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Effect of Thiram as A Seed-Dressing Fungicide on Growth and Enzymatic Activities of *Fusarium solani* on Legumes

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ABSTRACT

Many plant-pathogenic fungi constitute major constraints to legume production. Fungicides are the most important components in the management of fungal diseases. Imported legume seeds that are found in the local market and are used for cultivation in Al Madinah region are thiram-coated. The current study was conducted to (i) isolate and identify the common soil-borne fungi found in legume fields in Al Madinah Al Munawwarah, Saudi Arabia (ii) compare the effectiveness of coated and hand-treated thiram coated seeds with regard to seed germination, enzymatic activities of pathogens, and nodulation of the target plant. Six genera and eight species of fungi were morphologically and molecularly identified and were belonging to *Fusarium solani*, *F. equisetai*, *Gibberella moniliformis*, *Chochliobolus hawaiiensis*, *Aspergillus niger*, *Penicillium aculeatum, Rhizopus oryzae* and *A. flavus.*. *Fusarium solani* was the most frequently recovered fungus from the total fungi recovered. Among test plants species, alfalfa had the highest percentage germination in all treatments followed by faba bean and common bean. Alfalfa recorded the highest number of nodules under different treatments reaching to 49.48 nodules/root. The number of nodules dropped to11.33 nodules/root when the local seeds were treated with thiram. The same trend was noticed in the case of common bean. The fungicide thiram exerted significant reduction in mycelial growth of the isolated fungi when used at the recommended rates compared with the untreated control. *Fusarium solani* cellulolytic and pectinolytic activities decreased as the concentration of thiram increased from 375 to 3000 ppm.

Keywords: Enzyme activity, pesticides, plant-pathogenic fungi, Saudi Arabia, spore germination.

INTRODUCTION

Soil-borne plant pathogens can cause important diseases that are considered major problems in agriculture worldwide. Several pests and pathogens may attack cultivated plants during their growth in the field, and could pass through storage and handling on the plant products **(**Abd-Elgawad *et al*., 2010). Soil borne pathogens also reduce seedling emergence (Hwang *et al*., 2002) and establishment (Wang *et al*., 1999). In legumes, many fungi were identified to cause diseases and are considered major constraints to legume production (Coyne *et al*., 2003). On the other hand, other fungi including saprophytes may lower the seeds quality (Elwakil *et al*., 2009). Common legume seed-borne pathogens including *Alternaria* spp., *Ascochyta* sp., *Colletotrichum* sp., *Fusarium* sp., *Macrophomina phaseolina* (Rauf, 2000), *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *Rhizopus* sp., *Rhizoctonia* sp., *Botrytis* sp., *Stemphylium* sp., *Trichothecium* sp. *Epicoccum* sp., and *Cephalosporium* sp. (Alazab, 2009; Elwakil *et al*., 2009).

Fungicides are still the most important components in the management of fungal diseases despite of their

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adverse environmental hazards. They are formulated in several ways, depending on their physical characteristics and methods of application. Fungicides used for seed treatment should be formulated differently from those used as foliar spray (Vidhyasekaran, 2004). Thiram® (a.i. thiram belongs to dithiocarbamates) Shanghai Bosman Industrial Company, China is a well-known fungicide that has several trade names (Sharma *et al*., 2005). This fungicide has broad-spectrum activity against fungi with multi-site modes of action (Russell, 2005), and can be used as a foliar application to control some fungal diseases (Kunkur *et al*., 2007). Thiram was found effective against seed rot and/or damping-off in faba bean, chickpea, and lentil (Bishaw and Gastel, 2007).

In Al Madinah Al Munawwarah, Saudi Arabia, the imported legume seeds which were found in the local market, are thiram-coated. Thus, the objectives of the current study were to (i) isolate and identify the common soil-borne fungi found in legume fields in Al Madinah Al Munawwarah (ii) compare the effectiveness of thiram-coated and -hand-treated seeds with regard to seed germination, enzymatic activities of the most frequently isolated pathogenic fungus, and bacterial nodulation of the targeted host plant.

