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Seed borne mycoflora associated with rice seeds in south Gujarat

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ABSTRACT

The study aimed to isolate and identify seed-borne fungi associated with five stored grain cultivars of rice. The seeds samples of five rice varieties, GR-4, GNR-3, GNR-4, Gurjari and NAUR-1 were procured from Main Rice Research station, Navsari Agriculture University. The five seed samples was stored in cloth bag. The blotter paper and agar plate methods were used to identification of seed borne fungi. The fourfungal genera, *Aspergillus, Curvularia, Cheatomium Fusarium* was found to be prominently associated with different seeds of rice cultivars after 8 months of storage. The numbers of fungi were found to be increased during the storage period. The association of 10 fungus species *viz., Aspergillus candidus Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Aspergillus sp., Cheatomiumsp., Curvularialunata, Curvularia sp., Fusarium moniliforme* and *Fusarium* sp. were found. Among them, the most predominant was *Curvularialunata* (19.46%). The lowest (17.07%) of association of fungi was observed in cultivar GNR-3.

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INTRODUCTION

Rice is the seed of the grass specieryza sativa L. (Asian rice) or Oryza glaberrima L. (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Rice is the world's second most important cereal crop following only corn, based on production.Globally, rice is cultivated onworld around 157.8 million hectares (Anonymous, 2014a) and production of over 200 million metric tonnes. In India, was cultivated 44 million hectares with an annual production of 104 million tonnes in 2014-15 (Anonymous, 2014b). Rice is cultivated in Gujarat around 787 thousand hectares and production of 1616 thousand metric tonnes with productivity of 2054 kg/ha in 2013-14 (Anonymous, 2014c). In South Gujarat, rice is cultivated around 2918 hundred hectares and production of 6076 hundred metric tonnes with productivity of 2082 kg/ha during 2011-12 (Anonymous, 2013). South Gujarat is an important rice growing tract of the state belonging to Dang, Valsad, Navsari and Surat districts. These districts occupy maximum rice growing area of the state, where the crop is mainly grown in *Kharif* as well as in summer season.

More than 50 fungal pathogens have been reported to be seed borne in paddy (Agrawal, 1999) of the fungi

involved, species of Alternaria, Aspergillus, Ceratobasidium, Cercospora, Cochliobolus, Curvularia, Dreschslera, Fusarium, Gaeumannomyces, Microdochium, Penicillium, Pyricularia, Pythium, Rhizoctonia, Rhizopus, Sclerophthora, Trichoderma and Tricoconella are most common associates in paddy all over the world causing pre and post-infections and considerable quality losses viz., seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value have been reported (Miller, 1995 and Kavitha et al., 2005). Most of the storage fungi are species of Aspergillus and Penicillium. These fungi may decrease seed germiability, cause seed discolouration and reduce seed weight. They produce toxins that may be injurious to man and domestic animals (Neergaard, 1986).

MATERIAL AND METHODS

The experiment was conducted at the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari during 2015 - 2016. Five rice varieties selected for the study were Gurjari, GR-4, GNR-3, GNR-4 and NAUR-1 are most popular in South Gujarat region and are growing extensively. The experiment was conducted following Completely Randomized Design (CRD) with ten repetitions. The freshly harvested seeds of these five varieties was procured in Rabi season from the Main Rice Research Station, Navsari Agricultural University, Navsari. The seeds collected from the field were brought in P. G. Laboratory and stored at room temperature for use in study. The samples of rice variety was stored in three different type of bags were 1kg/bag with proper labelling with variety name and kept in room temperature.

Isolation:

Isolation of fungi associated with rice seeds were carried out from randomly taken 200 seeds from the composite seed sample of each variety by Standard Blotter Method and Agar Plate Method (ISTA, 1993) at monthly interval.

Standard blotter method :

Three pieces of filter paper were properly soaked in sterilized water and were placed at the bottom of a 9 cm well labelled plastic Petri dishes. Twenty (20) seeds per Petri dish were placed by using a pair of forceps and making sure that seeds are placed equidistantly under aseptic conditions. The lids of each Petri dish were held in place with gummy cellotape. The Petri dishes containing seeds were incubated at room temperature $(25^{0}\pm 2^{0}C)$ for 7 days under alternating cycles of light and darkness of 12 hours each.

