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THE PATHOLOGICAL ASPECTS OF THE SEEDLINGS OF FABA BEAN (*Vicia faba* L.) DUE TO SEED BORNE STORAGE FUNGI IN KOSI REGION

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Abstract: *It is axiomatic fact that the food and nutrition is the only main item for the survival of the living being on the earth. The plants are the directly & indirectly the sole source of the food. Vicia faba (family-fabaceae) seeds is commonly known as broad bean or faba bean and high protein content in seeds. It is also a tacit fact that these items are biotically or abiotically destroyed in nature to such an extent that their use in any form is horribly disagreeably either in the form of food or as seed for multiplication of plants. The findings of the present work that deals with the pathological aspects of the seedlings of faba bean (Vicia faba L.) due to seed borne storage fungi of the seed in kosi region have been very briefly described here-Totally 48 spp. Of fungi were isolated from the stored seeds. 17 Aspergillus spp. dominated. Mainly 20 spp. Of fungi were selected based on their high frequency, and were infested to the seeds and stored at 60, 70, 80 and 90% RH for 30 days at 30±1^{0}c. On storage of the seed with the spore of 20 spp. Of fungi at 80 and 90% RH for 30 days at 30±1^{0}c. the seedlings expressed the pathological symptoms such as–(a) radicle shorter, (b) radicle brown and shorter, (c) brown lesion on hypocotyle, (d) pink lesion on epicotyle, (e) brown lesion on the cotyledons and (f) blackening of the root tip. The percent seedlings affected by the noted pathological symptoms, were less due to storage of the seed with fungi at 80% RH while considerably more raised from the seeds stored at 90% RH. The percent seedlings affected by the noted pathological symptoms were maximum due to A. flavus. Besides the pathological symptoms on the radicle and on the cotyledons as noted earlier, these were noted in the aerial parts too. These were-(a) shortening of the plumule (b) fading of the plumule and (c) drying of the margin of the leaf. The acetone extract of the stained cotyledons due to 9 spp. Of storage fungi suppressed the germination of seed from 43 to 57%. The length of the radicle of germinated seed due to storage fungi were 7.6 mm to 9.8 mm against the control having 19.6 mm long radicle. The metabolite of the 9 storage fungi in Richard solution considerably suppressed the germination of the seed and the length of the radicle. The pathological symptoms in association with the seedlings of faba bean depend on many factors such as the RH (%) levels of storage, period of storage at particular RH, the nature of seed borne storage fungi and the nature of the seed comprising its anatomy and the biochemical composition. It is important to pathological symptoms quoted here were more in percent in the seedlings raised from the seeds stored at 90% RH and with less percent when at stored at 80% RH.*

Keywords: *RH (relative Humidity), Seed borne storage fungi, Broad bean.*

Introduction

Vicia faba L. is a rich protein legume seed and widely used for food as vegetable & pulses. It can be sown October-November as intercrop in the garden field. Each pod contains 3-8 seeds round to oval. It pods are cylindrical and compressed. (vicia faba L.) Broad bean plant bears pinnately compound leaves. It has normally white flowers with distinctive black centres. Faba bean is cultivated in winter for use of the green pod as vegetable pulses and flour for various purposes of throughout our country, particularly in kosi region of North Bihar.

- In this area the record of meteorological conditions conditions conductive to the decay/rotting of fleshy fruits in the market of said places is very important. The weather of kosi region in the summer is hot (max. temp 38^{0}C, min. temp. 20^{0}C). The rainy season is warm and greatly humid. (annual rainfall 125-

150cm). The winter is very cold and humid (max. temp. 25^0C and min temp. 4^0C)

- The main reasons of high humidity besides the rain the network of tributaries of the river kosi. This level of the humidity and warm/hot codition play role in the rotting of fleshy fruits stored with the stockiest and the retailers.
- It is worth noting that seed borne fungi are visualized with two aspects, one being the seed borne diseases in the standing crop commonly known as "seed pathology" as major discipline [1, 2] and another one being the role of seed borne storage fungi that invade the seed in the storage and cause its decay on getting suitable RH and temperature for their growth. This aspect is basically "seed deterioration" [2] and is different from the seed pathology.

Methodology

Methodology Involves: The methodology is mainly based on pot experiment which was carried out during October 2012 in the department of botany B.N. Mandal University Madhepura, Bihar.

1. Collection of the Seed: seed of local small variety of faba bean was collected from the farmers of the district Madhepura, Saharsa and Supaul. After collection the seed lots were kept in polythene pockets and labeled. These were brought to the laboratory and stored at 5-6^0C in a refrigerator.

