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Evaluation of seed-treating chemicals against seed-borne fungi on germinating wheat (*Triticum aestivum* L.) seeds

Sultana S, Mannan M, Islam R and Jahan N*

Agrotechnology Discipline, Life Science, Khulna University, Khulna-9208, Bangladesh

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Abstract

Seed-borne pathogens of wheat play a vital role in causing plant diseases and constrain wheat productivity in Bangladesh. An experiment was conducted to evaluate four seed-treating chemicals, namely Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC, against wheat seed-borne fungi in the plant protection laboratory, Agrotechnology Discipline, Khulna University Khulna, Bangladesh from January, 2021 to October, 2021. The experiment was carried out to isolate the associated seed-borne fungi in two wheat varieties and determine the efficacy of fungicides to control these seed-borne fungi of wheat. Wheat seed samples (Farmer's seed) of two (*Triticum aestivum* L.) varieties (BARI Gom-25 and BARI Gom-26) were collected from the Regional Agricultural Research Station, Jashore. The experiment was conducted following the complete randomized design (CRD) with four replications. Different seed-borne fungi, *Alternaria* spp., *Bipolari*s spp., *Curvularia* spp. and *Aspergillus* spp., were identified as associated fungi from these two varieties of seeds. The prevalence of different seed-borne fungi varied independently and individually across wheat varieties. In the case of germination between two wheat varieties, the highest germination percentage was recorded in BARI Gom-26. All the tested fungicides increased the germination percentage compared to untreated seeds. Knowin 50 WP showed a superior influence on the germination of the wheat variety BARI Gom-26 over untreated seeds using the Blotter method. Among the tested fungicides, Tilt 250 EC showed strong effectiveness in controlling all the fungi associated with wheat seeds.

Keywords – Seed-borne fungi – Germination – Pathogen – Blotter method

Introduction

Wheat (*Triticum aestivum* L.) is one of the widely grown cereal crops belonging to the family Poaceae, with a recorded 785 million metric tons of production in 2023 (Statista 2023). According to the Bangladesh Bureau of Statistics (BBS 2020), it is considered the second most staple food crop next to rice in Bangladesh. Besides human nutrition, wheat is also used as animal feed (Islam et al. 2020). It contains essential nutrients such as carbohydrates (59.40%), proteins (11.50), crude fibers (10.06%), fat (9.10%), and ash (1.80%) per 100g. These components offer essential amino acids, minerals, vitamins, beneficial phytochemicals, and dietary fibers required for a balanced human diet (Xu et al. 2023). By 2050, it is expected that the population will have doubled, necessitating an increase in food production to feed the expanding population (Olowe 2021).

In Bangladesh, the lower productivity of wheat is due to fungal diseases like wheat blast, leaf

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Corresponding Author: Nusrat Jahan ─ e-mail ─ nusratjahan190829@gmail.com

rust, Fusarium head blight, Septoria leaf blotch, stripe rust, spot blotch, tan spot, and powdery mildew, and poor management (Islam et al. 2020, Simón et al. 2021). There are nearly 200 diseases and pests in agriculture, and around 50 of them are economically important due to their ability to damage crops and reduce farmers' profitability. Approximately 20% of the wheat intended for food and feed is lost due to diseases annually. Additionally, seed-borne pathogens lead to changes in plant appearance and can diminish yields by 15–90% when untreated seeds are cultivated in fields (Wagan et al. 2022). The seed-borne pathogens may cause seed abortion, seed rot, seed necrosis or reduction in germination as well as seedling damage by systemic or local infection, resulting in the development of diseases at later stages of plant growth. Out of the 120 diseases affecting wheat, 42 are transmitted through seeds, and 35 are caused by fungi (Ghimire et al. 2023). The predominant seed-borne pathogen in cereal crops, including wheat, is *Bipolaris sorokiniana*, causing black point discoloration, leading to reduced germination, root decay, and spot blotch (Sharma et al. 2021). The seed-borne fungi in nature can exclusively be controlled only through the treatment of seeds.

