

Variability of *Corynespora cassiicola* Isolates Infecting Blackgram

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ABSTRACT

Corynespora cassiicola is an important leaf spot pathogen with wide host range including blackgram. Studies on *Corynespora* infecting blackgram were limited to the effect of weather parameters on disease severity and screening for resistance. It is necessary to understand the variability of pathogen in relation to its wider adoptability in nature. An investigation to understand the pathogen variability was conducted in blackgram. Six isolates of *C. cassiicola* obtained from different areas of blackgram cultivation were analysed for morphological variability including pigmentation, growth pattern, surface texture, margin, elevation, zonation, aerial hyphae, growth rate of the mycelium, length and width of the conidia and number of pseudosepta. The isolate B2 was statistically superior with 8.65 cm colony diameter after nine days of incubation at $28 \pm 1^\circ\text{C}$ whereas B6 (8.04 cm) and B1 (7.98 cm) were on par followed by 7.92 cm in B5 isolate. Average growth rate was found to be higher in B2 (0.376 cm h^{-1}) isolate followed by B6 (0.373 cm h^{-1}). The highest mean conidial length of $57.42 \mu\text{m}$ was recorded in B4 isolate followed by $57.17 \mu\text{m}$ (B6) and $51.1 \mu\text{m}$ (B5). The minimum width of conidia was observed in B-4 ($6.12 \mu\text{m}$) supervened by B-6 ($6.53 \mu\text{m}$) and B-5 ($7.25 \mu\text{m}$). Mean number of highest pseudosepta (5.12 ± 2.14) was found in B6 followed by 4.44 ± 2.08 in B5.

Key Words: Blackgram, *Corynespora cassiicola*, pseudosepta and variability

Blackgram/urdbean [*Vigna mungo* (L.) Hepper] is a vital pulse crop that has the highest supply of phosphoric acid among pulses and possesses 24% protein in its seed, fulfilling the dietary demand for protein (Duffus and Slaughter, 1980). It is legitimately referred to as “poor man’s meat” since it is a more affordable alternative to protein for the poor. Its therapeutic properties have been described in several medical systems, including Ayurveda and Unani. It is mostly used for medicinal purposes and has antihypertensive and antidiabetic effects (Soni *et al.*, 2016). Leaf spot disease in blackgram incited by *Corynespora cassiicola* (Berk. and Curt.) was first reported in 1959 from Bhubaneswar, India. In severe infection, many spots coalesce, leaves became yellow and shedding of the affected leaves occurred. Later Mallaiah *et al.* (1981) recorded the incidence of the disease on blackgram in Andhra Pradesh, for the first time. *Corynespora* leaf spot caused by *C. cassiicola* is one of the most detrimental and common fungal diseases impacting the production of blackgram (Singh 1990). *Corynespora* leaf spot caused yield loss ranging from 15-60% in blackgram (Singh *et al.*, 2010). Previous work on *Corynespora* infecting blackgram was concentrated on screening for resistance and effect of weather parameters (Sandeep Naik *et al.*, 2016;

Gunasri *et al.*, 2018). Understanding the pathogen helps to prevent the development and spread to other crops in the cropping system in view of broad host range. Since the information on the variability of *C. cassiicola* infecting blackgram is lacking, the present studies were conducted.

MATERIAL AND METHODS

A roving survey was conducted during *kharif* 2022 to assess the disease severity of *Corynespora* target spot in two major blackgram growing districts of Andhra Pradesh *i.e.*, Guntur and Krishna. In each district two mandals and in each mandal three villages were surveyed. Five plants at five locations *i.e.*, at four corners of each field and one at the centre were selected to record the severity of *Corynespora* target spot. The disease severity was assessed using 1-9 scale of Alice and Nadarajan (2007).

Per cent disease index (PDI) was calculated based on disease severity data from the formula given under

$$\text{PDI} = \frac{\text{Sum of all the numerical ratings}}{\text{Total number of leaves scored} \times \text{Maximum disease grade}} \times 100$$

Descriptive scale for *Corynespora* target spot of blackgram

Grade	Per cent infection	Reaction
1	No infection on leaves	Resistant (R)
2	0.1% to 5% infection on the leaf surface	Moderately resistant (MR)
3	5.1% to 10% infection on the leaf surface	Moderately resistant (MR)
4	10.1% to 15% infection on the leaf surface	Moderately susceptible (MS)
5	15.1% to 30% infection on the leaf surface	Moderately susceptible (MS)
6	30.1% to 40% infection on the leaf surface	Susceptible (S)
7	40.1% to 50% infection on the leaf surface	Highly susceptible (HS)
8	50.1% to 75% infection on the leaf surface	Highly susceptible (HS)
9	Above 75% infection on the leaf surface	Highly susceptible (HS)

