day 3rd at 200ppm**	
	Mean percent inhibition of day 3rd at 200ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	97.55%	97.27%
Chlorothalonil+Metalaxyl	85.55%	89.39%
Thiophanate methyl	82.22%	84.84%
Sulphur	62.22%	56.06%
Metalaxyl+Mancozeb	19.77%	42.42%

Fugicides	**Table: Mean percent inhibition of day 3rd at 300ppm**	
	Mean percent inhibition of day 3rd at 300ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	98.88%	97.87%
Chlorothalonil+Metalaxyl	92.22%	89.39%
Thiophanate methyl	91.11%	86.21%
Sulphur	88.88%	54.54%
Metalaxyl+Mancozeb	26.44%	43.78%

Fugicides	**Table: Mean percent inhibition of day 5th at 100ppm**	
	Mean percent inhibition of day 5th at 100ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	97.27%	97.85%
Chlorothalonil+Metalaxyl	81.81%	85.71%
Thiophanate methyl	80.30%	84.52%
Sulphur	40.60%	53.33%
Metalaxyl+Mancozeb	15.15%	33.33%

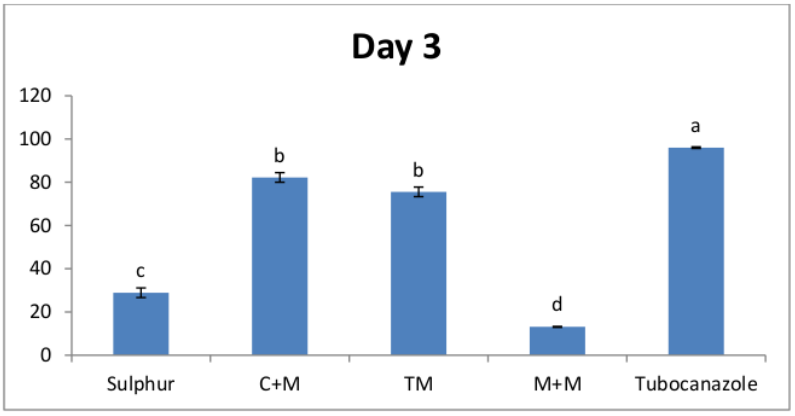
Fugicides	**Table: Mean percent inhibition of day 5th at 200ppm**	
	Mean percent inhibition of day 5th at 200ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	98.33%	98.09%
Chlorothalonil+Metalaxyl	86.51%	85.83%
Thiophanate methyl	84.84%	88.09%
Sulphur	65.15%	54.76%
Metalaxyl+Mancozeb	24.24%	34.52%

Fugicides	**Table: Mean percent inhibition of day 5th at 300ppm**	
	Mean percent inhibition of day 5th at 300ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	99.24%	97.61%
Chlorothalonil+Metalaxyl	92.72%	86.66
Thiophanate methyl	93.93%	85.35%
Sulphur	74.25%	55.95%
Metalaxyl+Mancozeb	37.87%	35.59%

Fugicides	**Table: Mean percent inhibition of day 7th at 100ppm**	
	Mean percent inhibition of day 7th at 100ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	98%	98.28%
Chlorothalonil+Metalaxyl	56.55%	62.76%
Thiophanate methyl	81.11%	83.80%
Sulphur	27.77%	38.09%
Metalaxyl+Mancozeb	17.11%	29.52%

Fugicides	**Table: Mean percent inhibition of day 7th at 200ppm**	
	Mean percent inhibition of day 7th at 200ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	98.77%	98.47%
Chlorothalonil+Metalaxyl	59.66%	63.80%
Thiophanate methyl	85.55%	84.76%
Sulphur	32.22%	41.90%
Metalaxyl+Mancozeb	27.77%	30.47%

Fugicides	**Table: Mean percent inhibition of day 7th at 300ppm**	
	Mean percent inhibition of day 7th at 300ppm	
	Aspergillus sp.	*Fusarium* sp.
Tubocanazole	99.44%	98%
Chlorothalonil+Metalaxyl	73.55%	64.76%
Thiophanate methyl	92.22%	84.76%
Sulphur	37.77%	41.90%
Metalaxyl+Mancozeb	41.11%	31.04%


