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Effectsof SelectedPlant Extracts on Seed Borne Fungi and Seedling Parameters of Wheat

Abubakar Ali Jama[1,2*], Mohamed Ibrahim Ahmed[3],Md. Mostafa Kamal[4],Yahyeabdullaahi Isse[2]

[1]*Department of Plant Pathology, Faculty of Agriculture,Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh*
[2]*Faculty of Agriculture, Zamzam University, Mogadishu, Somalia*
[3]*Faculty of Agriculture, University of Hargeisa, Hargeisa, Somalia*
[4]Department of Food Processing and Preservation, *Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh*
Corresponding Author:Abubakar Ali Jama

Abstract: *This study was conducted to investigate the effects of differernt medicinal plant extracts as fungicide to control seed borne fungi associated with wheat and also to study the seedling parameters. In this study, five treatments viz. T_1-Control (without treatment), T_2-Provax-200 WP, T_3-Basak, T_4-Allamanda leaves, and T_5-Allamanda flowers, were used. Results revealed that chemical fungicide Provax-200 WP worked well to control the seedborne fungi significantly. Among the plant extracts, Allamanda leaf and flower extracts and Basak also reduced the association of fungi in compare to control. In some cases, Allamanda gave statistically similar results with the Provax-200. This study could help the farmers by giving valuable information of medicinal plant extracts as a fungicide and its application to control seed brone fungi associated with wheat.*

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I. Introduction

Wheat (*Triticum aestivum*L.) is one of the top growing cereal crop belonging to the familyGramineae. According to the reports of FAO (2016), the total production of wheat in the world was 749 million tons, which is the third most producedcereal after maize and rice. In Bangladesh, wheat occupies the second position after rice (Jama et al., 2018)but, its yield is too low in comparison to the world wheat production (BBS, 2015). The production of wheat is decreasing due to the many reasons and among them abnormality in seed is a major constraint (Enikuomehin, 2005). Black point (kernel smudge or smudge) is a fungal disease that affects wheat, barley and rye which caused by various species of*Alternaria*, *Fusarium*, and*Helminthosporium* and possibly other fungal genera (Jama et al., 2018).It is reported by Hasan et al. (2005) that wheat is suffered by as much as 120 different diseases, among them, 42 are seed borne and fungi cause 35 diseases. Pathogens associated with the black-point disease has become one of the most serious problems of wheat, causing great losses in both yield and quality of wheat grains (Bhandari et al.,2003; Fernandez and Conner, 2011; Drazet al., 2016) and characterized by a dark discoloration of the embryo sides of the wheat and barley grains (Maket al., 2006). The favorable conditions for blackpoint diseases is comprised of low moisture content usually 20%, coupled with the high relative humidity (80-90%) (Toklu, 2007). In most countries where cereals are grown commonly, black point can result in reduced grain quality and value (Wang et al.,2003). Black point is frequently recognizing disease in all wheat growing region entire the world (Tokluet al.,2007) and delayed seedling emergence and reduced seedling vigour (Ozer, 2005).Recent research on wheat also reported that black pointed seed is also responsible for some dangerous animal diseases like esophageal cancer (Busman et al.,2012). Other pathogens are also associated with wheat seed discoloration which are*B.sorokiniana*, *A.alternata*, *Curvularia*, *Cladosporium*, *Epicoccum*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Trichoderma*etc. (Kolawoleet al.,2013; Islam et al., 2015; Pathak et al., 2013).Seed treatments are not only controls seed borne diseases but also improve the seed health of plants and crop yield. In this regard, botanical pesticides can play a significant role in management of seed borne diseases of wheat. A number of investigators identified several plant extracts for controlling many plant diseases (Hossainet al., 1997, Rahman 1998, Nargiset al., 2005).In the light of above information, the present study was undertaken to determine the efficacy of seed treating fungicide and selected plant extracts on fungal association with wheat seeds.

II. Materials and Methods

Collecton of plants parts

In this study, we used five treatments to study the in vitro effect of different medicinal plant extracts against fungi associated with wheat seeds. These treatsments were as follows: T_1 = Control; T_2= Provax-200 WP; T_3= Basak; T_4= Allamanda leaves, and T_5= Allamanda flowers. These plants were collected from the botanical gardens of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Preparationof plant extracts

The indigenous plant or plant parts used in the experiment were Allamanda (*Allamandacathertica*L.) leaves and flowers andBasak (*Justiciaadhatoda*). For extraction of juice, 100 g of respective parts of each plant was taken, washed in tap water and crushed in a mortar and pestle. The crushed materials were blended in an electric blender adding equal amount of sterile water for 1:1 solution. The blend was filtered through sterile cheese cloth. The liquid extract was used for seed treatment of wheat seed.