Pathogen Isolation

Laboratory experiment was carried out in the Department of Plant Pathology, Agricultural College, Bapatla. Leaf bits of five mm² with healthy and infected leaf portion were cut, surface sterilized using 1% sodium hypo chlorite solution for a minute and rinsed thoroughly three times with sterile distilled water to remove disinfectants and traces of chemical. Leaf bits were dried using blotter paper to remove excess moisture before transferring them aseptically onto PDA plates. The Petri plates were incubated at 27±1 °C (Silva *et al.*, 2003) in an incubator. Three-day old mycelial bits developed from diseased leaf bits were aseptically transferred to glass slide and observations were made to confirm their identity based on morphological characters (conidia). The obtained pathogen cultures were sub-cultured on PDA after confirmation. The fungus was identified based on cultural features given by Schlub *et al.* (2007), Conner *et al.* (2013) and Mmbaga *et al.* (2015).

Purification

Different isolates obtained from infected tissue were purified by single spore isolation method. Three millilitres of sterile distilled water was added to 10 day old culture tube to get spore suspension which was later diluted serially to get desired concentration and 100 µl was aseptically transferred for single spore isolation (Miyamoto *et al.*, 2009; Silva *et al.*, 2003) through spread plate method using 2% solidified water agar. After incubating at 27±1 °C for 4-5 h, a well isolated, germinated spore was located and marked using microscope. Circular disc of the medium corresponding to the marked single spore was picked up using a sterilized cork borer and was aseptically

transferred to PDA medium. Pure culture obtained was subcultured on PDA slants and incubated at 27±1 °C till the mycelium was fully grown over medium. The culture slants were then preserved in a refrigerator at 4 °C using the method proposed by Castellani (Figueiredo, 1967) for studying variables including Pigmentation (Top and Bottom), Growth pattern, Surface texture, Margin, Elevation, Zonation, Aerial hyphae, Growth rate of the mycelium, length and width of the conidia and number of pseudosepta.

Growth rates were evaluated by measuring the mycelial growth in Petri plates in two equilateral directions at three, six and nine days after incubation. Conidial length and width were evaluated by using optical microscopy (Lanscope) and photographed.

RESULTS AND DISCUSSION

Six isolates of *C. cassiicola* species were collected based on the symptoms, as pinhead sized reddish brown spots which turn brown with dark reddish brown margin (Parakhia *et al.*, 1989), from blackgram crop named as B 1-6, from the districts of Guntur, and Krishna after confirmation of pathogenicity (Table 1).

Colony Pigmentation

Variability among six isolates of *C. cassiicola* obtained from blackgram crop grown in PDA culture media is presented (Plate 1).

Various parameters were recorded among the isolates of black gram (Table 2). On the basis of colony texture, *C. cassiicola* isolates were grouped as fibrous (B1 and B5), undulate (B2), fluffy (B3), smooth (B4) and wrinkle (B6). Growth pattern was circular in all six isolates with margin as filiform (B4,

B5 and B6) and entire (B1, B2 and B3). Based on the elevation of colony the isolates were grouped as raised (B1, B2, B4 and B6), umbonate (B3) and crateriform (B5). The isolates were grouped based on the pigmentation as light grey (B1 and B2), black (B3, B4 and B6), light pink to dark pink with black coloured patches (B5). One to three zonations were observed in (B3, B2, B1 and B4) whereas No zonations were observed in B5 and B6 isolates. Colour of the mycelium varied from white (B1 and B3), light grey (B2) to grey (B4), strong brown (B6) and black and white patches (B5). Aerial hyphae were noticed in B3 and B5; other four isolates did not express aerial hyphae.

Ahmed *et al.* (2013) reported that okra isolate of *C. cassiicola* on PDA medium appeared as effuse, gray to light olivaceous green at immature stage and turned brown to dark blackish brown at maturity, often hairy or velvety. The appearance of mycelial colonies on

potato dextrose agar is woolly, cottony in 90 % of cases, and smooth (Prosper *et al.*, 2018). The colonies of *C. cassiicola* grown in PDA had uniform growth, developed an abundant aerial mycelium, and their morphology showed variation among isolates obtained from roselle (*Hibiscus sabdariffa*) and nine associated weeds (Ortega-Acosta *et al.*, 2020).