2. Maintenance of Relative humidity (RH): This particular RH was maintained in sealed desiccators using the solution of pure glycerol. This glycerol in pure form maintains zero percent RH. As it is diluted with distilled water the level of RH gradually increases and a time comes when there is no glycerol but only distilled water remains. Then it maintain 100% RH at 30 ± 1^0C [3]. The concentration of glycerol was measured in the form of specific gravity. Specific gravity of pure glycerol is 1.261 at 30 ± 1^0C.

Equivalent moisture of the seeds stored at varying

RH (%) levels	Moisture Content (%)
60	6.812
70	8.362
80	11.751
90	15.064

3. Isolation of seed borne storage fungi through the method of Blotter technique of Tempe (1963). The observation of the fungal growth on the seed was made after two days of setting the experiments. For clear visibility of the colonies of fungi on the seed surface, m Magnifying glass of 10x magnification was used.

4. **Infestation of Seed with the Spores of Storage Fungi:** The infestation of the seeds with the spores of storage fungi on the surface of the seed and its storage is the primary work for observing their effect on germination, affliction of pathological symptoms in the seedlings.

5. **Culture Media:** For the culture of most of the fungi especially belonging to Ascomycotina and Deuteromycotina czapek Dox Agar medium was used.

Composition of Czapek Dox Agar (CDA) Medium

Sodium nitrate ANALAR grade–2.00 g
Potassium dihydrogen phosphate–1.00 g
Crystalline magnesium sulphate–0.5 g
Potassium chloride–0.5 g
Sucrose-30.00 g
Crystalline ferrous sulphate–00.01 g
Agar–20.00 g
Distilled water–1000 ml

- The storage fungi were cultured on CDA slants at 28 ± 1^0C for 7 days. All the isolated seed borne fungi were preserved on the slants of CDA medium.
- The morphological and culture characteristics of the fungi were identified taking help of the standard publications [4-9].

Observation on the Pathological Aspects of the Seedlings: The effect of seed borne storage fungi on affliction of deviation of the pathological aspects from the normally regulated step, has been undertaken [10-14]. These are enough testimony of the role of seed borne storage fungi in inciting diseases in the seedlings. Pathological aspects in faba bean unexplored so far will be worked out due to their seed borne storage fungi.

Procedure: The seed lot possessing 98% germinability was infested with 20 spp of seed borne storage fungi and Stored at 80 and 90% RH on the ground that the suppression of seed germination in the lots stored at 60 and 70% RH was not so injurious due to the fact that the level of equivalent moisture at 60 and 70% RH was observed to be 6.812 and 8.362% respectively, really not conductive for the luxuriant growth of the fungi and consequently not causing considerable damage with respect to the storage

at 80 and 90% RH which raises the equivalent moisture of the seed to 11.751 and 15.064% respectively.

Germination of the Seed: The seeds were germinated in the sterilized moist blotters. These were permitted to grow for five days more for perceptible growth of the radicle. Following symptoms were recorded in association with the radicles of the seedlings besides staining of the cotyledons with Brown lesions. Pathological symptoms in association with the radicle and cotyledons

1. Seedling with shorter radicle (=SR)
2. Radicle of the seedlings brown and shorter (=RBs)
3. Brown lesion in hypocotyle (=BLH)
4. Pink lesion in epicotyle (=PLE)
5. Brown lesion in the cotyledons (BLC)

Brown lesions in the cotyledons were seen after removed of the seed coat. The percent value of the noted symptoms in association with seedlings due to the storage fungi stored at 80 and 90% RH for 30 days was recorded in

Table 01: Pathological symptoms in association with the radicle and cotyledon of faba bean due to storage of seeds with seed borne fungi at 80 and 90% RH for 30 days (expressed as percent value).

Storage Fungi	Pathological Symptoms*	RH (%)	
		80	**90**
A. flavus	SR	38	67
	RBS	32	56
	BLH	18	28
	PLE	13	21
	BLC	46	82
A.niger	SR	21	52
	RBS	23	48
	BLH	13	20
	PLE	09	14
	BLC	31	70
F.moniliforme	SR	18	47
	RBS	20	42
	BLH	11	18
	PLE	10	12
	BLC	26	65
Control	SR	-	-
	RBS	-	-
	BLH	-	-
	PLE	-	-
	BLC	-	-

Results

1. It appears that the seeds stored with seed borne fungi produced five types of pathological symptoms i.e.

SR = Shorter radicle
RBS = Radicle Brown and shorter
BLH = Brown lesion in hypocotyle
PLE = Pink lesion in epicotyle
BLC = Brown lesions in cotyledons
= Seedlings Symptomless

The Control seeds stored without seed borne fungi were seen symptomless Figure-1

2. Pink lesion was produced by Aspergillus and F. moniliforme.

3. Percent seedlings affected by the noted pathological symptoms were less due to the seed stored at 80% RH while considerably more raised from the seeds stored at 90% RH.