Agar plate method:

In agar plate method, 20 ml of potato dextrose agar was poured in glass Petri plate. After cooling, the ten seeds per Petri plate were kept at equidistance and incubated at room temperature $(25^0 \pm 2^0\text{C})$ under 12 hours alternating cycles of light and darkness for 7 days.

Identification:

The fungi were counted and identified by using identification manuals of Booth (1971), colony growth, colour, sporulation type and other basic characters as suggested by Barnett and Hunter (1972); Raper and Funnell (1973) and Mathur and Kongasdal (2003).

The per cent seed mycoflora and percentage frequency of various fungal species were calculated as follows (Agarwal, 1999 and Butt *et al.*, 2011).

Per cent seed mycoflora =	Total number of seeds on which the fungi were encountered	-x100
myconora –	Total number of seeds tested	- X100
Frequency of occurence(%)	No. of seeds on which a fungal species occures Total number of seeds examine	<u>-</u> x100

Purification:

The fungal growth of different fungi obtained on seeds was transferred on PDA Petri plates. Each fungal species isolated was further purified by hyphal tip method. Various cultures obtained were maintained on PDA slants and preserved in the refrigerator.

Seed germination:

The seed germination was determined by using paper towel method. The hundred (100) seeds from each of the cultivars were placed on damp 20 cm filter paper and covered by another filter paper. The base were folded on in and whole rolled up and secure with elastic bands and incubated upright in plastic trays at $25\pm 2^{\circ}$ C for 8 days. After incubation period, recorded the number of germinated seeds and then relationship between seed per cent mycoflora and germination was determined by using correlation analysis.

Seed vigour:

The seedling vigour test was conducted in sand (ISTA, 1993). Plastic trays (18õ9) were used. One hundred seeds were selected at random and sown on sand in each plastic tray in four lines (25 seeds/line). Percentage germination was counted at 14 DAS. After 20 days, shoot and root length were measured. Fifteen seedlings from each tray were randomly selected for measurement of shoot and root length. The seedling vigour was determined by using the following formula (Baki and Andersen, 1972).

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

Seed moisture content (%):

Moisture content of seeds of each category was determined at monthly interval to find out its effect on seed borne fungi. The seed moisture content was determined by air dry oven method (ASAE, 1986). Moisture content was determined by placing the seeds in hot air oven at 103 °C for 72 hr. the per cent moisture content was calculated by,

Per cent moisture content =
$$\frac{M_2 - M_3}{M_3 - M_1} \times 100$$

where,

 M_1 = Weight of container, M_2 = Weight of container + seeds before drying, M_3 = Weight of container + seeds after drying.

Seed weight (g):

The 1000 seeds of each variety of rice were randomly selected and weighted by the help of weighing balance at monthly interval for the seed weight.

RESULTS AND DISCUSSION

The initial (*i.e.* before) seed moisture was ranged from 10.50 per cent to 11.67 per cent in varietal seed

samples in Table 1. The lowest seed moisture (10.50%) was in coarse grain variety Gurjari and the highest (11.67%) was in long slender grain variety NAUR-1. Similarly, the 1000 seed weight was lower (19.72 g) in fine grain variety GR-4 and higher (22.12 g) in coarse grain variety Gurjari at the time of storage.

Effect on seed mycoflora:

The per cent seed mycoflora on various rice varieties was determined by the standard blotter method and Agar plate method at initial stage and at monthly interval upto 8 months of storage was depicted in Table 2. The blotter method yielded higher per cent seed mycoflora at initial (10.65%) and after 8 months of storage (67.11%) than agar plate method. Looking to the variety, highest seed mycoflora recorded from long slender grain variety NAUR-1 at initially (10.12%) and after 8 months of storage (66.15%). Whereas, lowest per cent seed mycoflora observed on bold grain variety GNR-3 at initial (6.78%) and after 8 months of storage (53.10%). The remaining three varieties viz., GR-4, GNR-3 (fine grain) and Gurjari (coarse grain) were at par with NAUR-1. The data of an interaction effect showed that lowest per cent seed mycoflora recorded from GNR-3 variety on blotter paper method (51.90%) after 8 months of storage and significant difference observed with NAUR-1 on blotter paper as well as agar plate method.