Qi *et al.* (2011) and Peiris *et al.* (2015) observed variation in colour, growth pattern, texture and shape of the culture of *C. cassiicola* inoculated to multiple hosts including rubber, papaya, tomato and eggplant. Cultural and morphological variability of *C. cassiicola* was also reported in soybean (Kurre *et al.*, 2019) where in different isolates depicted great variability in pigment production (brown, pale green, pale brown, dark brown, black pigment at the top portion, dark brown pigment and black pigment at the bottom portion) on PDA. Variations in different characters *viz.*, colony colour, colony growth, colony margin, surface appearance and conidia measurements of *C. cassiicola* from cotton were reported by Siva Prasad *et al.* (2021).

Growth rate

Colony diameter of six *C. cassiicola* black gram isolates was observed by inoculating seven days old culture of each isolate on PDA and recorded growth rate after three and nine days of incubation at $28 \pm 1^\circ\text{C}$. Growth of six isolates, in terms of colony

diameter is presented in Table 3. The isolate, B3 recorded higher colony diameter of 3.03 cm, significantly superior, at three days after incubation and followed by B2 (2.66 cm), B6 (2.63 cm) which were on par. B4 and B5 recorded 2.54 cm and 1.95 cm, respectively. Least growth of 1.58 cm colony diameter was observed in B1. After six days of incubation, B6 and B4 were superior with 5.52 cm and 5.51 cm followed by B2 (5.24 cm). Isolates B3 (4.67 cm), B5 (4.34 cm) and B1 (4.08 cm) exhibited after six days of incubation. The isolate B2 was statistically superior with 8.65 cm colony diameter after nine days of incubation at $28 \pm 1^\circ\text{C}$ whereas B6 (8.04 cm) and B1 (7.98 cm) were on par followed by 7.92 cm in B5 isolate. Among six black gram *C. cassiicola* isolates, average growth rate was found to be higher in B2 (0.376 cm h^{-1}) isolate followed by B6 (0.373 cm h^{-1}) and B3 (0.363 cm h^{-1}). Average growth rate was least (0.291 cm h^{-1}) in B1 isolate. Significant differences in the growth of different isolates of rubber (Akpaja *et al.*, 2015), soybean isolates (Kurre *et al.*, 2019) and cotton isolate of *C. cassiicola* in different media was reported by Siva Prasad *et al.* (2021).

Variability in conidia

The heterogeneity in conidial length was observed among different isolates of blackgram isolates and the ratios of length and width changed. Similar observations were reported earlier in cotton, blackgram, soybean etc. (Conner *et al.*, 2013, Peiris *et al.*, 2015, Soni *et al.*, 2016, Kurre *et al.*, 2019).

Conidial length

Population mean with respect to conidial length was observed as 49.98 ± 2.06 (12.32). Upper limit was $75.35 \mu\text{m}$ and lower limit was $24.6 \mu\text{m}$ (Table 4). There was significant variation observed in the conidial dimensions with respect to length of conidia. The length of conidia was in the range of $24.6\text{--}75.35 \mu\text{m}$ (Table 5, Figure 1 and 2). The maximum length was observed in B-6 ($76.65 \pm 28.9 \mu\text{m}$) followed by $54.61 \pm 8.98 \mu\text{m}$ in B-5 and $48.99 \pm 10.9 \mu\text{m}$ in B4. The minimum length of conidia was observed in B-1 ($32.32 \pm 4.5 \mu\text{m}$) followed by B-3 ($42.17 \pm 10.4 \mu\text{m}$) and B-2 ($45.1 \pm 10.48 \mu\text{m}$).

Conner *et al.* (2013) reported 50 to 209 μm long and 7 to 15 μm wide conidia in cotton. The maximum frequency of spore-length ranged between

25 and 74.9 μm was reported by Ahmed *et al.* (2013) in okra. Variation in conidial length (15–275 μm), width (3.75–12.50 μm) and shape (straight, curved, cylindrical and obclavate) was observed by Peiris *et al.* (2015) in *C. cassiicola* isolates from different host plants.