Materials and Methods

Isolation of fungal pathogens

Ten soil samples from the rhizosphere of common bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), and alfalfa (*Medicago sativa* L.), were collected from three locations in Al Madinah Al Munawwarah (Table 1). Soil samples were placed in clean plastic bags and kept in laboratory until used. The collected soil samples were sieved to remove various contaminants for the isolation of phytopathogens. One gram of air-dried soil was mixed with 9 ml of sterile distilled water, shacked for 15 min, and allowed to stand for 10 minutes. Serial dilutions were carried out ranging from 10^{-1} to 10^{-4} using sterile distilled water. One ml of each dilution was transferred to each of 5 Petri dishes with potato dextrose agar medium PDA (Oxoid LTD, Hampshire, England). Five replicates of each sample were used. Moreover, soil was spread directly on top of multiple Petri dishes containing the same medium to consider any missing fungal species. Samples were incubated at 28˚C for 7 days for estimating fungal growth and counting fungal colonies. The fungal hyphal tips of the recovered fungi were transferred to a fresh PDA plates and incubated for seven days at 28˚C. The fungal isolates were stored in 10% Glycerol (Nakasone *et al*., 2004) at 20˚ C.

Morphological and molecular identification

Fungi were slide-mounted in lactophenol blue and examined under a compound microscope (LEICA DME, United States) at 10-100X. The developing fungal colonies were identified up to the species level based on morphology (Booth, 1971; Moubasher, 1993). Molecular confirmation for all the identified fungi was conducted at the Fragment Analysis and DNA Sequencing Services, FADSS (Okanagan, British Columbia, Canada). Internal transcribed spacer (ITS) region was amplified using ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) primer set (White *et al*., 1990). Sequences were Blastn at the GenBank nucleotide database (http://www. ncbi.nlm.nih. gov/) and identity was confirmed. MEGA6 software was used to align and create the dendogram for the fungal isolates and reference accessions obtained from the genebank with 1000 bootstraps (Tamura *et al*., 2013).

Seed germination test

Seed germination test of the legumes was conducted to determine the ability of seeds to germinate under different thiram treatments: local non-treated seeds (LNTS), local thiram-hand treated seeds (LTHTS), imported coated thiram-treated seeds (ICTTS) and

imported coated thiram but washed seeds (ICTWS). For LTHTS, the seeds were surface-disinfected using 2% sodium hypochlorite, rinsed twice in sterile distilled water, and then treated with the recommended dose of the fungicide (3 g thiram, 30 % wettable powder kg $^{-1}$ seeds). The seeds were mixed thoroughly with the fungicide for 3-5 min, placed on sterile paper towels in open air to dry, and then kept in sterile plastic bags for further use. To wash ICTTS, seeds were submerged and agitated in a 0.2 % aqueous solution of unscented liquid soap for one minute to remove the fungicides from the seed coat, then rinsed twice (1 min each) in distilled water. Seeds were air-dried for 15 min then stored until used (Bowen *et al*., 2000). Germination tests were conducted according to "the between-paper (BP) method" of International Seed Testing Association rules (ISTA, 2008) for all samples. Each seed sample was subjected to the four different treatments. Whatman paper (15 cm in diameter) was placed between moist layers of two paper towels (20 cm x 20 cm) in a plastic bag. Six seeds were distributed evenly on the Whatman paper to avoid contact between seedlings during germination. The bags were gently closed and incubated in an upright position at 25˚± 1˚C. Percentage of seed germination was recorded throughout 12 days. Each treatment was replicated five times, and the experiments were repeated twice.

Effect of thiram on mycelial growth of the test *Fusarium* species

In order to examine the effect of thiram on the growth of selected isolates of different *Fusarium* spp. (*F. equisiti* (Corda) Sacc. 1, *F. equisiti* 2, and *F. solani* (Mart.) Sacc.), concentrations of 375, 750, 1500 and 3000 ppm were prepared. One sterile paper disk (Whatman, 1.3 cm in diameter) was immersed in each concentration and dried at 40˚C for 1 hour then was placed in the center of PDA Petri dishes (9 cm) inoculated with 0.5 ml conidial suspension (1 x10^4 spore ml^{-1}) of each fungus. The plates were incubated at 28˚C. Untreated filter paper disks immersed in sterilized distilled water served as control. After 5 days, inhibition zone was measured in centimeter as an indication of the effectiveness of the fungicide. Five replicates for each concentration were used and the experiment was replicated twice.

