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Evaluation of Seed Treatments with Fungicides and Bioagents in Controlling of Peanut Diseases.

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ABSTRACT

Field experiments were conducted in Khaled Ibn El-Waleed Village, Badr City, El-Behera Governorate to evaluate the efficacy of four fungicides (carboxin + thiram, thiophanate-methyl, thiram and tolclofos-methyl + thiram) and two bioagents (*Bacillus subtilis* and *Trichoderma harzianum)* against peanut fungal diseases during the summer season of 2014 and 2015, comparing with untreated control. All the tested compounds were applied at 1, 2 and 3g kg^{-1} of peanut seeds. The results clearly indicated that all these compounds particularly both tolclofos-methyl + thiram and carboxin + thiram fungicides, reduced pre- and post-emergence damping-off, 14 and 42 days after sowing (DAS), respectively, reduced rotted roots (120 DAS), increased survival (healthy) plants (120 DAS), and finally increased dry pod and seed yields. Generally, all the tested compounds gave better results at their high application rates and the chemical fungicides were the best.
Keywords: Peanut diseases, chemical control, biological control.

INTRODUCTION

Peanut (*Arachis hypogaea* L) belong to the legume or "bean" family (*Fabaceae*). It is known by many other local names such as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts (Seijo *et al*., 2007). It is one of the world's most important oilseed crops (Dwivedi *et al*., 2003). In Egypt, it is one of the most important and widely distributed crops and comes after cotton, rice and onion as an export crop (El-Deeb *et al*., 2002). Besides, it is considered one of the important field crops for improving sandy soil qualities as its root nodules bacteria can fix the atmospheric nitrogen (Salui and Bhatacharya, 1998). Consequently, cultivation of peanut in Egypt is mainly concentrated in the newly reclaimed land where El- Behera governorate is considered a major area (Atta-Alla *et al*., 2004). Generally, peanut growing plants and fruits are liable to several soil borne diseases such as damping-off, root rots, pod rots, crown rot, stem rot and wilt. In Egypt, damping-off, root rot and pod rot diseases are among the most destructive diseases attacking peanut (El-Deeb *et al*., 1985; Khalifa *et al*., 2006 and Khalifa *et al*., 2010) causing quantitative and qualitative losses to its yield which then becomes unprofitable (Hilal *et al*., 1994, Hassan and Frederic, 1995 and Mahmoud, 2004). Various methods for controlling such diseases were suggested worldwide. These included the use of resistant cultivars (Bahatia *et al*., 1996; Butzler *et al*., 1998), cultural practices (Helal *et al*., 1994; El-Deeb and Ibrahim, 1998), biological agents (Umamaheswar and Ramakrishnan 1994; Siddiqui *et al*., 2002; Bagwan,2011 and Mahmoud, 2015) and chemical fungicides (Frank *et al*., 1998; Rakholiya *et al*., 2011 and Mohapatra and Beher, 2012).

Therefore the present work was carried out to evaluate the effect of four fungicides and two bioagents (BCAs) on fungal pathogens of some soil borne diseases attacking peanut in the field.

MATERIALS AND METHODS

The field trials were carried out during the two consecutive summer seasons of 2014 and 2015 in a private farm at Khaled Ibn El-Waleed Village, Badr City, El-Behera Governorate. Peanut (*Arachis hypogaea* L.) seeds, Cv. Giza 6 cultivated in this study were obtained from Department of Legume Crop Research, Field Crop Research Institute, A. R. C., Ministry of Agric. and Land Reclaimation, Egypt.

