Print ISSN: 0976-2876 Online ISSN: 2250-0138

Available online at: http://www.ijsr.in

INDIAN JOURNAL OF SCIENTIFIC RESEARCH

DOI:10.32606/IJSR.V11.I1.00020

Received: 24-03-2019 Accepted: 12-05-2019 Publication: 31-08-2020

Indian J.Sci.Res. 11 (1): 125-130, 2020 Research Article

STUDY OF IMPACT OF SEED BORNE FUNGAL PATHOGEN ON SEED GERMINATION, SEEDLING BIOMASS AND CHLOROPHYLL CONTENTS OF FOUR DIFFERENT PULSE CROPS

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ABSTRACT

Different saprophytic fungi causes damage to seeds in storage. *Aspergillus flavus* was isolated from the seeds of horse gram, cow pea, green gram, black gram and red gram kept in the stores of farmers. The pathogen was maintained in the laboratory. Seeds of aforesaid pulse crops were purchased from the standard seed merchant. *In vitro* inoculation was done and seeds were cultivated in pre-sterilized soil kept in earthen pots. It was observed that percentage of inhibition of germination was maximum in horse gram 37.12 and minimum in green gram 23.80. Reduction in biomass of seedlings was highest in black gram 54.51% while it was minimum in horse gram 52.48%. The chlorophyll- a, b and total chlorophyll contents were also influenced. Maximum reduction in chlo-a content 57.14% was found in green gram, while minimum reduction in chlo-a 20.00% was found in cow pea. Similarly, highest percentage of reduction in chlo-b was found in case of Red gram 9.09% whiles the minimum 4.35% in black gram. Total chlorophyll contents were also observed. It was maximum in green gram 8.88% while minimum in black gram 4.25. It was further noted that, there was reduction in all the parameter considered for study in the present work. All these indicated that the pathogen had negative impact on the seed germination, seedling biomass and the chlorophyll contents, when it was inoculated in the healthy seeds of different pulse crops as mentioned above.

KEYWORDS: *Aspergillus flavus*, Seed Borne, Seedling Biomass, Seed Germination, Chlo-a and b, Negative Impact

From the survey of literatures, it was noted that there are numerous fungal species, which are found on storage seeds and cause damage to the seeds. The degradation may be qualitative that caused degradation in the nutritional values of the seeds on which they are found. In the present study seeds of most commonly used pulses were used for the study of the impact of the saprophytic fungus. Pulses are the second most important group of food crops, belonging to family *Leguminaceae*. They form an important and indispensable part of our daily diet. It is an important source of dietary carbohydrates, proteins, essential amino acids and micronutrients such as calcium, phosphorus and iron (Kandhare, 2015). In the present study, most commonly used pulses such as horse gram, cow pea, green gram, black gram and red gram were selected to observed the impact of *Aspergillus flavus* on percentage of seed germination, seedling biomass and chlorophyll contents among the seedlings obtained from the contaminated seeds.

There are several reports related with mycoflora, associates with seeds of pulses, vegetables and cereals. Some of them may be cited here. Christensen, (1965) reported deterioration of stored grains by saprophytic fungi. Vijaya and Karan (1981) reported deterioration of cowpea seeds in storage by *Aspergillus flavus*. Sinha and Prasad (1981) observed effect of fungal metabolites on seed germination and seedling growth of Mung. Thakur and Prasad (1983) observed effect of fungal metabolite on seed germination and seedling vigor of *Triticum vulgare*. Kumar and Singh (1983) reported seed borne fungi of Urad bean. Kumar and Patnaik (1985) reported seed borne fungus Alternaria alternata on pigeon pea. Singh and Prasad (1988) reported impact of seed mycoflora on seed quality and germination of *Helianthus annuus*. Patil *et al*; (1990) reported seed borne fungi on green gram.