Since approximately 90% of crops originate from seeds, they serve as a significant source for spreading diseases and allowing pathogens to persist from one season to another (Gupta et al. 2020). Seed treatment with chemicals prevents the decaying of seed by enhancing host resistance and managing Fusarium Head Blight and the associated T-2 toxin by controlling pathogens that carry them inside the seeds or existing in the soil where they are planted. It is the best economical method to maintain good seed health and ensure environmental safety as it uses very low doses of chemicals compared to foliar application. The seed-treating fungicides, viz., Vitavex-200 applied at 0.3% of seed weight, eliminates all seed-borne pathogens and increases germination by 25.70% over the control group. Many scientists have observed the effects of fungicides, biological agents and host resistance in managing and inhibiting pathogen growth *in vitro* using fungicides like as Carbendazim, Hexaconazle, Tebuconazle, and Sulphur (Bhuiyan et al. 2013, Musyimi et al. 2012).

Various fungicides, such as Mancozeb, Carbendazim, and a mixture of Mancozeb and Carbendazim, were examined to minimize seed-borne fungi (Ahmad & Zaidi 2018). The findings showed that different concentrations of these fungicides were notably effective in inhibiting the growth of *Alternaria alternata*, *Aspergillus flavus*, *Mucor species*, *Fusarium moniliforme* and *Rhizopus stolonifer*. Moreover, treating seeds with fungicides led to a significant increase in seed germination percentage and reduced fungal infestation (Mahal 2014). Given these findings, the current research aimed to identify the seed-borne fungi associated with wheat seeds and assess the efficacy of seed-treatment chemicals against wheat seed-borne fungi in laboratory conditions.

Materials & Methods

Experimental details

The experiment was conducted at the Plant Protection Laboratory, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh from January 2021 to October 2021. Four different fungicides (see Table 1) were used to evaluate the efficiency against seed-borne fungi on germinating wheat seeds at 25 °C. Wheat seed samples (Farmer's seed) of two (*Triticum aestivum* L.) varieties (BARI Gom-25 and BARI Gom-26) were collected from the regional agricultural research station, Jashore, Bangladesh.

Seed-treating chemicals used in this experiment

Four fungicides were selected as they were the most commonly used in Bangladesh and also due to their specific mode of action (Table 1). Using fungicides and their recommended doses (0.25%) against fungal pathogens in seed were selected based on the protocol provided by Islam et al. (2015).

Blotter method

According to the rules of the International Seed Testing Association (2001), the percentage of seed germination (health) and fungal association were determined. From each sample, 400 seeds were taken randomly for the test. Seeds were placed on three-layered blotting paper moistened with sterilized water in a glass petri dish (9 cm) (120 ºC for 12 hours in the oven) by using sterilized forceps. Each petri dish contained 25 seeds. Then, petri dishes were kept at room temperature (25 ℃) under 12/12 hours light and dark for seven days.

Table 1. List of seed-treating chemicals that were used in this experiment

Trade name	Chemical name	Mode of action	Concentration/ Dose
Knowin 50 Wp	Carbendazim	Systemic	250ppm
Sunvit 50 WP	Cupper oxychloride	contact	250ppm
Neuben 72 Wp	64% Mancozeb +8 % Metalaxil	contact	250ppm
Tilt 250 EC	Propiconazle	Systemic	250ppm

Preparation of fungicidal solutions for seed treatment

In this method, four different types of fungicides, viz., Knowin 50WP (0.25%), Sunvit 50 WP (0.25%), Neuben 72 WP (0.25%) and Tilt 250 EC (0.25%), were taken and poured 100 mL into four different conical flasks. According to Islam et al. (2016), 100 ml of distilled water was poured into each conical flask and shaken continuously to make a stock solution. Then, 250 ppm working solutions were prepared for each fungicide and used for seed treatment.

Seed treatment with chemicals

The four fungicides were tested at 250 ppm, and five replicates were carried out for each fungicide. The requisite amount of each fungicide and 100 seeds from each sample were taken and shaken mechanically for 10 minutes. Then, the excess chemical was drained off and moistened, and treated seeds were air dried. Seeds without any treatment served as the control (Islam et al. 2016). The efficacies of the four fungicides were evaluated by comparing them with an untreated control using the Standard Blotter Method (ISTA 2001). Fungi were isolated from the seeds using the Agar plate method (Habib et al. 2011). The seeds were placed on the petri dish where each plate contained 25 seeds and were incubated at 25±2 °C for 12 hours alternate cycles of light and darkness. After 7 days of incubation, seeds were observed under the stereomicroscope and the fungi were isolated using the Agar plate method. A temporary slide was prepared for the identification and confirmation of fungi. The culture characteristics, such as colony texture, color, and growth pattern, were carefully observed and documented. Furthermore, the fungi were identified up to the generic level by studying the spore morphology.

