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ORIGINAL RESEARCH ARTICLE



## Seeds infection of *Fusarium moniliforme* in different Rice varieties grown in mid-hills of Nepal

Poonam Pandey<sup>1</sup>, Ram Devi Timila<sup>2</sup> and Sandeep Airee<sup>3\*</sup>

<sup>1</sup>Himalayan College of Agricultural Sciences and Technology, Kathmandu, NEPAL, <sup>2</sup>Plant Pathology Divisions, NARC, Khumaltar, Lalitpur, NEPAL, <sup>3</sup>Agriculture and Forestry University, Chitwan, NEPAL

\*Corresponding author's E-mail: [sandepairee@gmail.com](mailto:sandepairee@gmail.com)

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### ABSTRACT

*Fusarium moniliforme* is one of the important seed-borne fungi responsible for foot rot disease in rice. The study was conducted at the Plant Pathology Division of NARC, Khumaltar from July- December 2019 to find out the level of seed infection of *F. moniliforme* in different varieties of rice from mid-hills of Nepal. A total of 20 seed samples of different varieties of rice with 240 seeds of each sample were tested following a deep-freeze blotter method distributing 80 seeds per replication and maintaining 3 replications. Seed to seedling transmission test was carried out under screen house conditions. Two hundred seeds of each highly infected five varieties from laboratory test data were planted in eight pots, twenty-five seeds per pot, and categorized into four replications. Component plating was done to determine the location of *F. moniliforme* infection in a seed. Data analysis was done using STAR at a 5% level of significance. There was a significant difference among all the varieties. The highest incidence of *F. moniliforme* infection was found in seeds of Khumal-9 variety and lowest in Fan-10 variety. From seed to seedling transmission test, Khumal-4 variety was found highly susceptible to foot rot among the 5 varieties planted. Transmission percentage of disease from seed to seedling was found ranging from 16.19-72.31%. Equally, Component plating concludes that seed coat, as well as endosperm, was the location of *F. moniliforme* infection in rice seed. Foot rot being one of the serious diseases of rice at present time researches should be done more on this for its effective management and control. Seed health status testification before taking seeds to the field should be done so that timely control like seed treatment could be applied to control the outbreak of the disease in the field.

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### INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crop after wheat and maize with a total harvested area of 167 million hectares, producing more than 782 million tons annually all over the world (FAO, 2018). In Nepal, the total area under rice cultivation during the year 2018/19 was 1.4 million hectares with annual production of 5.6 million tons of paddy with an average productivity of 3.76 Mt/ha (MOAD, 2018/19). Foot rot also known as "bakanae disease", caused by *Fusarium moniliforme* has

recently emerged as a significantly important disease in all the countries where rice is grown (Venturini *et al.*, 2013). *Fusarium moniliforme* is well represented among the communities of soil-borne fungi, in every type of soil all over the world. This species is also considered a normal constituent of the fungal communities in the rhizosphere of plants (Hassanein *et al.*, 2016). In the mid-hill region of Nepal incidence of foot rot in rice has been increasing. Initially, based on different morphological studies many plant pathologists reported *F. moniliforme* was the only species involved in causing foot rot disease of rice. But later

on three mating populations MP-A (anamorph *F. verticilloides*), C (anamorph *F. fujikuroi*) and D (anamorph *F. proliferatum*) of the *Gibberella fujikuroi* complex have been reported to associate with foot rot disease of rice (Bashyal and Aggarwal, 2013; Gupta et al., 2015).

Different local and improved varieties of rice are adversely affected by different fungal diseases. (Singh et al., 2019) The yield loss due to this disease has been reported to range from 3% to almost complete loss depending on extent of infection, variety and weather conditions. Typical symptoms of foot rot in rice plants are usually characterized with thin and elongated plants, the infected plants were taller than the normal height of the other plants in seedbed and field; formation of root lesion which dies before or after the emergence of the seedlings; surviving plant at maturity formed empty or partially filled sterile grains. It is considered to be a high threat because it causes significant seedling mortality and yield loss. The morphological architecture of the rice seeds shape and size, color, and smooth and roughness significantly correlated with the transmission of fungal pathogens (Akter et al., 2019).

