International Journal of Academic and Applied Research (IJAAR)
ISSN: 2643-9603
Vol. 3 Issue 8, August – 2019, Pages: 32-45

In Vivo Evaluation of Seed Dressing Fungicides against Karnal Bunt of Wheat

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Abstract: *Disease management is a key component of high-yielding wheat production. Seed treatment fungicides form low cost crop insurance. In order to assess the efficacy of different fungicides as a seed dressing against karnal bunt of wheat caused by Tilletia indica, an in vivo study was conducted at the experimental field of Agriculture Research Institute Quetta during 2014. In vitro study was carried out to isolate seed borne fungi from wheat grains collected from Tandojam city. In vivo study was comprised of six fungicides treatments (T_1 = Vibrax, T_2 = Thiophenate, T_3 = Definite, T_4 = Protocol, T_5 = Success and T_6 = Control) which was tested against karnal bunt of wheat in randomized complete block design with three replication. The results showed that wheat grains cultured on potato dextrose medium indicated colonies of seven fungal genera including Atlernaria, Aspergillus, Fusarium, Rhizopus and Tilletia presenting twelve fungal species. Among them, the Aspergillus flavus was dominant with 18.8%occurrence. The in vivo study revealed that in comparison to control seed dressing with Vibrax and Thiophenate exhibited maximum germination percentage (97.73 and 98.00), less disease incidence (7.96%), less infected spikes and spikelets, 65.23 grains spike^{-1}, tillers m^{-2} (413.67), biological yield (9114 kg ha^{-1}), grain yield (3851 kg ha^{-1}), seed index (43.03 g) and harvest index (45.18%). However, untreated seed showed maximum number of 197 infected spikes m^{-2} treatment^{-1} and 20.67 spikelets spike^{-1} respectively. Among the five fungicides, Vibrax and Thiophenate showed higher suppressiveness as a seed dressing and improved germination percentage, yield and yield components of wheat with less disease incidence and no affected spikes and spikelets. So, it can be inferred from this study that seed borne disease like karnal bunt of wheat can be controlled or managed by using systemic fungicides as seed dressing.*

Keywords: in vivo evaluation of seed dressing fungicides against karnal bunt of wheat

1. INTRODUCTION

Wheat is the staple food after rice in the world and first in Pakistan. According to the world's wheat statistics that 653 million tonnes of wheat produced from 217 million hectare during 2010 which account for 27% of the total production of cereal (FAOSTAT, 2013), in which the continent wise contribution was included Asia 45%, Africa 3%, America 17%, Europe 31% and Oceania 3%. The world top ten leading wheat producing countries are European union, China, India, United States, Russian Federation, Australia, Canada, Pakistan, Turkey and Ukraine and their total wheat production during 2012 was 135.0, 115.5, 88.3, 59.0, 56.8, 26.0, 26.1, 24.0, 19.4 and 14.0 million tonnes (FAO, 2012; Food Outlook, 2012) Wheat is the main source of protein and energy and it meet the 20.04% protein and 18.8% energy requirement of the total global supply (FAOSTAT, 2013). The total area of the country cultivated by wheat during 2012-13 was 8.64 million hectares with production of 24.3 million tonnes (GOP, 2013). However, wheat yield in Pakistan is low as compared to other countries and its present production is 2.5 to 3 tonnes ha^{-1} against the potential yield of 6 tonnes ha^{-1} (FAO, 2013). In Pakistan wheat crop provide more than 72% of dietary based caloric energy and its per capita consumption is 124 kg year^{-1} which is comparatively highest in the world (USDA Foreign Agriculture Service Report, 2010). This indicates the high importance of wheat crop in Pakistan. Wheat is grown by 80% of farmers in Pakistan that make 14% of value added in whole agriculture and 3.0% of GDP in 2009. There are many reasons of yield reduction in wheat including diseases, insect pests, and weeds infestations. The diseases such as powdery mildew caused by *Blumeria graminis* f.sp. *tritici* (Cunfer, 2002), the rust diseases (Saari and Prescott 1985) including stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*) and stripe rust or yellow rust (Chen, 2005) caused by *Puccinia striiformis*, tan spot caused by *Pyrenophora tritici-repentis* (Friesen *et al.* 2005), fusarium head blight is *Giberrella zeae* (*anamorph: F. graminearum*).

G. zeae (Goswami and Corby Kistler, 2004) and kernel bunt (*Tellicia indica*) pose a major threat to wheat production. Out of them, only leaf and stripe rust could affect production on approximately 60 (63%) and 43 (46%) million hectares if susceptible varieties are grown (Singh *et al.* 2005). The cereal nematodes include the sedentary cereal cyst nematode (CCN) (*Heterodera spp*.), which is complex, with several species and pathotypes and two species of migratory endoparasitic root lesion nematodes (RLN) (*Pratylenchus thornei*, *P. neglectus*). Both CCN and RLN have global distribution and can cause significant yield losses in cereals worldwide (Nicol *et al.* 2003).

Seed borne diseases of wheat like Kernel bunt (*Tilletia inidica)*, Loose smut (*Ustilago nuda* tritici), Head blight or scab (*Fusarium* spp*)* and Tundu or ear cockle (*Clavibacter tritici* and *Anguina tritici*) are considered as the constraints in wheat cultivation that affect crop yield and grain quality (Agarwal *et al*., 2008; Kumar *et al*., 2008). In addition, diseased seed may have toxic effects on humans and animals. The major impact of Karnal bunt of wheat is on quality and not yield reduction (Brennan *et al*., 1990; Pamela *et al.,* 2004). Symptoms appear after ears emerge. Plants are often stunted and sometimes have yellow streaks along the flag leaf. Infected ears are dark grey-green with slightly gaping glumes. Bunt balls replace all grains and, if broken, release millions of black spores smelling of rotten fish (Warham *et al*., 1986; Oxley *et al.,* 2014). Fungal hyphae concentrate in the inflorescence and spikelets, transforming the ovary into smutted tissues. The disease has been reported to occur in other parts of the world such as Pakistan, Nepal, Iraq, Iran, Afghanistan and Mexico (Begum and Mathur, 1989; Gill *et al*., 1983; Joshi *et al.,* 1983; Matsumoto and Bell, 1989; Singh, 1994; Warham, 1986). It was reported to occur in several limited areas of the Southwestern US (Bonde *et al*., 1997; Ykema *et al*., 1996) and now Karnal bunt pathogen is considered as a major threat to the wheat industry of the USA (Anon, 1991). The movement of wheat seeds from one country to another is strictly regulated through quarantine measures to restrict the entry of Karnal bunt infected wheat grains. Therefore, Karnal bunt acts as a genuine non-tariff trade barrier in commercial seed trade in the international markets and thus threatens wheat trade because export of Karnal bunt infected wheat grains would disseminate seed borne pathogens within and between regions, countries and continents (Kumar et al., 2008).

REVIEW OF LITERATURE

Important Wheat Seed-borne Fungi

Khanzada *et al.* (2002) stated Seed-borne fungal pathogen present externally or internally may cause seed abortion, seed rot and seed necrosis Some plant pathogenic fungi kill seedlings shortly after they emerge, whereas others cause Serious disease epidemics after being transmitted from seeds to seedlings. Seed-bone diseases also affect the growth and productivity of wheat.

