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EFFECT OF STORAGE FUNGI ON THE GROWTH OF SEEDLINGS OF BROAD BEAN (*Vicia faba* L.) AT VARYING RELATIVE HUMIDITY IN KOSI REGION

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Abstract: *The study was focused on the involvement of storage fungi in deterioration of the seeds as has been done earlier. The deviation in the enzymatic activities of the seed is expected to distract the chain of biochemical steps disarraying the whole physiology and biochemistry including the growth physiology presently intended to investigate it in the seedlings of broad bean due to storage fungi of the seed at varying RH.*

Effect of the seedlings of faba bean due to the storage fungi of the seed in kosi region, have been very briefly described here-(1). Altogether 48 spp of fungi were isolated from the stored seeds. Aspergillus spp (17) dominated. (2). Altogether 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. (3). Based on their high frequency in association with the seeds Aspergillus flavus. A niger and Fusarium moniliforme were selected to infest the local variety of broad bean (Vicia faba L; family-Fabaceae) Possessing 100% germinability and 7.62% moisture content. (4) As a result the germination of the seed was considerably reduced as the RH levels were raised. Similarly the rate of growth of the radicle was clearly attenuated as the RH of storage was raised employing A. flavus, A. niger and F. moniliforme. The rate of elongation of the plumule and the dry weight of the seedlings followed the same trend. (5) The noted fungi were cultured on Potato dextrose agar slants for 7 days at 28±1 ^{0}C. and their spore suspension was prepared in 5% of tween 20. (6). The rate of elongation of the radicle and plumule and increase in dry weight of the seedlings of broad bean raised from the seeds stored with storage fungi, was slower as compared to the control at all the RH levels opted here. of the three selected storage fungi based on their high frequency, A. flavus proved most deleterious with respect to the noted physiology followed in succession by A. niger and F. moniliforme. In the same way 80% RH of storage of the seed with mentioned fungi appeared most injurious followed in succession by 70 and 60% RH.

Keywords: *Broad bean, storage fungi, Growth of seedlings, RH (relative humidity)*

Introduction: The involvement of storage fungi in deterioration of seeds is well established [1-4] in most of the cases the deterioration of seeds has been confined up to the level of their germination and decay as parameter. But, recently alteration in the physiology and biochemistry of seeds and the seedlings.

- Vicia faba is one of the most ancient crops known to man. It is a annual herb and can be grown as inter crop in the garden field. It is also known as broad bean and is generally grown for green vegetable purpose. Its seeds are also used as pulses and flour is used for various purposes.
- Morphologically Vicia faba plant bears pinnately compound leaves, rachis ending in a twisted tendril or in a point. Leaflet entire, elliptical, flower white and pods are cylindrical and compressed.
- Vicia faba is cultivated in winter for use of the green pod as vegetable throughout our country, particularly in North Bihar and Kosi region.

Fig. of Vicia faba Plant in Pot

White flowers of Vicia faba

Green Pods of Broad Bean

Methodology

The methodology is mainly based on pot experiment which was carried out during October 2012 in the department of Botany Medhepura.

Methodology Involves

(a). Maintenance of Temperature: The temperature above the room was maintained in the ordinary incubators while below that was maintained in BOD Incubator. Sometimes a particular temperature was needed to maintain for enzymatic reaction. Sand bath or water bath was used for this purpose. If this maintenance was needed for long duration of a few hr, the temperature was first maintained with the help of heater and continued to maintain in an incubator after taking trial. Low temperature such as -10^0 C or so was maintained by deep freezer or using common salt in the ice.

(b). Maintenance of Relative Humidity (RH): The effect of particular RH is needed in course of working out the microbial deterioration of the seed. 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 $\pm1^0$C [5]. The concentration of glycerol was measured in the form of specific gravity. A list of maintaining 60, 70, 80 and 90% RH is given against the specific gravity of glycerol by the two noted authors.

Table-1: Maintenance of RH(%) by diluting pure glycerol in term of specific gravity.

Specific gravity	RH (%) levels maintained
1.192	60
1.168	70
1.135	90

Specific gravity of pure glycerol is 1.261at 30±1 ^{0}C

(c). Procurement of the Seed: Seeds were collected from local farmers of kosi region (Saharsa, Supaul & Madhepura). After collection, the seedlots were kept in polythene pockets and labelled. These were brought to the laboratory and stored at 5-6^{0}C in a refrigerator.

