

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 Partial Bunt of wheat caused by *Tilletia indica*, an in vivo study was conducted at the experimental field of Agriculture Research Institute Quetta during 2018. 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 100, T_2 = Thiophenate 540, T_3 = Definite, T_4 = Protocol, T_5 = Success and T_6 = Control) which was tested against Partial 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 *Atternaria*, *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 100 and Thiophenate 540 exhibited maximum germination percentage (98.83 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.

Keywords: seed dressing fungicides against Partial Bunt of wheat

INTRODUCTION:

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)[1], the rust diseases (Saari and Prescott 1985)[2] including stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*) and stripe rust or yellow rust (Chen, 2005)[3] caused by *Puccinia striiformis*, tan spot caused by *Pyrenophora tritici-repentis* (Friesen et al. 2005)[4], fusarium head blight is *Giberrella zeae* (anamorph: *F. graminearum*). *G. zeae* (Goswami and Corby Kistler, 2004)[5] and kernel bunt (*Tilletia 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)[6]. 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)[7].

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)[8]. In addition, diseased seed may have toxic effects on humans and animals. The major impact of Partial Bunt of wheat is on quality and not yield reduction (Brennan et al., 1990; Pamela et al., 2004)[9]. 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)[10]. 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)[11]. It was reported to occur in several limited areas of the Southwestern US (Bonde et al., 1997; Ykema et al., 1996)[12] and now Partial Bunt pathogen is considered as a major threat to the wheat industry of the USA (Anon, 1991)[13]. The movement of wheat seeds from one country to another is strictly regulated through quarantine measures to restrict the entry of Partial Bunt infected wheat grains. Therefore, Partial 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 Partial Bunt infected wheat grains would disseminate seed borne pathogens within and between regions, countries and continents (Kumar et al., 2008)[14].

The teliospores of the Partial Bunt fungus can survive in soil for more than 5 years (Krishna and Singh, 1983; Babadoost et al., 2004; Bonde et al., 2004)[15]. Although many control strategies have been suggested for the management of Partial 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)[16], however, the results were not convincing. The cheapest and the most feasible method of Partial Bunt control is the use of host resistance and breeding for varieties resistant to Partial Bunt disease.

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°C for 15 minutes. Petri dishes, beakers, test tubes, filter papers, and metallic materials such as spatula and forceps were sterilized using hot air oven at a temperature of 160°C 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 (HgCl₂). 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°C 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 Partial 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:

- T₁ = Vibrax 100 72 WP (Cymoxanil 8% + Mancozeb 64%)
 T₂ = Thiophenate 540 70 WP (Thiophanate-Methyl)
 T₃ = Definite 10 WDG (Difenoconazole)
 T₄ = Protocol 50% WP (Thiophanate-methyl 33.3% + Propiconazole 16.7%)
 T₅ = Success 72 WP (Chlorothalonil + Metalaxyl)
 T₆ = 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 Partial Bunt (*T. indica*), based on Aujla *et al.*, (1989) and Bonde *et al.*, (1996) which is given in Table 1 and 2.

Table: 1. Rating scale used to calculate the severity of Partial Bunt of wheat

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

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

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 m², weighed and computed for ha⁻¹ at harvest.

Grain yield: Grain yield from harvest of m² 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 Partial 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 100, T_2 = Thiophenate 540, T_3 = Definite, T_4 = Protocol, T_5 = Success and T_6 = Control) which was tested against Partial 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 *Alternaria*, *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 graminearum* with percent occurrence of 15.6 and 12.5%. While *Penicillium 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).

In vivo study

The application of fungicides through seed dressing significantly suppressed Partial 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

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

Fungi identified	Mean number of colonies (cfu)	Percent occurrence
<i>Alternaria alternata</i>	1.3	12.5
<i>Alternaria tenuis</i>	0.7	6.3
<i>Aspergillus flavus</i>	2.0	18.8
<i>Aspergillus nigar</i>	1.7	15.6
<i>Aspergillus parasiticus</i>	0.7	6.3
<i>Fusarium graminearum</i>	1.3	12.5
<i>Fusarium moniliforme</i>	0.3	3.1
<i>Mucor sp.</i>	0.7	6.3
<i>Penicillium sp.</i>	1.0	9.4
<i>Rhizopus sp.</i>	0.3	3.1
<i>Tilletia sp.</i>	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 Partial 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 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 (98.83 and 98.00) was recorded in treatment 1 and 2 where wheat grains were dressed with fungicides viz Vibrax 100 and Thiophenate 540 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 100 and Thiophenate 540 was at par from each other. The standard error (SE $\pm$) for this parameter was 1.19 and least significant difference (LSD) was 2.65. Out of six fungicides, two fungicides viz Vibrax 100 and Thiophenate 540 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).