Saberi *et al.* (2004) reported that infected or contaminated seeds serve as major source of inoculum for large number of plant pathogens which may infect the seeds and survive as spore or resting structures on or within the seeds. Wheat seed harbor several species of fungi, which can reduce seed quality and cause plant disease. Fungi carried on or within seeds

The teliospores of the Karnal bunt fungus can survive in soil for more than 5 years (Krishna and Singh, 1983; Babadoost *et al*., 2004; Bonde *et al.,* 2004). Although many control strategies have been suggested for the management of Karnal bunt disease and the strategies include seed treatment with hot water and solar energy, seed treatment with fungicides and soil drenching with fungicides (Anonymous, 2005), however, the results were not convincing. The cheapest and the most feasible method of Karnal bunt control is the use of host resistance and breeding for varieties resistant to Karnal bunt disease.

Control of Karnal bunt has now become a major concern in Pakistan due to scarcity or non-availability of resistance in commercial wheat varieties under cultivation. The gravity of the situation of the disease calls for evaluation of fungicitoxicants against the disease for its management. Epidemiological factors have great influence on the epidemic development of karnal bunt disease. Wheat is vulnerable to Karnal bunt fungus only during a 2-3 week windows of its' physiological development stages if the environmental conditions happen to be conducive during this short period for successful infection and the weather favourable for the disease development does not exist every year (Workneh *et al*., 2008). Although many control strategies have been suggested for the management of Karnal bunt disease and the strategies include seed treatment with hot water and solar energy, seed treatment with fungicides and soil drenching with fungicides (Workneh et al., 2008). Seed dressing fungicides have received a considerable attention for the last two decades and have proved inexpensive. The technique is also relatively a simple method of controlling many seedborne pathogens like *Septona nodorum* (Verma, 1983), *Puccinia gramillis* tritic! (Rakotondradon and Line, 1984), *P. recondita* and *P. striiformis* (Rakotondradon and Line, 1984), *Ustilago tritici* and *Urocystis agroperion* (Kausar, 1955), *Tilletia spp*. (Iren *et at*., 1982), *Mycosphaeretla gramillicola* (Brown, 1984), *Fusarium gramicanm*, (Diehl and Reis, 1983) and protects germinating seeds and seedlings against most of soil borne pathogens.

Keeping in view the importance of fungicides in management of wheat disease, the proposed study was conducted under field condition to investigate the efficacy of seed dressing fungicides against kernel bunt fungal disease caused by *Tilletia indica* and growth and yield of wheat with following objectives.

reduce seed germination, seedling emergence lead to less vigorous seedling.

Klyszejko *et al.* (2005) reported seed borne fungi in wheat include *Alternaria spp, Aspergillus spp, Cladosporium spp, Claviceps spp, Cochliobolus spp, Curvularia spp, Dilophosphora spp, Drechslera spp, Didymella spp, Fusarium spp, Gaeumannomyces spp, Gibberellazeae spp, Hymenella spp, Lasiopiplodia spp, Leptosphaeria spp, Leptosphaeria spp, Monographella spp, Mycosphaerella spp, Nigrospora spp, Pseudoseptoria spp, Puccinia spp, Pyrenophora spp, Sclerophthora spp, Setosphaeria spp, Tilletia spp, Urocystis spp* and *Ustilago spp.*

Hashmi and Ghaffar (2006) identified 21 species of fungi from wheat seeds by employing standard blotter method and deep freezing method; predominant species found were *Absidia spp, Alternaria spp, Aspergillus spp, A. candidus, A. flavus, A. niger, A.sulphureus, Cephalosprium spp, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera halodes, D. hawaiiensis, D. tetramera, Fusarium moniliforme, Fusarium oxysporum, F. pallidoroseum, F. subglutinans, Penicillium spp, Rhizoctonia solani and Rhizopus spp.*

Zare *et al.* (2006) reported the association of *Fusarium culmorum* (15.5%), *F. Graminearum* (13.1%), *B. sorokiniana* (24.4%), *Drecshslera tritici-repentis* (4.5%), *Alternaria alternata* (8.5%), *Cladosporium sphaerospermum* (24.2%), *Penicillium spp* (4.7%), *Aspergillus niger* (5%), *Aspergillus flavus* (9%) and *Rhizopus* (12%) with wheat seeds when assayed under blotter method. In the study, the number of colonies of *Aspergillus* and *Rhizopus* were increased by 27 and 64 %, respectively under agar method. Whereas the colonies of *Cladosporium*, *Penicillium*, *Fusarium* and *Phoma* were reduced in numbers.

Rehman *et al.* (2010) isolated some species of fungi from freshly harvested wheat seeds using agar plate method, eight genera and 13 species were isolated. These species were included *Alternaria alternate, Alternaria tenussima, Fusarium nivale, Fusarium graminearum, Fusarium heterosporum, Fusarium proliferatum, Fusarium sporotrichioides, Fusarium tricinctum, Fusarium semitecum, Aspergillus niger, Mucor spp, Rhizopus spp, Curvularia lunata, Bipolar specifera* and *Stemphylium herbarum.*

Habib *et al.* (2011) described the predominant fungal speices associated with wheat seeds when assayed under agar method compared to blotter method. These species were included *Alternaria alternata, Ulocladium alternariae, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Fusarium proliferatum, Cladosporium cladosporioides, Rhizopus spp*. and *Penicillium spp*

Hajihasani *et al.* (2012) collected 53 seed samples from harvested seed loads of irrigated wheat fields in Markazi province in the central of Iran. Isolation and identification of seed-borne fungi were conducted according to standard tests described by the International Seed Testing Association (ISTA). A total of 15 fungal species including *Tilletia laevis, Tilletia tritici, Ustilago tritici, Fusarium graminearum, Fusarium culmorum, Microdochium nivale, Bipolaris sorokiniana, Alternaria alternata, Curvularia sp., Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Penicillium sp., Mucor sp.* and *Rhizopus sp.* were identified in three wheat cultivars of Backcross Roshan, Alvand and C-78-14. The average of infection level in tested samples to both *T. laevis and T. tritici* was estimated as much as 7.1% in the province. The average of infection rate by *U. tritici* in seed samples was 1.3% while it was as much as 17.4% for both *F. culmorum* and *B. sorokiniana* in the province. The frequency of *A. niger* and *Penicillium sp.* was predominant with an infection range of 37.8 and 29.1%, respectively.

Pathak and Razia (2013) studied seed mycoflora associated with wheat on different media with a particular reference to Blotter and potato dextrose agar (PDA) procedures of ISTA. Seed-borne fungi, viz. *Fusarium moniliforme, Rhizopus spp., Mucor spp., Alternaria alternata, Aspergillus niger, Aspergillus flavus, Curvularia lunata, Drechslera spp, Alternaria spp.* and *Penicillium spp.*, were isolated from the variety HD264. Blotter method was found to be the best media for the isolation of mycoflora whether borne externally or internally. The effect of seed treatment with different chemicals and eco-friendly botanicals was analysed on germination, and growth, better percentage of seed germination and reduction in fungal pathogen were due to biochemical seed treatment.