Six commercial formulations of fungicides were used in this study which shown in table (1)

The experimental area was classified to equal plots of 21 m^2 (4.2 × 5m) that contain 7 rows (60 cm apart) for each and then designed in a complete randomized block design with 3 replicates for each treatment. Seeds were treated before sowing with the tested compounds at the rates 1, 2 and 3g product / Kg seeds according to the method described by Metwaly *et al*. (2006). The desired amount of each tested fungicide was thoroughly mixed with peanut seeds in plastic bags with arabic gum solution (1%) as sticker and shaked for 10 minutes to insure uniform coverage of seed with the tested compounds. Treated seeds were then allowed to dry at room temperature for 24 hours before sowing. Seeds treated with arabic gum solution were used as control. Treated and untreated seeds were planted with single seeds ≈ 28 cm apart (to comprise a total of 126 seeds / plot) in 10-5-2014 and 8-5-2015 during both growing seasons.

The following measurements were recorded during the growing seasons:

1- Number of pre-emergence damping-off 14 days after sowing (DAS).
2- Number of post-emergence damping-off 42 DAS.
3- Number of infected plants by rotted roots 120 DAS
4- Number of survival (healthy) plants 120 DAS.

After harvesting (120 days after planting) peanut plants were collected, then dry pod and seed yields (each at kg/ rep.) were determined**.**

Statistical analysis:

The obtained results were statistically analyzed according to Snedecor and Cochran (1969), and L.S.D values were obtained at 0.01 and 0.05.

Table 1. Trade name, common name and chemical name for tested fungicides.

Trade name and formulations	Common name	Chemical name (IUPAC)
Vitavax 200 75% W.P.	37.5% Carboxin+ 37.5% Thiram	5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide. Tetramethylthiuram disulfide; bis(dimethylthiocarbamoyl) disulfide
Topsin M 70% W.P.	Thiophanate-methyl	Dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate).
No-blight 50%W.P.	Thiram	Tetramethylthiuram disulfide; bis(dimethylthiocarbamoyl) disulfide
Rizolex-T 50%W.P.	20% Tolclofos-methyl + 30% Thiram	*O*-2,6-dichloro-*p*-tolyl *O,O*-dimethyl phosphorothioate. Tetramethylthiuram disulfide; bis(dimethylthiocarbamoyl) disulfide
Rhizo-N 30milion cell / g	*Bacillus subtilis*	Egyptian strains of bacteria *Bacillus subtilis* each one gram of the powder contains 30 million organisms.
Plant guard 30 million spores/cm^3	*Trichoderma harzianum*	Egyptian strains of fungus *Trichoderma harzianum* each one cm^3 of the liquid contains 30 million organisms.

All the tested fungicides were applied at 1, 2 and 3gkg^{-1} seeds.

RESULTS AND DISCUSSION

Effect of treatments on damping-off, root rot and survival plants:

Generally, data listed in tables (2, 3, 4 and 5) show that, all the tested fungicides, particularly tolclofos-methyl + thiram and carboxin + thiram, significantly ($p = 0.05$) reduced the number of pre-emergence damping-off, post-emergence damping-off, rotted roots and increased survival plants compared with the untreated control. However, *Trichoderma harzianum* at the rate of 1gkg^{-1} seed has the least effect and it exhibited insignificant effect against the pre-emergence damping-off during the first season, and against the post-emergence damping-off at the same rate during the two tested seasons (Table 2 and 3). The other fungicides showed an intermediate effect and the results were similar in two seasons. For example when tolclofos-methyl + thiram and carboxin + thiram were applied at 3g kg^{-1} seeds, the number of pre-emergence damping-off recorded 3.33 and 5.67 plants per replicate in the first season and 4.00 and 6.33 plants per replicate in the second season, respectively, while the corresponding values with *T. harzianum* at the same rate of application were 13.33 and 14.67 plants per replicate. Thiram, thiophanate methyl and *B. subtilis* at the same application rate (3g kg^{-1} seeds) gave 7.33, 9.33 and 11.00 plants per replicate in the first season and 8.33, 10.67 and 12 plants per replicate in the second season, respectively, indicating that these fungicides have an intermediate effect (Table 2). For the number of post-emergence damping-off, the same trend was observed in both seasons as tolclofos-methyl + thiram and carboxin + thiram at 3g kg^{-1} seeds, recorded values of 0.67 and 2.67 plants per replicate in the first season and 1.67 and 3.00 plants per replicate in the second season, respectively, while *T. harzianum* gave 6.00 and 7.67 plants per replicate in both seasons, respectively (Table 3). In the case of the number of rotted roots, results presented in Table (4) indicated that tolclofos-methyl + thiram and carboxin + thiram, at 2g kg^{-1} seeds recorded values ranged from 5.00 to 6.67 plants per replicate through both seasons while *T. harzianum* gave 11.00 and 12.00 plants per replicate in first and second seasons, respectively. For the number of survival plants, results in Table (5) indicated that tolclofos-methyl + thiram and carboxin+thiram were the most effective fungicides. These fungicides at 3gkg^{-1} seeds increased the survival plants to 119.33 and 113.67 plants per replicate in the first season and to 116.33 and 112.00 plants per replicate in the second season comparing to those of *T. harzianum* (97.00 and 93.00 plants per replicate), respectively.