Day 3
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Sulphur
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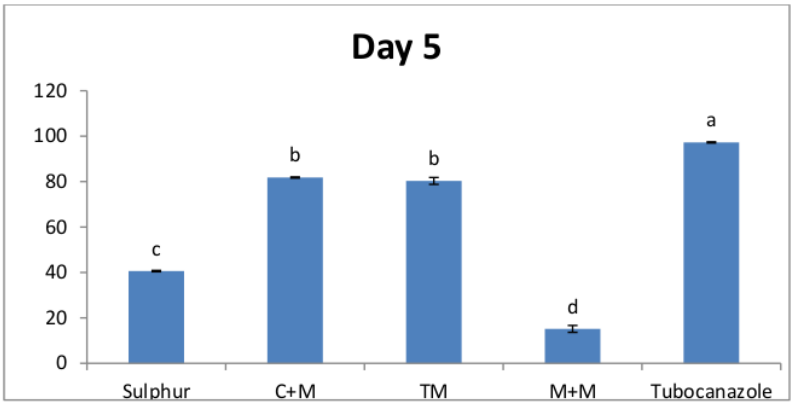
Tubocanazole


Day 5
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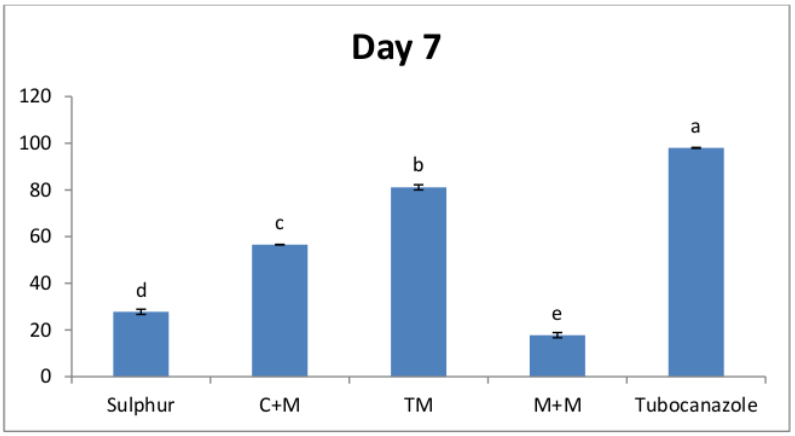
Tubocanazole


Day 7
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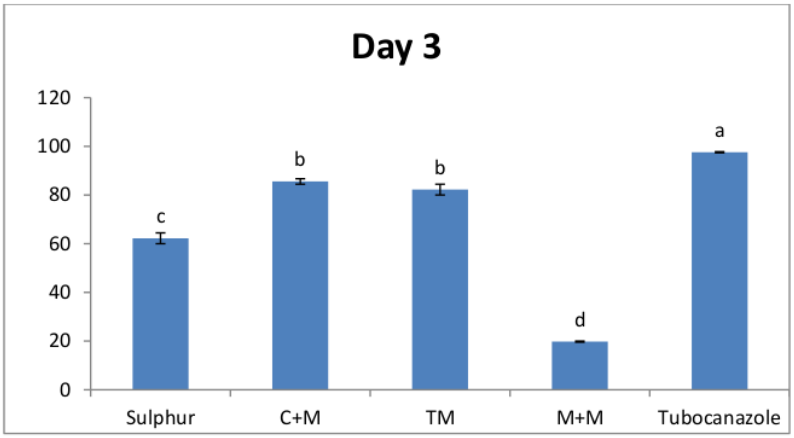
Sulphur
C+M
TM
M+M
Tubocanazole


Day 3
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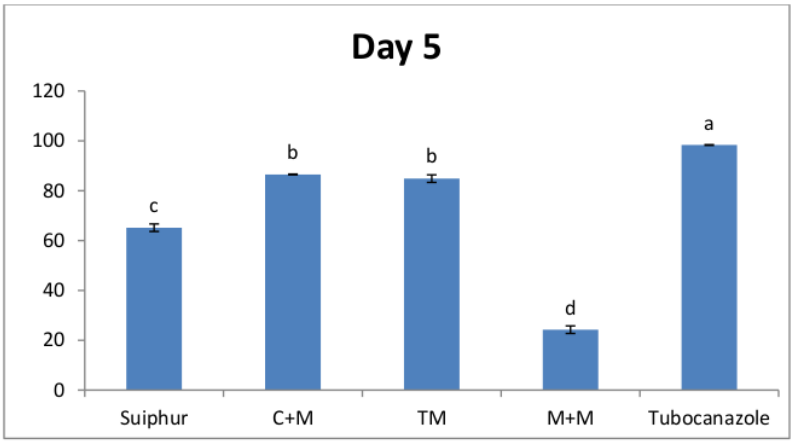
Tubocanazole


