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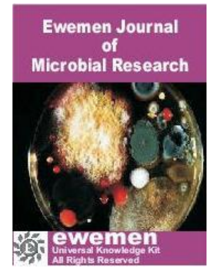

Full Length Research

EFFICACY OF TWO FUNGICIDES AGAINST SOME SOIL AND SEED BORNE FUNGI

EL-GALI Zahra Ibrahim

Department of Plant Protection, Faculty of Agriculture, Omer Al-Mukhtar University, El-Beida, Libya.

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**Corresponding Author's Email:*
Zelgali@yahoo.com

ABSTRACT

Seed borne pathogenic fungi can greatly affect seed quality and cause diseases that impact seedling production in nurseries. This investigation is the first report in Libya, aimed at *in vitro* evaluation of two fungicides Maxim (systemic) and Apron (non-systemic) on growth of *Botrytis cinerea*, *Macrophomina phaseolina* and *Rhizoctonia solani*. The use of seed-dressing fungicides with slurry methods and soil drench at various concentrations, for control of soil-borne fungi on bean seed *in vivo*, was also investigated. The results obtained showed that all the fungicides significantly reduced the linear growth of all tested fungi. Maxim was more effective in growth inhibition than Apron and *B. cinerea* was the most sensitive to tested fungicides than the other fungi. Fungicides Maxim and Apron, used separately as seed dressing at 2 ml/kg seeds and 3 g/kg seeds respectively, and soil drench at 2 ml/L. and 3 g/L of water respectively, before sowing significantly reduced seed decay, which reflected increased survival of seedling particularly in case of seed dressing at all concentrations.

Keywords: Linear growth, seed-dressing, soil drench, fungicides, Maxim, Apron.

INTRODUCTION

Seed occupies only a small niche in the overall agricultural economy, yet the importance of this commodity is greatly amplified by the fact that good quality seed is the basis of all future agricultural production. In recent years globalization of the seed industry has resulted in widespread and rapid distribution of seeds. With this movement of seed comes an increasing danger of the spread of seed-borne diseases and control of such diseases became a very pressing demand (Nene, 1999). Pathogenic fungi can infect seeds internally and destroy the endosperm and the embryo or contaminate the seeds and affect seedling germination and development (El-Gali, 2003; 2012). Seed borne diseases are important constraints to bean (*Phaseolus vulgaris* L.) production. The main fungal diseases on bean in Libya during spring-summer period are grey mold and damping of (*Botrytis cinerea*), charcoal rot (*Macrophomina phaseolina*) can reduce establishment by killing the developing bean plants and *Rhizoctonia solani*-infected plant-lets may develop, root rot, or stem canker which often leads to wilting and plant death in the severe cases (El-Gali, 2003; 2008).

The control of fungal diseases may be overcome with control strategies based on the adoption of appropriate cultural practices that can reduce the attacks of these pathogens. In the case of golf courses, with high agronomic maintenance level, these cultural practices may not be enough and chemical control may be necessary. The use of synthetic chemicals as antimicrobial for the management of plant diseases has undoubtedly increased crop protection (El-Wakil and Ghonim, 2000; Ibiam *et al.* 2008). Several studies had reported the use of common chemical fungicides for the successful control of seed pathogenic fungi. Shalaby *et al.* (1997) indicated that Homai 80%, Benlate 50% and Vitavax-Thiram were the most effective fungicides against the main soil-borne pathogens of maize and soybean such as: *Cephalosporium* spp., *Fusarium* spp., *M. phaseolina* and *R. solani* under laboratory conditions. Onuegbu (1999) showed that 100 ppm Dithane M-45 and 100 ppm Benlate-T completely inhibited the growth of *A. flavus* and *A. niger* in mung bean seeds. Tachigareen 30%, Topsin M-70% and Rizolex-T50% were also reported to be highly effective against pathogenic fungi *F. oxysporum*, *F. solani, R. solani* and *S. bataticola* (El-Shaer, 2002). Kiran *et al.* (2011) studied the antifungal activity of synthetic fungicide Bavistin and Thiram, against five seed borne fungi of maize *viz*., *Curvularia lunata*, *Dreschslera halodes*, *Alternaria alternata*, *Cladosporium cladosporioides* and *Rhizopus* sp., which were tested *in vitro*. Complete inhibition was observed against all the test fungi at 2% recommended concentration. The fungicides Mancozeb and Bavistin were examined against eight seed borne fungi namely, *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. oryzae*, *A. fumigatus*, *Fusarium moniliforme*, *F. solani* and *Penicillium* sp. isolated from maize grains. Antifungal activity was tested on PDA (Potato Dextrose Agar) medium. Mancozeb inhibited mycelial growth of all the test fungi significantly. Bavistin also inhibited all the test fungi except *F. solani* and *Penicillium* sp. (Shirurkar and Wahegaonkar, 2012). *In vitro* evaluation of new fungicide mixtures revealed that, carbendazim plus Mancozeb, and Hexaconazole plus Zineb, could control the *Sclerotium rolfsii* even at 250 ppm (Kumar *et al.* 2014). The antifungal susceptibility test was determined by suspension of Bavstin and Mancozeb against (*Fusarium solani, Aspergillus fumigatus, Alternaria solani* and *Helminthosporium spp.*) Both antifungi showed high activity against *Fusarium solani, Aspergillus fumigatus, Alternaria solani*, and *Helminthosporium* spp. (Masih *et al.* 2014).