Sunani et al. (2020) revealed that the infection of rice plants by *F. fujikuroi* can occur through three routes namely seed, seedlings (soil), and florets (air). Foot rot is an economically important rice-growing area of Asia as it causes significant yield loss estimated at approximately 20% (Cumagun et al., 2011). Desjardins et al. (2000) reported about 40% yield reduction of Khumal-4 rice variety in Nepal. Khumal varieties of rice are more susceptible to this disease although other varieties are also attacked. If the diseased seedlings do not die then also they are not able to give panicles which directly affect the yield and cause severe economic loss to the farmers. The disease is known to cause both qualitative as well as quantitative losses.

The application of Trichoderma as seed treatment alone and in a combination of fungicides was recommended most effective for the management of bakanae disease in rice (Pal et al., 2019). It is a major seed-borne disease of rice so it is important to know how different varieties of seeds differ in the level of infection so that we can get the best variety out of them against the disease. The infested seed is a primary source of inoculums and acts as the main means of spreading the disease from field to field (Anderson and Webster, 2005). Hence proper study is needed to know about the disease, its occurrence, epidemiology, disease cycle, and management also. This study established relevant information to determine seed infection difference in Khumal varieties of rice, determine the percentage of seed to the seedling transmission of *F. moniliforme* and finally find out the location of *F. moniliforme* infection in rice seed.

## MATERIALS AND METHODS

The experimental research was conducted in July- December 2019 at Plant Pathology Division of Nepal Agriculture Research Council, Khumaltar, Lalitpur.

### Collection of seed samples

Seed samples of 20 different varieties of rice grown in mid-hills were collected. Taichung variety was collected from the farmer's field, the remaining other 19 varieties were collected from the Agronomy division and Botany division of NARC, Khumaltar. The total collected varieties were Khumal-2, Khumal-3, Khumal-4, Khumal-5, Khumal-6, Khumal-7, Khumal-8, Khumal-9, Khumal-10, Khumal-11, Khumal-13, Palung-2, Machapuchhre-3, Lekali-1, Lekali-3, Chandannath-1, Chandannath-2, Fan-10, Chainung-242, and Taichung. These seed samples were stored in the refrigerator until the research was completed.

### Detection of *F. moniliforme*

The method used for the detection of *F. moniliforme* was Deep-freeze Blotter Method. In total 240 seeds of each variety were examined. The following points will highlight the major steps:

#### Preparation of petri plates

On a clean working table, 12 Petri plates were placed and were sterilized using cotton dipped in 70% alcohol. Then labeling was done on the lids of Petri plates by writing the varieties name, date, and replication number. Inside the Petri plates, the 3 layers of blotting paper dipped in sterilized water was placed. During moistening blotting paper, care was taken for trapping last drop of water in blotting paper.

#### Counting and plating of seeds

Randomly 240 seeds were taken from the seed sample. The counted seeds were plated in the prepared Petri plates. Among 12 Petri plates in each plate, 20 seeds were placed by making three replications. Each replication contained four Petri plates. Seeds were placed using clean and sterilized forceps.

#### Deep-freezing of the seeds

The Petri plates were incubated in the incubation room under 12 hour's alternate cycle of NUV light and darkness at  $22\pm 2^\circ\text{C}$  for 24 hours. Then the plates were placed in the deep freeze for 24 hours to inhibit the germination of the seed.

#### Incubation of the seeds

After 24 hours plates were taken out from deep freeze and brought back in the incubation room under 12 hours alternate cycle of NUV light and darkness at  $22\pm 2^\circ\text{C}$  and incubated for another 6 days. During incubation, watering with a dropper was done to avoid drying if necessary.

#### Examination of the incubated seeds

On the 8th day of plating and incubating the seeds were ready for the examination. Seeds were observed under the stereoscope one by one for the identification of *F. moniliforme* and other fungal growths. The conidia, conidiophores, and other morphological characters of the fungi were properly identified by preparing the temporary slides of the fungal growth and observing them in the compound microscope. At last, the percent of seed infection was calculated.

