

## **Influence of Seed Treatments with Four Fungicides on Seedling Emergence of Chickpea in Presence of *Alternaria alternata* and *Fusarium oxysporum*.**

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**Abstract.** Two genotypes of chickpea CLJ and E14 were tested in the laboratory, for percentage of seed germination. Each genotype was subdivided into two groups, clean and discolored seeds. The percentage of mortality was higher than 65% in the discolored group than in the clean ones in both genotypes.

Two isolates of *Alternaria alternata* and *Fusarium oxysporum* were detected in more than 90% of the discolored seeds of both genotypes. *Fusarium oxysporum* was associated with pre-emergence damping-off in the majority of the discolored seeds. Four fungicides were tested in the laboratory for the control of the fungi on PDA and as seed treatment. Bavistin significantly affected *F. oxysporum* followed by mancozeb and miceb while vinclozolin was the least effective fungicide. Bavistin unlike dithane and miceb, which significantly inhibited the growth of these two fungi, did not affect *A. alternata* at all. the systemic activity of bavistin was also observed in the seed coat, cotyledon, and embryo. In soil, artificially infested with *A. alternata* and *F. oxysporum*, seeds treated with bavistin, mancozeb and miceb significantly increased emergence over the untreated control, while vinclozolin was the least effective fungicide.

### **Introduction**

Chickpea (*Cicer arietinum*), is an important high protein pulse crop grown worldwide, though 90% of the production is concentrated in the Middle East and South East Asia [1]. Fungi play an important role in attacking this crop causing pre-emergence or post-emergence damping-off [1-9].

Previous researches have demonstrated the use of different fungicides in the control of seedling diseases and found them to be unsatisfactory. They concluded that the most effective and practical method of control is through the use of resistant cultivars [10]. On the other hand, when Mani *et al.* [11] treated chickpea seeds with either benlate, thiram, carbofuran or aftanol to test whether they improved seedling emergence in the presence of *Meloidogyne incognita*, *F. oxysporum* f. sp. *ciceri* and *F. solani*, they found that Benlate and carbofuran gave the highest seedling emergence. Haral *et al.* [12], reported that carbendazim plus *Rhizobium* spp. controlled many fungal diseases including *F. oxysporum* f. sp. *ciceri*; and significantly

increased nodulation and dry matter. Deo *et al.* [3], stated that seeds of *Cicer arietinum* and *C. kabulinum* were harvested, treated with fungicides and stored up to 12 months. Ceresan, dithane Z-78 (zineb) calcium propionate and ascorbic acid, considerably decreased the incidence of storage fungi and maintained good germination. Shukla *et al.* [14], reported that Bavistin at 0.5g/kg seeds improved germination by 16.5%, greatly reduced wilt incidence caused by *F. oxysporum* f. sp. *ciceri* on *C. arietinum* and increased grain yield by 23.7%. However, not enough data exist for the evaluation of fungicides on chickpea crop. Therefore, the objective of this study was to evaluate the efficiency of four fungicides as a seed treatment for chickpea.

### Materials and Methods

Two genotypes of chickpea seeds brought from two different locations were used in this study, CLJ-86 (Chickpea - local - Jouf) as a local genotype from the Kingdom of Saudi Arabia (K.S.A.), and E14-'85 as an entry from ICARDA (Syria). the latter was harvested in 1985 at King Faisal University Research Station, Alhasa, Eastern Province of K.S.A.

#### Germination of chickpea seeds in percentage, and the isolation of fungi

To test the percentage germination of the seeds of the two genotype chickpea samples in the laboratory, each sample was subdivided into two groups, clean and discolored seeds (based on seed coat color). Fifteen seeds of each group were surface sterilized with 1% sodium hypochlorite solution for 3 min. and then rinsed twice in sterilized distilled water. These were planted in 9cm petri dishes maintained with PDA and replicated five times. They were incubated at 24°C in an incubator supplied with two 20 W neon lamps and left on for six days to ensure sporulation. Percentage of germination was recorded every 24 hours for the whole period.