Table 2. Effect of fungicide seed treatments on the number of pre- emergence damping-off of peanut, after 14 days of sowing under field conditions during summer seasons of 2014 and 2015.

Treatments	season 2014 Rate of application (gm Kg^{-1} of seeds)			season 2015 Rate of application (gm Kg^{-1} of seeds)		
	1	2	3	1	2	3
Carboxin + Thiram	8.33	7.00	5.67	9.67	8.33	6.33
Thiophanate methyl	13.33	12.00	9.33	14.33	12.67	10.67
Thiram	11.00	10.00	7.33	12.00	10.67	8.33
Tolclofos- methyl + Thiram	7.67	5.67	3.33	9.00	7.00	4.00
Bacillus subtilis	15.00	13.33	11.00	16.67	15.00	12.00
Trichoderma harzianum	17.33	16.00	13.33	18.33	16.67	14.67
Untreated (check)		19.00			20.33	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	2.27		1.69	2.25		1.68
Rates (R.)	1.49		1.11	1.48		1.10
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates.

Table 3. Effect of fungicide seed treatments on the number of post- emergence damping–off of peanut, after 42 days of sowing under field conditions during summer seasons of 2014 and 2015.

Treatments	season 2014 Rate of application (gm Kg^{-1} of seeds)			season 2015 Rate of application (gm Kg^{-1} of seeds)		
	1	2	3	1	2	3
Carboxin + Thiram	5.00	3.33	2.67	6.33	4.67	3.00
Thiophanate methyl	7.00	5.67	5.00	8.67	7.33	7.00
Thiram	5.67	5.33	4.00	7.33	6.33	5.00
Tolclofos- methyl + Thiram	3.33	1.33	0.67	5.00	2.33	1.67
Bacillus subtilis	8.67	7.00	5.00	9.67	8.00	6.67
Trichoderma harzianum	10.00	8.33	6.00	11.67	10.00	7.67
Untreated (check)		10.00			12.33	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	1.66		1.24	2.16		1.62
Rates (R.)	1.09		0.81	1.42		1.06
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates.

Table 4. Effect of fungicide seed treatments on the number of rotted roots of peanut, after120 days of sowing under field conditions during summer seasons of 2014 and 2015.

Treatments	season 2014 Rate of application (gm Kg^{-1} of seeds)			season 2015 Rate of application (gm Kg^{-1} of seeds)		
	1	2	3	1	2	3
Carboxin + Thiram	7.67	5.67	4.00	9.33	6.67	4.67
Thiophanate methyl	10.00	8.33	5.67	11.33	9.67	7.00
Thiram	9.67	7.00	5.00	10.67	8.00	5.67
Tolclofos- methyl + Thiram	6.33	5.00	2.67	8.00	5.67	4.00
Bacillus subtilis	11.67	9.33	7.00	12.67	10.33	8.67
Trichoderma harzianum	12.33	11.00	9.67	14.33	12.00	10.67
Untreated (check)		15.00			17.33	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	2.17		1.62	2.23		1.67
Rates (R.)	1.42		1.06	1.46		1.09
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates.