Seed treatment with plant extract and fungicides

Wheat seeds(Bari Gom25)were dipped in each of the extract for 30 minutes. The liquid was drained off and the seeds were dried under shade before used in the laboratory for test. In this expirement, Provax-200wp (carboxin 37.5% + thiram 37.5%) 0.3% (on seed weight basis) were used as seed treating fungicide. Required amounts of fungicides and dry seeds were taken in a 500 ml conical flask. The flask was then shaken properly for coating of seed with fungicide. The treated seeds were then preserved in laboratory and used for seed health and seed germination test.

Seed health test of treated seed

Health status of all seed samples were analyzed for detection of seed borne fungi. The blotter method as mentioned by International Seed Testing Association (ISTA, 2001) was used for this study. Four hundred seeds were randomly taken from each of the treated seed samples. Twenty five seeds were placed on three layered moist blotter paper contained in each of 9 cm glass petridishes. The petridishes were placed on laboratory bench at room temperature for incubation under 12/12 hours light-darkness cycle. After 7 days of incubation, the seeds were examined under a stereobionocular microscope for the growth and detection of fungi. In case of confusion, temporary water mounts were prepared and observed under a compound microscope for identification of associated fungi. The fungal genera and species were identified using standard keys (Ellis, 1960; Benoit and Mathur, 1970; Booth, 1971; Chidambaram et al., 1973; Mathur and Kongsdal, 2003). We have also observed the seed germination, normal and abnormal seedlings, shoot and root length and weight to seed the effect of different treatments.

Seed germination tests for treated seed

Seed germination

Germination test was done in plastic trays filled with sterilized sand obtained from the riverbank of Punarvaba. Four hundred seeds were randomly taken from each sample for petridish. For germination test, fifty seeds were sown in each tray and the trays were then kept on the roof of academic building for 14 DAS. Care was taken to ensure the proper sunlight and moisture in soil of tray. Then data on percent seed germination, normal, abnormal, healthy and diseased seedlings were recorded according to International Seed Testing Association Rules (ISTA, 2001).

Categories of normal and abnormal seedlings

Normal seedlings

Normal seedlings were categorized by following points:

Intact seedling with all essential structures, well developed, complete in proportion and healthy. Seedlings with slight defects are (a) primary root defective but with limited damage or slight growth reduction (b) primary root defective but with sufficient well developed. Seedlings with secondary infection that would have fallen into category 2 or 1 but for infection by fungi or bacteria from sources other than parent seeds.

Abnormal seedlings

During recording the abnormalities of germinating seeds and seedlings, the following points were considered (Jama et al., 2018):

- Seminal roots missing/stunted or broken and decayed due to primary infection.
- Coleoptiles missing /split deformed or bent over.
- Shoot system (the mesocotyl if developed) broken/decayed
- Leaf missing /extending less than halfway of the coleoptile, shredded or deformed

- Seedling as a whole deformed spindly, discolored or decayed as a result of primary infection.
- Seedling with slight defects and seedlings with secondary infection.
- Blackened dead or decayed seed.
- The number of seeds that produced normal seedlings were counted and the percentage was calculated over the number of seeds placed for the test.

Measurement of plant shoots and root growth and weight

At first, the soil of the tray was watered to make it moist for easy uprooting of the plant. Then the plant along with root was lifted from the tray and dipped in a bucket of water. The root of each seedling was washed and cleaned carefully with gentle running tap water. The root portion was separated from shoot portion with sharp knife. Ten (10) seedlings were randomly selected from each tray and their individual shoot length was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of root to the largest available lateral root apex, fresh weight shoot and root was also determined with the help of digital balance.

Statistical analysis

Data on various parameters were subjected to analysis of variance following MSTAT-C computer programme and the means were compared by Duncan's New Multiple Range Test (DMRT).

III. Results and Discussion

Effects of plant extracts on pathogenic fungi

The prevalence of individual fungal pathogen included *B.sorokiniana, A. tenius, Culvularia* sp. *and Fusarium*sp. varied with respect of different treatments (Table 1). Out of the four major pathogenic fungi, *B.sorokiniana was* the most prevalent found frequently in all the treatments followed by *Fusarium* sp. *A. tenius* and *Culvularia* sp. However, all four individual pathogenicfungi were observed in higher amount (11.00, 3.00, 3.00, and 10.75) in the seeds which did not exposed to any plant extracts where provax-200 and allamanda extract reducedthe associaton of the fungi to 1.00, 1.25, 1.00 and 3.50.Similarly, Rahmanet al. (2006) found good effect ofthe extracts of onion, neem, allamanda, and durantain the mycelial growth of *Bipolarissorokiniana*.