Conidial width

Population mean of conidial width was 7.78 ± 2.06 (1.23) with upper limit of 10.31 μm and lower limit of 5.24 μm (Table 4, Fig. 2 and 3). The maximum width was observed in B-1 ($8.19 \pm 2.38 \mu\text{m}$) followed by B-2 ($8.27 \pm 1.08 \mu\text{m}$) and B-5 ($7.85 \pm 1.27 \mu\text{m}$). The minimum width was observed in B-6 ($7.39 \pm 0.79 \mu\text{m}$) supervised by B-4 ($7.31 \pm 1.08 \mu\text{m}$) and B-3 ($7.65 \pm 0.76 \mu\text{m}$) (Table 5). The above results were correlated with the observations of Soni *et al.* (2016) who reported that conidial size recorded in *C. cassiicola* in *Vigna mungo* was $40 \times 15 \mu\text{m}$. Cotton isolate of *C. cassiicola* contained 4 to 15 pseudosepta (Conner *et al.*, 2013).

Prosper *et al.* (2018) reported that the shape of conidia is cylindrical, straight for some and slightly curved for others; with average size of 11.77 to 52.87 μm in length and 1.66 to

5.71 in width in *C. cassiicola* causing leaf fall disease in rubber.

Number of pseudosepta

The number of pseudosepta varied from 1 to 7. Highest number of pseudosepta was recorded in B-6 (5.12 ± 2.14) succeeded by B-5 (4.44 ± 2.08) and lowest number of was observed in B2 ($0.96 \pm$

0.78), followed by B-1 (2 ± 0.91) and B-3 (2.16 ± 1.17) (Table 5).

Qi *et al.* (2011) observed differences in conidial shape oval, obclavate, cylindrical or Y; curved or straight, size (10.1–277.2 μm long; 1.3–17.1 μm wide) and the number of pseudosepta (0–18) when *C. cassiicola* was inoculated from rubber to rubber and other hosts like papaya, tomato and egg plant. Significant differences in the growth of different isolates of rubber on PDA were observed by Akpaja *et al.* (2015).

The maximum frequency of spore-length ranged between 25 and 74.9 μm was reported by Ahmed *et al.* (2013) in okra. Kurre *et al.* (2019) observed a high degree of variability in conidial morphology was observed among the isolates of soybean such as shape (oval, obclavate, cylindrical or Y; curved or straight), size (10.3–168.8 μm long; and 1.3–12.4 μm wide) and the number of pseudosepta (0–16). Toulet *et al.*, (2022) reported that the size of the conidia ranged from 6 to 120 μm in length and 2.4 to 12 μm in width, with an average of 19.90–46.80 μm long and 4.09–5.30 μm wide in leguminous crop soybean.

Variability studies in *C. cassiicola* isolates infecting blackgram revealed pigmentation, growth pattern, surface texture, margin, elevation, zonation, aerial hyphae, growth rate of the mycelium, length and width of the conidia and number of pseudosepta indicate cultural and morphological diversity to adapt to its wider host range.

Table 1. *Corynespora cassiicola* isolates from blackgram analysed for cultural and morphological characters

S. No.	Designation of the isolate	Village	Mandal	District
1	B-1	Telagapalem	Ponnuru	Guntur
2	B-2	Patchalatadiparru	Ponnuru	Guntur
3	B-3	Narakoduru	Chebrolu	Guntur
4	B-4	Vuyyuru	Vuyyuru	Krishna
5	B-5	Yakamuru	Vuyyuru	Krishna
6	B-6	Akunuru	Vuyyuru	Krishna

Table 2. Variation in cultural characters among *C. cassiicola* isolates of blackgram on PDA

S. No.	Isolate	Growth pattern	Surface texture	Margin	Elevation	Pigmentation at the bottom of the plate	Zonation	Aerial hyphae	Colour
1	B 1	Circular	Fibrous	Entire	Raised	Light grey	3	No	White
2	B 2	Circular	Undulate	Entire	Raised	Light grey	2	No	Light grey
3	B 3	Circular	Fluffy	Entire	Umbonate	Black	1	Yes	White
4	B 4	Circular	Smooth	Filiform	Raised	Black	3	No	Grey
5	B 5	Circular	Fibrous	Filiform	Crateriform	Pink with black patches	0	Yes	Black with white patches
6	B 6	Circular	Wrinkle	Filiform	Raised	Black	0	No	Strong brown

Table 3. Variability in the colony diameter of *C. cassiicola* isolates from blackgram

