Print ISSN 2227-1015
Online ISSN 2227-1015
https://jenvosci.com
Email: contact@jenvosci.com

Environmental Science (1): 68-77, 2022(December)
INSTITUTE OF ENVIRONMENTAL SCIENCE, UNIVERSITY OF RAJSHAHI

Open Access **RESEARCH PAPER**

EFFICACY OF CHEMICAL FUNGICIDES AND COW URINE ON SEED GERMINATION AND SEED-ASSOCIATED FUNGI THROUGH SEED TREATMENT OF TWO WHEAT VARIETIES

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Keywords: Wheat seeds, Blotter test, Cow-urine, Fungicides, Seed associated fungi, Seed germination.

Publication date: February 14, 2023

Abstract

The present experiment was conducted to evaluate the efficacy of three chemical fungicides i.e., Cupravit, Ridomil and Dithane M-45 and cow-urine on seeds of two wheat varieties as Prodip and Akbor. Through seed treatments *Bipolaris sorokiniana*, *Aspergillus* sp., *Trichoderma viride*, *Penicillium* sp. and *Rhizopus* sp. were detected by blotter method from the tested wheat seeds.Tested with fungicides and cow-urine remarkably increased the germination rate and also reduced seed associated fungal incidence over control. Higher seed germination (100%) was found at 2500 ppm of Ridomil in Prodip variety where 98.67% was in Akbor variety with Cupravit and Ridomil. Cow-urine also increased seed germination (36.99%) at 75% concentration. At 1500, 2000 and 2500 ppm of Cupravit, Ridomil and Dithane M-45 showed promising results to eradicate (100%) all associated fungi in both varieties. Moreover, among the three fungicides Cupravit had shown the best effect reducing seed associated fungal infection. Beside this, 75% concentration of cow urine were successfully controlled all associated fungi from the seeds of both varieties and increased germination.

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Introduction

Wheat is a major ingredient in such foods as bread, porridge, crackers, biscuits, Muesli, pancakes, pies, pastries, cakes, cookies, muffins, rolls, doughnuts, gravy, boza (a fermented beverage), and breakfast cereals. It is also used as animal feed. Poor farmers used wheat straw as fuel and also build their straw shade (Islam et al., 2015). In Bangladesh, the total production area 813000 acres was used for wheat cultivation and the total production is 1085000metric tons during 2020-2021 growing season (BBS 2021). Important crop diseases are often seed-borne which caused by fungi. Compared to other plant parasites, fungi cause serious diseases and crop production losses. Storage fungi are carried on seeds and can decrease germination power and seedling emergence (Ozer 2005). In addition, diseased seed was harmful to humans and animals. Concept of seed treatment is use and application of biological and chemical agents that basically can control seed borne infestation. Fungicides are used for seed treatment in the form of dusting, seed coating or wet treatment against seed associated fungi(Pedrini et al., 2017). Fungicidal seed treatment can help long periods storing seeds and improved seed emergence, plant vigour and reduce seed- and soil-borne fungal pathogens (Lamichhane et al., 2020), recent studies have raised some concerns regarding this practice (Vasanthakumari et al., 2019; You et al., 2020)Cow urine has medical properties which described in ancient medical science. The cow urine is considered as mobile medicinal dispensary which capable to cure many curable and incurable diseases (Pathak and Kumar, 2003.Ayurveda and modern researchers have also found its antimicrobial, antifungal and bio-enhancer properties. Few studies have also shown the efficacy of cow's urine in inhibiting the growth of pathogenic microorganisms like fungi and bacteria (Basak et al. 2001b, Kumar 2013, Kambar et al. 2013and Rana and De 2013). Thus, the current investigation has been carried out to evaluate the efficacy of some chemical fungicides and cow urine on seed germination and seed associated fungi of Prodip and Akbor varieties through seed treatment.

Materials and Methods.

The experiment was carried out at the Plant Pathology and Mycology Laboratory of the Department of Botany, University of Rajshahi Bangladesh.

Collection of seeds and health testing.

Sample of seeds of wheat (Prodip and Akbor) varieties were collected from Regional Wheat Research Center, Shaympur, Rajshahi (RWRC) during April 2013 to May 2014. Standard blotter method was used for health testing according to ISTA (1999) rules.

Chemical fungicides and cow urine used.

Three chemical fungicides *viz*. Cupravit 50WP, Ridomil WG and Dithane M-45 and cow urine were used.

Preparation of fungicides and cow urine.

