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EFFECTS OF FUNGICIDES ON THE SEED MICROFLORA OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

BY

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Fungicide treated groundnut seeds were stored for 0-63 days. Every 21 days interval, seed microflora were studied. Among the fungicides, used Dithane M45 and Emisan 6 showed best results to check the seed microflora associated with the groundnut seeds in comparison to control (untreated) seeds.

Key words : Fungicides, Microflora, seed, Arachis hypogaea.

INTRODCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oil seed crops in India. The crop can be grown throughout the year. The Kernels are liable to attack by several microorganisms (mainly fungi and bacteria) in fields and in storage. Among the fungi *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Fusarium* sp. *Mucor* sp. and *Rhizopus* sp. are most common (Chohan and Gupta 1968, 1971). In storage, most of them cause loss in seed viability. In the field, *A. niger* causes collar rot while *A flavus* causes afla root disease of seedlings. Further more. a toxin, aflatoxin produced by *A. flavus* is also toxin to animals and human beings. *Rhizopus* spp. always cause seed rotting. Many other microorganisms are also associated with the seeds which may not be pathogenic to the host, but play an important role on seed germination, plant stand and initial health of the seedlings.

Keeping in view of the damage caused by associated microorganisms with the seeds the associated microorganisms were isolated, choice of fungicides and the effect of fungicide treatments of seed microflora were evaluated.

Meterials and Methods

Seeds used

Groundnut seeds of local variety, spreading type were collected from different farmers of district Nadia, West Bengal and collected samples were mixed into one lot. The experiment was designed to know the extent of damage caused by

different seed microflora and prospective control measures using fungicides as seed treatment.

Fungicides used

Fungicide	Chemical nature	Usal dose	Manufacturer
Dithane M45	Zinc + manganous ethylene bisdithiocarbamate	0.4%	Indofil Chem. Ltd.
Dithane Z78	Zinc ethylene bisdithiocarbomate.	0.4%	—do—
Thiram	Tetra methyl thiorum disulphide	0.4%	May & Benker (India) Pvt. Ltd.
Captaf—75SD	N-trichloromethyl thio-4-cyclo-hexene-1, 2 dicar-boximide.	0.25%	Rallis India Ltd.
Blitox 50	Copper-oxychloride	0.4%	—do—
Bavistin 50 WP	Methyl-1-2-benzi midazola carbamate.	0.2%	BA SF India Ltd.
Emisan 6	Methoxyethyl mercury chloride.	0.25%	Excel Industries Ltd.

Studies on seed microflora

Seed microflora were studied in two procedures (a) without surface sterilization and (b) after surface sterilization. In former case, seed microflora were studied using usual blotter technique (Neergaard and Saad, 1962). 10 seeds in each Petreiplates were placed maintaining equal distance from one seed to other. Then the plates were incubated at 20°C for 20 h alternatively in dark and in light for 8 consecutive days in B.O.D. incubator. In the later case, seeds were surface sterilized with 0.1% mercuric chloride solution for 1 minute and rinsed in sterile distillted water for twice and incubated on PDA plates. For identification of the microflora the seeds were examined under binocular microscope and for specific cases they are identified under compound microscope. In both the cases 400 seeds were observed.

Seed treatment

Seeds were treated with the fungicides in two methods viz. (a) dry treatment and (b) wet (slurry) methods. Dry seed treatment was made by taking 200 g of seeds in a conical flask along with the required amount of fungicide and mixed thoroughly by shaking the flasks for 15 minutes in a wrist action shaker. Similarly, wet seed treatment was made by taking 200 g of seeds, fungicide and 25 ml of distilled water in a conical flask and shaken for 15 minutes. After this operation,

the seeds were coated with a thick layer of fungicide. These fungicide coated seeds were then dried in shade. In both the cases control (without fungicide) was also parllelly run for comparison.