### Seed to seedling transmission test

Among the total 20 varieties of rice 5 varieties in which the highest level of *F. moniliforme* infection was seen in the lab was selected for a seed to seedling transmission test under screen house condition. The experiment was conducted at the screen house of PPD and NARC, Khumaltar in CRD (Completely Randomized Design) with 4 replications. Each replication contained two pots having 25 seeds in each i.e. 50 seeds per replication. In total randomly selected 200 seeds of each variety were sown in 40 pots of size at the rate of 25 seeds per pot. The varieties selected were Khumal- 9, Khumal-8, Lekali-3, Khumal-4, and Machhapuchhre-3. The soil was sterilized in an autoclave before using it for sowing the seeds. Then the potting mixture was made by mixing chicken manure, sand, and sterilized soil. Germination was observed after 5 days of sowing seeds. After 75% of the germination of the seeds, the data reading was taken for this test. Data was taken for four weeks in 7 days interval. Dead seedling in which the symptoms of rot at the base (Collar) were observed was taken out from pot and plating was done in the lab for the confirmation of the disease.

### Isolation and culture of the pathogen

The diseased samples were plated in the laboratory in a moist chamber and the water agar medium. At first, the sample was cut into small pieces and surface sterilized using 4% NaOCl. The sample was dried in laminar airflow and then plated. After plating the plates were incubated in an incubator at 24°C. After 3 days the growth was observed in both moist chambers as well as water agar medium. For culture, the seed in which fungal growth of *F. moniliforme* was seen; the mycelia were taken out and put it into Potato Dextrose Agar (PDA) media and PDA slant. Then the plates and slant were placed inside the incubator. After 2-3 days growth was observed in the plates and slant. Then when the mycelia grew fully it was observed under the microscope for the morphological character of spores or conidia.

### Component plating

For component plating 4 varieties (Khumal-9, Khumal-8, Khumal-4, and Lekali-3) were selected twenty-five seeds of each variety were randomly taken and soaked in sterile distilled water for forty-eight hours at room temperature in separate small beakers. After 48 hours the seeds were taken out from water and surface sterilized using 4% NaOCl for only 10 seconds. Seed coat and endosperm were separated using forceps and blade. Then in two separate Petri-plates, endosperms and seed coats were plated using a deep-freezing blotters method. After 7 days each endosperm and seed coat were observed under the stereoscope. Also for the proper identification temporary slides were prepared and observed in the compound microscope.

### Data analysis

The data analysis was done using Statistical Tool for Agricultural Research (STAR) software at a 5% level of significance.

## RESULTS AND DISCUSSION

### Detection and incidence of *F. moniliforme*

The incidence of pathogens differed among varieties although some varieties showed the nearly similar percent of infection (Table 1). The highest percentage of infection was found in Khumal-9 variety showing 37.91% and lowest infection was on Fan-10 variety with 2.91% of infection as shown in Table 1. Fungal growth was observed directly on the seed coat. The growth of the fungus was white, cottony, and powdery as seen on the stereoscope. On compound microscope microconidia as well as macro-conidia of *F. moniliforme* were seen. Most of the time only microconidia were present while on few observed sporodochia consisted of macro-conidia. Macro-conidia observed were 3-5 septate, straight, and hooked at the apex. Morphologically, hyphae of *F. moniliforme* are hyaline, branched, and septate (Karov et al., 2009). Micro conidia were clavate shaped and slightly flattened at the base without septa. Micro-conidia is club or oval-shaped with a flattened base and 0 to 1-septate (Karov et al., 2009; Pradeep et al., 2013). Some other *Fusarium* species were also detected in the seeds in a very low percentage. Ahmed et al. (2013) detected 6.33, 6.33, and 4.66% of the incidence of *F. moniliforme* in three varieties of rice Joya, BR6, and Panjam, respectively by Standard blotters method which showed no significant difference among varieties. Butt et al. (2011) also reported 11, 6, 4, 5, and 3% of *F. moniliforme* incidence in the 5 rice varieties Basmati kernel, Basmati-385, Basmati-198, Basmati-370, and KS.282, respectively by standard blotters method. These incidences were low compared to the varieties in this research but similar to some of the varieties with low infection.