4. The percent seedlings affected by the noted pathological symptoms were maximum due to A. flavus.

Figure-1

A=Normal radicle, B=Brown lesions in the cotyledon, C, D=redicle shorter, E=Pre-emergence damping off

The Seedlings afflicted with the pathological symptoms in association with the radicle and the cotyledons. The seedling afflicted with the pathological symptoms (Table-01) were cultured in sterilized sandy soil. The autoclaved soil was taken in plastic pots with the help of sterilized steel spoon and the seeds producing brown radicles and the stained cotyledons, were cautiously sown in the soil after watering with autoclaved tap water. The surface of the soil was covered with nearly 0.5 cm thick sterilized cotton wool. These were left for five days by the emergence of the plumule. In the mean time light watering was maintained with sterilized tap water alternate day. After five days the cotton wool was removed and the seedlings were permitted to grow in the natural light (13hr light, 11 hr darkness, in the month of November, 2011). The seedlings were permitted to grow for another five days and pathological symptoms in the aerial part of the seedlings were noted.

Table-2: Pathological symptoms in aerial part of the seedlings of faba bean due to seedborne storage fungi (expressed as per cent value)

Pathological symptoms in the radicle			Pathological symptoms in the aerial part of the seedlings	
SR	PS	=	Plumule shorter in height	84
RBS	FP	=	Fading of Plumule	24
	BR	=	Blackening of root	
BLH	FP			40
	BR			36
	DML		Drying of margin of leaf	08
	RBD		Root Blackish and dried	06
PLE	FP			50
	BR			48
	DML			12
	BD			08
BLC	PS			64
	FP			26
	BR			20
	DML			24

PS=Plumule shorter in length-upto 4.2 cm; FP=Fading (drying of plumule; BR=Blackening of the root system; DML=Drying of margin of the leaf.

1. The seedlings whose radicle was brown in colour produced plumule which after some growth faded. After growth of the roots to some extent, these became black and the plumule was slow in growth and ultimately faded.
2. The margin of the leaftet became dry in less number of the seedlings produced from the seeds whose cotyledons were severely stained with brown lesions.

Observation of the Toxic Effect of the Seed Borne Storage Fungi: Observation on the effect of acetone extract of cotyledons stained with brown lesions due to seed borne storage fungi.

Procedure: For the purpose noted above 50 g of the stained cotyledons due to A.flavus, A.niger, F. moniliforme, A.alternata, A.sydowi, Anidulans, M.echinata, A.chevalieri and A.terreus besides unstained control cotyledon were extracted three times with 25 ml of acetone each time by grinding in mortar with pestle. The extract was filtered on wheatman no. 1 filter paper on Buchner funnel. The extract was centrifuged at 10,000 rpm for 5 min. The supernatant was dried at 60^0C under reduced pressure taking in a breaker of 200 ml capacity. 50 ml of conductivity water was added to the residue in the beaker. 100 surface sterilized seeds possessing 100% germinability were soaked in the extract of the fungus stored seeds for 12 hr at 30 ± 1^0C. After this treatment the seeds were set in sterilized moist blotters for germination. 10 seeds in each blotter were used 1 seed in the centre and 9 in the periphery and incubated at 30 ± 1^0C for 10 days on alternate day light moistening of the blotters with autoclaved tap water.

The result with anomaly, if any, was recorded in Table-03 in term of percent value of the following aspects.

Table No-03: Effect of the acetone extract of the stained cotyledons of faba bean due to seedborne storage fungi on the normal seed (expressed as percent value)

Seed borne Storage fungi	Germination (%)	Shorter radicles	
		*Percent value	Length (in mm)
A.flavus	57	100	7.6
A.niger	48	90	9.5
F.moniliforme	43	85	9.8
Control	100	Nill**	Nill**

**Figures were rounded to their whole number; **Radicles long- 19.6 mm*

1. The acetone extract of the stained cotyledon due to all the storage fungi suppressed the germination of the seed between 43 to 57% there was no suppression of germination of the control seed treated with the extract of the fungus free seed.
2. It appears worth noting that 80-100% of the germinated seeds produced shorter radicles ranging from 7.6 mm to 9.8 mm against the control which produced seedlings having redicles upto 19.6 mm in length.

