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TREATMENT OF FRENCH BEAN SEEDS TO CONTROL THREE SEED BORNE FUNGAL DISEASES

BY

S. GANGOPADHYAY*

Indian Agricultural Research Institute, Regional Station, Katrain (Kulu Valley), Himachal Pradesh

French bean seeds presoaked for 24 hrs. in water followed by treating in 100 ppm suspension of Terramycin proved to be most effective in controlling three seedborne fungi, i. e. *Ascochyta fabae*. *Whetzelenia slcerotiorum* and *Isariops. is griseola* and increasing seed yield. The next best was 100 ppm Benlate. Hot water treatment at 44°C for 45 minutes showed increased germination rate and enhanced yield but hot air treatments were not encouraging.

INTRODUCTION

A wet environment does not immediately impair the ability of bean seeds to germinate (Orphanos, 1966) but germination is subnormal in seeds from infected pods and seedling growth remains poor due to fungal (Diaz, 1965) and bacterial (Grogan, 1967) infection. In Tanzania, bean crop was completely destroyed due to circular leaf spot incited by *Phaeoisariopsis griseola*. In Cambridge (U.K.), french bean seeds carry 2-15% infection due to *Ascochyta fabae* (Hewett, 1973). *Sclerotinia* wilt incited by *Sclerotinia sclerotiorum* reduced 1. 3% bean yield in Ontario (Canada) in 1969-70 (Haas *et al.*, 1972). French bean seeds, collected from different ecological regions in India, revealed that 8.0 to 59.2% infection

*Present address— Scientist-S.2 Central Rice Research Institute, Cuttack-753006 (Orissa)

might be possible through seeds only due to fungi (Gangopadhyay and Kapoor, 1975). Severin (1965) tried 43 pesticides including mercury compounds for seed treatment but in majority of the cases seed yield was low. Ruschel (1966) also disfavoured seed treatment with mercury compounds as they reduced nodulation and seed yield. The present paper reports studies on the practicability of different heat treatments and chemotherapeutics to control three major seed borne fungal diseases of french bean.

MATERIALS AND METHODS

Naturally infested seeds of french bean (*Phaseolus vulgaris* : cv, pusa parvati) were collected from infested plants on the basis of visual symptoms (Gangopadhyay *et al*., 1971). For each treatment 300 seeds were treated separately in hot water for 30,45 and 60 minutes at each of the temperature of 40°, 42° and 44°C. The second set of experiment was done at higher temperatures i. e. 48°, 50°, and 52°C for shorter duration *viz*. time 25, 30 and 35 minutes at each temperature taking 300 infested seeds from the same lot. Each treatment was carried out in BIJAU ADCO thermostat hot water bath. The seeds were dipped in cold water (10°C) for 15 minutes immediately after each treatment. For hot air treatment 300 seeds were kept separately in incubators adjusted to 40, 42 and 44°C fanning the hot air uniformly with hair drier for 5, 6 and 7 hours . Similarly for higher temperatures and shorter durations 300 seeds were kept in incubators at 48, 50 and 52°C fanning uniformly for 3,4 and 5 hours for each of the temperatures separately.

Three hundred seeds (presoaked in sterile distilled water for 12 hrs.) from the same lot packed loosely in cheese cloth were dipped in each of the antibiotic solution prepared in 0.005 M phosphate buffer(PH7),namely : Chlorotetracycline hydrochloride—100 ppm (Achromycin), Aromatic polyene produced by *Streptomyces cinnamoneus*—100 ppm (Aureofungin), Chloramphenicol—100 ppm (Rampheuical), Streptomycin with fortified Procaine Penicillin—100 ppm