Storage of treated seeds

Fungicide treated seeds were stored in polythene bags separately at room temperature for 3 months. Periodically (at 21 days interval) seeds from different polythene bags were studied to observe the effect of fungicides on seed microflora and also on the viability of seeds.

RESULTS AND DISCUSSION

Studies of seed microflora

From the data in Table 1 it was observed that groundnut seeds under study were highly contaminated with *Aspergillus niger* (64 percent), *A. flavus* (35 percent) *M. phaseolina*. *Fusarium* sp. and unidentified fungi which were the lowest in population. It was also noted that seeds were also highly contaminated by bacteria (57.0 percent). In case of surface sterilized seeds, all the above mentioned microflora were observed but their population were reduced. But still a higher percentage of seeds (44 percent) showed *A. flavus*, *Penicillium* sp., *M. phaseolina*, *Rhizopus* sp. and unidentified fungi. Bacterial population was markedly reduced after surface sterilization with 0.1 percent mercuric chloride solution. This observation was also in agreement with the reports of Barbosa (1968), and Chohan and Gupta (1968).

Table 1. *Seed microflora associated with groundnut*

Microflora observed with the seeds	*Percentage of seeds infected*	
	Unsterilized	sterilized
Aspergillus niger	64.0	44.0
Aspergillus flavus	35.0	13.5
Rhizopus sp.	56.0	20.0
Penicillium sp.	38.0	6.5
Macrophomina phaseolina	7.0	1.5
Fusarium sp.	4.5	1.5
Unidentified fungi	1.5	0.5
Bacteria	57.0	7.0

Effect of fungicides on the microflora of groundnut seeds

The exprimental data on the effect of fungicides on the microflora of groundnut seeds are given in Tables 2 and 3.

From the data in Table 2 for dry seed treatment and in Table 3 for wet seed treatment, it was clear that all the fungicides reduced the population of seed

Table 2. *Effect of fungicides (dry seed treatment) on microflora of groundnut seeds.*

Period of Storage (days)	Fungicides	Percentage of seed infestation			Seed microflora
		Fungi	Bacteria	Total	
1	2	3	4	5	6
0	Dithane M45	20.00	6.66	20.00	*Rhizopus, Aspergillus niger, A. flavus*, Unidentified fungi, Bacteria.
	Dithane Z78	70.00	0.00	70.00	*A. niger, A. flavus, Rhizopus, M. phaseolina.*
	Thiram	30.00	3.33	30.00	*Rhizopus, A. niger, A. flavus*, Bacteria.
	Captaf	50.00	0.00	50.00	*Rhizopus, M. phaseolina*
	Blitox 50	56.00	0.00	56.66	*A niger, A. flavus, M. phaseolina.*
	Bavistin	63.33	40.00	96.66	*Rhizopus*, Bacteria.
	Emisan 6	26.66	10.00	26.66	*Rhizopus*, Bacteria.
	Control (Untreated)	100.00	53.33	100.00	Unidentified fungi, *A. niger, A. flavus, Penicilium, Rhizopus, M. phaseolina, Fusarium*, Bacteria.
21	Dithane M45	26.66	10.00	26.66	*Rhizopus, A. niger, A. flavus*, Bacteria.
	Dithane Z78	60.00	10.00	66.66	—do—
	Thiram	20.00	13 33	30.00	—do—
	Captaf	40.00	13.33	40.00	*Rhizopus, A. niger*, Bacteria.
	Blitox 50	66.66	0.00	66.66	*A. niger, A. flavus, Rhizopus, Penicillium M. phaseolina.*
	Bavistin	66.66	50.00	96.66	*Rhizopus*, Bacteria.
	Emisan 6	6.66	6.66	10.00	—do—
	Control (Untreated)	100.00	56.66	100.00	*Rhizopus A. niger, A. flavus, M. phaseolina, Penicillium, Fusarium*, Unidentified fungi Bacteria.
4	Dithane M45	10.00	16.66	20.00	*Rhizopus, A. flavus*,
	Dithane Z78	80.00	6.66	80.00	*Rhizopus, A. niger, A. flavus*, Bacteria.
	Thiram	50.00	10.00	50.00	—do—
	Captaf	56.66	30.00	56.66	—do—
	Blitox 50	50.00	3 33	50.00	—do—
	Bavistin	66.66	36.66	86.66	*Rhizopus*, Bacteria.
	Emisan 6	10.00	6.66	16.66	—do—
	Control (Untreated)	100.00	46.66	100.00	*Rhizopus, A. niger, A. flavus*, Unidentified fungi, *M. phaseolian, Penicillium, Fusarium*, Bacteria.