Day 5
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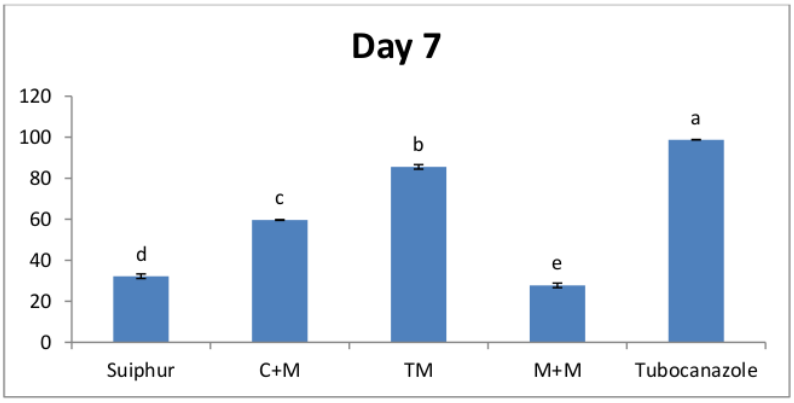
Tubocanazole


Day 7
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Suiphur
C+M
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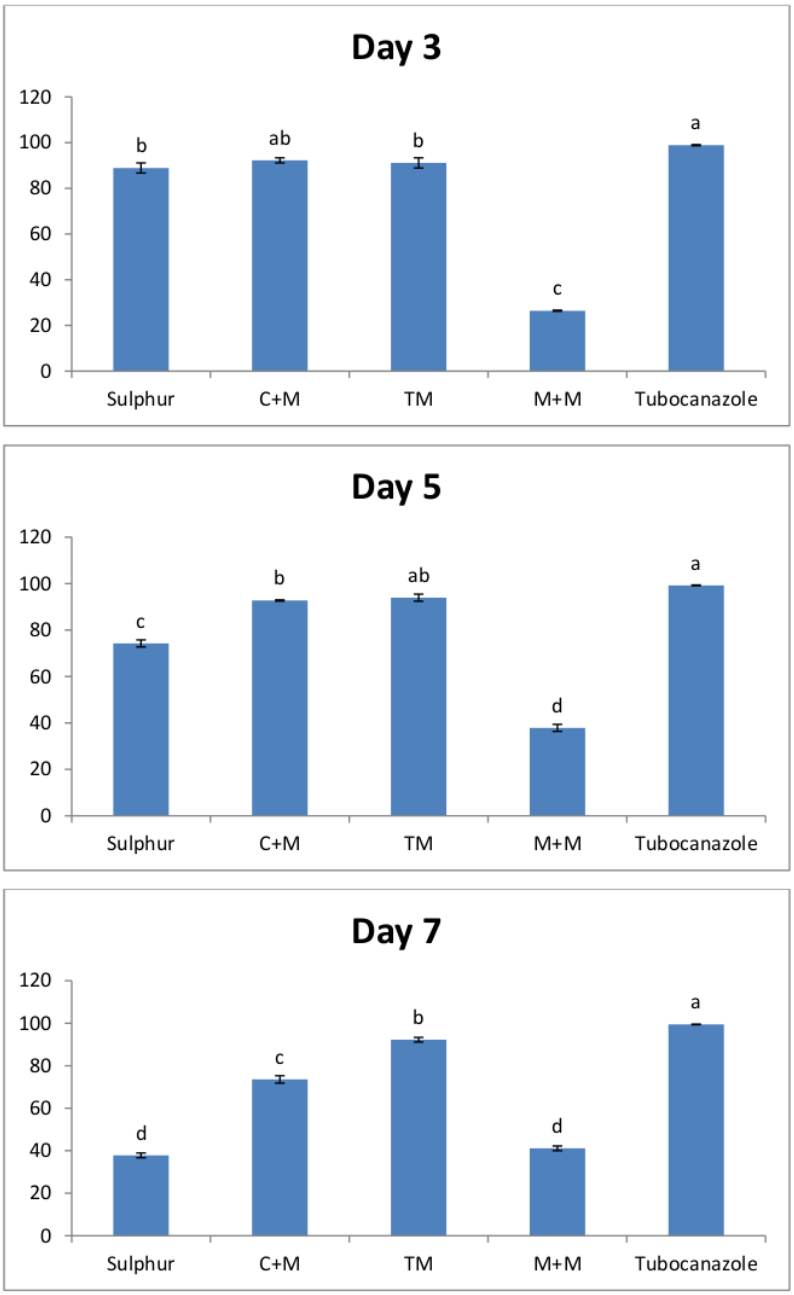
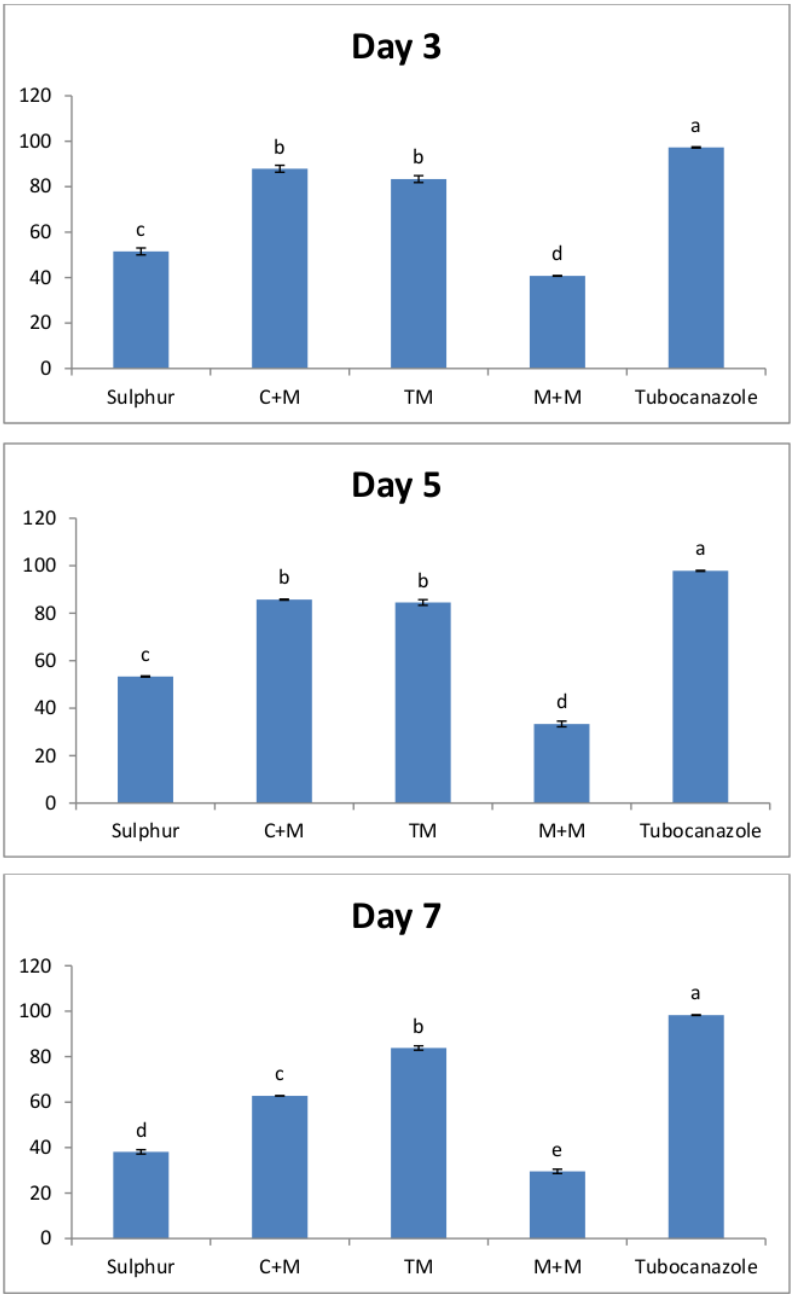
M+M
Tubocanazole

