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RESEARCH PAPERS

Response of some chickpea cultivars to foliar, seed and soil inoculations with *Botrytis cinerea*

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Summary. Twenty-four cultivars of chickpea were evaluated for their susceptibility to the grey mould fungus *Botrytis cinerea* using foliar, seed and soil inoculation at 2, 4 and 8 g fungus per plant, per kg seed or per kg soil. At 8 g the inoculum caused necrotic lesions to all 24 cultivars with foliar inoculation, to 23 cultivars except cv. CH-2007-22 with seed inoculation and to 17 cultivars with soil inoculation, and reduced yield by 7–43% (foliar inoculation), 3–34% (seed inoculation) and 3–26% (soil inoculation). Foliar or seed inoculation with 4 g of the fungus significantly reduced the yield of all cultivars tested except CH-2007-22 with foliar inoculation, and 4 cultivars with seed inoculation. Soil inoculation at 4 g fungus kg^{-1} soil, significantly reduced the yield of eight cultivars. Foliar and seed inoculations at 2 g of the fungus significantly reduced the yield of 16 and 7 cultivars of chickpea respectively; but soil inoculation at this concentration, did not significantly reduce yield in any cultivar. The greatest significant decline in yield was recorded with foliar inoculation in the cv. BG-256, 43% at 8 g, 40% at 4 g and 26% at 2 g inoculum level. The cv. CH-2007-22 was tolerant to *B. cinerea* as it exhibited only 3–7% yield loss at 8 g inoculum. The fungal population, especially that on the phylloplane, increased exponentially from January to March and declined drastically in April. At the high inoculum level of 8 g fungus kg^{-1} soil, *B. cinerea* may initiate infection through the soil. There was a positive correlation between disease severity and yield decline, and a disease severity above 2 significantly reduced yield.

Key words: Botrytis grey mould, gram, screening, germplasm, pot culture.

Introduction

Grey mould caused by *Botrytis cinerea* Pers. ex Fr. is one of the most destructive diseases of chickpea (*Cicer arietinum* L.) throughout the world, especially in Bangladesh, Nepal, and western Australia, and also in areas with a cool, cloudy and humid climate. In India Botrytis grey mould (BGM) is quite common in the Tarai region of Uttar Pradesh, in Bihar, West Bengal, eastern India etc., and it appears every year in moderate to severe form depending on the prevalence of favourable environmental conditions and on the cultivar (Grewal and Laha, 1983). Under prolonged cool and humid conditions the fungus first infects the lower leaves and then progresses upwards causing defoliation, rotting of tender branches and pods with shriveled seeds (Joshi and Singh, 1969; Haware and McDonald, 1992). The disease causes considerable damage to chickpea in India (Tripathi and Rathi, 2000; Pande *et al*., 2006a) with an annual yield loss of 50% or more. Yield loss may reach as much as 100% if favourable conditions prevail during the vegetative and reproductive growth stages of the crop (Grewal and Laha, 1983; Pande *et al*., 2006a).

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In view of the economic importance of chickpea, the seriousness of the disease and the yield loss associated with it, farmers in India employ some management methods, especially fungicides (Rewal, 1987; Pandey, 1988; Haware *et al*., 1997; Lindbeck *et al*., 2009) and also biopesticides (Arne and Jonas, 1980; Liu *et al*., 2004). However, both these methods require additional input and are not completely satisfactory. Host plant resistance is the most economic and eco-friendly means to control BGM. Rewal (1987) screened several chickpea cultivars and lines against *B. cinerea*, and found two lines, ICC 1069 and ICC 5035 that were resistant to the fungus with a disease rating of 3 on a 0–10 scale. Similarly, one line ICC 12339, was also resistant to grey mould (Pande *et al*., 2006a).

To exploit host plant resistance, reliable screening techniques in the field and in a controlled environment are essential. In general, field screening is used for the large-scale screening of cultivar and breeding material, while controlled environment screening, especially greenhouse screening, serves to confirm field resistance to different pathogens, and to pathotypes/races. This last type of screening is also used to carry out inheritance and race identification studies.

In the present study, the resistance/tolerance of some commonly grown chickpea cultivars to *B. cinerea* was evaluated at different inoculum levels using foliar, seed and soil inoculation.

Materials and methods

Procurement of chickpea cultivars and *B. cinerea*

Greenhouse whole plant screening was used to evaluate the resistance/tolerance to *B. cinerea* of some commonly grown chickpea cultivar: BG-256, Avrodhi, GNG-469, Pant G-186, Digvijay, DCP-92-03, CH-2007-22, CH-2007-23, CH-2007-25, CH-2007-26, CH-2007-36, CH-2007-44, CH-2007-46, CH-2007-50, CH-2007-52, CH-2007-54, CH-2007-58, CH-2007-60, CH-2007-62, CH-2007-63, CH-2007-64, CH-2007-72, CH-2007-73 and CH-2007-74. The chickpea cultivars were procured from the Indian Institute of Pulse Research, Kanpur, India. A pure and identified culture of *B. cinerea* Pers. ex. Fr. was procured from the Division of Mycology and Plant Pathology, IARI, New Delhi, India.

Inoculum of *B. cinerea*

Inoculum of *B. cinerea* was prepared using single-spore culture and a standard serial dilution method. A spore suspension of the fungus was prepared in sterile distilled water and diluted to 10^{-4}. The suspension from the final dilution was pipetted on potato dextrose agar (PDA) in a Petri dish (0.3 mL dish^{-1}) under a laminar flow. Three dishes were maintained. Inoculated Petri dishes were incubated at 25±2°C for 3–5 days in a biological oxygen demand (BOD) incubator. After incubation, fresh Petri dishes with PDA were inoculated each with a single small colony of 3–6 mm developed from a single spore of the fungus. The dishes were incubated for 5 days. Colonies from the dishes were identified on the basis of their morphological characters (Gilman, 2001). After confirming the identity of *B. cinerea*, the fungus was grown on sorghum seeds as well as on potato dextrose (PD) broth to obtain sufficient biomass for plant inoculation.