Senbeta1 and Abdella (2014) conducted study to assess the prevalence of fungi associated with stored wheat grains under the storage conditions of farmers in Shashemene and Arsi Nagelle districts from June to July, 2013. Stratified random sampling technique was used to collect wheat grains from the study sites. A Malt Extract and Potato Dextrose Agar were used for the isolation of fungi. Fungi were identified based on the microscopic examination of spore morphology and culture characteristics of the isolates. A total of 898 fungal isolates belonging to five genera and three unidentified taxa were obtained. The isolated mycoflora were dominated by the morphotaxa of Aspergillus (45.54%) and Penicillium (29.18%), respectively. It was concluded that stored wheat from the study areas could be contaminated by storage fungi and therefore, awareness creation and training should be given to the farmers on better and improved storage techniques.

Seed dressing fungicides

Warham *et al.* (1989) carried out screening of 47 products, wheat seed treatments that reduced teliospore germination of

T. indica appeared to be limited in the length of their activity. Most were effective for up to 6 months, but only a few for longer. Of those with a longer period of activity, triphenyltin hydroxide (use now discontinued in USA and elsewhere), methoxyethylmercury acetate and ethylmercury chloride were effective for up to 18 months. However, with the possible exception of the mercurial compounds, none was capable of killing *T. indica* teliospores when applied to infected seeds. It was concluded that no existing chemical seed treatment can ensure that wheat seed is not carrying viable *T. indica* teliospores.

Arshad *et al.* (1995) evaluated seed dressing and spray fungicides either alone or in combination under artificially inoculated field conditions of karnal bunt of wheat to see their efficacy in reducing the disease infection and increasing grain yield and 1000-kernel weight. The seed dressing fungicides were: Duter at the rate of 2 g/kg seed, Daconil at the rate of 2 g/kg seed and spray fungicides Dithane M-45 at the rate 2g/l; Tilt at the rate of 1 ml/l. Disease severity was the lowest in Daconil + Tilt treated plots. Moreover, yield was the second highest and the 1000-kernel weight the highest in this treatment. Boot stage inoculation caused the highest infection of karnal bunt and reduced yield and kernel weight more significantly (P=0.05) than the seed or no-inoculation.

Bryson *et al.* (2002) evaluated different fungicides as seed dressing against the karnal bunt of wheat.The treatments comprised Bavistin 50 WP (carbendazim), Vitavax 75 WP (carboxin), Thiram 75 WS (thiram), Vitavax 200 WP (carboxin 3637.5% + thiram 37.5%), Vitavax 200 FF (carboxin 17.5% + thiram 17.5%), Pulsor 2F (thifluzamide), Tilt 25 EC (propiconazole), Contaf 25 EF (hexaconazole) and Raxil 2DS (tebuconazole). Raxil 2DS reduced Neovossia indica [Tilletia indica] teliospore germination between 89.60 and 100%. Raxil, Tilt, and Pulsor were very effective even at 1 g ml^{-1} kg^{-1} seed with a 47.68-74.03% reduction in teliospore germination. Thiram combined with Vitavax 200 WP and 200 FF also significantly controlled teliospore germination compared to the control after 45 and 65 days of seed treatment.

Khanzada *et al.* (2002) observed the effect of seed dressing fungicide for the control of seed-borne mycoflora of wheat that maximum germination of wheat seedling was higher with seed treated with Baytan followed by Metalaxy plus, Benlate, Captain and Dithane M-45 respectively. There was no significance difference in the germination of seeds treated with Derosal and the greatest root length was observed in seed of all varieties treated with Baytan and Vitavax followed by Benlate. The root length was significantly decreased in seeds treated with Rizolex and Derosal, but it was higher than the untreated seeds of all the seeds in all varieties treated with Baytan followed by Metalaxy plus and Benlate. Whereas, shoot length was significantly decreased in seedlings obtained from seed treated with Rizolex. Shoot length of seedlings obtained from Captain, Dithane M-45, Derolex was also greater than the seedlings germinated from untreated seeds.

Jatav *et al.* (2003) investigated the effects of Karnal bunt pathogen on yield components, germination potentials and tillering capacities of wheat. Several infected seeds did not germinate due to the complete loss of embryo, whereas point infected seeds germinated and produced tillers. In 1999/2000, 2000/2001 and 2001/2002, the rate of germination of infected seeds was 91, 88 and 90%, respectively (compared to 96, 95 and 95% in healthy seeds); Point infection of seeds reduced tillering by 29.17, 30.00 and 29.42% and yields by an average of 55.2, 56.6 and 53.8%, respectively. The reduction in yield was due to the decreased size of ear heads and seeds, and death of tillers before producing ear heads. The point infected seeds exhibited germination greater than the minimum prescribed level; however, these seeds should not be used for sowing because the pathogen, introduced into the field via infected seeds, may remain viable in the soil for several years and may provide the inoculum to cause infection in the subsequent years.

Borgen (2004) reported that in conventional agriculture the disease is controlled exclusively by fungicide seed treatment, but in organic farming these fungicides are not accepted. Previous studies in India have shown that seed treatment with extracts of Canabis sativa [Cannabis sativa], Eucalyptus globulus, Thuja sinensis and Datura stramonium was fully effective against the disease under field conditions. Later, in vitro studies have shown that also germination of spores of the Karnal bunt pathogen (Neovossia indica [Tilletia indica]) could be prevented by these plant extracts. The experiment was repeated in Denmark with extracts from the same species grown in Denmark, which has climate conditions that are very different from India. In this experiment, the same seed treatments had no or very limited effect on the frequency of the disease. The treatments were compared with indigenous methods from Europe including salty brine, Thuja leaves and lime. These methods had a significant, but insufficient effect on disease suppression.

Sharma and sharma (2004) evaluated the efficacy of Milstin (carbendazim, 50% WP), Profit (tricyclazole, 75% WP), Controll (hexaconazole, 5% EC), Vitavax (carboxin, 75% WP), Bavistin (carbendazim, 50% WP), Raxil (tebuconazole, 2% WP) and Tilt (propiconazole, 25% EC) against loose smut (*Ustilago segetum*) and karnal bunt (*Tilletia indica*) of wheat (cv. PBW 343), and paddy bunt (*T. barclayana*) in Ludhiana, Punjab, during the kharif of 2002 and rabi of 2002-03. Post-inoculation foliar sprays were given at 2, 8 and 15 days after inoculation. Seeds were also inoculated with the aforementioned fungicides. Pooled results showed that Tilt (recommended fungicide) and Controll were the most effective against karnal bunt (94.7 and 88.0% control, respectively) and paddy bunt (93.7 and 89.8%). Vitavax, Raxil and Controll were equally effective against loose smut (99.36,

95.57 and 99.87% control). Tilt as seed treatment was phytotoxic, resulting in stunting, curling, and yellowing of emerging seedlings. Controll is a promising fungicide against loose smut, karnal bunt and paddy bunt, and can be used as an alternative to recommended fungicides.