Table 5. Effect of fungicide seed treatments on the number of survival plants of peanut, after 120 days of sowing under field conditions during summer seasons 2014 and 2015.

Treatments	season 2014 Rate of application (gm Kg^{-1} of seeds)			season 2015 Rate of application (gm Kg^{-1} of seeds)		
	1	2	3	1	2	3
Carboxin + Thiram	105.00	110.00	113.67	100.67	106.33	112.00
Thiophanate methyl	95.67	100.00	106.00	91.67	96.33	101.33
Thiram	99.67	103.67	109.67	96.00	101.00	107.00
Tolclofos- methyl + Thiram	108.67	114.00	119.33	104.00	111.00	116.33
Bacillus subtilis	90.67	96.33	103.00	87.00	92.67	98.67
Trichoderma harzianum	86.33	90.67	97.00	81.67	87.33	93.00
Untreated (check)		82.00			76.00	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	3.60		2.69	3.79		2.84
Rates (R.)	2.36		1.76	2.48		1.86
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates.

Generally, the increment of application rates significantly reduced the number of pre-emergence damping-off, post-emergence damping-off, rotted roots and increased the number of survival plants. For example, in the second season, increasing the application rate of carboxin + thiram from 1 to 2 and 3 g kg^{-1} seeds significantly reduced the number of pre-emergence damping-off, from 9.67 to 8.33 and 6.33 plants per replicate, the number of post-emergence damping-off, from 6.33 to 4.67 and 3.00 plants per replicate, the number of rotted roots from 9.33 to 6.67 and 4.67 plants per replicate and increased the number of survival plants from 100.67 to 106.33 and 112.00 plants per replicate, respectively.

However, certain exceptions were watched. For example no significant differences were observed between the effects of 1 and 2 g kg^{-1} rates of thiram against pre-emergence damping-off, which recorded 11.00 and 10.00 plants per replicate, in the first season, respectively (Table 2).

The high efficacy of tolclofos-methyl + thiram and carboxin+thiram in reducing pre-emergence, post-emergence damping-off and root rot diseases may be due to the high activity of these fungicides on fungal pathogens of the seed and root rot diseases including genera of *Aspergillus, Fusarium, Macrophmina* and *Rhizoctonia*.

Observations made in the present study on the effect of the tested fungicides on controlling damping-off and root rot diseases of peanut plants are consistent with those described by several authors. Gangopadhyay *et al*. (1996) found that carbendazim and thiram at rate 2g/kg seed significantly improved seed germination and reduced collar rot disease incidence compared with control. El-Deeb and Ibrahim (1998) recorded that Pre- and post- emergence damping-off and pod rot diseases were significantly decreased by using seed dressing fungicides (Rizolex- T, Benlate, Vitavax - T and Topsin M). EL-Wakil and Ghonim (2000) reported that Rizolex-T50% was the best fungicide in reducing root rot and wilt disease of peanut under greenhouse and field experiments. Abdel-Ghany (2001) found that treatment of peanut seeds with Plantguard (*Trichoderma harzianum*) and Rhizo-N (*Bacillus subtilis*) reduced root rot incidence and increased survived plants and pod yield compared to control. Hussain, Zeinab (2005) found that Vitavax T followed by Rizolex T recorded the highest effect in reducing damping- off, wilt and root rot diseases and gave the highest percentage of healthy survival plants compared with other treatments and control. Metwally *et al*. (2006) found that treated peanut seeds cv. Giza 5 with Plantguard *(Trichoderma harzianum* 3×10^6 spore/ ml),Rhizo-N (*Bacillus subtilis* 3×10^6 c.f.u/ml) reduced percentage of damping-off, wilt and peanut root rot diseases and consequently increasing percentage of healthy survival plants. Abd-El-Khair *et al*. (2016) found that in field experiments, soil application with *Bacillus pumilus*(Rb14), *Bacillus subtilis* (Rb18) and *Bacillus subtilis* (Rb28), *B. pumilus* (Bp) and *Bacillus subtilis* (Bs) significantly reduced the incidence of damping- off and root rot of peanut.