Fifteen seeds from clean and discolored groups were sterilized, planted in PDA plates, and replicated as before and used to identify the fungi responsible for causing their damage. For a period of six days after planting, fungi were isolated from ungerminated and slightly germinated seeds. Pure culture of these fungi were kept in PDA test tubes at 4°C for further studies.

#### Pathogenicity tests

Pathogenicity test was carried out in the laboratory using *Alternaria* sp. and *Fusarium* sp. isolated from the above mentioned discolored seeds of the chickpea of both genotypes and maintained in 9 cm PDA plates. Content of two petri dishes including agar and fungi were homogenized in 40 ml of sterile water for 1 min., then a suspension of  $1 \times 10^8$  (conidia/ml) was prepared from each fungus by using hemocytometer. Clean seeds of both genotypes were used in this experiment. Fifteen seeds of chickpea were immersed in the conidial suspension of each of the two

isolated fungi then the seeds were planted in Jiffy pots and replaced in 16 × 22 cm seed tray containing water. These were replicated five times. Untreated clean seeds of both chickpea genotypes served as control. Pathogenicity of each fungus was recorded after six days and was based on the severity of the fungus growing on the seeds and measured as follows:

- + for low infection (slight infection of mycelial growth with normal seedling emergence)
- ++ for medium infection (white or grey profuse mycelial growth with a slight seedling emergence)
- +++ for heavy infection (heavy mycelial growth and an inhibited germination of seeds).
- ++++ for complete rotten seeds and pre-emergence damping-off.

### Fungicide tests

Fungicides used in this study were as follows:

- 1- bavistin (50% WP), carbendazim (methoxycarbonylamino)-benzimidazole.
- 2- dithane M-45, mancozeb (manganese ethylene bis dithiocarbamate) with zink salt 80%.
- 3- miceb 80% super (manganese 16% + zink 2% + ethylene bis dithiocarbamate 62%).
- 4- ronilan, vinclozolin, 3- (3, 5 -dichlorophenyl) -5 -ethenyl -5 methyl -2, 4 -oxazolidinedione.

### Fungicides effect on *Alternaria* sp. and *Fusarium* sp.

In order to examine the effect of the fungicides on the growth of these isolates, sterile distilled water was prepared containing the fungicides in final concentrations (commercial formulations) of 50, 150, 250 mg/l for each of the four fungicides. Four replicate sterile filter paper disks (Whatmann, 13 mm, A.A. assay) were immersed in each solution and dried at 40°C for 1 hr. Each disk was then placed in the centre of a PDA petri dish (15 ml/plate) seeded with a conidial suspension of 1 × 10 (spores/ml) of each of the two fungi. The plates were incubated at 22 ± 2°C. Untreated filter paper disks immersed in sterilized distilled water served as control. After five days, zones of inhibition were measured as an indication of the effectiveness of the fungicides.

### Systemic activity of the fungicides

The systemic activity of the fungicides was examined by a method similar to that used by Russel and Mussa [15]. twenty gr of clean chickpea seeds (CLJ-86) were surface sterilized in 1% sodium hypochlorite for 5 min. and rinsed twice in sterile water prior to being soaked in bavistin and dithane in concentrations of 100, 200, 300, 400, 500, 600 and 700 mg/l and left for 24 hr in that solution. The other fungicides were ignored because they are non-systemic similar to dithane. The seeds were then divided into groups, each with four replicates. In the first group, the seeds were

washed and plated directly on PDA 9 cm petri dishes seeded as before with conidial suspension of  $1 \times 10$  conidia/ml of each fungus. For the second group, the seed coats were aseptically removed after washing and plated separately on the seeded PDA plates. The remaining cotyledons and embryos were washed for further 3 min. in running water and then plated. Untreated seeds, seed coats, cotyledons and embryos from such seeds served as control. The plates were incubated as before and zones of inhibition were measured after five days. As a further check on the absorption or translocation of these two fungicides, treated seeds were placed on moist filter paper in sterile Petri dishes for 24 hr. Then these were washed, aseptically cut in half, and the seed coat removed. The cup-shaped half seed coats were placed on PDA plates so that the inside of the cup faced upwards. Agar plugs, 5 mm diameter, were cut either from 10 day old culture of the isolated fungi of *Alternaria* sp. or *Fusarium* sp. and divided into quarters, one of which was placed on the inner surface of each cup. After 72 hr observations were made on the extent of the fungal growth on both the agar plugs and the seed coats. Untreated seeds served as control.