Table 2 continued

1	2	3	4	5	6
	Dithane M45	20.00	23 33	40.00	*Rhizopus, A. flavus* Bacteria.
	Dithane Z78	80.00	20.00	80.00	*Rhizopus, A. niger, A. flavus, M. phaseolina.*
63	Thiram	56.66	10.00	56.66	*Rhizopus, A flavus,* Bacteria.
	Captaf	40.00	43.33	56.66	*Rhizopus,* Bacteria.
	Blitox 50	70.00	0.00	70.00	*Rhizopus, A. niger, A. flavus, M. phasolina.*
	Bavistin	50.00	40.00	86.66	*Rhizopas,* Bacteria.
	Emisan 6	0.00	6.66	96.66	Bacteria.
	Control	100.00	46.66	100.00	As in previous tables.
Untreated					

All the results are averages of 100 seeds with 3 replications.

Table 3. *Effect of fungicides (wet seed treatment) on microflora of groundnut seeds.*

Period of Storage (days)	Fungicides	Percentage of seed infestation			Seed microflora
		Fungi	Bacteria	Total	
1	2	3	4	5	6
	Dithane M45	53.33	20.00	63.33	*Rhizopus, A. niger, A. flavus, M. phaseolina,* Bacteria.
	Dithane Z78	83.33	13.33	83.33	All except *M. phaseolina.*
0	Thiram	60.00	16.66	73.23	—do—
	Captaf	56.66	23.33	60.00	All with *Penicillium.*
	Blitox 50	93.33	0.00	93.33	*Rhizopus, A. niger, A. flavus, M. phaseolina.*
	Bavistin	70.00	50.00	100.00	*Rhizopus, A. niger, A. flavus,* Bacteria.
	Emisan 6	23.33	40.00	60.00	*Rhizopus, A. flavus,* Bacteria.
	Control (Untreated)	100.00	56.66	100.00	*Rhizopus, A. niger, A. flavus, M. phaseolina, Penicillium, Fusarium,* Bacteria, Unidentified fungi.
	Dithane M45	16.66	13.33	30.00	*Rhizopus, A. niger,* Bacteria.
	Dithane Z78	60.00	33.33	80.00	*Rhizopus, A. niger, A. flavus,* Bacteria.
21	Thiram	6.66	16.66	16.66	*A. niger,* Bacteria.
	Captaf	13.33	30.00	40.00	*Rhizopus, A. niger,* Bacteria.
	Blitox 50	40.00	20.00	56.66	*A. niger, A. flavus, M. phaseolina* Bacteria.
	Bavistin	33.33	53.33	86.66	*Rhizopus,* Bacteria.
	Emisan 6	0.00	50.00	50.00	Bacteria.
	Control (Untreated)	100.00	56.66	100.00	*Rhizopus A. niger, A. flavus, M. phaseolina, Penicillium, Fasarium,* Bacteria, Unidentified fungi.