Effect of fungicides on yield:

The results in Tables (6 and 7) show the effect of fungicide seed treatments, at 1, 2 and 3 g kg^{-1} seeds, on pod yield (kg /rep.) and seed yield (kg/rep.), respectively, compared with the untreated control.

The results indicated that all the tested fungicidal treatments significantly ($p= 0.05$) increased the pod and seed yields (kg / rep.) comparing with the untreated control. The measured values of pod yield of all fungicidal treatments ranged between 6.50 for *T. harzianum* and 9.17 for tolclofos-methyl + thiram in the first season and between 6.40 for *T. harzianum* and 8.88 for tolclofos methyl+thiram in the second season comparing with 5.67 and 5.33 for the untreated control in the first and second seasons, respectively (Table 6). For seed yield, the corresponding values were 4.50 for *T. harzianum* and 6.62 for tolclofos-methyl + thiram in the first season and 4.44 for *T. harzianum* and 6.49 for tolclofos-methyl + thiram in the second season comparing with the untreated control which gave 3.92 and 3.70 in the first and second season, respectively (Table 7). As stated previously with other evaluation parameters, tolclofos-methyl + thiram and carboxin + thiram were the most effective fungicides in this respect whereas *T. harzianum* was the lowest effective one. For example the application of tolclofos-methyl + thiram and carboxin + thiram at 3gkg^{-1} seeds resulted in pod yield values 9.17 and 8.67 kg / rep. in the first season and 8.88 and 8.58 kg / rep. in the second season, respectively, whereas the corresponding pod yield values of *T. harzianum* 7.27 and 7.12 kg / rep. (Table 6). On the other hand the corresponding seed yield values of the same fungicides at the same rate of application were 6.62 and 6.22 kg / rep. in the first season and were 6.49 and 6.19 kg / rep. in the second season while those of *T. harzianum* were 5.10 and 5.02 kg / rep. (Table 7).

Table 6. Effect of fungicide seed treatments on the pod yield (kg rep^{-1}) of peanut, under field conditions during summer seasons 2014 and 2015.

Treatments	season 2014 Rate of application (gm Kg^{-1} of seeds)			season 2015 Rate of application (gm Kg^{-1} of seeds)		
	1	2	3	1	2	3
Carboxin + Thiram	7.83	8.50	8.67	7.65	8.37	8.58
Thiophanate methyl	7.17	7.33	7.83	7.02	7.22	7.72
Thiram	7.37	7.87	8.33	7.27	7.75	8.17
Tolclofos methyl + Thiram	8.00	8.67	9.17	7.83	8.51	8.88
Bacillus subtilis	6.67	7.17	7.63	6.50	7.03	7.43
Trichoderma harzianum	6.50	6.83	7.27	6.40	6.68	7.12
Untreated (check)		5.67			5.33	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	0.62		0.46	0.67		0.49
Rates (R.)	0.41		0.30	0.43		0.32
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates.

Table 7. Effect of fungicide seed treatments on the seed yield (kg rep^{-1}) of peanut, under field conditions during summer seasons 2014 and 2015.

Treatments	**season 2014 Rate of application (gm Kg^{-1} of seeds)**			**season 2015 Rate of application (gm Kg^{-1} of seeds)**		
	1	**2**	**3**	**1**	**2**	**3**
Carboxin + Thiram	5.53	6.03	6.22	5.42	5.97	6.19
Thiophanate methyl	5.00	5.15	5.53	4.91	5.10	5.49
Thiram	5.16	5.56	5.94	5.12	5.50	5.84
Tolclofos methyl + Thiram	5.66	6.16	6.62	5.56	6.11	6.49
Bacillus subtilis	4.63	5.03	5.37	4.53	4.96	5.27
Trichoderma harzianum	4.50	4.78	5.10	4.44	4.70	5.02
Untreated (check)		3.92			3.70	
L.S.D.at =	1 %		5 %	1 %		5 %
Treatments (T.)	0.46		0.34	0.49		0.36
Rates (R.)	0.30		0.22	0.32		0.24
T .R	N. S		N. S	N. S		N. S