Examination of incubated seeds and recording of infectious fungi

Numbers of germinated seeds were recorded along with the seed-borne fungi after seven days of incubation. The observation was made from each dish, and data were entered in working recording sheets immediately after the examination of the dish (Mathur & Kongsdal 1994, ISTA 2007), and the results were expressed in percentage. Reproduction characters of each fungus were observed using the compound microscope and identified with the help of keys (Chidambarain et al. 1973). Image of conidia of fungi was taken using Carl Zeiss Software.

Calculation of germination percentage in the blotter method

Four hundred seeds were used in each treatment, and within the four hundred seeds germination percentage was calculated using the following formula:

$$Germination(\%) = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ placed\ in\ the\ experiment} \times 100$$

Calculation of reduction percentage of fungal association

The reduction percentage of fungal association was calculated based on the Islam et al. (2015) protocol using the following formula:

$$Reduction\ of\ fungal\ association\ (\%) = 100\ - Total\ number\ of\ \%\ seed\ borne\ fungi$$

Experimental design and statistical analysis

The experiments were conducted following the Completely Randomized Design (CRD). Analysis of variance and the mean differences were conducted to determine the efficacy of the treatments using the Statistical Tool for Agricultural Research program (STAR, IRRI, Philippines) software. The noticeable and significant differences among the results were compared by the Least Significant Difference (LSD) Test.

Results and Discussion

Identification of seed-borne fungi

After seven days of incubation, the yielded fungi were detected and identified (Table 2). The yielded fungi were identified as *Alternaria* spp. *Bipolaris* spp. *Curvularia* spp. and *Aspergillus* spp. Only the most prevalent or abundant ones were considered.

Effect of seed treating chemicals against the associated fungi on BARI Gom-25

It was observed that the treated seeds showed a significantly higher rate of germination than the untreated seeds (control) (Figure 1). Germination ranged from 92% to 98%. The highest germination (98%) was reported from Knowin 50 WP-treated seeds, and the lowest (92%) was found in the control treatment. However, according to the gazette published by Seed Wing, Ministry of Agriculture, Government of the People's Republic of Bangladesh (2010), the minimum percentage of germination for seed standards is 96%. Therefore, the germination percentage should be increased by treating seeds. The percentage of germination was positively linearly correlated with the seed-treating chemicals. Their relationship could be explained by the regression equation Y=93.5+0.8X, where Y = estimated germination and X = regression coefficient. The correlation coefficient was r = 0.301 (Fig. 2).