Various types of seed treatments can control soil-borne pathogens. El-Wakil and Ghonim (2000) used five fungicides against root-rot and wilt of peanut as seed dressing and soil treatment. They found that Rizolex-T50% was the best fungicide in reducing disease infection under greenhouse and field experiments, followed by soil treatment with Chlorotosep and Amconil. Moreover, Vitavax Thiram and Rizolex T were the more efficient to reduce the percentage of pod rot infection against *F. oxysporum, F. solani*, *M. phaseolina, S. rolfsii* and *R. solani* under both artificial and natural infections. El-Shaer, (2002) indicated that the fungicides Tachigareen 30%, Topsin M-70% and Rizolex-T50% significantly decreased plants rot and increased the plants survival, crop yield and increment yield in lentil plants. Ibiam *et al*, (2000) and (2006), reported that seed dressing fungicides; Benlate, Apron plus 50 Ds, Fernasan-D, Dithane M-45 and Bavistin, controlled seed-borne fungi of rice *Fusarium moniliforme*, *Bipolaris oryzae*, *Trichoderma hazianum*, *Curvularia lunata*, *Fusarium oxysporum* and *Chaetomium globossum* which both causes damage to rice both in the field and storage. They also reported that theses fungicides improved seed germination *in vitro*, seedling emergence *in vivo* and yield of the crop. The objective of this research work was to investigate the *in vitro* antifungal activity of two fungicides on growth of *B. cinerea*, *M. phaseolina* and *R. solani* as well as evaluate the protective effects of the most effective one against the fungal invasion of bean seeds and seedlings under greenhouse conditions.

MATERIAL AND METHODS

Fungal material

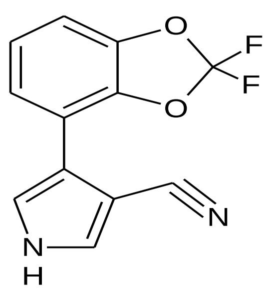
Three isolates of *B. cinerea*, *M. phaseolina* and *R. solani* were used in the study. The fungi were isolated from samples of white bean seeds naturally infected with seed pathogens.

Fungicides

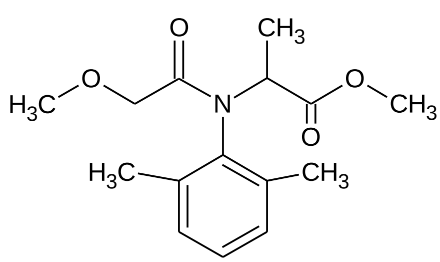
Two fungicides Maxim (systemic) and Apron (non-systemic) were obtained from the market and used in the form of solutions (fs) and wettable powder (wp), respectively. The specification of the fungicides including names, chemical composition, formulation, manufacture source, and method of application are as stated in Table (1). The effects of two fungicides against soil-borne fungi were investigated *in vivo* and *in vitro*.

