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FIELD EFFICACY OF THE COMMERCIAL FORMULATION OF THE ANTAGONISTIC TRICHODERMA HARZIANUM ON CHICKPEA WILT CAUSED BY FUSARIUM OXYSPORUM

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ABSTRACT:
Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato) is one of the major yield limiting factors of chickpea (*Cicer arietinum* L.). For eco-friendly and sustainable management of the disease, *Trichoderma harzianum* as commercial product (Biocont-T), used as seed coat and jointly amended in peat moss were evaluated against the pathogen. The study was carried out in the fields of Girdarasha research station (8.8 Km south of Erbil), College of Agriculture, Salahaddin University. A moderate Ascochyta-resistant chickpea cultivar (Flip 6-15) was used. The results showed that seed treatments with *T. harzianum* and peat moss amendments were significantly reduced the disease incidence and severity. An increase of growth rate, plant height, biological and seed yield was also occurred. Three quantities (500, 1000, 1500 g) of peat moss showed enhancements in terms of disease incidence and severity reduction and increased the growth rate and other plant agronomic parameters as compared to untreated control. Treatment efficiency towards the yield and percentage of disease inhibition (PDI%) between treatments were measured. Using 10 Bcnt, 1000 Ptms-Tri and 1500 Ptms treatments were the most efficient treatments to enhance yield. For PDI %, 1000 Ptms-Tri, 10 Bcnt1500 Ptms and 500Ptms-Tri, were showed high disease inhibition in the field.

KEYWORDS: Chickpea, Cicer arietinum, Fusarium wilt, Fusarium oxysporum f. sp. ciceris, Trichoderma harzianum, Biological control.

1. INTRODUCTION

Chickpea (*Cicer arietinum L.*) is currently the world's second most important grain legume after common bean (*Phaseolus vulgaris L.*) (Faostat, 2015). Chickpea is grown in over 50 countries, extending from subtropical and temperate regions, for its rich in protein seeds (Jukanti *et al.*, 2012). In 2013, the cultivated area of chickpea was reached to 13.5 mha with production of 13.1 MT (Faostat, 2015).

Due to susceptibility, several diseases have been attributed to cause low production in chickpeas, which include fungal diseases, bacterial and viral diseases. Chickpea wilt disease is usually caused by two or more pathogens and is referred to as a multi-pathogenic disease or a disease complex. Fusarium wilt caused by (*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato) is one of the main chickpea limiting yield factors. The disease is widespread in chickpea growing areas in the world (Haware and Nene, 1982).

Losses due to the disease is ranging from 5-10 % (Dubey *et al.*, 2007), which in years of severe epidemics may increase to 60 - 70 % (Jalali and Chand, 1992). Representative symptoms are rapid drooping of leaves and petioles, no external rotting of roots and black internal discoloration involving xylem and pith. Additionally, it can affect the crop at any stage of growth (Dubey and Singh, 2004).

There are several disease management measures that have been employed to manage and control chickpea Fusarium wilt, which include crop rotation, pathogen-free seed, removal of plant debris, and fungicide seed treatment (Nene and Reddy, 1987). More recently, there has been an international attempt to the use of eco-friendly methods for controlling pests and diseases. Application of potential harmful chemical sprays are viewed with displeasure in many countries (Harman *et al.*, 2004).

Since the pathogen is both seed and soil borne, drenching with fungicides is very expensive and impractical. *F. oxysporum* f. sp. *ciceris* is a facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crop's residues, buried in the soil for up to five to six years (Haware *et al.*, 1986). Therefore, integrated disease management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen pollution, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998).

Among biological agents, *Trichoderma*, have attracted the attention of their multipronged action against a range of plant pathogens (Harman *et al.*, 2004). Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma*, these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities (Harman, 2006). *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular (Monte, 2001).

