Menoufia J. Plant Prot., Vol. 5 November (2020): 145 –154 https://mjpam.journals.ekb.eg/

EVALUATION OF SOME CHEMICAL FUNGICIDE ALTERNATIVES AGAINST *FUSARIUM OXYSPORUM* F. SP. *CAPSICI* THE CAUSAL ORGANISM OF WILT DISEASE OF PEPPER (*CAPSICUM ANNUM* L.)

M. M. Ammar[(1)], E. Z. Khalifa[(1)], S. M. H. Kamel[(2)] and Sara M. A. Kheder

(1) Faculty of agriculture, Menoufia Univ.

(2) Institution of Plant Pathology, Agricultural Research Center (ARC), Giza, Egypt.

Received: Oct. 25 , 2020 Accepted: Nov. 8 , 2020

ABSTRACT: The inhibitory effect of the plant water extracts, essential oils and some nano-particles material against the linear growth of *Fusarium oxysporum* f. sp. *capsici*; the causal organism of wilt in pepper (*Capsicum annum* L.) was evaluated *in vitro*. The plant water extracts of sweet pepper, hot pepper, garlic and mint were tested. Also, the role of essential oils of clove, mustard, mint and thyme were estimated. Meanwhile, the nano-particle materials of Calcium oxide (CaO) and Zinc oxide (ZnO) were tested against the fungal growth. Results indicate that the sweet pepper extract gradually reduced the mycelial growth of the pathogen followed by the hot pepper extract comparing with the garlic and mint extract. Increasing the tested concentrations gradually reduced the pathogen growth. Complete reduction in mycelial growth was recorded at concentration 9% of all extracts. The fungal mycelial growth was reduced significantly by the application of any tested oil. Clove oil gave the best results in reducing the fungal growth in comparison with the other tested oils. Increasing the concentration of the tested oils showed more efficiency in reducing the fungal growth and vice versa. The obtained results indicate that the tested nano-particles material CaO showed superior inhibitory effect against the growth of the pathogenic fungi at concentration 200 ppm compared with ZnO. the present study showed the possibility of usage plant water extracts, essential oils and nano-particles materials to control the pathogen.

Key words: Pepper, wilt, *Fusarium oxysporum*, water extracts, essential oils and nano-particles.

INTRODUCTION

Pepper plants were attacked by many fungal diseases which cause yield reduction and death of the er plants. Fusarium wilt caused by *Fusarium oxysporum* f. sp*. capsici* is considered one of the most important and dangerous disease among them all (Williams et al., 2004). It was noticed that the most important symptoms of this pathogen is causing a visible vascular discoloration in xylum tissues. The optimum growth temperature of *Fusarium oxysporum* f. sp*. capsici* is quite high (27℃). The present investigation was carried out at both Agricultural Botany Department, Faculty of Agriculture, Menufia university and Agricultural Research Center (ARC), Giza, Egypt. It was directed to study Fusarium wilt of pepper caused by *Fusarium oxysporum* f. sp*. capsici,* including the available control techniques *in vitro* experiments. twenty isolates were isolated from pepper plants showing wilt disease symptoms, collected from different locations of three Egyptian governorates, (Menufia, Behira and Kafr El-Sheikh). Plant water extracts of sweet pepper, hot pepper, garlic and mint at different concentrations were tested against F. oxysporum. Results showed that all plant extracts gave good

levels of control the pathogen (Jeenny 2002), sweet pepper extract showed the best efficiency.

Essential oils of clove, mustard, mint and thyme were tested with many concentrations against the pathogen growth (Viuda *et al.,* 2006). All of the oils inhibited the pathogen growth but clove oil showed the best effective oil.

Some Nano-particle materials Calcium oxide (CaO) and Zinc oxide (ZnO) also showed an inhibitory effect on the pathogen growth (Sawai and Yoshikawa, 2003). Calcium oxide (CaO) showed the most effective treatment.

MATERIALS AND METHODS

Isolation of the causal organisms and associated fungi was conducted from the collected diseased parts of pepper plants (2-3mm) following the method adopted by Agrios (1978).

The wilted roots were cut into small pieces washed thoroughly with running tap water, then washed in distilled sterile water, and immersed in 3% sodium hypochlorite for 3 minutes and washed several times in distilled sterile water. Finally, they were platted between two layers sterilized filter papers. The sterilized samples were transferred onto autoclaved PDA medium in 9 cm diameter Petri dishes, incubated at 25℃ and daily examined visually to record initiation of the in vitro growth of the causal organism. The same steps were applied with the stem base infected samples. The developed fungal colonies were microscopically examined and identified. Purification of the isolated fungi was obtained by picking hyphal tips and single spore techniques which were transferred in aseptic conditions onto PDA medium and incubated at 25℃ for 4-7 days and kept in a fridge till usage.