Table 1: The listed fungicides and their chemical structure

Brand name / Formulation	Application rate	Common name	IUPAC Chemical name	Company
Maxim 3.5% fs.	2 ml/kg seeds	Fludioxonil	4-(2,2-difluoro-1,3- benzodioxol-4-yl)-1H-pyrrole-3- carbonitrile	Novartis
Apron 35% wp	3 gm/kg seeds	Metalaxyl	Methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-DL-alaninate	Jingma; Novartis; Rallis



Fludioxonil



Metalaxyl

Application of fungicides *in vitro*

The effects of fungicides on linear growth of the pathogenic fungi were determined on PDA medium amended with the 4 concentrations (0, 0.5, 1.0 and 1.5 ppm active ingredient) of the two fungicides using Poisoned Food Technique (Nene and Thapliyal, 1993). Inhibition index was estimated for each of the different concentrations of the tested fungicides used. The desired concentrations of each fungicide were prepared by adding the calculated amounts of any of the tested fungicides of each concentration to 20 ml of previously autoclaved PDA medium just before solidification (45 ˚C) under sterilized conditions and mixed thoroughly to obtain homogeneous poisoned media. The poisoned media were poured into 9.0 cm sterilized Petri-dishes and five replicates were made for each concentration. Medium without fungicide was used as control. After the medium had solidified, the dishes were inoculated in the center with an equal discs (5 mm in diameter) taken from 7 days old culture of each fungus. Petri-dishes were incubated at 22 ˚C. The linear growth (cm) was periodically measured until the growth of the fungus in one dish-reached to the edge. Percentage of toxicity was calculated according to the formula suggested by Nene and Thapliyal (1993) as follows:

$$I = 100\{\frac{(C - T)}{C}\}$$

where,

I = Percent inhibition in growth of test pathogen, C = Radial growth of pathogen in control, T = Radial growth of pathogen in treatment

Application of fungicides *in vivo*

This experiment was carried out to investigate the efficiency of the tested fungicides as seed-dressing and soil drench to control seed-borne fungi. Two fungicides were applied to the bean seed at less concentration than the recommended dose, at recommended dose and at above of recommended dose.

Plastic pots (25 cm in diameter) were used. Pots were sterilized by immersing in 5% formalin solution for 15 minutes and left several days before being used, then filled with sterilized sand and clay soil (1:1 v/v). The soil was infested with the pathogenic fungi grown on sand barley water (1: 3: 3) at the rate of 2% (w/w). The pots were irrigated for one week before sowing. Surface sterilized seeds of bean were planted, at the rate of 15 seeds per pot.

Slurry method

Fungicide seed slurries were prepared mixing the amount of Apron in 3-4ml of water in a 500-ml Erlenmeyer flask. About 200 seeds were added to the flask, mixed properly for two minutes and air-dried for 30minutes on sterile tray to enable the seeds absorb the fungicides at 23-25C° (Kaiser and Hannan, 1988). In case of Maxim, seeds were wetted immediately with fungicide till coating and after treatment they were spread on sterile trays and air-dried for 30 minutes. The control was soaked in sterile distilled water for 1hr, and air-dried the same way as the other treatments. Four doses (1, 2, 3 and 4 ml/kg seeds or gm/kg seeds) of the tested fungicides were applied by two methods (a) & (b) as stated below:

a) Seeds were treated, 24- 48 hours prior to sowing with the tested fungicides

b) The soil drench with the tested fungicides (doses ml or gm per liter of water) immediately before sowing.

Disease assessments

Disease effects due to *B. cinerea*, *M. phaseolina* and *R. solani* in the different treatments were assessed at 20 days after planting as decayed seeds, death of seedlings after emergence and seedling survival. These parameters were calculated and expressed as percentages.

