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RESEARCH ARTICLE

SEED BORNE INFECTION AND MANAGEMENT OF ALTERNARIA SPECIES IN WHEAT CULTIVARS

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ABSTRACT

The research was carried on "Incidence of the seed borne infection and management of Alternaria species in wheat cultivars" at Laboratory of Bright Mid-West Agriculture and Forestry Campus, Birendranagar, Surkhet, Nepal from December 2019 to March 2020. The experiment was laid out in Completely Randomize Design (CRD). In the present investigation, seed samples of seven different varieties of wheat (Triticum aestivum L.) seeds viz. Gautam, Virkuti, Aaaditya, Wk1204, NL1172, NL1177 and NL297 were collected from National Wheat Research Program (NWRP) Bhairahawa, Rupandehi, Nepal and Nepal Agriculture Research Council (NARC), Botany division, Khumaltar, Lalitpur, Nepal. Seed samples were categorized by different varieties and treatment in different day's interval. Seed treatment with, Dhanuka M-45, Acmes tin, Trichoderma viridae and control (without treatment) was done to see their effect on seed borne infection of Alternaria in wheat. The recorded parameters among the sampled plants were germination percentage, disease incidence percentage and incidence of Alternaria on the seeds. The variety 'Aditya' germinated maximum (97.00%) and variety NL-297 germinated minimum (0.25%) without treatments. However, Variety 'Virkuti' germinated maximum (95.5%) after treatment. Whereas, Variety NL-297 showed no seed-borne infection (0.00%) and variety 'NL-1177' showed the maximum disease severity (12.75% at 5DAI, 24.75% at 7DAI and 32.25% at 9DAI respectively) without treatment. Similarly, in treated seeds variety NL-1177 showed the maximum disease incidence percentage (6.75%) at 5DAI, variety Gautam (9.37%) at 7DAI and variety NL-1177 (9.87%) at 9DAI. whereas variety Gautam showed the minimum disease incidence (3.50%) after 9DAI, Variety 'Virkuti' (6.62%) at 7DAI and Variety 'Gautam' (8.0%) at 9DAI respectively. With the treatment variety Gautam could reduce the disease incidence by 60% at 5DAT, 50.02% at 7DAT and 68.93% at 9DAT.Similarly, variety Virkuti reduce in disease incidence by 13.53% at 5DAT, 15.12% at 7DAT and 10.41% at 9DAT. The Variety NL-1177 reduced disease incidence by 47.05% at 5DAT, 70.22% at 7DAT and 69.39% at 9DAT respectively. The results also showed that the control measure of Trichoderma viridae found significant performance (99.84%) in controlling seed borne pathogens and increasing germination of wheat seeds and the Variety Aditya was found as best in germination (97%) with lower seed borne Alternaria and the variety NL-297 was found non-disease severity (0.00%).

KEYWORDS

Triticum aestivum, Alternaria, Disease incidence, Trichoderma viridae

1. INTRODUCTION

The cultivation of wheat (Triticum aestivum L.) stands as a fundamental practice in Nepal's agricultural landscape, holding the third position in both cultivation area and production volume among staple food crops (Wiese, 1987). Its nutritional value and economic significance underscore its importance, contributing significantly to global food calories and serving as a dietary staple for about 40% of the global population. Despite its prominence, wheat cultivation in Nepal faces multifaceted challenges, including biotic factors like diseases, abiotic stress conditions, inadequate crop management, and socio-economic constraints (Dubin and Bimb, 1994; Mudwari, 1998). Wheat productivity in the country has encountered obstacles despite the introduction of high-yielding varieties in the mid-1960s, which significantly expanded its cultivation area and production (PPD 2007). With wheat cultivation frequently integrated into rice cultivation, forming the Rice-Wheat (R-W) rotation, over 84% of the nation's wheat area is represented.

Numerous wheat varieties have been released by institutions like the Department of Agriculture (DoA) and Nepal Agriculture Research Council (NARC), showcasing high-yield potentials such as Pasanglhamu, Annapurna-3, Annapurna-1, and Kanti, tailored for diverse ecosystems. However, diseases, particularly Alternaria triticina, present a significant challenge to achieving optimal wheat production levels in Nepal. This fungal pathogen poses a substantial threat to wheat crops, resulting in considerable yield losses that can reach up to 60% in severe infections (Prasada and Prabhu, 1962). Its prevalence spans various countries and is both soil and seed-borne, with seed transmission playing a crucial role in fungal inoculum propagation across seasons (Kumar and Arya, 1973; Kumar and Arya, 1976). Addressing the prevalence and transmission dynamics of seed-borne fungi like Alternaria in Nepal's wheat fields remains a critical research focus. Given the substantial annual yield losses attributed to seed-borne fungi, especially within the Alternaria complex, understanding their dynamics comprehensively is pivotal for devising effective management strategies (Dugan and Peever, 2002).