In both the methods, the av. seed mycoflora have showed the significant differences among the varieties depicted in Table 3. The lowest av. seed mycoflora 39.40 per cent and 24.12 per cent was recorded from the variety GNR-3 on blotter paper and agar plate methods, respectively. The variety GR-4 was at par with GNR-3. The highest av. per cent mycoflora 51.34 per cent and 46.16 per cent noticed in variety NAUR-1 on blotter paper and agar plate method, respectively. The per cent seed mycofloral increasing trend from initial to 8 months of storage was noticed in variety NAUR-1 and lowest

Table	Table 1 : Initial seed moisture and seed weight in various ricevarieties						
Sr. No.	Variety	Grain character of variety	Initial seed moisture (%)	Initial seed weight (1000 g)			
1.	Gujarat rice - 4 (GR-4)	Fine grain	10.82	19.72			
2.	Gujarat Navsari rice -3 (GNR-3)	Bold grain	11.57	21.87			
3.	Gujarat Navsari rice - 4 (GNR-4)	Fine grain	11.10	20.15			
4.	Gurjari	Coarse grain	10.50	22.12			
5.	Navsari Agriculture University rice -1 (NAUR-1)	Long slender grain	11.67	21.07			

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Sr. No.	Treatments		ed mycoflora*
51.100.	Treatments	(Initial)	(After 8 months)
Method (M)			
1.	Blotter paper (M ₁)	4.12 (10.65)**	55.28 (67.11)***
2.	Agar plate (M ₂)	2.88 (8.28)	49.92 (58.11)
S.E.±		0.20	1.56
C.D. (P=0.05)		0.59	4.97
/ariety (V)			
l.	GR-4 (V ₁)	2.99 (8.92)	51.88 (63.13)
2.	GNR-3 (V_2)	2.60 (6.78)	46.99 (53.10)
3.	GNR-4 (V_3)	3.15 (9.92)	50.47 (59.11)
1.	Gurjari (V ₄)	2.73 (7.47)	47.09 (53.12)
5.	NAUR-1(V ₅)	3.65 (10.12)	54.92 (66.15)
S.E.±		0.31	2.62
C.D. (P=0.05)		0.92	7.87
Interaction effect	(A×B)		
Ι.	V_1M_1	2.05 (4.20)	47.90 (55.06)
	V_1M_2	2.14 (4.57)	47.93 (55.10)
3.	V_2M_1	2.19 (4.79)	46.09 (51.90)
l.	V_2M_2	2.25 (5.06)	47.32 (54.06)
5.	V_3M_1	2.38 (5.66)	48.20 (55.11)
5 .	V_3M_2	2.38 (5.66)	49.00 (56.15)
<i>.</i>	V_4M_1	3.02 (9.12)	51.67 (61.11)
8.	V_4M_2	3.16 (9.98)	51.67 (61.11)
).	V_5M_1	3.25 (10.56)	56.45 (69.10)
10.	V_5M_2	3.30 (10.89)	54.34 (66.06)
S.E.±		0.37	2.40
C.D. (P=0.05)		1.12	7.20
C.V. (%)		35.07	17.29

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*Mean of five replications

*** Square root retransformed values are given in parentheses ****ARCSIN retransformed values are given in parentheses

Sr. No.	Variety	Av. seed myc	oflora (%)*
51.110.	v ai ici y	Blotter paper	Agar plate
1.	GR-4	39.22 (40.01)**	35.20 (33.20)**
2.	GNR-3	36.21 (39.40)	29.68 (24.12)
3.	GNR-4	42.21 (45.12)	39.75 (40.10)
4.	Gurjari	38.95 (39.56)	30.12 (25.13)
5.	NAUR-1	45.77 (51.34)	43.21 (46.16)
S.E.±		1.06	0.98
C.D. (P=0.05)		3.20	2.95
C.V. (%)		24.55	22.67

