https://doi.org/10.29321/MAJ.10.A03915

Studies on seed borne infection of Anthracnose on *Dolichos lab-lab* by *Colletotrichum lindemuthianum* and its Control*

by

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Synopsis: Anthracnose of *Dolichos lab-lab* var. *typicus* is caused by the fungus *Colletotrichum lindemuthianum* (Sacc & Magn.) Briosi and Cavara *(Glomerella lindemuthianum* (Sacc & Magn.) Shear. The disease is found to be both externally and internally seed borne. Seed borne infection of *Dolichos lab-lab* by *Colletotrichum lindemuthianum* is reported for the first time in the present investigations. Among the various seed treatments tried for the control of the disease, Dow 9-B has recorded significantly greater germination than control. Among the systemic chemicals, treating the seeds with zinc chloride solution has recorded highest germination. In respect of the others, one hour soaking appears to be the optimum. Soaking the seeds in water at a temperature of 60° C for 10 to 30 minutes appears to be the optimum without impairing seed viability.

Introduction: Among the various diseases infecting *Dolichos lab-lab.* anthracnose is most important. The disease is caused by the fungus *Colletotrichum lindemuthianum.* A perusal of the literature shows that there are no reports of seed borne infection of *Dolichos lab-lab* by the anthracnose pathogen. Several workers have however reported the seed borne infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum* (Hubling, 1942 Doyer, 1943).

Materials and Methods: Investigations were conducted to find out whether the seeds in infected *Dolichos lab-lab* pods were also affected by the anthracnose disease, using eight varieties of *Dolichos lab-lab.* DL. 244, DL. 453, DL. 692, DL. 269, DL. 389, DL. 9413, DL. 279 and DL. 259. Twenty seeds were taken from each variety and incubated in petri dishes. Seeds showing growth of *Colletotrichum lindemuthianum* were recorded after a week.

In another set of experiments, to find out whether the cotyledons were also infected by this disease, seeds were collected from the above 8 varieties and incubated in petri dishes after removing the seed coat. After one week cotyledons infected with *Colletotrichum lindemuthianum* were recorded.

Different seed treatments were tried for the control of external infection of seed. The most susceptible variety DL. 453 was taken for this study. Each treatment having 10 seeds was replicated 3 times. Seeds were collected from diseased pods and treated with the different fungicides at the rate of 1 gram per lb. of seed and sown in pots containing sterilised soil. The fungicides used were 1. Dow 9-B, 2. Tillex, 3. Semesan, 4. Agrosan, 5. Ceresan, 6. Cerenox, 7. Phygon, 8. Spergon, 9. Arasan, 10. Fernasan, 11. Filt 406 and 12. Brassicol. Germination counts were taken after a week.

Investigations were carried out to find out the efficacy of systemic chemicals for the control of seed infection of anthracnose disease on *Dolichos lab-lab* with 12 treatments, replicated five times. The following chemicals were used in this experiment.

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*Received on 25—2—1964.

1.	Terramycin	... 100 ppm.
2.	Streptomycin	... 100 ppm.
3.	Griseofulvin	... 100 ppm.
4.	Captan	... 1000 ppm.
5.	Dithane	... 1000 ppm.
6.	Salicylic acid	... 5 ppm.
7.	P–amino benzoic acid	... 5 ppm.
8.	Calcium Chloride	... 2 ppm.
9.	Zinc chloride	... 2 ppm.
10.	Nickel chloride	... 2 ppm.
11.	Phenyl mercury acetate	... 1 ppm.

Diseased seeds collected from DL. 453 were taken for this experiment. The seeds were soaked in solutions of systemic chemicals for different durations, 15, 6, 3 and 1 hour and sown in pots containing sterilised soil. Diseased seeds were also soaked in water for similar durations to serve as control. Germination counts were recorded after a week.

Investigations were carried out to find out the effect of hot water treatment against the internally seed borne infection of *Colletotrichum lindemuthianum* on *Dolichos lab-lab*. Seeds were collected from diseased pods and loosely bundled in a muslin cloth. The temperatures tested were 55°C, 60°C, 65°C, 70°C, 75° C and 80°C. Constant temperatures were maintained in a thermostatically controlled water bath. The loosely bundled seeds were immersed inside the bath for 10, 20 and 30 minutes. The treatments were replicated four times. After the hot water treatment, the seeds were sown in pots containing sterilised soil. Germination counts were recorded after a week.

Results: *Seed borne infection of Dolichos lab–lab.*: The results of this experiment are presented in table I.

TABLE I.

Comparison of Seed infection in different varieties of Dolichos lab-lab.