Growth inhibition of *Aspergillus* sp. at day 3rd, 5th and 7th at 300 ppm



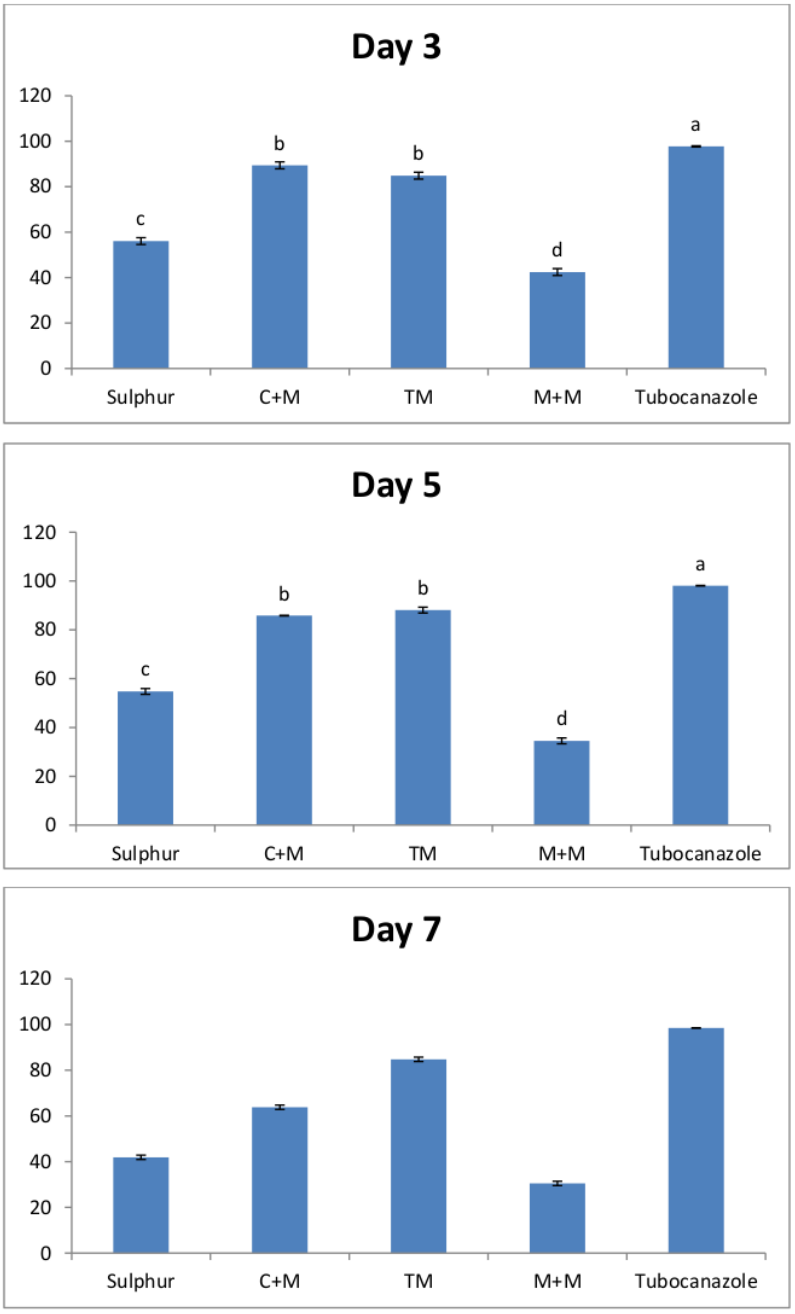
Growth inhibition (mean±SE) of mycelial of *Fusarium* sp. at 100 ppm at 3rd, 5th and 7th day after treatment. Mean sharing letter are not significantly different at P<0.05 (Table 4.20).