- Pathogenicity test :-

Sterilized pots were inoculated with fungal inoculums at the rate of 3% of soil (w:w). soil was mixed thoroughly and watered every other day for a week before planting to ensure the systemic distribution of the pathogen.

Pepper seedlings cv. Top star aged 60 days used in this experiment, three pepper seedlings were planted in each pot and watered as required (Jamiokowska 2008).

Three replicates were used for each isolate. Wilt symptoms were estimated after 30, 60, 90 days from the planting, then record the results and choose the most aggressive isolate of the fungal isolates.

- Identification of the pathogen:-

The most aggressive isolate was identified at the department of mycological researches, plant pathology institute (ARC), Giza.

- Control experiments :-

1- Role of fruit pepper extract of either sweet or hot pepper cultivars in controlling pepper wilt:-

Two pepper cultivars fruits were assigned for this study: sweet pepper cv. California wonder and hot pepper cv. El-sudany paprika. The two pepper extracts were screened for their fungi toxicity against the tested fungi.

100 gm of each cultivar were washed in running tap water then minced in 100 ml distilled sterile water in an electric mixture for 5 minutes then autoclaved and filtered through sterilized filter paper. Amounts of 3,6, and 9 ml of each extract were added to flasks containing 100 ml potato dextrose agar (PDA) medium just before pouring in Petri plates. Equal discs of 5 mm in diam. Of the pathogen were inoculated in the center of the plate. three plates were served as replicates for

each concentration. This experiment was carried out in completely randomized design with three replicates on PDA medium. The tested plates were incubated at 28c and preserved till the control plates reached maximum growth.

Efficacy % of each extract was determined according to the equation adopted by (Fokkema, 1973).

$$\text{Efficiency \%} = \frac{\text{Control - treatment}}{\text{Control}} \times 100$$

2- Using of some essential oils on the growth of the pathogen :-

In this experiment four essential oils were selected to control the pathogen i.e. mint, thyme, mustard and clove. concentrations of 500,600 and 700 ppm of each oil were used in this experiment. added to flasks containing 50 ml potato dextrose agar (PDA) medium.

Three replicates for each concentration were inoculated with 5mm diameter discs of the pathogen then incubated at 28c until reached the completely growth of the control treatment. Efficacy of each oil was measured according to the equation adopted by (Fokkema, 1973).

3- Appling of Nano-particles materials on the growth of the causal organism:-

Two nano-particles materials were selected to control the pathogen i.e. Cao and Zno with concentrations 100,200 and 300 ppm of each material. PDA medium was prepared and autoclaved in 121c for 30 minutes. After the prepared medium reached 45c, the ZnO NPs were added with the concentrations (100 , 200 and 300 mg Zn/l). Three replicates were used for each concentration, inoculated with discs of the pathogen in the center of the plate then incubated at 28c and daily examined until reach the completely growth of the pathogen in the control. The inhibitory effect of nano-particles materials was measured according to the equation adopted by (Fokkema, 1973).

- ### Preparing of the greenhouse experiments:-

Glass bottles of 500 ml size, containing 30 gm clean sand, 90 gm sorghum seeds and 120 ml water were autoclaved for 30 minutes, each was inoculated with the fungal isolates then incubated at 28℃ for 20 days. Twenty cm diameter pots were dipped in 5% formalin solution for 5 minutes to be sterilized then left in the open air to dry. These pots were filled with sand and loam soil, infested with fungal inoculum at the rate of 3% of the soil (w:w) soil was mixed with the inoculum and moistened with water every other day for one week before planting to ensure the systemic distribution. Control treatments including the plant water extracts (sweet pepper, hot pepper, garlic and mint extracts), Essential oils (clove, mustard, mint and thyme) and nano-particles (CaO and ZnO) were applied under greenhouse conditions in addition to the positive control (blank), the negative control (infested with the pathogen) and the fungicide as a control. Three replicates were used for each treatment and daily irrigated for three months. the selected fungicide was Mon-cut 25%, it was added according to the recommended dosage to compare it with other treatments.

- ### Anatomical studies:-

After applying of the obtained control treatments on the pepper plants under greenhouse conditions, micrometric slides of pepper roots were taken to identify the action of the pathogen inside *xylum* tissues. In addition to make a horizontal cutting in these roots to show the vascular discoloration.

