Evaluation Seed-born Fungi of Rice [*Oryza sativa* L.] and that Effect on Seed Quality

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Abstract

Poor seedling establishment in rice is one of the problems in Guilan province as the second most important rice-growing locations in Iran. The aim of this study was to evaluate the contamination occurrence of rice seed in five locations of Guilan province to seed-borne fungi and their effects on germination characteristics. Five seed sample of Hashemi variety obtained from leading farmers from each location, and their germination characteristics and the rate of contamination to *Fusarium moniliforme, Bipolaris oryzae, Aspergillus niger, A. flavus, Penicillium sp., Alternaria padwickii* was evaluated using the PDA and standard blotter methods. The assessment results showed that among the seed-borne fungi, two species *A. niger* and *A. flavus* exhibited the highest severity in both methods. Samples from Rasht and Zeybaknar locations showed the most contamination to *A. flavus* and *A. niger*. A positive and significant linear relationship was observed between daily precipitation and relative humidity with severity of *A. flavus* and *A. niger* fungus. *A. niger, A. padwickii*, daily precipitation and average minimum temperature had the most influence on germination characteristics. Among the studied factors, *A. niger* had high negative impact compared to other factors on all rice seed germination characteristics. Among the study locations, quality of seeds in Rostam-Abad in terms of germination percentage, germination rate, germination uniformity and electrical conductivity had the best state. The results of this study showed that the low precipitation and low daily average humidity along with higher number of sunshine hours in the Rostam-Abad caused reduction in activity and abundance of fungi, led to improve the quality of produced seed.

Keywords: Seed-borne fungi; Rice; Seed quality; Climatic conditions

Introduction

Seed is the primary basis of crop production and is the most important available input factor for smallholder farmers [1]. In most parts of the world, smallholder farmers use their produced seed for next year planting, consequently, they attempt to stock their own produced seed for several months to several years. These seeds are often of poor quality, impure and contaminated with pathogens [2]. Seed contamination of pathogens during storage could reduce seed vigor, germination, and cause negative effect on appearance and chemical composition of seeds in addition to accelerate seed deterioration, it can also inhibit germination, transmission of the pathogen from seed to seedling or main plant leading to reduction in crop yield and threatened food security [3].

Food security of the world depends on sufficient production of small seed cereals as the cheapest source that provided about 70 of absorbable energy [4]. Based on seed production, rice is the second small seed cereals, with area under cultivation about 154 million hectares, with a global production of 720 million tons of paddy [5].

Rice is very important in Iran and it provides substantial part of the diet, especially in the three Northern provinces; Guilan, Mazandaranand and Golestan. Total rice cultivation area in the country is approximately 600 thousand hectares. Guilan province with 230 thousand hectares of rice fields with an average of 0.8 hectares of land owned by each farmer has the second place in rice production. In this province, from total rice seeds which are used by farmers only about 5 percent are considered as certified seed and the other 95 percent is produced by farmers and keep maintained in On-farm storages until sowing [6].

Different research studies have stated that in poor storage conditions, pathogens are the most important factors which not only cause the seed aging and deterioration during storage, but also seed and seedling rotting or abnormal production of seedlings in nursery [7,8]. Several reports indicated that the majority of these pathogens that lead to the production of abnormal seedlings are seed-borne fungi [9]. The rate of damage of these fungi depends on their genus and species, rate of density, fungi invaded, environmental conditions, cultivar susceptibility and interaction of these factors on cultural practice [10].

More than 100 species of fungi have been identified on rice seeds so far. However, their severity depends on the time of sampling, location and varieties are different [11-13]. Through an assessment of rice seed contamination to different seed-born fungi, 8 genera including 12 species of fungi were identified which among them *Bipolaris oryzae* with 59% and *Alternaria padwickii* with 53% had the highest severity [13]. Ahmed et al. [9] in their study on rice seed contamination identified 9 species of seed-born fungi including; *Fusarium oxysporum, F. moniliforme, Bipolaris oryzae, Alternaria padwickii, Curvularia lunata, Aspergillus flavus, A. niger, Penicillium sp.* and *Nigrospora oryzae*. They

