

## **Impact of Seed-borne *Colletotrichum lindemuthianum* on French Bean Seed Quality**

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French bean seed samples were collected from different parts of Tamil Nadu including both tropical and temperate region including four cultivars viz., Arkakomal, Premier, Rajmash DPU 88-4 and Watex. The variety Watex was found to maximum per cent seed infection of *C. lindemuthianum* with more discolorations. Among the various techniques adopted for detection, agar plate method was found to be best and yielded maximum per cent seed infection. Direct correlation was obtained between seed discoloration and per cent seed infection. The pathogen was found to be present mostly in seed coat and cotyledon and rarely in embryonic axis. The artificial inoculation of 45-day-old plants resulted in maximum disease incidence, resultant seed infection and minimum seed yield /plant and 100 grain weight.

Key words: *Colletotrichum lindemuthianum*, French bean

French bean (*Phaseolus vulgaris* L.) is a highly priced proteinaceous vegetable crop of India being severely affected by anthracnose incited by *Colletotrichum lindemuthianum*. As an important seed-borne pathogen, it inflicts considerable damage to the crop, reduces the seed quality and yield. Goulart (1988) and Tylkowska and Dorna (1990) described anthracnose incidence and seed transmission of the pathogen. The severely affected pods shows characteristic brown lesions with white centre, surrounded by a pale brown to dark brown area of various size (Ansleme and Champion, 1981) and the pathogen survive mostly in cotyledons (Zaumeyer and Thomas, 1957). Keeping in mind, an attempt was made to standardize the detection of seed borne *C. lindemuthianum*, its site of infection, impact on seed quality and seedling vigour and susceptibility of plant age on disease incidence and seed transmission in four popularly cultivated french bean cultivars of Tamilnadu.

## **Materials and Methods**

### *(i) Collection of seed samples*

French bean (each 1 kg) variety viz., Arkakomal (Hosur, Madurai, Melchengam, Ooty); Premier (Gudiatham, Hosur, Kodaikonal and Tirupathur); Rajmash DPU-88-4 (Kelamangalam, Nanjanadu, Ooty and Yercaud) and Watex (Hosur, Kelamangalam, Nanjanadu and Ooty) harvested during the previous season were collected and used for the detection and isolation of *Colletotrichum lindemuthianum*.

(ii) *Detection and isolation of seed-borne C. lindemuthianum*

Detection of seed-borne *C. lindemuthianum* was made as per standard blotter method (ISTA, 1985), 0.1% 2,4-D blotter method (Neergaard Paul and Saab Adib, 1962), deep freeze method (Limonard, 1966) and agar plate method (Mew and Misra, 1974). The fungus was isolated from the naturally infected french bean seeds by transferring the acervulus into Potato Dextrose Agar (PDA) under aseptic condition, with the help of steriobinocular microscope. The pathogen was further purified by single spore isolation and maintained on PDA slants.

(iii) *Impact of seed-borne C. lindemuthianum on germination*

This experiment was conducted by following the procedure outlined by ISTA (1985) in sand medium. One hundred seeds of each seed sample were sown in a germination tray contains sterilized moistened sand and incubated in a germination room, maintained at  $25 \pm 2$  °C and  $90 \pm 3$  per cent RH. Four replications were maintained for each seed sample. The number of normal seedlings, abnormal seedlings and dead seeds in each replications were counted ninth day after sowing and per cent of germination, abnormal seedling and dead seed were calculated and the results were tabulated.

(iv) *Assessment of C. lindemuthianum infection in discoloured seeds*

Infected seed lots of different variety viz., Arkakomal, Premier, Rajmash DPU-88-4 and Watex showing varying degrees of discolouration were used in this study. Each seed sample was homogenized as per sampling technique (ISTA, 1985); divided into four working samples and treated as four replications. In each replication, 100 seeds were counted separately and the per cent of discoloured seed was worked out. Then the discoloured seeds were plated on blotter paper. The percentage of *C. lindemuthianum* associated with discoloured seeds was recorded.

(v) *Effect of C. lindemuthianum seed infection on seed size and weight*

In order to find out the effect of seed infection on quality of french bean seeds, a pot culture experiment was conducted. Thirty-day-old french bean varieties viz., Arkakomal, Premier, Rajmash DPU 88-4 and Watex raised in earthen pots were sprayed with *C. lindemuthianum* spore suspension ( $10^6$  spore /ml) along with Tween 20. Suitable control was also maintained. Finally, the seeds were harvested separately, processed and dried to bring down the moisture content to less than 9 per cent. The seeds harvested from these plants were used for assessing *C. lindemuthianum* infection as mentioned earlier.