Effects of thiram on cellulase and pectinase activities

In this experiment, *Fusarium solani* was selected because it was the most recovered fungus from Al Madinah Al Munawarah's soils and considered as common soil-borne fungus. Carboxymethyl cellulose (CMC) medium consisted of (g l^{-1}): 7.5 g CMC, 7.5 g sucrose, 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$ was used. The different concentrations of the fungicide were added to the medium to prepare final concentration of 375, 750, 1500 and 3000 ppm in addition to the control. Pectin medium has the same composition as CMC medium but pectin replaces CMC. In seeds medium, 7.5 g l^{-1} of the ground seeds of each of the three test plants (alfalfa, faba bean and common bean) replaced cellulose or pectin under different treatments. One mycelial disc (1 cm in diameter) of each fungus was inoculated in each flask. Three replicates were used for each treatment. All flasks were incubated at 28˚C.

For the assay method, pectinolytic and cellulolytic activities of the fungus were determined in the growth medium after 10 days through viscosity reduction method using viscometer (Lee *et al*., 2007). It was based on the function of enzymes which make a solution less viscous. In the current study, five ml of 1% carboxymethyl cellulose (CMC) were added to 4 ml citrate buffer (pH 4.6) and 2 ml of enzyme extract (prepared by growing on Czapek Dox broth, but the carbon source was replaced with 1% carboxyl methyl cellulose for cellulose and 1% pectin for pectinase). The mixture was left for about 15 min at 35˚C then in a boiling water bath for five minutes

to stop the enzyme action. A boiled sample served as blank. The average of three readings was recorded. The above steps were repeated on the blank and distilled water. Pectinolytic and cellulolytic activities were measured in term of percentage of decrease in viscosity (Abdel-Razik, 1970).

Greenhouse experiment

Greenhouse experiment was conducted to investigate the effects of thiram-treated seeds on plant growth rate and bacterial nodulation of the three test legumes.

Preparation of inocula

***Fusarium* inoculum**

Sand-corn meal medium was used in order to get optimum growth of the fungus *Fusarium solani*. Sand was sieved, washed and then dried. Twelve flasks (500 ml each) were filled with 200 g of sand: white corn (1:1 w/w). All flasks were sterilized at 1.5 kg/cm^2 pressure (121˚C) for 15 min. Twenty milliliters of sterilized distilled water was added to each flask. Five-day old cultures of *Fusarium solani* were inoculated to the flasks under aseptic condition, incubated at 28º C for 10 days, and kept until use.

Rhizobia inoculum

Soil inoculums (0.4 g Rhizobacterien L^{-1} water) (Hebei New Century Zhoutian Biotechnology, Hebei, China) were added into all pots after planting by spraying the surface soil before irrigation. The rhizobacteria concentration was 10 billion/g.

Pathogenicity test

To examine the efficiency of thiram in controlling *Fusarium solani in vivo* under greenhouse conditions, 1:1, w/w compost (peats) and sandy soil mixture was used. Soil was amended with 20 g *Fusarium* inoculum and 8 ml of *Rhizobium* inoculum for each 2 kg of soil. The amended soil was transferred to plastic pots (20 cm diameter) and watered until run-off a day before sowing the seeds of the different treatments. All seeds were disinfected by 2% NaOCl and left to dry for about one hour. Five replicate pots for each treatment were performed, each with six seeds. The growth rate as plant height (cm/day), the number of leaves (leaves/plant), the number of nodules (nodules/root) and root length (cm/day) for each plant were recorded throughout 45 days. The experiments were arranged in a randomized complete block design and each experiment was replicated twice.

Statistical analysis

Analysis of variance (ANOVA) was performed using the PROC ANOVA in Statistical Analysis System (SAS 9.3, SAS Institute Inc., NC, USA). Regression analysis was also used to create some ANOVA tables for the different measurements. Differences in means for the different dependent variables were compared using Fisher's least significant difference (LSD) test ($\alpha = 0.05$).

Results

Fungal isolation and identification

A total of 123 fungal isolates (related to six genera and eight species) were recovered from the rhizosphere of common bean, faba bean and alfalfa in three investigated locations in Al Madinah Al Munawarah. These isolated fungi were belonging to: *Fusarium solani, F. equisetai*., *Gibberella moniliformis* Wineland, *Chochliobolus hawaiiensis* Alcorn, *Aspergillus niger* Tiegh., *Penicillium aculeatum* Raper & Fennell, *Rhizopus oryzae* Went & Prins. Geerl. and *A. flavus* Link (Figure 1). *Fusarium* spp. were the most recovered fungus with 44.72% compared to the other fungi. Moreover, *F. solani* had 40.65% of the recovered *Fusarium* spp. The sequences of the recovered fungi were deposited in the gene bank under accession numbers (KF274669- KF274683).