All values shown in the table are averages of three replicates

Moreover, the increment of fungicide application rates was accompanied by increment in pod and seed yield but with no significant differences in some cases. For example, thiram at 1, 2 and 3 g kg^{-1} seeds resulted in pod yield values of 7.37, 7.87 and 8.33 in the first season and of 7.27, 7.75 and 8.17 in the second season, respectively (Table 6). In addition, the same fungicide at the same rates gave seed yield values of 5.16, 5.56 and 5.94 in the first season and of 5.12, 5.50 and 5.84 in the second season, respectively (Table 7). However, as example there are no significant differences between pod yield values (7.17 and 7.33) that recorded when thiophanate methyl was applied at 1 and 2 g kg^{-1} seeds (Table 6). Similarly, no significant differences were observed between seed yield values (6.03 and 6.22) that produced when carboxin + thiram was applied at 2 and 3 gkg^{-1} seeds (Table 7).

Fungicide seed treatments against fungi affecting the peanut growth and yield resulted in increment and improvement the peanut growth and consequently increase yield and yield components. These results are in agreement with those obtained by several authors. Abdel-Ghany (2001) studied the effect of seed treatments with four fungicides namely Maxim, Topsin-M, Rizole-T and Monceren against peanut root rot disease under greenhouse and field condition. Results indicated that Maxim was the best effective fungicide in reducing root rot incidence of peanut. It gave the highest percentage of survived plants and increased pod yield and100seed weight followed by Topsin-M. El-Deeb *et al*. (2002) found that treating groundnut seeds cv. Giza-5 with the fungicides Vitavax-T [carboxin-thiram], Rizolex-T [tolclofos methyl- thiram] and Topsin-M70 [thiophanatemethyl] reduced the percentage of root and pod rots in both the greenhouse and the field. In addition, all treatments increased pod yield compared to the non-treated check. Rakholiya *et al*. (2011) found that minimum disease incidence and maximum pod yield were recorded in the treatment of peanut seeds with vitavax 200 wp at 4.0g/kg seed followed by ipconazole 3.8 FS (0.1ml) + thiram 75wp (2.5g/kg seed) and vitavax 200wp 3.0g/kg seed.

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تقييم معاملات البذور بمبيدات الفطريات والمركبات الحيوية فى مكافحة أمراض الفول السودانى.

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أجريت التجارب الحقلية فى قرية خالد بن الوليد – مدينة بدر - محافظة البحيرة لتقييم فاعلية أربعة من مبيدات الفطريات و إثنين من المركبات الحيوية فى مكافحة أمراض الفول السودانى الفطرية فى الحقل خلال صيفى ٢٠١٤ و ٢٠١٥ وذلك بمعدلات ١,٢,٣ جم / كجم من البذور بالمقارنة بالغير معامل. أوضحت النتائج أن المبيدات المختبرة والتى أستخدمت كمعاملات للبذور قد أدت الى خفض سقوط البادرات قبل الإنبثاق وبعد الإنبثاق (١٤يوم، ٤٢ يوم) وكذا عدد النباتات المصابة بأعفان الجذور (١٢٠يوم) وزادت عدد النباتات السليمة (١٢٠ يوم) ونتج عن هذة المكافحة أن زادت أوزن القرون الجافة مما أدى إلى زيادة المحصول الناتج كذلك أشارت النتائج الى أن المبيدات الكيماوية أعطت نتائج أفضل من المركبات الحيوية وتميز فى ذلك مبيدى تولكلوفوس ميثيل + ثيرام و كاربوكسين + ثيرام وفى جميع الأحوال كان هناك علاقة طردية بين معدلات المبيدات المستخدمة والنتائج الإيجابية المتحصل عليها.