For soil and seed inoculation the fungus was mass cultured on sorghum seeds. The seeds were soaked overnight in a 5% sucrose and chloramphenicol (30 mg L^{-1}) solution. The seeds were then transferred to conical flasks of 500 mL capacity and autoclaved at 15 kg cm^{-2} pressure at 120°C for 15–20 minutes two times with a gap of 12 hours to ensure sterilization. Thereafter, they were inoculated with the pure culture of *B. cinerea* under aseptic conditions and incubated for 8–10 days on a rotatory shaker (120 rpm) inside a BOD incubator at 25±2°C to provide optimum conditions for growth. To achieve uniform colonization and sporulation of the BGM fungus, the flasks were shaken for 30 minutes daily during the incubation period.

For foliar inoculation, the fungus was cultured on PD broth in 500 mL conical flasks from which the mycelial mat was collected and ground with double distilled water in a mixer grinder. The fungal suspension was standardized to 2.5×10^4 cfu ml^{-1}. Colonization of the BGM fungus on the inoculated PDA dishes with the serially diluted suspension took 2–3 days. During this period the suspension was kept in a deep freezer at 5°C to restrict propagation. A spore/cfu suspension of $10^{4–5}$ has been found effective in causing infection (Pande *et al*., 2006a). Infection with *B. cinerea* may be initiated by hyphae as well as by spores, and the

former cannot be counted in the suspension under the microscope. The serial dilution method was used to standardize the suspension, and this method does not differentiate between hyphae and spores, hence the inoculum load was denoted by the cfus.

Inoculation of *B. cinerea*

Field experiment

For soil application, sorghum seeds colonized with *B. cinerea* at 2, 4 and 8 g kg^{-1} soil were mixed in the soil in pots, and for seed application, sorghum seeds colonized with the fungus were first ground in sterilized grinder and then applied to the seeds at 2, 4 and 8 g kg^{-1} seeds. On the leaves the fungus was applied by uniformly spraying one-month-old seedlings with a suspension of *B. cinerea* using an atomizer (2.5×10^4 cfu mL^{-1}) at 2, 4 and 8 g $plant^{-1}$. After spraying the plants were bagged in moistened polyethylene bags for 24 hours to maintain high humidity.

Pot experiment

The experiments were conducted in clay pots of 30×30 cm size. The pots were each with 3 kg mixture of steam sterilized soil and farmyard manure (3:1). Chickpea seeds irrespective of the manner of treatment were applied with commercial *Rhizobium* of chickpea strain and seeds were inoculated with *B. cinerea* depending on the manner of treatment. Thereafter, 3–5 seeds were sown. After sowing, pots were sprinkled with water. Two weeks later, the seedlings were thinned to one plant pot^{-1}. For each treatment three replicates were maintained including an uninoculated control. Pots were placed on the roof, where they received uniform light, and they were arranged in a completely randomized block design. Moreover, plants were placed in four groups: uninoculated, inoculated soil, inoculated seed and inoculated leaves, and these groups were separated by transparent polythene sheets to avoid cross-contamination from neighbouring pots. At harvest, 4 months after sowing, the plant dry weight and the number of pods $plant^{-1}$ were determined.

Quantification of disease and of the *B. cinerea* population

Disease severity was recorded 3 months after sowing, while the rhizosphere and phylloplane population of *B. cinerea* was estimated monthly till harvest. Disease severity was determined on a 0–10 scale (0, 0%; 1, 1–9%; 2, 10–19%; 3, 20–29%; 4, 30–39%; 5, 40–45%; 6, 50–59%; 7, 60–69%; 8, 70–79%; 9, 80–89%; 10, 90–100% leaves showing BGM). The disease percentage was calculated as:

$$\text{Disease \%} = \frac{(\text{No. of leaflets showing symptoms per plant}) \times 100}{\text{Total No. of leaflets per plant}}$$

The phylloplane population of the grey mould fungus was estimated monthly using the serial dilution technique. This population indicated the number of *B. cinerea* spores on the leaves, not those embedded inside the leaf tissues. To estimate the phylloplane population the infected leaves were collected. One-g discs were cut from the leaves of inoculated plants showing symptoms and were put in a conical flask with 10 mL sterilized water. The mixture was stirred over a magnetic stirrer for five minutes and the suspension was diluted (1:10) by adding 1 mL of suspension to 9 mL sterilized water and was then further diluted to 10^{-4}. From this suspension, 0.1 mL was spread over PDA in Petri dishes and incubated at 25±2°C for 4–5 days. The dishes were examined and the colonies of the BGM fungus were identified on the basis of color and morphological characters. The colonies were counted under a colony counter to determine the phylloplane population (cfu g^{-1} leaf) of the fungus. Similarly, the rhizosphere population of the fungus was estimated monthly by sampling soil from the rhizosphere of the plants and passing the soil through a coarse sieve. One g of soil was taken and the number of cfu g^{-1} soil was estimated using the dilution technique.

Statistical analysis

The data on plant growth variables were subjected to two-factor analysis of variance (ANOVA), considering cultivars as factor one and fungus inoculations as factor two, whereas the data on the fungus population was analyzed by single-factor ANOVA. The LSD was calculated at three probability levels, $P \leq 0.05$, 0.01 and 0.001. The data on disease severity and phylloplane population, and on disease severity and yield decline were regressed to determine the correlation between the variables.