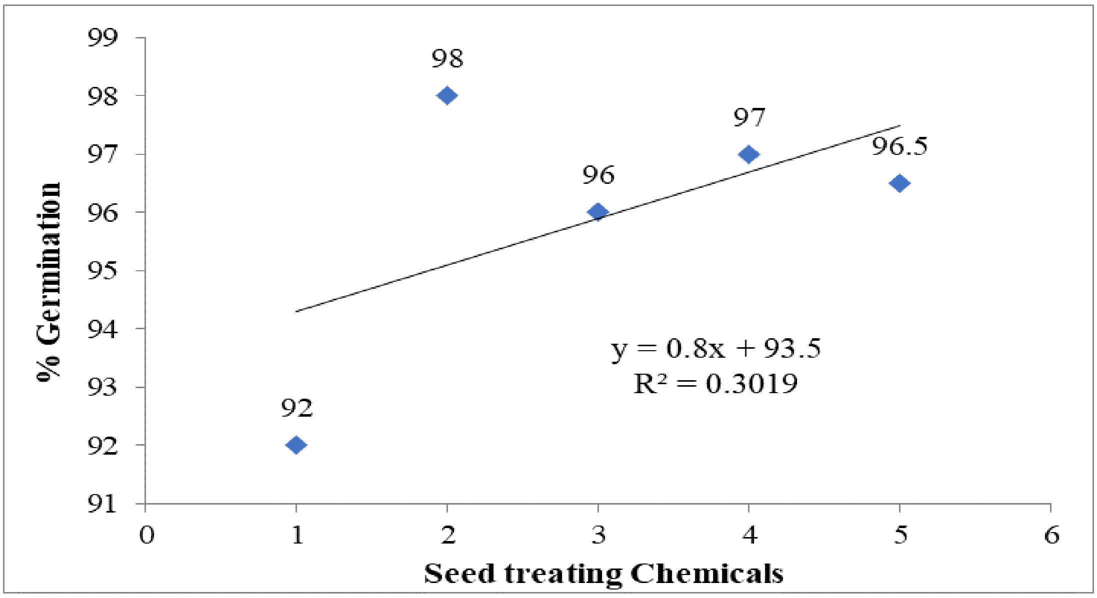
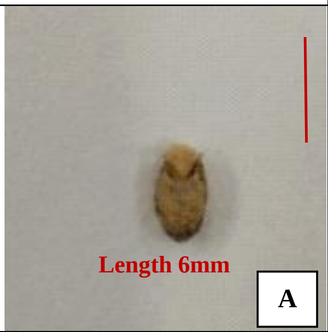
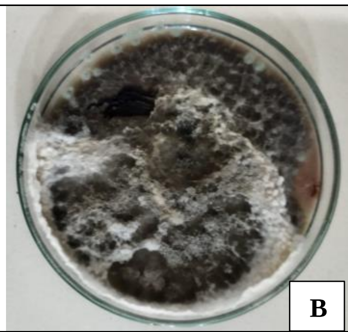
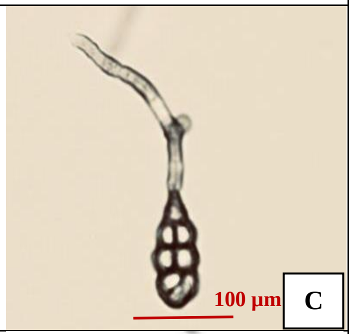
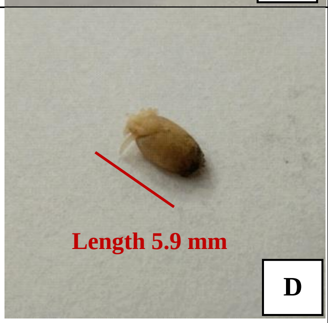
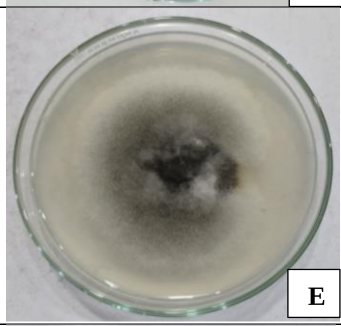
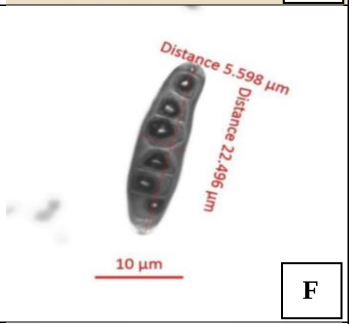
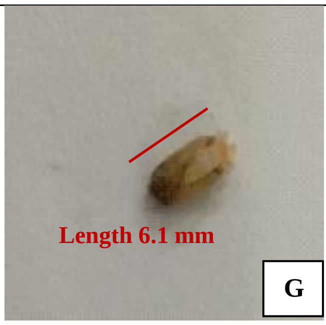
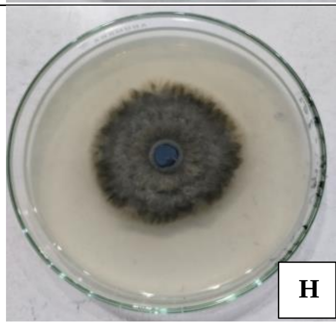
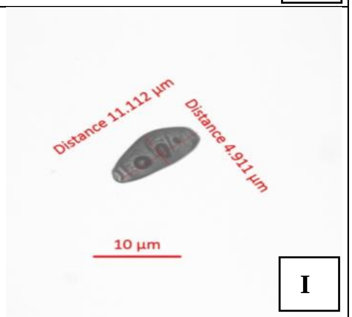
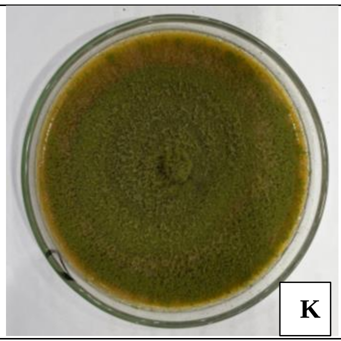
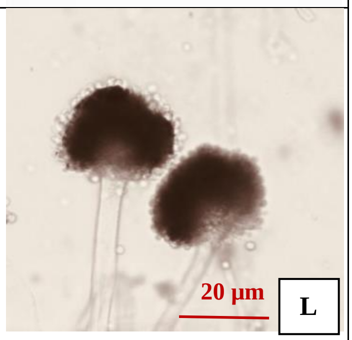