Germination test

In all the test plants, LNTS treatment had the least germination seeds number, while LTHTS followed by ICTTS had the highest germination % (Figure 2).

During the germination experiment, some contaminating fungi appeared on test plants. These fungi were isolated, purified and identified. The isolated fungi were *A. flavus, A. niger, P. aculeatum* and *Fusarium solani* with 6.01%, 33.91%, 18.26%, and 41.74%, respectively (Table 2).

Effect of thiram on the mycelial growth of *Fusarium* spp.

The isolated fungi varied in their response to the different concentrations of thiram. In general, for the three *Fusarium* spp. (*F. equisiti* 1, *F. equisiti* 2, and *F. solani*), inhibition zone was increased as thiram concentrations increased (Table 3). Moreover, *F. equisiti* had higher inhibition zone compared to *F. solani*. Analysis of variance showed high significance for the model tested (<0.0001) at α=0.05 (Table 3).

Effects of thiram on cellulase and pectinase activity

Using CMC and pectin as substrate, cellulase had lower percentage of loss in viscosity compared to pectinase at all thiram concentrations (Table 4). There was a significant differences between the two substrates and among thiram concentrations at α = 0.05. Moreover, loss of viscosity was decreasing as thiram concentrations increased (Table 4).

Similar to the CMC substrate, percentage of loss in viscosity in cellulase activity was less than in pectinase in both local and imported plant seeds with high significant difference (P<0.05) (Table 5) when using seeds as substrate. In all test plants, loss in viscosity pectinase activity was higher than that in cellulose (Table 5). Generally, using imported seeds as substrate showed higher loss in viscosity for both enzymes compared to local seeds, however, no significant differences were found (Table 5).

Greenhouse experiment

Pathogenicity test

Growth rate

In the greenhouse experiment, the growth rate (mm/day) for alfalfa was the highest in the ICTWS and ICTTS treatments with high significant differences, while the least was for LTHTS (Tables 6 and 7). On the other hand, the highest growth rate for common bean was for ICTTS and LNTS and the least was for ICTWS with high significant differences. For faba bean, growth rate was higher in LNTS treatment but low in LTHTS and high significant differences were found among treatments.

Number of nodules

In all the test plants, the number of nodules (nodules/root) was the highest in the LNTS treatment (Table 6). Number of nodules in faba bean was about two to five folds of the other test plants at the different treatments considered. Significance was found among the different treatments in alfalfa and faba bean (Table 7).

Percentage of stand plants

Number and percentage of stand plants growing in Fusarium infested soil under different treatments were estimated (Table 8). In general, alfalfa had higher stand plants percentage. Moreover, the imported thiram-coated seeds had higher stand plant percent followed by imported thiram-coated washed seeds in the three plants. On the other hand, the local non-treated seeds treatment had the least stand plants percent in the three test plants. The variance among plants and treatments were significantly different (Table 8).

Discussion

This is the first study that deals with isolating fungi from legume plants rhizosphere and from legume seeds found in the local market in AL Madinah Al Munawwarah. Previous studies in Saudi Arabia were conducted in Makkah and the eastern part of the kingdom to isolate fungi from legume seeds. In Makkah, *A. niger*, *Alternaria alternata* (Fr.) Keissl., *A. flavus, F. moniliforme, P. expansum, F. oxysporium* and *A. terreus* recorded the highest percentage of occurrence and frequency on legume seeds (Alazab., 2009). On the other hand, in the eastern kingdom of Saudi Arabia,

Rhizoctonia solani, Pythium aphanidermatum, and *Sclerotinia sclerotiorum* were the most isolated fungi from legume seeds (Al-Abdalall, 2010).

The present work was conducted to evaluate and compare the effectiveness of film-coating legume seeds with thiram and those treated manually with the same fungicide in protecting the seeds from the damage that may be caused by seed- and soil-borne phytopathogens. Three legume seeds were employed in this study namely; alfalfa, common bean and faba bean. Seed coating is a mechanism of applying certain materials as an external cover to protect seeds from biotic stresses and can affect seed growth or rhizospheric soil. It can influence the microenvironment of each seed and affect pathogen survival (Carisse, 2010).