### Seed treatments

To examine the efficiency of the fungicides in controlling *Alternaria* sp. and *Fusarium* sp. in vivo, a sterilized soil used for these treatments containing 14: 7 v/v sacks (capacity of 10 l each) peat moss and sand, respectively. These were amended with 50 gr of urea plus 100 gr superphosphate plus 20 gr iron plus 10 gr of zinc, copper and magnesium total for the mix. The amended soil was transferred to plastic pots (11 × 11 × 12 cm) of capacity 1.1 l. Also seeds of the two genotype samples were divided into two groups, clean and discolored ones. These seeds were surface sterilized as before and treated with a slurry of 0.058 gr/20 gr w/w seeds for each of the four fungicides. Four treatments were carried out as follows: The first treatment comprised the application of the four fungicides to the clean and discolored seeds of the two genotypes and planted in the amended pots.

In the second treatment, the seeds were treated with the fungicides, planted as before, then the soil was inoculated with 5 ml of a spore suspension containing  $1 \times 10$  (conidia/ml) of each of the two fungi.

In the third treatment, the seeds were planted first, then the soil was inoculated as before with *Alternaria* sp. and *Fusarium* sp.

The fourth treatment comprised the untreated control and this included the clean and discolored seeds of both samples of chickpea.

In all treatments, each pot was planted with four seeds and randomized in a shaded area outside the Department of Crop Production, College of Agriculture, King Faisal University. All treatments were replicated three times and watered regularly. Reading of percentage of germination was recorded after the second week of planting.

## Results

### Testing the percentage of germination of seeds

Results show that germination of clean seeds of both chickpea genotypes was significantly increased reaching a maximum of 96% for E14 and 93.3% for CLJ after 144 hr. On the other hand, the discolored seeds of both genotypes showed a very low percentage of germination; 38.7% for E14 and 33.3% for clj.

### Isolation of fungi

Table 1 represents the data on the isolation of two fungi only, *Alternaria alternata* (Keissler) identified according to Ellis [16] and *Fusarium oxysporum* (Schlecht) according to Booth [17].

Although *A. alternata* and *F. oxysporum* were isolated from clean seeds of both groups, it showed that 49% of *A. alternata* and 51% of *F. oxysporum* were isolated from the discolored seeds of genotype E14, and 55% of *A. alternata* and 45% of *F. oxysporum* from genotype CLJ. There are significant difference between these fungi isolated from clean and discolored seeds of both genotypes. The discolored seeds showed grey profuse mycelial growth for *A. alternata* and white profuse mycelial growth for *f. oxysporum*. However, examination by the unaided eye and

**Table 1.** Percentage of infected clean and discolored seeds of chickpea in PDA plates at 24°C after one week.

Chickpea genotype	Type of seeds <sup>b</sup>	Fungus <sup>a</sup>	Means %
E14	Discolored	<i>Alternaria</i>	49
		<i>Fusarium</i>	51
	Clean	<i>Alternaria</i>	9
		<i>Fusarium</i>	7
CLJ	Discolored	<i>Alternaria</i>	55
		<i>Fusarium</i>	45
CLJ	Clean	<i>Alternaria</i>	15
		<i>Fusarium</i>	3

a fungus isolated from discolored chickpea seeds.

b clean unstained seeds were separated by the unaided eye from stained ones (discolored ones).

stereobinocular microscope showed seed with evident lesions, brown to dark brown discolourations of various sizes and shapes and small wrinkled seeds.