Fig. 1 – Effect of seed treating chemicals on germination percentage of BARI Gom-25. 1 – Control (No treatment), 2 – Knowin 50 WP, 3 – Sunvit 50 WP, 4 – Neuben 72 WP, 5 – Tilt 250 EC.

Table 2. List of associated fungal genera with their identifying characteristics. Identification key: Illustrated Genera of Imperfect Fungi (Barnett 1972); Basic Plant Pathology Method (Dingra & Sinclair 1986).

Sl. No.	Identified fungal genera	Infected seed	Pure culture	Reproductive structures of Identified Fungi
1	*Alternaria* spp.			
2	*Bipolaris* spp.			
3	*Curvularia* spp.			
4	*Aspergillus* spp.			

In *Alternaria* spp., the maximum association (15.50) was observed in the control treatment, while the minimum was found using Tilt 250 EC (2.00) and Neuben 72 WP (2.50), both of which were statistically similar (Table 3). The lowest association of *Bipolaris* spp. (1.0) was observed when seeds were treated with Tilt 250 EC, followed by Neuben 72 WP (2.25), which was statistically similar to Sunvit 50 WP (2.75). The highest association of *Bipolaris* spp. (12.50) was found in the control treatment. *Curvularia* spp. was observed with the lowest association (0) when seeds were treated with Tilt 250EC, and the highest association of *Curvularia* spp. (7.25) was found in the control treatment. The chemicals Knowin 50 WP (2.50) and Sunvit 50 WP (2.25) gave a similar result by controlling *Curvularia* spp. When considering *Aspergillus* spp., the maximum association (13.25) was observed in the control treatment, while the minimum was found using Tilt 250 EC (2.00), which was statistically similar to Neuben 72 WP (2.00). The performance of Knowin 50 WP (5.00) was statistically similar to Sunvit 50 WP (5.00).

Table 3. Effect of seed treating chemicals against fungi associated with BARI Gom-25 at 250 ppm concentration.

Treatments	Seed-borne fungi			
	Alternaria spp.	*Bipolaris* spp.	*Curvularia* spp.	*Aspergillus* spp.
Control (no treatment)	15.50a	12.50a	7.25a	13.25a
Knowin 50 WP	7.00b	4.00b	2.50b	5.00b
Sunvit 50 WP	5.75c	2.75c	2.25b	5.00b
Neuben 72 WP	2.50d	2.25c	1.00c	2.00c
Tilt 250 EC	2.00d	1.00d	0.00d	2.00c
Level of significance	**	**	**	**
LSD	0.64	0.64	0.61	0.33

** =1% level of significance. Letters indicate the significant differences of fungal association rates. Association rates that do not share the same letters are significantly different.

Effect of seed treating chemicals against the associated fungi on BARI Gom-26

The effect of different treatments on germination percentages of BARI Gom-26 ranged from 94% to 98.50%. The highest germination (98.50%) was reported in Knowin 50 WP-treated seeds, followed by Neuben 72 WP (97.75%), Tilt (97.25), Sunvit 50 WP (96.50%), and the lowest, 94% was found in the control treatment. The germination percentage was positively linearly correlated with the seed-treating chemicals. Their relationship could be explained by the regression equation Y = 95.07 + 0.575X, where Y = estimated germination and X = regression coefficient. The correlation coefficient was r = 0.277 (Fig.2).

