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Assessment and management of microbial pathogens associated with chickpea (*Cicer arietinum* L.) grains

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Abstract

Chickpea (Cicer arietinum L.) is most important grain crop which have great economic value throughout the world. Chickpea seeds may carry different pathogens directly and indirectly. This research was planned for management and assessment of seed borne microbial pathogens of chickpea grains from different storage conditions. Different samples of chickpea were collected from the different storage conditions of district Bhakkar from Punjab, Pakistan. The efficiency of four different fungicides namely; Carbendazim 50% WP, Azoxystrobin 23% SC, Mancozeb 75% WP, Chlorothalonil 75% WP with two different concentrations 1000ppm and 500ppm was evaluated. Results against fungus infection indicated that the decrease in concentration of fungicides also decreased their efficacy. Result also indicated that higher efficacy was noted in Carbendazim fungicide followed by Azoxystrobin, Mancozeb and lower efficacy was noted by Chlorothalonil.

Key words: *Chickpea (Cicer arietinum L.),* fungicides, microbial pathogens

Introduction

Chickpea (*Cicer arietinuin L.*) is the third most essential pulse crop in whole world. Its number comes after beans and peas. This legume crop is grown on approximately 9.5-million-hectare area in the world. Overall production was 11.09 million tonnes (FAO, 2019). In developing countries of world, this crop is considered as a cheap and important legume crop for rural and urban population (Sharma *et al*., 2016). It plays a significant role in enhancement of soil fertility through organic nitrogen fixation (Rao *et al*., 2007). It grows as a spring crop in North and East Africa, West and South Asia (Soltani *et al*., 2006). Its seeds are used for eating purpose in the form of fried, green vegetable, as snake food and flour (Baloch and Zubair, 2010). Its seed consists of 59.0 percent carbohydrates, 3.1 percent fiber, 29.1 percent proteins, 4.01 percent ash and 5.02 percent oil (Maheri-Sis *et al*., 2008). Chickpea has also some medical importance. It contains hypercholesteraemic ingredient for the control of cholesterol level. Sour taste of these legumes is due to malic and oxalic acids present in leaves secretions and lower the blood cholesterol level (Nene *et al*., 1996).

Chickpea is regularly attacked by fungi during both pre and post-gather essentially influencing its efficiency. The degree of seed harm depends on upon natural conditions, for example, high relative humidity, dew, and temperature over 25 °C (Barz and Welle, 1992). The fungi species that infect chickpeas can be potential mycotoxin producers. The fundamental species mycotoxin makers belong to the genera Aspergillus, Penicillium, Fusarium and Alternaria. Mycotoxins can be extremely poisonous. The production of various toxins depends on, transmission of pathogen, type of toxin, its percentage, the health, the age and plant species and the possible synergistic impacts between mycotoxins (Yadav *et al*., 2011). *Different fungal pathogens and microflora can be transferred through different sources from the long distance. Literature reports that, at world level when seeds are imported and exported may be infected with pathogens due to several reasons. Chickpea seeds may carry diverse pathogens directly and indirectly. Infected seeds have pathogens which have ability to remain in the field for long period, and it can appear*

again and again when temperature and environment is suitable for their growth and multiplication (Agarwal, 2011). These pathogens are mainly soil borne and/or seed borne in nature.

These fungal pathogens cause various infections in seeds such as seed abnormality, abortion, reduce the germination of seed and increase the rate of abnormal seedlings. At numerous stages of growth, the chickpea seeds can be infected by various biotic and abiotic factors, which reduce the purity, germination, growth, production quality and marketing of chickpea. Throughout the world chickpea is affected with various diseases due to fungal and bacterial pathogens. In Pakistan chickpea is infected by seed borne fungi during suitable environment (Khanzada et al., 2002). There are different seed-borne fungi like Bipolaris, Fusarium, Alternaria, Rhizopus and Penicillium. These fungi are reported as frequent disease-causing agent in different crop seeds throughout the world (Rehman et al., 2011). Seed borne fungi play a vital role in the germination of seed. It is responsible for the death of pre and post germinated seed. Seed borne fungi caused lower seed vigour, decreased germination rate and caused variation in morphology (Niaz and Dawar, 2009). Root rot and wilt complex in chickpea is brought about by a few pathogens like Sclerotium rolfsii, R. solani, F. oxysporum f. sp. ciceri have been considered as the important pathogens. The disease can show up at any stage of plant development, indications in an exceedingly susceptible cultivar can build up within 25 days in the wake of planting till as late as pudding stage (Nene et al.,2011). It is hard to deal with these diseases using synthetic compounds.