Cupravit 50WP, Ridomil WG and Dithane M-45 were prepared at the rate of 500, 1000, 1500, 2000 and 2500ppm concentrations. At 15, 30, 45, 60 and 75% concentrations of cow-urine solution were also prepared adding 85,

70, 55, 40 and 25 ml sterile distilled water, respectively (Basak and Lee 2001b).

Seed treatment.

All seeds are sterilized with 1% Sodium hypo chloride (NaOCl) solution for 2 minutes while in control, the seeds were dipped in distilled water only. Then, the seeds were dipped for 30 minutes by the prepared different concentrations of chemical fungicides (500, 1000, 1500, 2000 and 2500ppm) and cow urine (15, 30, 45,60 and 75%)separately. After 30 min. the treated seeds were soaked on blotting paper and placed on moist blotter to determine seed germination, vigor index and seed associated fungi.

Testing of seed germination.

For seed germination test blotter method was followed. Three layers' moist blotter papers were placed each 9 cm petridish. In each petridish, randomly selected 25 seeds of Prodip and Akbor varieties were placed at same distance. The petridishes were then incubated at room temperature for seven days. After that number of germinations of the seed were counted, shoot and root length were measured and vigour index were calculated using the following formula:

Seed germination (%)= Number of seeds germinated/Total number of seeds used×100

Vigour index= (Mean of root length + Mean of shoot length) × Percentage of seed germination

Measurement of seed-associated fungi and infection percentage.

In the blotter test, after seven days a number of fungi seed and infected seeds were counted. The infection percentage was calculated following the formula:

Infection %= No. of infected seeds by individual fungus /Total number of seeds used× 100

Identification of the fungi were confirmed by studying their morphological and microscopic characteristics following the standard methods of Booth, (1971) and Alexopoulos, 1979.

After that number of germinations of the seed, shoot and root length were measured and vigour index was calculated using the following formula:

Germination (%)= Number of seeds germinated/Total number of seeds used×100

Vigour index= (Mean of root length + Mean of shoot length) × Percentage of seed germination

After seven days number of fungi per seeds was also counted and infection percentage was calculated following the formula

Infection %= No. of infected seeds/Total number of seeds used× 100

Identification of the fungi were confirmed by studying their morphological and microscopic characteristics standard methods of Booth (1971) and Ellis (1971).

Statistical analysis.

Present experiments were conducted in triplicate consistency of results and statistical purpose. Mean and standard error (M±SE) were measure using Microsoft Excel software 2013. $P<0.05$ was considered statistically significant in ANOVA test.

Results and Discussion.

Efficacy of fungicides on seed germination and vigour index of two wheat varieties.

Three chemical fungicides and cow-urine were tested on seed germination and vigour index in Prodip and Akbor varieties. Results revealed that the highest seed germination was recorded in Prodip variety, which were treated with Ridomil (100%) followed by Cupravit (98.67%) and Dithane M-45 (97.33%)at 2500 ppm.(Table 1). When considered Akbor variety, the maximum seed germination (98.67%) was observed at 2500 ppm of Cupravit and Ridomil and they enhance germination by 44.59% over untreated seeds and the lowest was 76% at 500 ppm of Ridomil. These results support the findings of Mahal (2014) who reported the percent of seeds germination were higher (98 and 95%) when the seed of *Lens arietinum* (lentils) and *Lathyrus sativus* were treated with Captan and Dithane 45 fungicides, respectively. The maximum shoot (9.08cm) and root length (11.30 cm)was found at 2000 ppm of Dithane M-45 and at 2500 ppm of Cupravit, respectively in Prodip variety. The maximum and lowest vigor index was observed 1973.40 at 2500 ppm of Cupravitin Prodip variety and 1327.47 at 500 ppm of Ridomil in Akbor variety. These results are correlated with Islam et al. (2015) who recorded highest germination percentage (80.50%) of sourav variety over control using Captan and Vivavax 200.

Efficacy of fungicides on seed associated fungi of wheat.

Rhizopus sp., *Aspergillus* spp., *Fusarium* sp.,*Bipolaris sorokiniana* and *Penicillium* sp. were isolated from fungicides treated seeds of Prodip variety (Table 1) and the most dominant was *B. sorokiniana* (12%).Tested with fungicides not only promoted the seed germination but also reduced seed associated fungi. At 1500, 2000 and 2500 ppm of Cupravit, Ridomil and Dithane M-45 successfully eradicate all isolated fungi from Prodip and Akbor varieties. Beside this, *Rhizopus* sp., *Fusarium* sp. and *Penicillium*sp. were remarkably controlled at 1000 ppm of Cupravit. Cupravit was the most effective to reduce seed associated fungi and also higher concentration was most effective. These observations are agreed with Suharti et al. (2020) who found benomyl and mancozeb fungicides effectively suppress 100% fungal growth. Nuraini and Latiffah (2019) also showed benomyl and difenoconazole inhibit mycelial growth of seven *Colletotrichum* spp. causing chilli anthracnose.