### Pathogenicity tests

Results of the pathogenicity tests experiment show that *A. alternata* infected clean seeds of chickpea E14 after 48 hr, it took 96 hr to cause medium infection (white or grey profuse mycelial growth of the fungus observed to grow on the surface of the seed but the seeds kept germinating) for CLJ and heavy infection was observed for both genotypes after 144 hr but this did not prevent germination. This is contrary to the effect of *F. oxysporum* which heavily infected genotype E14 after 72 hr and CLJ after 96 hr and the germination of seeds were significantly decreased. The seeds infected with *F. oxysporum* turned soft and the seed coats started to slough off after 96 hr and 120 hr for the genotypes E14 and CLJ respectively. The explanation offered is that the severe effect of *F. oxysporum* is due to the fact that this fungus is associated with damping-off.

### Fungicides test

The effect of the four fungicides on the growth of the two fungi is presented in Table 2. Bavistin significantly inhibited the growth of *F. oxysporum* at a very low dose of 100 mg/l, but failed to inhibit the growth of *A. alternata*. On the other hand, dithane and miceb significantly inhibited the growth of both fungi, the former also showed good response at a level of 250 mg/l for both fungi. In contrast, ronilan gave poor control to both fungi.

### Systemic activity of the fungicides

Results of the systemic activity of the fungicides are given in Table 3. Bavistin showed considerable activity in both seed coat and cotyledon regions; while dithane was restricted to the seed coat only and did not penetrate to the cotyledons. On the other hand, bavistin did not affect *A. alternata* or inhibit its growth. However as the

Table 2. Growth inhibition zones of *Alternaria alternata* and *Fusarium oxysporum* (F) in PDA plates after treatment with four fungicides measured in cm.\*

Fungicide	Bavistin		Dithane		Miceb		Ronilan	
	Fungi		Mean inhibition zones (cm)					
Con mg/l	A	F	A	F	A	F	A	F
Control	0.0a	0.0d	0.0d	0.0d	0.0c	0.0d	0.0a	0.0a
100	0.0a	4.5c	1.9c	1.8c	1.3b	1.7c	0.1a	0.3a
150	0.0a	5.8b	3.2b	2.9b	1.8a	2.2b	0.2a	0.4a
250	0.0a	6.6a	5.3a	6.1a	2.0a	2.7a	0.3a	0.4a
L.S.D				0.47				

\* Figures within a column followed by the same letter are not significantly different at the 0.05% level.

concentration increased, the effect of bavistin on *F. oxysporum* was more distinct of the inhibition zones in the three treated regions of the whole seed, seed coat and cotyledons. Dithane significantly inhibited the two fungi growig in seed and seed coat only and the inhibition zones increased with the increase of its concentrations. Although the systemic activity of bavistin was evident in penetrating the cotyledon, there was no difference between bavistin and dithane in all concentrations to produce stronger inhibition zones in case of the whole seed and the seed coat.

**Table 3.** Activity of bavistin (B) and dithane (D) measured in cm on PDA plates, seeded with  $1 \times 10^6$  conidia of *A. alternata* (A) and *F. oxysporum* (F) after 5 days.

Fungicides		B+F	D+A	D+F
Concn mg/l	Portion of seed	Mean inhibition zones (cm)		
100	Whole seed	2.4	2.3	2.4
	Seed coat	1.4	1.6	1.2
	Cotyledon	1.2	0.0	0.0
200	Whole seed	2.9	3.3	2.7
	Seed coat	2.7	1.8	1.8
	Cotyledon	1.8	0.0	0.0
300	Whole seed	3.2	4.2	3.2
	Seed coat	2.9	2.5	2.5
	Cotyledon	2.2	0.0	0.0
400	Whole seed	3.8	4.7	4.0
	Seed coat	3.4	2.3	2.9
	Cotyledon	2.9	0.0	0.0
500	Whole seed	5.2	5.4	4.5
	Seed coat	3.7	3.8	3.5
	Cotyledon	3.3	0.0	0.0
600	Whole seed	5.4	6.0	5.0
	Seed coat	4.3	4.4	4.1
	Cotyledon	3.6	0.0	0.0
700	Whole seed	6.1	6.2	6.0
	Seed coat	4.4	5.6	4.7
	Cotyledon	3.8	0.0	0.0

Data on measurements of inhibition zones (x) were transformed using the formula  $\sqrt{x + 1/2}$  according to Snedecor and Cochran [18].