Data Analysis

All experiments were conducted on the basis of completely randomized designs (CRD). Each treatment was replicated at least five times. For statistical analysis, data were subjected to the analysis of variance (ANOVA) using Co Stat Program. Angular transformed values were used for data analysis. Statistical comparisons among means were performed using Duncan's multiple range test (DMRT)

RESULTS AND DISCUSSION

In vitro study

The results of the in vitro studies of the effect of different fungicide (Maxim and Apron) concentration on the linear growths of the three tested fungi *B. cinerea*, *M. phaseolina* and *R. solani* using Poisoned Food Technique (PFT) are shown in Figures 1 and 2, and Table 2. The linear growths (Fig. 1 and 2) were measured for each of the different concentration of the tested fungicides and data were statistically analyzed and presented in Table 2. The behaviors of tested pathogenic fungi under the effect of fungicides were different. Generally, treatment with fungicides decreased the growth of tested fungi, compared to control. The growth of the tested isolates decreased with increase in the concentration of fungicides. *B. cinerea* was more sensitive to the tested fungicides followed by *M. phaseolina* and *R. solani.* Maxim was effective in checking the mycelia growth of the tested pathogenic fungi than Apron.

Table (2) revealed that growth rates of the tested fungi decreased and significantly differed depending upon type and concentration of the fungicide and the

Figure 1: Inhibition of linear growth of pathogenic fungi by Maxim at different concentrations

(1) = 0.5 ppm, (2) = 1.0 ppm, (3) = 1.5 ppm

(B) = *B. cinerea*, (M) = *M. phaseolina*, (R) = *R. solani*

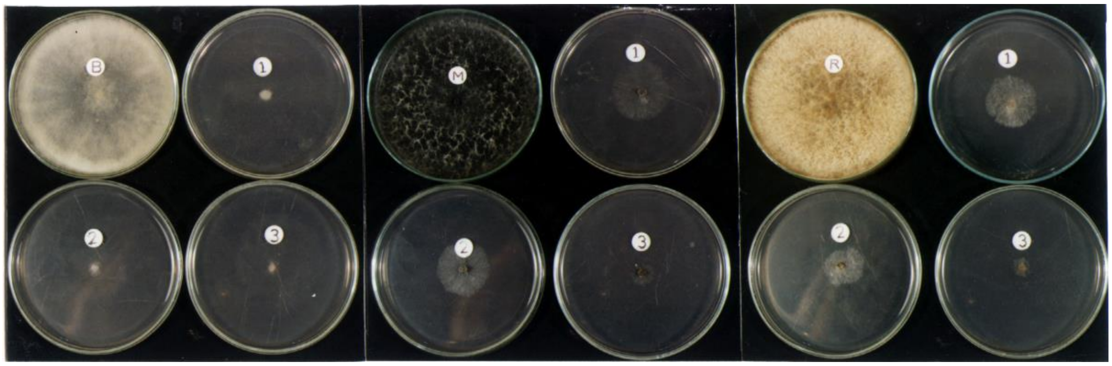


Figure 2: Inhibition of linear growth of pathogenic fungi by Apron at different concentrations

(1) = 0.5 ppm, (2) = 1.0 ppm, (3) = 1.5 ppm

(B) = *B. cinerea*, (M) = *M. phaseolina*, (R) = *R. solani*

tested fungus isolate. Both fungicides reduced linear growth of all tested fungi. Maxim was the most effective fungicide against tested fungi than Apron. Maxim was the most effective against *B. cinerea*, *M. phaseolina* followed by *R. solani*. *B. cinerea*, was more sensitive (94% to 100% inhibition) to Maxim fungicide as the concentration increased from 0.5 to 1.5ppm than the other fungi. Shalaby, *et al.* (1997) attributed the differences in fungicides actions to the selective actions of fungicides on a given fungus due to the differences in physiology and metabolism. The results of this study were approximately in agreement with results of Manu et al (2012). He reported that systemic fungicides like Avatar, Nativo and Vitavax power showed complete inhibition of *S. rolfsii* at all the concentrations tested in finger millet. Whereas the contact or non-systemic fungicide, mancozeb, was found to exhibit inhibition only at higher concentrations. Other studies had shown that the tolerance of fungi towards the fungicides is correlated with ability to synthesize extracellular melanin under fungicidal stress (Amany *et al.*, 2003). Melanized cell poses increased resistance to environmental stress and melanin may be the anti-inhibitory factor. Natural occurrence or the induction of melanin pigment secretion may be the mechanism of defense against the toxic effect of the fungicide by pathogenic fungi (Fogarty and Tobin, 1996).

