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Efficacy of seed dressing fungicides against seed borne diseases in wheat crops

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Abstract

Wheat (*Triticum aestivum* L) seeds are mainly grown for food and cash purpose. Seeds play a significant part in the spread of diseases in plant communities, however these are seed borne, responsible in reducing the yield of wheat crops in an ecosystems. Gemstar super 325 SC (Azoxystrobin+Difenoconazole); Flumax 60% EC (Fluzanium + Metalyxal); Cymoxanil + Mancozeb 72% WP (Cymoxanil + Mancozeb) and Aliette 80% WP (Fosityle Aluminium) was applied @ 150, 200 and 250 ppm concentrations to wheat seeds and seedlings. The effect of fungicides and concentration along their relationship showed highly significant (P<0.01) results after 5, and 7 days, however the correlation of fungicide and concentration showed non-significant (P>0.05) investigations after three days of incubations respectively. Our results found that Flumax 60% EC fungicide were recorded maximum affect on the pathogens in an ecosystem of wheat seeds. Our results recorded strong *Co-efficient of determination* (R^2) with highly significant (P<0.01) relationship of concentrations and fungicides seven days after incubations. The treated seeds used to promote the establishment of good seedlings that minimized the further spread of pathogens in the crops. The main seed borne and soil borne pathogens are, Loose Smut (*Ustilago tritici*), Flag Smut (*Urocystis tritici*) Karnal bunt (*Neovossia indica*), Fusarium Rot (*Fusarium oxysporum*), Pythium root Rot (*Phythium debarianum*) and *Rhizoctonia* root Rot (*Rhizoctonia solani*). At the end, it is concluded that treated seeds with Flumax 60% EC killed seed borne

Keywords: Efficacy, pathogens, seed borne, fungicides, wheat crops, Pakistan

Introduction

Wheat (*Triticum aestivum* L.) belongs to Poaceae family is an important food crop of Pakistan and ranking in 8th positions in the world to produce wheat after Europe, China, India, America, Russia, Canada and Australia in 2016. It is cultivated on 8.690 million hectares in the country, with the total production of 24.303 million ton (Federal Bureau of Statistics, 2016). Wheat is rich source of protein and other micronutrients which serves as a chief source of plant protein in human being than other cereal crops (Peter, 2009). Additionally, wheat is more adjustable to a wide range of environmental conditions as compared to other cereals. Due to this property wheat cultivation is possible in various parts of the world (Nutr *et a1.*,2016). Wheat has become the staple cereal worldwide, due to its taste nutritive quality, price and availability. A rising economy worries about rations and health are also play a role to the increased wheat consumption. Additionally, the utilization of wheat corresponds with socio economic a topographic factor of the state. Wheat is more consumed in the developing countries (Anonymous, 2003). Per capita utilization of wheat surpasses 150 kg per annum in Middle East from 66 to 70 countries, while in South

Asian countries like India; it is used 66 to 70 kg annually (FOA, 2011). Whereas, per capita consumption of wheat at Central America, Sub Saharan Africa regions is very low because of the reason that they mainly depend on other cereals. This data shows the global cultivation and utilization of wheat crop.

Wheat is the rich source of fibers, copper, magnesium, anti-oxidants, iron, zinc, vitamin B, starchy carbohydrates, proteins, vitamin E, antioxidants, phyto-nutrients and unsaturated fats Wheat is consumed as white flour or sometimes in the form of whole grain (Shewry and Hey 2015). Allocation of nutritive wheat is found to be confined to the specific parts of wheat (FAO 2007). For that reason, it is advised to use the whole grain as food than white flour only (Shewry 2007). Foods prepared from whole wheat grain are more advantageous for our body, providing the essential micronutrients. Additionally, the wheat protein "gluten" serves as a main source of cereal protein meeting the daily requirement level of essential amino acids (Henderson et al. 2007). Similarly, minerals are another most important group of nutrients, which are present in wheat. Selenium is an essential mineral nutrient in cereals that is found in the form of selenocystein in several enzymes. Although selenium is also present in other cereal crops, but wheat serves as the nutritional source of selenium to the majority of countries in world (Yu, *et al*., 2005).

The seed borne pathogens may cause seed rotten, necrosis, quality that reduced germination ability (Neergaard, 1977). However, treated seeds with effective fungicides used to promote the establishment of good seedlings and to minimize the further spread of pathogen (wiese, 1984).