Sl. No.	Varieties	Percentage of seed infection				Mean infection **	SE of mean	CD P= 0·05
		Rep. I	Rep. II	Rep. III	Rep. IV			
1.	DL. 244	80	100	100	100	83 36		
2.	DL. 453	100	100	100	100	90·00		
3.	DL. 692	100	60	100	60	70·39		
4.	DL. 269	100	100	100	100	90·00		
5.	DL. 389	40	100	80	60	60·86	7·67	22·56
6.	DL. 9413	60	80	100	60	63·75		
7.	DL. 279	100	60	100	60	70·39		
8.	DL. 259	100	100	100	100	90·00		

** Transformed value

Conclusion : 2, 4, 8, 1, 3, 7, 6, 5

The results show that the seeds were infected by the fungus *Colletotrichum lindemuthianum*. There was variation in the range of seed infection among the different varieties. Seed borne infection severely reduced germination. Healthy seeds recorded 95% germination while diseased seeds recorded 20% germination. Seedlings raised from diseased seeds were stunted in growth while those raised from healthy seeds were vigorous.

Cotyledonary infection of Dolichos lab-lab.: The resuts of this experiment are presented in table II.

TABLE II

Comparison of Cotyledonary infection in different varieties of Dolichos lab-lab.

Sl. No.	Varieties	Percentage of cotyledonary infection				Mean transformed value	S. E. of mean	C. D. P. 0·05
		Rep. I	Rep. II	Rep. III	Rep. IV			
1.	DL. 244	60	10	10	100	44·41		
2.	DL. 453	100	100	80	100	83·36		
3.	DL. 692	20	20	60	40	35·78		
4.	DL. 269	40	40	40	40	39·23	7·17	21·09
5.	DL. 389	40	60	20	60	41·83		
6.	DL. 9413	20	40	20	20	29·73		
7.	DL. 279	20	40	20	40	32·90		
8.	DL. 259	60	40	60	40	45·00		

Conclusion: 2, 8, 1, 5, 4, 3, 7, 6

The results showed that the cotyledons of all the varieties were infected by the fungus indicating that the disease was internally seed borne. There was variation in the range of cotyledonary infection. Comparison of tables I and II shows that in all the varieties seed infection was more than the cotyledonary infection.

Seed treatment for the control of Anthracnose: Seed treatment with different fungicides showed that among the various seed treatments, only the treatment with Dow 9–B recorded significantly greater germination than control (no treatment). Dow 9–B (Phenoxy compound) recorded mean germination of 49·14 (transformed value) while control recorded 23·85. Mercurial compounds like Tillex, Semesan, Agrosan and Ceresan recorded better germination than the untreated control. The results are presented in table III.

TABLE III

Effect of Seed treatment on germination of Dolichos lab-lab. Seeds.

Sl. No.	Treatment	Percentage of germination			Mean transformed value	S. E. of mean	C. D. P. 0·05
		Rep. I	Rep. II	Rep. III			
1.	Dow. 9-B ...	30	60	80	49 14		
2.	Tillex ...	20	40	60	38·85		
3.	Semesan ...	40	40	20	35·01		
4.	Agrosan ...	20	40	40	35·01		
5.	Ceresan ...	30	60	20	36·85		
6.	Cerenox ...	30	30	40	35·22		
7.	Peygon ...	0	10	30	I7·22		
8.	Spergon ...	20	30	30	30·99	6·48	18·77
9.	Arasan ...	20	10	0	15·00		
10.	Fernasan ...	30	20	10	26·07		
11.	Flit 406 ...	30	0	10	17·22		
12.	Brassicol ...	20	20	40	30·78		
13.	Control ...	20	10	20	23·85		

Conclusion: 1, 2, 5, 6, 3, 4, 8, 12, 10, 13, 7, 11, 9

Seed treatment with systemic chemical for the control of Anthracnose on Dolichos lab-lab.: The results of the above experiment are presented in tables IV, V, VI and VII.

TABLE IV

Comparison of systemic chemicals.