The species of *Trichoderma* have been studied against the wilt pathogen and have showed greater potential in managing chickpea wilt under field condition (Poddar *et al.*, 2004). Use of

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reduced doses of bio-agents combination with other chemical fungicides has been highlighted for a better sustainable agricultural management (Andrabi *et al.*, 2011). Several factors affect the ability of *Trichoderma* to provide systemic disease control. Some studies focused on the role of substrates, including peat moss as primary agro-input, in which plants are grown, resistance of the host to disease, and the ability of introduced *Trichoderma* inoculum to spread under commercial conditions (Hoitink *et al.*, 2006).

Considering those points, the present study was aimed to use *Trichoderma harzianum* as biocontrol with its combinations in different quantities of peat moss and the way of their mixing to manage the chickpea wilt disease caused by *F. oxysporum* f. sp. *ciceris*.

2. MATERIALS AND METHODS

2.1 Field trial location

The experiment was conducted in the Girderasha research fields belong to the College of Agriculture, Salahaddin University (about 5 km south of the city of Hawler). A moderate susceptible chickpea cultivar Flip 6-15, resistant to Ascochyta blight, was sown in a naturally contaminated soil with the fungus *Fusarium oxysporum*. The fungus is causes wilt in chickpea and has been isolated in infected plant and soil samples from the previous year (June 2015).

2.2 Experimental design and treatments

The field experiment was designed to a randomized complete block design (RCBD)s in which seed was drilled in 25 cm spaced rows (the distance between rows), while the plant-to-plant distances (the distance between seeds) was 10 cm. The net plot size of the experiment was 2 x 1.20 m in a rate of 5 row per plot and 20 seeds per row (100 seeds per plot). The treatments were replicated 3 times with having1 m distance between treatments (plots) and between replicates (blocks). A commercial product of *Tricoderma harzianum*, Biocont-T (Ain Almasa, Saudi Arabia) is used as the bioagent and antagonistic fungus of the pathogen *Fusarium oxysporum*. The treatments used in the experiment were shown in Table (1).

Peat moss is used in treatments 3 - 6 by dispersing and mixing with the rhizosphere layer of the soil in each row of the treatment. The experiment was started in the beginning of February 2016 and watered as required in case of no rainfall.

2.3 Measured parameters

The chickpea wilt disease was assessed and was measured by evaluating parameters shown in (Table 2) directly on the plant.

Table 1. The treatments and their combinations used in the field experiment.

Treatment No.	Treatment symbol	Description
1	5Bcnt	5 g Biocont-T/kg chickpea seed*
2	10Bcnt	10 g Biocont-T/ kg chickpea seed
3	500Ptms-Tri	500 g peat moss amended with *Trichoderma*
4	1000Ptms-Tri	1000 g peat moss amended with *Trichoderma*
5	5Bcnt1500Ptms	5 g Biocont-T + 1500 g peat moss**
6	10Bcnt1500Ptms	10 g Biocont -T+ 1500 g peat moss
7	500Ptms	500 g peat moss only
8	1000Ptms	1000 g peat moss only
9	1500Ptms	1500 g peat moss only
10	UntCtrl	Untreated control-chickpea seeds only

*Chickpea seeds were firstly coated with a layer of liquid date jam then mixed with Biocont-T product.
**The commercial product of Biocont-T was mixed with peat moss just before sowing.

Table 2. The parameters were considered for data recording.

No.	Parameter	Details (time and measurement)
1	Disease incidence (DI)	At flowering and pod formation
2	Disease severity	At flowering and pod formation
3	Growth rate (GR)	After 1 month from growing
4	Plant height(PH)	At 80 % of flowering (average of 10 plants/treatment replication)
5	Biological yield (BY)	After harvesting for each treatment replication
6	Grain yield(GY)	Average yield of 10 plant per treatment replication
7	Yield efficiency (YE)	Percentage measured after calculating grain yield
8	PDI	Calculated after measuring disease incidences

2.4 Disease assessment and data analysis

Wilt disease incidence was measured by using the following formula used by (Khan *et al.*, 2004):

$$\text{Wilt incidence (\%)} = \frac{\text{No. of wilted plants in a microplot}}{\text{Total No. of plants in a microplot}} \times 100$$

