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# DETECTION OF *ASPERGILLUS* AND *FUSARIUM* SP. FROM COMMERCIAL MARKETS OF SARGODHA, PUNJAB, PAKISTAN

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**A B S T R A C T**

Different fungal species attack on vegetables and cause severe post-harvest losses. The objective of this research was to find out the cause of post-harvest diseases occurred in summer and *in-vitro* management by using different fungicides at various concentrations. Surveys of different markets of Sargodha were conducted for the collection of infected vegetables including potato, tomato, pumpkin, cucumber and eggplant. The fungi was isolated on PDA media and identified on the basis of morphological characteristics. 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 noted by tebuconazole while, least percent inhibition was noted by metalaxyal+mancozeb at all concentrations. The same trend was found in mycelial growth of Fusarium sp. that was significantly inhibited by all fungicides. It is concluded that tebuconazole is the most effective fungicide against post-harvest fungi i.e. Aspergillus sp. and Fusarium sp. at 300 ppm concentration.

**Keywords**: Postharvest, Fungi, In-vitro, Management, Fungicides.

## INTRODUCTION

Fungi 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 are widely attacked by fungal pathogens which reduce their shelf life and limit the commercial worth. It is estimated that 20-25% loss occurred by post-harvest pathogens during handling of harvested fruit and vegetable sand it is increasing in developing countries due to poor management practices (Hailu and Derbew, 2015).

Due to poor post-harvest handling; the storage and marketing quality of fruits and vegetables greatly affected. This results in rotting and development of microorganisms which are activated as the fruits and vegetables change their physiological states (Yahya *et al*., 2016). World food resources are monitored by international agencies and acknowledge that to meet the future need of food is to reduce the post-harvest losses (Gautam *et al*., 2017). Vegetables are at risk from mechanical damage because of their structure and quite soft surface related with their high moisture content. In postharvest handling injury could occur at any point. Diseases and disorders also caused high cash loss for vegetable crops. Damage by insect is relatively of minor importance as compared to decay caused by micro organism (Noman *et al*., 2018).

Losses due to decay and rotting are difficult to determine, these losses differ broadly with goods, area

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of production and season (Zhang *et al*., 2019). The losses in developing and tropical countries on perishable commodities are 50% or more (Khanzada and Shah, 2012). These vegetables are the source of primary and secondary foods and are the valued mean of energy and micronutrients (Sani *et al*., 2014).

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

**MATERIALS AND METHODS**

Present research was carried out under laboratory conditions at Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha (Pakistan), 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 specifically on five vegetables including cucumber, pumpkin, eggplant, potato and tomato in the vegetable market. Infected vegetables were collected in polythene bags and labeled with necessary information i.e. area, name of vegetable, name of disease, market name and date. Samples brought into laboratory in an ice box to maintain the temperature for the pathogen. The samples were stored in refrigerator at 4°C until use.

**Incidence of disease:** Five different markets of Sargodha were visited (Sabz Mandi, Choti Mandi, Qainchi More, 49 Tail and Gol Chowk) to record the disease incidence on five vegetables. In each survey, whole markets were visited to calculate the disease incidence. The incidence of disease was calculated as follows:

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

Data was recorded by visual examination of symptoms on different vegetables.

**Isolation of fungal pathogens:**

**Culture medium (PDA):** Different fungal pathogens were isolated and multiplied on potato dextrose agar (PDA).

**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 distilled water. Samples were placed on tissue paper for drying. 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 and Shamsi, 2014).

**Sub culturing:** When growth of fungi initiated on PDA media a bit of mycelia was transferred to new petri plate having PDA. Fungi, that colonized were observed under microscope and transferred to new petri plates for purification and multiplication. Plates were incubated at 25±2ºC and growth was observed 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. The samples were placed in boxes and these boxes placed on the laboratory bench at room temperature. On the 4$^{th}$ 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 999 ml of distilled water. This stock solution was used for preparation of different concentration.

**Preparation of fungicidal concentration:** Three different concentrations were prepared for each fungicide by using stock solution. 100 ppm concentration was prepared by adding 100 ml of stock solution in 900 mL distilled water. For 200 ppm concentration, 200mL of stock solution was taken and dissolved in 800mL of distilled water. 300ml of stock solution was dissolved into 700ml of distilled water for preparation of 300ml concentration.

**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 3 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:** In Sabz Mandi, the maximum disease incidence (20%) was recorded in tomato followed by brinjal (6.25%), pumpkin, cucumber and potato (5%) each (Table 1). Tomato showed highest disease incidence in all other places i.e. Choti Mandi, Qinchi More, Gol Chowk and 49 Tail.

Table 1. 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 |
| Choti Mandi | 5 | 18.33 | 5 | 5.6 | 4.4 |
| Qainchi More | 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 |

**Infection percent of Aspergillus and Fusarium sp. in Sabz Mandi:** It was reported that the maximum attack of *Aspergillus* sp (73.33%) was on cucumber, while minimum on tomato (53.33%) Table 2. The attack of *Fusarium* sp. was maximum 46.67% on tomato while minimum (33.33%) on tomato.