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2. Materials and Methods

2.1 Location

All the experiments were conducted at Laboratory of Bright Mid-Western Agriculture and Forestry Campus, Birendranagar, Surkhet, Nepal.

2.2 Experimental period

The experiments were conducted during the period from December, 2019 to March, 2020.

2.3 Collection of seed samples

The seed samples of seven wheat (Triticum aestivum L.) cultivars were collected from National Wheat Research Program (NWRP) Bhairahawa, Rupandehi and Agriculture Botany Division Khumaltar, Lalitpur, Nepal for the experiments.

2.4 Varieties included

The wheat cultivars selected for the study were Gautam, Virkuti, Aaditya, Wk1204, NL1172, NL1177 and NL297.

2.5 Dry inspection

The collected seed samples were examined following dry inspection method. The percentage of apparently healthy, diseased, shriveled, discolored and mechanically injured seeds were recorded.

2.6 Detection of seed-borne fungi (Blotter method)

Health of all the seed samples used were explained for detection of fungi by Blotter Method following the International rules of Seed Testing Association (ISTA, 2001).

2.6.1 Procedure followed (Blotter method)

In this method, three layers of filter paper (Whatman No.1) were soaked in sterilized water by placing them in a bowl by holding with forceps. Excess of water was drained out by lettis the water to drop and before getting the last drop off placed in the 9cm diameter glass petri-dish. Four hundred seeds from each sample were taken randomly and then placed on the moist filter paper in four replicate petri-dishes at the rate of 25 seeds per plates. The petri-dishes with the seeds were then incubated at 25 ± 2 ºC. Time to time watering was done whenever felt necessary to keep the filter paper moist.

2.7 Germination test

Four hundred seeds were taken from the seed sample according to the rules of ISTA, 2001. Twenty five seeds were plated in each petri-dish and thus 24 petri-dishes were used for plating 400 seeds of each sample. Each petri-dish was considered as one replication. The filter papers (Whatman No.1) were soaked in distilled water and placed at the bottom of 9 cm diameter plastic petri-dish and then 25 seeds were placed on the top of filter paper. Evaporation of water was minimized by tightly fitting the lids of the petri-dishes. The petri-dishes were placed in incubation room maintaining the temperature at 25±2°C. Seeds produced both plumule and radical after incubation were considered as sprouted seeds. The result was expressed as percentage (in different plates).

2.8 Inspection of incubated seed samples and identification of fungi

Incubated seeds were observed under stereomicroscope at 16x and 25x magnification. The incidence of seed born fungi (Aterneria triticina) was detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined (Khan, 1998). Temporary slides were prepared from the fungal colony and observed under compound microscope. The fungi were identified with the help of keys suggested (Ellis, 1976; Neergaard, 1979). The culture was incubated at 25±1° C for 5-9 days. Temporary or semi-permanent slides were also prepared from the fungal colony and observed under compound microscope. The fungi were identified with the help of different books, manuals and publications (Ellis, 1976). The results were presented as percent incidence for individual pathogen.

2.9 Treatments

A total of 4 treatments (two Chemicals, One Biological and One control) were used as follows:

T1= Control

T2= Dhanuka M-45

T3= Acmestin

T4= TrichodermaViride

2.10 Seed Treatment with Chemical

2.10.1 Dhanuka M-45

Required amount of Dhanuka M-45 (2 g/kg) and seeds from each sample were taken in a 250 ml conical flask and were shaken mechanically for 10 minutes for proper coating of fungicides. To check the efficacy of Dhanuka M-45, treated seeds were tested using the Standard Blotter Method with slight modification (ISTA, 1996).

2.10.2 Acmestin

Required amount of Acmestin (2 g/kg) and seeds from each sample were taken in a 250 ml conical flask and were shaken mechanically for 10 minutes for proper coating of fungicides.

2.11 Seed Treatment with Bio-control Method (Trichoderma viridae)

Required amount of Trichoderma viridae (1 ml/gm) and seeds from each sample were taken in a 250 ml conical flask and were shaken mechanically for 10 minutes for proper coating of fungicides.

2.12 Data collection

2.12.1 Primary data

The research was based on primary data and the primary data has been collected regularly from the own lab research and experiments at Laboratory of Bright Mid-Western Agriculture and Forestry Campus, Birendranagar, Surkhet, Nepal.