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stated that the most common fungal contamination in examined seed samples was related to *Bipolaris oryzae* and *Alternaria padwicki*. Uma and Wesely [14] by studying on 5 seed varieties after ending storage period in India, identified seeds contamination to *Aspergillus flavus, A. niger, Penicillium citrinum, Alternaria padwickii* and *Rhizopus oryzae* in which, *A. flavus* with 18% and *A. niger* with 17.6% had the highest severity . Van Du et al. [15] studied the seed-borne fungi which were identified from samples of rice varieties in different locations in Vietnam and reported that there was a significant difference for fungus severity among the cultivars, but not for different locations of seed production. In their study, contamination to seed-born fungi in improved varieties was more than local varieties causing reduction in germination and seedling height percentage in these varieties. Among seed-borne fungi, the most severity in different varieties was related to *A. padwickii*. *A. padwickii* has led to reduction of germination stronger than others fungus. Karami et al. [16] studied the effect of *Fusarium spp.* and brown spot [*Bipolaris oryzae*] on seed germination of high yield rice varieties and reported that germination percentage and seedling growth on studied cultivars has declined due to fungal contamination.

The quality of used seeds in the establishment of normal seedlings is important. The contamination of seeds to seed-borne fungi during storage by reducing seed quality affects on seed germination and establishment of seedling. By considering the self-consumption of rice seed in the rice seed production system in Guilan, and inappropriate condition during on-farm storage, it seems that performing targeted basic-applied research in this field is necessary. Hence, the present study was carried out to evaluate the contamination of rice seed for Hashemi variety, which is commonly used by farmers in different locations of Guilan province, to seed-borne fungi and its effect on germination characteristics.

Methods and Materials

Guilan province located between 38˚-27´-7″ to 36˚-36´3″ north latitude and 50˚-26´-42″ to 48˚-34´-25″ east of the Greenwich meridian, between the heights of the Alborz Mountains and humidity resource of the Caspian Sea. However, among various locations of rice production in Guilan, in terms of the rate of precipitation, temperature, relative humidity and sunshine hours are different [17]. Hence, this evaluation was carried out in the spring of 2013 by using of seed samples collected from 5 different locations of Guilan including Amlash, Zeybaknar, Talesh, Rasht and Rostam-Abad. Meteorological data in the studied locations of Guilan province during six months of plant growth period [2012/03/01 to 2012/09/01] and the seed development is presented in Table 1. From each location after the end of seed harvesting, five

seed samples of Hashmi which is the local dominant variety in Guilan province randomly received from the leading farmers. All samples stored in similar condition in cold storage at 5°C for 6 months.

Seeds germination characteristics were evaluated by using standard germination test in a completely randomized design with four replications in Petri-dishes with 9 cm diameter. For this reason, the first seed surface dis contaminated for the purpose of determining the presence of internal fungi in seed with sodium hypochlorite [5%] for 1 min and then washed 3 to 5 times with distilled water [18]. Then, after laying one Whatman filter paper at the bottom of each Petri-dish, 25 seeds of each sample were placed on filter paper and after adding 5 ml of distilled water, to retain moisture Petri dish lid were placed on them. Then Petri-dishes were transferred to growth chamber at 25°C, with normal light and relative humidity of 70 ± 5 percent [19]. During the experiment [7 days] once in every 8 hours germination seeds were counted, based on root let criteria in size of 2 mm or more [20]. Using of obtained data, germination characteristics including germination percentage, germination rate, germination uniformity, D10, D50 and D95 [in order of time that it takes to cumulative germination percentage reach its maximum 10, 50 and 95] were calculated by Germin program [21]. In order to perform the electrical conductivity test [electrical conductivity which represented the amount of material was leakage from seed], the seeds washed with deionized water to remove contaminated surface of seeds, then 5 g of seeds immerse in 50 ml of distilled water at 25°C and after 24 hours electrical conductivity of solution measured by conductivity meter device model LF 90 SER-NA31245385 [22].