Results

Disease symptoms and severity

All 24 cultivars of chickpea leaf-inoculated with *B. cinerea* developed characteristic lesions which appeared at the flowering stage and became more pronounced at the pod formation stage. The disease severity (on the 0–10 scale) however varied greatly with the cultivar, dose and mode of inoculation. Three cultivars, BG-256 (ds 6.3, 5.4, 2.6), CH-2007-63 (ds 6.1, 5.6, 2.5) and CH-2007-23 (ds 6, 5.5, 2.4) developed the strongest symptoms of the disease ($P \leq 0.05$) whereas mildest symptoms occurred on CH-2007-22 (ds 2.1, 0.8, 0), CH-2007-50 and CH-2007-52 (ds 2.2, 1.6, 0) after foliar inoculation with 8 g, 4 g or 2 g of the fungus (Table 1). Seed inoculation was less effective than foliar inoculation in causing symptoms ($P \leq 0.05$). Five seed-inoculated cultivars, BG-256, CH-2007-63, CH-2007-23, DCP-92-03 and GNG-469 showed symptoms of BGM at 8, 4 and 2 gram inoculation level per kg seed ranging in severity from 1.5 to 5.3 (Table 1). Soil inoculation with the BGM fungus was the least effective, causing only very mild symptoms in six cultivars at 8 g inoculum per kg siol: BG-256, CH-2007-63, CH-2007-23, Avrodhi, GNG-469 and DCP-92-03 (disease severity 2.1–2.6) while at 4 or 2 g of inoculum there were no symptoms (Table 1). As regards overall disease severity, the cultivar BG-256 was highly susceptible and the cultivars

Table 1. Disease severity (0–10 scale) [a] of chickpea cultivars with soil, seed and foliar inoculation of *Botrytis cinerea*.

Cultivars [b]	Soil inoculation (g plant^{-1}) [c]				Seed inoculation (g kg^{-1} seed) [c]				Foliar inoculation (g plant^{-1}) [c]			
	Control	2	4	8	Control	2	4	8	Control	2	4	8
BG-256	0	0	1.6 d	2.6 d	0	1.6 c	2.7 d	5.3 i	0	2.6 d	5.7 q	6.3 n
Avrodhi	0	0	1.5 c	2.5 d	0	1.5 c	2.6 d	4.9 h	0	2.4 d	5l	5.4 j
GNG-469	0	0	1.1 b	2.1 c	0	1.5 c	2.2 c	5 h	0	2.1 c	5.2 m	5.6 k
Pant G-186	0	0	0	1.3 ab	0	1.1 a	1.3 ab	1 a	0	1.2 a	1.6 b	2.3 ab
Digvijay	0	0	0	1.4 ab	0	1.5 c	1.5 b	1.1 a	0	1.5 b	1.7 c	2.8 c
DCP-92-03	0	0	1a	2.1 c	0	0	1.6 b	5.1 h	0	1.5 b	5.4 n	5.9 l
CH-2007-22	0	0	0	0	0	0	0	0	0	0	0.8 a	2.1 a
CH-2007-23	0	0	1.5 c	2.5 d	0	0	2.6 d	5.1 h	0	2.4 d	5.5 o	6l m
CH-2007-25	0	0	0	0	0	1.1 a	0	4.2 g	0	0	4.3 k	5 i
CH-2007-26	0	0	0	1.4 ab	0	1.1 a	1.5 b	3.6 f	0	1.4 ab	3.7 i	4.1 g
CH-2007-36	0	0	0	1.3 ab	0	0	1.4 b	2.5 d	0	1.3 ab	2.9 g	3.8 f
CH-2007-44	0	0	0	1.2 a	0	1.2 ab	1.1 a	2.3 d	0	1a	2.7 f	3.2 de
CH-2007-46	0	0	0	1.4 ab	0	0	1.5 b	4.2 g	0	1.4 ab	4.3 k	5 i
CH-2007-50	0	0	0	0	0	0	0	1 a	0	0	1.6 b	2.2 a
CH-2007-52	0	0	0	0	0	1 a	0	1 a	0	0	1.6 b	2.2 a
CH-2007-54	0	0	0	1.2 a	0	0	1.3 ab	4 g	0	1.2 a	4.1 j	4.4 h
CH-2007-58	0	0	0	1a	0	0	1.1 a	2.9 e	0	1 a	3 h	3.1 d
CH-2007-60	0	0	0	1.1 a	0	0	1.1 a	2.5 d	0	1 a	2.9 g	3.8 f
CH-2007-62	0	0	0	0	0	1.5 c	0	1.3 ab	0	0	2.1 d	3 d
CH-2007-63	0	0	1.5 c	2.5 d	0	0	2.6 d	5.2 hi	0	2.5 d	5.6 p	6.1 lm
CH-2007-64	0	0	0	0	0	0	0	1 a	0	0	1.6 b	2.3 ab
CH-2007-72	0	0	0	1.1 a	0	0	1 a	2 c	0	1 a	2.5 e	3.1 d
CH-2007-73	0	0	0	1 a	0	0	1 a	1 a	0	1 a	1.6 b	2.3 ab
CH-2007-74	0	0	0	1 a	0	0	1 a	1 a	0	1 a	1.6 b	2.3 ab
$P \leq 0.05$			0.07	0.22		0.17	0.22	0.29		0.21	0.10	0.19

[a] Disease severity: 0, 0%; 1, 1–9%; 2, 10–19%; 3, 20–29%; 4, 30–39%; 5, 40–45%; 6, 50–59%; 7, 60–69%; 8, 70–79%; 9, 80–89%; 10, 90–100%.

[b] Source of BG-256: IARI New Delhi, Pant G-186 is GBPUAT Pantnagar and for the remaining cultivars is IIPR Kanpur, India; each value is the mean of three replicates.

[c] Means followed by different letters in column are significantly different from each other at $P \leq 0.05$.

CH-2007-22, CH-2007-52 had a considerable degree of tolerance against *B. cinerea* ($P \leq 0.05$).

Plant growth and yield

Foliar inoculation

Most cultivars were susceptible to foliar inoculation with *B. cinerea*. Foliar inoculation reduced plant dry weight by up to 47% of and plant yield by up to 43% except for cultivar CH-2007-22, which was not significantly different from the control except at the 8 g inoculum level. BG-256 was the most susceptible, with reductions in yield and dry weight of 43 and 47% respectively at 8 g, 40 and 40% at 4 g, and 26 and 27% at 2 g. The least susceptible cultivar was CH-2007-22, with reduction of 8% in dry weight and 7% in yield at the 8 g of inoculum level, and a reduction of 3% in yield at the 2 g inoculum level (Tables 2 and 3).

Seed inoculation

Cultivars were susceptible to seed inoculation with *B. cinerea* at all inoculum levels (Tables 2 and 3). At 8 g fungus kg^{-1} seed necrotic lesions occurred on all cultivars except CH-2007-22; while in cvs.