2.12.2 Secondary data

In some constant, secondary data has been also collected as a review from different sources such as books, annual publication of NARC, articles and journals related to Wheat, Proceedings, Magazines, library of NARC and various institutional publication related to the topic etc.

2.13 Analysis of data

The design of experiment was CRD (Completely Randomized Design). The recorded data on various parameters under the present study were statistically analyzed using R-STAT statistical-package program. The level of significance and analysis of variance along with the Least Significant Difference (LSD) was done (Gomez and Gomez, 1984).

3. Results and Discussion

The percentage of disease incidence at different days after incubation showed highly significant with respect to different treatments (Table 1). At 5 days after incubation NL-1177 cultivar showed highest (12.75%) disease incidence which was followed by cultivar Gautam (8.75%). Whereas, NL-297 showed no disease incidence. At 7 days after incubation, NL-1177 cultivar showed highest (24.75%) disease incidence which was followed by cultivar Gautam (18.75%). The cultivar NL-297 was found free of disease infestation. At 9 days after incubation NL-1177 cultivar showed highest (32.25%) disease incidence which was followed by cultivar Gautam (25.75%) and cultivar NL-297 had no infection. The cultivar NL-1177 was found the most susceptible to Alternaria species whereas NL-297 was immune to Alternaria species infestation. The cultivars Gautam and Virkuti were at par with disease incidence.

Table 1: The percentage of seed-borne disease incidence (Alternaria species) at different Days after Incubation (DAI) at room temperature

Treatments	Disease incidence (%)		
	5 DAI	7 DAI	9 DAI
Gautam	8.75b	18.75b	25.75b
Virkuti	6.5b	7.8c	9.6c
Aditya	0.25c	1.50de	1.75de
WK-1204	0.75c	1.75de	3.25cde
NL-1172	2.00c	4.75cd	5.50cd
NL-1177	12.75a	24.75a	32.25a
NL-297	0.00c	0.00e	0.00e
Mean	3.89	8.17	10.85
SEM (±)	0.69	0.905	0.99
$LSD_{0.05}$	3.13	3.39	4.39
CV (%)	54.13	27.90	27.25
F-Probability	2.42e-07	1.47e-11	1e-11
Significant Level	***	***	***

Alternaria species was most frequently isolated from the Wheat samples of different cultivars (Duan et al., 2007; Rajput et al., 2005; Al-Rokibah, 1996). It was observed that highest germination percentage was found in cultivar Aditya (97.00%) which was at par with WK-1204 (96.00%) and Virkuti (95.50%). The lowest germination percentage was found in cultivar NL-297 (0.25%).

Table 2: Germination percentage of Wheat cultivars at 9 days after incubation at room temperature

Cultivars	Germination %
Gautam	72.50c
Virkuti	95.50a
Aditya	97.00a
WK-1204	96.00a
SNL-1172	4.00d
NL-1177	83.25b
NL-297	0.25e
Mean	64.07
SEM(±)	0.97
$LSD_{0.05}$	2.91
CV (%)	3.05
F-Probability	2e-16
Significant Level	***

A group researchers reported that germination of wheat seeds was negatively correlated to seed infection (Khokon et al., 2005). Some researcher reported that fungal and bacterial incidence and frequency of occurrence on wheat seeds, as well as their impact on seed germination were incidence was 47.12, 12.28 and 6.76%, respectively (Ammara et al., 2001). The frequency of occurrence of seed borne pathogens and their impact on seed germination varied from cultivar to cultivar. The highest germination percentage was found in cultivar Virkuti (95.50%) followed by Gautam (81.50%) and NL-1177 (68.0%).

Table 3: Germination percentage of treated Wheat cultivars after 9 days of incubation at room temperature

Cultivars	GAT %
Gautam	81.5b
Virkuti	95.5a
NL-1177	68.0c
Mean	81.66
SEM(±)	2.66
$LSD_{0.05}$	8.16
CV (%)	9.58
F-Probability	3.96e-06
Significant Level	***

A group researcher reported that seed germination and seedling growth decreased with the increase in susceptibility to a variety of infection (Zhimin et al., 1998). A studied that high percentage of grains fails to germinate in the field due to fungi (Khanum et al., 1987). The germination of healthy grains was 55-96.5% and that of diseased grains 34.5-71%. At least 5 fungal species were involved in the diseases complex in which Alternaria alternata was the most important. At 5 day after incubation, the cultivar NL-1177 showed highest disease incidence (6.75%) which was followed by cultivar Virkuti (5.62%) whereas the significant low disease seen in cultivar Gautam (3.50%). It was observed that seed treatment significantly reduced the disease (Alternaria species) incidence as compare to control. Trichoderma viridae was found to check the pathogen completely at 5 DAI followed by Dhanuka M-45 and Acmestin. At 7 day after incubation, the cultivar Gautam showed highest disease percentage (9.37%) followed by cultivar NL-1177 (7.37%) whereas low disease percentage found in the cultivar Virkuti (6.62%). It was highest (15.5%) in control which was followed by Acmestin (12.66%) and minimum disease in Trichoderma viridae (0.16%). At 9 day after incubation, the cultivar NL-1177 showed highest disease percentage (9.87%) and low disease percentage found in cultivar Gautam (8.0%). The highest disease (18.16%) was found in care of untreated seeds.