Table. 2. Infection percent of *Aspergillus* and *Fusarium* sp. in Sabzmandi

| Host | 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 |

**Interaction percent of Aspergillus and Fusarium sp. in Choti Mandi:** The maximum attack of Aspergillus sp. was recorded on pumpkin while of *Fusarium* sp. on brinjal (Table 3).

Table 3. Infection percent of Aspergillus and Fusarium sp. in Choti Mandi

| Host | 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 |

**Infection percent of *Aspergillus* and *Fusarium* sp. in Qainchi More:** It was reported that the attack of *Aspergillus* sp. was maximum 80% on cucumber, while of *Fusarium* sp. was maximum 50% on tomato (Table 4).

Table 4. Infection percent of Aspergillus and Fusarium sp. in Qainchi More

| Host | Aspergillus sp. (%) | Fusarium sp. (%) |
|---|---|---|
| Potato | 70 | 30 |
| Tomato | 50 | 50 |
| Cucumber | 80 | 20 |
| Brinjal | 50 | 50 |
| Pumpkin | 70 | 30 |

**Infection percent of Aspergillus and Fusarium sp. in Gol Chowk:** Aspergillus infection was maximum on cucumber followed by pumpkin, potato, brinjal and tomato. The infection of *Fusarium* was maximum on tomato followed by brinjal, potato, pumpkin, and cucumber (Table 5).

Table 5. Infection percent of Aspergillus and Fusarium sp. in Gol Chowk

| Host | *Aspergillus* sp. (%) | *Fusarium* sp. (%) |
|---|---|---|
| Potato | 60 | 40 |
| Tomato | 50 | 50 |
| Cucumber | 80 | 20 |
| Brinjal | 60 | 40 |
| Pumpkin | 70 | 30 |

**Infection percent of Aspergillus and Fusarium sp. in 49 tail:** From 49 tail, the attack of *Aspergillus* species was maximum on cucumber and minimum on brinjal while the attack of *Fusarium* species was maximum on brinjal and minimum on cucumber (Table 6).

Table 6. Infection percent of Aspergillus and Fusarium sp. in 49 tail

| Host | 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 |

**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 7).

Table 7. Symptoms observed

| Host | Symptoms observed | |
|---|---|---|
| | Aspergillus | 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 |

**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 8).

Table 8. 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% |

**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 9).

Table 9. 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% |

**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 10).

Table 10. 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% |

**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 11).

Table 11. 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% |

**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 12).

Table 12. 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% |

**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 13).

Table 13. 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% |

**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 14).

Table 14. 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% |

**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 15).

Table 15. 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% |

**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 16).

Table 16. 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% |

**DISCUSSIONS**

Post-harvest diseases are severe threat to vegetables. Aspergillus and Fusarium spp.are two most important pathogens of vegetable diseases that incite losses up to 20-30% every year (Noman *et al*., 2018). 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 it is easily accessible way to control of plant diseases. In this study, we evaluated different fungicide products against fungal isolates. For in-vitro evaluation of 5 fungicides at 3 concentrations food poisoning technique was used in this research (Khanzada and Shah, 2012).

Present research was planned to evaluate different

fungicides against *Aspergillus* and *Fusarium* spp. at different concentrations at different time intervals. Tebuconazole is broad spectrum systemic fungicide and has been found very effective against Aspergillus and Fusarium (Ewekeye *et al*., 2013). Genanew (2013) evaluated ten different fungicides including and reported that tebuconazole gave significant inhibition by using food poisoning technique against Aspergillus and Fusarium. It was also noted that the tebuconazole efficacy increased by increasing concentration i.e. 300 ppm. 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, tebuconazole and thiophanate methyl was the most effective fungicide at different concentrations. They also reported that tebuconazole and thiophanate methyl efficacy was increased gradually by increasing concentration and efficacy was also increased by time. Sultana and Ghaffar (2013) conducted in vitro experiments to evaluate the efficacy of fungicides at various concentrations 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 tebuconazole and thiophanate methyl completely inhibited the mycelial growth of fungus. Sani *et al*., (2014) used food poisoning technique to check the efficacy of different fungicides against *Ilyonectria radicicola,* a soil borne fungus. 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 controls the mycelial growth of both species at different concentrations and at different days interval. These results are similar to the studies conducted previously (Elshahawy *et al*., 2016). metalaxyl+mancozeb had been found effective against wilt disease of pepper (Sahi *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. Genanew (2013) 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 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 acts to upward and downward in plant system. This fungicide controls the spore formation as well as it stimulates the plant defense system. Data of this research is similar to the study conducted by (Khanzada and Shah, 2012). Sahi *et al*., (2012) 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 and 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.

**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.

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|---|---|---|
| Yasir Iftikhar | : | Wrote manuscript |
| Muhammad Noman | : | Conduct research trials |
| Muhammad A. Zeshan | : | Conceive the idea of research and supervised study |
| Ashara Sajid | : | Helped in the conduct of experiments |
| Qaiser Shakeel | : | Helped in manuscript writing |
| Muhammad U. Ghani | : | Make statistical analysis |
| Rizwan Mahmood | : | Make tables, figures, and graphs |
| Tariq A. Hamid | : | Finalize the manuscript |