Table 2. Chickpea cultivars receiving foliar, seed and soil inoculation with 2, 4 and 8 g inoculum level of *Botrytis cinerea*: effect on yield (pods $plant^{-1}$) of chickpea. Each value is the mean of three replicates.

Inoculum level (g)	Mode of inoculation	BG-256	Avrodhi	GNG-469	Pant G-186	Digvijay	DCP-92-03	CH-2007-22	CH-2007-23	CH-2007-25	CH-2007-26	CH-2007-36	CH-2007-44
0 (Control)		21.7	23	25	29	28	21.3	24.3	25	19.3	20	27	24
2	Foliar	16^{***a}	18.7^{**}	20^{***}	27^{**}	26^{**}	17^{***}	24	20^{***}	18.3	18.3^{*}	24.7	21.7^{**}
2	Seed	19.3^{**}	20.7	22.7^{**}	27.2^{*}	25.7^{**}	19.3^{**}	24	22.7^{**}	19	19.3	26	23.3
2	Soil	21	22.3	24.3	28.3	27.3	20.7	24.3	24.3	19	20	26.7	23.7
4	Foliar	13^{***}	15.7^{***}	16.7^{***}	26^{***}	25^{***}	14^{***}	23.6	16.3^{***}	16^{***}	17^{***}	23.7^{*}	21^{***}
4	Seed	15.7^{***}	18.3^{***}	19.7^{***}	26.7^{**}	25.7^{**}	16.7^{***}	23.3	19.7^{***}	18	18^{**}	24.3^{*}	21.7^{**}
4	Soil	19.3^{**}	20.7	22.7^{**}	27.3^{*}	26^{**}	19.3^{**}	24	22.7^{**}	19	19.3	26.3	23.3
8	Foliar	12.3^{***}	15^{***}	16.3^{***}	25.7^{***}	24.7^{***}	13.3^{***}	22.7^{*}	15.3^{***}	15.3^{***}	16.3^{***}	23.3^{**}	20^{***}
8	Seed	14.3^{***}	17^{***}	18.3^{***}	26.4^{***}	25.3^{***}	15.3^{***}	23.7	18^{***}	17^{**}	17.7^{**}	24.3^{*}	21.7^{**}
8	Soil	16^{***}	18.7^{**}	20^{***}	26.7^{**}	25.7^{**}	17^{***}	23.7	19.7^{***}	18	18.3^{*}	24.7	22^{**}
LSD													
$P \leq 0.05$		1.4	2.4	1.4	1.4	1.4	1.2	1.4	1.4	1.4	1.4	2.5	1.4
$P \leq 0.01$		1.9	3.3	2	2	1.9	1.7	1.9	1.9	1.9	2	3.4	2
$P \leq 0.001$		2.6	4.5	2.7	2.7	2.6	2.3	2.6	2.6	2.5	2.7	4.6	2.7
F- value [b]													
A (df=3)		227.5^{***}	158.9^{***}	194.3^{***}	26.67^{***}	26.6^{***}	158.9^{***}	NS	173.6^{***}	42.6^{***}	31.8^{***}	NS	34.7^{***}
B (df=3)		73.9^{***}	43.8^{***}	59.1^{***}	11.9^{**}	17.3^{**}	43.8^{***}	NS	59.1^{***}	NS	7.8^{*}	11.2^{**}	3.2^{**}
A×B[c] (df=9)		47.3^{***}	10.3^{***}	38.6^{***}	4.2^{**}	4.6^{**}	44.6^{***}	2.4^{*}	48.3^{***}	9.1^{***}	6.5^{***}	2.4^{*}	7^{***}

continued on the next page

[a] Values followed by an asterisk are significantly different from the control at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{**}P \leq 0.001$.
[b] NS, not significant otherwise significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$; 'A' indicates dose, 'B' mode of inoculation and
[c] A×B, interaction between dose and mode of inoculation.

Table 2. Continued

Inoculum level (g)	Mode of inoculation	CH-2007-46	CH-2007-50	CH-2007-52	CH-2007-54	CH-2007-58	CH-2007-60	CH-2007-62	CH-2007-63	CH-2007-64	CH-2007-72	CH-2007-73	CH-2007-74
0 (Control)		22.3	28	21.7	19	27.3	24.7	29	24	17.3	18	28.7	25
2	Foliar	20.3*	26**	20.3	17.7	25.3*	22.7	27*	17.7***	17	16.7	26.7*	23**
2	Seed	21.3	27	21.3	18.3	26.3	24	28	21.3***	17	17.7	28.3	24.7
2	Soil	21.7	27.3	21.3	18.7	27	24.3	28.7	23.3	17.3	18	28.7	25
4	Foliar	18.7***	25.3**	20*	16***	24**	22*	26**	15.7***	15.7*	15.7**	26**	22.7**
4	Seed	20**	26**	20.3	17.3*	25*	22.3*	27*	17.7***	16.7	16.3*	26.3*	23**
4	Soil	21	26.7	21.3	18.3	26.7	24	28.3	21.7**	17	17.7	28.3	24.7
8	Foliar	17.7***	25.7***	19.7*	15.3***	23***	21**	25.3***	14.3***	15**	15**	25.7**	22.3***
8	Seed	19.7**	25.7**	20*	16.7**	24.7**	22.3*	26.7*	17***	15.3*	16*	26.3*	22.7**
8	Soil	20.3*	26.3*	20.3	17.3*	25*	22.7	27.3	18.3***	15.7*	16.7	26.3*	23**
LSD													
$P \leq 0.05$		1.7	1.4	1.6	1.4	1.8	2.2	1.8	1.4	1.6	1.7	1.8	1.4
$P \leq 0.01$		2.3	1.9	2.2	2	2.5	3	2.5	2	2.2	2.3	2.5	1.9
$P \leq 0.001$		3.1	2.6	3	2.7	3.4	4	3.4	2.7	3.1	3.1	3.3	2.6
F- value [b]													
A (df=3)		36.3***	17.3**	8.4*	9.4*	15.5**	NS	4.9*	220.2***	26.3***	7.6*	21.8**	14.8**
B (df=3)		6.4*	8.3*	NS	NS	NS	5.3*	NS	95.8***	7.4*	NS	10.9**	11.1**
A×B[c] (df=9)		6.2***	3.1*	3.6*	5.2**	5.1**	3.1*	3.6*	45.4***	6.2***	3.5*	8.9***	4.8**

BG-256, Avrodhi, GNG-469, DCP-92-03 and CH-2007-63 the pods remained hollow and yield and dry weight went down by 33 and 34, 26 and 27, 27 and 27, 28 and 29, 29 and 32% respectively. With a 4 g inoculum level, yield or dry weight went down significantly in cultivars except CH-2007-22 and CH-2007-52 (Tables 2 and 3). With 2 g inoculum only CH-2007-63 showed hollow pods and a 26% reduction in yield and plant dry weight (Tables 2 and 3).