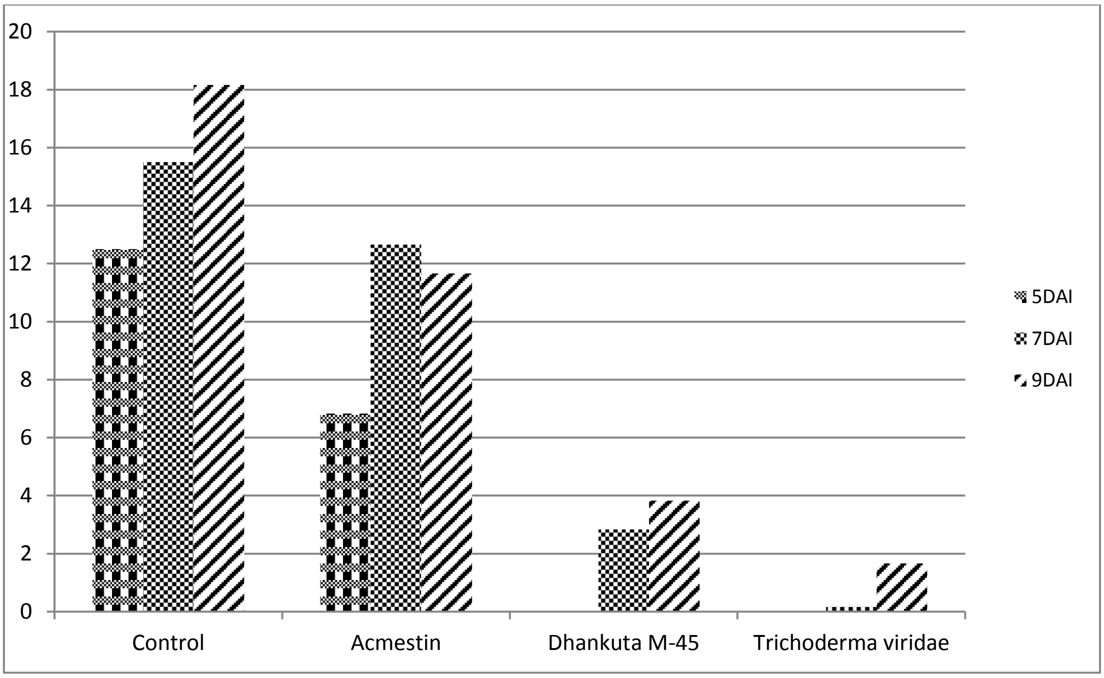

Figure 1: Effect of chemicals and Trichoderma viridae seed treatment on the incidence of Alternaria species

T. viride is the most effective mycoparasite and is able to penetrate hyphae of Alternaria alternata in wheat and can therefore be successfully used to control leaf blight of wheat (El-Hasan et al., 2008). Trichoderma spp. has been found to be effective in reducing the severity of foliar disease in wheat plants when compared to untreated plants (Sabatini et al., 2002). Trichoderma spp. may play an important role in reducing seedling disease incidences in plant stands in the field (Hasan and Alam, 2007). The number of tillers per row was found to have increased by 53% after using treated seeds with bio-control agents (Knudsen et al., 1995). A group researchers observed that the product of biological control agents (T.viridae) increased germination and plant height in wheat (Sawant et al., 2003). Bio-control agent Trichoderma viridae can also increase the number of healthy grains/ear as well as the yield of wheat (Sliesaravicius et al., 2006). After using bio-control agents, the yield of winter wheat was increased by 160% and the1000-grain weight by 4% (Knudsen et al., 1995).

The fungi which are exclusively seed-borne in nature can be controlled only through the treatments of seeds. Seeds are treated with chemicals like Dhanuka M-45, Acmestin, and biological method as Trichoderma to prevent their decay after planting by controlling pathogens carried on them, present inside the seeds or existing in the soil where they will be planted. Different chemicals and botanicals control measures are used for seed borne fungi of wheat. So far an appreciable amount of work has been done on the control of seed-borne pathogens of wheat by fungicidal seed treatment (Singh and Chauhan, 1995).