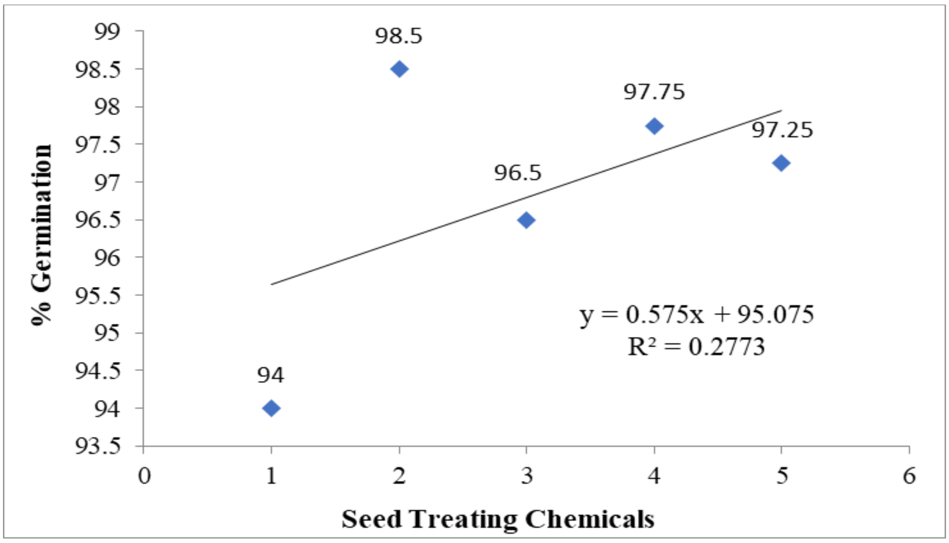


Fig. 2 – Effect of different seed treatments on germination percentage in BARI Gom-26. 1 – Control (No treatment), 2 – Knowin 50 WP, 3 – Sunvit 50 WP, 4 – Neuben 72 WP, 5 – Tilt 250 EC.

The effect of various fungicidal treatments on associated seed-borne fungi is shown in Table 4. For *Alternaria* spp., the control treatment exhibited the highest association rate (12.75), whereas Tilt 250 EC demonstrated the lowest (2.00), followed by Neuben 72 WP (3.75), which was statistically comparable to Sunvit 50 WP (4.00). Conversely, treating seeds with Tilt 250 EC resulted in the lowest association rate with *Bipolaris* spp. (0.50), while the control treatment showed the highest (11.25). Similar performance was observed with Neuben 72 WP (2.50), Sunvit 50 WP (2.75), and Knowin 50 WP (3.25) against *Bipolaris* spp. The lowest association of *Curvularia* spp. (0) was observed when seeds were treated with Tilt 250 EC, whereas the highest of *Curvularia* spp. (5.25) was found in the control treatment. Statistically different performances were observed when using Neuben 72 WP (1.00), Sunvit 50 WP (2.25) and Knowin 50 WP (3.00) against *Curvularia* spp. In the case of *Aspergillus* spp., the highest association (8.25) was observed in the control treatment, while the minimum was found using Tilt 250 EC (2.00). Statistically different results were observed when using Neuben 72 WP (3.00), Sunvit 50 WP (3.50) and Knowin 50 WP (3.75) treatments against *Aspergillus* spp.

Table 4. Effect of seed-treating chemicals against fungi associated with BARI Gom-26 at 250ppm concentration

Treatments	Seed-borne fungi			
	Alternaria spp.	*Bipolaris* spp.	*Curvularia* spp.	*Aspergillus* spp.
Control (no treatment)	12.75a	11.25a	5.25a	8.25a
Knowin 50 WP	5.00b	3.25b	3.00b	3.75b
Sunvit 50 WP	4.00c	2.75b	2.25c	3.50bc
Neuben 72 WP	3.75c	2.50b	1.00d	3.00c
Tilt 250 EC	2.00d	0.50c	0.00e	1.00d
Level of significance	**	**	**	**
LSD	0.4766	0.80	0.47	0.61

** =1% level of significance. Letters indicate the significant differences of fungal association rates. Association rates that do not share the same letters are significantly different.

Reduction of fungal association on BARI Gom-25

The maximum reduction in fungal association on BARI Gom-25 was observed using Tilt 250 EC (95%), followed by Neuben (93.25%). The lowest reduction was observed in the control (51.5 %). Sunvit 50 WP was also effective, reducing fungal infection in seeds by 84.25%. Knowin 50 WP showed the lowest reduction of fungal association (81.5%) among the four tested chemicals. It was clear that Tilt 250 EC was significantly effective as a seed-treating chemical in reducing fungal associations (Figure 3).