Wilt severity percentage was measured by using the following equation:

$$\text{Wilt severity (\%)} = \frac{\text{No. of wilted branches in a plant} \times 100}{\text{Total No. of branches in a plant}}$$

The percentage of yield efficiency was measured using the following equation:

$$\textbf{Yield efficiency (\%)} = \frac{\textbf{Yield of treatment} - \textbf{Yield Untreated Control}}{\textbf{Yield treatment}} \times \mathbf{100}$$

The percentage of disease inhibition (PDI) is calculated using the following formula used by (Nikam *et al.*, 2007):
PDI (%) = (DIUC – DIIT) * 100 / DIUC, where;
PDI % = percentage of disease inhibition
DIUC = disease incidence in untreated control
DIIT = disease incidence in the interesting treatment

2.5 Statistical analysis

Data analysed using StatgraphicsXV5 to find ANOVA table and means compared using Fischer's least significant difference (LSD) test at P = 0.05. Data were square root transformed when necessary to minimize the variability to achieve normal distribution.

3. RESULTS

3.1 Disease Incidence

A significant low disease incidence percentage (DI %) was recorded when plots treated with *Trichoderma* amended at 1000 g peat moss (18.99 %), followed by 10Bcnt1500Ptms (19.63%) and 500Ptms-Tri (24.40 %). DI % among Biocont-T used directly on chickpea seeds were (25.15 %) for 10Bcnt and (34 %) for 5Bcnt. While the similar DI % was observed for the three levels of sole peat moss treatments (1500Ptms, 1000Ptms, and 500Ptms) which were (31.28, 31.83 and 34.29 %), respectively. Whilst highest DI % was for the untreated control (50.80 %) (Figure 1).

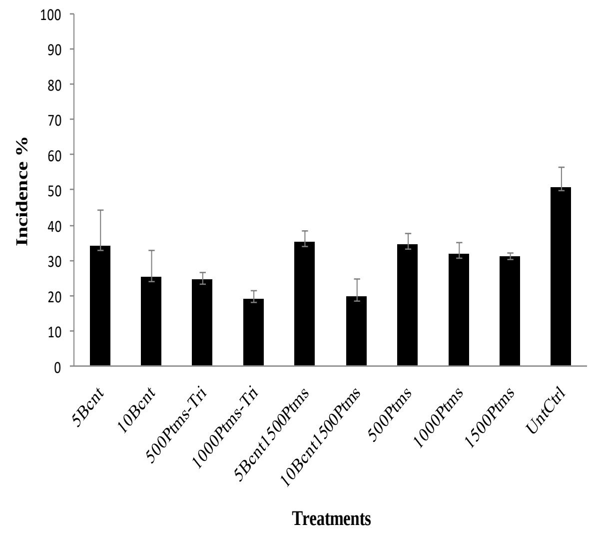

Figure 1. Disease incidence parentage for chickpea plants treated by *T. harzianum* amended with peat moss caused by *F. oxysporum* f. sp. *ciceris*. Error bars represent the standard deviations, LSD at *P*=0.05.

3.2 Disease Severity

A significant reduction of disease severity was observed in plots when treated with 10Bcnt1500Ptms (54.33 %) followed by 5Bcnt1500Ptms (70 %), 1000Ptms-Tri (75.30 %) and 500Ptms-Tri (78.33 %), respectively. However, there were no statistical difference resulted between peat moss treatments compared to untreated control (Figure 2).

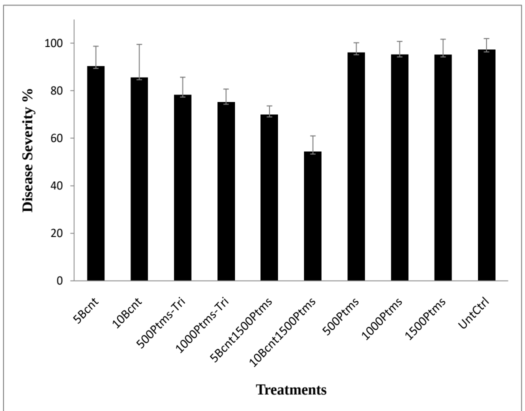

Figure 2. Disease severity percentage for chickpea plants treated by *T. harzianum* amended with peat moss caused by *F. oxysporum* f. sp. *ciceris*. Error bars represent the Standard deviation, LSD at *P*=0.05.

