TOXICITY OF SEED BORNE STORAGE FUNGI OF COWPEA (*Vigna sinensis* L.)-EFFECT OF FUNGICIDAL DRESSING ON GROWTH OF ROOT AND AERIAL PARTS OF THE SEEDLING

Monalisha Saha*
*University Department of Botany, Ranchi University,Ranchi,Jharkhand,India

ABSTRACT: ***One of the prerequisite for healthy crop and good agriculture production system is healthy seed, health of the seeds are greatly depend on storage condition and proper seed treatment before storage. Seed borne pathogens are responsible for causing various abnormalities in the seed and also result in reducing the yield significantly, if infected seeds are planted in the field. Fungicides have been developed to protect plants against diseases, which cause serious problems such as loss of germination, abnormalities in seedling growth, abnormalities in root and aerial parts and change in biochemical activities. It has been noticed that the storage fungi has effect on the seeds of Cowpea. The deterioration has been reflected in multiple directions. Here the effect of storage fungi has been assessed on root and aerial part of seedling. The storage fungi of the seed not only deviate its physiology and biochemistry but they afflict disease in the seedlings The aim was to test the abnormalities in the root and aerial part of seedling of fungal treated seeds and to study the effect of fungicidal treatment on root and aerial part under different moisture condition.***

The study was conducted under three moisture level; 6.82% 7.68% and 9.87% and results are compared with control treated seeds. The fungicides used were five commonly used fungicides, they are Thiram, Captan, Bavistin, Difolatan and Dithane M-45. The fungicidal dressing probably destroyed the fungal spores and protected the seeds from deterioration. As regards the pathological symptoms in the root and aerial part of the seedlings raised from the seeds dressed with fungicides, 0.35 Bavistin and Difolatan can be recommended followed by Captan with the caution, the seeds must have less than 9.87% moisture.

Key Words : Cowpea seeds, storage fungi, root part, aerial part, seed deterioration, fungicidal dressing, moisture level.

INTRODUCTION:

Legume seeds have comparatively higher protein content than non-legume plant. The high protein content makes them desirable crops in agriculture. The seeds of legumes are second only to cereals as the most important source of food for humans and animals. But there are several factors which are responsible for their low production. Among them, diseases play an important role. Seed-borne diseases have been found to affect the growth and productivity of crop plants[1,2,3]. Presence or absence of seed borne fungi on seed surface is one of the important aspects that determine the quality of seed[4].

Considering all the protein sources that might help tribal belt of Jharkhand's poor and malnourished population, none seems more promising or more practical than grain legumes, which famously deliver the amino acids needed to grow and repair tissues of brain , nerve and muscles as well as help to construct the enzymes necessary for normal life function.

Luckily there is a grain legume which can suit every local soil and climatic condition of Jharkhand, is cowpea. Its widespread occurrence and importance in the lives of most tribal and rural population and its intensive use as dietary protein source makes it a preferred choice of crop to study upon.

Cowpea (*Vigna sinensis* L.) is one of the most important indigenous legume crops of India, thrives in low to m oderate rainfall zones. Seed-borne fungi are the limiting factors among others in the production of cowpea in India. Seed-borne fungi invade cowpea grains while still in the field or during storage causing seed rotting, mycotoxin contamination and loss of seed viability. The seed infection leads to low germination of seeds, and thus reduces yield loss both quantitatively and qualitatively[5,6]The type of infection of seeds and the fungi involved are dependent on many factors. Few literatures are available with regards to seed-borne fungi of cowpea and their control [7,8,9,10,11,12,13,14]The deterioration of seeds due to storage fungi is well established.[15]

Seeds of cowpea namely Pusa-1 is cultivated in almost all the villages of Ranchi district. The stored seed was collected from the farmers and seed dealers of Ranchi. The fungi were isolated and identified by standard methods of culturing. The seeds of cowpea were infested with the isolated storage fungi. These fungi afflict seeds deterioration which are reflected by abnormalities in the root and aerial part of the seedling.