In vitro potato dextrose agar method [PDA] and standard blotter method were employed to identify the seed-borne fungi of *Fusariummoniliforme, Bipolarisoryzae, Aspergillusniger, Aspergillusflavus, Penicillium sp.*, *Alternariapadwickii* and to determine their contamination percentage. In both methods, seeds were soaked for 5 minute at sodium hypochlorite [1%] solution and immediately washed with distilled water 3 to 5 times in order to prevent fungal pathogens infection [23]. In PDA method for each sample was performed with four replications, in each Petri dish poured some prepared of PDA then with sterile forceps under the hood 10 seeds were placed on the in PDA [23] (Figure 1). After six days Petri dishes were placed at a temperature of 22°C incubator, seed born fungi were identified and contamination rate of seeds to these factors were evaluated [24]. The standard Blotter method for each sample was carried out with 400 seeds, in each Petri dishes, three layers of Whatmann filter papers soaked with distilled water and were placed with 25 seeds. Petri dishes [16 Petri dishes for per sample] for incubating placed in temperature of 22°C then 6 days in alternating cycles of 12 hours near

	Seed development period [heeding to harvest maturity]	Maximum temperature average [°C]		Minimum temperature average [°C]		Daily sunny hours		Daily precipitation [mm]		Daily relative humidity	
		Seed develop. period	2012/03/01 to 2012/09/01	SEED develop.period	2012/03/01 to 2012/09/01	Seed develop. period	2012/03/01 to 2012/09/01	Seed develop. period	2012/03/01 to 2012/09/01	Seed develop. period	2012/03/01 to 2012/09/01
Amlash	2012/07/05 to 2012/08/15	31.15	27.02	21.03	16.85	7.75	6.26	1.02	3.04	72.42	73.90
Zeybakenar	2012/06/30 to 2012/08/10	29.88	26.34	21.75	18.48	7.95	6.87	3.70	2.05	73.45	74.23
Rasht	2012/06/30 to 2012/08/10	30.34	27.29	21.43	17.99	6.92	6.41	3.10	2.89	81.22	80.91
Talesh	2012/07/05 to 2012/08/15	30.54	25.89	22.17	17.83	7.36	6.01	1.20	2.72	69.01	74
Rostam-Abad	2012/08/05 to 2012/09/10	30.05	28.79	22	19.43	8.30	7.24	0.10	0.50	65.80	63.99

Table 1: Meteorological information for different locations of seed production of Guilan province during seed development period and six months of year in 2012.

Figure 1: Potato Dextrose Agar method [PDA].

Figure 2: Standard blotter method.

ultraviolet light and 12 hours in darkness. At the seventh day grown fungi on seeds germination were identified by using of a stereo microscope and number of infected seeds in each seed sample marked and calculation was performed as contamination percentage [24] (Figure 2).

Data analysis conducted by stepwise regression, ANOVA and multiple comparison using LSD test was performed by using of SAS [25] software.

Results and Discussion

The results showed differences between evaluated locations from contaminated rate point of view to seed-borne fungi and also different severity of contaminated seeds to various fungi included *Fusarium moniliforme*, *Bipolaris oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium sp.*, *Alternaria padwickii* (Table 2). In examined seed samples, among rice seeds borne fungi, *Aspergillus niger* and *Aspergillus flavus* in both evaluated methods [Standard blotter method and PDA method] had the most severity than the other fungi species (Table 3), these data confirms the results reported by Uma and Wesely [14] which they stated that *A. flavus* and *A. niger* fungi as the most important storage fungi could be active in seed moisture content below 20 percent. Trung et al. [26] by examination on the 25 seed samples of rice in Vietnam mentioned that genus of fungus Aspergillus, Fusarium and Penicillium were the most common fungi. Reddy et al. [27] in their study on stored rice for more than a year from 18 different ecosystems in India were stated that Aspergillus is the most important fungal contamination on rice seeds.

Among the five different evaluated locations, samples from Rasht and Zeybakenar had the most contamination to rice seed-borne fungi, and the most severity fungi were *A. flavus* and *A. niger* (Table 3). Reddy et al. [27] stated that weather conditions before harvesting could be the most important factors that led to invasion of fungal species. Mosa-Nejad et al. [28] reported that the rate of precipitation, daily minimum humidity, daily maximum temperature and sunshine hours could be the most important factors affecting the distribution of rice blast in Guilan province so that suitable climatic conditions could considerably help to spore population of blast fungi and occurrence of blast disease in farm during seven to ten days after occurrence of suitable conditions. Aghili et al. [29] by study on the rice fungal contamination in Mazandaran province stated that increasing average humidity in the west of Mazandaran could be the reason for the severity of fungal contamination of the seeds. Also, the results showed that under high humidity conditions, fungi of genus Aspergillus had the most severity which corresponded with the obtained results in this research.