Sharma and Basandrai (2004) conducted field experiments during 1995-96 and 1996-7 in Dhaulakuan, Himachal Pradesh, India, to determine the efficacy of propiconazole (0.05%), 10% oxadixyl + 54% copper oxychloride (0.05%), triadimefon (0.05%), myclobutanil (0.05%), difenconazole [difenoconazole] (0.05%) and hexaconazole (0.05%), and leaf extracts of Vitex negundo (25%), Cassia fistula (25%), Azadirachta indica (25%), Eucalyptus tereticornis (25%) and Lantana camara (25%) against Karnal bunt (Neovossia indica [Tilletia indica]) disease of wheat (cultivars HD 2009 and WL711). Disease incidence was significantly less in all the treatments in both cultivars during 1995-96 and 1996 97. The plots sprayed with propiconazole, triadimefon, A. indica, C. fistula and difenconazole developed 0.97, 1.30, 2.30, 2.69 and 2.79% mean disease incidence, respectively, on WL-711. On HD 2009, the plots treated with propiconazole, difenconazole, A. indica, myclobutanil, triadimefon and C. fistula showed 2.17, 2.38, 3.80, 3.79, 4.31 and 4.36% disease incidence, respectively. This is thought to be the first observation on the efficacy of aqueous plant extracts against N. indica. All the leaf extracts were non-phytotoxic and markedly differed in their fungi toxicity.

Sharma *et al*. (2005) conducted greenhouse experiments to determine the efficacy of new fungicides against the karnal bunt of wheat and durum wheat, caused by *Tilletia indica*. The treatments comprised: 0.05, 0.10, 0.20, 0.40 and 0.80% Folicur (tebuconazole); 0.05, 0.10 and 0.20% Contaf (hexaconazole); 0.05, 0.10 and 0.20% Tilt (propiconazole); 50, 100 and 200 g a.i. thifluzamide/ha; and the control, applied at 48 h after fungal inoculation. Infected and healthy grains were counted in the inoculated ear heads and the percent infection was calculated. Folicur at 0.20%, Contaf at 0.10%, Tilt at 0.10% and 100 g a.i. thifluzamide ha^{-1} resulted in more than 90% karnal bunt control, while Folicur at 0.40% and 0.80%, and Contaf at 0.20% resulted in 100% bunt control.

MATERIALS AND METHODS:

Seeds treated with fungicide control many seed transmitted diseases caused by fungal pathogens. Seed treatment is a key factor in establishing a healthy and vigorous stand which optimizes the chances of obtaining high yield. This study was comprised of two phases, the first one was to collect wheat seed from local market at Tandojam and cultivated them on PDA medium for isolation and identification of pathogenic fungi while the second phase was to evaluate in vivo efficacy of seed dressing fungicides for its effect on growth and yield of wheat. The experimental details are as under:

Collection of seed sample

Wheat seed samples were collected from local markets here at Tandojam during 2014. The collected seed samples were properly packed in paper envelop, labeled and brought into the laboratory of Plant Pathology Faculty of Crop Protection Sindh Agriculture University Tandojam for the assessment of fungi responsible for causing seed borne diseases in wheat using potato dextrose medium.

Sterilization

Autoclavable materials such as agar were aseptically sterilized in an autoclave at 121^0C for 15 minutes. Petri dishes, beakers, test tubes, filter papers, and metalic materials such as spatula and forceps were sterilized using hot air oven at a temperature of 160^0C for 1 hour. The wire loops were also sterilized until red hot and allowed to cool before using 70% alcohol to wipe the work tops to prevent contamination.

Preparation of PDA medium

Potato dextrose agar was used for the cultivation of seed borne fungi as Table 1 showing the PDA formula based on one liter volume. Potato infusion was made by boiling 200 grams of sliced (washed but unpeeled) potatoes in 1 litre distilled water for 30 minutes and then decanting or straining the broth through cheesecloth. Distilled water was added such that the total volume of the suspension was made up to 1 litre. 20 grams dextrose and 15 grams agar powder was then added and the medium was sterilized by autoclaving at 15 pounds per square inch (100 kPa) for 15 minutes. After sterilization, pH of PDA medium was adjusted upto 5.6.

Isolation of fungi from wheat seeds

The collected seeds samples were washed with distilled water and surface sterilized with 5% mercuric chloride ($HgCl_2$). Then were rinsed again with sterilized distilled water to avoid the toxic effect of mercuric chloride and plated on three layered tissue paper for drying. The surface sterilized seed were plated on potato dextrose agar (PDA) @ 10 seeds per petri dish. The dishes were incubated at 25^oC for 7 days under 12 hours cycling of light and darkness. The distinct fungal growth colonized on PDA medium was purified and identified on the basis of their morphological characteristics by using the key developed by Booth (1971) and Nelson *et al.* (1983) as well as with the help of hand book "The Isolation and Identification of Fungi" by Frank .M. Dugan. Pure cultures were obtained by several transfers of the colony growth from PDA plates to clean PDA plates aseptically.

Identification

The pure culture isolates obtained from the diseased tomato seeds were used for identification purposes. Each isolate was subjected to colony and microscopic examinations during which their structural features were observed. Identification of fungi was based on the growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive

structures according to (Barnett and Hunter, 1999; Alexopoulos *et al*., 2002).

Table 1. Potato Dextrose Agar (Formula / Liter)

Ingredients	Quantity (g)
Potato Infusion from 200 g	4*
Dextrose	20
Agar	15
Final pH	5.6±0.2 (at 25°C)

*4.0 g of potato extract is equivalent to 200 g of infusion from potatoes

In vivo study

In vivo study was conducted on experimental field at Agriculture Research Institute Sariab Quetta during 2014-15 to examine the most effective fungicides against karnal bunt disease caused by *Tilletia indica* for its effect on growth and yield of wheat. The experiment was based on completely randomized block design with six treatments and three replications using plot size of 8 x 6 m. The treatments were consisted of the following fungicides along with control:

T_1 = Vibrax 72 WP (Cymoxanil 8% + Mancozeb 64%)
T_2 = Thiophenate 70 WP (Thiophanate-Methyl)
T_3 = Definite 10 WDG (Difenoconazole)
T_4 = Protocol 50% WP (Thiophanate-methyl 33.3% + Propiconazole 16.7%)
T_5 = Success 72 WP (Chlorothalonil + Metalaxyl)
T_6 = Control

Inoculum of *Tilletia indica*

The already prepared inoculum of *Tilletia indica* will be obtained from the Directorate of Agriculture Research Plant Protection, Agriculture Research Institute Sariab Quetta that was isolated by plant pathologist (Faisal Adnan) during 2014.

Seed dressing and sowing

The seeds of the wheat variety Zardana were contaminated with 5 g spores of *Tilletia indica* per kg seeds (Kietreiber, 1984). Then seeds were treated by tested fungicides. After treatment the seeds were stored at 5°C. Samples were removed for sowing of field tests 4 days after seed treatment. Germinating tests were conducted 1 week later. Seeds were dressed with inoculum of *Tilletia indica* during 24 hours before sowing using fungicides as per treatments. The seeds were then sown in plot with seed rate of 120 kg ha^{-1} keeping row to row distance of 20 cm. All the agronomic practices were performed as per procedure.

Disease incidence (%)

At maturity the kernels were picked from each plot and hand threshed. The incidence of the disease was calculated by using the following formula:

$$\text{Disease incidence} = \frac{\text{Number of bunted grains in 10 karnals}}{\text{Total number of grain in 10 karnals}} \times 100$$

To determine the severity of the disease a rating scale was used for Karnal bunt (*T. indica*), based on Aujla *et al*., (1989) and Bonde *et al.,* (1996) which is given in Table 1 and 2.