In order to control seed borne pathogens the application of chemicals are used as seed treatment which are more cheap and effective. The treated seeds killed seed borne pathogens by providing protective layer around the seed and seedlings. The previous suggested that the diseases are controlled by the effective use of fungicides (Sharvelle, 1979, Singh *et al.,* 1984). Randhawa *et al.* (1985) conducted an experiment on the stored grains with vitavax, captan and thiram and conclude that thiram was more effective in good germination capability. El-Tayed *et al.* (1987) evaluated that benlate and dithane are more effective fungicide as seed dressing against *Fusarium roseum, Alternaria alternata. Rhizoctonia* is a fungus that attacks the roots of wheat crops. Take-all produced by (*Gaeumannomyces graminis var tritici*), that is a fungal disease-causing nutrient-deficiency symptom in the tops, and it disturbs the flow of water to the tops and cause stunting growth and the premature death of the plant. Unfortunately, this pathogen is a saprophyte survived through the parasitism relationship and remains in the stem bases and in dead roots and also affect our next wheat crop. Take-all is maybe the utmost-considered root disease of any crop and it is considered as a root disease worldwide (Asher *et. al*,.1981). The plan of the present experiment was conducted to assess the effectiveness of different new chemistry fungicides against seed borne and soil borne pathogens in wheat crops during Rabi 2018-2019.

Materials and Methods

This field experiment was managed at the Plant Pathology Research area, Department of Plant Pathology University of Agriculture Faisalabad during Rabi season (2018-19) to manage seed and soil borne disease of wheat through seed dressing fungicides. The experiment was executed by randomized complete block design (RCBD) with three replications in field conditions. The wheat varieties Galaxy- 2013, Faisalabad-2008, Gold-2016, Johar-2016 and Anaj-2017 were used in the experiment.

Table 1: List of fungicides with active ingredients

Sr. No.	Fungicides	Active ingredients
1	Gemstar super 325 SC	Azoxystrobin+Difenoconazole
2	Flumax 60% EC	Fluzanium + Metalyxal
3	Cymoxanil + Mancozeb 72% WP	Cymoxanil + Mancozeb
4	Aliette 80% WP	Fosityle Aluminium

Whereas SC (Soluble concentrates), EC (Emulsify able Concentrates), WP (Wet able power)

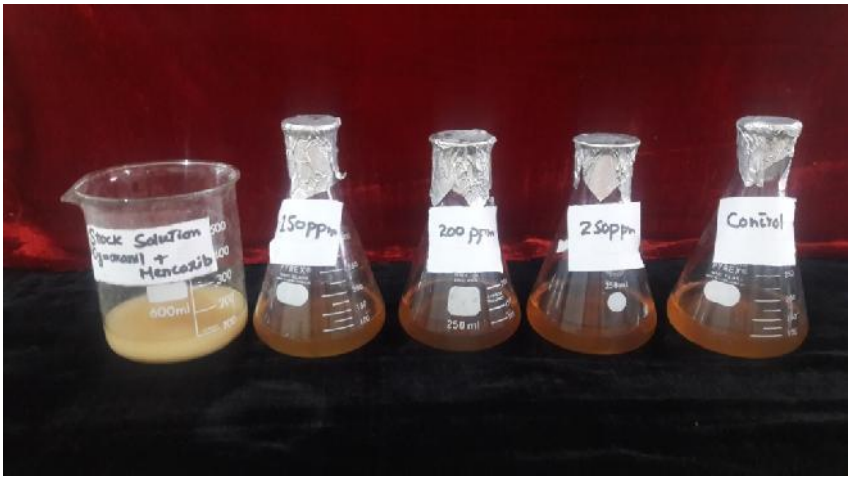

Figure 1 showing stock solution prepared by different concentrations

Four unique fungicides like Gemstar super, Cymoxanil, Alliete and Flumax were utilized for the in-vitro evaluation of *F. oxysporum*. Distinctive dosages of fungicides were utilized for in vitro assessment by harm nourishment strategy. Stock arrangements of fungicides were set up in refined water. Stock arrangement were prepared by expansion of dynamic elements of fungicides and put in 100 ml of water with the assistance of micropipette. Fungicides were poured to the PDA medium at centralization of 50, 100, and 150 ppm. Petri plates PDA medium without fungicides filled in as controls (Bahoo, 2020). With the assistance of stopper borer 1cm plates shape the immaculate culture of *F. oxysporum* and put on the PDA containing petri plates. Petri plates were hatched at 25°C and settlement distance across was measured when development of the test organism was finished in charge. Information was recorded following 3, 6 and 9 days and was subjected to factual examination keeping in mind the end goal to exhibit the outcomes in scientific expressions.