Effect of cow-urine on germination and vigour index of two wheat varieties.

Cow-urine was also tested on seeds of two wheat varieties and the results are presented in Table 3. The highest seed germination (97.33%) was found at 75% concentration in Prodip variety followed by Akbor variety (93.33%) and increased germination range 36.99 to 15.10% over the control (Table 3). The shoot length and vigour index of Prodip variety was higher (10.40 cm and 2163.97) than Akbor variety (10.33 cm and 2106.15). These finding completely agrees with Ghosh et al. (2018) who observed cow urine solution increased the shoot length and root length of wheat.

Efficacy of Cow-Urine Solution on seed associated fungi of wheat.

The efficacy of the cow-urine solution was tested against seed-associated fungi of Prodip and Akbor varieties (Table 4). Cow-urine has an inhibitory effect against seed-associated fungi. Total five genera were identified as *Aspergillus*spp., *Trichoderma viride*, *Rhizopus* sp., *Penicillium* sp. and *Bipolaris sorokiniana* in Prodip variety. In all cases, *Aspergillus* spp. showed dominance in cow-urine-treated seeds. It was also observed that 60 and 75% concentrations of cow urine completely eliminated all the fungi from the seeds of both varieties and45% concentration also controlled all the fungi except *B. sorokiniana.* This result is agreed with Amin et al. 2013 who recorded 40, 50, 60, and 70% concentrations of cow urine suppress sclerotial germination and mycelial growth of *Sclerotium rolfsii* causing fruit rot of Betel Vine. Ghosh et al. (2018) also observed cow urine solution showed the antifungal activities and inhibitory activity against *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* sp., *Penicillium* sp. *Alternaria* sp. *Mucor* sp., *Fusarium* sp. and *Macrophomina* sp. of wheat seeds.

Conclusion

From the results it reveals that 2500ppm of Ridomil showed the best performance for increasing seed germination of wheat over control. At2000 and 2500ppm concentrations of tested fungicides and at 60 and 75%cow urine solution completely eradicated seed-associated fungi of both ssvarieties and enhanced germination rate. So, cow urine can be an as promising source for the enhancement of seed germination and as well as controlling seed-associated fungi and also eco-friendly.

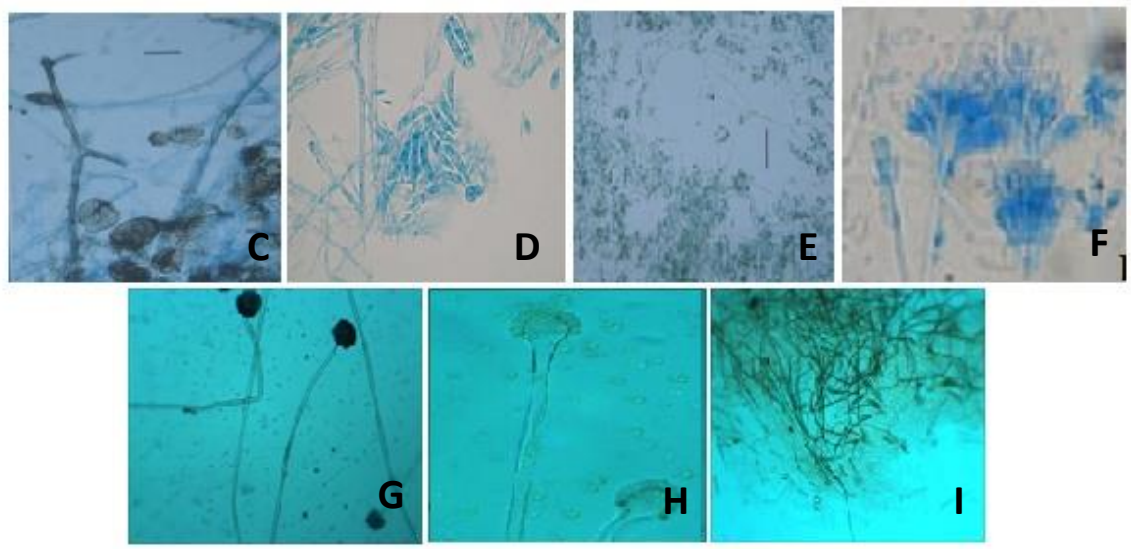
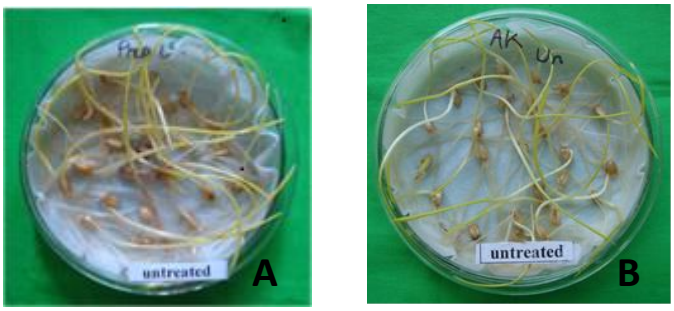