Generally, alfalfa seeds showed the highest germination percentage followed by faba bean and common bean seeds under different treatments. The thiram hand-treated seeds had the highest germination percentage as compared to imported thiram treated or washed seeds or local non-treated seeds (control). Previous work reported that moisture uptake and seed viability have been reduced Daran 8600 (polyvinylidene chloride, PVDC)-coated soyabean seeds compared to the non-coated ones (Henning 1990). Thus, as in this work, the influence of seed coatings on water uptake by the seedlings varies depending on germinated seeds. Coating may sometimes reduce seed germination because it forms a physical barrier to primary root protrusion (Kavak and Eser, 2009). Thiram is one of the most suitable fungicides that inhibit fungal growth *in vitro*. It reduces wilt incidence and Singh and Jha (2003) and can persist in soil for a long period (Nikam *et al*., 2007). Rathod *et al*. (2010) reported that fungicides were helpful in controlling of seed-borne fungi of groundnut seeds when they studied the effect of fungicide on *A. flavus* and observed that thiram was one of the most fungicides inducing inhibitory effect.

Aspergillus niger followed by *R. oryzae*, *P. aculeatum* and *A. flavus* were the fungal contaminants of the germinated seeds in Petri dishes under different treatments. Imported thiram treated seeds and local thiram hand-treated seeds had the lowest contamination (0.02 and 0.03 CFU/100g seeds, respectively) as compared to the control (local non-treated seeds) which was loaded with 0.59 CFU/100g. Seed-borne infection led to infection of the radical and plumule and resulted in stunting and death of seedlings. The variation in seed infection may be due to the variation in the moisture content of the seeds; as the moisture content increases, the fungi incidence increases (Francisco and Usberti, 2008). Similar results were reported by Chisholm and Coates (1997) who evaluated the germination percentages and fungi incidences in three leguminous seeds during storage.

Duan *et al*. (2007) reported that species of *Aspergillus*, *Penicillium* and *Fusarium* are responsible for most spoilage and germ damage of stored grains and legumes during storage. They cause reduction in cooking or baking quality, and nutritive values, produce undesirable odors and color, and change the appearance of stored food grains grade. In addition, they produce mycotoxins which are health hazard for man and animals, make products unacceptable for edible purposes or lower their market grade. Fungal infestation of seed coat decreases viability of seeds, or may cause the appearance of abnormal seedlings.

The results reported in the present study indicated that there was a gradual increase in the inhibition of the *Fusarium* spp. when the concentration of thiram increased to 3000 ppm. Similar finding were reported by Patel *et al*. (2005) and Banyal *et al*. (2008) for other fungi. However, complete inhibition of *F. oxysporum* mycelia growth was observed in Richard medium (Sharma, 2006).

Cellulases and pectinases are known to destroy or

degrade cellulose in plant cell walls during pathogenesis of certain bacteria and fungi (Jia *et al*., 2009). In the present study, the cellulolytic and pectinolytic activities (determined as percentage loss in viscosity of CMC and citrus pectin, respectively), detected in the medium of the tested fungal pathogen *F. solani*, had progressively declined as the concentration of the fungicide increases to 0.00 and 32.00% at 3000 ppm, respectively (Table 4). The high activities of the cellulolytic and pectinolytic enzymes recorded in the control samples indicate the high virulence of the pathogen. A number of plant pathogenic organisms are capable of producing multiple groups of cellulases that act to hydrolyze the β-1,4-D-glycosidic bonds within the cellulose molecules (Moreira *et al*., 2005). *Fusarium solani* is considered as one of the most potent fungi in degradation of cellulose materials (Wood and McCrae, 1977). Sahab *et al*., (2007) reported that the addition of benomyl drastically affected the β-glusidases and CMC-ase production in the mycelium and culture filtrate of *F. oxyspo*rum. Munoz-Leoz *et al*., (2011) observed a reduction in β-glucosidase activity in soils exposed to tebuconazole.

The growth rate of the seedlings of the tested seeds (alfalfa, common bean and faba bean) varied under different treatments. Common bean seeds had the highest growth rate. As well, this study demonstrates that the imported thiram treated seeds even when washed had the highest growth rate as compared with the local untreated or manually thiram treated seeds. Ouf (1993) showed that the germination of *Convolvulus arvensis* and *Rumex dentatus* seeds was stimulated at the lower doses of benomyl fungicide and the stimulation was more pronounced as the soaking period was extended. However, the higher doses were inhibitory to germination of both seeds.