Soil inoculation

Cultivars were fairly susceptible to soil inoculation with *B. cinerea* at all inoculum levels except 2 g (Tables 2 and 3). With 8 g fungus kg^{-1} soil, a few necrotic lesions were seen on BG-256, CH-2007-63, CH-2007-23, Avrodhi, GNG-469 and DCP-92-03, whereas on BG-256, CH-2007-63 and CH-2007-23 the pods remained hollow, and reduction in the yield and dry weight were 26 and 27, 23 and 26 and 21 and 24% respectively. At 2 g fungus in the soil, the fungus did not significantly reduce the dry weight or yield of any cultivar ($P \leq 0.05$), but at 4 g eight cultivars had reductions in yield and dry weight (shown in parentheses): BG-256 (11 and 13%), GNG-469 (9 and 11%), Pant G-186 (6 and 7%), Digvijay (7 and 8%) , DCP-92-03 (9 and 8%), CH-2007-23 (9 and 7%), CH-2007-50 (5 and 8%) and CH-2007-63 (10 and 12%) (Tables 2 and 3).

Table 3. Chickpea cultivars receiving foliar, seed and soil inoculation with 2, 4 and 8 g inoculum level of *Botrytis cinerea*: effect on dry weight (g plant^{-1}) of chickpea.

Inoculum level (g)	Mode of inoculation	BG-256	Avrodhi	GNG-469	Pant G-186	Digvijay	DCP-92-03	CH-2007-22	CH-2007-23	CH-2007-25	CH-2007-26	CH-2007-36	CH-2007-44
0 (Control)		8.3	10	9.3	12.3	12.6	8.3	9.6	10	7.6	8	12.3	10.3
2	Foliar	6.1*** [a]	8***	7.5***	11.3**	11.5*	6.5**	9.5	7.6***	7.2	7.2*	11.2*	9.3
2	Seed	7.2***	8.7***	8.2*	11.4**	11.5*	7.6	9.5	9.2	7.4	7.8	11.5	9.9
2	Soil	7.9	9.6	9	11.9	12.2	7.9	9.4	9.6	7.4	7.8	11.9	10
4	Foliar	5***	6.6***	6.1***	10.8***	11.1**	5.4***	9.3	6.5***	6.3***	6.8**	11*	9*
4	Seed	6***	7.9***	7.3***	11.1***	11.4*	6.4***	9.4	7.6***	7*	7.2*	11.2*	9.4
4	Soil	7.2***	8.8**	8.3*	11.4**	11.5*	7.6	9.3	9.3	7.3	7.6	11.6	9.7
8	Foliar	4.4***	5.8***	5.4***	10.4***	10.5***	4.7***	8.8*	5.7***	5.7***	6.2***	10***	8.2***
8	Seed	5.5***	7.3***	6.8***	11***	11.3**	5.9***	9.3	7.1***	6.6**	7*	11.1*	9.2*
8	Soil	6.1***	8***	7.4***	11.2***	11.4*	6.5***	9.1	7.6***	7*	7.2*	11.2*	9.4
LSD													
$P \leq 0.05$		0.6	0.7	0.9	0.6	1	1	0.8	1.1	0.7	0.8	1.1	1.1
$P \leq 0.01$		0.8	1	1.2	0.8	1.3	1.4	1.1	1.5	1	1.2	1.6	1.6
$P \leq 0.001$		1.1	1.3	1.6	1.1	1.8	1.9	1.5	2	1.3	1.6	2.1	2.1
F- value [b]													
A (df=3)		132.4***	120.8***	31.0***	30.1***	NS	11.6**	NS	157***	12.4**	5.9*	NS	7.9*
B (df=3)		18.1**	17.8**	13.0**	NS	NS	NS	NS	6.5*	NS	NS	NS	NS
A×B[c] (df=9)		37.8***	31.3***	17.4***	6.8***	3.0*	11.9***	NS	16.6***	7.6***	3.8**	2.6*	4.5**

continued on the next page

Phylloplane population

The phylloplane population of *B. cinerea* varied with the method and level of inoculation and with the cultivar infected (data shown on only two cultivars, Figure 1). The phylloplane population increased on all cultivars from January to March, but showed a marked decline in April irrespective of the method of inoculation. The greatest phylloplane population irrespective of the mode of inoculation, occurred with an 8 g inoculum level. With foliar inoculation at the 8 g inoculum level, the cfu count of *B. cinerea* in March was highest on CH-2007-54 (5.7×10^6 cfu g^{-1} leaf), followed by CH-2007-63 (5.6×10^6 cfu g^{-1} leaf) and BG-256 (5.5×10^6 cfu g^{-1} leaf) and it was lowest on Pant G-186 (2.6×10^6 cfu g^{-1} leaf), preceded by CH-2007-22 (2.7×10^6 cfu g^{-1} leaf). With seed inoculation at the 8 g level, the phylloplane population was greatest on BG-256 (3.1×10^6 cfu g^{-1} leaf), followed by CH-2007-63 (3×10^6 cfu g^{-1} leaf) and CH-2007-54 (2.1×10^6 cfu g^{-1} leaf). On plants that received soil inoculation, the phylloplane population of the fungus was almost 3–4 times lower than it was with foliar inoculation; however, the trend of variation was identical to that with the foliar treatment, particularly at the 8 g inoculum level. At inoculum levels of 4 and