Statistical Analysis

The data was analyzed statistically by one way ANOVA keeping view P = 0.05; however *Co-efficient of determination* (R^2) was calculated between each fungicide used with different level of concentrations. The Co-efficient of determination showed the strong positive relationship between two variables recorded better model fitness (Iqbal and Feng, 2020; Iqbal, et. al. 2019a; Iqbal, et. al. 2019b)

Results and Discussion

Examination of incubated seeds

After the incubation petri plates were analyzed under a stereo-binocular magnifying lens for the colonization of seed by different fungi. The initial growth pattern and number of fungal growths were recorded; however the isolations were also prepared from these growths to detect the kind of fungi.

Fig 2: Infection of different fungi at seedling stage

Morphological characters of isolated fungi

Alternaria alternata

The main symptoms of the colony were grayish black and mycelium was consisted of the chain of conidia. The conidial color was dark brown, both beak and without beak detected under microscopic examinations. Conidia had transverse and longitudinal septation, however the length of beak was varied.

Fusarium oxysporum

The morphology of this pathogen was white to cream colored mycelium and its growth was observed on the seed. Colony color was initially white to cream but later on it became dark pink. When slides were observed under compound microscope two types of conidia were observed micro and macro conidia. Micro conidia were small without septation, but some have one septation. Macro conidia were large, hyaline crescent shape having 3-5 septation.

Aspergillus flavus

Colony color was yellowish green in start but later on it became green. The pure colony observed under microscope that consist of conidiophore and conidia head. Conidiophore was long, hyaline bearing globose conidia head. Conidial head was yellowish green.

Aspergillus niger

The color of the colony color was dark brown. Conidiophores were long, erect, hyaline, bearing globose brown to black rough conidia. The size of conidia was 3-5 micrometer in length.

