Eradication of Fusarium oxysporum f. sp. ciceri Transmitted in Chickpea Seed

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ABSTRACT

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The pathogen of chickpea wilt, *Fusarium oxysporum* f. sp. *ciceri*, was transmitted internally through seeds of chickpea collected from diseased plants. The fungus was found in the hilum region. Seed treatment with Benlate T (benomyl and thiram) completely eradicated the pathogen.

Additional key words: *Cicer arietinum*.

After Padwick (10) described chickpea wilt [which is caused by *Fusarium oxysporum* Schiech. emend. Snyd. & Hans. f. sp. *ciceri* (Padwick) Snyd. & Hans.] from India in 1940, several workers reported its occurrence in other countries (1, 2, 3, 7, 8, 9). Seed transmission of the wilt fungus was suspected by Erwin and Snyder (3). Evidence is presented here to show that the fungus can be seedborne, and a method to eradicate the fungus from seed is described.

MATERIALS AND METHODS

Collection of seed.—Seeds of three chickpea (*Cicer arietinum* L.) cultivars, JG-62, Chafa, and P-436, were collected from plants that wilted prior to maturity, and from plants that remained healthy until maturity. To avoid contamination during harvesting and threshing, seed was removed from dried pods of individual plants by hand in the laboratory and air-dried in petri dishes for 2 days at room temperature. The seed then was bulked in six lots (seed from healthy and diseased plants of the three cultivars) and stored in paper bags at 5 C.

Testing procedures.—In all experiments, unless stated otherwise, (i) 400 seeds of each cultivar were used (4) and (ii) seed was surface-sterilized by a dipping for 2 min in 2.5% sodium hypochlorite (diluted Clorox-5.25%). The blotter test (4) was used to measure seed germination. Seeds were plated in plastic petri dishes on top of three layers of moistened, sterilized blotters, and incubated at 20 C for 8 days. For detecting *F. oxysporum* f. sp. *ciceri* in seed, two agar plate procedures were followed. In the first procedure, surface-sterilized seed was plated on potato-dextrose agar (PDA). In the second procedure, seed was plated on Czapek-Dox agar which contained, in addition to normal ingredients, 500 mg PCNB, 25 mg malachite green, 750 mg Dicrysticin-S (Strepto-penicillin of Sarabhai Chemicals Ltd., Baroda, India), and 2 g yeast extract per liter of medium (11). The plates then were incubated at 20 C for 8 days in a cycle of 12 hr of near-UV light (Philips TL 40/05) followed by 12 hr of darkness. The "seedling-symptom" test was made to confirm transmission through seed. Surface-sterilized seed (2 min exposure in 0.1% mercuric chloride) were planted in autoclaved (121 C for 2 hr), fine, riverbed sand in 15-cm-diameter plastic pots in a net house located 25 kilometers from the experimental fields. Germination was recorded and plants were observed up to 40 days.

Pathogenicity tests.—Isolations were made on PDA from plants showing wilting in the growing-on test. To determine pathogenicity, healthy seedlings were dipped in conidial suspensions of the isolates and transplanted into autoclaved riverbed sand in pots. To avoid chances of seedborne infection, the seedlings for the pathogenicity test were raised in sterile sand from surface-sterilized seed of healthy plants of cultivar JG-62.

Histopathology of seed.—Shrivelled seed collected from diseased plants of JG-62 were fixed in formalin-propionic acid-alcohol, dehydrated in tertiary butyl alcohol series (5), and embedded in paraffin wax. Sections were cut 12 μm thick by rotary microtome, stained with 1% methyl violet in 50% alcohol for 10 min, washed briefly in 50% alcohol, and stained for 2 min in 1% eosin in 50% alcohol. Sections were differentiated in a mixture of turpentine, cedar oil, and phenol crystals (2:1:2, w/w), washed in xylene, and mounted. Microphotographs were taken under phase contrast.