### Incidence of other seed-borne fungi

Numbers of other fungi were also observed in abundant amounts in the seeds. *Alternaria* species, *Bipolaris* spp., *Curvularia* spp., *Microdochium* spp., *Nigrospora* spp., *Aspergillus* spp., *Rhizopus* spp. and *Pyricularia* spp. were the different seed-borne pathogens detected on seeds in the laboratory as per the modified deep-freeze blotters method. Among all these *Alternaria* spp. occurred in the highest amount. The blast disease pathogen, *Pyricularia oryzae* was detected in seed samples of three varieties such as Khumal-10, Khumal-11, and Lekali-3 (Table 2). However, blast disease susceptible variety Taichung did not show seed infection in the present study. Ahmed et al. (2013) recorded nine species of fungi on the seeds of three varieties of rice. The identified fungi were *Fusarium oxysporum*, *F. moniliforme*, *Bipolaris oryzae*, *Aspergillus flavus*, *Alternaria padwickii*, *Curvularia lunata*, *Aspergillus niger*, *Penicillium* sp. and *Nigrospora oryzae* which were similar to the majority of the pathogens observed in this study except that *F. oxysporum* and *Penicillium* sp. were not observed. (Archana and Prakash, 2013) isolated 27 fungal species on samples collected in different Indian states; the predominating fungi isolated being *B. oryzae* (82.1%) and *Alternaria padwickii* (63.4%). Shawki et al. (2020) reported that *F. moniliforme* was the most frequent fungus

**Table 1.** Incidence of *F. moniliforme* in different varieties of rice seeds (%).

S.N	Variety	No. of infected seeds	Mean infection %
1.	Khupal-2	19	7.92ghij
2.	Khupal-3	9	3.75ij
3.	Khupal-4	62	25.83abcd
4.	Khupal-5	21	8.75fghij
5.	Khupal-6	28	11.67defghij
6.	Khupal-7	17	7.08ghij
7.	Khupal-8	81	33.75ab
8.	Khupal-9	91	37.92 a
9.	Khupal-10	21	8.75fghij
10.	Khupal-11	55	22.92jbcdef
11.	Khupal-13	12	5hij
12.	Chandannath-1	47	19.58bcdefgh
13.	Chandannath-2	48	20.00bcdefg
14.	Lekali-1	44	18.33cdefghi
15.	Lekali-3	65	27.08abc
16.	Chainung-242	40	16.67cdefghij
17.	Taichung	23	9.58efghij
18.	Palung-2	47	19.58bcdefgh
19.	Machhapuchhre-3	57	23.75abcde
20.	fan-10	7	2.92j

Means with the same letter are not significantly different. CV=29.14% and P-Value= 0.000; Highly Significant difference ( $p < 0.05$ ) was observed among the varieties based on the HSD test at a 5% level of significance.

associated with sugar beet seeds of most varieties followed by *Aspergillus spp.*, and *Penicillium spp.* *Verticillium spp.*, *Alternaria alternata* and *Chaetomium sp.* moderately occurred, while *Cladosporium spp.* and *Bipolaris spp.*

#### Percentage of seed to seedling transmission of *F. moniliforme*

Among 5 varieties planted in the pots highest transmission of the pathogen from seed to seedling was shown by Khupal-4 which is the most susceptible variety to foot rot disease. It showed 72.31 % of transmission. While the lowest transmission was seen in Khupal-9 with only 9.46% of transmission. However, seed infection percent was found higher in Khupal-9 than in Khupal-4. The lesser seedling transmission even after having higher seed infection incidence could be due to varietal reaction. Transmission of a pathogen from seed to seedling was studied by observing the symptoms and the mortality of seedlings in the pot caused by *F. moniliforme*. The confirmation of transfer was shown by the growth of *F. moniliforme* mycelia in the plated samples of seedlings and observation of its microconidia in a compound microscope. The seedling infection started after 10-12 days of the germination of seeds. Germination of 76.5 to 97.5% was observed from 23.75 to 37.92% infected seed lots (Table 3).