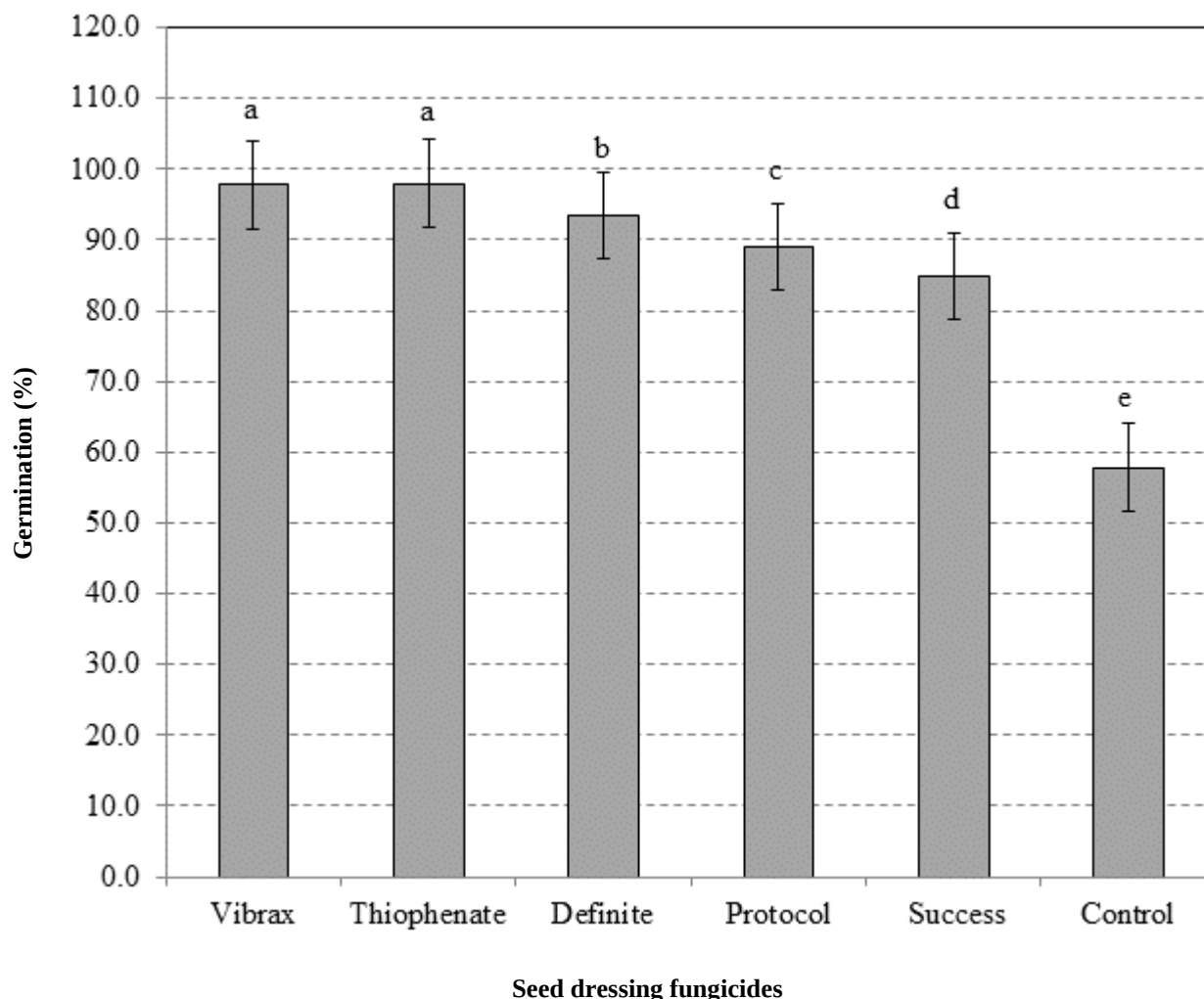


Fig.1. Germination percentage of wheat seeds as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

Disease incidence (%)

The statistical analysis for disease incidence as affected by seed dressing fungicides against Partial 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 100, Thiophenate 540 and Definite) suppressed Partial 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 100 and Thiophenate 540. The standard error ($SE \pm$) for this parameter was 1.48 and least significant difference (LSD) was 3.31. Out of six fungicides, three fungicides viz Vibrax 100, Thiophenate 540 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 Partial Bunt of wheat effectively and without using any fungicides have resulted higher percent of disease incidence as indicated in the control (Fig.2).