It was established that thiram improves seed quality. For example, maize seeds were found to have the highest germination and emergence % when thiram and carboxim were combined (Southwell *et al*., 2003). Similarly, Xue (2003) reported that germination and emergence of pea increased by 33 and 29% when thiram was used. On the other hand, Aamil *et al*. (2004) reported that thiram-treated seeds of chickpea (*Cicer arietinum* L.) at a high rate dramatically affected plant viability and seed germination dramatically.

In the present study, the number of bacterial nodules varied according to the cultivated legume and the treatment. Alfalfa recorded the highest number of nodules under different treatments reaching to 49.48 nodules/root when the local non-treated seeds were used. The number of nodules dropped to 11.33 nodules/root when the local seeds were treated with thiram. The same trend was noticed in the case of common bean. Disruption of the signaling between legume and rhizobia may have occurred after treating with the fungicide. This may cause blocking the communication between *Rhizobium* NodD receptors and legumes derived phytochemicals (Fox *et al.*, 2007). El-Bahrawy and Ghazal (1989) found that the application of an insecticide (temik) at different concentrations generally affected the formation of efficient nodules, whereas the number of nodules was reduced. It was also found that dry weight of plant and symbiotic nitrogen fixation were increased at a certain concentration. On the other hand, the numbers of nodules indicate no relation to Terracur (Phensulfothion) nematicide concentrations. Moreover an increase in numbers of nodules, dry weight of plant, and symbiotic nitrogen fixation was observed. Hamdi *et al*. (1978) found that the survival of rhizobia was enhanced when rhizobia-pelleted peanut seeds were stored at room or in a refrigerator for 10 days. Protection was more obvious for peanut seeds when kept at low temperature for many fungicides including thiram, but was not helpful for the rhizobia to survive.

Conclusion

Eight species within six genera of soil-borne fungi were recovered from legume-cultivated areas in Al Madinah Al Munawwarah. *Fusarium solani* was the most recovered fungus with 44.35%. The fungicide thiram exerted significant reduction on mycelial growth of the isolated fungi when used at the recommended rates compared with control. Moreover, cellulolytic and pectinolytic activities decreased as the concentration of thiram increased for controlling the most recovered fungus *F. solani* fungus. In the greenhouse experiment, the growth rate was the highest in the ICTWS and ICTTS for alfalfa, in the ICTTS and LNTS for common bean, and in LNTS treatment for faba bean. On the other hand, the number of nodules was higher in alfalfa followed by faba bean and common bean but was obviously reduced compared with thiram untreated seeds.

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Table 1: Frequency of fungal isolates (CFU X 10^3 g/air dry soil) recovered from the rhizospheric soil of three farms at Al Madinah Al Munawarah, Saudi Arabia.

Fungal isolate	Location			Total count	Percentage of total count
	24˚11'28''N 39˚37'7''E	24˚33'52''N 39˚35'11''E	24˚37'5''N 39˚54'26''E		
Aspergillus flavus	3	4	2	9	7.31
A. niger	8	5	9	22	17.89
Chochliobolus hawaiiensis	0	2	0	2	1.63
Fusarium equiseti	2	2	1	5	4.07
F. solani	19	14	17	50	40.65
Gibberella moniliformis	2	1	3	6	4.87
Penicillium aculeatum	6	9	4	19	15.45
Rhizopus oryzae	5	4	1	10	8.13
Total	45	41	37	123	100

Table 2: Frequency of fungi recovered from the germinated seeds (CFU/ 100 seeds) at different treatments.