In the present study with regard to fungal contamination rate as a dependent variable, and climatic factors including; daily relative humidity, number of daily sunny hours, daily precipitation and minimum average temperature were entered to the regression model, respectively. Stepwise regression analysis showed that the daily humidity, the amount of daily sunshine hours, daily precipitation and minimum average temperature are the most important climatic factors that affected the distribution of different types of the evaluated seed-borne fungi except *Fusarium moniliforme* and *Bipolaris oryzae* (Table 4). The stepwise regression analysis on climatic factors affecting severity of fungi *A. flavus* and *A. niger* determined that precipitation and daily humidity could be the most influential factors in distribution of rice

	DF	*Fusarium moniliforme*	*Pyricularia oryzae*	*Bipolaris oryzae*	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium sp.*	*Alternaria padwickii*	% total infection
Methode	1	33.40*	143.41**	138.44**	2892.18**	442.50ns	367.90**	6.59ns	2304.59**
Location	4	142.33**	42.80**	176.32**	11491.03**	868.48**	221.06**	156.27**	10789.87**
Location × Methode	4	46.96**	38.09**	16.28**	244.76**	139.65**	188.09**	30.45**	884.97**
Rep[Methode* Location]	30	0.94	0.41	3.01	62.44	18.76	2.38	0.73	123.74
Error	160	31.23	6.65	33.18	626.53	275.62	19.42	10.14	435.55
Coefficient Variance		8.67	6.83	6.61	7.85	7.76	6.66	6.95	3.37

ns: non-significant, * and **: Significant at 5% and 1% probability levels, respectively.

Table 2: Analysis of variance for seed-born fungi in rice seed samples in different locations of seed production of Guilan province.

Seed production location	Method	Number of Sample	Fusariummoniliforme	Pyryicularia oryzae	Bipolarisoryzae	Aspergillusniger	Aspergillusflavus	Penicillium sp.	Alternariapadwickii	%total infection
Amlash	PDA	5	0g*	2.51b	6.2b	5.14f	16.33ab	2.06e	0g	19.40d
	Blotter	5	0.25f	0e	4.68bc	3.32f	11.94dc	1.31ef	0.90e	17.79d
Zeybakenar	PDA	5	2.06d	0e	6.15c	35.14c	17.29a	3.12bc	0g	39.17b
	Blotter	5	0.85e	0.45d	3.73d	23.99d	15.52ab	2.57bc	1.31d	30.09c
Rasht	PDA	5	7.1a	2.47c	1.05f	50.62a	14.26bc	1.05f	6.15a	56.67a
	Blotter	5	2.72bc	0e	1.56e	41.79b	17.19a	1.76d	3.78b	61.80a
Talesh	PDA	5	0g	4.78a	8.21a	14.26e	11.24de	12.3a	2.11c	28.48bc
	Blotter	5	1.15d	0.45d	5.79bc	11.14e	6.25f	2.16dc	0g	20.21d
Rostam-Abad	PDA	5	2.11c	0e	5.39bc	18.04e	9.12e	4.13b	0g	32.46bc
	Blotter	5	2.21b	0e	2.92d	4.94f	2.47g	1.31ef	0.45f	12.35e

*Mean in each column followed by similar letters are not significantly different at 5% level, according to LSD Test.