In International Seed testing Association reported that almost 12 seed samples of chickpea varieties were checked to study various seed borne fungi and isolated Mycoflora were including *Alernaria tenuis, A. niger and C. lunata*. Various fungicides were tested to control the fungus and Mycoflora associated with chickpea of which Baytan, Captan and Vitavax showed good results to control the seed borne fungi (Magan *et al* 1988). The seeds ought to be treated with fungicides (2 g thiram + 1 g carbendazim kg-1 seed) before planting for lessening seed and soil borne transmittable disease. Phosphorus solubilizing bacteria (PSB) have been identified, which improve accessibility of phosphorus to plants. Therefore, seed treatment with PSB is prescribed. Where chickpea is being established for the first time, the seeds should be treated with Rhizobium culture. The seeds are treated first with fungicides and after that with PSB and Rhizobium, following the system prescribed by seed providers. The way of culture treated seeds should be dried in the shade and planted as quickly as time allows from there on (Gaur *et al*., 2010). Six different fungicides are used for control of fungi and Mycoflora and check the efficacy of these all fungicides on the germination and growth of chickpea and wheat seeds. These fungicides including Mancozeb, captain and tilt give the most efficient result against the Mycoflora. After these all treatments of fungicides the growth and germination percentage of chickpea seeds increases (Javaid, 2009). The objective of this study was the *management of microbial seed borne pathogens in chickpea through different fungicides under in vitro conditions.*

Materials and methods

Sample collection:

Three different samples infected with microbes of chickpea seeds were collected from different areas of Bhakkar, area of Punjab, Pakistan. Sample was collected to identify the seeds of required suitable size for research. These samples were collected from different warehouses and storage houses of Bhakkar region. To identify the seed lot, required quantity of seed samples were collected from that region. Sample, which was collected known as primary sample, so for research purpose we need composite samples. After making composite sample, working sample was collected from composite sample. From working sample submitted sample was made to perform experiment. These sample then used for research purpose and sample must labeled and maintained for whole research.

Methods for microbial pathogens identification

Mycoflora was identified by two methods w were:

Blotter paper

Potato dextrose agar

Blotter paper method

Procedure

All apparatus's including petri plates, blotter paper autoclaved by putting into laminar flow. Laminar flow was used to avoid contamination. Labeled the samples with name and date on petri plates. Blotter paper was dipped into the distilled water for moisture and growth of Mycoflora, blotter paper with Mycoflora was placed into the petri plates. Moisture given to blotter paper due to the best growth of Mycoflora. After that 400-500 seeds were separated from working samples. Almost 6 grains were placed into the petri plates at same distance to stop blend. After that chickpea seeds with low moisture was used for Mycoflora growth, and then these 9 petri dishes were tightened with the help of tape. These petri dishes having Mycoflora was placed in incubator at suitable temperature of 25-28°C for further growth. This temperature is suitable for the growth of Mycoflora.

Potato dextrose agar

Potato was used in Potato dextrose agar. To prepare the media, 300g potatoes starch was made with nutrient agar, water and dextrose. First potatoes were peeled and cut into small pieces then washed with rinse water. This solution was placed into 1litter of water for boiling purpose for almost 1 hour. Process was continued till the mash formed. Then squeezed mash with muslin cloth. Dissolve 20 g agar into the little amount of water and 20 g dextrose was mixed into the potatoes mash with 1 litter for media preparation. Sterilized it at 121°C and 15 p.s.i for 15 minutes. After preparing the media placed it into the petri dishes. Grains were placed into petri dishes for fungal growth. After that these plates were tighten for avoiding entering external air to stop contamination. When these petri dishes sealed and air tightened with tapes was kept into incubator for growth of Mycoflora at 25-28°C. Fungi growth was observed after 8 days from chickpea grains with the blotter paper method, while Mycoflora growth was observed after 5 days by using PDA method.

Bioassay against Mycoflora on PDA media:

To check the efficacy of fungicide against fungus. Four different fungicides were used on the growth of fungus on PDA paper. Petri dishes of PDA paper with fungus were treated with four different fungicides. Fungicides used in my research work was Carbendazim 50% WP, Azoxystrobin 23% SC, Mancozeb 75% WP, Chlorothalonil 75% WP. These fungicides were used against fungi to check the control effect of these fungicides on fungi. These fungicides were used in two different concentrations of 1000ppm and 500ppm.