(Streptopenicillin). Demeclocycline hydrochloride – 100 ppm (Ledermycin) Oxytetracycline hydrochloride—100 ppm (Terramycin), Tetracycline hydrochloride— 100 ppm (Tetracycline) and a systemic fungicide Methyl -1-butyl (carbamoyl-2 - benzimidazole- Carbamate) — 100 ppm (Benlate) in water, for 45 minutes in each. Controlled seeds were kept soaked in water only and sown under identical conditions . Hundred seeds for eace treatment were sown in one plot at a depth of 1.5 cm and distance from seed to seed has maintained at 15 cm in rows and 120 cm. Row to row distance was 45 cm. There were three replicates for each treatment. Every replication of the different treatments was followed by a row of untreated infested seeds for optimum disease development under field condition. Seeds were sown within 24 hrs. of treatment. Non-germinated seads of treated and untreated lots were tested about the existence of *Isariopsis griseola, Ascochyta fabae* and *Whetzelinia sclerotiorum* (*S sclerotorum*) by agar plate method. *I. griseola was* isolated and grown on Lima Bean Agar (Tuite, 1969) as it does not sporulate on PDA. Healthy germination of seeds were counted on the basis of seedling grnwth upto 18 cm free from above mentioned three fungi. Lesions on hypocotyl and cotyledonary leaves were considered as diseased ones. Such seedlings were examined further to note these fungi. Plants were allowed to grow upto seed stage and all these tests were carried out for 3 years. Data presented in all the Tables are average of 1973-75.

RESULTS

Hot water treatment : Bean seeds treated at 44°C for 45 minutes (Table 1) was better than those of other treatments. About 85.5% germination and increased seed yield (900 gm per plant). were noted after this treatment. Isolation of fungi i.e. *I. griseola, W. sclerotiorum* and *A fabae* from the non – germinated seeds was also very low. Minimum fungal population was observed after treatment at 44°C for 60 minutes but percentase germination of seeds was only 41.2.% and yield was 180 gm per plant. Low germination rate and yield were due to heat injury.

Table 1. *Effect of hot water treatment (lower temperature for longer duration) on the 3 seed borne fungal diseases of french bean*

Temperature (°C)	Duration (mins)	Healthy seed germination (%)	Average seed yield/plant (kg)	Frequency of fungi isolated from non germinated seeds *I. griseola* (%)	*W. sclerotiorum* (%)	*A. fabae* (%)
40	30	30.7	0.150	70.5	30.8	69.5
	45	59.1	0.450	38.8	50.2	42.7
	60	71.7	0.850	20.5	15.0	10.0
42	30	51.8	0.205	65.2	18.5	40.9
	45	63.3	0.425	32.5	45.0	40.1
	60	75.5	0.900	18.2	26.2	8.5
44	30	65.5	0.670	39.5	40.2	36.5
	45	85.5	0.980	11.6	10.5	7.9
	60	41.2	0.180	8.0	2.8	1.6

Table 2. *Effect of hot water treatment (higher temperature for shorter duration) on the 3 seec dorne fungal diseases of french bean*

Temperature (°C)	Duration (mins)	Healthy seed germination (%)	Average seed yield plant (kg)	Frequency of fungi isolated from non gerimrated seeds *I. giseola* (%)	*W. sclerotiorum* (%)	*A fabae.* (%)
48	25	50.7	0.195	40.5	27.1	31.9
	30	30.5	0.160	38.2	20.5	16.5
	35	21.9	0.105	10.5	13.7	8.1
50	25	44.2	0.200	30.2	18.5	20.4
	30	25.6	0.125	19.0	11.7	9.5
	35	16.5	0.90	8.5	5.8	3.1
52	25	29.6	0.140	11.8	8.8	7.1
	30	20.2	0.110	8.5	6.5	5.9
	35	8.5	0.045	3.5	3.2	1.5

Though the disease severity of the crop was not assessed the disease symptoms were noted on visual odservation. This was also confirmed from the non-germinated seeds. Yield was always found to be directly correlated with germination. For further confirmation, the respective temperatures were increased to 48, 50 and 52°C and the respective durations of treatment were decreased to 25, 30 and 35 minutes This set of experiment showed very poor

performance from rhe germination and yield point of view and the frequency of fu ıgal population form the non-germinated seeds was also very. low (Table 2), This might be bue to excessive heat injury caused to embryo as phytotoxic symptoms such as stunted growth and shrivelled cotyledonary leaves were observed, These plants also gave very poor yield.