Table 4: The interaction of percentage of disease (Alternaria species) at different DAI in wheat cultivars

Interaction	Percent of disease Occurrence		
Variety*Treatment	**5 DAI**	**7 DAI**	**9 DAI**
NL-1177*Control	15a	16.0ab	19.5a
Virkuti*Control	13a	14.0abc	19.5a
Gautam*control	9.5b	16.5a	15.5ab
NL-1177*Acmestin	8.5b	10.5bc	13.5b
Virkuti*Acmestin	7.5b	10.0c	10.5b
Gautam*Acmestin	4.5c	17.5a	11.0b
NL-1177*Dhanuka M-45	3.5c	2.5d	5.0c
Virkuti*Dhanuka M-45	2.0cd	2.5d	3.0c
Gautam*Dhnuka M-45	0.3d	3.5d	3.5c
Gautam*Trichoderma viridae	0.0d	0.00d	2.0c
NL-1177*Trichoderma viridae	0.0d	0.5d	1.5c
Virkuti*Trichoderma viridae	0.0d	0.0d	1.5c
Mean	**5.29**	**7.79**	**8.83**
SEM (±)	**0.622**	**1.32**	**0.99**
$LSD_{0.05}$	**2.84*** **	**5.31*** **	**4.70*** **
CV (%)	**24.69**	**31.32**	**24.45**

At 5th days after treatment the minimum disease occurrence were shown by the interaction Gautam, NL-1177 and Virkuti with Trichoderma viridae (0.00%) whereas maximum disease occurrence was shown by the interaction NL-1177 with Control (15%). At 7th days after treatment the minimum disease occurrence were shown by the interaction Gautam and Virkuti with Trichoderma viridae (0.00%) whereas maximum disease occurrence was shown by the interaction Gautam with Control (16.5%). Similarly, at 9th days after treatment the minimum disease occurrence were shown by the interaction NL-1177 and Virkuti with Trichoderma viridae (1.5%) whereas maximum disease occurrence were shown by the interaction NL-1177 and Virkuti with control (19.5%).

Table 5: The percentage reduction of seed-born inoculums of Alternaria species after treated the seeds at different DAI

Varieties	Disease reduction percentage								
	5th day		Reduction incidence%	7th day		Reduction incidence%	9th day		Reduction incidence%
	Untreated seeds	Treated seeds		Untreated seeds	Treated seeds		Untreated seeds	Treated seeds	
Gautam	8.75	3.5	60	18.75	9.37	50.02	25.75	8	68.93
Virkuti	6.5	5.62	13.53	7.8	6.62	15.12	9.6	8.6	10.41
NL-1177	12.75	6.75	47.05	24.75	7.37	70.22	32.25	9.87	69.39

Varieties	Disease reduction percentage		
	5th day	**7th day**	**9th day**
Gautam	60	50.02	68.93
Virkuti	13.53	15.12	10.41
NL-1177	47.05	70.22	69.39

After treatment, variety Gautam reduced in disease incidence by 60% at 5day, 50.02% at 7day and 68.93% at 9day. Similarly, variety Virkuti reduce in disease incidence by 13.53% at 5day, 15.12% at 7day and 10.41% at 9day. And Variety NL-1177 reduced in disease incidence by 47.05% at5day, 70.22% at 7day and 69.39% at 9 day respectively.

4. Conclusion

The conclusion drawn from this research underscores the significance of seed treatment in mitigating seed-borne infections, particularly Alternaria species in wheat cultivars. The study revealed significant variations in seed-borne infections among the tested wheat cultivars, with certain varieties demonstrating higher susceptibility to the pathogen. Notably, seed treatment with Trichoderma viridae, Dhanuka M-45, and Acmestin emerged as effective methods in suppressing Alternaria species on wheat seeds. Trichoderma viridae, in particular, exhibited robust potential in completely inhibiting the pathogen within five days across all tested cultivars. Dhanuka M-45 and Acmestin also demonstrated substantial control efficacy, significantly reducing the incidence of Alternaria over a nine-day incubation period. The findings strongly advocate for the pivotal role of seed treatment in reducing seed-borne pathogens, enhancing seed germination, and bolstering seed vigor in wheat plants. In conclusion, this research underscores the crucial importance of seed treatment in curbing seed-borne pathogens, especially Alternaria species, which have a profound impact on wheat germination and plant vigor. The study emphasizes the necessity of implementing seed treatment strategies to alleviate seed-borne infections and optimize wheat productivity.

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