Table 3. Continued

Inoculum level (g)	Mode of inoculation	CH-2007-50	CH-2007-52	CH-2007-54	CH-2007-58	CH-2007-60	CH-2007-62	CH-2007-63	CH-2007-64	CH-2007-72	CH-2007-73	CH-2007-74
0 (Control)		13	7.6	8.6	11.3	10.6	12.6	11	12	9	13	9
2	Foliar	12**	7	7.7**	10.3*	9.7	11.9	8.1***	11.6	8.3	12.2	8.5
2	Seed	12.2*	7.3	8.4	11	10.3	12.1	9.6*	11.7	8.8	12.7	8.8
2	Soil	12.6	7.3	8.4	11	10.2	12.3	10.5	11.7	8.8	12.7	8.8
4	Foliar	11.6***	6.5*	7.6**	10.1**	9.5*	11.2**	6.8***	10.8***	7.9*	11.6*	8.1*
4	Seed	12**	6.9	8*	10.3*	9.7	11.8*	8***	11.4*	8.1*	12.1	8.3
4	Soil	12**	7.2	8.2	10.8	10	12.1	9.7*	11.5	8.7	12.6	8.7
8	Foliar	10.6***	5.9**	7.2***	9.2***	8.6***	10.2***	5.9***	10.5***	7.2***	10.8**	7.7**
8	Seed	11.9**	6.8	7.9*	10.2*	9.6*	11.5*	7.5***	10.8***	8*	11.9*	8.2*
8	Soil	12.1**	7	8*	10.3*	9.7	11.8*	8.1****	11.1**	8.2	12.1	8.3
LSD												
$P \leq 0.05$		0.7	1.1	0.6	0.9	1	0.8	1.1	0.6	0.9	1.1	0.8
$P \leq 0.01$		0.9	1.5	0.8	1.2	1.3	1.2	1.5	0.8	1.3	1.6	1.3
$P \leq 0.001$		1.3	2.1	1.1	1.6	1.8	1.6	2.1	1.1	1.7	2.7	1.8
F- value												
A (df=3)		44.2***	NS	NS	4.7^{a}	NS	7.0^{a}	24.7***	16.3**	4.7^{a}	NS	7.5^{a}
B (df=3)		NS	NS	NS	NS	NS	NS	8.1^{a}	NS	NS	NS	NS
A×B^{c} (df=9)		7.9***	NS	4.1**	4.3**	2.8^{a}	33.7***	19.3***	5.6**	4.2**	2.5^{a}	2.5^{a}

[a, b, c,] See Table 2.

2 g the variation was the same as at 8 g, but in soil-inoculated plants the population was almost below the detection threshold. The greatest phylloplane population over all was recorded on BG-256 and the lowest on CH-2007-22 (Figure 1).

Rhizosphere population

The rhizosphere population also varied with the time, the inoculum level and the chickpea cultivar. From January to March the cfus increased, but in April the rhizosphere population declined drastically irrespective of the mode of inoculation and the inoculum load. In March, at 8 g per kg soil inoculum level the highest population was on BG-256 (3.9×10^6 cfu g^{-1} soil), followed by CH-2007-63 (3.6×10^6 cfu g^{-1} soil), and the lowest population was on CH-2007-22 and CH-2007-52 (1.4×10^6 cfu g^{-1} soil) (data presented for only two cultivars, Figure 2). In that same month at 4 and 2 g inoculum per kg soil from 2×10^5 cfu g^{-1} soil, the maximum increase in cfus was seen on BG-256 with 2.2 and 1.4×10^6 cfu g^{-1} soil, and the minimum on CH-2007-22 with 1.2 and 1×10^6 cfu g^{-1} soil respectively. With 8, 4 or 2 g seed inoculation the maximum and minimum rhizosphere populations were recorded on the same cultivars as with the soil inoculations, but the population was reduced by more than half. The soil population of *B. cinerea* applied by foliar spray showed the same trend of increase and decrease, but the cfu counts were 4–5 times lower