Grade of infection	0	1	2	3	4
Numerical values	0	0.25	0.50	0.75	1.00
Number of grains	200	75	50	25	10
Multiplication with numerical values	0x200	0.25x75	0.50x50	0.75x25	1.0x10
Value after multiplication	0	18.75	25	18.75	10
Gross total	0.00 + 18.75 + 25 + 18.75 + 10 = 72.25				
Total grains	200 + 75 + 50 + 25 + 10 = 360				
Coefficient of infection	72.25x 100 ÷ 360 = 20.14				

Table: 1. Rating scale used to calculate the severity of Karnal bunt of wheat

Infection category	Symptoms	Coefficient of infection
0	Healthy	0
1*	Well developed point infection c*. 25 % seed bunted.	0.25

2	Infection spreading along the groove c. 50 % seed bunted.	0.50
3	Three-quarters of the seed converted to sorus. c. 75 % seed bunted.	0.75
4	Seed completely converted to sorus. c. 100 % seed bunted.	1.0

*categories combined for use in calculations of coefficients of infection

Table: 2. An example of the calculation of coefficient of infection (from Aujla *et al.*, 1989)

Observations

When crop to reach to maturity the following observations will be recorded:

1. Number of fungi isolated from wheat seeds and their percent occurrence.
2. Germination percentage
3. Record of disease incidence
4. Total number of spike infected with *Tilletia indica* treatment^{-1}
5. Number of affected spikelet spike^{-1}
6. Number of grains spike^{-1}
7. Number tillers m^{-2}
8. Biological yield (kg ha^{-1})
9. Grain yield (kg ha^{-1})
10. Seed index
11. Harvest index

Seed index: 1000 grains weighed on an electric balance after sun drying.

Harvest index: Harvest index was calculated through formula

Harvest index = Grain yield/biological yield x 100

Biological yield: Biological yield was weighed from harvest of m^2, weighed and computed for ha^{-1} at harvest.

Grain yield: Grain yield from harvest of m^2 was threshed, weighed and computed for ha^{-1}

Statistical Analysis

The data thus obtained were analyzed statistically for analysis of variance using SPSS 12.00 software (SPSS Inc. Chicago, IL, USA) based on completely randomized block design and LSD test for comparison of mean in case of significance were calculated at $p< 0.05$ level of probability.

Fig. 1. An infected spike of wheat showing the symptoms of Karnal bunt

Fig. 2. Infected grains of wheat showing the symptoms of Karnal bunt

RESULTS:

In order to assess the efficacy of different fungicides as a seed dressing against karnal bunt of wheat caused by *Tilletia indica*, an in vivo study was conducted at the experimental field of Agriculture Research Institute Quetta during 2014. In vitro study was carried out to isolate seed borne fungi from wheat grains collected from Tandojam city. The percent occurrence of seed borne fungi are presented in Table 2 and Appendix-1.

In vivo study was comprised of six fungicides treatments (T_1 = Vibrax, T_2 = Thiophenate, T_3 = Definite, T_4 = Protocol, T_5 = Success and T_6 = Control) which was tested against karnal bunt of wheat in randomized complete block design with three replication. The effect of seed dressing fungicides was observed for seed germination, disease incidence, total number of spike infected with *Tilletia indica* treatment^{-1}, number of affected spikelet spike^{-1}, number of grains spike^{-1}, number tillers m^{-2}, biological yield (kg ha^{-1}), grain yield (kg ha^{-1}), seed index and harvest index of wheat as exhibited in Figure 3-13 and Appendix II-XII.

Isolation of fungi from wheat grains

The data regarding the number of fungal species isolated from wheat grains are presented in Table 2 and Appendix-I. The wheat grains cultured on potato dextrose medium showed colonies of seven fungal genera including *Atlernaria, Aspergillus, Fusarium, Rhizopus* and *Tilletia.* Among them, the Alternaria presented two species such as *Alternaria alternata* and *Alternaria tenuis,* Aspergillus genera reflected three species including *Aspergillus flavus, Aspergillus niger* and *Aspergillus parasiticus*, *Fusarium* genera presented two species as *Fusarium graminearum* and *Fusarium moniliforme,* while all the remaining genera revealed single species. The maximum number of mean colonies of 2.0 cfu was recorded for *Aspergillus flavus* with 18.8% occurrence followed by 1.7 and 1.3 cfu for *Aspergillus niger* and *Fusarium gramiearum* with percent occurrence of 15.6 and 12.5%. While *Pencillium sp.* presented 9.4% occurrence. However, minimum percent occurrence of *Fusarium moniliforme and Rhizopus sp.* indicated only 3.1% occurrence each respectively. But, in case of *Mucor* and *Tilletia sp.* the percent occurrence was 6.3%. Though, *Aspergillus* and *Rhizopus spp.* are storage fungi (saprophytes).

Table 2. Fungal species identified from tomato seed by culturing on potato dextrose agar

Fungi identified	Mean number of colonies (cfu)	Percent occurrence
Alternaria alternata	1.3	12.5
Alternaria tenuis	0.7	6.3
Aspergillus flavus	2.0	18.8
Aspergillus nigar	1.7	15.6
Aspergillus	0.7	6.3
Fusarium	1.3	12.5
Fusarium	0.3	3.1
Mucor sp.	0.7	6.3
Penicillium sp.	1.0	9.4
Rhizopus sp.	0.3	3.1
Tilletia sp.	0.7	6.3
Total	**10.7**	**100.0**

Germination percentage

The data regarding germination percentage of wheat seeds as affected by seed dressing fungicides against karnal bunt of wheat showed significant differences as presented in Fig.1 and Appendix-III. The analysis of variance for germination percentage was highly significant with mean square value of 680.21^{**} and 1.68% coefficient of variance (Appendix-III). Generally, the germination percentage was ranged from 100 to

In vivo study

The application of fungicides through seed dressing significantly suppressed karnal bunt disease of wheat caused by *Tilletia indica* when evaluated under in vivo condition and improved all growth traits such as germination percentage, disease incidence, number of spikes infected with *Tilletia indica* treatment^{-1}, number of affected spikelets spike^{-1}, number of grains spike^{-1}, number tillers m^{-2}, biological yield (kg ha^{-1}), grain yield (kg ha^{-1}), seed index and harvest index of wheat as exhibited in Fig. 1-10 and Appendix-III-XII. 55% with mean value of 86.8% as shown in Appendix-II. The LSD test for comparison of mean ($p<0.05$) revealed that maximum germination percentage (97.73 and 98.00) was recorded in treatment 1 and 2 where wheat grains were dressed with fungicides viz Vibrax and Thiophenate followed by 93.37% in treatment 3 when Definite was used in seed dressing and minimum germination percentage of 57.87% was observed in control plot where no fungicides was applied. Statistically, the germination percentage in Vibrax and Thiophenate was at par from each other. The standard error (SE±) for this parameter was 1.19 and leas significant difference (LSD) was 2.65. Out of six fungicides, two fungicides viz Vibrax and Thiophenate effectively protected wheat grains from any fungal infection and showed highest germination percentage. However, germination percentage was reduced in control when wheat grains were not dressed with fungicides. This explicitly suggests that seed dressing with fungicides have improved wheat seed germination substantially and without using any fungicides have resulted poor germination as indicated in the control (Fig.1).