Hashmi and Thrane (1990) isolated *Fusarium* spp. from different seeds and observed impact of mycotoxins and other secondary metabolites. Shankar and Rao, 1995 also reported impact of culture filtrates of selected mycoflora on seed germination and seedling growth. Kumar and Singh (2001) reported fungi causing seeds and seedling diseases in pigeon pea. Singh *et al*; (2004) observed pathogenic potential and control of seed mycoflora of ground nut. Arshad *et al*; (2005), Singh *et al*; (2005); Embaby *et al*; (2006); Arshad *et al*; (2006); Mali *et al*; (2008); Abdullah and Abdalall (2008); Mallesh *et al*; (2008); Charjan *et al*; (2010); Kayata and Agarwal (2010); Dumbrel *et al*; (2011); Singh *et al*; (2011); Singh *et al*; (2011); Patil *et al*; (2012); Ismile *et al*; (2012); Gangokar and Ayodhya (2013); Zakaria *et al*; (2014); Sadhu (2014); Chilkarni and Giri (2014); Irshad *et al*; (2015); Patekar *et al*; (2017); Khandhare (2019) all have reported seed borne fungi of different crops, including pulses, vegetables, cereals etc. In addition there are report regarding impact of seed borne fungi on seed germination, growth and biomass of different crops.

MATERIALS AND METHODS

These seed samples of each of five commonly grown pulses such as, horse gram, cow pea, green gram, black gram and red gram were purchased from the

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authorized seed traders of Hajipur, District Vaishali (Bihar). Ten seeds of each sample of test species were placed in pre-sterilized Petri plates lined with three well-moistened blotting papers. Each treatment was replicated thrice. The Petri plates were incubated at 26 ± 1^0C for seven days. Fungal growth was observed. They were isolated and identified. Here most abundant species which was identified as *Aspergillus flavus*, was cultured on Potato Dextrose Agar Medium. This was maintained in the laboratory by frequent sub culturing on the same medium.

To observe the impact of *Aspergillus flavus* on percentage of seed germination, seedling biomass and chlorophyll contents, fresh seeds of above pulse crops were used. First of all these seeds were washed properly in running tap water and then surface sterilization was done with 0.1% $HgCl_2$ for 2-3 min. Above seeds were taken out and rinsed thrice with pre-sterilized distilled water. Water adhering on the surface of the seeds was removed with the help of pre-sterilized and dried tissue paper. Above seeds were placed in a well cleaned 250 ml conical flasks separately. In the mean time spore suspension was prepared by harvesting spores from 10 days old culture of *Aspergillus flavus*. By serial dilution with sterilized distilled water, the number of spores/ml suspension was adjusted to $1X10^5$. This was done after counting the spores in 1 ml suspension of spore from the stock suspension. For treatment 0.5 ml of spore suspension was used for 5 g of seeds. The flasks were shaken manually for uniform contact of surface of the seeds with the spores. Above seeds were sown in the pre-sterilized soil which was placed into earthen pots. These seeds were placed in the soil at the depth of nearly 5 mm to 10 mm. Soil surface of the pots were covered with pre-sterilized thin layer of cotton wool. All the pots were placed on the roof of the laboratory in natural condition. Observation was made on alternate day to observe the seed germination in the pots.

Percentage of germination was calculated as

% germination = No. of germinating seeds / Total no. of seeds sown X 100

At least10 seedlings were allowed to growth per pots. These pots were kept moistened by spraying pre-sterilized tap water on every alternate day. The seedlings grew vigorously. New leaves were formed. After 4 weeks the biomass of the seedlings was determined.

Determination of Biomass

The pots were moistened with pre-sterilized distilled water and seedlings were carefully uprooted so that no part of the root should be left in the soil. They were carefully washed with tap water to remove the soil particles. A set of three seedlings in three replicates form each lot were kept in butter paper pocket and dried in an incubator at 60^0C for 48 hours. It was placed in desiccators containing $CaCl_2$ for next 48 hours. The weight was taken. The weight of per seedling in mg was taken. In this way biomass was calculated and the mean of the data was placed in table-2 for discussion.

Determination of Chlorophylls

Cotyledonary leaves form 15 days old seedlings were collected in a clean polybag. These leaves were washed under running tap water and were dried with well sterilized tissue paper. The petiole and mid ribs were carefully removed with the help of a sterile blade. Now 150 mg of the leaflets was weighed and chlorophyll was extracted. Leaves were homogenized in 15 ml acetone (80%) in the mortar with the help of pestle. Extract was filtered through muslin cloth and the residue was used for extraction again. This was repeated thrice and the extracts were pooled together and centrifuged at 5000 rpm for 15 min. the supernatant was taken and used for the determination of OD. The OD was determined at 645 nm and 663 nm with the help of UV Spectrophotometer. The amount of chlorophyll-a mg/g fresh weight of leaves was calculated by the formula proposed by Wiltham *et al*; 1971.