Seed treatment with fungicides.—Systemic fungicide formulations used were Benlate 50 W.P. (benomyl), methyl 1-(butylacarbamoyl)-2-benzimidazolecarbamate, 50%, E. I. Du Pont de Nemours, Wilmington, DE 19898; Belate T (30% benomyl + 30% thiram), also Du Pont; and Bavistin W.P. (carbendazim), methyl-1H-benzimidazole-2-ylcarbamate, BASF India Ltd., Bombay 400 011, India. Unless stated otherwise the fungicide dosage was 2.5 g of the commercial formulation

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per liter of water. The seeds were dipped in fungicide suspensions for 5 min and air-dried for 30 min. Dry seed dressing was done by mixing requisite quantities of fungicides with seed and shaking thoroughly in petri dishes. The treated seeds then were plated on modified Czapek-Dox agar or sown in autoclaved sand.

RESULTS

Detection of the pathogen.—*Fusarium oxysporum* f. sp. *ciceri* was present in the seeds from diseased plants of all three cultivars (Table 1 and Fig. 1-A). Percentage of seedborne *F. oxysporum* f. sp. *ciceri* was higher in JG-62 and P-436 than in Chafa and it was not isolated from seeds of healthy plants. The number of *F. oxysporum* f. sp. *ciceri* isolations was greater on the selective medium than on PDA. The pathogenicity of the *F. oxysporum* f. sp. *ciceri* isolates was confirmed, and it resembled the strain commonly found in fields at ICRISAT. In cultivars JG-62 and P-436, percentage germination of seeds collected from diseased plants was somewhat lower, but in Chafa, it was as good as that of seed from the healthy plants (Table 1).

Wilt detection in seedling-symptom test.—Wilt incidence in seedlings of diseased JG-62 and P-436 was considerably higher than in those of Chafa (Table 2). Surface sterilization of seed with mercuric chloride prior to planting reduced wilt incidence to a slight extent in two cultivars and no wilting occurred in surface-sterilized seeds of Chafa. There was a slight reduction in germination of seed collected from diseased plants of all three cultivars. *Fusarium oxysporum* f. sp. *ciceri* was

TABLE 1. Detection of *Fusarium oxysporum* f. sp. *ciceri* in seeds of three healthy and wilted chickpea cultivars

Cultivar	Germination[a] (%)	Colonies of *F. oxysporum* f. sp. *ciceri*	
		PDA[b] (%)	Modified Czapek-Dox agar (%)
JG-62			
H	100	0	0
W	93	18	22
Chafa			
H	100	0	0
W	100	2	2
P-436			
H	99	0	0
W	89	19	27

[a]Blotter test.
[b]Abbreviations: PDA = Potato-dextrose agar; H = seeds from healthy plants; and W = seeds from wilted plants.

TABLE 2. Incidence of wilt caused by *Fusarium oxysporum* f. sp. *ciceri* in three chickpea cultivars

Cultivar	Treatment	SHP[a]		SWP[a]	
		Germination (%)	Wilt (%)	Germination (%)	Wilt[b] (%)
JG-62	SS[c]	99	0	96	14.5
	NS	100	0	87	18.3
Chafa	SS	100	0	95	0.0
	NS	100	0	95	0.5
P-436	SS	98	0	88	19.8
	NS	96	0	90	21.7

[a]Abbreviations: SHP = seeds from healthy plants, and SWP = seeds from wilted plants.
[b]Plants were observed for wilting up to 40 days after sowing.
[c]Abbreviations: SS = surface-sterilized with mercuric chloride (0.1%) for 2 min and then sown, and NS = not surface-sterilized.