(d) Methods of Isolation of Seedborne Storage Fungi: Blotter technique of Tempe (1963) was adopted for the isolation of seedborne storage fungi. The preparation of sterilized moist blotter which is used for the isolation of the associated fungi; Adopting the ISTA (1966) 400 seeds are to be examined. For this purpose 10 seeds per moist blotter was used. 01 seed was placed in the centre and 09 seeds in the periphery per moist blotter with the help of spatulate forceps nearly at equal distance. Forty moist blotters were utilized for setting 400 seeds. It is to be mentioned especially that the seeds collected from different place of kosi region, were mixed together before setting the seeds in the sterilized moist blotters.

The seeded moist blotters were incubated at 28±^{0}C for 7-10 days. If it appeared that the moisture of the blotting circles set in the petri dishes had dried, these were remoistened with autoclaved tap water with the help of sterilized pipette.

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 Magnifying glass of 10X magnification was used. This helped in differentiating colonies of different fungi very close together. The fungi growing on the seed surface, were transferred to czapek Dox Agar

medium in petri dishes and slides were made conventionally by staining with 2.0 of cotton Blue in Loctophenol and mounting in the latter. The margin of the cover slip was sealed with nail polish. With the help of the morphological and culture characteristics the fungi were idenfified taking help of the standard publications [6-11].

(e). 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	2.00g
Potassium dihydrogen phosphate	1.00g
Crystalline magnesium sulphate	0.5g
Potassium chloride	0.5g
Sucrose	30.00g
Crystalline ferrous sulphate	00.01g
Agar	20.00g
Distilled water	1000ml

For the growth of chaetomium spp and formation of their perithecia yeast Extract agar (YEA) medium was used based on the recommendation [12].

Composition of Yeast Extracts Agar Medium

Yeast extract	4.00g
Malt extract	10.00g
Dextrose	4.00g
Agar	15.00g
Distilled water	1000ml

This medium was found clearly effective in producing perithecia in comparison to CDA medium.

(f). Calculation of the Frequency or Percent Frequency

$$\text{Frequency}=\frac{\text{Number of seed harbouring the fungus}}{\text{Total number of seed examined}} \times 100$$

(g). Preservation of the Culture: All the isolated seedborne fungi were preserved on the slants of CDA medium except chaetomium spp which were preserved on the slants of YEA medium.

(h). **Infestation of the 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 and growth of the seedlings. Before infestation of the seed, following experiments were necessarily done.

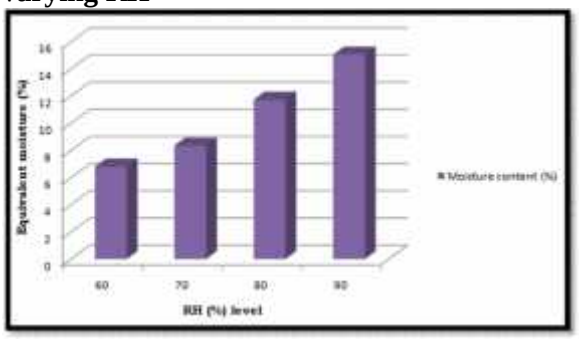
(i). Culture of the Storage Fungi: The storage fungi were cultured on CDA slants at 28 ± 1^0C for 7 days.

Table-2: Equivalent moisture of the seeds stored at varying RH

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

(ii). Preparation of Spore Suspension: The spore suspension was prepared in 5% of tween 20 (Sao et al; 1989). The number of spores perml of suspension was adjusted to $1X10^4$ by counting with the help of haemocytometer. 20 g of the noted seedlot after surface sterilization with 0.1% mercuric chloride solution for 1 min and removal of the adherent water with dry sterilized blotting sheets, was infested with 1 ml of the suspension and stored over diluted solution of glycerol to maintain 60, 70 and 80% RH in sealed desiccators at 30 ± 1^0C for 30 days.

Equivalent moisture of the seeds stored at varying RH



About Kosi Region: Kosi region lies in the northern most side of Bihar state touching the territory of Nepal. Three Districts are there namely Supaul, Saharsa and Madhepura. The record of meteorological conditions conductive to the decay/rotting of fleshy fruits in the market of said places is very important. The weather in the summer is hot (max. temp 38^0C, Min. tem 20^0C). the rainy season is warm and greatly humid due to frequent and torrential rainfall (Annual rainfall125-150 cm). The winter is very cold and humid (Max. temp. 25^0C and Min. temp.4^0C). The main reason of high humidity besides the rain the network of tributaries of the river kosi. This level of the humidity and warm condition play role in the rotting of fleshy fruits stored with the stockists and the retailers.