Total chlo-a (mg/g fresh weight) = [12.7(D663)-2.69(D645) X V/1000XW

Chlo-b (mg/g fresh weight) = [22.9(D645) – 4.68(D663) X V/1000XW

Total Chlo-a + Chlob = [20.2(D645) + 8.02(D663) X V/1000XW

Where V = Volume of extract in ml.

W = Fresh weight of the leaves g

D = Density at noted wavelength

Determination of Chlorophylls Contents

Data for Chlo-a, Chlo-b and total chlorophylls were placed in table-3 for analysis.

For the determination of percentage of seed germination, biomass of seedlings and chlorophylls contents, the healthy seeds of all the five pulse crops were shown as control. These data have been also placed in respective tables- such as Talbe-1, Table-2 and Table-3.

RESULTS AND DISCUSSION

Calculation of germination percentages of five different pulses on 15^{th} day of showing after artificial inoculation was done.

Seed germination test was followed for seedling biomass as indicate by the dry weights and chlorophyll contents of the above seedling. From the table-1, it was noted that percentage of germination in the control ranged between 68.10 to 75.20. The highest percentage of germination was found in case of red gram 75.20 while the minimum in case of black gram that was 68.10. Similarly, percentage of germination among the

inoculated seeds ranged between 44.10 to 56.70. Here green gram had 56.70% germination and Black gram 44.10%. Here the percentage of inhibition of germination due to inoculation of the fungal spores was maximum in case of horse gram which was 37.13 and minimum 23.80 in case of green gram. The discrepancy in the percentage of inhibition may be due to the seed structure and its composition. Impact of fungal metabolites on seed germination and seedling growth of Mung has been reported by Singh and Prasad (1981); Thakur and Prasad (1983) in case of *Triticum vulgare*; Singh & Prasad (1988); Sharma and Rao (1995) in case of gram seeds, Debnath *et al*; (2012) in case of maize seeds, Patil *et al*; (2012) in case of some pulses. Khokhar *et al*; (2013) in case of wheat seeds, Gauruba *et al*; (2014) in cse of maize seeds. Khandare (2015) in case of chick-pea. All these workers have concluded that there were considerable reductions in the percentage of germination of seeds of different plants when treated with culture filtrates of the fungi, isolated from the respective seeds. Findings of the present work therefore, corroborate with the findings of the above workers.

Biomass of seedlings raised form the seeds inoculated with fungal spores was also determined. Mean of the data was placed in table-2. Here also we get discrepancy in the biomass as expressed in mg/dry weight was found in the healthy and treated seedlings. The percentage loss in biomass among the treated seedlings in comparison to control was noted. Maximum percentage of loss 54.51 was found in Black gram and minimum 52.48 in case of horse gram.

The loss in biomass may be due to the action of the secondary metabolites of the fungus, which reduces different biochemical reactions in the inoculated seedlings. Impact of culture filtrates and mycotoxins on seedling vigor has been reported by different workers. Thakur and Prasad (1983) observed impact of fungal metabolites on seed germination and seedling vigor of *Triticum vulgare*. Shankar and Rao (1995) observed the impact of culture filtrates of selected seed mycoflora on seedling vigor of pulses. Domijan *et al*; (2005) observed impact of ochratoxin on dry bean, for germination and seedling growth. Khayum *et al*; (2006) observed effect of seed mycoflora of Soybean on seedlings growth characters. Mallesh *et al*; (2008) observed impact of seed mycoflora on seed germination and seedling vigour in case of Pigeon pea. Jalander and Gachande (2012) observed impact of soil fungi on seed germination and seedling growth of some pulses and cereals. Mogle and Maskg (2012) also reported impact of seed mycoflora on seed germination and seedling vigor index of cowpea. Chaudhary *et al*; (2018) observed effect of seed mycoflora in case of Mung bean, on seed germination and seedling vigor. Wani and Alum (2018) reported effect of seed borne fungi on seed germination and seedling vigour of pea seeds. All these findings are in agreement with the finding of present work as here also there was reduction in biomass of the seedlings raised form the inoculated seeds with fungal spores.