Table 1.Pathogenic fungi associated with wheat seed treated with different plant extracts

Treatments	*Bipolaris*sp.	*Alternaria* sp.	*Curvularia*sp.	*Fusarium*sp.
T_1-Control	11.00 a	3.00 a	3.00 a	10.75 a
T_2-Provax-200	1.00 c	1.25 b	1.00 c	3.50 b
T_3-Basak	5.25b	2.00 ab	2.00 b	6.50 b
T_4-Allamanda leaf	5.50 b	3.25 a	2.00 b	6.75 b
T_5-Allamanda flower	5.25 b	2.25 ab	2.00 b	6.00 b
LSD	**	**	**	**
CV %	1.73	4.29	1.26	3.22
Same letter(s) within the same column do not differ significantly at 5% level of significance. ** Highly significance (p≤ 1%).				

In addition, the extracts of neem and allamanda also showed profound effect in increasing seed germination. Similar to allamanda, bishkatali (*Polygonum hydropiper*), garlic (*A. sativum*), zinger (*Z. officinale*), and neem (*A. indica*) extracts were also found effective against seed-borne infections of wheat caused by *A. tenuis*and *B. Sorokiniana*(Rahman et al., 1999). However, it is established that the seed treating chemical including Vitavax-200, Bavistin and Captan were very effectinve against seed-borne fungi of wheat seeds Islam et al.(2015). Though the seed treating chemical can reduce the pathogenic contamination from seeds, but also have the negative effect on environment and human health. Hence, plant extracts like allamanda can be a good alternative to chemical for the treatment of wheat seeds.

Effects of plant extracts on saprophytic fungi

After the treatment of wheat seed with different treatment, the prevalence of individual fungal pathogen including *Aspergillus* sp.,*Epicoccum* sp., *Rhizopus* sp.,*Nigrospora* sp.and *Phoma* sp. are reduced significantly (Table 2). The allamanda leaf extract reduced the association of *Aspergillus*sp. from 2.52 to 1.50, *Epicoccum*sp. 2.88 to 2.00, *Rhizopus* 3.37 to 2.00, *Nigrospora*sp. 2.00 to 1.65 and *Phoma*sp. 2.00 to 1.63. Similar to our findings, garlic clove (*Allium sativum*) and allamanda leaf extracts were also reported to reducing seed borne fungi including *Colletotrichumdematium, Macrophominaphaseolina, Cercospora*spp, *A.niger, A. flavus* and *Penicillium* spp. (Akter 2008). The seed treatment with mancozeb increased the germination percentage and reduced seed mycoflora in seed (Abatiet al., 2014). However,seed treatment with fungicide has become an

important practice for ensuring initial plant stand in establishing crops. Although seed treating fungicides reduced the association of saprophytic fungi with seeds in compare with plant extracts, but plant extracts can reduce the fungi contamination without causing any negative effect on environment and human being.

Table 2.Saprophytic fungi associated with wheat seed treated with different plant extracts

Treatments	*Aspergillus* sp.	*Epicoccum*sp.	*Rhizopus* sp.	*Nigrospora*sp.	*Phoma*sp.
T_1-Control	2.50 a	2.88 a	3.37 a	2.00 a	2.00 a
T_2-Provax-200	1.00 b	1.00 d	1.00 c	1.00 c	1.00 d
T_3-Basak	1.50 b	2.48 b	2.37 b	1.68 b	1.70 b
T_4-Allamanda leaf	1.50 b	2.00 c	2.00 b	1.65 b	1.63 b
T_5-Allamanda flower	1.50 b	1.98 c	2.13 b	1.63 b	1.43 c
LSD	**	**	**	**	**
CV %	2.95	5.63	6.11	6.18	5.13

Same letter(s) within the same column do not differ significantly at 5% level of significance.
** Highly significance (p≤ 1%)