*Mean of eight months

Retransformed values are given in parentheses

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The present results of prevalence of fungi varied with respect to rice varieties are followed the results of Habib *et al.* (2012) who reported the higher percentage of seed borne fungi in fine varieties as compared to coarse varieties of rice and the rice variety mostly affected have long ridge, respectively. In the present study, the two common methods blotter paper and agar plate methods were used for the detection of seed mycoflora and the results are in coherence with findings of the earlier researchers. Blotter method was the most suitable method for detecting *A. niger, A. flavus, Alternria alternata, A. terreus, Cheatomium globosum* and *Curvularialunata* on rice seeds Archana and Prakash (2013). Moreover, the blotter paper method resulted high number of fungi as compared to agar plate method was also reported by Ashfaq et al. (2015).

Frequency occurrence of associated fungi:

At initial stage in blotter paper method a total of 3 fungal genera were found to be associated with the seed samples of different rice varieties before storage. The associated fungi were Aspergillus flavus, Aspergillus niger, Curvularialunata, Curvularia sp., Fusarium moniliforme and Fusarium sp. The fungus Curvularialunata (25.71%) was the most predominant followed by Fusarium moniliforme (18.10%), Aspergillus niger (17.14%), Curvularia sp. (15.24%), Aspergillus flavus (14.29%) and Fusarium sp. (9.52%).Curvularialunata, Fusarium moniliforme, Aspergillus niger and Aspergillus flavus were the most frequently isolated fungi irrespective of the rice varieties.

Table 4: I	Frequency occurrence of ass	sociated fung	i with seeds of	various rice va	arieties after 8	month of stora	age (blotter	r paper method)
Sr. No.	Fungus/ variety	GR-4	GNR-3	GNR-4	Gurjari	NAUR-1	Total	Frequency (%)
1.	Aspergillus candidus	5	3	8	3	4	23	6.89
2.	Aspergillus flavus	5	6	4	5	7	27	8.08
3.	As pergillus nidulans	4	5	3	4	5	21	6.29
4.	Aspergillus niger	7	8	6	5	8	34	10.18
5.	Aspergillus sp.	7	5	7	2	4	27	7.49
6.	Cheatomium sp.	5	3	8	6	5	24	8.08
7.	Curvularialunata	11	10	12	15	17	65	19.46
8.	Curvularia sp.	4	5	6	5	7	27	8.08
9.	Fusarium moniliforme	10	8	14	10	14	56	16.77
10.	Fusarium sp.	7	4	7	5	6	29	8.68
Total		65	57	75	60	77	334	
Frequency	(%)	19.46	17.07	22.46	17.96	23.05		-

Sr. No.	Fungus/ variety	GR-4	GNR-3	GNR-4	Gurjari	NAUR-1	Total	Frequency (%)
1.	A spergillus candidus	2	4	3	5	5	19	6.51
2.	A spergillus flavus	3	2	4	3	2	14	4.79
3.	A spergillus nidulans	5	3	5	4	5	22	7.53
4.	A spergillus niger	4	5	5	6	2	22	7.53
5.	Aspergillus sp.	3	2	5	5	6	21	7.19
6.	Cheatomium sp.	5	3	6	5	3	22	7.53
7.	Curvularia lunata	12	13	11	11	17	64	21.92
8.	<i>Curvularia</i> sp.	4	5	8	7	8	32	10.96
9.	Fusarium moniliforme	9	10	9	11	12	51	17.47
10.	Fusarium sp.	5	6	4	5	5	25	8.56
Total		52	53	60	62	65	292	
Frequence	v (%)	17.80	18.15	20.55	21.23	22.26		

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The rice variety GNR-3 showed higher per cent frequency (28.57%) in comparision to other varieties. The lower (13.33%) being noticed in NAUR-1 variety.