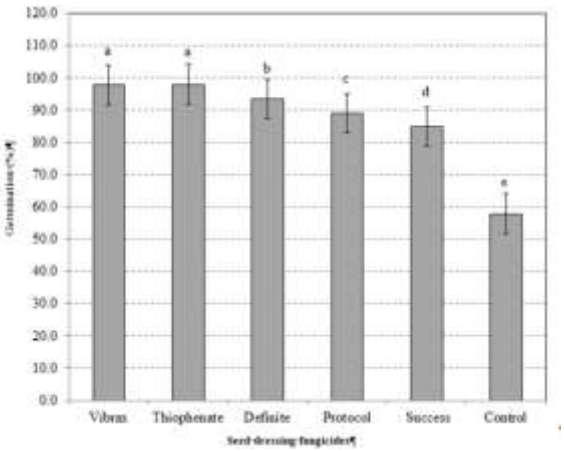

Fig.1. Germination percentage of wheat seeds as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

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Disease incidence (%)

The statistical analysis for disease incidence as affected by seed dressing fungicides against karnal bunt of wheat showed significant differences as presented in Fig.2 and Appendix-IV. The overall disease incidence was ranged from 81.2 to 7.4% with mean value of 26.9% (Appendix-II). The LSD test for comparison of mean ($p<0.05$) exhibited that maximum disease incidence (81.2%) was observed in control. Among the fungicides, three fungicides (Vibrax, Thiophenate and Definite) suppressed Karnal bunt effectively and no disease incidence was noted in these treatments. However, fungicides Protocol and Success did not prove effective as compared to other fungicide against wheat bunt but as compared to control they showed 27.43 and 26.03% disease incidence. Statistically, the disease incidence in two seed dressed fungicides (Protocol and Success) were at par from each other as well as at par differences in Vibrax and Thiophenate. The standard error (SE±) for this parameter was 1.48 and least significant difference (LSD) was 3.31. Out of six fungicides, three fungicides viz Vibrax, Thiophenate and Definite effectively protected wheat grains from any fungal infection and showed no disease incidence of karnal bunt. This explicitly suggests that seed dressing with fungicides have controlled karnal bunt of wheat effectively and without using any fungicides have resulted higher percent of disease incidence as indicated in the control (Fig.2).

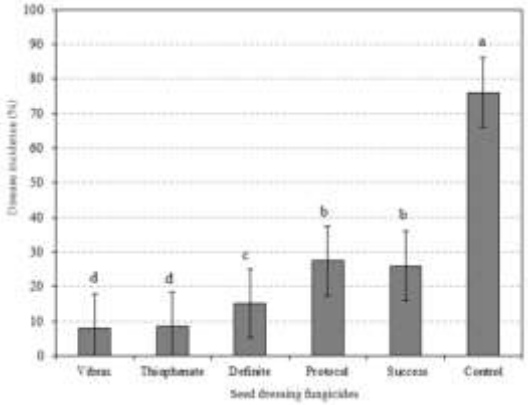

Fig.2. Disease incidence (%) of wheat seeds as affected by seed dressing fungicides gainst karnal bunt of wheat caused by *Tilletia indica*

Number of spikes infected with *Tilletia indica* treatment^{-1}

The data regarding number of spikes m^{-2} of wheat infected with *Tilletia indica* is shown in Fig.3 and Appendix-V. The analysis of variance exhibited statistically significant differences for this parameter as given in Appendix-V. The overall infected spikes per treatment and m^{-2} was ranged from 211.9 to 11.4 with mean value of 75.6 (Appendix-II). The LSD test for comparison of mean ($p<0.05$) exhibited that maximum of 197.0 infected spikes m^{-2} treatment^{-1} was observed in control treatment where wheat grains were not dressed with fungicides. Among the fungicides, three fungicides (Vibrax, Thiophenate and Definite) suppressed Karnal bunt effectively and showed less number of infected spikes in these treatments. However, fungicide Protocol did not prove effective as compared to other fungicide against wheat bunt but as compared to control Protocol showed 103.33 spikes m^{-2} treatment^{-1} followed by Success which indicated 86.33 spikes m^{-2} treatment^{-1} as affected by karnal bunt (*Tilletia indica*). Statistically, the infected spikes m^{-2} treatment^{-1} in three seed dressed fungicides (Vibrax, Thiophenate and Definite) non-significant differences. The standard error (SE±) for this parameter was 3.93 and least significant difference (LSD) was 8.76. Out of six fungicides, three fungicides viz Vibrax, Thiophenate and Definite effectively protected wheat grains from any fungal infection and showed no infected spike caused by karnal bunt. This explicitly suggests that seed dressing with fungicides have controlled karnal bunt of wheat effectively and without using any fungicides have resulted in higher number of affected spikes m^{-2} treatment^{-1} as indicated in the control (Fig.3)

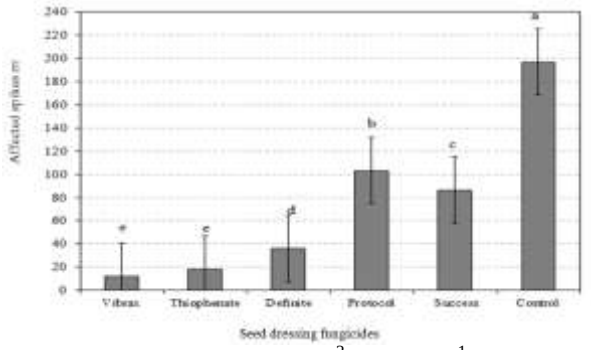

Fig.3. Number infected spikes m^{-2} treatment^{-1} as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Number of affected spikelet spike^{-1}

The data regarding number of spikelets spike^{-1} of wheat infected with *Tilletia indica* is shown in Fig.4 and Appendix-VI. The analysis of variance exhibited statistically significant differences for this parameter as given in Appendix-VI. The overall infected spikelets spike^{-1} was ranged from 22.5 to 1.0 with mean value of 6.8 (Appendix-II). The LSD test for comparison of mean ($p<0.05$) manifested that maximum of 20.67 infected spikelets spike^{-1} was observed in control treatment where wheat grains were not dressed with fungicides. Among the fungicides, three fungicides viz Vibrax, Thiophenate and Definite suppressed Karnal bunt effectively and less number of infected spikelets were recorded in these treatments. However, fungicide Success did not proved effective as compared to other fungicide against wheat bunt but as compared to control Success showed 8.0 infected spikelets spike^{-1} followed by Protocol which indicated only 6.67 spikelets spike^{-1} as affected by karnal bunt (*Tilletia indica*). Statistically, the infected spikelets spike^{-1} in three seed dressed fungicides (Vibrax, Thiophenate and

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Definite) revealed non-significant differences. The standard error (SE±) for this parameter was 0.53 and least significant difference (LSD) was 1.18. Out of six fungicides, three fungicides viz Vibrax, Thiophenate and Definite effectively protected wheat grains from any fungal infection and showed no infected spikelets spike^{-1} caused by karnal bunt. This explicitly suggests that seed dressing with fungicides have controlled karnal bunt of wheat effectively as healthy spikelets spike^{-1} and without using any fungicides have resulted in greater number of affected spikelets spike^{-1} as indicated in the control (Fig.4).