3.3 Growth rate (Seedling emergence):

The results in figure (3) illustrate that the treatments had no significant effect on the Growth rate (GR). However, 5Bcnt and 1000Ptms-Tri were provided highest seed germination rate (94.58 %).

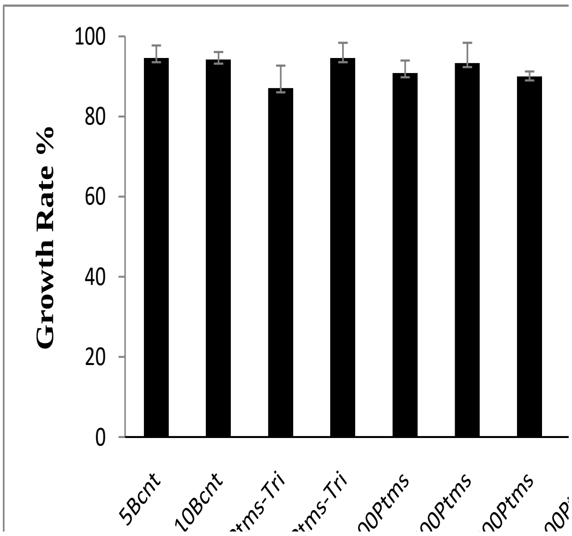

Figure 3. Seedling emergence for chickpea plants treated by *T. harzianum* amended with peat moss caused by *F. oxysporum* f. sp. *ciceris*. Error bars represent the Standard deviation, LSD at *P*=0.05.

3.4 Plant height

According to the results presented in table (3), all used treatments had no pronounced effect on the chickpea plant height. Nevertheless, 1000Ptms, 500Ptms and 5Bcnt treatments had higher plant heights compared with the untreated control.

3.5 Biological yield

Significant increase in biological yield (BY) was obtained when plots treated with 10Bct (6600 kg/ha), followed by peat moss treatments 500Ptms, 1000Ptms and 1500Ptms (5866.67, 5655.56 and 5333.33 kg/ha), respectively. However, other treatments showed less efficiency in increasing BY (Table 3).

3.6 Seed yield (kg/ha)

All treatments were increased the seed yield (SY) significantly with contrast to untreated control (2155.56 kg/ha) (Table 3). The highest seed yield was harvested in 10Bcnt (3444.44 kg/ha), followed by other treatments that ranged from 2244.44 to 2733.33 kg/ha.

3.7 Harvest index (HI)

The treatments have also affected on harvest index (HI). The highest HI was 57.22 with the use of 1000Ptms-Tri followed by 10Bcnt (53.18) and 5Bcnt1500Ptms (52.70) (Table 3).

3.8 Treatment efficiency %

All treatments were played significant role in increasing crop yield production in the field, the most efficient treatment was10Bcnt (37 %), followed by 1000Ptms-Tri (21 %) (Table3). While other treatments showed less efficacy percentage ranged 4 - 20 %.

Table 3. The response of Biocont-T, peat moss and their mixtures on chickpea plant height, biological yield, seed yield, and yield efficiency % in the field of chickpea plants caused by *F. oxysporum* f. sp. *ciceris*.