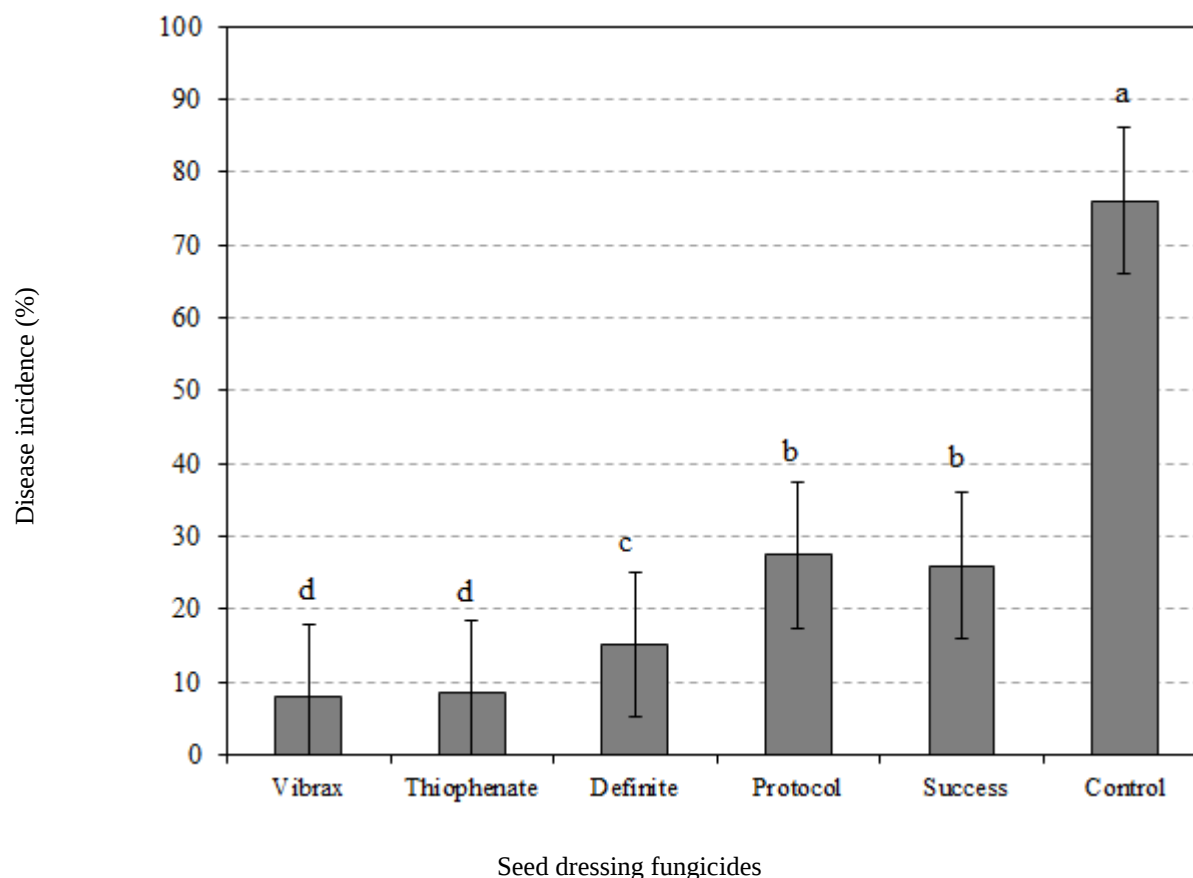
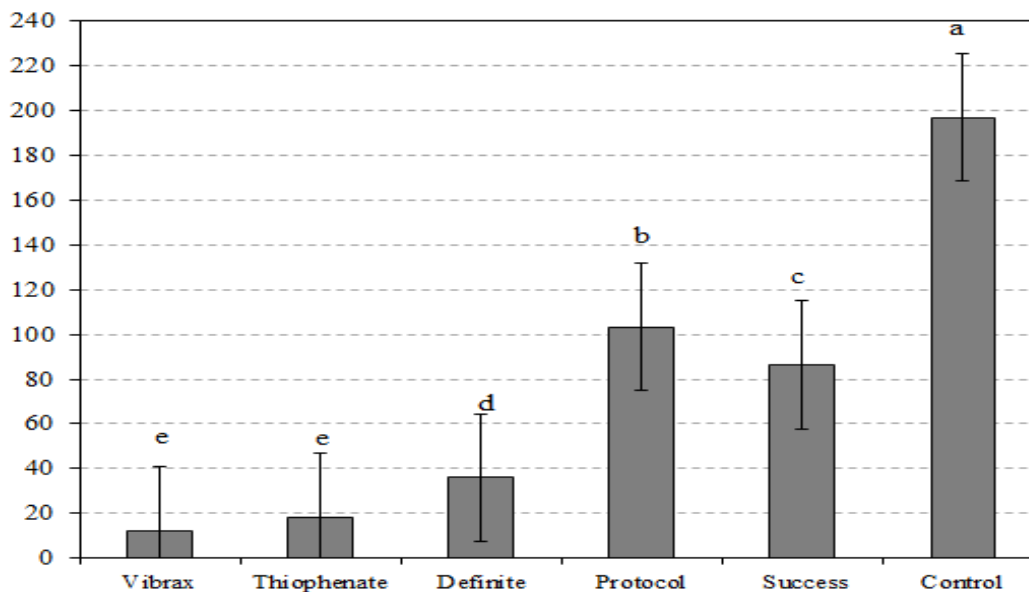