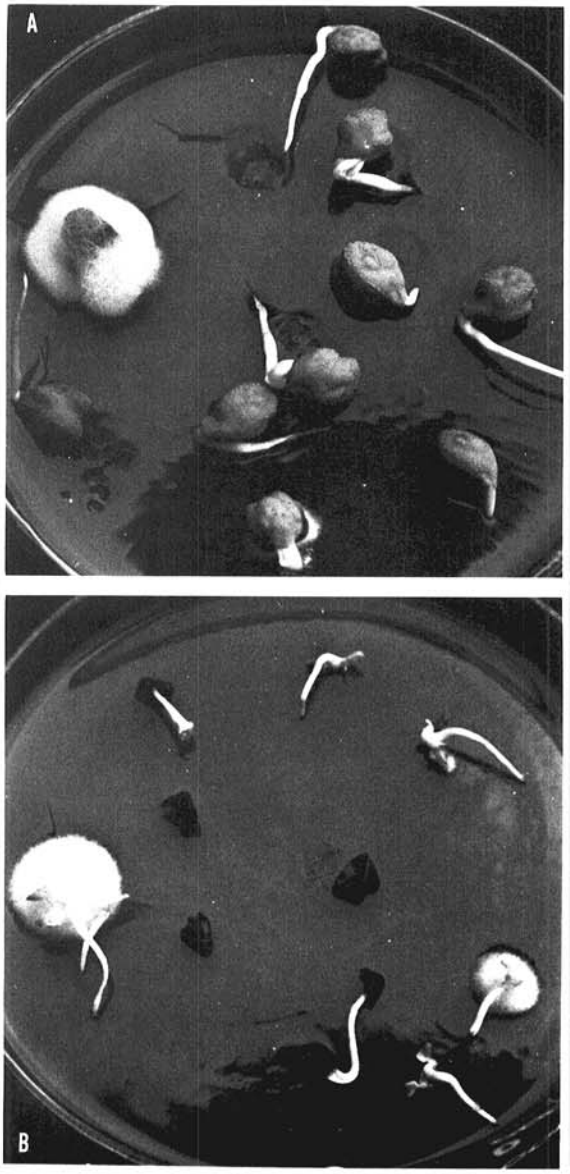

Fig. 1-(A, B). Detection of *Fusarium oxysporum* in chickpea seed. **A)** Surface-sterilized seeds of cultivar JG-62 plated and incubated on modified Czapek-Dox agar and **B)** seed bits taken from hilum region and plated on Czapek-Dox agar showing emerging *F. oxysporum*.

isolated from wilted seedlings and its pathogenicity was confirmed.

Histopathology of seed.—Longitudinal sections of seeds collected from both diseased and healthy plants of JG-62 were examined. Although no fungal structures were seen in the embryo, chlamydospore-like structures were seen in the hilum region of seeds collected from diseased plants. These structures were thick-walled, spherical, and closely packed. They stained intense red compared with violet-stained host cell walls. Hyphal cells connecting chlamydospore-like structures also were observed. No such structures were observed in the hilum region or elsewhere in seeds from healthy plants. To confirm these observations, minute bits (one bit from one seed) from the hilum region of dry seeds (collected from diseased plants) were separated with a razor blade and these bits were plated on the modified Czapek-Dox agar medium after surface sterilization. Nineteen of 100 bits plated in this fashion yielded *F. oxysporum* f. sp. *ciceri* (Fig. 1-B).

Seed treatment with fungicides.—*Fusarium oxysporum* f. sp. *ciceri* could not be detected from seed treated with Benlate T. Benlate alone, and Bavistin both reduced the incidence of this pathogen in seed relative to the nontreated check (Table 3). All three fungicides improved germination.

Dry and wet seed treatments were carried out with Benlate T at 2.5 g, 2.0 g, and 1.5 g per kg of seed or in one liter of water, respectively. It was observed that 1.5 g/kg dry seed treatment or 1.5 g/liter as a wet seed treatment also completely eradicated *F. oxysporum* f. sp. *ciceri* from seed. Another experiment in which 0.15% Benlate T formulation was compared with a mixture of Benlate and thiram at the equivalent rates, the latter was found to be equally effective; i.e., complete eradication was obtained.

DISCUSSION

The above results clearly demonstrate that *F. oxysporum* f. sp. *ciceri*, the chickpea wilt pathogen, is internally seedborne. Whereas seeds collected carefully from healthy plants did not yield *F. oxysporum* f. sp. *ciceri*, seeds from wilted plants yielded *F. oxysporum* f. sp. *ciceri* (Table 1) both on PDA as well as on the selective medium (modified Czapek-Dox agar). Presence of *F. oxysporum* f. sp. *ciceri* in the seeds was further confirmed by observing wilt in seedlings raised from surface-sterilized seeds collected from field-wilted plants (Table 2). Additional evidence of the internal presence of *F. oxysporum* f. sp. *ciceri* was obtained through histopathological studies in which chlamydospore-like structures were observed in the hilum region of seed collected from diseased plants. The presence of *F. oxysporum* f. sp. *ciceri* in the hilum region was confirmed by obtaining the fungus from seed bits plated on the selective medium.