Hot air treatment: Both lower air temperature for longer duration and higher air temperature for shorter duration did not prove worth recommending. The results in Table 3 show that maximum germination took place at 44°C for 7

Table 3. *Effect of hot air (lower temperature for longer duration) on the 3 seed borne fungal diseasee of frencn bean*

Temperature (°C)	Duration (hrs.)	Healthy seed germination (%)	Average seed yield plant kg)	Frequency of fungi isolated from nongerminated seeds *I griseola* (%)	*W. sclerotiorum* (%)	*A. fabae* (%)
40	5	30 8	0.155	71.5	60.8	70.5
	6	38.9	0 185	58.9	60.2	61.7
	7	51.2	0.220	45.7	48.2	32.5
42	5	35.8	0.170	60 2	51.2	50.7
	6	40 5	0 195	40.8	40.9	42.5
	7	53.5	0.250	23.7	30.5	21.5
44	5	35.5	0.170	50.8	37.6	38.2
	6	45.0	0.205	31 7	25.5	19 8
	7	55 7	0.2 5	20.9	20.5	12.5

hours while the seed yield was only 275 g/plant and frequency of *A. fabae* was minimum followed by *I. griseola* followed by *W. sclerotiorum*. At 40°C for 5 hours, fungal population was very high confirming that this treatment could not eliminate the three fungi completely, It suggests that the failure of germination was mainly due to infection caused by these 3 spp. of fungi

While the air temperature was increased and duration was decreased (Table 4) it showed reverse results. At 52°C germination was as low as 6.2% which was thought to be due to heat injury to the cotyledon or embryo. Hot air treatment at 48°C even for 3 hours was also not encouraging as the germination was only 45.5%.

borne diseases of bean. No phytotoxic symptom was observed except in case of chloramphenicol. This set of experiment further confirmed that failure of germination of french bean seeds was mainly due to the association of these 3 fungi as their frequency was highest in control while the germination was only 0.5%. Three years' data thus confrmed that bean seeds should always be soaked in water for 24 hrs. followed by soaking in 100 ppm terramycin for 45 minutes

DISCUSSION

Wicks (1970) advocated the treatment of seeds of all in hot water before sowing' irrespective of size. Keyworth (1975) reported about reduction of germination of larger seeds after treating in hot water. The results of present study suggests that the bean seeds may be treated safely at 44°C for 45 minutes in order to erradicate the aforesaid fungi. Maude (1965) tested the effect of hot air on *A. pisi* infected pea seeds using a fairly simple hot steam (air mixing). This treatment failed to erradicate the fungi from the infected seeds, The present investigation also could not bring forth any good recommendation regarding hot air treatment.

Chloramp henical treated seeds inhibited the axis growth of bean (Kein, 1971). Stunted growth of plants and yellowing of leaves were also noted. Mc. Millan (1970) reported that bean seeds treated with Benlate gave good control against fungal diseases but could not erradicate all the fungi. In this finding, though Benlate - treated seeds germinated to the extent of 80, 5% still 2.5, 6.0 anb 8.1% *W. sclerotiorum, A. fabae* and *I. griseolar* respectively were recorded. This populatiou may increase after few years by cumulative effect. Remembering the Swann Committee recommendation (1969) the present study recommends that the french bean seeds should be soaked in water for 24 hours and then in 100 ppm Terramycin solution for 45 minutes. The next best is 100 ppm Benlate. If heat therapy is to be used then bean seeds should be treated in hot water bath at 44°C for 45 minutes.

Table 4. *Effact of hot air (higher temperature for shorter duration) on the 3 seed borne fungal diseases of french bean*

Temperature (°C)	Duration (hr)	Healthy seed germination (%)	Average seed yield/plant (kg)	Frequency of fungi isolated from non germinated seed *I. griseola* (%)	*W. sclerotiorum* (%)	*A. fabes.* (%)
48	3	45 5	0.190	51.5	53.2	60.9
	4	43.2	0.180	58.1	60.5	61.2
	5	31.0	0.125	50.2	41.7	42.6
50	3	30.2	0.105	32.7	31.9	38 5
	4	26.6	0.080	21.9	25.7	28.3
	5	18.5	0.065	20.5	32.8	30.5
52	3	19.6	0.051	20.2	12.7	21.5
	4	12.5	0 039	18.1	19.5	13.6
	5	6.2	0.018	9.5	11.2	8.5

Chemotherapeutic treatments: Terramycin completely eliminated fungal infection (Table 5) and increased seed germination upto 100% and the average yield to 105 times more than that of control. The higher yield may be due to disease free seeds. In this case also yield was directly correlated with the percentage of germination and negatively correlated with the frequency of fungal population. Benlate, a systemic fungicide considerably increased germination (80.5%) as well as seed yield (1.005) gm/plot) markedly reduced fungal population (Table 5). Achromycin proved to be least effective in controlling three seed