Chlorophyll contents of the seedlings raised from seeds treated with fungal spores were also determined. Form the table-3, it may be observed that remarkable reductions in Chlo-a, Chlo-b and total chlorophyll contents were found among the seedlings which were treated than that of the control. Maximum loss in chlo-a of green gram seedling was 57.14% while in chlo-b it was in red gram (9.09%). Similar was the case for total chlorophyll contents. Here maximum loss was in case of green gram, where the loss was 8.88%. Loss in chlorophylls of different pulses showing discrepancy may be due to leaf structure and chlorophyll contents.

Loss in chlorophyll contents of different plants, where seeds were infested with fungal pathogens have been reported by Al-Askar *et al*; (2013); Ahmad and Jutta (2015) and Ahmad and Zaidi (2018). These workers have concluded that seed borne mycoflora also include toxigenic strains. These pathogens may produce phytotoxins that may disturb the synthesis of chlorophyll pigments or may degrade the synthesized pigments. Due to this there are reductions in total chlorophyll contents among the seedlings of infected seeds. In the present study seeds were inoculated with *Aspergillus flavus* and there is reduction in the chlorophylls contents of the inoculated seedlings in comparison of the healthy seeds. Therefore, findings of the present work are in agreement with the aforesaid workers.

Table 1: Showing impact of *Aspergillus flavus* on percentage of seed germination of aforesaid seeds of pulse crops

S.N.	Name of the crop	C	I	% inhibition
1	Horse gram	70.30 ± 0.02	44.20 ± 0.04	37.13
2	Cow pea	72.20 ± 0.06	48.60 ± 0.06	32.69
3	Green gram	74.40 ± 0.08	56.70 ± 0.04	23.80
4	Black gram	68.10 ± 0.04	44.10 ± 0.02	32.24
5	Red gram	75.20 ± 0.04	50.40 ± 0.08	32.98

Table 2: Biomass of seedlings raised form the seeds inoculated with the spores of *Aspergillus flavus* of different pulse crops

Expressed as mg/ seedling. Mean dry weights+ SE				
S.N.	Name of the crops	Control	Inoculated	% loss in biomass
1	Horse gram	28.20 ± 0.04	13.40 ± 0.08	52.48
2	Cow pea	23.40 ± 0.08	11.10 ± 0.02	52.56
3	Green gram	30.60 ± 0.06	14.20 ± 0.04	53.60
4	Black gram	27.70 ± 0.04	12.60 ± 0.06	54.51
5	Red gram	28.10 ± 0.02	13.20 ± 0.04	53.02

Table 3: Chlorophyll contents of seedlings raised from treated seeds of the pulse crops with spore suspension of *Aspergillus flavus*. (Chlorophyll expressed in mg/g of leaves)

Name of Pulse Crop	**Chlorophyll-a**		**% loss**	**Chlorophyll-b**		**% loss**
	C	I		C	I	
Horse gram	0.07± 0.004	0.05 ± 0.033	28.57	0.44 ± 0.004	0.42 ± 0.008	4.55
Cow pea	0.05 ± 0.003	0.04 ±0.003	20.00	0.42 ± 0.005	0.40 ±0.006	4.76
Green gram	0.07 ±0.003	0.03 ± 0.002	57.14	0.43 ± 0.006	0.40 ± 0.006	6.25
Black gram	0.07 ± 0.004	0.05 ±0.003	28.57	0.46 ± 0.008	0.44 ±0.004	4.35
Red gram	0.05 ± 0.002	0.03 ± 0.002	40.00	0.44 ± 0.006	0.40 ± 0.006	9.09
	Percentage loss in total chlorophylls					
Species	Control			Inoculated		% loss
Horse gram	0.46 ± 0.004			0.43 ± 0.008		6.52
Cow pea	0.44 ± 0.006			0.41 ± 0.006		6.81
Green gram	0.45 ± 0.004			0.41 ± 0.004		8.88
Black gram	0.47 ± 0.008			0.45 ± 0.004		4.25
Red gram	0.42 ± 0.006			0.41 ± 0.006		2.38

CONCLUSION

Seed borne mycoflora on the stored seeds of different crops, reduces, germination, seedling growth and chlorophyll contents. All these are important factors for loss in yield. Therefore, seeds which are stored without treatment should not be used for cultivation.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, K.R. College, Gopalganj for providing laboratory and library facilities for the present research work.

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