The dressing of seeds, before storage, with different fungicides like Thiram, Captan, Bavistin, Difolatan and Dithane M-45 can help in reducing seed deterioration, reducing pathological symptoms and improving biochemical activities on seed.

MATERIAL AND METHODS:

The stored seeds were collected from the farmers and seed dealers of Ranchi, after collection and labeling, the packets were stored at 5-7 ˚C in refrigerator till the time of the use of seeds for isolation.The seeds of cowpea were infested with the isolated storage fungi possessing their frequency 15% and above. The infested seed lots was stored in triplicate over glycerol solution to maintain 60, 70 and

80% R.H. at 30°C. in sealed desiccators for a period of 30 days.The growth of the radical was permitted for the next seven days to observe pathological symptoms in the root and aerial parts.

For fungicidal treatment fifty gram of seed lot having different moisture level was allotted each for 0.1, 0.2 and 0.3 % concentration of: Thiram, Captan, Bavistin, Difolatan and Dithane M-45. For dressing the seed with fungicides, the requisite amount was taken over, and the seeds are kept in dry conical flasks and manually shaken for 5 minutes. These fungicides are commonly used for seed dressing in storage. The control lot of the seed was undressed or not treated with fungicides. The dressed seed lots were kept in polythene packets and stored in the room temperature for observation.

RESULT AND DISCUSSION:

There are many reports referring to storage fungi and seed borne fungi associated with cowpea seed. In one of the earliest reports concerning seed borne fungi associated with cowpea seed[16], the authors' analyzed the seeds collected from local markets in Ludhiana, India for seed born fungi. It has been reported that 16 major diseases (bacteria) and (fungal) afflict cowpea in the four ecological zones of cowpea production in Africa[17]. It is very likely that some of these field diseases are carry-over diseases from storage. The impact of seed-borne fungi in the raising of seedling in all crops has been reported. Seed fungi have also been reported to induce changes in the nutrient contents of associated seeds[18].The present paper study the abnormalities in the root and aerial part of seedling raised from fungicide treated seeds.

Abnormalities in the root of the seedlings raised from the fungicide treated seeds

The result of the abnormalities in the root of the seedlings raised from the fungicide treated seeds as shown in **Table 1** It appears that the seeds having 6.82 ,7.68% and 9.87% moisture and treated with 0.1, 0.2 and 0.3% concentration of fungicides were symptomless. The control seedlings raised from the seeds having 7.68% moisture content were found afflicted with shortening of the root and root with brown lesions.0.2% Bavistin appeared most effective and as good as 0.3% of its concentration. Other fungicides in 0.2% concentration were found appreciable and 0.3% of their concentration checked the appearance of any pathological symptoms.

Table 1:Abnormalities in the roots of the seedlings raised from the fungicides treated seeds if any (expressed as % value)

			Moisture level % of seeds*		
Fungicides	**Conc.**	**Abnormalities***	**M_1**	**M_2**	**M_3**
Captan	0.1	RS	--	--	9
		RBL	--	--	3
		MGJ	--	--	--
		DO	--	--	2
	0.2	RS	--	--	3
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
	0.3	RS	--	--	--
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
Bavistin	0.1	RS	--	--	6
		RBL	--	--	1
		MGJ	--	--	--
		DO	--	--	--
	0.2	RS	--	--	--
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
	0.3	RS	--	--	--
		RBL	--	--	--

		MGJ	--	--	--
		DO	--	--	--
Difolatan	0.1	RS	--	--	8
		RBL	--	--	3
		MGJ	--	--	--
		DO	--	--	--
	0.2	RS	--	--	--
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
	0.3	RS	--	--	--
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
			Moisture level % of seeds*		
Fungicides	**Conc.**	**Abnormalities***	**Mı**	**M_2**	**M_3**
Dithane M-45	0.1	RS	--	--	8
		RBL	--	--	3
		MGJ	--	--	--
		DO	--	--	--
	0.2	RS	--	--	3
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
	0.3	RS	--	--	--
		RBL	--	--	--
		MGJ	--	--	--
		DO	--	--	--
CONTROL		RS	--	3	15
		RBL	--	2	7
		MGJ	--	--	6
		DO	--	--	5