Table 5. *Effect of different chemotherapeutics on the 3 seed borne fungal diseases of french bean*

Chemotherapeutants	Concentration (ppm)	Healthy seed germination (%)	Average seed yield/plant (kg)	Frequency of fungi isolated from non germinated seeds *I. griseola* (%)	*W. sclerotiorum* (%)	*A. fabae* (%)
Achromycin	100	17.5	0.050	72.8	60.5	60.8
Aureofungin	100	21.7	0.125	60.5	63.8	49.4
Chlorampheinicol	200	65.7	0.850	13.9	21.2	7.4
Ledermycin	100	50.3	0.655	24.7	28.3	29.4
Streptopencillin	100	31.1	0.255	51.7	55 4	30.9
Terramycin	100	100	1.575	00	00	00
Tetracyclin	100	5.3	0.520	30.4	30.8	16.4
Benlate	100	80.5	1.005	8.1	2.5	6 0
Control	—	0.5	0.015	88.2	80.5	92.7

borne diseases of bean. No phytotoxic symptom was observed except in case of chloramphenicol. This set of experiment further confirmed that failure of germination of french bean seeds was mainly due to the association of these 3 fungi as their frequency was highest in control while the germination was only 0.5%. Three years' data thus confrmed that bean seeds should always be soaked in water for 24 hrs. followed by soaking in 100 ppm terramycin for 45 minutes

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REFERENCES

Diaz, P. C., De Armas, E. and Barros A. 1965. Angular leaf spot of bean caused by *Isariopsis griseola* in the Lake Valeusia basin. *Agron. Trop. Maracay.* **14**: 261-275.

Gangopadhyay, S., Wyllie, T. D. and Luedders, V. B. 1971. Charcoal rot disease of soybean transmitted by seeds. *Plant Dis. Reptr.* **54**: 1088-1091.

Gangopadhyay, S. and Kapoor, K. S. 1975. Studies on the seed borne mycoflora of tropical and temperate vegetables in Kuiu Vallev. *Seed Research.* **3** (2) : 113-115.

Grogan, R. G. and Kimble, K. A. 1975. The role of seed contamination in the transmission of *Pseudomonas phaseolicola* to *Phaseolus vulgaris*. *Phytopathology.* **57** : 28-31.

Haas, T. H. and Bolwyn, B. 1972. Ecology and epidemiology of sclerotinia wilt of white leaves in ontario. *Can. J. Plant. Sci.* **52** : 525-528.

Hewett. P. D. 1963. The field behaviour of seed borne *Ascochyta fabae* and disease control in field beans. *Ann. appl Biol.* **74** : 287-295.

Keyworth, W. G. 1975. The control of seedborne diseases of temperate and tropical vege able crops. *Advances in Mycology and Plant Pathology,* New Delhi. 311-314.

Keni, S., Barenbolz, H, and Budnik, A. 1971. The initiation of growth in isolated lima bean axes, Physiological and fine structural effects of actinomycin D, cycloheximide and chloramphenicol. *Plant and Cell Physiol.* **12** : 41-60.

Maude, R.B. 1966. seed borne diseases of peas and their control. *Pl. Path.* **15** : 187-189.

Mc. Millan, R. T. Jr. 1970. Post harvest control of *Sclerotinia sclerotiorum* of pole beans *Proc. Fla. St. Hort. Soc.* **82** : 139-140.

Orphanos, P. I. and Heydocker, W. 1967. The danger of wet seed beds to germination. *Rep. Sch. Agric. Univ. Nottingham* 1966-67 : 73-76.

Ruschel, A.-P. and Dacosta, W. F. 1966. Symbiotic fixation of atmospheric nitrogen in bean plants. III. Influence of some insecticides and funglcides. *Psquisa. agrcpec brasiliera* **1** : 147-149.

Severin, V. 1965. Studies on the chemical control of common blight of leaves. *An. Sect. Prot. Plant. Bucuresti.* **3** : 87-96.

Swann. M 1969. Report on the joint committee on the use f antibiotics in animal husbandry and veterinary medicine. London.

Wicks, T. J. 1970. The control of seed borne vegetable diseases. *Ext. Bull. S. Aust. Dep. Agric.* 21. *70* : 8.