RESULTS AND DISCUSSION

- **Effect of plant water extracts on the growth of the pathogen:-**

Results in Table (1) showed that the concentrations of 6, 9 and 12 ml\ L of each sweet pepper, hot pepper, garlic and mint water extracts significantly reduced the linear growth of *Fusarium oxysporum* f. sp. *Capsici* in comparison of control (Morsy *et al.,* 2009) (Table1). Results also showed that the most effective water extract was sweet pepper extract as it reduced the growth of the pathogen by 63.76% at the concentration 12 ml and 63% at the concentration 9 ml followed by hot pepper extract which reduced the growth of the pathogen by 62.56% at the concentration 12 ml. In the meantime, garlic and mint water extracts were the least effective extracts which reduced the growth the pathogen by 47.13 and 42.11% respectively at 12 ml. In all cases all of the three concentrations reduced the growth of the pathogen, increasing the concentration gave more efficiency in reducing the fungal growth (Abd El-Hakam, 2017 and Al-Reza et al., 2010).

Results in Table (2) indicated the effect of the applied plant water extracts had an effective results which showed that sweet pepper extract was the most effective water extract as it caused 0% of dead pepper plants which means 100% inhibition of the pathogen, followed by garlic extract which caused 75% inhibition of the pathogen then hot pepper extract which caused 67.45% inhibition of the pathogen, in the meantime mint extract was the least effective one as it caused 58.25% inhibition of the pathogen comparing with fungicide (Mon-cut 25%), the blank and the negative control which caused 75%,100% and 0% respectively inhibition of the pathogen as presented in Table (2).

Table (1): Effect of plant water extracts on the growth of the pathogen.

Plant extract	Concentrations ml \ L	Radial growth	Inhibition %
Sweet pepper	6	4.71	47.67
	9	3.33	63.00
	12	3.27	63.76
Hot pepper	6	4.62	48.67
	9	4.00	55.56
	12	3.37	62.56
Garlic	6	5.42	39.78
	9	5.00	44.44
	12	4.76	47.13
Mint	6	6.62	26.44
	9	5.50	38.89
	12	5.21	42.11
Control	-	9	0.00

LSD at 5%	0.403

Table (2): Effect of plant water extracts on the growth of the infected pepper plants after 30, 45, 60, 75 and 90 days from transplanting under greenhouse conditions.

Plant extract	After 30 days	After 45 days	After 60 days	After 75 days	After 90 days
Hot pepper	0.33	1.00	1.00	1.00	1.33
Sweet pepper	0.00	0.00	0.00	0.00	0.00
Garlic	0.33	0.33	0.67	0.67	1.00
Mint	0.33	0.67	1.00	1.33	1.67
Fungicide	0.00	1.00	0.67	0.67	1.00
Blank	0.00	0.00	0.00	0.00	0.00
Control(-)	1.00	1.33	0.51	3.00	4.00
LSD at 5%	0.725	0.632	0.821	0.534	0.646

- **Effect of nano-particles (Calcium oxide and Zinc oxide) on the growth of the pathogen:-**

Two of nano-particles materials (Calcium oxide and Zinc oxide) were tested against the pathogen at concentrations of 100, 200 and 300 ppm as presented in Table (3). The obtained results showed that CaO was more efficiency in reducing the fungal growth in comparison with control. On the other hand ZnO showed less effect than CaO. After the microscopic examination of the dishes treated with both of the nano-particles materials, it was noticed that these materials completely destroyed the pathogen growth which mean that the mycelium of the pathogen became unable to form germs (Xue et al., 2014).

Results in Table (4) showed that CaO significantly controlled the pathogen by 100% so it was considered the most effective treatment comparing with ZnO which controlled the pathogen by 66.75% and the fungicide which controlled the pathogen by 83.25%.

- **Effect of essential oils on the growth of the pathogen:-**

Four essential oils (clove, mint, mustard and thyme) were applied at the concentrations 500, 600 and 700 ppm against *Fusarium oxysporum* f. sp. *capsici.*

Results in Table (5) clear that all concentrations of all tested oils significantly inhibited the growth of the pathogen compared the control. The best effective oil is clove (700 ppm) which inhibited the fungal growth by 93.30% this was followed by mint (700 ppm) which inhibited the growth by 92.50%. however, mustard and thyme oils showed the least efficiency on growth inhibition at the same concentration (700 ppm) as they inhibited the radial growth of the pathogen by 87.10% and 85.50% respectively. In all cases increasing the concentration of any used oil caused

more inhibition of the fungal growth (Ponce et al., (2004).

Results in Table (6) showed that all tested essential oils had an inhibitory effects against the pathogen (*Fusarium oxysporum* f. sp*. Capsici*) under greenhouse conditions. Clove and mustard oils had higher inhibitor effect on the fungal pathogen than mint and thyme oils. Percentage of dead plants treated with clove and mustard oils were 25% and 50%. However, mint and thyme oils gave the same effect as the percentage of dead plants treated with them was 58.25% after 90 days from transplanting under greenhouse conditions. It was also noticed that the percentage of dead plants in pepper plants which treated with fungicide was 16.75% . Meanwhile the plank and the negative control were 0% and 100% respectivly.