No.	Treatments	Plant height (cm)	Biological Yield (kg/ha)	Seed Yield (kg/ha)	HI (%)	% Efficiency (Yield)
1	5Bcnt	34.5	5266.67	2526.67	47.85	15 %
2	10Bcnt	31.9	6600.00	3444.44	53.18	37 %
3	500Ptms-Tri	30.9	5311.11	2606.67	49.01	17 %
4	1000Ptms-Tri	33.9	4800.00	2733.33	57.22	21 %
5	5Bcnt1500Ptms	30.4	4955.56	2622.22	52.70	18 %
6	10Bcnt1500Ptms	31.3	4822.22	2400.00	50.39	10 %
7	500Ptms	34.8	5866.67	2244.44	42.47	4 %
8	1000Ptms	35.7	5655.56	2533.33	47.81	15 %
9	1500Ptms	32.2	5333.33	2693.33	51.41	20 %
10	UntCtrl	29.2	5044.44	2155.56	43.55	0 %
	LSD= 0.05	7.15	382.65	954.38		
	Each value is an average of three replicates, LSD at *P*=0.05.					

3.9 Percentage of disease inhibition

The evaluation of disease inhibition percentage (PDI %) were calculated and the results showed a greatest inhibition by 1000Ptms-Tri (62.62 %) and 10Bcnt1500Ptms (61.36%) for the causal agent *F. oxysporum* f. sp. *ciceris* in the field (Figure 3), followed by 500Ptms-Tri (51.97%) and 10Bcnt (50.49%). However, the PDI % for peat moss treatments were 32.5 - 38.43 %.

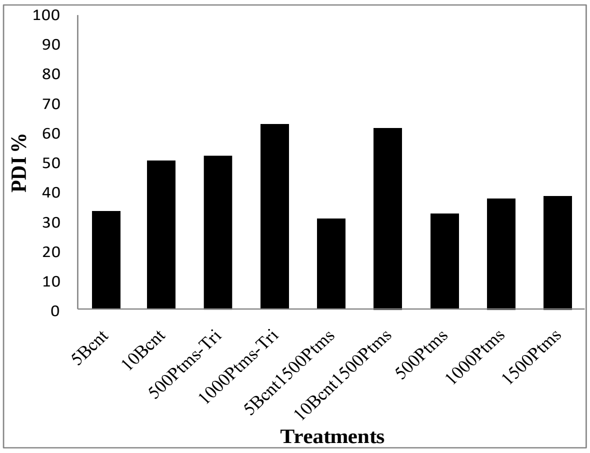

Figure 4. Percentage of disease inhibition (PDI %) for chickpea plants treated by *T. harzianum* amended with peat moss caused by *F. oxysporum* f. sp. *ciceris*.

4. DISCUSSION

In the present study, disease assessments were measured to evaluate the infield suppression of *F. oxysporum* by the commercial formulation of *T. harzianum*, Biocont-T. Obviously, all treatments in table (1) were significantly reduced the percentage of disease incidence in chickpea plants. Microorganisms that can grow in the plant rhizosphere showed the ideal capacity to be used as biocontrol agents, this agent present in the front line for the root against the pathogens (Alabouvette *et al.*, 1993). *Trichoderma harzianum* is an active colonizer in soil (Tronsmo and Dennis, 1978), because of their direct ability to parasitize (mycoparasite) on other fungi, produce antibiotics like trichodermin, gliotoxins, viridin, cell wall-degrading enzymes (Lorito *et al.*, 1993), and certain biologically active heat-stable metabolites like ethyl acetate (Mohiddin *et al.*, 2010) demonstrated as one of sufficient potential biocontrol. Some of treatments, 10Bcnt1500Ptms, 5Bcnt1500Ptms and 1000Ptms-Tri, reduced the disease severity percentage significantly. Several studies reported that β-1-3 glucanase are the main polysaccharides of fungal cell wall and they also propose chitinase and β-1-3 glucanase involve as key enzymes in the lysis of phyto-pathogenic fungal cell wall during the antagonistic action of *Trichoderma*. Therefore, fungal cell wall degrading enzymes of *Trichoderma* spp. are of particular importance in plant defence mechanisms (KüÇük *et al.*, 2007, Piegza *et al.*, 2014, Singh *et al.*, 2007).