(iv-a) *On seed weight*

In order to find out the effect of *C. lindemuthianum* on seed infection, 100 seed weight was taken individually for both infected and healthy seeds in all the four varieties under test and five replications were maintained in each. The percentage of seed infection was also recorded by agar plate method.

(iv-b) *On seed size*

The length and breadth of both infected and healthy seeds in the four varieties were measured with the help of a screw gauge. Five replications were maintained in each variety. One hundred seeds formed one replication.

(vi) *Site of infection*

To find out the location of *C. lindemuthianum* infection in infected french bean seeds, seed component plating method (Kunwar et al., 1985) was followed.

One hundred and twenty-five asymptotic and symptomatic seeds were soaked separately in sterilized double distilled water with one seed per well (Template) for 6 h. Soaked seeds were then dissected aseptically into three components viz., the seed coat, cotyledon and embryonic axis. Each component was surface sterilized with 0.1 per cent Sodium hypochlorite for five minutes and then washed in two changes of sterile double-distilled water. The components of individual seeds were plated on Petri plates containing three layers of moistened filter paper and incubated as per ISTA (1985). Twenty-five seeds formed one replication and five replications were maintained in each variety.

(vii) *Effect of plant age on seed infection and seed transmission of C. lindemuthianum*

Highly susceptible french bean variety Watex was used in this experiment. The plants were maintained in pots with twenty plants per replication. Spore suspension of the pathogen ( $10^6$  spore/ml concentration) was sprayed on 15-, 30-, 45- and 60-day-old plants. Four replications were maintained. The per cent disease incidence, per cent pod infection were recorded 15 days after last spray. The seeds were harvested and per cent seed infection in the resultant seeds, seed yield/plant and 100 seed weight were recorded.

## Results and Discussion

Sixteen french bean samples consisting of four varieties were collected from different parts of Tamil Nadu and were subjected for seed health testing to assess the per cent occurrence of *C. lindemuthianum* seed infection. Among the four techniques followed, agar plate method and blotter technique gave better results when compared to 2,4-D and deep freeze method (Table 1). In conformity with the above findings, Tytkowska and Dorna (1990) reported that potato dextrose agar medium with streptomycin gave better result for the detection of *C. lindemuthianum* compared to roll towel method. The maximum seed infection was noticed in seed samples collected from temperate and sub tropical regions (Ooty, Nanjanadu, Kodaikanal, Yercaud and Hosur) compared to tropical regions (Gudiatham, Madurai, Melchengam, Tirupathur and Kelaman-gaklam). Similar findings on high rate of anthracnose incidence and seed transmission of *C. lindemuthianum* in french bean was also reported by Arya et al. (1986) and Davis (1988). Out of four varieties tested, the variety Watex was found to show maximum seed infection compared to others.