Significant difference was found among seedling infection of the varieties ( $P=0.0051 < 0.05$ ) based on LSD test at 5% level of significance. Dhakar et al. (2017) did the pot culture and in-vitro experiment to confirm the seed transmission nature of *Alternaria alternata* and *F. moniliforme* in the wheat and proved that the pathogenic seed borne mycoflora viz., *A. alternate* and *F. moniliforme* were seed transmissible in nature, 18.17 % of

seedling mortality by *F. moniliforme* in wheat was recorded in pot culture. This was in confirmation with the finding of this research which also showed that *F. moniliforme* transmit from seed to seedlings and cause seedling mortality in rice (Akter et al., 2019) The effect of colour on the transmission of *B. oryzae* was recorded as the highest in number in case of bright seeds (8.5%) and spotted seeds (dark brown) (17.5%).

#### Location of *F. moniliforme* in the seed of rice

From component plating it was found that both seed coat and endosperm is the site of *F. moniliforme* infection in the seed. Total seed coat and endosperm infection by *F. moniliforme* were 28% and 32% respectively (Table 4). Other pathogen *Alternaria spp.*, *Cladosporium spp.*, *Curvularia spp.* were also observed in small number. The table 4 shows the location of *F. moniliforme* in component parts of rice seed. Kumar et al. (2015) recorded the maximum recovery of *F. moniliforme* in lemma (91.25%) followed by palea (78.75%), endosperm (58.75%) and embryo (35.00%) in Basmati CSR 30 by component plating. This was not in confirmation with the results from this work which showed high infection in rice endosperm than rice husk which may be due to difference in process of plating because rice seeds were surface sterilized before separation of components due to which only husk were sterilized not endosperm. Surface sterilization reduces about 10 % of the seed infection of *Fusarium* species in rice seeds (Desjardin et al., 2000). Isolation and culture of *F. moniliforme* *F. moniliforme* isolated from seed and cultured on PDA showed dense white cottony growth of mycelium in about 5 days of plating. Micro-conidia were observed under a compound microscope from the temporary slide preparation.

**Table 2.** Incidence of different seed-borne fungi in seed samples of different varieties of rice.

Varieties	Incidence percent								Total infection %
	A	B	C	M	N	Asp	P	R	
Khumal-2	3.33	0.00	7.08	0.42	11.25	0.00	0.00	0.00	22.08
Khumal-3	4.17	2.08	11.67	0.42	27.50	0.00	0.00	0.42	46.26
Khumal-4	15.83	1.67	1.67	0.00	17.50	0.00	0.00	0.00	36.67
Khumal-5	2.50	0.00	5.42	0.00	15.00	0.00	0.00	0.00	22.92
Khumal-6	5.00	0.83	5.83	0.42	5.83	3.33	0.00	0.00	21.24
Khumal-7	1.67	2.50	5.83	0.42	11.67	0.00	0.00	0.00	22.09
Khumal-8	19.17	0.83	1.67	0.00	2.50	0.00	0.00	0.00	24.17
Khumal-9	4.17	0.00	3.33	0.42	3.33	0.00	0.00	0.00	11.25
Khumal-10	5.42	0.42	4.58	0.00	0.83	0.42	0.83	0.00	12.50
Khumal-11	63.33	0.00	5.00	0.00	0.83	0.00	1.25	0.42	70.83
Khumal-13	61.25	5.42	11.67	0.00	3.33	0.83	0.00	0.00	82.50
Chandannath-1	5.83	1.25	6.25	1.25	1.25	1.67	0.00	0.83	18.33
Chandannath-2	5.83	1.67	10.83	2.08	2.92	5.42	0.00	0.42	29.17
Lekali-1	6.25	2.08	5.00	1.25	0.00	2.92	0.00	1.25	18.75
Lekali-3	3.33	1.25	5.83	4.17	6.25	0.83	0.42	0.42	22.50
Chainung-242	66.25	2.08	11.25	0.00	10.83	0.00	0.00	0.42	90.83
Taichung	2.08	2.08	4.17	2.50	0.00	6.25	0.00	7.50	24.58
Palung-2	2.50	0.42	9.58	0.42	16.25	0.00	0.00	0.00	29.17
Machapuchhre-3	7.92	1.67	21.25	0.83	7.08	0.00	0.00	0.00	38.75
fan-10	22.92	5.42	12.92	0.00	9.58	0.00	0.00	0.00	50.84

Note: A (*Alternaria spp.*), B (*Bipolaris spp.*), C (*Curvularia spp.*), M (*Microdocium spp.*), N (*Nigrospora spp.*), Asp (*Aspergillus spp.*), P (*Pyricularia spp.*), and R (*Rhizopus spp.*).