TABLE 3. Effect of fungicidal seed treatment on seedborne *Fusarium oxysporum* f. sp. *ciceri*

Fungicide[a]	Germination[b]		Infection			
			Modified Czapek-Dox agar		In sand[c]	
	JG-62 (%)	P-436 (%)	JG-62 (%)	P-436 (%)	JG-62 (%)	P-436 (%)
Benlate	100	98	5	2	2	0
Benlate T	100	100	0	0	0	0
Bavistin	98	98	6	7	3	2
Check	90	89	31	46	16	27

[a]Fungicide formulated at 2.5 g/liter and applied as a wet seed treatment.
[b]Blotter test.
[c]Plants were observed for wilting up to 40 days after sowing.

The possibility of the contamination of chickpea seed surface by *F. oxysporum* f. sp. *ciceri* was pointed out by Erwin and Snyder (3). Westerlund et al. (12) failed to obtain evidence of seed transmission of pathogenic *F. oxysporum*. They were unsuccessful in detecting *F. oxysporum* f. sp. *ciceri* in washings from chickpea seed or in seed plated on acidified PDA. It is not clear from their paper if the seeds they used for these tests were obtained from wilted plants. Likewise the name of the cultivar from which the seed was obtained was not mentioned. It is important to know the cultivar, because there seems to be clear differences between cultivars with regard to seed transmission (Table 1, 2). The extent of seed transmission in cultivar Chafa was considerably less than in JG-62 and P-436.

Another important finding is the demonstration that Benlate T (a mixture of benomyl and thiram) can completely eradicate seedborne *F. oxysporum* f. sp. *ciceri*. Since it can be eradicated by seed treatment *F. oxysporum* f. sp. *ciceri* in chickpea seed should not adversely affect international movement of seed needed for germplasm exchange. Earlier work showed that benomyl is effective in reducing seedborne *Ascochyta rabiei*, the causal organism of chickpea blight (6).

LITERATURE CITED

1. BENLLOCH, M. 1949. Phytopathological observations in the year 1948 (in Spanish). Bol. Patol. Veg. Entomol. Agric. Madrid 16:203-242.
2. ECHANDI, E. 1970. Wilt of chickpeas or garbanzo beans (Cicer arietimum) incited by Fusarium oxysporum. Phytopathology 60:1539 (Abstr.).
3. ERWIN, D. C., and W. C. SNYDER. 1958. Yellowing of garbanzo beans. Calif. Agric. 12:6-16.
4. INTERNATIONAL SEED TESTING ASSOCIATION. 1966. International rules for seed testing. Proc. Int. Seed Test. Assoc. 31:1-152.
5. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
6. KAISER, W. J., M. OKHOVAT, and G. H. MOSSAHEBI. 1973. Effect of seed-treatment fungicides on control of Ascochyta rabiei in chickpea seed infected with the pathogen. Plant Dis. Rep. 57:742-746.
7. KUZ'MINA, G. 1966. Antibiotics against wilt (in Russian). Zashch. Rast., Mosc. 11:31.
8. MALIK, S. A., and M. A. KHAN. 1943. Parasitic fungi of the Northwest Frontier Province. Indian J. Agric. Sci. 13:522-527.
9. MANUCHERI, A., and MESRI. 1966. Fusarium wilt of chickpea (in Farsi). Iran J. Plant Pathol. 3:1-11.
10. PADWICK, G. W. 1940. The genus Fusarium.III. A critical study of the fungus causing wilt of gram (Cicer arietinum L.) and of the related species of the sub-section Orthocera, with special relation to the variability of key characteristics. Indian J. Agric. Sci. 10:241-284.

11. SINGH, R. S., and H. S. CHAUBE. 1970. A technique for estimation of hyphal and conidial propagules of Fusarium spp. in soil. Labdev. J. Sci. Technol. 8:172-174.

12. WESTERLUND, F. V. JR., R. N. CAMPBELL, and K. A. KIMBLE. 1974. Fungal root rots and wilt of chickpea in California. Phytopathology 64:432-436.