Statistical analysis

Collected data of all these experiments was analyzed by using factorial under Complete Randomized Design (CRD). All collected data was be analyzed for mean and compared by using LSD test with 5% probability level.

Results

The isolation and characterization of different mycoflora was carried out in Mycology and Biocontrol Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad.

Identification of different mycoflora from seed samples

Identification of different mycoflora from seed samples was done. For this purpose, fungi from chickpea seeds collected from different storage places were purified and identified up to a species level on the basis of morphological characters using glass

slides. On the basis of various morphological characters, using different isolation methods various prevalent fungi were found including Fusarium oxysporum, *Alternaria alternata, Aspergillus flavus, Penicillium* sp. and *Rhizopus* sp. By using different isolation methods, various mycroflora mentioned above were isolated (Table 1).

Microflora isolated from seeds i.e. Fusarium oxysporum, *Alternaria alternate, Aspergillus flavus, Penicillium* sp. and *Rhizopus* sp. incidence in seeds, isolation methods and their interaction showed significant difference (Table 2). Similarly, Table 2 demonstrated that in blotter paper method highest value of colony diameter was recorded *Fusarium oxysporum* followed by *Rhizopus spp., Aspergillus flavus, Penicillium spp. and Alternaria alternata*. Somehow similar patterns were showed by different fungi on agar paper method in which PDA was used as culture media. In PDA, again highest growth was demonstrated by Fusarium oxysporum followed by *Aspergillus flavus, Penicillium spp., Alternaria alternate* and *Rhizopus spp*. Similarly over all better growth was shown by PDA method as compared to the blotter paper method.

Table 1. The mean comparison of values for mycoflora growth on PDA and Blotter paper

	Aspergillus flavus	*Penicillium spp*	*Alternaria alternata*	*Rhizopus spp.*	*Fusarium oxysporum*	Mean
Blotter paper	5.06A	3.795DE	3.564E	6.96663A	7.11337A	5.2998B
PDA	6.6AB	6.12337B	6.01337B	4.29C	7.00337A	6.0060A
Mean	5.83B	4.959185C	4.788685C	5.628315B	7.05837A	

The results showed that *Fusarium oxysporum* was one of the most prevalent fungus isolated from the chickpea seeds. However, two types of bacteria were isolated from the seeds of chickpea.

In vitro efficacy of different fungicides with different concentrations on PDA against *Fusarium oxysporum*

In vitro efficacy of different fungicides depending upon their concentrations was assessed against the fungus *Fusarium oxysporum* isolated from the samples of chickpea seeds. These fungicides were used in laboratory under control conditions. The results indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect whereas "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (84.60 ± 0.40a) effect and with concentration of 500ppm showed (56.8 ± 0.37e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (77.00 ± 0.31b) and (54.2 ± 0.37f) effects respectively. "Mancozeb 75% WP" with concentration of 1000ppm and 500ppm showed (75.20 ± 0.37c) and (50.2 ± 0.37g) effects respectively. Chlorothalonil 75% WP with concentration of 1000ppm showed (72.20 ± 0.37d) and with concentration of 500ppm showed effect of (46.2 ± 0.37h).

Table 2. In vitro efficacy of different fungicides with different concentrations on PDA against *Fusarium oxysporum*

Treatments	**Concentrations**	**Efficiency**
Carbendazim 50% WP	1000ppm	84.60 ± 0.40a
Azoxystrobin 23% SC	1000ppm	77.00 ± 0.31b
Mancozeb 75% WP	1000ppm	75.20 ± 0.37c

Chlorothalonil 75% WP	1000ppm	72.20 ± 0.37d
Carbendazim 50% WP	500ppm	56.8 ± 0.37e
Azoxystrobin 23% SC	500ppm	54.2 ± 0.37f
Mancozeb 75% WP	500ppm	50.2 ± 0.37g
Chlorothalonil 75% WP	500ppm	46.2 ± 0.37h