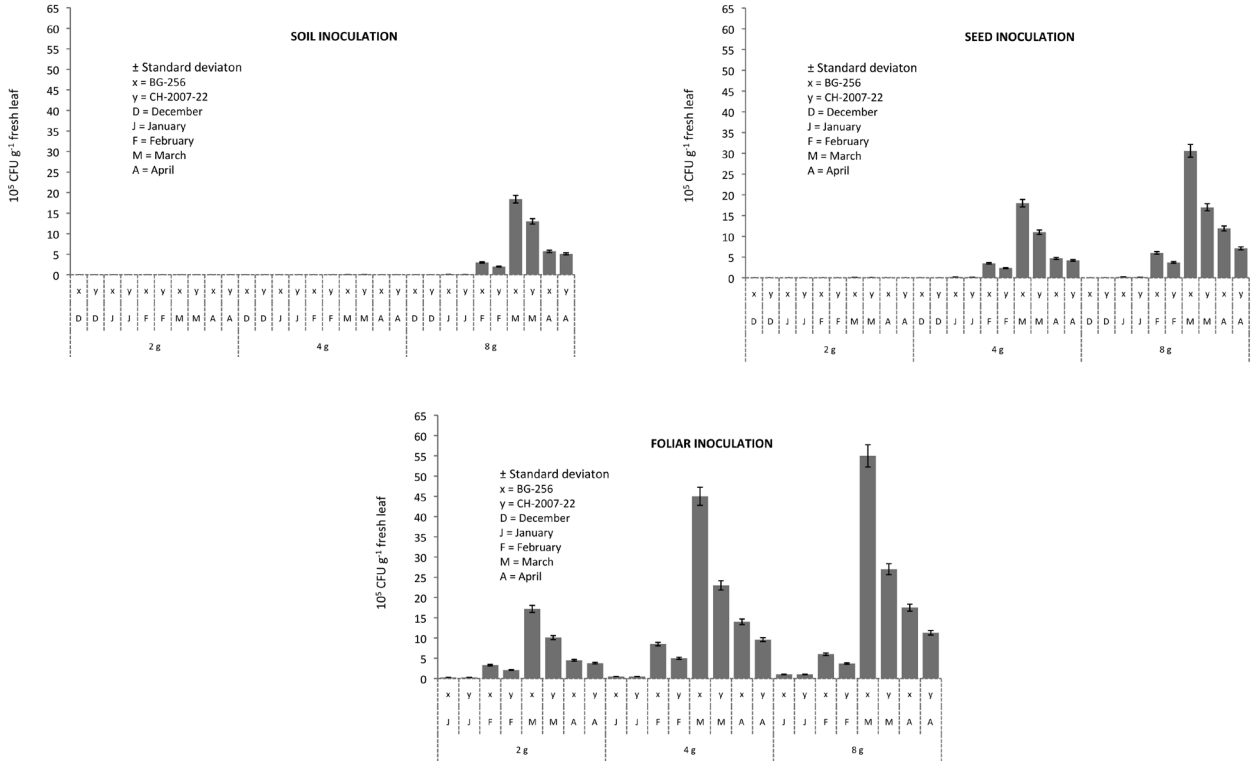

Figure 1. Phylloplane population of *Botrytis cinerea* in the most tolerant and susceptible chickpea cultivars subjected to soil, seed and foliar inoculation with *B. cinerea*.

than with soil inoculation. The highest and lowest rhizosphere populations also occurred in BG-256 and CH-2007-22, as with the phylloplane population (Figure 2).

Discussion

Botrytis cinerea is a foliar pathogen and under natural conditions is carried to the foliar parts of plants by wind, water, insects etc. (Elad *et al*., 2004). In infection, spores of the fungus penetrate through the leaf cuticle or the stomata (Pandey, 1988). In the study, foliar inoculation with the fungus produced severe symptoms including seedless pods and small or shriveled seeds. Pods and leaves also showed numerous round grayish lesions. These symptoms have been reported on chickpea (Haware and McDonald, 1992). Soil inoculation of the fungus also caused mild symptoms in a few cultivars, although severity was much less. The fungus can survive as a saprophyte in the soil or in infected plant debris but there are few reports of infection being caused through the roots. The seed-borne transmission of *B. cinerea* on chickpea has however been reported (Cother, 1977; Laha and Grewal, 1983), and infested seeds can cause BGM to spread into new regions. Laha and Grewal (1983) reported that 8–19% of BGM was due to seed infestation. Soil inoculation at 8 g inoculum kg^{-1} soil or seed inoculation with 4 g inoculum kg^{-1} seed caused grey mould of mild severity and seed inoculation with 8 g inoculum caused fairly severe grey mould on six cultivars, the most severe being on BG-256.

With seed inoculation there was a negative correlation between disease severity and yield, with a correlation coefficient of 0.73 (Figure 3). With four cultivars, BG-256 (highly susceptible) and CH-2007-22, CH-2007-50, and CH-2007-52 (all tolerant) the correlation coefficient was 0.9 (data not

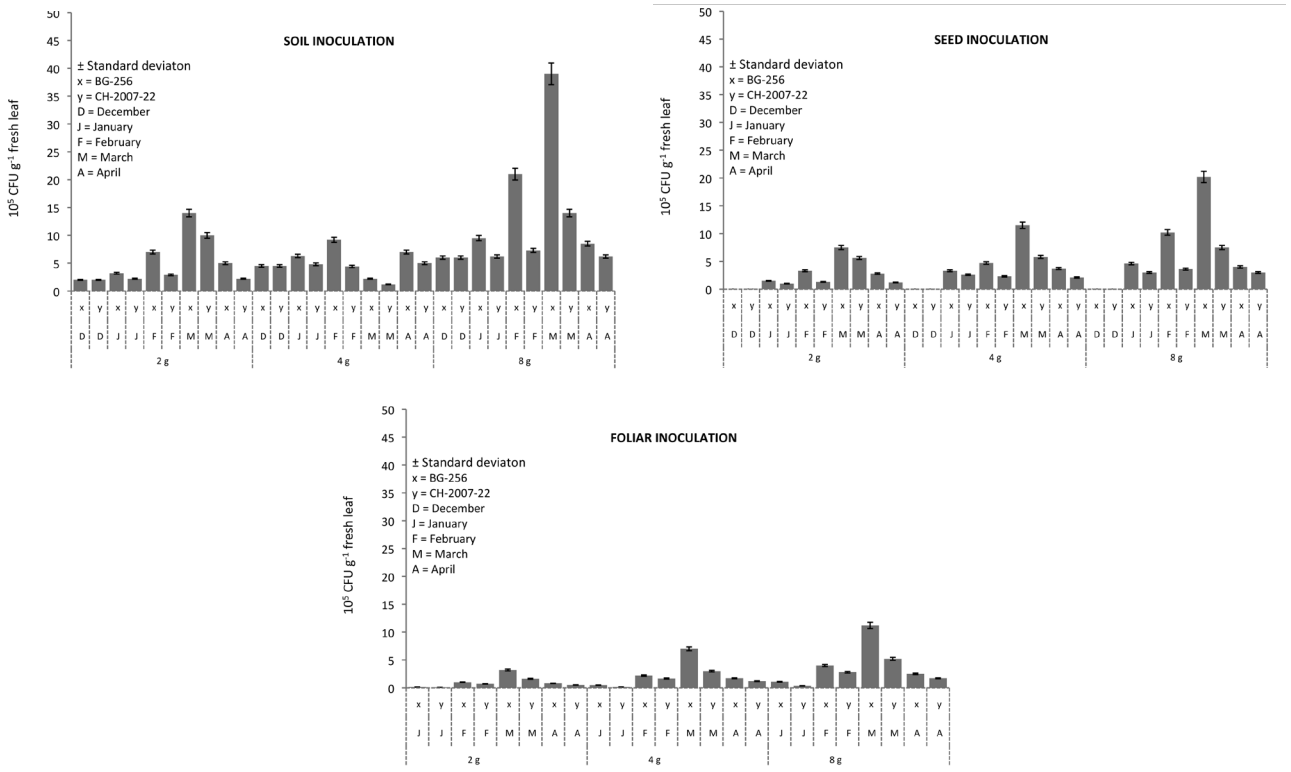

Figure 2. Correlation between disease severity and yield decline of chickpea cultivars subjected to foliar and seed inoculation with *Botrytis cinerea*.

shown). This infection may have been caused by infected seeds, since higher inoculum levels of *B. cinerea* occurred in the soil around the seeds. The chickpea seeds took around two weeks to germinate and to cause their plumule to emerge, this may have been enough for the fungus to penetrate and infect the emerging seedlings in a way similar to a seed-borne infection as reported by Maden (1987).