Table 3: Mean comparison of seed-born fungi at rice seed samples in different locations of seed production of Guilan province based on PDA and Blotter methods.

dependent variables	Methode	independent variables	MS	a ± Es	b ± Es	R^2	Pr-F
A. niger	PDA	daily precipitation	4161.44	9.69 ± 7.16	4.26 ± 9.53	0.87	0.035
	Blotter	dailyrelative humidity	3430.59	6.97 ± 0.92	3.06 ± 8.65	0.95	0.009
A. Flavos	PDA	-	-	-	-	-	-
	Blotter	dailyrelative humidity	585.53	3.94 ± 3.87	1.73 ± 3.57	0.91	0.051
Alternaria	PDA	daily sunny hours	110.06	12.09 ± 35.25	1.57 ± -4.39	0.95	0.010
	Blotter	daily precipitation	16.92	6.53 ± 14.39	0.85 ± 1.72	0.85	0.054
Blast	PDA	daily sunny hours	37.93	11.05 ± 21.55	1.44 ± -2.58	0.92	0.086
	Blotter	-	-	-	-	-	-
Penisilum	PDA	minimum temperature average	209.68	62.92 ± -149.46	2.9 ± 7.09	0.90	0.022
	Blotter	-	-	-	-	-	-
tootal	PDA	dailyrelative humidity	2176.18	69.44 ± -95.47	0.95 ± 1.80	0.93	0.072
	Blotter	dailyrelative humidity	3068.91	65.07 ± 178.14	2.88 ± 6.22	0.97	0.0009

Intercept [a], the values of the slope of the regression line [b], mean squares [MS], coefficient of determination [R2], The level of significance [Pr-F].

Table 4: Analysis of stepwise regression with consideration of seed-born fungi as dependent variables and climatic factor as independent variables.

seeds to this fungi. Regression analysis showed that there is linear relationship and positive significant difference between precipitation and daily humidity rate with severity of fungi *A. flavus* and *A. niger*, these functions 87 to 95 percent justified the variation in these characteristics. According to the obtained regression function, it could be stated that for *A. niger* fungi in PDA method, for every unit increase with increasing daily precipitation and the standard blotter method for per unit increase with increasing daily humidity, the severity of *A. niger* will be increase 9.53% and 8.65%, respectively. Furthermore, obtained regression function for *A. flavus* fungus by standard blotter method for per unit increase in daily humidity severity of fungi *A. flavus* will increase to 3.57 percent (Table 4).

The results for evaluating the effect of climatic factors on the severity of the *Alternaria padwickii* fungus, showed that in PDA method, there was significant negative linear relationship between the amount of daily sunshine hours and severity of Alternaria fungi and in standard blotter method there was positive and significant linear relationship between precipitation rate and severity of the Alternaria fungus. Obtained functions explain 95% and 85% changing percentage in severity of *Alternaria padwickii* fungus. According to obtained regression function, it could be stated that for Alternaria fungus in PDA method, for every per unit reduction of daily sunshine hours and for standard blotter method for every per unit increase of daily precipitation, severity of this fungus will increase 4.39 percent and 1.72 percent respectively (Table 4).

To examine the characteristics of germination and EC as the dependent variable, climatic factors and fungus factors pathogenic arranged individually then entered into the regression model (Table 5). Among the studied factors *A. niger*, *Alternaria padwickii*, daily precipitation and minimum average temperature had the most impact on the characteristic of studied germinating seed samples. These effects could probably be due to the influence of precipitation and daily minimum average temperature on seed aging before harvest [30] or the influence of *A. niger* and *A. padwickii*, on seed deterioration with various changes including the loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis, and DNA degradation during storage [31]. Makun et al. [32] showed that *A. niger* and Fusarium fungus were more abundant in rainy weather and dry weather conditions, respectively. Jalander and Gachande [33] by study on the effect of different fungal species of seed-borne fungi of Aspergilluson germination and seedling growth of Bean and Cereals reported that *A. niger* caused reduction in germination percentage, growth of the plumule and radicle. They reported that this effect could be resulted from further metabolic production by *A. niger* fungi than *A. flavus* and *A. nidulans*.