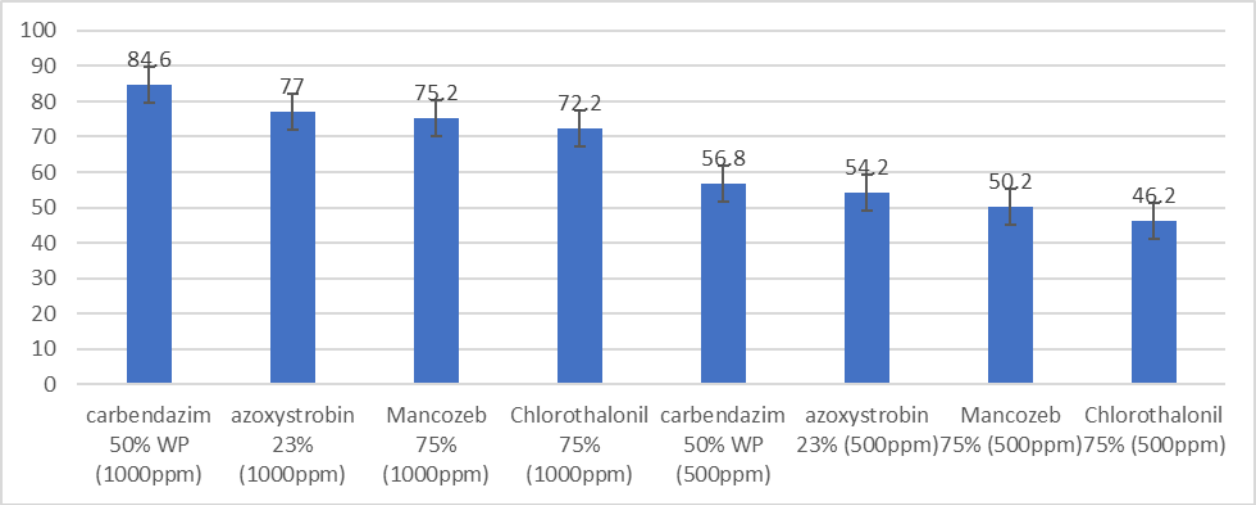

Fig 1. Response of different fungicides against *Fusarium oxysporum*

This is the graph of different fungicides against *Fusarium oxysporum.* Result indicated that with the decrease in concentration efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower efficacy than Carbendazim. And Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin.In vitro efficacy of different fungicides depend upon their concentrations and different seed samples of chickpea where it is going to apply. These fungicides were used in laboratory under control conditions. Result indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect. "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (81.40 ± 0.51a) effect and with concentration of 500ppm showed (56.00 ± 0.32e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (76.40 ± 0.40b) and (53.60 ± 0.24f) effects respectively. "Mancozeb 75% WP" with concentration of 1000ppm and 500ppm showed (74.00 ± 0.45c) and (49.20 ± 0.37g) effects respectively. "Chlorothalonil 75% WP" with concentration of 1000ppm showed (70.20 ± 0.58d) and with concentration of 500ppm showed effect of (45.20 ± 0.37h).

Table 3. In vitro efficacy of different fungicides with different concentrations on PDA against *Aspergillus flavus*

Treatments	Concentrations	Efficiency
Carbendazim 50% WP	1000ppm	81.40 ± 0.51a

Azoxystrobin 23% SC	1000ppm	76.40 ± 0.40b
Mancozeb 75% WP	1000ppm	74.00 ± 0.45c
Chlorothalonil 75% WP	1000ppm	70.20 ± 0.58d
Carbendazim 50% WP	500ppm	56.00 ± 0.32e
Azoxystrobin 23% SC	500ppm	53.60 ± 0.24f
Mancozeb 75% WP	500ppm	49.20 ± 0.37g
Chlorothalonil 75% WP	500ppm	45.20 ± 0.37h