Plant	Fungus	Treatment[1]				
		LNTS	LTHTS	ICTTS	ICTWS	Total
Faba bean	*Aspergillus flavus*	6	0	1	0	7
	Aspergillus niger	12	1	1	3	17
	P. aculeatum	6	2	0	0	8
	F. solani	24	0	0	2	26
	Total	48	3	2	5	58

Plant	Fungus	Treatment[1]				
		LNTS	**LTHTS**	**ICTTS**	**ICTWS**	**Total**
Common bean						
	Aspergillus flavus	0	0	0	0	0
	Aspergillus niger	9	3	3	4	19
	P. aculeatum	7	0	4	2	13
	F. solani	14	0	0	3	17
	Total	30	3	7	9	49
Alfalfa						
	Aspergillus flavus	0	0	0	0	0
	Aspergillus niger	3	0	0	0	3
	P. aculeatum	0	0	0	0	0
	F. solani	5	0	0	0	5
	Total	8	0	0	0	8

[1] LNTS: local non-treated seeds, LTHTS: local thiram hand-treated seeds, ICTTS: imported coated thiram-treated seeds, ICTWS: imported coated thiram washed seeds. There were 10 replicates, each contains 10 seeds/bag. The numbers represented the recovered fungal isolates out of 100 seeds.

Table 3: Effects of different concentrations of thiram on mycelial growth and hydrolytic exam activity of the *Fusarium* spp. determined as width of inhibition zone (mm) and their ANOVA analysis

Fungal species	Thiram concentration (ppm)				
	0	**375**	**750**	**1500**	**3000**
Fusarium equiseti 1 (KF274671)	0.00±0.00	18.8±2.75	21±0.02	20.3±1.22	26.15±1.87
F.equiseti 2 (KF274672)	0.00±0.00	21.7±0.82	21.5±0.97	22.8±1.60	24.5±2.08
F. solani	0.00±0.00	12.8±1.49	20.4±1.17	21.8±0.63	23.15±0.85
Analysis of variance					
Source	df	F	Pr>F		
Model	24	191.81	.000		
Concentration	4	239.45	.000		
Fungus	2	6.53	.002		
Replicate	9	00.90	.527		
Concentration * Fungus	8	3.52	.001		
Error	126				

Table 4: Enzyme activities of *Fusarium solani* using carboxymethyl cellulose (CMC) and pectin as substrate.

Substrate	Concentration of thiram					F value	Sign.[1]
	0	375	750	1500	3000		
CMC (Cellulase)	6.67±0.94	2.14±0.87	4.94±2.97	1.28±1.82	0.07±0.12	7.097	0.006
Pectin (Pectinase)	33.02±9.12	32.63±5.61	26.26±12.20	7.42±2.29	1.57±1.57	3.986	0.035

[1] Concentrations were significant at $\alpha = 0.05$. Three replicates were used for each concentration.

Table 5: Enzyme activities of *Fusarium solani* using local and imported seeds as substrate.

Enzyme	Substrate (seeds)	Treatment		F value	Sign.[1]
		Local	Imported		
Cellulase	Faba bean	1.54±0.005	1.66±0.12	0.468	0.532
	Alfalfa	1.82±0.26	3.09±0.57	2.340	0.200
	Common bean	2.04±0.18	11.71±5.38	1.434	0.297
Pectinase	Faba bean	37.33±3.76	21.93±6.46	2.39	0.197
	Alfalfa	44.12±0.532	50.69±7.14	0.376	0.573
	Common bean	19.99±8.29	39.33±3.16	7.37	0.05

[1] Treatments were significant at $\alpha = 0.05$. Three replicates were used for each plant.

Table 6: Growth rate (mm/day) and number of nodules (nodules/root) of the test plants under different treatments.

Test plant[1]	Growth rate				Number of nodules			
	LNTS[2]	LTHTS[3]	ICTTS	ICTWS	LNTS	LTHTS	ICTTS	ICTWS
Alfalfa	23.00±14.0	21.00±17.0	45.00±20.0	46.00±20.0	49.48.00±5.	11.33±2.7	8.98±3.8	19.76±7.
Comm	47.00±15.0	40.00±13.0	47.00±15.0	32.00±10.0	29.00±4.03	22.00±0.0	13.00±0.	14.00±2.
Faba	32.00±20.0	23.00±10.0	26.00±9.00	27.00±14.0	190.00±0.0	132.00±0.	88.00±0.	33.26±2.

[1] The total number of plants / treatment was 30 plants.

[2]LNTS: local non-treated seeds, LTHTS: local thiram hand-treated seeds, ICTTS: imported coated thiram-treated seeds, ICTWS: imported coated thiram washed seeds.

[3]Thiram concentration used in this treatment was 3000 ppm.

Table 7. ANOVA for the growth rate (cm/day) and number of nodules (nodules / root) of the test plants under different treatments.