The growth and yield response of the 24 chickpea cultivars showed that most of them were susceptible to the fungus. Tripathi and Rathi (2000) conducted extensive field tests of chickpea cultivars/lines infected with *B. cinerea* and found that many lines were susceptible. Pande *et al*. (2006b) reported that 156 out of 211 chickpea cultivars were susceptible to a *B. cinerea* suspension sprayed on the leaves in a controlled environment test. Soil inoculation of the fungus at an 8 g inoculum level caused a significant decline and the rhizosphere population of the fungus gradually increased over time and reached its peak in March irrespective of the mode of inoculation after which it declined. Dry and hot weather coupled with approaching plant maturity may have caused the drastic decline in the fungus population in April. The phylloplane population of *B. cinerea* was much greater on cultivars inoculated by leaf sprays and these cultivars exhibited more sever BGM symptoms compared to cultivars that were inoculated through soil or seed. This difference was confirmed by regression analysis of the phylloplane population (foliar inoculated) and disease severity, which detected that there was a positive correlation between these variables, with a correlation coefficient of 0.8 (Figure 4). Since *B. cinerea* is not a systemic pathogen, any small population in the soil may have been built up by infected leaf fragments and spores falling as result of rains that occurred in January or February. During foliar spraying, some inoculum may also have reached the soil directly. The phylloplane population was obviously much greater in plants that received foliar inoculation than in plants that were inoculated through the soil. Although the correlation coefficient between disease severity and the phylloplane

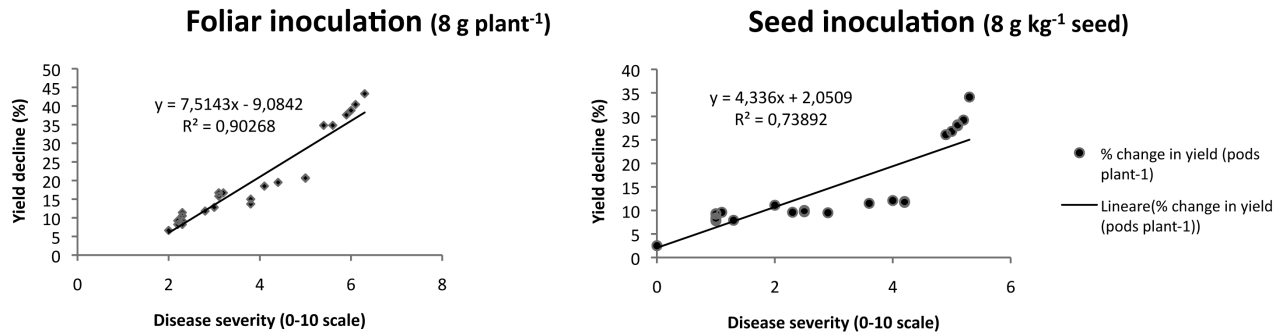

Figure 3. Correlation between disease severity and yield decline of chickpea cultivars subjected to foliar and seed inoculation with *Botrytis cinerea*.

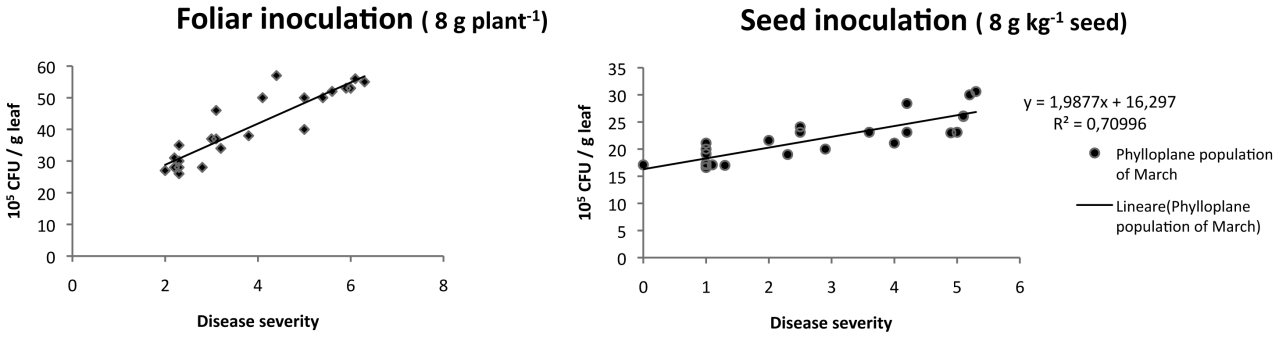

Figure 4. Correlation between disease severity and phylloplane population after foliar and seed inoculation with *Botrytis cinerea* on chickpea.

population was greater with foliar inoculation (0.8) and that between disease severity and seed inoculation was less (0.7), that between disease severity and soil inoculation was very low (0.2, data not shown). The study found that an inoculum level > 8 g kg^{-1} soil, > 4 g kg^{-1} seed, or > 2 g $plant^{-1}$ of *B. cinerea* initiates infection through the soil, seed or leaves respectively. Also, two cultivars, CH-2007-22 and CH-2007-52, but particularly CH-2007-22 demonstrated a considerable degree of tolerance and did not significantly reduce plant growth or yield at any inoculum level. The study found that there was a positive correlation between disease severity and yield loss, and that disease severity may be used as an indicator of BGM management. Disease control measures may be employed on crops showing a disease severity ≥ 2. The study also showed that whole plant screening in the glasshouse is an efficient method to evaluate the susceptibility and tolerance of crop cultivars against *B. cinerea*.

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