Fig.2. Disease incidence (%) of wheat seeds as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

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 100, Thiophenate 540 and Definite) suppressed Partial 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 Partial Bunt (*Tilletia indica*). Statistically, the infected spikes m^{-2} treatment⁻¹ in three seed dressed fungicides (Vibrax 100, Thiophenate 540 and Definite) non-significant differences. The standard error ($SE \pm$) for this parameter was 3.93 and least significant difference (LSD) was 8.76. Out of six fungicides, three fungicides viz Vibrax 100, Thiophenate 540 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 Partial 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).

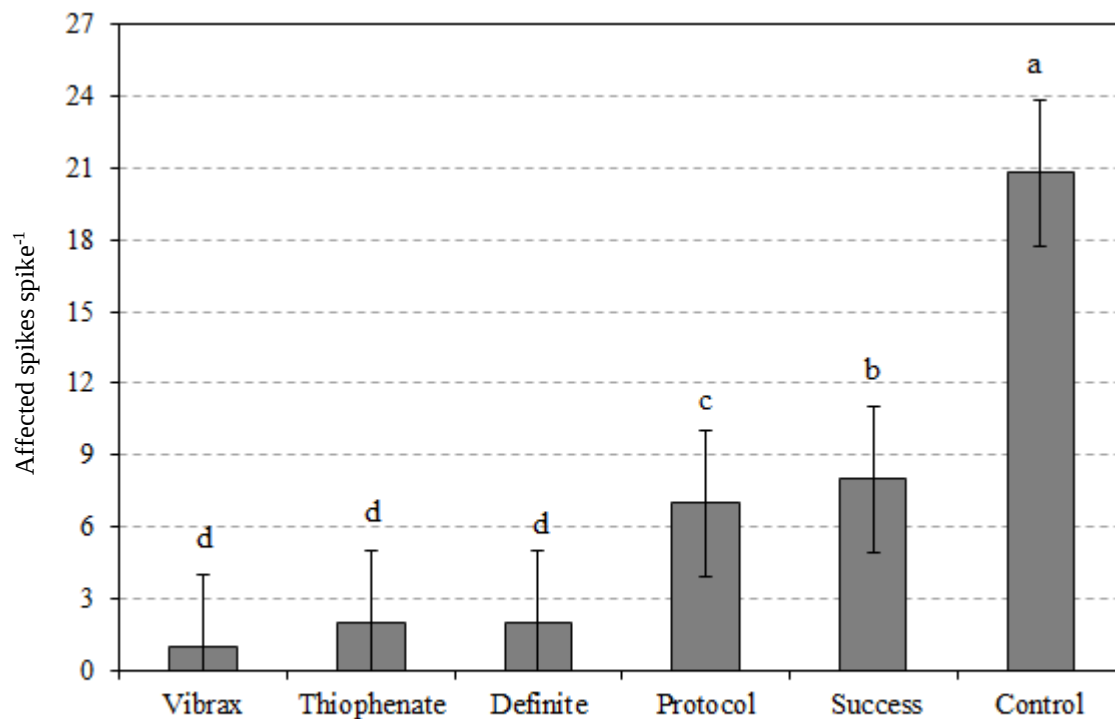


Seed dressing fungicides

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

Number of affected spikelet spike $^{-1}$

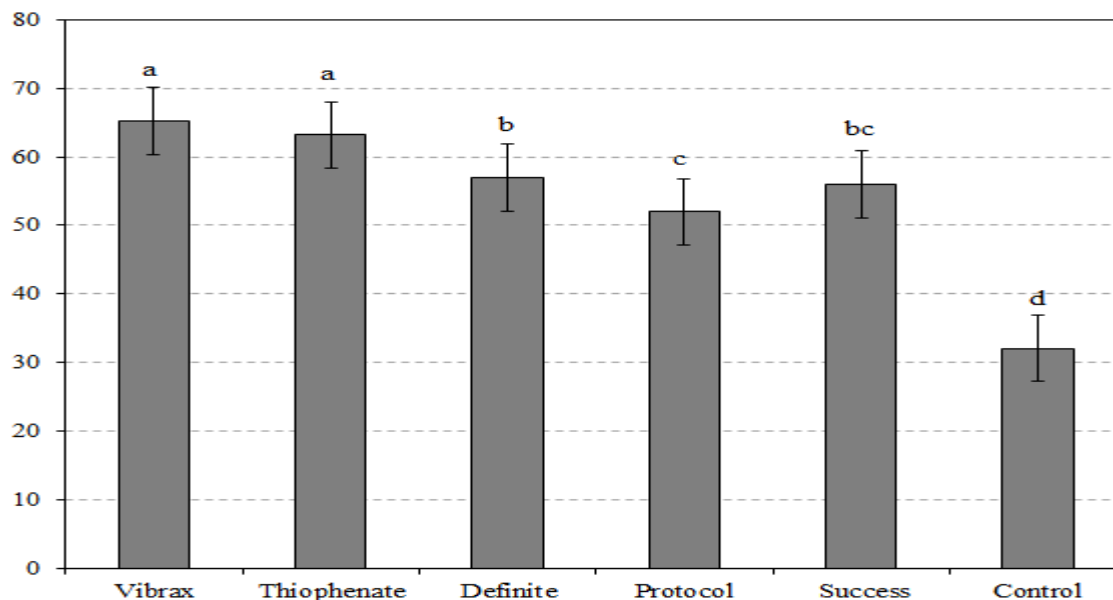
The data regarding number of spikelets spike $^{-1}$ of wheat infected with *Tilletia indica* is shown in Fig.4 and Appendix-VI. The analysis of variance exhibited statistically significant differences for this parameter as given in Appendix-VI. The overall infected spikelets spike $^{-1}$ was ranged 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 100, Thiophenate 540 and Definite suppressed Partial 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 Partial Bunt (*Tilletia indica*). Statistically, the infected spikelets spike $^{-1}$ in three seed dressed fungicides (Vibrax 100, Thiophenate 540 and Definite) revealed non-significant differences. The standard error ($SE \pm$) for this parameter was 0.53 and least significant difference (LSD) was 1.18. Out of six fungicides, three fungicides viz Vibrax 100, Thiophenate 540 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 Partial 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).



Seed dressing fungicides
Fig.4. Number infected of spikelets spike⁻¹ as affected by seed dressing fungicides against Partial 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 100 fungicides. Among the fungicides, four fungicides viz Vibrax 100, Thiophenate 540, Definite and protocol increased grains spike⁻¹ by controlling Partial 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 100 and Thiophenate 540) showed non-significant differences. The standard error ($SE \pm$) for this parameter was 1.97 and least significant difference (LSD) was 4.38. Out of six fungicides, three fungicides viz Vibrax 100, Thiophenate 540 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 Partial 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).



Seed dressing fungicides

Fig.5. Number grains spike⁻¹ as affected by seed dressing fungicides against Partial 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 100 fungicides. Among the fungicides, two fungicides viz Vibrax 100 and Thiophenate 540 increased number of tillers m⁻² by controlling Partial 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 540, 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 ($SE \pm$) for this parameter was 8.67 and least significant difference (LSD) was 19.32. Out of six fungicides, two fungicides viz Vibrax 100 and Thiophenate 540 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 Partial 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).