RS – Root Shorter RBL – Root with Brown Lesions
MGJ – Mycelial growth at the junction of epicotyls and hypocotyle DO – Damping Off
M_1 = 6.82 M_2 = 7.68 M_3 = 9.87

Abnormalities in the aerial parts of the seedlings raised from the fungicide treated seeds

The result of the abnormalities in the aerial parts of the seedlings raised from the fungicide treated seeds are shown in **Table 2.** No pathological symptoms in the aerial parts of the seedlings of cowpea were observed raised from moisture on the seeds having 6.82% ,7. 68% & 9.87%moisture. Even the control seeds having 6.82% moisture could not produce seedling with any pathological symptom. Of course, those produced from the seeds having 7. 68% moisture produces symptoms of shorter seedling and necrotic lesions in 3% of seedling. Bavistin was found to be most effective followed by Difolatan. 0.3% concentration of all the fungicides was effective in checking the appearance of any pathological symptoms.

Table 2: Abnormalities in the aerial parts of the seedlings raised from the fungicide treated seeds, if any (expressed as % value)

			Moisture level % of seeds*		
Fungicides	**Conc.**	**Abnormalites***	**M1**	**M_2**	**M_3**
Thiram	0.1	SS	--	--	16
		CNS	--	--	7
		CT	--	--	5
		FLT	--	--	--
	0.2	SS	--	--	4
		CNS	--	--	3
		CT	--	--	5
		FLT	--	--	--
	0.3	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
Captan	0.1	SS	--	--	11
		CNS	--	--	3
		CT	--	--	6
		FLT	--	--	--
	0.2	SS	--	--	3
		CNS	--	--	2
		CT	--	--	--
		FLT	--	--	--
	0.3	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
Bavistin	0.1	SS	--	--	4
		CNS	--	--	2
		CT	--	--	--
		FLT	--	--	--
	0.2	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
	0.3	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
Difolatan	0.1	SS	--	--	6

		CNS	--	--	3
		CT	--	--	--
		FLT	--	--	--
	0.2	SS	--	--	2
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
	0.3	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--

			Moisture level % of seeds*		
Fungicides	**Conc.**	**Abnormalites***	**M1**	**M_2**	**M_3**
Dithane M-45	0.1	SS	--	--	7
		CNS	--	--	4
		CT	--	--	--
		FLT	--	--	--
	0.2	SS	--	--	4
		CNS	--	--	2
		CT	--	--	--
		FLT	--	--	--
	0.3	SS	--	--	--
		CNS	--	--	--
		CT	--	--	--
		FLT	--	--	--
CONTROL		SS	--	8	23
		CNS	--	3	10
		CT	--	--	8
		FLT	--	--	--

CONTROL = Seedling longer more than 5 cm without any pathological symptoms.
SS - Seedling shorter in length (Up to 5cm above ground) CNS - Cotyledon showing blackish brown necrotic spots CT - Cotyledons twisted FLT - First leaf twisted
*** *$M_1 = 6.82$* * *$M_2 = 7.68$* * *$M_3 = 9.87$***

It is well established fact that the seeds are invaded by the fungi during storage. The effect of storage fungi on the cowpea seeds resulted in suppression in their germination. These fungi afflict seed deterioration which are reflected by various physiological, biochemical and cytological changes in seeds. Seed borne storage fungi though saprophytic in nature, affect biochemical disorder and pathological symptoms in the seedlings of crop plants.The relative humidity (RH) and temperature are very important for the occurrence of the seed borne fungi. The former raise the equivalent moisture of the seed for luxuriant growth of the storage fungi whereas the latter helps in long term survival. It has been noticed that the storage fungi has effect on the seeds of Cowpea. The deterioration has been reflected in multiple directions. Here the effect of storage fungi has been assessed on root and aerial part of seedling. The storage fungi of the seed not only deviate its physiology and biochemistry but they afflict disease in the seedlings. The seedlings were raised in autoclaved soil and the pathological symptoms were reported in the seedlings of Cowpea due to some storage fungi. Attention was paid to interpret the symptoms in the root and the aerial part of the seedlings of Cowpea due to storage fungi. The percent of the seedling affected by these pathological symptoms were different depending upon the seed borne fungi.