Fig. 2: Photographs showingconidia with conidiophore of different fungi.C: *Rhizopus* sp. D: *Fusarium* sp. E: *Trichoderma viride*F: *Penicillium* sp. G: *Aspergillus niger* H: *Aspergillus flavus* and I: *Bipolaris sorokiniana*



Prodip variety Akbor variety

Fig. 1:Photograph showing seed germination rate and infected seed of (A.Prodip and B. Akbor) wheat varieties.

Table 1: Effects of fungicides on seed germination, shoot length, root length and vigour index in Akbor and Prodip varieties.

Seed	Fungicides	Concentrations (ppm)	Germination (%) Mean±S.E.	Increased germination (%) over control	Shoot length(cm) Mean±S.E.	Root length(cm) Mean ±S.E.	Vigour index.
Prodip	Cupravit	500	76±2.31	21.05	8.90±0.15	10.90±0.15	1504.80
		1000	81.33±1.33	26.23	9.07±0.12	10.27±0.03	1572.38
		1500	88±2.31	31.82	8.93±0.28	10.97±0.12	1751.20
		2000	94.67±1.33	36.62	8.77±0.12	10.93±0.23	1865.00
		2500	98.67±1.33	39.19	8.70±0.15	11.30±0.21	1973.40
	Ridomil	500	77.33±1.33	22.41	8.40±0.32	9.70±0.12	1399.67
		1000	80±2.31	25	7.77±0.13	9.70±0.12	1397.33
		1500	89.33±1.33	32.83	7.77±0.13	9.70±0.12	1560.30
		2000	96±2.31	37.5	9.03±0.09	11.03±0.24	1926.40
		2500	100±0	40	8.77±0.09	10.90±0.15	1966.67
	Dithane M-45	500	81.33±1.33	26.22	8.20±0.15	10.03±0.09	1482.92
		1000	88±2.31	31.82	8.13±0.09	9.50±0.23	1551.73
		1500	89.33±1.33	32.83	7.77±0.13	9.70±0.12	1560.30
		2000	93.33±1.33	35.71	9.07±0.12	10.27±0.03	1804.38
		2500	97.33±1.33	38.35	8.90±0.23	11.07±0.09	1943.36
Control			60±2.31		4.03±0.12	3.33±0.09	
F value (LSDp≤0.05)			27.33(6.69)		8.603(0.49)	17.907(0.44)	
Akbor	Cupravit	500	80±2.31	31.66	8.77±0.12	10.83±0.09	1568.00
		1000	82.67±1.33	33.87	8.77±0.15	10.93±0.23	1628.60
		1500	88±2.31	37.88	8.40±0.32	9.70±0.12	1592.80

		2000	90.67±1.33	39.7	7.77±0.13	9.70±0.12	1583.70
		2500	98.67±1.33	44.59	8.13±0.09	9.50±0.23	1739.88
	Ridomil	500	76±2.31	28.07	7.77±0.13	9.70±0.12	1327.47
		1000	82.67±1.33	33.87	8.90±0.15	10.90±0.15	1636.87
		1500	88±2.31	37.88	9.07±0.12	10.27±0.03	1701.33
		2000	94.67±1.33	42.25	8.90±0.15	10.90±0.15	1874.47
		2500	98.67±1.33	44.59	9.07±0.12	10.27±0.03	1907.62
	Dithane M-45	500	78.67±3.53	30.51	8.13±0.09	10.03±0.09	1429.17
		1000	85.33±1.33	35.93	8.20±0.15	9.50±0.23	1510.34
		1500	88±2.31	37.88	9.07±0.12	10.27±0.03	1701.33
		2000	93.33±1.33	41.42	7.77±0.15	9.37±0.09	1599.05
		2500	97.33±1.33	43.83	7.77±0.13	9.70±0.12	1700.03
Control			54.67±1.33		3.67±0.03	3.27±0.03	
F value (LSDp≤0.05)			27.33(6.69)		12.130(0.44)	16.745(0.40)	

* Mean of three replications.