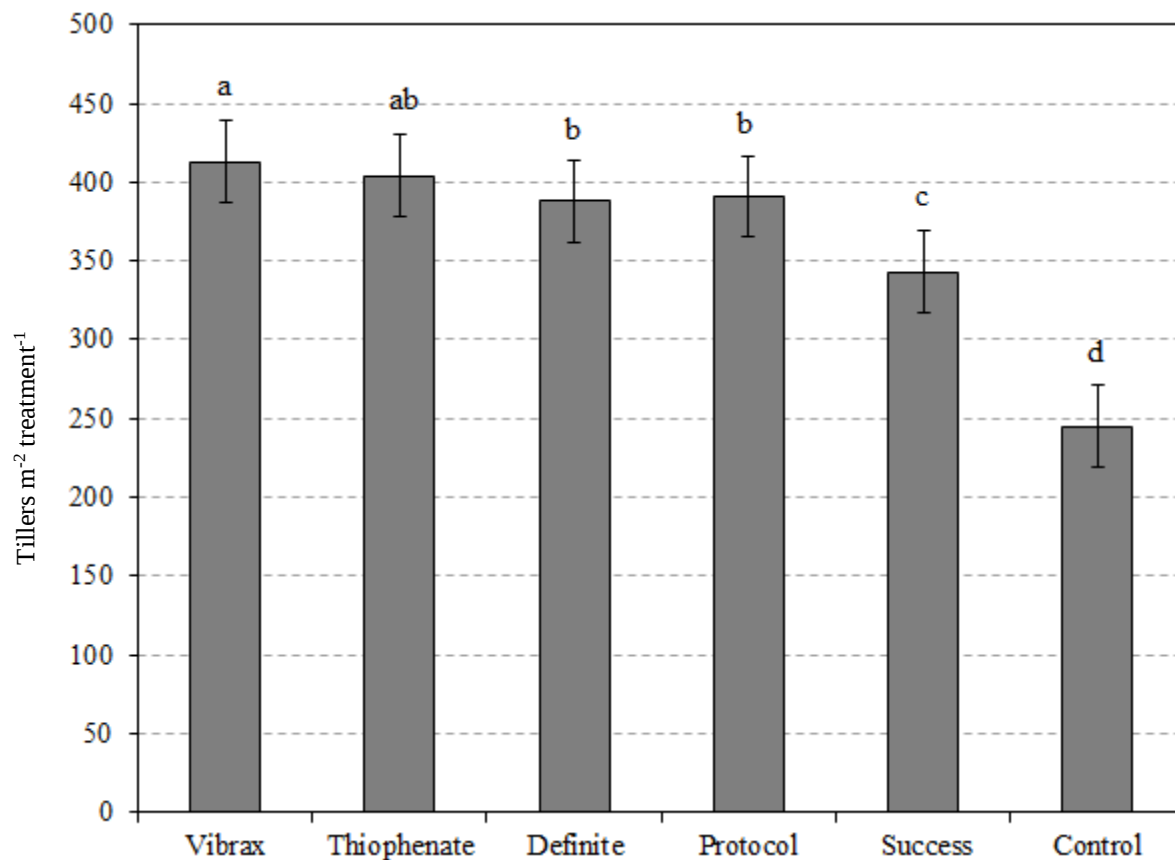


Fig.6. Number tillers m⁻² as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

Biological yield (kg ha⁻¹)

The data regarding biological yield of wheat as affected by seed dressing fungicides against Partial 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 100 fungicide