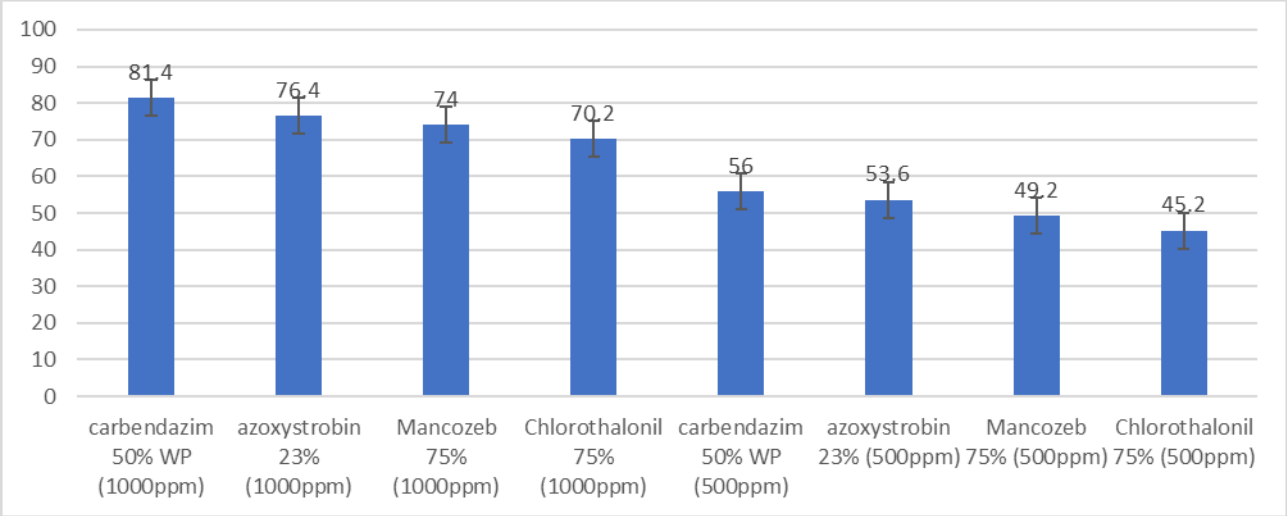

Fig 2. Response of different fungicides against *Aspergillus flavus*

This is the graph of different fungicides against *Aspergillus flavus* . Result indicated that with the decrease in concentration efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower efficacy than Carbendazim. And Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin.

In vitro efficacy of different fungicides depend upon their concentrations and different samples of chickpea seeds where it is going to apply. These fungicides were used in laboratory under control conditions. Result indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect. "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (80.60 ± 0.51a) effect and with concentration of 500ppm showed (55.60 ± 0.24e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (75.00 ± 0.32b) and (52.60 ± 0.51f) effects respectively. "Mancozeb 75% WP" with concentration of 1000ppm and

500ppm showed (73.4 ± 0.25c) and (48.20 ± 0.37g) effects respectively. "Chlorothalonil 75% WP" with concentration of 1000ppm showed (69.20 ± 0.37d) and with concentration of 500ppm showed effect of (44.20 ± 0.37h).

Table 4. In vitro efficacy of different fungicides with different concentrations on PDA against *Rhizopus sp*.

Treatments	Concentrations	Efficiency
Carbendazim 50% WP	1000ppm	80.60 ± 0.51a
Azoxystrobin 23% SC	1000ppm	75.00 ± 0.32b
Mancozeb 75% WP	1000ppm	73.4 ± 0.25c
Chlorothalonil 75% WP	1000ppm	69.20 ± 0.37d
Carbendazim 50% WP	500ppm	55.60 ± 0.24e
Azoxystrobin 23% SC	500ppm	52.60 ± 0.51f
Mancozeb 75% WP	500ppm	48.20 ± 0.37g
Chlorothalonil 75% WP	500ppm	44.20 ± 0.37h

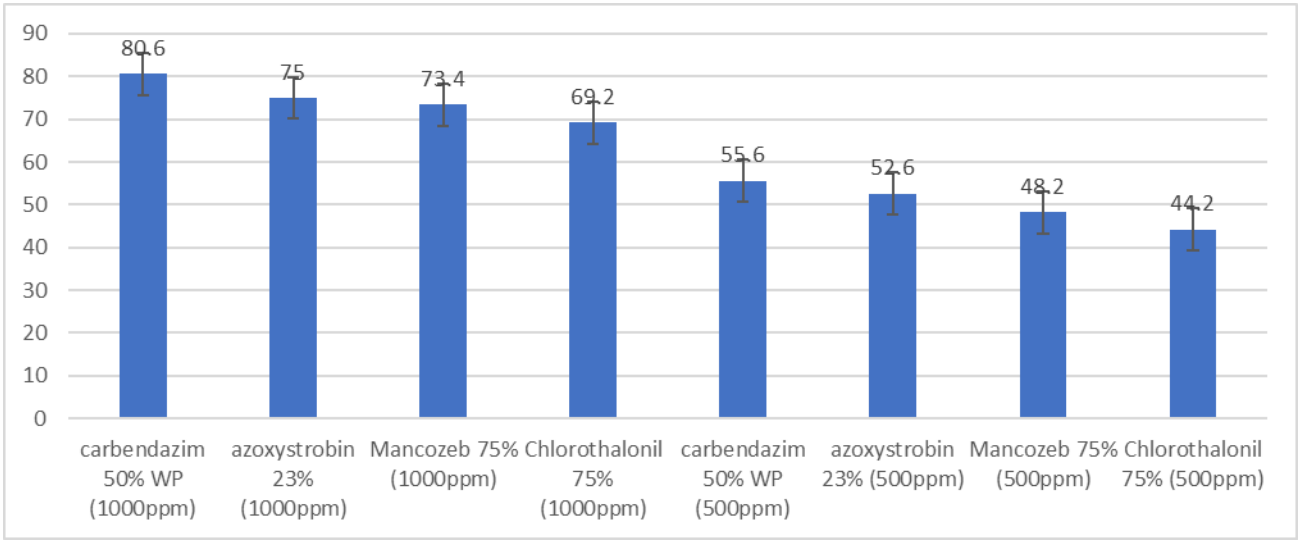

Fig 3. Response of different fungicides against *Rhizopus sp*.