Map of Bihar State

Presenting the location of Saharsa, Madhepura and Supaul districts of Kosi Division

Germination of Seed (Faba Bean): Germination of the seed on storage with fungi at varying RH at 30±1^{0}Cfor 30 days.

Procedure: The surface of the seed was observed after storage to record any change in colour and texture. After expiry of the storage, the seeds were germinated using autoclaved moist paper towels. These were set in BOD incubator for 7 days by light moistening of the towel with autoclaved distilled water with the help of all-glass sprayer. After counting of the germinated seed the percent germination was recorded.

Table 03: Percent germination of the seed on storage with fungi at varying RH at 30±1^{0}C for 30 days period.

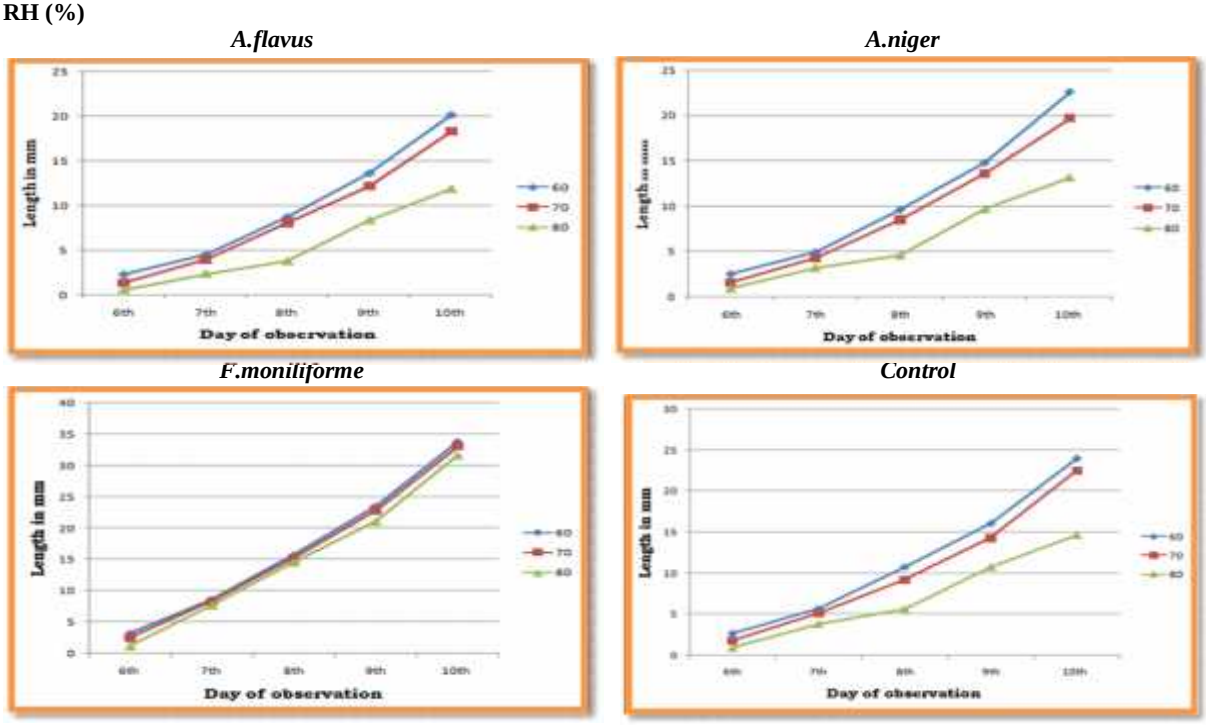
Storage fungi	RH(%)			
	60	70	80	90
A. flavus	94	85	55	30
A. niger	95	88	62	36
E. moniliforme	95	90	65	38

Observation of the Rate of Elongation of the Radicle: After expiry of the storage 20 seeds in five replicates each for the storage fungi besides the control and the levels of RH, were set for germination using autoclaved moist paper towel at 30±1^{0}C for a period of 6 days. The paper towels were moistened alternate day lightly with autoclaved distilled water. 10 seed of each replicate were randomly earmarked by labeling on the 6th day when the radical from the seed has just emerged and its length was recorded daily in mm. of scale up to 10th day. The length of the radicle of each replicate of earmarked seeds was polled together (say it was total X mm). In this way the length of five replicates was pooled together (as $X_1+X_2+…..X_5=X$) and was divided by 50 to get the value of the length of one radicle which was recorded in Table.