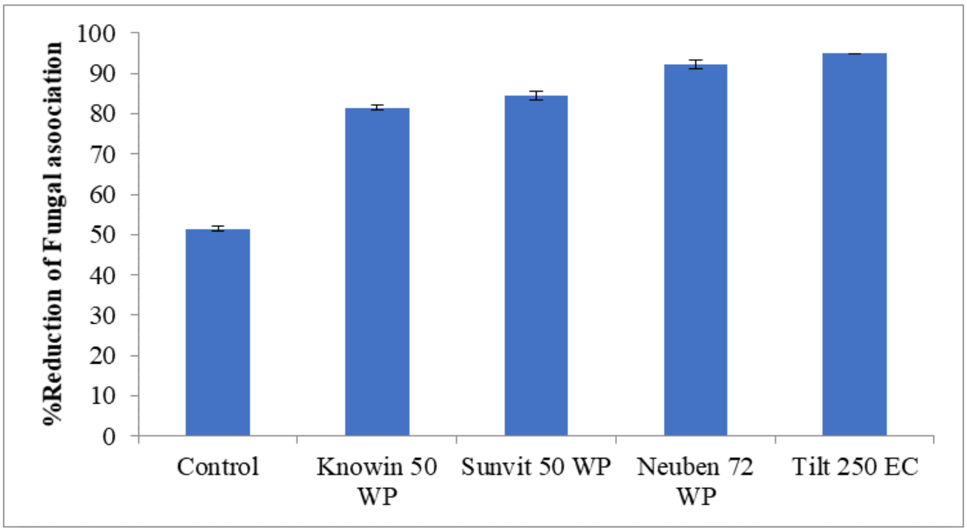


Fig. 3 – Effect of seed treatment on reduction of fungal association on BARI Gom-25.

Reduction of fungal association on BARI Gom-26

For BARI Gom 26, the highest fungal association was observed in the control treatment (37.5%), and the lowest was observed when seeds were treated with Tilt 250 EC (3 %). The maximum reduction in fungal association in BARI Gom-26 was 97% with Tilt 250 EC. Neuben (89.75%) and Sunvit 50 WP (87.50%) were also effective in reducing fungal associations. Knowin 50 WP observed the lowest reduction among all the tested chemicals, with an 85% reduction percentage in fungal associations (Fig. 4).

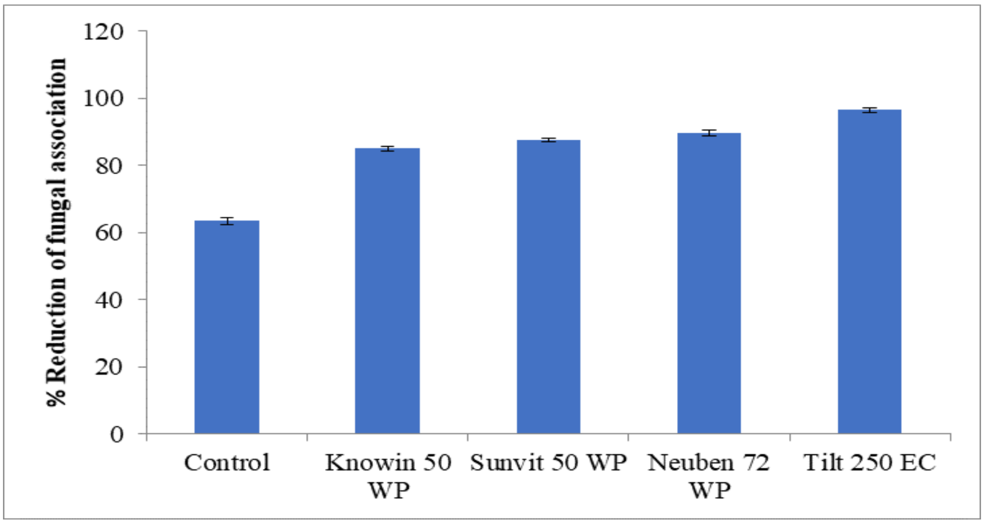


Fig. 4 – Effect of seed treatment on reduction of fungal association on BARI Gom-26

In this experiment, the highest germination rate was observed with the seed treatment using Knowin 50 WP, while the lowest was reported with treatments using Sunvit 50 WP and Tilt 250 EC. Among the treatments, Knowin 50 WP exhibited a superior influence in wheat germination over untreated control in the Blotter method. Germination rates in the Blotter method improved by up to 6% in BARI Gom-25 and 4.5% in BARI Gom-26 compared to the untreated control. These results are consistent with the studies by Naznine et al. (2016) and Islam et al. (2015), who reported increases in germination ranging from 4 to 12.5 % over the untreated control. The minimum germination percentage was observed in seeds treated with Tilt 250 EC.