**Table 1**Occurrence of seed borne *C. lindemuthianum* in french bean culture

Variety	Place of collection	Seed infection (%)*			
		Standard blotter	1 % 2,4-D	Deep freeze	Agar plate
Arkakomal	Hosur	70.4 <sup>h</sup>	28.8 <sup>e</sup>	14.4 <sup>e</sup>	76.0 <sup>f</sup>
		(57.21)	(32.31)	(22.28)	(61.20)
	Madurai	24.8 <sup>d</sup>	9.6 <sup>c</sup>	5.6 <sup>c</sup>	40.0 <sup>c</sup>
		(29.68)	(17.65)	(13.69)	(39.96)
Premier	Melchengam	21.6 <sup>c</sup>	4.0 <sup>a</sup>	1.6 <sup>a</sup>	68.0 <sup>d</sup>
		(27.15)	(10.21)	(4.62)	(55.71)
	Ooty	84.0 <sup>j</sup>	64.8 <sup>l</sup>	28.8 <sup>l</sup>	88.0 <sup>j</sup>
		(66.76)	(53.65)	(32.36)	(72.00)
	Gudiatham	23.2 <sup>d</sup>	4.0 <sup>a</sup>	3.2 <sup>b</sup>	36.0 <sup>b</sup>
		(28.23)	(10.21)	(7.90)	(36.65)
	Hosur	64.0 <sup>f</sup>	24.8 <sup>d</sup>	7.2 <sup>d</sup>	78.0 <sup>g</sup>
		(53.19)	(29.77)	(15.56)	(62.40)
Rajmash DPU-88-4	Kodaikanal	68.0 <sup>g</sup>	36.0 <sup>f</sup>	14.4 <sup>e</sup>	68.0 <sup>d</sup>
		(55.63)	(36.81)	(22.08)	(55.71)
	Tirupathur	10.4 <sup>a</sup>	5.6 <sup>b</sup>	1.6 <sup>a</sup>	68.0 <sup>d</sup>
		(18.42)	(10.31)	(4.62)	(55.89)
	Melchengam	18.4 <sup>b</sup>	9.6 <sup>c</sup>	3.2 <sup>b</sup>	24.0 <sup>a</sup>
		(25.26)	(17.33)	(7.90)	(28.80)
	Nanjanadu	78.4 <sup>l</sup>	62.4 <sup>h</sup>	21.6 <sup>g</sup>	82.0 <sup>h</sup>
		(62.40)	(53.23)	(27.60)	(65.36)
Watex	Ooty	88.0 <sup>k</sup>	69.6 <sup>i</sup>	24.0 <sup>h</sup>	86.0 <sup>l</sup>
		(73.30)	(56.62)	(29.17)	(70.67)
	Yercaud	60.8 <sup>c</sup>	45.6 <sup>g</sup>	18.4 <sup>f</sup>	70.0 <sup>e</sup>
		(51.35)	(42.46)	(24.91)	(57.51)
	Hosur	91.2 <sup>l</sup>	94.4 <sup>l</sup>	68.0 <sup>k</sup>	98.0 <sup>l</sup>
		(73.01)	(78.05)	(55.65)	(86.31)
	Kodaikanal	84.8 <sup>i</sup>	92.0 <sup>k</sup>	63.2 <sup>i</sup>	96.0 <sup>k</sup>
		(67.14)	(74.10)	(52.73)	(82.63)
	Nanjanadu	98.4 <sup>m</sup>	98.4 <sup>n</sup>	88.88 <sup>m</sup>	100.0 <sup>m</sup>
		(85.39)	(85.39)	(72.63)	(90.00)
	Ooty	98.4 <sup>m</sup>	96.8 <sup>m</sup>	76.8 <sup>l</sup>	100.0 <sup>m</sup>
		(85.39)	(82.10)	(61.56)	(90.00)

\* Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

The seed samples collected from higher elevation recorded poor per cent seed germination and seedling vigour compared with seed samples collected from plains (Table 2). Skirpta et al. (1986) reported similar reduction in seed germination and inhibition of seedling development of soybean seeds infected with *C. truncatum*.

Regarding the association of *C. lindemuthianum* with discoloured seeds, the cultivar Watex recorded maximum per cent discoloured seeds with *C. lindemuthianum*.