Table-4: Rate of elongation of the radicle of faba bean raised from the seeds stored with seedlborne storage fungi at varying RH (%) (expressed as length in mm of scale)

Storage fungi	RH (%)	Day of observation				
		6th	7th	8th	9th	10th
A.flavus	60	2.3	4.5	8.7	13.6	20.1
	70	1.4	4.0	8.1	12.2	18.3
	80	0.6	2.4	3.8	8.4	11.9
A.niger	60	2.5	4.9	9.6	14.8	22.6
	70	1.6	4.3	8.5	13.6	19.7
	80	0.9	3.2	4.6	9.7	13.2
F.moniliforme	60	2.7	5.7	10.8	16.1	24.0
	70	1.8	5.1	9.2	14.3	22.5
	80	1.0	3.9	5.7	10.8	14.7
Control	60	3.2	8.6	15.7	23.5	33.8
	70	2.5	8.3	15.2	22.9	33.1
	80	1.2	7.7	14.6	21.1	31.7

Rate of elongation of the radicle of faba bean raised from the seeds stored with seedlborne storage fungi at varying RH (%)



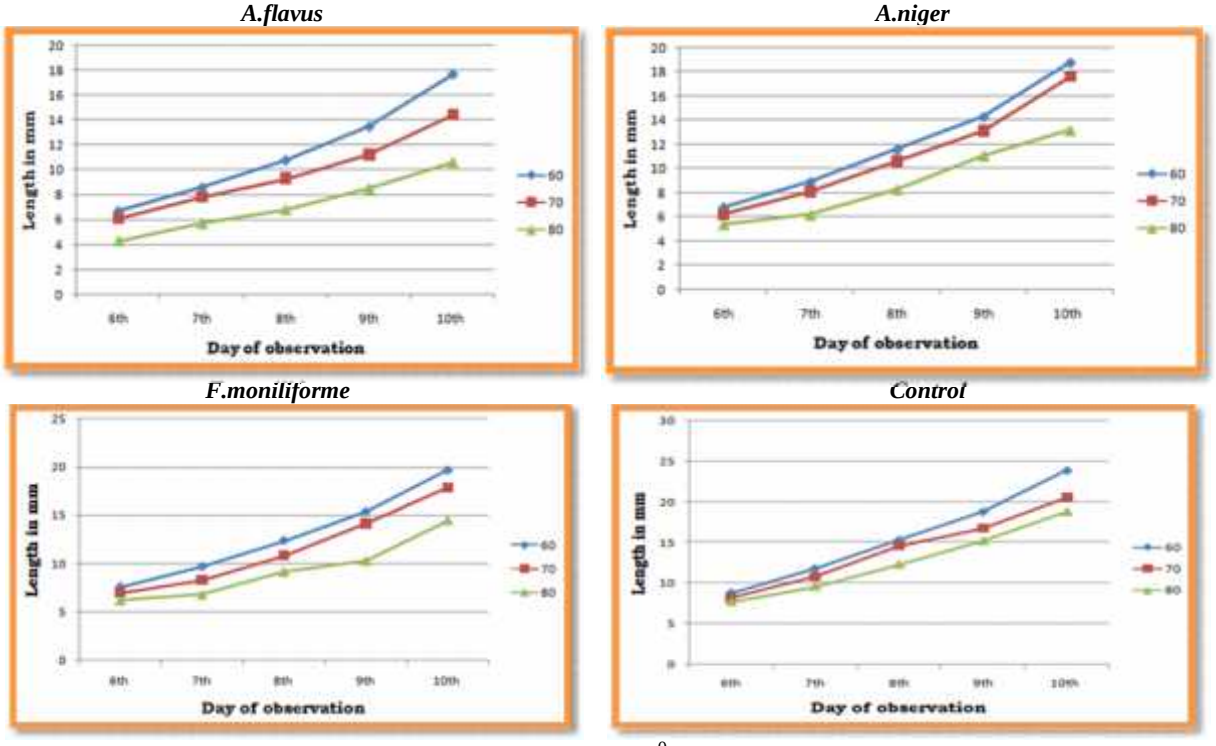
Observation of the Rate of Elongation of the Plumule: The seeds stored as noted earlier, were used for raising the seedlings in suitably sieved garden soil autoclaved at 20 psi for 20 min for two consecutive days. The autoclaved soil was filled in previously sterilized plastic pots with the vapour of absolute alcohol. The dimension of the pots was 15 cm. top diameter, 10 cm. base