S. No.	Blackgram Isolate	Colony diameter (cm)			Growth rate (cm h ⁻¹)			Average growth rate (cm h ⁻¹)
		3 DAI	6 DAI	9 DAI	3 DAI (cm h ⁻¹)	6 DAI (cm h ⁻¹)	9 DAI (cm h ⁻¹)	
1	B 1	1.58 (1.25d)	4.08 (2.02e)	7.98 (2.82b)	0.022	0.028	0.037	0.029
2	B 2	2.66 (1.63b)	5.24 (2.28b)	8.65 (2.94a)	0.037	0.036	0.04	0.038
3	B 3	3.03 (1.74a)	4.67 (2.16c)	7.58 (2.75c)	0.042	0.032	0.035	0.037
4	B 4	2.54 (1.59b)	5.51 (2.34a)	7.46 (2.73c)	0.035	0.038	0.035	0.036
5	B 5	1.95 (1.39c)	4.34 (2.08d)	7.92 (2.81b)	0.027	0.03	0.037	0.031
6	B 6	2.63 (1.62b)	5.52 (2.34a)	8.04 (2.83b)	0.037	0.038	0.037	0.037
	CD (p≤0.05)	0.15	0.18	0.22				
	SEm±	0.053	0.06	0.078				
	CV%	2.01	1.1	0.84				

*Mean of three replications

*Values in parenthesis are arc sine transformed values

*Values with the same alphabets are statistically not significant.

Table 4. Population means of *Corynespora* conidial dimensions from blackgram

S. No.	Character	Population mean (µm)	Upper limit (µm)	Lower limit (µm)	Range (µm)
1	Conidial length	49.98±2.06(12.32)	75.35	24.6	24.6-75.35
2	Conidial width	7.78±2.06(1.23)	10.31	5.24	5.24-10.31