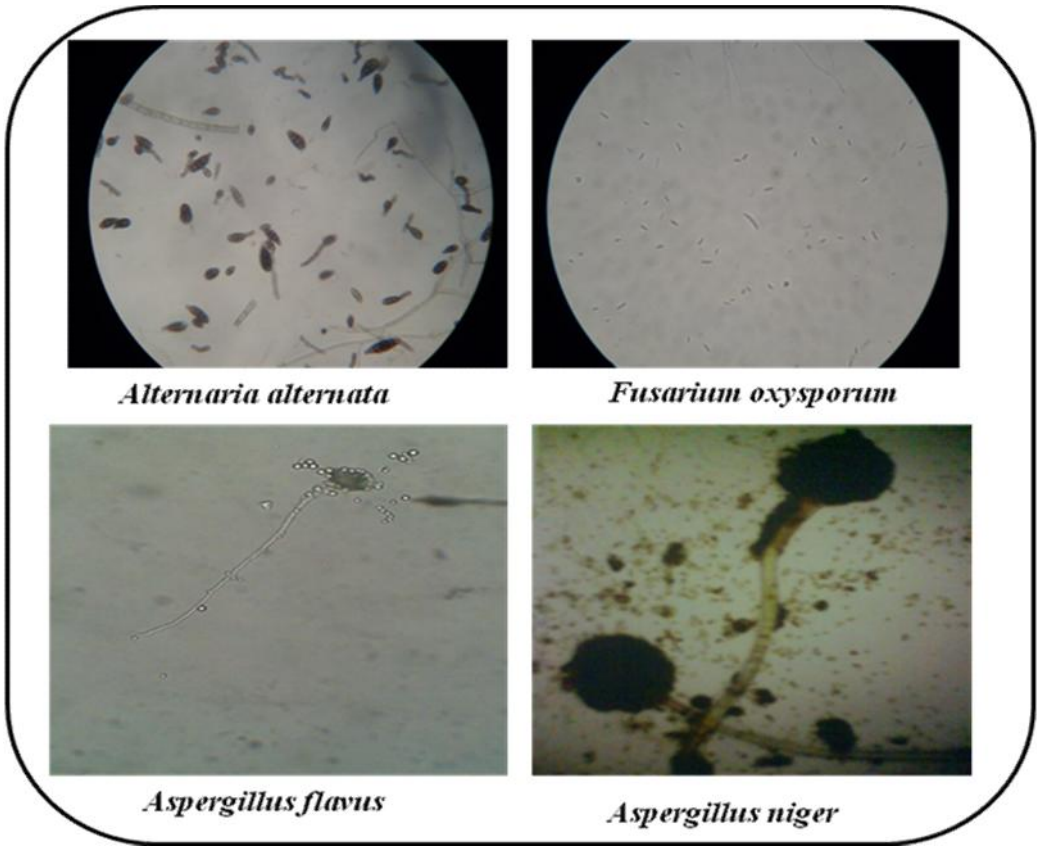

Figure 3: Morphological characters of various kinds of isolated fungi

Impact of various fungicides against *Fusarium oxsporum* 3 days after Incubation under in vitro conditions

The experiment was conducted to evaluate the efficacy of five distinct fungicides (Gemstar super, Flumax, Aliette and cymoxanil+ Mancozeb) were assessed against *Fusarium Oxsporum*. The petri plates without fungicides were filled in as control. The information was recorded following three, five and seven days after fungicides applications. The width of mycelial development was measured in cm. We made 150, 200, 250 ppm doses and these were utilized for every fungicide. Three days of brooding period viability of all fungicides against *Fusarium Oxsporum* changed perpetually. Flumax at all three focuses demonstrated factually huge outcome with mycelial development restraint 0.51 cm. Gemstar super focused 250 ppm was additionally indicated factually noteworthy outcome with mycelial development hindrance 0.55cm at 200 ppm fixation (Table 2). However the Alliete, and cymoxanil were demonstrated middle of the road impact to restrain the mycelial advance on *Fusarium Oxsporum* (Figure 5A). The result recorded strong *Co-efficient of determination* (R^2) with non significant (P>0.05) relationship of concentrations and fungicides (Table 3) three days after incubations.