diameter and 12 cm. depth. A set of five pots were made ready for each fungus and control and the levels of RH. The soil of the pot was moistened with autoclaved tap water and 20 seeds per pot were sown 1 cm deep nearly at equal distance. The soil surface was covered with approximately 0.5 cm thick cotton wool. The soil was watered lightly every alternate day with autoclaved tap water temporarily removing the cotton wool. After emergence of the plumules from the surface of the soil, 10 of them were randomly earmarked as noted for the radicle and their length was measured in mm starting from the surface of the soil as noted for the radicle and recorded in table

Table-05: Rate of elongation of the plumule of faba bean raised from the seeds stored with seedborne storage fungi at varying RH (%) (expressed as length in mm of scale)

Storage fungi	RH (%)	Day of observation				
		2nd	4th	6th	8th	10th
A.flavus	60	6.7	8.6	10.8	13.5	17.7
	70	6.1	7.8	9.3	11.2	14.4
	80	4.3	5.7	6.8	8.5	10.6
A.niger	60	6.8	8.9	11.6	14.3	18.8
	70	6.2	8.1	10.6	13.1	17.6
	80	5.4	6.2	8.3	11.1	13.2
F.moniliforme	60	7.6	9.7	12.4	15.4	19.7
	70	7.0	8.3	10.9	14.2	17.9
	80	6.2	6.8	9.2	10.3	14.5
Control	60	8.8	11.8	15.3	18.8	23.9
	70	8.2	10.8	14.6	16.8	20.6
	80	7.7	9.6	12.3	15.2	18.8

Rate of elongation of the plumule of faba bean raised from the seeds stored with seedborne storage fungi at varying RH (%)



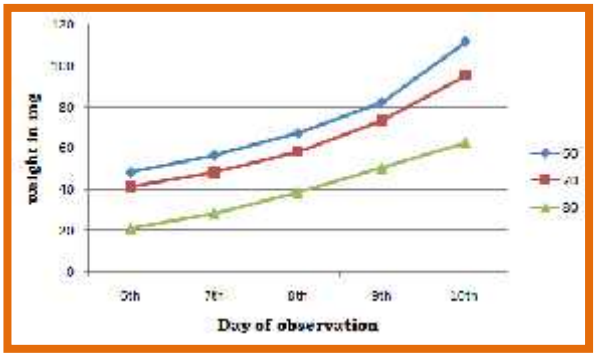
Observing the Rate of Increase in Dry Weight of Seedlings: Ten of the seedlings grown in the soil as noted earlier were randomly taken out cautiously to avoid damage of the root, washed thoroughly, adherent water was removed with blotting sheets and set for drying in an oven at 80^0C for 36 hr and then cooled over fused calcium chloride to their constant weight. The calcution for dry weight of one seedlings was made as noted earlier for the length of the radicle. The dry weight was recorded in Table.

Table-06: Rate of increase of the dry weight of the seedlings of faba bean raised from the seeds stored with seedborne storage fungi at varying RH (%) (expressed as mg weight per seedling)

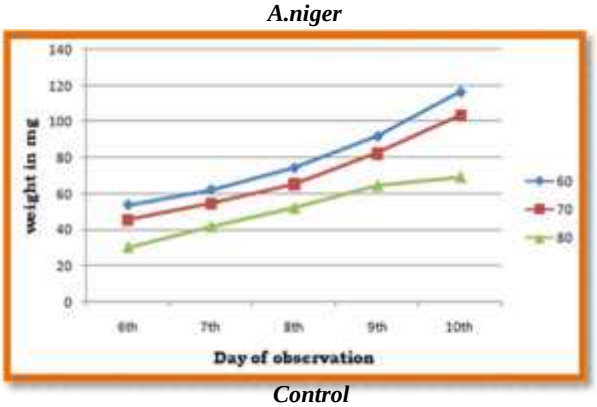
Storage fungi	RH (%)	Day of observation				
		2nd	4th	6th	8th	10th
A.flavus	60	48.4	56.7	67.2	82.3	111.5
	70	41.3	48.5	58.4	73.6	95.1
	80	21.2	28.1	38.5	50.5	62.6
A.niger	60	53.7	62.2	74.3	91.9	116.6
	70	45.5	54.4	65.3	82.3	103.4
	80	30.3	41.8	52.1	64.5	69.3
F.moniliforme	60	62.3	72.6	83.5	101.7	121.3
	70	54.1	61.7	71.3	92.2	111.8
	80	35.1	46.2	58.3	73.1	89.3
Control	60	78.6	89.5	118.3	142.2	172.6
	70	68.2	75.7	93.5	124.7	158.0
	80	60.1	69.2	79.1	109.5	132.2