Sl. No.	Treatments	Mean germination transformed value	S. E. of mean	C. D. P. 0·05
1.	Terramycin (100 ppm.) ...	38 20		
2.	Streptomycin (100 ppm.) ...	39·29		
3.	Griseofulvin (100 ppm.) ...	35·55		
4.	Captan (1,000 ppm.) ...	41·31		
5.	Dithane (1,000 ppm.) ...	43·22		
6.	Salicylic acid (5 ppm.) ...	34 10		
7.	P-amino benzoic acid (5 ppm.) ...	39·81	3·34	9·26
8.	Calcium chloride (2 ppm.) ...	45·06		
9.	Zinc chloride (2 ppm.) ...	47·65		
10.	Nickel chloride (2 ppm.) ...	41·02		
11.	Phenyl mercury acetate (1 ppm.) ...	41·65		
12.	Control (no treatment) ...	25·97		

Conclusion: 9, 8, 5, 11, 4, 10, 7, 2, 1, 3, 6, 12

A comparison of systemic chemicals (table IV) showed that the treatment with zinc chloride solution recorded the highest germination which was significantly more than in terramycin, griseofulvin, salicylic acid and control. Treatment with calcium chloride was superior to the last three mentioned above. Treatment with any of the other systemic chemicals except salicylic acid recorded markedly greater germination than control.

TABLE V.

Comparision of duration

Duration	Mean germination	S. E. of mean	C. D. P. 0·05
15 hours	19·53		
6 hours	45·64	1·93	5·35
3 hours	40·60		
1 hour	51·84		

Conclusion: *(1 hour, 6 hours, 3 hours, 15 hours)* A comparison of different durations (Table V) of treatment showed that the consensus of preference appeared to be for a duration of one hour of soaking. The differences in germination were not marked between 3 hours and 6 hours of soaking. There was a significant reduction in germination when the duration of soaking was extended to 15 hours, indicating phytotoxicity.

TABLE VI.

Results of interaction between systemic chemicals and duration.

Sl. No.	Treatments	Duration
1.	Terramycin 100 ppm.	1, 6, 3, 15
2.	Streptomycin 100 ppm.	1, 6, 3, 15
3.	Griseofulvin 100 ppm.	6, 3, 1, 15
4.	Captan 1,000 ppm.	1, 6, 3, 15
5.	Dithane 1,000 ppm.	6, 1, 15, 3
6.	Salicylic acid 5 ppm.	1, 3, 6, 15
7.	P-amino benzoic acid 5 ppm.	1, 3, 6, 15
8.	Calcium chloride 2 ppm.	1, 6, 3, 15
9.	Zinc Chloride 2 ppm.	6, 1, 3, 15
10.	Nickel Chloride 2 ppm.	3, 1, 6, 15
11.	Phenyl mercury acetate 1 ppm.	1, 3, 6, 15
12.	Control (no treatment)	6, 1, 3, 15

The interaction between systemic chemicals and duration (Table VI) showed that only in dithane, the duration of soaking had apparently no influence on germination. In respect of the others, a duration of one hour soaking appeared to be the optimum and was on a par with 3 hours or 6 hours of soaking or both. Soaking for 15 hours seemed to be the least favourable.

TABLE VII.

Results of interction between duration and treatment.

Duration		Treatments
15 hours	...	5, 8, 4, 11, 1, 9, 2, 3, 6, 7, 10, 12
6 hours	...	9, 3, 5, 8, 10, 1, 2, 7, 4, 12, 11, 6
3 hours	...	10, 11, 7, 3, 6, 9, 4, 5, 2, 1, 8, 12
1 hour	...	8, 2, 9, 4, 7, 6, 11, 1, 10, 5, 3, 12

The interaction between duration and treatments (Table VII) showed that the best results were obtained when the duration of soaking was 15 hours for dithane, 6 hours for zinc chloride and griseofulvin and 1 hour for calcium chloride, streptomycin, captan and p—aminobenzoic acid.

Hot water treatment for the control of Anthracnose: Treating the seeds at high temperature viz., 80° C resulted in complete failure of germination. Therefore, the data regarding this temperature are not included for statistical analysis. The data pertaining to other temperatures were subjected to statistical analysis and are presented in Table VIII, IX, and X.

TABLE VIII.

Comparison of temperatures.

Sl. No.	Temperatures	Mean germination transformed value	S. E. of mean	C. D. P. 0·05
1.	55° C	59·57		
2.	60° C	85·57		
3.	65° C	76·72	3·82	10·86
4.	70° C	69·42		
5.	75° C	61·82		
6.	Control	42·81		

Conclusion: 2, 3, 4, 5, 1, 6

A comparison of the results under various temperatures (Table VIII) showed that the soaking of seeds in water at a temperature of 60°C appeared to be the optimum without any deleterious effect on the seeds. 60°C was on a par with 65°C but recorded significantly greater germination than the rest. There was significantly least germination in seeds soaked in water at room temperature. At 55°C, the infection appeared to be only partly removed if at all, while 70°C and 75°C seemed to have affected the germination of seeds.

TABLE IX.

Results of interaction between temperature and duration.