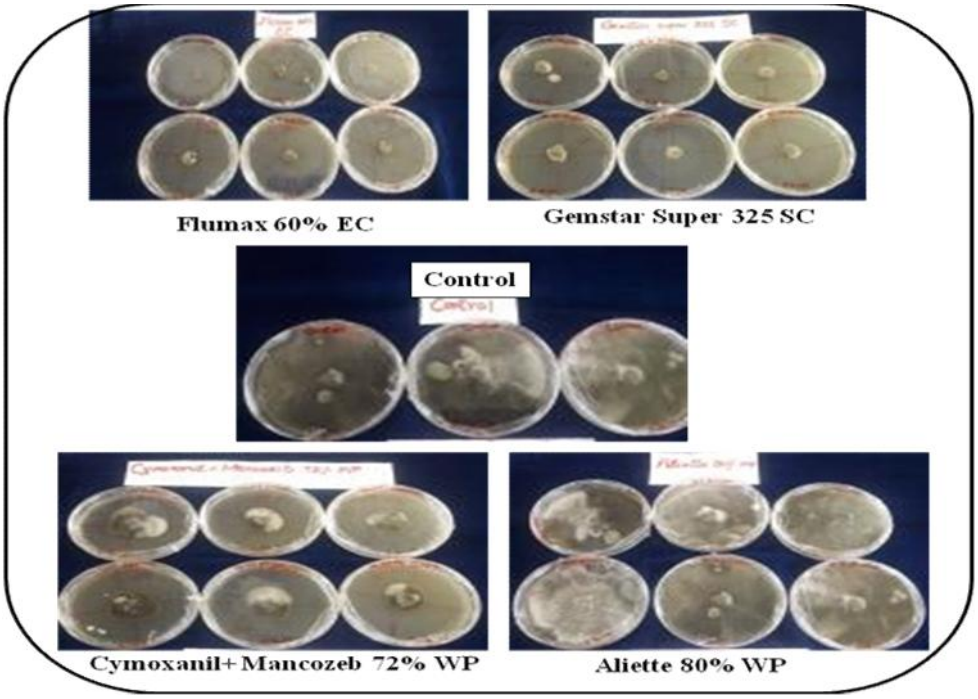

Fig: 4 showing mycelial growth of pathogens 3 days after incubations

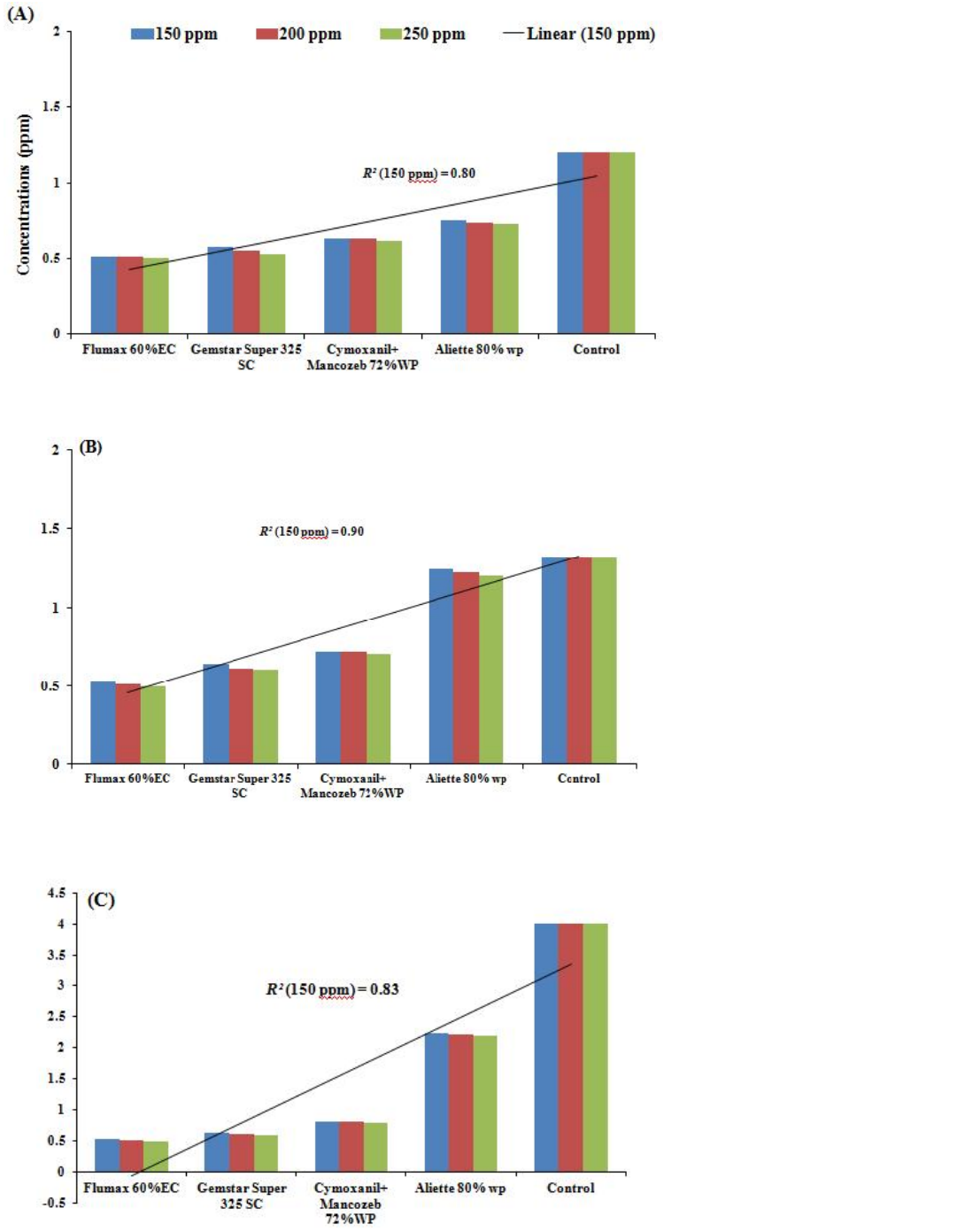

Fig 5: Fungicides effect on colony growth of *Fusarium oxisporum* after 3 days (A), 5 days (B) and 7 days (C) of incubation

Table 2: Comparative efficiency of different fungicides against *Fusarium oxsporum* at different incubation periods

Fungicides	Incubation Period								
	3 days			5 days			7 days		
	150ppm	200ppm	250ppm	150ppm	200ppm	250ppm	150ppm	200ppm	250ppm
Flumax 60% EC	0.51gh	0.51h	0.50h	0.53i	0.51j	0.50j	0.53i	0.51j	0.50j
Gemstar Super 325 SC	058e	0.55f	0.53g	0.64g	0.61h	0.60h	0.64g	0.61h	0.60h
Cymoxanil+ Mancozeb 72% WP	0.63d	0.63d	0.62d	0.72e	0.72e	0.70f	0.82e	0.82e	0.80f
Aliette 80% WP	0.75b	0.74bc	0.73c	1.24b	1.22c	1.20d	2.24b	2.22c	2.20d
Control	1.20a	1.20a	1.20a	1.32a	1.32a	1.32a	4.00a	4.00a	4.00a