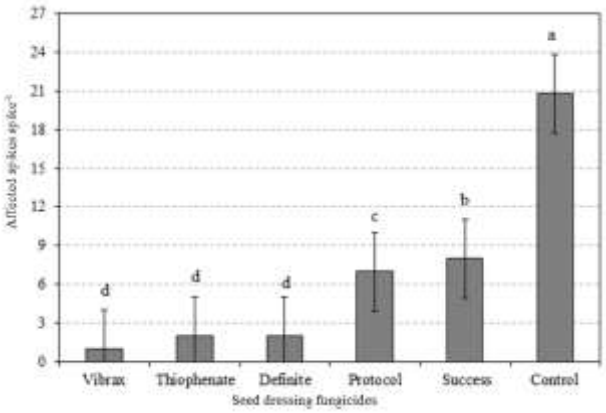

Fig.4. Number infected of spikelets spike^{-1} as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Number of grains spike^{-1}

The data regarding number of grains spike^{-1} of wheat infected with *Tilletia indica* is shown in Fig.5 and Appendix-VII. The analysis of variance showed statistically significant differences for this parameter as given in Appendix-VII. The overall infected grains spike^{-1} was ranged from 78.0 to 25.9 with mean value of 54.7 (Appendix-II). The LSD test for comparison of mean ($p<0.05$) revealed that maximum of 65.23 grains spike^{-1} was recorded in treatment 1 where wheat grains were dressed with vibrax fungicides. Among the fungicides, four fungicides viz Vibrax, Thiophenate, Definite and protocol increased grains spike^{-1} by controlling Karnal bunt effectively where no infected spikelet was recorded in these treatments. However, fungicide Protocol did not proved effective as compared to other fungicide against wheat bunt but as compared to control Protocol showed 52.0 grains spike^{-1}. Statistically, the grains spike^{-1} in two seed dressed fungicides (Vibrax and Thiophenate) showed non-significant differences. The standard error (SE±) for this parameter was 1.97 and least significant difference (LSD) was 4.38. Out of six fungicides, three fungicides viz Vibrax, Thiophenate and Definite effectively protected wheat grains from any fungal infection and showed greater number of grains spike^{-1} which not affected by karnal bunt. This explicitly suggests that seed dressing with fungicides have controlled karnal bunt of wheat effectively as healthy and greater number of grains spike^{-1} and without using any fungicides have resulted in reduction of grains spike^{-1} as indicated in the control (Fig.5).

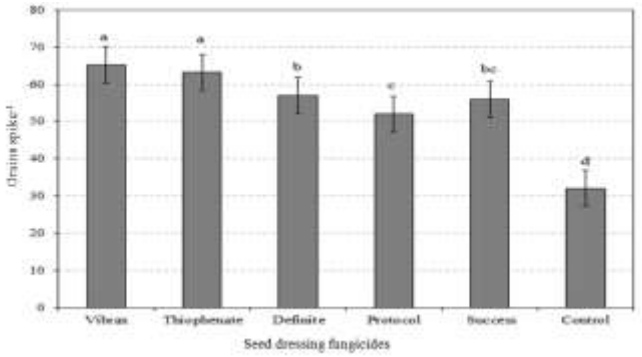

Fig.5. Number grains spike^{-1} as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Number tillers m^{-2}

The data regarding number of tillers m^{-2} of wheat infected with *Tilletia indica* is shown in Fig.6 and Appendix-VIII. The analysis of variance showed statistically significant differences for this parameter as given in Appendix-VIII. The overall number of tillers m^{-2} was ranged from 485.0 to 204 with mean value of 364 (Appendix-II). The LSD test for comparison of mean ($p<0.05$) showed that greater number of tillers m^{-2} (413.67) was recorded in treatment 1 where wheat grains were dressed with vibrax fungicides. Among the fungicides, two fungicides viz Vibrax and Thiophenate increased number of tillers m^{-2} by controlling Karnal bunt effectively where no infected spike was recorded in these treatments. However, fungicide Success did not proved effective as compared to other fungicide against wheat bunt but as compared to control success showed 343.0 tillers m^{-2}. Statistically, the tillers m^{-2} in three seed dressed fungicides (Thiophenate, Definite and Protocol) showed non-significant differences from one another. Whereas, the minimum (244.67) number of tillers m^{-2} was recorded in control plot where grains were not dressed with fungicides.The standard error (SE±) for this parameter was 8.67 and least significant difference (LSD) was 19.32. Out of six fungicides, two fungicides viz Vibrax and Thiophenate effectively protected wheat grains from any fungal infection and showed greater number of tillers m^{-2} which were not affected by karnal bunt. This explicitly suggests that seed dressing with fungicides have controlled karnal bunt of wheat effectively that resulted in healthy and greater number of tillers m^{-2} and without using any fungicides have reduced number of tillers m^{-1} as recorded in the control plot (Fig.6).

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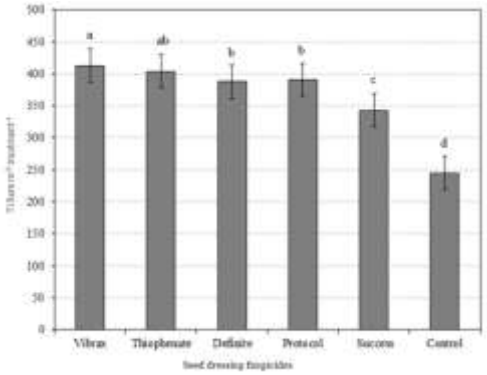

Fig.6. Number tillers m^{-2} as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Biological yield (kg ha^{-1})

The data regarding biological yield of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica* is depicted in Fig.7 and Appendix-IX. The analysis of variance showed statistically highly significant differences for this parameter as given in Appendix-IX. The overall biological yield (kg ha^{-1}) was ranged from 9432 to 6583 kg ha^{-1} with mean value of 8153 kg ha^{-1} (Appendix-II). The LSD test for comparison of mean ($p<0.05$) showed that higher biological yield (9114 kg ha^{-1}) was found in treatment 1 where wheat grains were dressed with Vibrax fungicide followed 8513 kg ha^{-1} was exhibited by Thiophenate seed dressed fungicide. While, lower biological yield (6858 kg ha^{-1}) was noted in control plot where seed were not dressed with fungicides. Statistically, all seed dressed fungicides produced significantly different biological yield. The standard error (SE±) for this parameter was 8.76 and least significant difference (LSD) was 19.52. When compared to control, it become evident that seed dressing with fungicides have controlled karnal bunt of wheat effectively that resulted in non-significantly higher biological yield and without using any fungicides have reduced biological yield of wheat as recorded in the control plot (Fig.7).