Growth rate percentage was increased in plots treated with 5Bcnt and 1000Ptms-Tri. The present results are supported by the remarks that *Trichoderma* species produces growth factors that increase the rate of seed (Benítez *et al.*, 1998). Previous studies also observed enhanced seed germination with treatment of *Trichoderma* spp. in several other host pathogen systems (Kumar and Dubey, 2001, Dubey and Patel, 2001, Poddar *et al.*, 2004). All treatments in the present work were increased chickpea plant height in the open field. Compared to untreated control, 1000Ptms, 500Ptms, 5Bcnt and 1000Ptms-Tri were showed maximum plant height. The present findings are in agreement with that of Arora *et al.* (1992) who reported that root colonization by *Trichoderma* strains frequently enhances root and shoot growth of chickpea plants. Significant increase in biological weight, seed yield and HI were obtained where plots treated with 1000Ptms-Tri and 10Bct. This finding is supported by several studies that reported about the reduction in disease incidence and disease severity lead to higher yield in *Trichoderma*-treated seeds and soil (Singh *et al.*, 2007, Poddar *et al.*, 2004, Dubey and Patel, 2001).

Three levels of commercial peat mosses (500, 1000, 1500 kg) were used alone and amended jointly with *T. harzianum* (Table 1). The reduction performance of peat mosses, in terms of disease incidence, were (500Ptms 16.51 %, 1000Ptms 18.97 % and 1500Ptms 19.52 %⇓). Moreover, peat mosses also reduced disease severity percentage significantly and increased the growth rate percentage, plant height, biological and seed yield. These findings are supported by previous studies that reported about the suppressive of *Rhizoctonia solani* when compost was used (Tuitert *et al.*, 1998). Peat moss, organic matter in general, considered a principle medium for microorganisms, including Trichodermal colonization and it supply nutrition to the crops. Consequently, the nutrition will enhance the yield and productivity (Tuitert *et al.*, 1998).

Finally, the yield efficiency for the treatments to control *F. oxysporum* was compared. The 10Bcnt, in which 10 g of

Trichoderma was mixed with seeds, was showed the highest activity, followed by 1000Ptms-Tri, in which *Trichoderma* was amended in 1000 g of commercial peat mosses. This observation is in agreement with the work of Huber and Sumner (1996), they reported that the suppression of *R. solani* by organic matter amendments has been correlated with increased the capacity of antagonistic soil microbial activity. In terms of disease inhibition percentage (PDI %), the 1000Ptms-Tri, 10Bcnt1500Ptms, 500Ptms-Tri and 10Bcnt were significantly inhibited the occurrence of Fusarium wilt disease the causal agent *F. oxysporum* f. sp. *ciceris* in the potted chickpea field. Dubey *et al.* (2007) was observed the inhibition of *F. oxysporum* both in *vitro* and in open field by *T. harzianum* and other Trichodermal species.

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كاریگەری كێلگەیی پێكهاتەی ئامادەكراوی دژەكەڕوو، ترایكۆدێرما هارزیانەم، لەسەرنەخۆشی سیسبوونی نۆكی تووشبوو بە فیوزاریەم ئۆكسیسپۆرەم

كورتیا لێكولینێ:

نەخۆشی سیسبوونی فیوزاریومی نۆك كە بەهۆی (*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato) تووشدەبێت، یەكێكە لە هۆكارە سەرەكییەكانی كەمبوونەوەی بەرووبوومی نۆك. بۆ كۆنترۆلكردنی ئەم نەخۆشییە بە ڕێگایەكی ژینگەدۆستانە، دژە كەڕوی (*Trichoderma harzianum*) لە شێوەی ئامادەكراوی (Biocont-T) بە داپۆشینی تۆوو یان بە تێكەڵاوی لەگەڵ پیتمۆس هەڵسەنگاندنی لە دژی نەخۆشییەكە بۆ كرا. بۆ ئەم مەبەستە توێژینەوەیەكی كێڵگەیی لە بنكەی گردەڕەشەی سەر بە كۆلیژی كشتوكاڵی زانكۆی سەلاحەدین (٨،٨ كم خوارووی هەولێر) ئەنجامدرا. چەشنە نۆكی لەجۆری فلیپ ٦-١٥ ، كە تاڕاددەیەك بەرەنگارە لە دژی نەخۆشی ئەسكۆكایتا، بەكار هات. دەرەنجاماكانی توێژینەوەكە دەریانخست كە مامەلەكانی ترایكۆدێرما و لەگەڵ پیتمۆس بە شیوەیەكی بەرچاو برِ و تووندی نەخۆشییەكەیان كەمكردەوە. هەوەها لەهەمان كاتتدا ڕێژەی شینبوون و بەرزی ڕووەكەكان و بەرووبوومی بایۆلۆجی و بری بەرهەمی تۆو زۆربوون. بەكارهێنانی سێ بڕ لە پیتمۆس (500, 1000, 1500 g)، بەبەراورد لەگەڵ مامەڵەی كۆنترۆڵ، برِ و تووندی نەخۆشییەكە كەمم بووەوە هاوكات ڕێژەی شینبوون و پارامیتەرەكانی تری ڕووەكەكە زیادیانكرد. هەروەها لە توێژینەوەكەدا هەریەكە لە كارایی مامەڵەكان و ڕێژەی كەمكردنەوەی نەخۆشی (PDI %) پێوران. بەكارهێنانی هەریەكە لە مامەڵەكانی (10 Bcnt, 1000 Ptms-Tri and 1500 Ptms) باشترین كاریگەرییان هەبوو لە سەر باشكردنی بری بەری تۆو. سەبارەت بە ڕێژەی كەمكردنەوەی نەخۆشییەكەش، هەریەكە لە مامەڵەكانی (1000 Ptms-Tri, 10 Bcnt1500 Ptms and 500Ptms-Tri) باشترین كاریگەرییان هەبوو لە سەر ڕێژەی كەمكردنەوەی نەخۆشییەكە.

ألكفاءة ألحقلية للمستخلص ألتجاري للفطر ألحيوي *Trichoderma harzianum* على ذبول ألحمص ألمتسبب عن *Fusarium oxysporum*

خلاصة البحث:

ان مرض الذبول الفيوزاريومي المتسبب عن (*Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato) من اهم الامراض المحددة لانتاج الحمص. لمكافحة المرض, تم استعال وتقييم احدى الطرق المكافحة المحبة للبيئة هي المكافحة الاحيائية باستعمال الفطر (*Trichoderma harzianum*) والمحضر على شكل (Biocont-T) لتغليف البذور و بالخلط مع بيتموس. نفذت التجربة الحقلية في مركز ابحاِث كردةرةشة التابعة لكلية الزراعة لجامعة صلاح الدين (8.8 كم جنوب اربيل). وتم زراعة صنف الحمص من نوع فليب 6-15 , المقاوم نسبيا لمرض لفحة الاسكوكايتا. اظهرت نتائج التجربة ان استعال ترايكوديرما مع البيتموس ذو تاثير معنوي على تقليل نسبة و شدة المرض. وفي نفس الوقت زادت نسبة نمو البذور و ارتفاع النبات و الحاصل البايولوجي و حاصل البذور. ان استعال الكميات الثلاثة (500, 1000, 1500 g) من البيتموس ادت الى الزيادة في نسبة الانبات و مقايس الحاصل وفي نفس الوقت قللت نسبة و شدة المرض. و تم ايضا قياس كفاءة المعاملات ونسبة تقليل المرض (PDI %). وجدت ان كل من المعاملات (10 Bcnt, 1000 Ptms-Tri and 1500 Ptms) ذات كفاءات عالية على تحسين الانتاج البذور, و فيما يتعلق بنسبة تقليل المرض, فان المعاملات (1000 Ptms-Tri, 10 Bcnt1500 Ptms and 500Ptms-Tri) كانت ذو احسن فعالية على نسبة تقليل المرض.