This is the graph of different fungicides against *Rhizopus sp.*. Result indicated that with the decrease in concentration efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower

efficacy than Carbendazim. And Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin.

In vitro of different fungicides depend upon their concentrations and different samples of chickpea seeds where it is going to apply. These fungicides were used in laboratory under control conditions. Result indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect. "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (94.40 ± 0.51a) effect and with concentration of 500ppm showed (66.20 ± 0.66e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (86.80 ± 0.74b) and (63.40 ± 0.51f) effects respectively. "Mancozeb 75% WP" with concentration of 1000ppm and 500ppm showed (84.80 ± 0.58c) and (59.00 ± 0.45g) effects respectively. "Chlorothalonil 75% WP" with concentration of 1000ppm showed (81.60 ± 0.68d) and with concentration of 500ppm showed effect of (55.80 ± 0.58h).

Table 5. In vitro efficacy of different fungicides with different concentrations on PDA against *Alternaria alternata*

Treatments	Concentrations	Efficiency
Carbendazim 50% WP	1000ppm	94.40 ± 0.51a
Azoxystrobin 23% SC	1000ppm	86.80 ± 0.74b
Mancozeb 75% WP	1000ppm	84.80 ± 0.58c
Chlorothalonil 75% WP	1000ppm	81.60 ± 0.68d
Carbendazim 50% WP	500ppm	66.20 ± 0.66e
Azoxystrobin 23% SC	500ppm	63.40 ± 0.51f
Mancozeb 75% WP	500ppm	59.00 ± 0.45g
Chlorothalonil 75% WP	500ppm	55.80 ± 0.58h

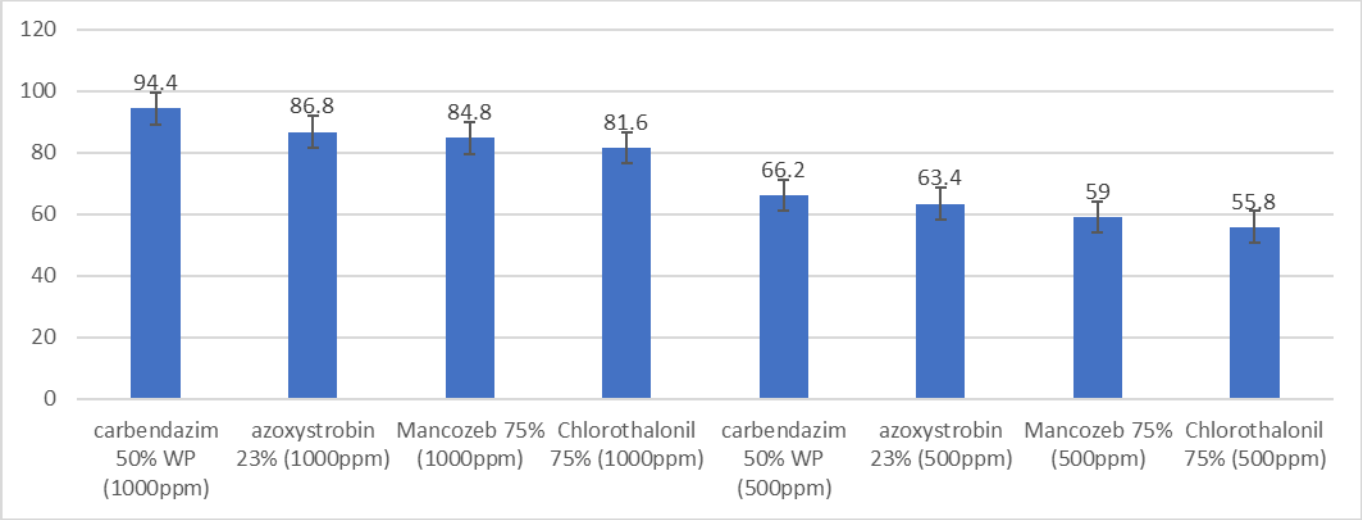

Fig 4. Response of different fungicides against *Alternaria alternata*.