Effect of the metabolite of seed borne storage fungi on the germination of seed of faba bean and length of the radicle. For the purpose noted above a liquid medium, the Richard solution was prepared.

Composition of Richard Solution

Sucrose-50.0 g
Potassium nitrate-10.0 g
Ferric chloride-0.02 g
Magnesium sulphate crystal-2.5 g
Potassium monohydrogen phosphate-5.0 g
Distilled water-1000 ml

150 ml of the medium was taken in conical flasks of 250 ml capacity and autoclaved at 15 psi for 15 min. The said medium was inoculated with at bit culture of 4 mm diameter actively growing on CDA medium for 7 days at 30 ± 1^0C. The culture was incubated at 30 ± 1^0C for 10 days.

Preparation of the Metabolite: After expiry of the incubation period the culture was filtered on Buchner funnel using sterilized whatman no.-1 filter paper. The metabolite was used to soak the surface sterilized seeds possessing 100% germinability at the rate of 50 ml of the metabolite per 100 seeds for 12 hr at 30 ± 1^0C.

The seeds were germinated as noted earlier using sterilized moist blotters. The record of germination and the length of the radicle was maintained after the expiry of 10 days in table 04.

Tabe-4: Effect of the metabolite of seed borne storage fungi on the germination of faba bean seed and the length of the radicle (expressed as per cent value)

Seed borne storage fungi	Germination(%)	Length of the radicle (in mm)
A.flavus	41	4.7
A.niger	47	5.2
F.moniliforme	50	5.4
Control	100	9.7

1. It appears that the percent germination of seed is suppressed considerably much due to the treatment of seeds with the metabolite of seed borne fungi. Maximum suppression of seed germination was observed due to A. flavus. The germination of the control seed was 100%.
2. The length of the radicle of the seedlings raised from the seeds soaked in the metabolite was apparently low as compared to the control whose radicle was definitely longer.

Discussion

The RH levels of storage that is 80 and 90% are very suitable to raise the equivalent moisture of the seed to such extent that the luxuriant growth of seed borne fungi is easily permitted. The percent value of the disease symptoms in the seedlings indicate that 90% RH is the most suitable for copious growth of the fungi and consequently causing its deplorable degree of loss of germination of the seed and suppressing the growth of the radicle.

The high level of moisture of the seed is highly expected to promote the fungal growth luxuriantly by secreting copious amount of cutinolytic [15], cellulolytic [16], pectionolytic [17-18] enzymes that facilitate their colonization on the seed surface and beneath. The storage fungi derive nutrition from the hydrolytic products.

It seems important to for a particular pathological symptoms many seedborne storage fungi are involved and also a particular fungus sp. Incite more than one pathological symptoms in association with the seedlings. By this observation one can simply conclude that the seedborne storage fungi in course of their invasion of the seed and their subsequent colonization secrete some sort of metabolite whose effect is adverse on the growth physiology of the seedlings. This does not matter which is the seed borne storage fungus. Presented clearly summarized adverse effect of such fungi on the biochemistry of the seedlings and pathological symptoms afflicted to them [12 & 14].

Food Security

1. Recently attempts have been made to ameliorate the ill effect or detriment of the storage fungi by invigorating the seeds of crop plants in the solution of chemicals such as KH_2 PO_4, $FeSO_4$, $ZnSO_4$, $MgSO_4$, urea, ammonium sulphate and ammonium molybdate before sowing in the field. Consequently, hopeful results have been noted in rectifying the deviated biochemistry

and physiology of the seed and the seedlings [13].

2. For the control of the growth of storage fungi Thiram and captan have been/are being used extensively. Smearing of Benlate and Bavistin before sowing the seed has been shown to check damage of the seedlings by storage and soil borne fungi [19]
3. Food grade antioxidants have also been used for the control of Aspergillus section Flavipes [20]. Besides the application of salicylic acid and citric acid to the seed [21] and methyleugenal [22]
4. Drying of the seed before storage and the 10% moisture level or less have been regarded as safe for many crop seeds.
5. In the godowns dry air must pass to protect the seed from fungal growth on it.
6. Precaution on rainy season – the effect of RH thus caution not to leave the seeds in open during the rains when it goes high generally from last week of June to August in Kosi region.

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