In vivo study

Percentage of seed decay, seedling emergence and seedling survival were determined, data were statistically analyzed and presented in Tables 3 and 4. Obtained data indicated that the fungicides, Maxim and Apron, used either as seed-dressing or soil drench, significantly reduced seed decay, seedling emergence caused by the pathogenic fungi and increased the number of surviving plants compared with control treatment. The results also showed that the two fungicides significant decreased seed decay incidence and increased the number of seedling survival when used as seed dressing more than in case of soil drench treatment (Tables 3and 4).

In seed dressing, Maxim was the most effective fungicide even at lower doses (1 ml/kg seeds). Seedling survival recorded 90.60%, 88.00% and 82.70% in case of infection by *B. cinerea*, *M. phaseolina* and *R. solani* respectively, whereas, it recorded 67.90%, 61.30% and 64.00% in soil drench by the same fungi, respectively. Apron fungicide was less effective as it gave the highest percentage of seedling survival at 4 gm/kg seeds. Percentage of seedling survival recorded were 66.00%, 61.20% and 64.00% compared with 59.80%, 56.00% and 51.30% in both treatments respectively after infection with *B. cinerea*, *M. phaseolina* and *R. solani*, respectively (Tables 3 and 4). From the results in Tables 3 and 4, treatment with fungicides seems to decrease seed decay and increase seedling emergence and survival. Also, disease incidence decreased with increase in the concentration of fungicides. Maxim gave higher reduction in disease incidence than Apron. The results were in agreement with results of many earlier investigators and proved that certain fungicides reduced seed-borne fungi on bean (Abou-Neama, 1978; Fancelli and Kimati, 1986; Thakur *et al.*, 1991; Raffat, 1992; Issa, 1998). Yehia *et al.* (1979) and El-Deeb *et al.* (1985), showed that Daconil 2787 and Benlate 50% w.p. were superior in controlling root-rot.

Table 2. Effect of different concentrations of fungicides on the inhibition zones of tested fungi.

Fungicide	Conc (ppm)	Mean diameter colony (cm)			% inhibition growth		
		B. cinerea	*M. phaseolina*	*R. solani*	*B. cinerea*	*M. phaseolina*	*R. solani*
Maxim	0.00	9.00	9.00	9.00	0.00	0.00	0.00
	0.50	0.38	1.22	1.62	95.80 (78.17)	86.40 (68.36)	82.00 (64.90)
	1.00	0.00	0.64	0.90	100.00 (90.00)	92.90 (74.55)	90.00 (84.26)
	1.50	0.00	0.50	0.50	100.00 (90.00)	94.40 (76.31)	94.40 (76.31)
Apron	0.00	9.00	9.00	9.00	0.00	0.00	0.00
	0.50	0.94	1.98	1.88	89.60 (71.19)	78.00 (62.03)	79.10 (62.80)
	1.00	0.72	1.52	1.40	92.00 (73.57)	83.10 (65.73)	84.40 (66.74)
	1.50	0.38	0.72	0.50	95.80 (78.17)	92.00 (73.57)	94.40 (76.31)
LSD at 0.05 for:							
Fungicides (F):			0.30			0.31	
Concentration (C):			0.43			0.43	
Fungi (N):			0.37			0.38	
F × C:			0.88			0.89	
F × N:			0.98			0.99	
C × N:			1.13			1.15	
F × C × N:			1.15			1.40	

Each value is mean of five replicates
Values between brackets are the arcsine square root transformation for inhibition percentage

Table 3: Effect of different doses of fungicides seed dressing on seed borne fungi