Growth inhibition of *Fusarium* sp. at 3rd, 5th and 7th day at 100 ppm



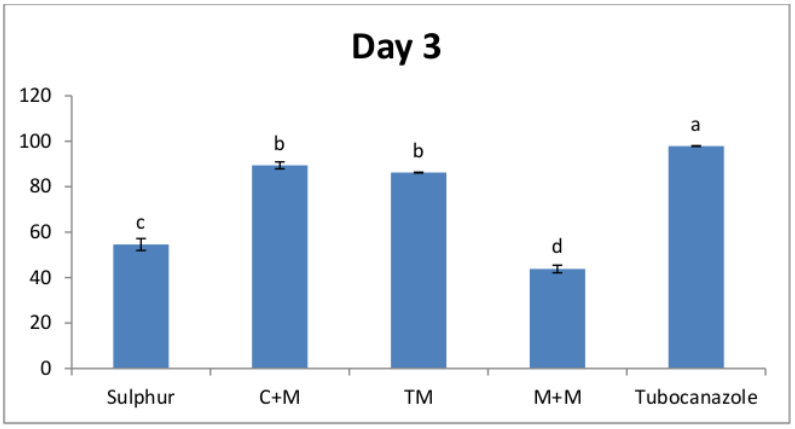
Growth inhibition (mean±SE) of mycelial of *Fusarium* sp. at 200 ppm at 3rd, 5th and 7th day after treatment. Mean sharing letter are not significantly different at P<0.05 (Table 4.21)

Growth inhibition of *Fusarium* sp. at 3rd, 5th and 7th day at 200 ppm



Growth inhibition (mean±SE) of mycelial of *Fusarium* sp. at 300 ppm at 3rd, 5th and 7th day after treatment. Mean sharing letter are not significantly different at P<0.05 (Table 4.22).

Growth inhibition of *Fusarium* sp. at 3rd, 5th and 7th day at 300 ppm


Day 3
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20
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c
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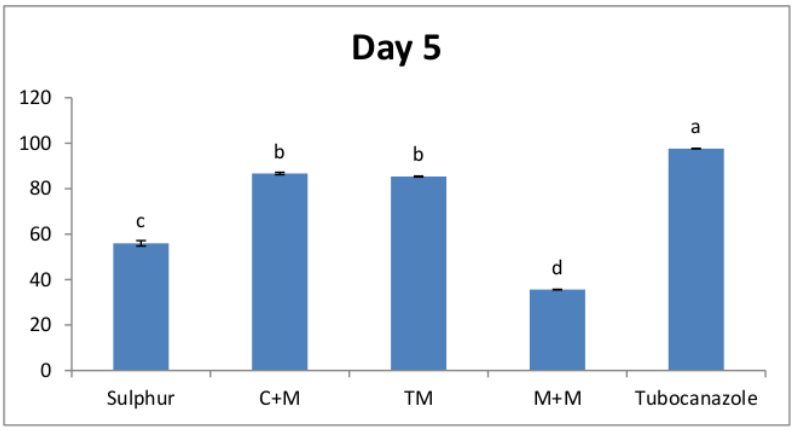
Sulphur
C+M
TM
M+M
Tubocanazole


Day 5
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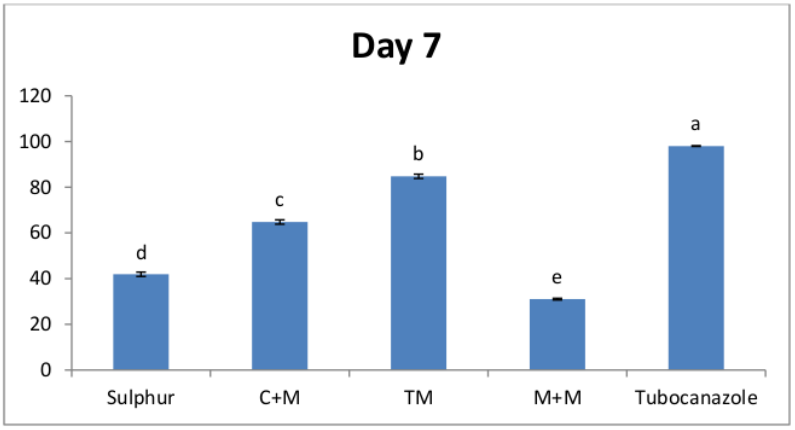
d
a
Sulphur
C+M
TM
M+M
Tubocanazole


Day 7
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Sulphur
C+M
TM
M+M
Tubocanazole

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