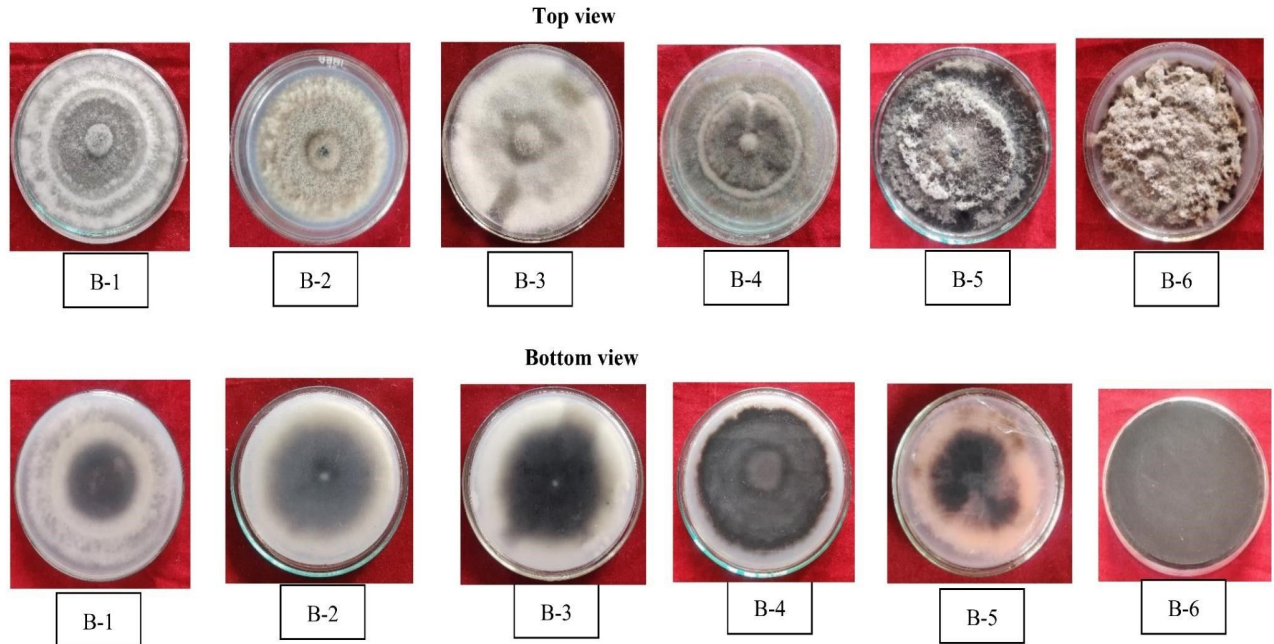


Plate 1. Variability in pigmentation of *Corynespora cassicola* in blackgram

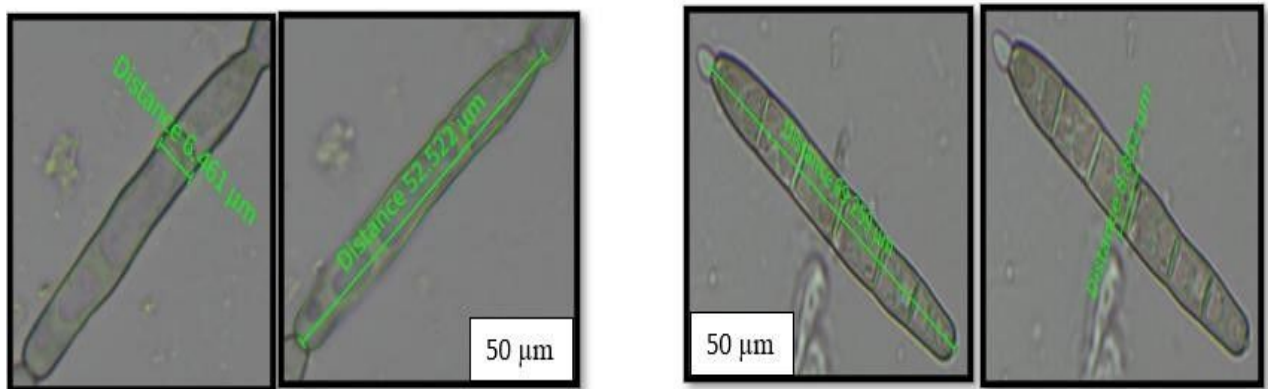


Fig 1. Variability in conidial measurements of *Corynespora cassicola* isolates in blackgram

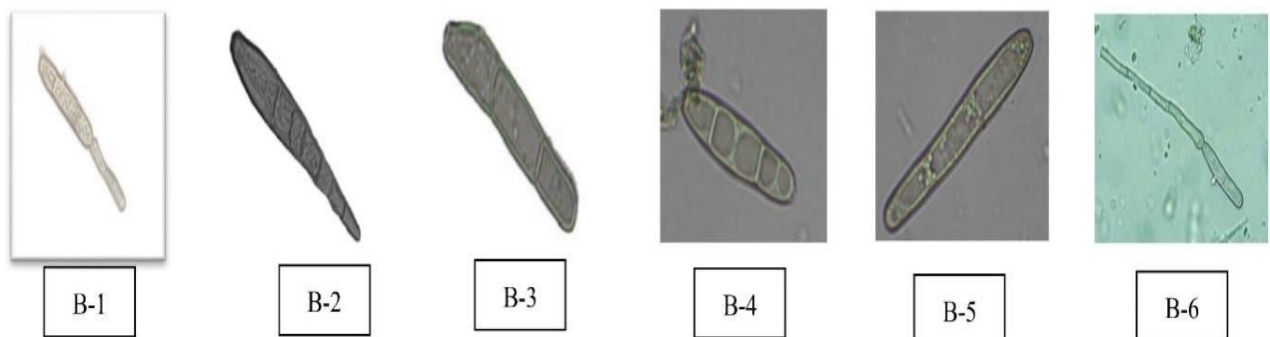


Fig 2. Variability in conidial morphology of *Corynespora cassicola* in blackgram

Table 5. Variability in conidial dimensions of *C. cassiicola* isolates from blackgram

S. No.	Blackgram Isolate	Conidial Dimensions (μm)				Pseudosepta Population Mean	L:W ratio
		Length	Population Mean	Width	Population Mean		
1	B 1	37.59 ^d	32.32 \pm 4.5	8.66 ^a	8.19 \pm 2.38	2 \pm 0.91	4.34
2	B 2	42.08 ^c	45.1 \pm 10.48	7.44 ^c	8.27 \pm 1.08	0.96 \pm 0.78	5.65
3	B 3	33.67 ^c	42.17 \pm 10.4	7.96 ^b	7.65 \pm 0.76	2.16 \pm 1.17	4.23
4	B 4	57.42 ^a	48.99 \pm 10.9	6.12 ^f	7.31 \pm 1.08	2.52 \pm 1.15	9.38
5	B 5	51.1 ^b	54.61 \pm 8.98	7.25 ^e	7.85 \pm 1.27	4.44 \pm 2.08	7.05
6	B 6	57.17 ^a	76.65 \pm 28.9	6.53 ^e	7.39 \pm 0.79	5.12 \pm 2.14	8.75
SEm \pm		0.18		0.05			
CV%		4.82		3.71			
C.D. ($P \leq 0.05$)		0.57		0.17			

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