(B+A) Bavistin treatments did not affect *A. alternata*.

After five days, the mycelium growth of *A. alternata* and *F. oxysporum* completely colonized the excised seed coat cups from non-treated seeds. There was no growth on any of the seed coats from fungicide treated seeds except in case of bavistin with *A. alternata*. Growth had ceased on the agar plugs within the cups from bavistin treatment with *F. oxysporum*, but there was active growth on the plugs in the cups from dithane treated seeds plus both fungi.

### Seed treatments

Bavistin, dithane and miceb significantly reduced pre-emergence damping-off and seed rot of the treated seeds of genotype E14 and CLJ (Tables 4, 5, 6). Treat-

**Table 4.** The effect of seed treatments of chickpea genotypes E14 and CLJ with four fungicides on the percentage germination after two weeks (2W) in artificially infested soil with *A. alternata* (A) and *F. oxysporum* (F).

Treatment	Clean seeds						Discoloured seeds					
	C*		A		F		C*		A		F	
	%		%		%		%		%		%	
	E14	CLJ	E14	CLJ	E14	CLJ	E14	CLJ	E14	CLJ	E14	CLJ
Control	83.3	83.3	58.3	50.0	33.3	66.7	25.0	50.0	33.3	25.0	16.7	33.3
Bavistin	91.7	91.7	83.3	66.7	83.3	83.3	75.0	75.0	66.7	41.7	75.0	66.7
Dithane	100.0	83.3	91.0	83.3	100.0	83.3	75.0	66.7	83.3	66.7	91.7	58.3
Miceb	91.7	83.3	75.0	66.7	66.7	75.0	58.3	66.7	66.7	58.3	58.3	50.0
Ronilan	75.0	83.3	58.3	50.0	41.7	33.3	25.0	41.7	41.7	33.3	25.0	25.0

\* C Control

**Table 5.** Mean values of the effect of the different fungicide treatments on percentage of germination of chickpea genotypes E14 and CLJ after two weeks in artificially infested soil with *A. alternata* and *F. oxysporum*.

Treatment	Chickpea genotype	
	E14	CLJ
Control	41.65c	51.38a
Bavistin	79.17b	70.85c
Dithane	90.27a	73.60c
Miceb	70.8 b	66.67bc
Ronilan	44.45c	55.54ab
LSD	9.01	10.27

Figures within a column followed by the same letter are not significantly different at the 0.05% level.

ment of seeds with bavistin resulted in more significant germination of both clean and discoloured seeds of both genotypes than the untreated seeds. Higher percentage of pre-emergence damping-off was observed among the discoloured seeds of both

**Table 6.** The mean response of two chickpea genotypes E14 and CLJ inoculated with *A. alternata* and *F. oxysporum* given in terms of percentage of germination.

Treatment	Chickpea genotype	
	E14	CLJ
Control	54.2d	66.65d
<i>Alternaria alternata</i> (A)	45.8bc	45.80b
<i>Fusarium oxysporum</i> (F)	41.7b	29.15a
Bavistin (B)	83.4ij	83.40f
B + A	75.0gh	75.00e
B + F	79.2hi	75.00e
Dithane (D)	87.5j	87.50f
D + A	87.5j	87.50f
D + F	95.5k	95.60g
Miceb (M)	66.7ef	66.70d
M + A	70.9fg	70.90e
M + F	75.0gh	75.00e
Ronilan (R)	54.2d	58.30c
R + A	50.0cd	50.00b
R + F	33.4a	29.15a
LSD	7.01	7.96

Figures within a column followed by the same letter are not significantly different at the 0.05% level.

genotypes in the untreated control. Seeds inoculated with either *F. oxysporum* or *A. alternata* significantly increased the percentage of pre-emergence death of the clean seed. Although, significant increase in germination of clean seeds of the untreated control was noticed after two weeks, bavistin dithane and miceb gave even higher increase in the percentage of germination. On the other hand, dithane gave an outstanding results when the infested seeds with both fungi were treated with this chemical. All three chemicals gave good protection against *F. oxysporum* and improved the germination of the seeds, compared to ronilan which failed to give good protection for either the discolored seeds or the infested seeds of the two fungi.