This is the graph of different fungicides against *Alternaria alternata* Result indicated that with the decrease in concentration efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower efficacy than Carbendazim. And Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin.

These fungicides were used in laboratory under control conditions. Result indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect. "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (91.80 ± 0.58a) effect and with concentration of 500ppm showed (65.00 ± 0.45e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (86.00 ± 0.63b) and (62.80 ± 0.37f) effects respectively. "Mancozeb Efficacy of different fungicides depend upon their concentrations and varieties of chickpea 75% WP" with concentration of 1000ppm and 500ppm showed (83.80 ± 0.37c) and (58.80 ± 0.37g) effects respectively. "Chlorothalonil 75% WP" with concentration of 1000ppm showed (80.20 ± 0.58d) and with concentration of 500ppm showed effect of (55.00 ± 0.32h).

Table 6. In vitro efficacy of different fungicides with different concentrations on PDA against *Penicillium spp.*

Treatments	Concentrations	Efficiency
Carbendazim 50% WP	1000ppm	91.80 ± 0.58a
Azoxystrobin 23% SC	1000ppm	86.00 ± 0.63b
Mancozeb 75% WP	1000ppm	83.80 ± 0.37c
Chlorothalonil 75% WP	1000ppm	80.20 ± 0.58d
Carbendazim 50% WP	500ppm	65.00 ± 0.45e
Azoxystrobin 23% SC	500ppm	62.80 ± 0.37f
Mancozeb 75% WP	500ppm	58.80 ± 0.37g

Chlorothalonil 75% WP	500ppm	55.00 ± 0.32h

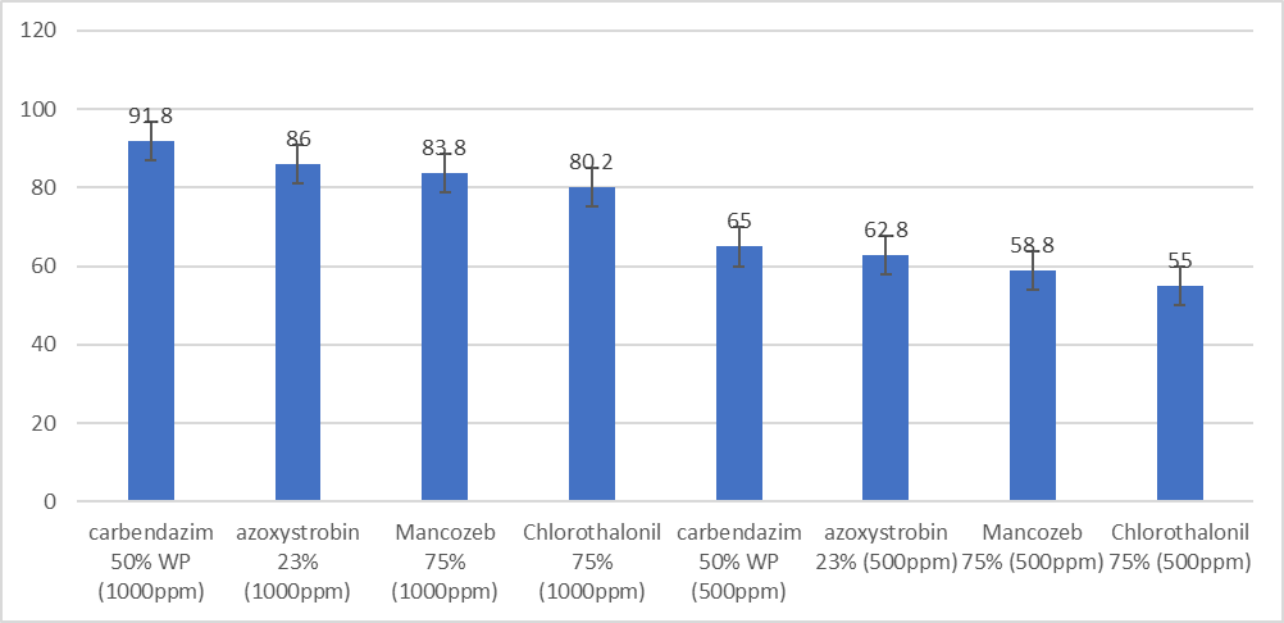

Fig 5. Response of different fungicides against *Penicillium spp.*

This is the graph of different fungicides against *Penicillium* sp. Result indicated that with the decrease in concentration efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower efficacy than Carbendazim. And Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin.