Source	df	Growth rate (cm/day)						Number of nodules (nodules/root)					
		Faba bean		Common		Alfalfa		Faba bean		Common		Alfalfa	
		F	Pr > F[b]	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr >	F	Pr > F
Experiment	1	0.15	0.6981	19.3	<0.000	17.32	<0.000	10.0	0.0024	0.0	0.821	0.73	0.3948
Treatment [1]	3	58.6	<0.000	57.1	<0.000	274.4	<0.000	34.9	<0.000	2.3	0.084	23.6	<0.000
Pot	4	9.87	<0.000	8.31	<0.000	28.64	<0.000	1.81	0.1380	1.2	0.315	1.56	0.1966
Treatment*po	1	16.9	<0.000	14.1	<0.000	6.82	<0.000	1.81	0.0664	0.9	0.527	1.07	0.4026

[1] Treatments were: LNTS: local non-treated seeds, LTHTS: local thiram hand-treated seeds, ICTTS: imported coated thiram-treated seeds, ICTWS: imported coated thiram washed seeds.

b Treatments were significant at $P < 0.05$.

Table 8: Percentage of stand plants growing in *Fusarium* infested soil under different treatments.

Plant	Treatment[1]	Number of stand plants[2]	Percentage	F -value	P-value
Alfalfa	LNTS	11±0.84	36.67	169.41	0.000
	LTHTS	17±0.71	56.67		
	ICTTS	29±0.45	96.67		
	ICTWS	27±0.55	90.00		
Faba bean	LNTS	6±0.71	20.00	80.46	0.000
	LTHTS	12±0.84	40.00		
	ICTTS	22±0.84	73.33		
	ICTWS	18±0.55	60.00		
Common bean	LNTS	6±0.55	20.00	92.82	0.000
	LTHTS	13±0.32	43.33		
	ICTTS	24±1.30	80.00		
	ICTWS	19±0.71	63.33		

[1] LNTS: local non-treated seeds, LTHTS: local thiram hand-treated seeds, ICTTS: imported coated thiram-treated seeds, ICTWS: imported coated thiram washed seeds.

[2] The total number of plants / treatment was 30 plants.

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تأثير الثيرام كمبيد فطري مغلف للبذور على النمو والنشاط الانزيمي لفطر *Fusarium solani* في البقوليات

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ملخص

تعد العديد من الامراض الفطرية معوقات رئيسية لإنتاج البقوليات، كما تعد المبيدات الفطرية المكونات الأكثر أهمية في معالجة هذه الأمراض. لوحظ بأن بذور البقوليات المستوردة التي تم العثور عليها في السوق والتي يتم استخدامها للزراعة في منطقة المدينة المنورة هي بذور مغلفة بمبيد الثيرام، لذا تهدف هذه الدراسة إلى (أ) عزل وتعريف الفطريات الاكثر شيوعاً في حقول البقوليات في المدينة المنورة (ب) مقارنة فعالية البذور المغلفة بالثيرام مع تلك المعاملة يدوياً بالثيرام فيما يتعلق بإنبات البذور والأنشطة الإنزيمية التي تفرزها مسببات الأمراض الفطرية الأكثر شيوعاً، وبتكوين العقد البكتيرية في النبات العائل المستهدف. تم تعريف ستة أجناس وثمانية أنواع من الفطريات بناءً على الصفات الشكلية والجزيئية، وقد كانت تنتمي إلى *Fusarium solani* و *F. equisetai* و *Gibberella moniliformis* و *Chochliobolus hawaiiensis* و *Aspergillus niger* و *Penicillium aculeatum* و *Rhizopus oryzae* و *A. flavus*. كان *F. solani* الفطر الأكثر عزلاً من بين الفطريات المعزولة. ومن بين أنواع النباتات المدروسة، كان لنبات البرسيم أعلى نسبة إنبات في كل المعاملات يليه الفول والفاصولياء. كان للمبيد الفطري الثيرام عند الجرعة الموصي بها تأثير تثبيطي على نمو ميسيلوم الفطريات المعزولة مقارنة بمعاملة الشاهد. انخفضت النشاطات الأنزيمية المحللة للسيليولوز والبكتين في فطر *F. solani* مع زيادة تركيز الثيرام.

الكلمات الدالة: النشاط الانزيمي، المبيدات الزراعية، فطريات ممرضة للنبات، السعودية، نمو الجراثيم.

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