Table 2: Effects of fungicides on seed associated fungi in Prodip and Akbor varieties.

Seed	**Fungicides**	**Concentrations (ppm)**	**Infection (%) of seed associated fungi**				
			***Penicillium* sp.**	***Aspergillus* spp.**	***Rhizopus* sp.**	***Fusarium* sp.**	***Bipolaris sorokiniana***
Akbor	Cupravit	500	4	8	8	8	8
		1000	0	0	4	0	0
		1500	0	0	0	0	0
		2000	0	0	0	0	0
		2500	0	0	0	0	0
	Ridomil	500	0	0	4	8	12
		1000	0	0	0	0	4
		1500	0	0	0	0	0
		2000	0	0	0	0	0
		2500	0	0	0	0	0
	Dithane M-45	500	8	4	8	4	4
		1000	0	0	0	0	0
		1500	0	0	0	0	0
		2000	0	0	0	0	0
		2500	0	0	0	0	0
Control			47	60	46	30	32
F value ($LSD_{p\leq0.05}$)			51.23 (3.27)				
	Cupravit	500	8	4	8	4	4
		1000	4	0	4	0	0
		1500	0	0	0	0	0
		2000	0	0	0	0	0
		2500	0	0	0	0	0
	Ridomil	500	8	8	8	0	12
		1000	4	4	4	8	8

		1500	0	0	0	0	0
		2000	0	0	0	0	0
		2500	0	0	0	0	0
	Dithane M-45	500	8	16	8	8	12
		1000	0	8	4	4	8
		1500	0	0	0	0	0
Prodip		2000	0	0	0	0	0
		2500	0	0	0	0	0
Control			39	49	33	32	40
F value ($LSD_{p\leq0.05}$)			60.15 (2.68)				

* Mean of three replications

Table 3: Effects of different concentrations of cow-urine solution on seed germination, shoot length, root length and vigour index in Prodip and Akbor varieties.

Seed	Treatment	Concentrations of cow-urine (%)	Germination (%) Mean ± S.E.	Increased germination (%) over control	Shoot length (cm) Mean±S.E.	Root length (cm) Mean±S.E.	Vigour index
Prodip	Cow-urine	15	76 ± 2.31	19.30	10.13 ± 0.23	12.07 ± 0.32	1687.20
		30	81.33 ± 1.33	24.59	9.73 ± 0.12	12.13 ± 0.26	1778.42
		45	86.67 ± 1.33	29.24	10.10 ± 0.26	11.80 ± 0.26	1898.07
		60	93.33 ± 1.33	34.29	10.40 ± 0.23	12.07 ± 0.26	2096.81
		75	97.33 ± 1.33	36.99	10.17 ± 0.26	12.07 ± 0.26	2163.97
Control			61.33 ± 1.33		4.57 ± 0.07	4.10±0.06	531.73
F value ($LSD_{p\leq0.05}$)			30.14(4.97)		1.50(0.64)	0.53(0.64)	
Akbor	Cow-urine	15	70.67±3.53	15.10	9.90±0.17	12.27±0.15	1566.52
		30	78.67±1.33	23.73	9.77±0.20	12.37±0.15	1741.23
		45	82.67±1.33	27.42	9.87±0.20	12.00±0.17	1807.72
		60	86.67±1.33	30.77	10.33±0.15	12.37±0.15	1967.41
		75	93.33±1.33	35.71	10.27±0.15	12.30±0.17	2106.15
Control			60.00±2.31		3.93±0.03	3.27±0.12	432.00
F value ($LSD_{p\leq0.05}$)			18.50(6.23)		2.11(0.55)	0.93(0.50)	

* Mean of three replications.

Table 4:Effects of different concentrations of cow-urine solution on seed associated fungi in Prodip and Akbor variety

Seed	Treatment		Infection (%) of seed associated fungi				
		Concentrations of cow-urine(%)	*Aspergillus* spp.	*Tricoderma viride*	*Penicillum* sp.	*Bipolaris sorokiniana*	*Rhizopus* sp.
Prodip	Cow-urine	15	12	8	8	4	4
		30	8	4	4	0	4
		45	0	0	0	0	0
		60	0	0	0	0	0
		75	0	0	0	0	0
Control			52	24	44	42	43

F value ($LSD_{p\leq 0.05}$)			11.21(4.10)				
Akbor	Cow-urine	15	8	8	4	8	0
		30	4	4	4	4	4
		45	0	0	0	4	0
		60	0	0	0	0	0
		75	0	0	0	0	0
Control			48	20	32	57	40
F value ($LSD_{p\leq 0.05}$)			10.21(2.34)				

* Mean of three replications.

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