Rate of increase of the dry weight of the seedlings of faba bean raised from the seeds stored with seedborne storage fungi at varying RH (%)

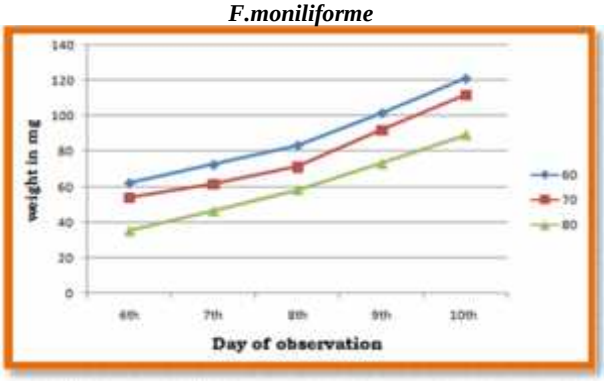
A.flavus



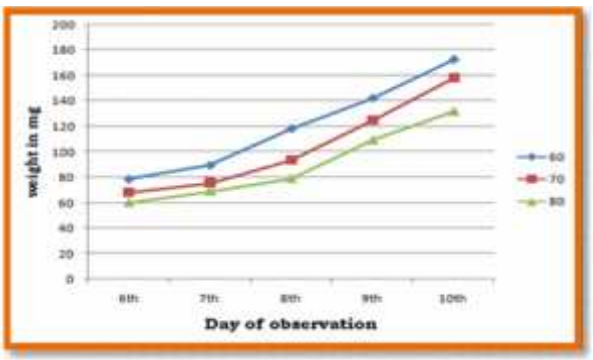
A.niger



F.moniliforme



Control



Results and Discussion

The rate of elongation of radicle and plumule and the increase in dry weight of the seedlings raised from the seeds stored with storage fungi of the seed were slower as compare to the control at all the RH levels of storage. A. flavus appeared to be the most deleterious in these respects followed in succession by A. niger and F. moniliforme. AS regards the effect of RH of storage of seeds, 80% RH proved most injurious followed in succession by 70 and 60% RH. The slow growth of the radicle and plumule of the seedling and tardy increase in their dry weight due to storage of seeds with fungi indicate their injurious effect on the growth physiology. This might be due to the toxic secretion by the storage fungi in the seed, more at 80% RH than at lower level of RH due to the fact that at high RH the equivalent moisture of the seed increases creating suitability for luxuriant growth of the fungi. In such condition more amount of toxic principle is expected to be secreted in the seeds causing more harm to the seedlings. In the comparable conditions of storage and similar findings [13], have authentically established the involvement of toxic metabolite of Aspergillus ruber in multiple deterioration of pea seeds. Also, the acetone extract of A.niger stored lablab bean FD_5 seeds [14], and Memnoniella echinata stored radish seed [15] have been found to suppress the growth of the seedlings. Similarly, the metabolite of A. flavus in Richard solution has been reported to suppress the growth of mustard seedlings [16].

Food Security: Today's agricultural processes are mechanized resulting in the injury of the seed especially at harvest, threshing and winnowing. Such seeds are quite prone to the microbial attack in storage.

Drying of the Seed before Storage: The seed after separation from the mother plant is dried in the sun before storage but the farmers have no authentic kit for the test of determining the moisture level in the seed. The seed moisture is highly important factor in affliction of seed deterioration by the storage fungi besides the RH level of storage. The 10% moisture level or less have been regarded as safe for many crop seeds but varies from crop to crop [1,17].

The Built of the Godowns: In the godowns dry air must pass to protect the seed from fungal growth on it.

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.

Food Grade Antioxidants: Food grade antioxidants have also been used for the control of Aspergillus section Flavips [18]. 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 soilborne fungi [19].

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