### Discussion

Results of the in-vitro inhibition trials showed that one systemic fungicide bavistin and two non-systemic fungicides dithane and miceb were effective in preventing fungal growth of *F. oxysporum*. The superiority of bavistin in preventing the fungal growth of *F. oxysporum* over the other two fungicides is explainable in terms of its systemic nature which extends its effectiveness for a longer duration. Further more it is spread over a wider area of the plant material. The fact that bavistin was ineffective in preventing the fungal growth of *A. alternata* is not beyond expectation as this lack of effect on this fungus is in harmony with a previous finding [19]. Dithane and miceb gave good results in preventing fungal growth of both fungi and this was confirmed

in all trial experiments. It is suggested that these two fungicides may be absorbed into the seed coat, without passing through it. Seeds of untreated discolored groups of both genotypes gave low percentage of germination. Although *A. alternata* grew profusely in the germinating seeds, *F. oxysporum* seemed to be the fungus which reduced the percentage of germination and this confirmed the results given by ICARDA [2,3].

The in-vivo control experiments showed the three chemicals could prevent *F. oxysporum* from causing serious pre-emerging damping-off developing in an inoculum rich medium. Ronilan, however failed to prevent infection presumably because of its low activity against the two pathogens besides its non-systemic action.

It is concluded, that the three fungicides; bavistin, dithane and miceb can successfully be used in seed treatments to protect both genotypes of chickpea from attack by *F. oxysporum*; while the last two fungicides protected the seeds from being attacked by *A. alternata*. Of the three chemicals, bavistin could be regarded as the best, because of its greater systemic activity. Ronilan cannot be recommended due to its low activity against these pathogens. Nevertheless, further investigations into these chemicals would seem to be warranted, especially as they appear to have strong activity on the two fungi studied.

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## تأثير معاملة بذور الحمص بأربعة مبيدات فطرية على ظهور البادرات في وجود فطري الترناريا الترناتا وفيوزاريوم أوكسيسبورم

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المملكة العربية السعودية

ملخص البحث. تم اختبار نسبة الإنبات لاثنتين من التراكيب الوراثية لمحصول الحمص هما CLJ و E-14 معملياً. وقد قسمت بذور كل من تلك السلالتين إلى مجموعتين سليمة وملونة المظهر الخارجي. وقد كانت نسبة الموت أكبر من ٦٥٪ في المجموعة ملونة المظهر الخارجي. وقد تم عزل فطري الترناريا الترناتا وفيوزاريوم أوكسيسبورم في أكثر من ٩٠٪ من البذور الملونة لكل من التركيبين الوراثيين. كما شوهد أن فطر الفيوزاريوم أوكسيسبورم كان مرتبطاً مع موت البادرات لمعظم البذور الملونة.

اختبر تأثير أربعة من المبيدات الفطرية معملياً لمقاومة الفطريات المعزولة والمنهارة على بيئة البطاطس والدكستروز وأيضاً كمعاملة للبذور. أوضحت النتائج أن مبيد بافستين أثر معنوياً على فطر الفيوزاريوم أوكسيسبورم ويلييه مانكوزيب ثم مايسب بينها فينكلوزولين كان أقلهم تأثيراً. لم يتأثر فطر الترناريا الترناتا مطلقاً بمبيد البافستين بينما تأثر نمو كل من الفطرين المختبرين معنوياً باستخدام المبيدات الأخرى الديثين والمايسب. كما لوحظ أن للبافستين تأثير جهازي على الفطريات المختبرة في كل من غلاف البذرة والفلقة والجنين.

أوضحت نتائج معاملة البذور في التربة المعدة صناعياً بالفطر الترناريا الترناتا أو الفطر فيوزاريوم أوكسيسبورم بواسطة أي من المبيدات الفطرية بافستين، مانكوزيب، مايسب أن نسبة الإنبات تزداد معنوياً بمقارنتها بالبذور غير المعاملة. بينما كانت معاملة البذور بالمبيد الفطري فينكلوزولين أقلها تأثيراً.