Whereas SC (Soluble concentrates), EC (Emulsify able Concentrates), WP (Wet able power), ppm (parts per million), different letter in a column means significant result at P = 0.05 level

In Vitro effect of different fungicides against *Fusarium Oxsporum* after 5 days of incubation

The result found that five days of brooding period adequacy of all fungicides against *Fusarium oxsporum* differed constantly. The flumax fungicides recorded three fixations indicated measurably huge outcome with mycelial growth hindrance i.e. 0.51 cm (Table 2). The Gemstar super applied @ 250 ppm indicated factually huge outcome with mycelial development restraint 0.60 cm. While Alliete, and cymoxanil were demonstrated transitional impact to control the mycelial advance of *Fusarium oxsporum.* The result recorded strong *Co-efficient of determination* (R^2) with highly significant (P<0.01) relationship of concentrations and fungicides (Table 3) five days after incubations.

Table 3: Analysis of varience showing relationships of fungicides with concentrations at different incubation periods

Treatments	Incubation Periods									
	3 Days				5 Days			7 Days		
	DF	SS	MS	F	SS	MS	F	SS	MS	F
Concentration	2	0.003	0.0015	**	0.0052	0.0026	**	0.0052	0.0026	**
Fungicides	4	2.7794	0.6949	**	4.8592	1.21481	**	80.0638	20.0159	**
Conc* Fung	8	0.0023	0.0003	*NS*	0.002	0.00025	**	0.002	0.0002	**
Error	30	0.0034	0.0001		0.0019	0.00006		0.0019	0.0001	
Total	44	2.7881			4.8684			80.0729		

Whereas Conc means concentrations, Fung means fungicides, DF (degree of freedom), SS (sum of square), MS (mean square), P<0.01 (**), P >0.05 (NS), P <0.05 (*)

In Vitro effect of fungicides against *Fusarium Oxsporum* after 7 days of incubation

The Flumax fungicides at all three ppm concentrations indicated critical outcome with mycelial development hindrance 0.51cm. Gemstar super at fixations 250 ppm was additionally demonstrated factually critical outcome with mycelial development restraint 0.60 cm (Table 2). The Alliete, and cymoxanil fungicides were indicated middle of the road impact in diminishing the mycelial movement of *Fusarium Oxsporum*. Similarly, the result recorded strong *Co-efficient of determination* (R^2) with highly significant (P<0.01) relationship of concentrations and fungicides (Table 3) seven days after incubations.

Our results are in line with the researchers who reported that pathogens of wheat crops can be controlled by Penconazole (Desai et al., 2002). These results are also with the agreement that Cyproconazole and Triadimenol fungicides were observed to involve for the management of ailment (Souza et al., 2003).

The results found that seed treatment with benomyl + thiram eradicated (*F. graminearum*) (*Gibberellazeae*), Fenapronil, H 719 + imazalil + thiabendazole, CGA 64251 + imazalil + thiabendazole, and imazalil gave better management of pathogens resulted good control (Diehl *et al*. 1983)**.** These results are in line with the researcher's who reported that fungal pathogens attack on the wheat crops against seed borne pathogens (Islam *et al.* 2015).

Carbendazim is very effective fungicide against *A.alternata* pathogen showed very little resistance against this fungicide. Thus, he recommended it to control diseases caused by *A. alternate.* Seed treatment is also one important way to manage the seed borne diseases. Three fungicides Thiram, Captan and metalaxyl are used to control the seed borne, root rot and other diseases. Thiram and captan are used as foliar fungicides and sprayed. The mixture of metalaxyl and Thiram is used broadly as protectant against fungal diseases (Nikanth 2012).

The previous studies found that *Alternaria, Aspergillus and Fusarium* pathogens found in the form of pre-harvest fungal pollutant that affected refining process and become potential risk of toxic materials. The other studies found that *A. alternate* fungus may be the reason of black-point disease in ecosystems processes (Logrieco *et al*., 2003, Iqbal et al., 2014).

It is concluded that significant effect of flumax fungicide gave significantly high outcomes at all days at these three concentrations. These results followed by Gemstar super and cymoxanil, however Aliette was not recorded better control against mycelial growth of *Fussarium oxysporum*.

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