After 8 months of storage, the total 4 fungal genera were found to be associated with seeds of rice varieties was given in Table 4. The number of fungi was found to be increased during the storage period. A total of 10 fungus species viz., Aspergillus candidus Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Aspergillus sp., Cheatomium sp., Curvularialunata, Curvularia sp., Fusarium moniliforme and Fusarium sp. were found to be associated. Among them the most predominant was Curvularialunata (19.46%), followed by Fusarium moniliforme (16.77%). A least incidence 6.29% was recorded of Aspergillus nidulans. The highest (23.05%) per cent frequency was observed in variety NAUR-1 and lowest (17.07%) in GNR-3.

At initial stage in agar plate method a total of 3 fungal genera were found to be associated with the seed samples of different rice varieties before storage. The associated fungi were Aspergillus flavus, Aspergillus niger, Curvularialunata, Curvularia sp., Fusarium moniliforme and Fusarium sp. The fungus Fusarium moniliforme (25.93%) was the most predominant followed by, Curvularialunata (24.07%), Fusarium sp. (16.67%), Aspergillus niger (15.74%), Curvularia sp. (10.19%) and Aspergillus flavus (7.41%). Fusarium moniliforme, Curvularialunata, Fusarium sp. and Aspergillus niger were the most frequently isolated fungi irrespective of the rice varieties. The rice variety Gurjari showed higher per cent frequency (29.63%) in comparision to other varieties. The lower (12.96%) being noticed in NAUR-1 variety.

After 8 months of storage, the total 4 fungal genera were found to be associated with seeds of rice varieties was given in Table 5. The number of fungi was found to be increased during the storage period. A total of 10 fungus species were identified viz., Aspergillus candidus Aspergillus flavus, Aspergillus nidulans, Aspergillus niger, Aspergillus sp., Cheatomium sp., Curvularialunata, Curvularia sp., Fusarium moniliforme and Fusarium sp. Among them the most predominant fungus species were recorded Curvularialunata (21.92%), followed by Fusarium moniliforme (17.47%). The least incidence was recorded of Aspergillus candidus (6.51%). The highest (22.26%) per cent frequency was observed in variety NAUR-1 and lowest (17.80%) in GR-4.

The results of isolation of various fungal species are also agreement with the results of earlier research workers isolated from different varieties of rice. They reported the Curvularialunata, Curvulariaoryzae, Aspergillus niger, A. flavus, A. terreus, A. fumigatus, A. candidus, A. ochraceus, Alternaria alternata, Helminthosporium sp., F. moniliforme, F. oxysporum, F. solani, Cheatomium globosum, Trichoconispadwickii, Penicillium spp., Cladosporium sp. by Elangovan et al. (1999). The isolation of various seed borne fungi was isolated from rice seeds was carried out earlier by various workers, Fusarium moniliforme, Fusarium sp., Cheatomium sp. and Aspergillus niger (Butt et al., 2011; Archana and Prakash, 2013), Aspergillus candidus and Aspergillus nidulans (Paderes et al., 1996), Aspergillus flavus and Aspergillus sp., Curvularialunata and Curvularia sp. (Gupta, 2010).

Effect on seed moisture :

The av. per cent seed moisture has showed the significant differences among the varieties in Table 6. The lowest av. seed moisture 11.13 per cent was recorded

Sr. No.	Variety	Seed moisture (%)*
1.	GR-4	19.97 (11.15)**
2.	GNR-3	20.20 (11.18)
3.	GNR-4	19.75 (11.13)
4.	Gurjari	20.07 (11.14)
5.	NAUR-1	21.05 (12.90)
S.E.±		0.25
C.D. (P=0.05)		0.75
C.V. (%)		2.31

⁶Mean of eight months

**Retransformed values are given in parentheses

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from the fine grain variety GNR-4. The variety Gurjari, GR-4 and GNR-3 was at par with GNR-4. The highest av. per cent seed moisture 12.90 per cent noticed in long slender grain variety NAUR-1. The highest increasing trend was noticed in variety NAUR-1 and lowest being

in variety GNR-4.