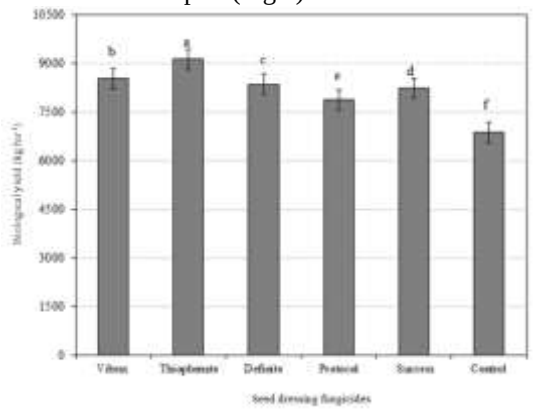

Fig.7. Biological yield (kg ha^{-1}) of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Grain yield (kg ha^{-1})

The data regarding grain yield of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica* is presented in Fig.8 and Appendix-X. The analysis of variance showed statistically highly significant differences for this parameter as shown in Appendix-X. The overall grain yield (kg ha^{-1}) was ranged from 4242.6 to 2159.3 kg ha^{-1} with mean value of 3318.1 kg ha^{-1} (Appendix-II). The LSD test for comparison of mean ($p<0.05$) manifested higher grain yield (3851.3 kg ha^{-1}) in treatment 1 where wheat grains were dressed with Vibrax fungicide followed 3768.7 kg ha^{-1} was exhibited by Thiophenate seed dressed fungicide. While, lower grain yield (2360.7 kg ha^{-1}) was found in control plot where seeds were not dressed with fungicides. Statistically, grain yield was at par from each other at Definite and Success seed dressed. The evaluated fungicides efficacy manifested that Protocol fungicide for seed dressing was less effective as compared to other fungicides in grain yield production but higher over control. The standard error (SE±) for this parameter was 40.29 and least significant difference (LSD) was 89.78. When compared to control, it become obvious that seed dressing with fungicides have controlled karnal bunt of wheat effectively that resulted in significantly higher grain yield and without using any fungicides have reduced grain yield of wheat as recorded in the control plot (Fig.8).

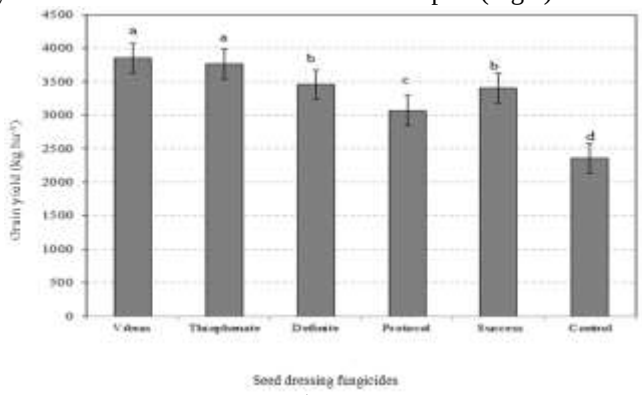

Fig.8. Grain yield (kg ha^{-1}) of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Seed index (g)

The statistical analysis for seed index (g) of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica* is presented in Fig.9 and Appendix-XI. The analysis of variance showed statistically highly significant differences for this parameter as given in Appendix-XI. The overall seed index (g) was ranged from 45.7 to 32.7 g with mean value of 39.3 g (Appendix-II). The LSD test for comparison of mean ($p<0.05$) manifested greater seed index of 43.03 g in treatment 1 where wheat grains were dressed with Vibrax fungicide followed 40.93 g seed index in Thiophenate seed dressed fungicide. While, lower seed index (34.60 g) was found in control plot where seeds were not dressed with fungicides. Statistically, seed index was at par from each other at Thiophenate and Success seed dressed. The

evaluated fungicides efficacy manifested that Protocol fungicide for seed dressing was less effective as compared to other fungicides in respect of seed index but higher over control. The standard error (SE±) for this parameter was 0.29 and least significant difference (LSD) was 0.65. When compared to control, it become evident that seed dressing with fungicides have controlled karnal bunt of wheat effectively that resulted in significantly higher seed index and without using any fungicides have resulted in reduced seed index of wheat as recorded in the control plot (Fig.9).

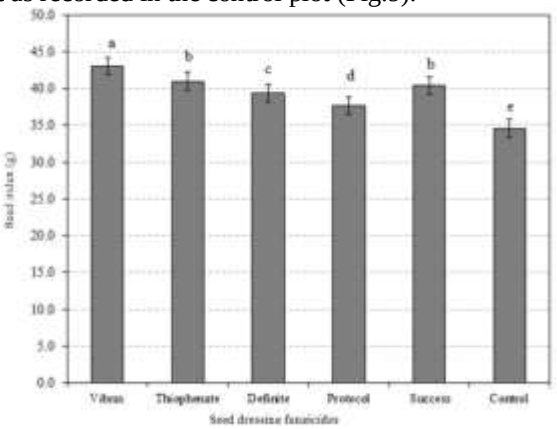

Fig.9. Seed index (g) of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Harvest index (%)

The statistical analysis for harvest index (%) of wheat under the influence of seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica* is shown in Fig.10 and Appendix-XII. The analysis of variance indicated statistically highly significant differences for this parameter as given in Appendix-XII. The overall harvest index was ranged from 48.5 to 32.8% with mean value of 40.5% (Appendix-II). The LSD test for comparison of mean ($p<0.05$) revealed higher harvest index of 45.18% in treatment 1 where wheat grains were dressed with Vibrax fungicide followed 42.06% harvest index in Thiophenate seed dressed fungicide. While, minimum harvest index (34.38%) was found in control plot where seed were not dressed with fungicides. The evaluated fungicides efficacy manifested that Protocol fungicide for seed dressing was less effective as compared to other fungicides in respect of harvest index but higher over control. The standard error (SE±) for this parameter was 0.63 and least significant difference (LSD) was 1.41. When compared to control, it become evident that seed dressing with fungicides have controlled karnal bunt of wheat effectively that resulted in significantly greater harvest index and without using any fungicides have resulted in reduced harvest index of wheat as recorded in the control plot (Fig.10).

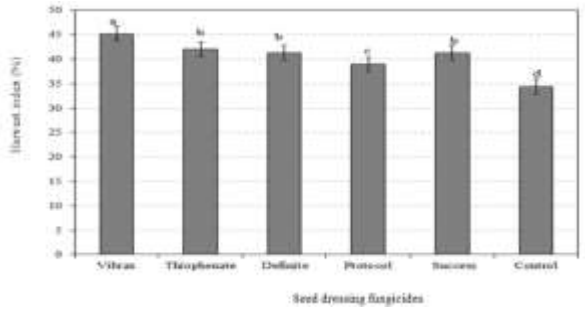

Fig.10. Harvest index (%) of wheat as affected by seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica*

Conclusions:

The isolation of fungal pathogens from wheat seeds showed *Aspergillus flavus* as the dominant fungal pathogen with higher percent occurrence. The results obtained from the in vivo study conducted for the efficacy of seed dressing fungicides against karnal bunt of wheat caused by *Tilletia indica* suggested that among the five fungicides, Vibrax and Thiophenate showed higher suppressiveness as a seed dressing and improved germination percentage, yield and yield components of wheat with less disease incidence and minimum affected spikes and spikelets. So, it can be inferred from this study that seed borne disease of wheat can be controlled or managed by using systemic fungicides as seed dressing.

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