Effects of plant extracts on seed health of wheat

Significance variations in germination percentage, normal and abnormal seedling wereobserved among all the treatment used to treat the wheat seeds (Table 3).The highest germination percentage both at 7 and 14 DAS(95.00% and 99.00%) was observed in the seeds treated with Provax-200 which was followed by seed treated with Allamanda flower (91.00%) and Basak (89.00%) where lowest ovserved in control(78.00% and 83.00%).Percentage of normal seedlings at 14 DAS was also significantly varied among all the treatments.Higher numbers of normal seedlings (95.00%)were found with the treatment of Provax-200, which was followed by Allamanda flower (83.00%) and Basak(83.00%) while the lowest number observed in control treatment (75.00%). On the other hand, at 14 DAS, control showed the maximum number of abnormal seedling (8.00%) and Provax-200 gave minimum abnormal seedling(4.00%) followed by the treatment Allamanda leaf(6.00%) and Basak (7.00%).Islam (2007) also used garlic clove, allamanda leaf, neem leaf and marigold leaf to control seed borne fungi of wheat. Where garlic clove (*Allium sativum*) and allamanda reduced maximum number of seed borne fungialong with the increasing of germination.Rather than plant extracts, Rufinoet al. (2013) concluded the positive effect of fungicide on germination of wheat seeds. However, Yeasmin (2012) observed significant reduction of seed borne fungi up to 100% over the control, where Provax, garlic and allamanda extract gave statistical similar effect against seed borne pathogen of rice.

Table 3.Germination, percentage, normal and abnormal seedling after different days of sowing

Treatments	Germination 7 DAS	Germination 14 DAS	Normalseedlings 14 DAS	Abnormalseedlings 14 DAS
T_1-Control	78.00 b	83.00 c	75.00 c	8.00 a
T_2-Provax-200	95.00 a	99.00 a	95.00 a	4.00 d
T_3-Basak	83.00 ab	89.00 b	82.00 b	7.00 b
T_4-Allamanda leaf	86.00 ab	86.00 bc	79.00 bc	7.00 b
T_5-Allamanda flower	91.00 ab	89.00 b	83.00 b	6.00 c
LSD	**	**	**	**
CV %	6.15	2.01	3.06	1.93

Same letter(s) within the same column do not differ significantly at 5% level of significance.
** Highly significance (p≤ 1%)

Shoot and root growth of wheat seedling treated with different plant extracts

The highest shoot length (26.47 cm) was observed in seeds treated with Provax-200,which was statistically similar to the Allamanda flower (25.64 cm) followed by Basak(24.53 cm) and the lowest shoot length (23.90 cm) was recorded in control (Table 4).

Table 4.Shoot and root growth of wheat seedling treated with different plant extracts

Treatments	Shoot length (cm)	Shoot Weight (g)	Root length(cm)	Root Weight (g)
T_1-Control	23.90 c	0.26 c	6.84 c	0.16 b
T_2-Provax-200	26.47 a	0.31 a	12.40 a	0.29 a
T_3-Basak	24.53 c	0.28 b	8.68bc	0.19 ab
T_4-Allamanda leaf	25.42 ab	0.28 b	11.34 ab	0.22 ab
T_5-Allamanda flower	25.64 ab	0.27bc	11.84 a	0.18 ab
LSD	**	**	**	**
CV %	1.32	1.75	1.55	2.82

Same letter(s) within the same column do not differ significantly at 5% level of significance.
** Highly significance (p≤ 1%)

The highest shoot weight (0.31 g) was obtained from Provax-200 followed by Basakand Allamanda leaf (0.28 cm) and the lowest (0.26 g) was obtained from control.On the other hand, the highest root length (12.40 cm) was recorded from Provax-200, which was statistically similar to the Allamanda flower (11.84 cm) followed by Basak (8.68 cm), wherethe lowest root length (6.84 cm) was obtained in control treatment.In the similar fashion, the highest root weight (0.29g) was recorded in treatment with Provax-200,which was statistically similar to Allamanada leaf (0.22 g) and Basak (0.19 g) whereas the lowest root weight (0.16 g) was recorded in control treatment. Ourfindings were also similar to Mostafa (2004) who showed aximum plant heights, branch, leaf, shoot length and root length in wheat plant treated with Allamanda extract gave.

Conclusion

Wheat is an important and promising crop having good potential as a cereal in Bangladesh. However, the yield of wheat is very low in compare to other wheat growing countries because of different seed bronefungi associated with wheat. Therefore, an investigation was carried out to know the association of seed borne fungi and the effect of different plant extract on those associations. Results indicated that seed treating fungicide Provax-200 reduced the association of all the associated seed born fungi in compare with all other plant used in this experiment. However, Allamanda leaf and flower extracts and Basak also reduced the association of fungi in compare to control. In some cases, Allamanda gave statistically similar results with the Provax-200. This study recommendation to carryout further comprehensive research on the health status of wheat seeds and the effect of different medicinal plant extracts on the association of fungi and their management.

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