Correlation of seed mycoflora with seed moisture:

The rice variety wise correlation co-efficient values in Table 7 were exhibited the highly positive correlation

Sr. No.	Variety	Correlation co-efficient "r"
51.10.	vallety	Av. per cent seed moisture*
1.	GR-4	0.98575
2.	GNR-3	0.92917
3.	GNR-4	0.99224
4.	Gurjari	0.75632
5.	NAUR-1	0.75271

* indicate significance of value at P=0.05 (± 0.70477)

Table 8 : Effects on seed germination and vigour index					
Sr. No.	Variety	Seed germination (%)*	Seed vigour index		
1.	GR-4	76.19 (94.30)**	1461.34		
2.	GNR-3	79.40 (96.16)	1582.69		
3.	GNR-4	73.82 (92.89)	1445.14		
4.	Gurjari	76.38 (94.41)	1526.58		
5.	NAUR-1	70.86 (89.91)	1495.03		
S.E.±		1.83	23.25		
C.D. (P=0.05)		4.25	66.74		
C.V. (%)		6.92	4.38		
*Mean of eigh	t months	** Retransformed values are g	given in parentheses		

Sr.	Variety	Correlation co-	efficient "r"
No.	Vallety	Germination (%)	Vigour index
1.	GR-4	-0.95941	0.73549
2.	GNR-3	-0.90398	0.98605
3.	GNR-4	-0.96723	0.82705
4.	Gurjari	-0.98864	0.99329
5.	NAUR-1	-0.93049	0.95980

*indicate significance of value at P= $0.05 (\pm 0.70477)$

Sr. No.	Variety	Seed weight (g)*
1.	GR-4	19.84
2.	GNR-3	22.08
3.	GNR-4	20.37
4.	Gurjari	22.51
5.	NAUR-1	21.53
S.E.±		-
C.D. (P=0.05)		NS
C.V. (%)		0.45

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of seed moisture with seed mycoflora.

The present study results were more or less similar to results were reported by earlier studies Paderes *et al.* (1996). They have reported that the seed mycoflora increases with the increase in seed moisture and results in seed rotting and loss in seed viability. A high fungal population of the species of *Aspergillus* was noted in rice stored at high moisture content levels (Paderes *et al.*, 1996). Moreover, the higher variation in the moisture content and higher incidence of *Aspergillus flavus* and *Rhizopus stolonifer* found on rice seeds stored under ambient conditions (Gupta, 2010). The significant relationship was reported between the fungi and seed moisture contents during the study of store fungi and seed health of rice (Misra *et al.*, 1995).

Effect on seed germination and seed vigour index:

The significant difference was observed for the per cent seed germination and vigour index of various rice varieties given in Table 8. The per cent seed germination was found decreased during 8 months of storage in five varieties of rice. The higher percentage of seed germination was recorded in GNR-3 (96.16%), followed by Gurjari (94.41%) and GR-4 (94.30%). The lowest seed germination percentage was recorded in NAUR-1 (89.91%). The GNR-4 was at par with NAUR-1.

The vigour index was found increased during storage, highest vigour index were recorded in bold seeds of GNR-3 (1582.69), followed by Gurjari (1526.58) and the lowest vigour index was recorded in fine seeds of GNR-4 (1445.14). The remaining two varieties *viz.*, NAUR-1 and GR-4 were at par with GNR-4. The per cent seed germination trend from initial to 8 months storage noticed the highest decreasing trend in variety NAUR-1 and lowest being in variety GNR-3. Whereas, the highest vigour index was found to be increased in bold grain variety GNR-3 and lowest being in fine grain variety GNR-4.

Correlation of seed mycoflora with seed germination :

The correlation of per cent seed mycoflora with per cent seed germination and vigour index were mentioned in Table 9. It is very conspicuous from the correlation co-efficient values, the per cent seed mycoflora was found highly significantly negatively correlated with per cent seed germination irrespective to rice varieties. Whereas, the vigour index have shown highly significantly positive correlation with per cent seed mycoflora.

The present results are in confirmation with Imolehin (1983). He reported the highly negative significant correlation between seed infection by mycoflora and seed germination in the laboratory for all the cultivars tested. Similarly, the seed germination was decreased with increased of the seed infection irrespective of their source in rice was found by Islam *et al.* (2012).

Effect on 1000 seed weight (g):

The data regarding the seed weight in Table 10 revealed that there was no significant difference in seed weight during storage in tested rice varieties.

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