**Table 3.** Germination, Seed and seedling infection of *F. moniliforme*.

Varieties	Germination %	Seed infection%	Mean seedling infection %	Transmission %
Khumal-4	86.5	25.83	18.68 a	72.31
Khumal-8	85.5	33.75	15.89 a	47.08
Khumal-9	97.5	37.92	3.59 b	9.46
Machapucchre-3	81.5	23.75	4.52 b	48.92
Lekali-3	76.5	27.08	11.62 ab	16.69

Means with the same letter are not significantly different; CV=51.40% and P- value = 0.0051.

The pigment produced was kind of reddish on PDA by *F. moniliforme*. Maximum growth of the fungus was observed on potato dextrose agar (Aurangzeb et al., 2003; Sunder et al., 1997). Pradeep et al. (2013) reported that the best mycelia growth of *F. moniliforme* in PDA showing pink cottony irregular growth and pigment reddish brown which was similar to the results of culture obtained in this research. The deep freezing method was found to be most suitable for the detection and isolation of *F. moniliforme* from rice seeds (Khan et al., 1996). Improved modern protocols based upon PCR, ELISA, etc. would be available for the detection of all seed-borne pathogens and may supersede conventional detection methods (Kumar et al., 2020).

### Symptoms observed

In seedlings sown on pot different symptoms were observed like the growth of pinkish mycelium at collar region, yellowing of leaves. Infected Seedlings gradually dried and then died. When pulled gently seedlings come out showing root rot symptoms. Different types of symptoms are seen caused by *F. moniliforme* in rice starting from pre-emergence seedling death to grain infection at maturity (Ou, 1985). Such seedlings die either before or

after transplanting (Karov et al., 2009). Other symptoms are the formation of pink sporodochia at the palea and lemma junction of the damaged grains (Gupta et al., 2015). Several reports had implicated *Aspergillus* and *Fusarium* species in the decay of cereals and pulses (Habib et al., 2012; Dania and Arabambi, 2015; Taylor and Ngaujah, 2016). The symptoms varied in radial growth, the presence of macro conidia, and virulence sixty-three different *Fusarium*-infected rice samples collected from rice fields of different states of India (Bashyal et al., 2020).

### Conclusion

Research results showed that most of the varieties differed in their seed infection level while some varieties were nearly similar in their seed infection level. Among the twenty varieties, Khumal-9 variety showed the highest seed infection with (37.92%) while Fan-10 variety showed the least infection (2.92%) in seed pathology test done in a laboratory. In seed to seedling transmission test, among five varieties tested Khumal-4 variety was found to transmit the disease in the highest amount with 72.31 % of transmission. The disease (foot rot)

**Table 4.** Site of rice seed infected by *F. moniliforme*.

Varieties	No. of seeds tested	<i>F. moniliforme</i> infection (%)	
		Seed coat	Endosperm
Khupal-4	25	20	16
Khupal-8	25	4	4
Khupal-9	25	0	4
Lekali-3	25	4	8
Total	100	28	32

killed the seedlings by gradually drying and rotting their roots. It was concluded that both seed coat and seed endosperm are the habitats for *F. moniliforme* pathogen in rice seed. Infected seed may bring the outbreak of the disease in the field as it acts as the source of inoculums. Hence, the higher the incidence of seed infection, the higher the rate of disease transmission. However, the varietal reaction and also the presence of other fungi may affect the transmission rate. Health status testing of rice seed is very important to determine whether the seed lot needs treatment with appropriate chemicals before sowing to control the disease incited by the pathogen in a seed. Seed health testing and treatment should be done as preventive measures in all the varieties of rice grown in Nepal to know their level of infection as foot rot is a seed-borne disease.

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