The seeds of Cowpea if stored with seed borne fungi pathological symptoms were recorded in the root region of the seedlings. The root of control seedlings were found symptomless and were evidently longer in length as compare to the fungus stored seed. The pathological symptoms afflicted to the root region of seedlings were roots shorter between 15-26mm (RS), Roots with brown lesions (RBL), Mycelial growth at the junction of the epicotyl and Hypocotyl (MGJ) and Damping off like symptoms (DO).

Pathological symptoms in the seedlings were also observed in the aerial part due to storage of the seed borne fungi with the seeds of Cowpea. The pathological symptoms as observed were Seedlings shorten in length (upto 5 cm above ground), Cotyledons showing blackish brown necrotic spots with clearly visible / slightly visible fungal growth (CNS), Cotyledone twisted (CT) and First leaf twisted (FLT). Shorter seedlings (upto 5 cm) were common due to all the storage fungi. Cotyledons with necrotic spots were observed due to most of the fungi. Twisting of cotyledons and the first leaf were also noticeable symptoms due to most of the storage fungi.

The aim was to test the germination of cowpea seeds treated with several fungicides and also study the prevention of abnormalities in the seedling under various fungicides. The study was conducted under three moisture level; 6.82% 7.68% and 9.87% and results are compared with control treated seeds. The fungicides used were five commonly used fungicides, they are Thiram, Captan, Bavistin, Difolatan and Dithane M-45 and untreated seeds.Here the Cowpea seed lot having different moisture level was allotted each for 0.1, 0.2 and 0.3% concentration of Thiram, Captan, Bavistin, Diflotan and Dithane M-45. These fungicides are commonly used for seed dressing in storage. The control lot of the seed was undressed or not treated with fungicide.Increase in moisture content of seeds stored under ambient condition is an important factor responsible for early decline in seed germinability. High moisture of the seeds provides suitable environment for the fungal growth in storage, there by the germination was adversely affected in control seed lots. The fungicidal dressing probably destroyed the fungal spores and protected the seeds from deterioration.As regards the pathological symptoms in the root and aerial part of the seedlings raised from the seeds dressed with fungicides, 0.35 Bavistin and Difolatan can be recommended followed by Captan with the caution, the seeds must have less than 9.87% moisture

CONCLUSION:

One of the major goals of this research is to study the pathological effect on root and aerial part of cow seedling under various seed born fungi and effect of fungicidal treatment on storage fungi. The cowpea seeds longevity, though is an inherited trait, is greatly influenced by their storage conditions, mainly relative humidity and temperature. Of the two factors, relative humidity which is directly proportional to seed moisture content is reported to exert a greater influence on seed longevity than temperature. In recent years there has been notable increase in chronic diseases among people due to consumption of contaminated cowpea seed containing mycotoxine produced by storage fungi. Fungicidal treatment with proper storage of seeds not only helps in increasing the self life of seed but also help in prevention of mycotoxine growth.

Cowpea to a large extent has remain an under exploited crop in India where relatively large gains can be made with relatively little investment. It is mainly grown by poor farmers in developing countries and hence received little attention from research standpoint. It is indeed known as orphan crop which need continuous support from government and local social organization in its research and marketing and promotion. The major challenge is to apply the knowledge gain from this research to help the malnourished tribal population of Jharkhand to get food security by proper storage of cowpea seed and economic security by proper promotion of this poor man's food to general public.

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