The present study investigated the climatic factors during seed development stage [heading to harvest maturity], and daily average precipitation and minimum average temperature were entered in to the regression model. The results showed that daily precipitation with D10 and minimum average temperature with EC had negative linear and significant relationship. Obtained functions were 56% and 89%, respectively and justified these traits of variation. According to the obtained regression function, it could be stated that for per unit increase in precipitation, D10 will decrease as much as 2.08 hours and per unit increase in minimum average temperature, EC will decrease at a rate of 9.54 micro Siemens per gram. Effect of minimum temperature

dependent variables	Methode	Independent variables	MS	a ± Es	b ± Es	R²	Pr-F
Percent of germination	PDA	*A. padwickii*	576.15	1.60 ± 96.11	0.35 ± -1.14	0.80	0.003
		A. niger	322.77	1.79 ± 97.01	0.04 ± -0.09	0.91	0.001
	Blotter	*A. niger*	619.20	1.81 ± 97.88	0.06 ± -0.21	0.83	0.002
Speed of germination	PDA	*A. niger*	0.000013	0.0003 ± 0.014	0.000007 ± -0.000024	0.89	0.005
	Blotter	*A. niger*	0.000017	0.0002 ± 0.014	0.000009 ± -0.000036	0.88	0.0009
Germination uniformity	PDA	*A. niger*	1662.03	2.93 ± 26.72	0.07 ± 0.26	0.95	0.001
	Blotter	*A. niger*	2192.56	2.56 ± 26.49	0.08 ± 0.40	0.96	0.0002
D10	PDA	*A. niger*	157	1.49 ± 54.6	0.03 ± 0.08	0.86	0.042
		daily precipitation	159.72	1.78 ± 57.48	0.90 ± -2.08	0.56	0.010
	Blotter	*A. niger*	171.54	1.42 ± 54.71	0.04 ± 0.11	0.89	0.033
		daily precipitation	159.72	1.78 ± 57.48	0.90 ± -2.08	0.56	0.010
D50	PDA	*A. niger*	525.85	1.86 ± 66.80	0.04 ± 0.15	0.70	0.004
	Blotter	*A. niger*	687.17	1.66 ± 66.69	0.05 ± 0.22	0.84	0.0008
D95	PDA	*A. niger*	2685.75	3.57 ± 87.64	0.09 ± 0.33	0.87	0.001
	Blotter	*A. niger*	3321.92	3.18 ± 87.66	0.11 ± 0.49	0.86	0.0002
EC	PDA	Minimum temperature average	379.51	66.4 ± 247.14	3.06 ± -9.54	0.89	0.004
	Blotter	Minimum temperature average	379.51	66.45 ± 247.14	3.06 ± -9.54	0.89	0.004

Intercept [a], the values of the slope of the regression line [b], mean squares [MS], coefficient of determination [R2], The level of significance [Pr-F].

Table 5: Analysis of stepwise regression with consideration of characteristics of germination as dependent variables and climatic factor and seed-born fungi as independent variables.

	DF	Gmax	R50	GU	D10	D50	D95	EC
Location	4	238.64**	0.000006*	909.88**	263.92**	251.44*	1525.5**	549.1**
Rep[Location]	15	16.69ns	0.000002ns	9.78ns	7.44ns	11.55ns	34.69ns	0.91ns
Error	80	90.24	0.000002	199.32	37.71	83.64	293.16	36.45
Coefficient Variance		10.07	10.33	12.43	10.84	12.97	17.83	14.86

ns: non-significant, * and **: Significant at 5% and 1% probability levels, respectively.

Table 6: Analysis of variance for percent of germination [Gmax], germination rate [R50], uniformity of germination [GU], D10, D50 and 95[in order of time that it takes to cumulative germination percentage reach its maximum 10, 50and95] and electrical conductivity [micro Siemens. cm^{-1}. g^{-1}] in different locations of seed production of Guilan province.

increase could be caused by reducing activity of *A. niger*. Similar studies have also stated that unfavorable climatic factors during grain filling, to grain chalkiness, specification changes in viscosity, gelatinization temperature and amylose content leads to reduced grain and seed quality [34-37]. Pantuwan [34] reported that although high humidity in the reproductive phase and grain filling phase resulted to increase in grain amylase content, but due to the prolonging period of grain filling period, seed quality reduced by increasing fungi activities. Mobasser et al. [38] by studying wheat seed contamination with seed-borne diseases in cold climatic zone of Iran reported a significant difference between and among provinces and towns with respect to *T. leavis* infection. Moreover, the data showed clear dispersal of these two species [*T. caries* and *T. leavis*]. Khalili aqdam et al. [39] stated that environmental condition caused difference in seed vigor between seed lots of soybean. This difference could due to seedling dry weight, normal seedling percent and more importantly, electrolyte leakage during the electrical conductivity test. Herrera et al. [40] at the study of temperature and precipitation as environmental impact on some wild species of the Grass showed that this factors by changing grain filling period effecting the seed germination and seed vigor.