followed 8513 kg ha⁻¹ was exhibited by Thiophenate 540 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 $\pm$) 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 Partial 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).

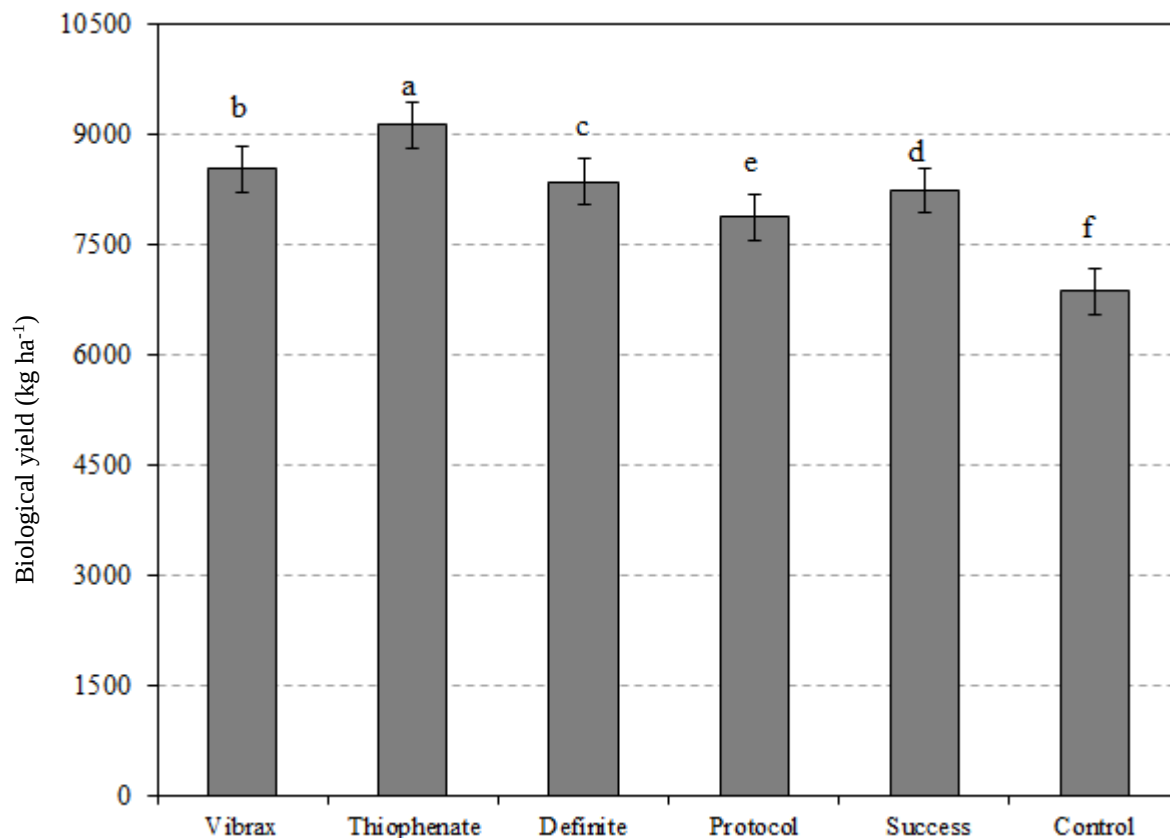
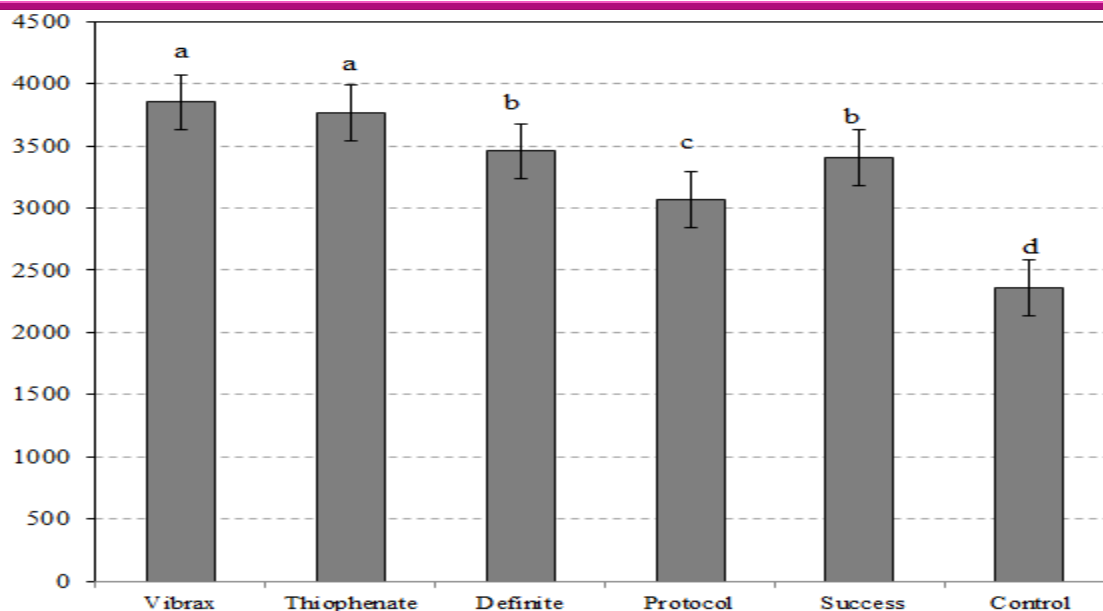