Sl. No.	Temperature			Duration
1.	55° C	...	...	30, 20, 10
2.	60° C	...	...	10, 20, 30
3.	65° C	...	...	30, 10, 20
4.	70° C	...	...	20, 10, 30
5.	75° C	...	...	10, 20, 30
6.	Control	...	...	10, 30, 20

TABLE X.

Results of interaction between duration and temperatures.

	Duration			Temperatures
	10 minutes	...	...	2, 3, 4, 5, 1, 6
	20 minutes	...	...	2, 4, 3, 5, 1, 6
	30 minutes	...	...	3, 2, 1, 4, 6, 5

The interaction between temperature and duration (Table IX) and duration and temperature (Table X) showed that the duration of soaking was not of any consequence at temperatures of 60°C and 65°C and also at the room temperature. But at 55°C the temperature being somewhat inadequate to reduce infection, soaking for the longer duration of 30 minutes recorded significantly more germination than for the other durations of 10 minutes and 20 minutes. At 70°C and 75°C soaking for the longer duration of 30 minutes apparently affected the seeds adversely, resulting in significantly less germination than the other two durations of 10 minutes and 20 minutes.

Discussion: Though several workers have reported the seed-borne anthracnose infection of *Phaseolus vulgaris*, (Schaffnit and Boming, 1925, Hubbling, 1942, Dayer 1943 and staples, 1958) seed borne infection of anthracnose on *Dolichos lab-lab*. is being reported for the first time in the present investigations. There is, however, great variation in the range of seed infection among the different varieties.

The infection of the seeds severely reduces the germination. Jorgensen (1934) has stated that bean *(P. vulgaris)* seeds heavily spotted over the surface with anthracnose germinate better than those having only one inconspicuous lesion close to the hilum.

The present investigation clearly indicated that the anthracnose of *D. lab-lab.* is internally seed-borne. All the varieties tried were infected by this fungus. Dl. 453 which showed the highest percentage of seed infection also recorded the maximum percentage of cotyledonary infection.

Several workers have tried seed treatment of beans for the control of anthracnose disease. Disinifection of bean seed with uspulun has been suggested by Kreuzpointer (1922), Schienpflung (1929), Claus and Mosig (1922), Schienpflung (1929), Claus and Mosig (1925), Schaffnit and Boning (1925), Labner (1925) and Bodnar (1926). Frohberger (1956) has reported the superiority of phenyl mercury acetate in the elimination of *Colletotrichum lindemuthianum* from bean seed. In the present investigations seed treatment against seed borne anthracnose of *Dolichos lab-lab.* has shown that Dow 9-B. (phenoxy compound) has recorded significantly greater germination than control.

Grumer and Mach (1955) have reported that bean anthracnose can be successfully eliminated by immersing the seeds for 24 hours in 5% solution of streptomycin. In the present investigations treating the diseased seeds in zinc chloride solution recorded the highest germination which is significantly more than in terramycin, griseofulvin, salicylic acid and control but on a par with calcium chloride, dithane, phenyl mercury acetate, captan, nickel chloride, p–aminobenzoic acid, and streptomycin. The consensus of preference appears to be for a duration of one hour of soaking. The differences in germination are not marked between 3 hours and 6 hours of soaking. There is a significant reduction in germination when the duration of soaking is extended to 15 hours, indicating phytotoxicity. In the case of dithane, the duration of soaking has apparently no influence on germination.

A perusal of the literature shows that there are no reports of hot water treatment of *Dolichos lab-lab.* seeds. Uerich (1958) has suggested that bean seeds soaked for 15 hours in water at 18° C, 22° C and then treated at 47–48°C for 25 minutes give 21–24% more plants free from anthracnose. In the present investigations treating the seeds of *Dolichos lab-lab.* at high temperature viz., 80°C has resulted in complete failure of germination. Soaking the seeds in water at a temperature of 60°C for 10 to 30 minutes appears to be the optimum treatment without any perceptible deleterious effect on the seeds. Treating the seeds at 70°C and 75C° reduces the germination of the seeds, while soaking the seeds at 55°C is inadequate to control the disease.

Acknowledgement: Grateful acknowledgements are due to the University of Madras, for granting permission to publish this work which formed part of the thesis submitted to the University for the award of M. Sc. degree.

The author is greatly indebted to Dr. K. Ramakrishnan, Professor of Plant Pathology, Agricultural College and Research Institute, for the valuable guidance throughout the progress of this investigation and help in the preparation and presentation of this paper. I am also thankful to the Mycologist and Associate Professor of Plant Pathology, for the encouragement given throughout the entire course of this investigation. I also thank Sri K. A. Seshu, statistical Assistant, for helping me in the statistical analysis of the data.

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