Tabie 3 continued

1	2	3	4	5	6
	Dithane M45	16.66	16.66	30.00	*Rhizopus, A. niger* Bacteria,
	Dithane Z78	66.66	20.00	66.66	*Rhizopus, A. niger, A. flavus,* Bacteria.
42	Thiram	0.00	30.00	30.00	Bacteria.
	Captaf	10.00	10.00	20.00	*Rhizopus*, Bacteria.
	Blitox 50	23 33	16 66	30.00	*Rhizopus, A. niger,* Unidentified fungi, Bacteria.
	Bavistin	56 66	50 00	76.66	*Rhizopus*, Bacteria.
	Emisan 6	0.00	50.00	50 00	Bacteria.
	Control (Untreated)	100.00	46.66	100.00	*Rhizopus, A. niger, A. flavus, M. phaseolina, Penicillium, Fusarium* Bacteria Unidentified fungi.
	Dithane M45	26.66	13.33	36 66	*Rhisopus A. niger*, Bacteria.
	Dithane Z78	80.00	23.33	100.00	*Rhisopus A. niger, A. flavus,* Bacteria.
63	Thiram	0.00	13.33	13.33	Bacteria.
	Captaf	20.00	13.33	36.66	*Rhisopus, A. niger, A. flavus,* Bacteria.
	Blitox 50	60.00	6.66	60.00	*Rhisopus, A. flavus, M. phaseolina*, Bacteria.
	Bavistin	50 00	40 00	80.00	*Rhisopus*, Bacteria.
	Emisan 6	0.00	30.00	30 00	Bacteria.
	Control	100.00	46.66	100 00	*Rhisopus, A. niger, A. flavus, Penicillium.* Unidentified fungi, Bacteria.

Results are averages of 100 seeds with 3 replications.

microflora in comparison to untreated control in both dry as well as wet treatments. Among the fungicides used Dithane M-45, Thiram, Captaf and Emisan 6 showed very promising results. Bavistin showed a very poor performance in controlling the growth of seed microflora. Blitox 50 and Dithane Z-78 showed fairly good result in reducing the seed infestation.

In case of dry seed treatment Emisan 6 effectively reduced the percentage of seed infection i.e. as low as 6.6 percent after 63 days of storage. All the fungi except *Rhizopus* sp. were eliminated after 42 and 63 days of treatment. The bacterial population was reduced to a great extent which was 6.6 percent after 63 days of storage. Though Bavistin did not showed good result still it was able to eliminate all the microflora excepting *Rhizopus* sp. and bacteria. The bacterial infection totally suppressed by seed treatment with Blitox 50 and Captaf soon after treatment, but in case of Captaf it again reappeared. Dithane M-45 had checked the microbial growth on the seed to a considerable extent (20 percent) upto 42 days

of storage but then it increased. *A. niger* was completely eliminated by Dithane M-45 after 42 days of storage but not the *A. flavus*. *Rhizopus* sp. and bacteria were not eliminated but others had eliminated after 42 days. In all the treatments except Blitox 50 had eliminated *M. phaseolina* after 21 days of starage. *Penicillium* sp. was also eliminated by all the fungicides except Blitox 50. Growth of *Rhizopus* sp. was found to be checked upto 42 days of storage when treated with Blitox 50 but reappeared after 63 days. *Fusarium* sp. and unidentified fungi were completely eliminated by all the treatments.

In case of wet seed treatment results are not promising soon after treatment. But after 21 days of storage Thiram, Captaf and Dithane M-45 showed good results, particularly Thiram which had reduced the percentage of seed infection to the extent of 13 33 percent after 63 days of storage followed by Dithane M-45 (36.66 percent) and Captaf (36.66 percent). Though Emisan 6 treated seeds showed 30 percent seed infection after 63 days of storage but it is not so promising because the bacterial infection was not checked like dry seed treatment excepting soon after Blitox 50 treatment where it reappeared afterwards. Like Bavistin and Emisan 6 *M. phaseolina* and *Penicillium* sp. were also checked by all fungicides except Blitox 50. Results of the present investigation are in agreement with the reports of Aulakh and Sunnar (1920) Kadian and Suryanarayana (1971). and Methew and Sharma (1971).

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