In vitro efficacy of different fungicides depends upon their concentrations and different seed samples of chickpea where it is going to apply. These fungicides were used in laboratory under control conditions. Result indicted that "Carbendazim 50% WP" fungicide with concentration of 1000ppm showed highest effect. "Chlorothalonil 75% WP" with concentration of 500ppm showed minimum effect. "Carbendazim 50% WP" with concentration of 1000ppm showed (90.20 ± 0.58a) effect and with concentration of 500ppm showed (64.20 ± 0.49e) effect. "Azoxystrobin 23% SC" with concentration of 1000ppm and 500ppm showed (85.00 ± 0.45b) and (60.80 ± 0.37f) effects respectively. "Mancozeb 75% WP" with concentration of 1000ppm and 500ppm showed (82.80 ± 0.37c) and (57.00 ± 0.45g) effects respectively. "Chlorothalonil 75% WP" with concentration of 1000ppm showed (78.40 ± 0.40d) and with concentration of 500ppm showed effect of (54.00 ± 0.45h).

Discussion

Chickpea is very important crop not in world but also in Pakistan. It is used as legume crop in Pakistan. There were many researches done to enhance its yield value that the need of population can fulfil. But due to some reasons its yield decreased in past years. This crop become the host of some insect pest. But some fungus infection and bacterial infection was also done to this crop. Its seed has also much importance, but this fungal infection and bacterial infection caused seed borne diseases in seed of chickpea. Due to these diseases its yield decreased at high level. These diseases become a big threat for farmers. So, researchers done a work on it and find the management techniques to save seeds from these diseases. They used fungicides to control fungus infection.

There were many researchers that worked on it to control these fungal and bacterial disease on seed. Singh and Jha, (2003) assessed seven fungicides, for example Thiram, Bavistin, Blitox, Captaf, Indofil M45, Ridomil MZ and Kitazin against chickpea wilt under in vitro (each at 0.1% focus) what's more, in vivo as seed treatment each at 2.5 g kg-1 seed, with the exception of Kitazin (1.0 ml kg-1) also, as soil soaking each at 0.3% and found Thiram and Bavistin as the most appropriate fungicides in inhibiting the mycelial development of *Fusarium oxysporum* under in vitro. Ayyub, (2001) discovered eleven fungicides such as Benlate, Folicur and Derosal, as the best against mycelial development of Fusarium shrink. Moderate reaction was seen in results that, Topas-l00 and Tilt, though, Daconil, Antracol, Apron and Polyram combi were discovered least successful.

In this research work, I used four different fungicides on the growth of fungus on blotter paper and PDA paper. Petri dishes of blotter paper and PDA paper with fungus was treated with four different fungicides.Agar plate and Blotter paper method were used for isolation of different seed borne Mycoflora of chickpea seeds collected from three different districts.Five seed borne pathogens i.e.*Aspergillus flavus,Penicillium spp,Alternaria Alternata,Rhizopus spp* and *Fusarium oxysporum* Fungicides used in my research work were Carbendazim 50% WP, Azoxystrobin 23% SC, Mancozeb 75% WP, Chlorothalonil 75% WP. These fungicides were used against fungi to check the control effect of these fungicides on fungi. These fungicides were used in two different concentrations of 1000ppm and 500ppm. Results against fungus infection indicated that with the decrease in concentration of fungicides efficacy also decreased. Result also indicated that higher efficacy was noted in Carbendazim fungicide and lower efficacy was noted in Chlorothalonil. While Azoxystrobin fungicide showed higher efficacy then Chlorothalonil and Mancozeb and showed lower efficacy than Carbendazim. Mancozeb fungicide showed higher efficacy then Chlorothalonil and showed lower efficacy than Carbendazim and Azoxystrobin. Result for bacterial infection indicated that with the decrease in concentration of antibiotics efficacy also decreased. Result also indicated that higher efficacy was noted in Kanamycine antibiotic and lower efficacy was noted in Streptomycin. While Ampicillin antibiotic showed higher efficacy then streptomycin and Chlorampenicol and showed lower efficacy than Kanamycine. And Chlorampenicol antibiotic showed higher efficacy then streptomycin and showed lower efficacy than Kanamycine and ampicillin.

Conclusions

Results against fungus infection indicated that the decrease in concentration of fungicides also decreased their efficacy. Result also indicated that higher efficacy was noted in Carbendazim fungicide followed by Azoxystrobin, Mancozeb and lower efficacy was noted by Chlorothalonil.

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