Fig.7. Biological yield (kg ha⁻¹) of wheat as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

Grain yield (kg ha⁻¹)

The data regarding grain yield of wheat as affected by seed dressing fungicides against Partial 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 100 fungicide followed 3768.7 kg ha⁻¹ was exhibited by Thiophenate 540 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 ($SE \pm$) 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 Partial 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).



Seed dressing fungicides

Fig.8. Grain yield (kg ha⁻¹) of wheat as affected by seed dressing fungicides against Partial 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 Partial 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 100 fungicide followed 40.93 g seed index in Thiophenate 540 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 540 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 ($SE \pm$) 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 Partial 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).

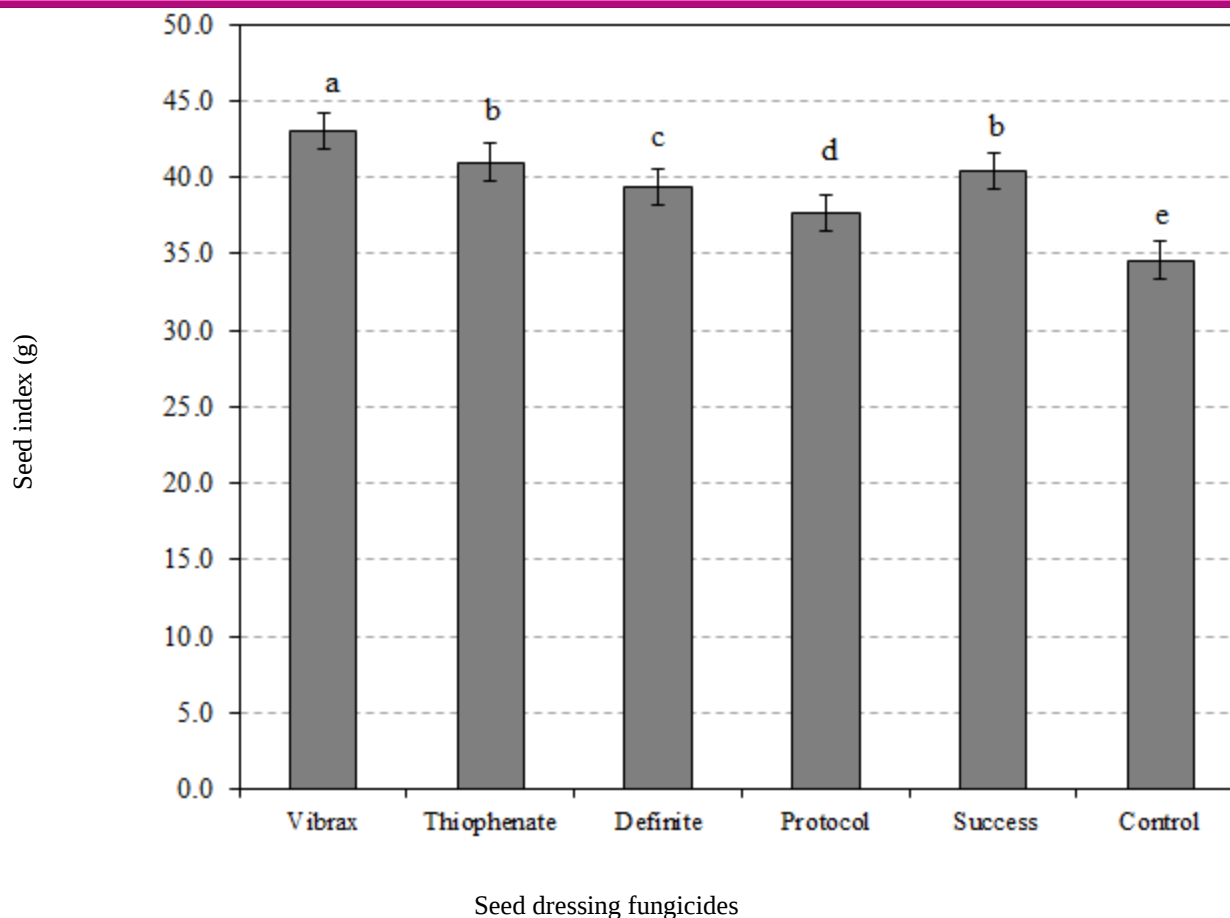


Fig.9. Seed index (g) of wheat as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

Harvest index (%)

The statistical analysis for harvest index (%) of wheat under the influence of seed dressing fungicides against Partial 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 100 fungicide followed 42.06% harvest index in Thiophenate 540 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 ($SE \pm$) 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 Partial 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).

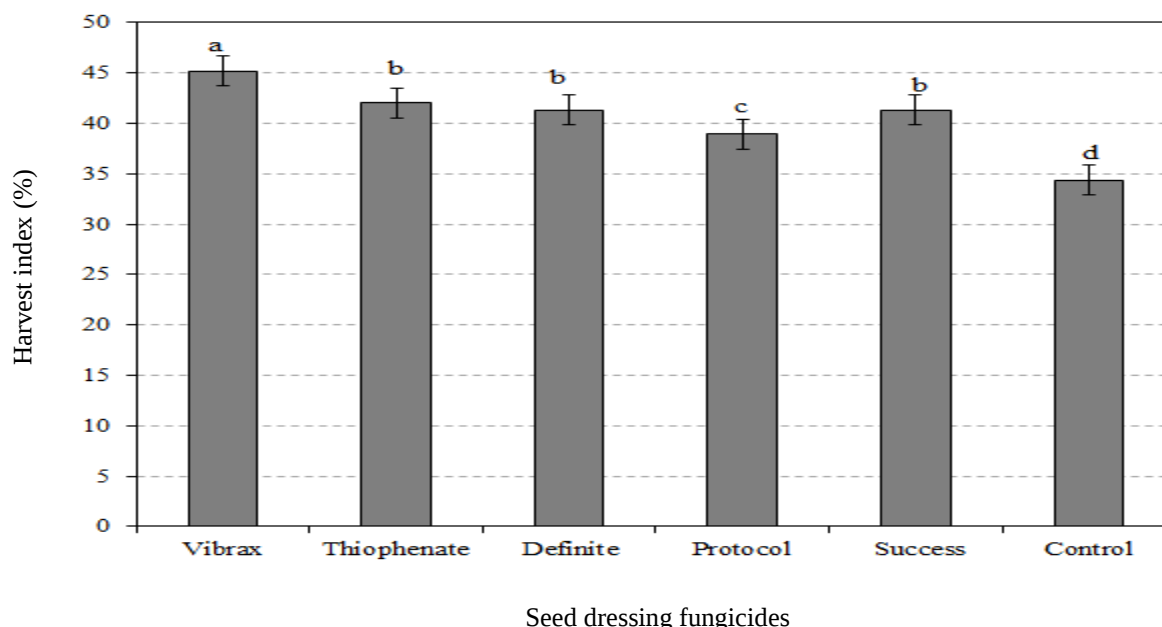


Fig.10. Harvest index (%) of wheat as affected by seed dressing fungicides against Partial Bunt of wheat caused by *Tilletia indica*

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 Partial Bunt of wheat caused by *Tilletia indica* suggested that among the five fungicides, Vibrax 100 and Thiophenate 540 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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