**Table 2**Effect of *C. lindemuthianum* seed infection on french bean seed germination

Variety	Place of collection	Germination (%)	Abnormal seedlings* (%)	Dead seed* (%)
Arkakomal	Hosur	49.6 <sup>b</sup> (44.75)	37.6 <sup>b</sup> (37.74)	12.8 <sup>a</sup> (20.65)
		72.0 <sup>a</sup> (58.17)	17.6 <sup>c</sup> (24.66)	10.4 <sup>ab</sup> (16.77)
	Melchengam	95.2 <sup>a</sup> (78.81)	3.2 <sup>a</sup> (9.23)	1.6 <sup>c</sup> (4.62)
		44.8 <sup>c</sup> (41.86)	48.0 <sup>a</sup> (43.85)	7.2 <sup>ab</sup> (15.24)
Premier	Gudiatham	84.8 <sup>a</sup> (67.53)	12.8 <sup>a</sup> (20.65)	2.4 <sup>a</sup> (5.59)
		73.6 <sup>a</sup> (59.51)	20.0 <sup>a</sup> (26.12)	6.4 <sup>ab</sup> (12.93)
	Kodaikanal	73.6 <sup>a</sup> (59.51)	17.6 <sup>a</sup> (24.31)	8.8 <sup>a</sup> (16.99)
		72.8 <sup>a</sup> (59.29)	16.0 <sup>a</sup> (23.16)	11.2 <sup>a</sup> (17.15)
Rajmash DPU-88-4	Kelamangalam	76.8 <sup>a</sup> (61.38)	17.6 <sup>b</sup> (24.56)	5.6 <sup>a</sup> (13.49)
		56.0 <sup>b</sup> (48.47)	32.8 <sup>a</sup> (34.80)	11.2 <sup>b</sup> (19.39)
	Ooty	52.0 <sup>b</sup> (46.16)	35.2 <sup>a</sup> (36.29)	12.0 <sup>b</sup> (20.06)
		72.0 <sup>b</sup> (58.38)	20.8 <sup>b</sup> (26.77)	7.2 <sup>a</sup> (15.24)
Watex	Hosur	60.0 <sup>a</sup> (50.81)	24.8 <sup>b</sup> (29.68)	15.2 <sup>a</sup> (22.68)
		50.4 <sup>b</sup> (45.24)	29.6 <sup>ab</sup> (32.91)	20.0 <sup>a</sup> (26.37)
	Nanjanadu	51.2 <sup>b</sup> (45.69)	30.4 <sup>ab</sup> (33.43)	18.4 <sup>a</sup> (24.81)
		43.2 <sup>b</sup> (40.93)	38.4 <sup>a</sup> (38.13)	18.4 <sup>a</sup> (24.81)

\* Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

The infected seeds showed characteristic brown spots with whitish center surrounded by a pale brown to dark brown margin. Arkakomal recorded lowest seed infection (Table 3). The results indicated that there was direct correlation exist between discoloured seeds and seed infection. Similar observations on the association of *C. lindemuthianum* in discoloured seeds of french bean have been reported by several workers (Rodriguez, 1984; Goulart, 1988; Tytkowska and Dorna, 1990).

**Table 3**Association of *C. lindemuthianum* with discoloured french bean seeds

Variety	No. of seeds tested	Discoloured seeds* (%)	<i>C. lindemuthianum</i> associated with discoloured seeds* (%)
Arkakomal	400	37.25 <sup>c</sup> (37.61)	20.50 <sup>d</sup> (26.91)
Premier	400	58.75 <sup>b</sup> (50.04)	36.50 <sup>c</sup> (37.17)
Rajmash DPU-88-4	400	60.50 <sup>b</sup> (51.06)	40.75 <sup>b</sup> (39.67)
Watex	400	67.50 <sup>a</sup> (55.25)	47.25 <sup>a</sup> (43.42)

\* Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

The results from the effect of *C. lindemuthianum* seed infection on seed quality revealed that there was a significant reduction in seed size and weight in all four varieties. Among the four varieties, Watex recorded the maximum per cent seed infection and reduction in seed size and weight, while Arkakomal recorded minimum seed infection with least reduction in seed size and weight (Table 4). The loss in seed weight and shriveling of seeds due to fungal infection was reported by Gaikward et al. (1993) in soybean and by Subramanian (1988) in rice. It was observed that upon the extent of seed infection there was a reduction in length and breadth of seeds.

**Table 4**Effect of *C. lindemuthianum* seed infection on seed size and weight

Variety	Seed infection (%)	Length (mm)*			Breadth (mm)*			100 Seed weight (g)*		
		Healthy	Infected	Reduction over healthy seed	Healthy	Infected	Reduction over healthy seed	Healthy	Infected	Reduction over healthy seed
Arkakomal	57.0 <sup>a</sup> (49.02)	13.41 <sup>c</sup>	11.39 <sup>c</sup>	15.06	7.75 <sup>a</sup>	6.64 <sup>*</sup>	14.32	34.94 <sup>*</sup>	33.07 <sup>*</sup>	5.35
Premier	59.0 <sup>ab</sup> (50.20)	14.63 <sup>b</sup>	12.15 <sup>b</sup>	16.95	7.34	6.11 <sup>*</sup>	16.76	36.22 <sup>*</sup>	33.75 <sup>*</sup>	6.82
Rajmash DPU-88-4	66.0 <sup>ab</sup> (54.33)	15.48 <sup>a</sup>	12.81 <sup>b</sup>	17.25	6.55	5.60 <sup>*</sup>	14.50	38.29 <sup>*</sup>	34.51 <sup>*</sup>	9.87
Watex	70.0 <sup>a</sup> (56.80)	12.61 <sup>d</sup>	10.11 <sup>a</sup>	19.83	5.75	4.27 <sup>*</sup>	25.74	31.40 <sup>*</sup>	26.58 <sup>*</sup>	15.35

\* Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

In the present study, seed coat and cotyledon showed maximum infection of *C. lindemuthianum*, while the embryonic axis showed very low infection. The seed samples of all the four varieties recorded maximum infection of *C. lindemuthianum* on seed coat. The variety Watex had higher seed infection leads to higher seed coat and cotyledonary infection and low infection of embryonic axis (Table 5). Franca Neti and West (1989) also made similar observation in soybean that the *C. truncatum* infection was mainly restricted to seed coats.

**Table 5***C. lindemuthianum* infection in seed and seed parts

Variety	Seed infection (%)*	Seed coat infection (%)*	Cotyledonary infection (%)*	Embryonic axis infection (%)*
Arkakomal	57.00	75.2 <sup>bc</sup> (60.35)	66.4 <sup>a</sup> (54.83)	11.2 <sup>b</sup> (19.08)
Premier	59.00	70.4 <sup>c</sup> (57.21)	54.4 <sup>b</sup> (47.54)	10.4 <sup>b</sup> (18.63)
Rajmash DPU-88-4	66.00	80.0 <sup>ab</sup> (63.66)	60.8 <sup>ab</sup> (51.25)	8.0 <sup>b</sup> (16.22)
Watex	70.00	86.4 <sup>a</sup> (68.58)	67.2 <sup>a</sup> (55.11)	19.2 <sup>a</sup> (25.78)

\* Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

The result on the susceptible age at which the anthracnose incidence occurs in a severe form revealed that the susceptibility increased with the increase in age of plants and later it declined. The plants received inoculum on 45th day recorded the maximum disease incidence with maximum resultant seed infection and minimum seed yield/plant and 100 seed weight (Table 6). This was in agreement with the earlier findings of Sindhan and Bose (1981). They observed that the plants at all ages were susceptible to the disease, but the young plants were less susceptible in comparison to older plants and the lower leaves were more vulnerable to infection than younger ones. Higher susceptibility of the older plants may be due to the decrease in total sugar and phenolic content as observed in brown sarcon by Chahal (1986).

With this it is concluded that the results emerged from the above investigation have brought out the following points:

(i) Agar plate method by using potato dextrose agar was found to be an ideal technique to detect *C. lindemuthianum* from french bean.

(ii) The seeds collected from temperate regions were found to record more seed borne infection than tropical regions.

(iii) A direct correlation was observed between per cent discoloured seed and seed infection which caused lesser seed weight.

**Table 6**Effect of plant age on disease incidence and seed transmission of *C. lindemuthianum*

Plant age	Disease incidence (%) <sup>*</sup>	Pod infection (%) <sup>*</sup>	Resultant seed infection (%) <sup>*</sup>	Seed yield/plant (g) <sup>*</sup>	100 Seed weight (g) <sup>*</sup>
15	10.15 <sup>d</sup> (18.58)	17.27 <sup>c</sup> (24.37)	7.87 <sup>d</sup> (16.18)	15.17 <sup>a</sup>	30.83 <sup>a</sup>
30	47.40 <sup>c</sup> (43.50)	36.31 <sup>b</sup> (37.01)	19.66 <sup>c</sup> (26.28)	13.93 <sup>b</sup>	28.95 <sup>b</sup>
45	70.77 <sup>a</sup> (57.27)	66.87 <sup>a</sup> (54.88)	56.75 <sup>a</sup> (48.91)	8.83 <sup>d</sup>	24.29 <sup>d</sup>
60	65.13 <sup>b</sup> (59.79)	59.70 <sup>a</sup> (56.21)	48.77 <sup>b</sup> (44.29)	9.80 <sup>c</sup>	25.10 <sup>c</sup>

<sup>\*</sup> Mean of five replications

(Figures in paranthesis are arc sine transformed values) In a column, means followed by a common letter are not significantly different at 5% level by DMRT

(iv) Pathogen was found to be associated mostly in seed coat and cotyledons.

(v) The 45-day-old plants were highly susceptible to the infection and lead to maximum disease incidence and resultant seed infection.

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