Discussion

The seed treatment with Knowin 50 WP revealed a significant effect in increasing the germination of wheat seeds. This result is consistent with the study by Bhuiyan et al. (2013), who reported that Bavistin reduced seed-borne infection and enhanced seed germination compared to the control. However, the increment of germination percentages with Tilt 250 EC was unsatisfactory, as it led to a reduction in seed germination in the Blotter method. Naznine et al. (2016) reported a reduction in germination percentage with Tilt 250 EC-treated seeds, whereas Singh (2011) reported a 66.6% reduction in germination when using Tilt 250 EC as the seed treatment.

Identification of seed-borne fungi from wheat seeds was conducted using the standard blotter method. The results indicated that seed-borne fungi could be destructive during seed germination or cause mortality soon after the emergence of seedlings, highlighting the necessity for chemical treatments before planting. Among the identified seed-borne fungi, *Alternaria* spp. was the most frequent during incubation, followed by *Bipolaris* spp., *Aspergillus* spp., and *Curvularia* spp. Management of seed-borne fungi with fungicides positively affects the production of better emergence and vigorous of seedlings as seed protectants. The chemical treatments of the seeds are a common and effective method for controlling most seed-borne pathogens. Based on the results, it is evident that the seed-treating chemical, Tilt 250 EC, shows a remarkable effect in reducing fungal associations in BARI gom-26 (97%) and BARI gom-25 (95%), consistent with the findings of Magar et al. (2020) (Fig. 4 & Fig. 5). Additionally, Kakraliya et al. (2018) reported a remarkable effect of fungicides, botanical and bio-agents against *Alternaria triticina* (89.72%) under both field and *in vitro* conditions. Gupta et al. (2013) reported the effects of seven fungicides, azoxystrobin, carbendazim, hexaconazle, mancozeb, propiconazole, triadimefon and tricylazoleviz against *Bipolaris oryzae* in the laboratory. They also disclosed that fungicides are highly effective in the management of seed-borne fungi. Neuben 72 WP also appeared effective in controlling the seed-borne fungi of wheat. Similarly, Neuben 72 WP controls fungal association in wheat, BARI Gom-25 (93.25%) and BARI Gom-26 (89.75%) (Pun et al. 2020, Suharti et al. 2020, Hasan et al. 2012) The fungicide Knowin 50 WP is effective in increasing germination of wheat seeds, while Tilt 250 EC is effective in reduction of fungal associations. Chemical seed treatments offer several advantages, including effective protection against seed-borne diseases, improved crop yield, and cost efficiency by reducing the need for extensive field applications. In developed countries, 100% of seed treatments are practiced (Birah et al. 2014). The potential for unintentional poisoning, the limited dose capacity, the shortened shelf life of the treated seeds, and the worker's chemical exposure when treating bigger batches of seeds are among the drawbacks (Shakeel et al. 2019). It is clearly depicted from the study of Islam et al. (2015) that seeds treated with Vitavax 200, Bavistin and Captan were found effective against seed-borne fungi. These findings are also in agreement with our results that seed-borne fungi can be controlled using Knowin 50 WP, which was found to have a strong effect against seed-borne fungi. The selection of proper fungicides may help farmers in Bangladesh to control seed-borne diseases.

Conclusion

The study evaluated various fungicides for their effectiveness in enhancing seed germination and reducing fungal association in BARI Gom-26 and BARI Gom-25 wheat varieties using the blotter method. Among the tested fungicides, Knowin 50 WP emerged to be the most effective, significantly increasing germination percentages by 4.5–6.0% compared to the control, with germination rates of 98.50% and 98% in BARI Gom-26 and BARI Gom-25, respectively. The identified fungi included *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp., and *Aspergillus* spp., with a higher association observed in BARI Gom-25. Notably, Tilt 250 EC exhibited a significant effect in reducing fungal association, achieving reductions of 2.75–13.5% compared to other fungicides, with 97% and 95% reductions in BARI Gom-26 and BARI Gom-25, respectively. Overall, the results underscore the effectiveness of Knowin 50 WP in enhancing seed germination and Tilt 250 EC in reducing fungal association, suggesting their potential for effective disease management in wheat cultivation.

Conflict of interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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