Among the examined seed-borne fungus, for germination percentage, Alternaria and *A. niger* were entered into models and for other studied traits only *A. niger* was entered into the regression model. As a negative impact, *A. niger* fungi affects on all rice seed germination characteristic more than all other fungi. In accordance with the results of the present study, Islam et al. [41] stated that there is negative and significant correlation [R=-97%] between rate of fungal

contaminations and germination percentage in different rice cultivars. Ijaz et al. [42] reported *A. niger* as the most damageable storage fungi among fungal pathogens which were introduced, leads to lower quality and seed germination.

The results of ANOVA (Table 6) showed significant differences among different seed germination characteristics in the different locations of rice seed production in Guilan province. Based on germination percentage, germination rate, germination uniformity and electrical conductivity among the studied locations, Rostam-Abad location had highest seed quality and Rasht and Zeybaknar locations had the lowest quality of seed production (Table 7). According to the findings of this study, it could be stated that climatic conditions such as the lowest precipitation rate, daily humidity average and number of daily sunshine hours in Rostam-Abad reduce the activity and severity of seed-borne fungi, leading to an improvement in seed quality. Several studies have also reported the similar results [43-46]. According to their studies, diversity and difference in severity of pathogenic fungi in the rice seed in various locations were attributed to the climatic conditions and crop management. Also, they stated that during seed development, severity of farm fungi [different species of Aspergillus, Fusarium and Penicilliumin] influenced by certain factors such as high relative humidity and environments temperature as well as high levels of seed moisture during harvest which could increase the contamination to these fungi and reduce seed quality during Storage.

Conclusions

The results showed that there was large variation in the diversity and

Seed production Location	Gmax	R50	GU	D10	D50	D95	EC
Amlash	98a*	0.0148ab	29.55ab	53.92c	67.43c	87.84b	47.38a
Zeybakenar	93.4b	0.0145b	41.09a	53.18c	70.46b	103.52a	38.72d
Rasht	90.6c	0.0139c	38.39b	57.74b	73.36a	100.44a	43.68b
Talesh	91.6bc	0.0136c	33.03c	62.27a	74.64a	102.9a	39.85c
Rostam-Abad	97.8a	0.015a	24.29e	55.94b	66.55c	85.29b	33.51e

*Mean in each column followed by similar letters are not significantly different at 5% level, according to LSD Test.

Table 7: Mean comparison for percent of germination [Gmax], germination rate [R50], uniformity of germination [GU], D10, D50 and 95 [in order of time that it takes to cumulative germination percentage reach its maximum 10, 50 and 95] and electrical conductivity [micro Siemens. cm^{-1}. g^{-1}] in different locations of seed production of Guilan province.

severity of fungal contamination to seed-borne fungi in seed samples. Among seed-borne fungi, *Aspergillus niger* and *Aspergillus flavus* had the most severity rate compared to others. Among the climatic factors, daily humidity, daily sunshine hours, daily precipitation and daily minimum average temperature were the most important factors that effected the distribution of studied seed-borne fungi types except *Fusarium moniliforme* and *Bipolaris oryzae*. Evaluation of germination characteristics of rice seeds under the influence of climatic factors on seed development stage and seed-borne fungi showed that there was a significant negative linear relationship between the average of daily precipitation amount with D10 and the average of minimum temperature with EC. Also among studied seed-borne fungi, for germination percentage, Alternaria and *A. niger* fungus and for other germination traits only *A. niger* were significant. From negative impact *A. niger* fungi compared with others fungus implied all characteristics on rice seed germination. Among the five different locations, samples from Rasht and zeybaknar had the highest contamination to seed-borne fungi pathogens, also the most severity of *A. flavus* and *A. niger* fungi were observed in these locations. Samples from Rostam-Abad had the least seed-borne fungi; this could be due to low precipitation rate and daily mean average humidity per day and number of sunshine hours in the studied location.

Acknowledgements

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