Fungicide	Dose kg/seeds	*B. cinerea*			*M. phaseolina*			*R. solani*		
		Seed decay	Seedlings emergence	Seedlings survival	Seed decay	Seedlings emergence	Seedlings survival	Seed decay	Seedlings emergence	Seedlings survival
Control	0.00	50.60	49.30	46.60	40.00	60.00	40.00	22.60	77.30	33.30
Maxim 2ml/kg seeds	1.00	6.66	93.30	90.60	10.6	89.30	88.00	10.60	89.30	82.70
	2.00	5.30	94.60	94.60	5.30	94.60	93.30	6.70	93.30	86.60
	3.00	0.00	100.00	100.00	2.70	97.30	97.30	4.00	97.30	93.30
	4.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	96.00
Apron 3g/kg seeds	1.00	33.30	66.60	53.30	26.60	73.30	46.60	20.00	80.00	40.00
	2.00	15.90	83.90	57.60	21.20	78.60	50.10	18.60	81.30	54.60
	3.00	12.00	87.90	62.90	16.00	84.00	56.00	13.30	86.60	60.00
	4.00	10.60	89.30	66.00	13.30	86.60	61.20	10.60	89.30	64.00
LSD 0.05 for:			Seed decay			Seedling emergence			Seedling survival	
Fungicide (F)			1.10			1.70			1.60	
Dose (D)			1.70			2.60			2.60	
Fungi (N)			1.30			2.10			2.00	
F × D:			1.90			1.70			1.70	
F × N:			N.S			N.S			N.S	
D × N:			2.40			3.80			2.30	
F × D × N:			3.40			N.S			N.S	

Values are mean of 5 replicates
Percentage of infection transformed data by the arcsine square before analysis.
NS: Not-significant.

Such fungicides when covering seed surfaces prevented the fungi from attacking the seeds and protected the emerging seedling against the causal organisms. Also, El-Wakil and Ghonim (2000) reported that the seed dressing fungicides were the most effective against *Fusarium oxysporum*, *F. solani*, *M. phaseolina*, *Sclerotium rolfsii* and *R. solani* on peanut seeds. He attributed that the long period of persistence of fungicides around the seeds in case for seed dressing protected the seed against any microorganisms in soil. According to Ibiam *et al.* (2006), the systemic fungicides would have inactivated or killed the pathogens in the seeds or the seedlings, as the seeds germinated and thereby increases the resistance of the seeds or seedlings, or must have interfered with pathogenic process, thus, blocking the development of symptoms in the seeds or seedlings.

Table 4: Effect of soil drench by fungicides at different doses to control seed borne fungi on bean seeds

Fungicide	Dose kg/seeds	*B. cinerea*			*M. phaseolina*			*R. solani*		
		Seed decay	Seedlings emergence	Seedlings survival	Seed decay	Seedlings emergence	Seedlings survival	Seed decay	Seedlings emergence	Seedlings survival
Control	0.00	50.60	49.30	46.60	40.00	60.00	40.00	22.60	77.30	33.30
Maxim 2ml/kg seeds	1.00	30.60	69.30	67.90	34.60	65.30	61.30	19.90	80.00	64.00
	2.00	18.60	81.30	77.30	20.00	80.00	73.30	16.00	84.00	69.30
	3.00	15.90	84.00	80.00	13.30	86.60	80.00	12.00	88.00	78.60
	4.00	10.60	89.30	88.00	8.00	91.00	86.60	9.30	90.60	81.30
Apron 3g/kg seeds	1.00	45.30	53.30	46.80	33.30	66.60	44.00	21.30	78.70	36.00
	2.00	38.70	61.30	51.60	26.60	73.30	50.60	17.30	82.60	41.70
	3.00	29.30	70.60	55.10	20.00	80.00	53.30	15.90	83.90	46.20
	4.00	18.60	81.30	59.80	15.90	84.00	56.00	13.30	86.60	51.30

LSD 0.05 for:	Seed decay	Seedling emergence	Seedling survival
Fungicide (F)	1.40	1.50	1.20
Dose (D)	1.70	2.00	1.80
Fungi (N)	1.40	1.80	2.20
F × D:	3.70	4.80	3.40
F × N:	4.10	5.40	N.S
D × N:	4.60	6.30	N.S
F × D × N:	N.S	N.S	N.S

Values are mean of 5 replicates
Percentage of infection transformed data by the arcsine square before analysis.
NS: Non-significant

CONCLUSION

Referring to these results it could be concluded that the linear growth of all tested fungi *B. cinerea*, *M. phaseolina* and *R. solani* was decreased under fungicides toxicity. Seed dressing with two fungicides, Maxim and Apron resulted in a significant increase in the seedlings survival in the infected seeds at recommended dose, but the fungicides should be used with caution as it is costly, and excessive use can be hazardous to health and the environment.

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