Table (3): Effect of nano-particles materials (Calcium oxide and Zinc oxide) on the growth of the pathogen:-

Nano-particle material	Concentration (ppm)	Radial growth	Inhibition
CaO	100	5.26	41.56
	200	4.33	51.89
	300	3.23	64.11
ZnO	100	4.43	50.78
	200	3.63	59.67
	300	2.30	74.44
Control	-	9	0.00
LSD at 5%	0.341		

Table (4): Effect of nano-particles materials on the infected pepper plants after30, 45, 60, 75 and 90 days from transplanting under greenhouse conditions:-

Nano-particle material	After 30 days	After 45 days	After 60 days	After 75 days	After 90 days
CaO	0.00	0.00	0.00	0.00	0.00
ZnO	0.33	0.33	0.67	0.67	1.33
Fungicide	0.00	0.33	0.33	0.33	0.67
Blank	0.00	0.00	0.00	0.00	0.00
Control(-)	1.00	1.33	0.51	3.00	4.00

LSD $_{at\ 5\%}$	0.325	0.367	0.425	0.486	0.532

Table (5): Effect of essential oils on the growth on the growth of the pathogen:-

Essential oil	Concentration (ppm)	Radial growth	Inhibition %
Clove	500	1.30	85.50
	600	0.70	92.20
	700	0.61	93.30
Mint	500	1.46	83.78
	600	1.06	88.20
	700	0.67	92.50
Mustard	500	2.13	76.33
	600	1.40	84.40
	700	1.16	87.10
Thyme	500	2.67	70.30
	600	2.53	71.89
	700	1.30	85.50
Control	-	9	0.00
LSD at 5%	0.240		

Table (6): Effect of essential oils on the growth of the infected pepper plants after 30, 45, 60, 75 and 90 days from transplanting under greenhouse conditions:-

Essential oil	After 30 days	After 45 days	After 60 days	After 75 days	After 90 days
Mustard	0.33	0.33	1.00	1.67	2.00
Mint	0.67	0.67	1.33	2.00	2.33
Thyme	0.67	0.67	1.33	1.67	2.33
Clove	0.33	0.33	0.67	0.67	1.00
Fungicide	0.00	0.33	0.33	0.67	0.67
Blank	0.00	0.00	0.00	0.00	0.00
Control(-)	1.00	1.33	2.00	3.00	4.00
LSD at 5%	0.433	0.452	0.422	0.534	0.632

- Anatomical studies:-

The effect of different of treatments on internal structure of pepper stems showed in Table (7) and Figures (1-4), the results indicated that the ground tissue and conductive tissue as well as total

stem area increased with the all treatments under study. The maximum increasing of these areas recorded under treatment of CaO by about 89.86 %, 223.37 % and 140.51% as compared with the control plants (Guillemond *et al.,* 2005, Ortiz *et al.,* 2014 and Moustafa *et al.,* 2018).

Table (7): Effect of the most effective control treatments on the histological measurements of the infected pepper plants.

Character slide no.	Cortex	Pith	Ground Tissue	Phloem tissue	Xylum Tissue	Conductive tissue	Total area	x. v. diameter
Control	3.94	1.41	5.52	0.53	2.00	2.61	7.48	0.031
Clove oil	5.04	2.36	6.95	1.02	4.31	5.15	11.16	0.046
Sweet pepper ex.	7.18	3.51	10.28	1.15	5.36	6.42	15.48	0.053
CaO	7.40	3.53	10.48	1.59	6.94	8.44	17.99	0.054
LSD at 5%	0.325	0.523	0.654	0.633	0.543	0.334	0.725	0.213

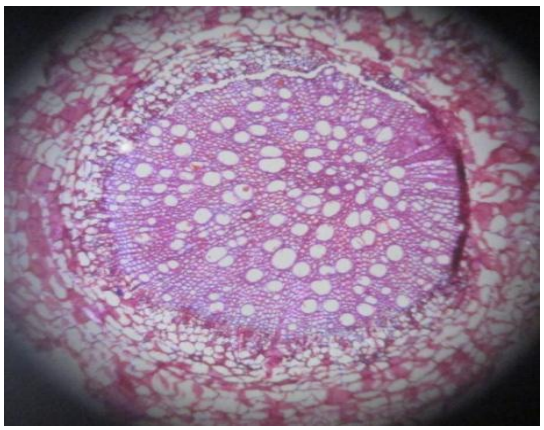
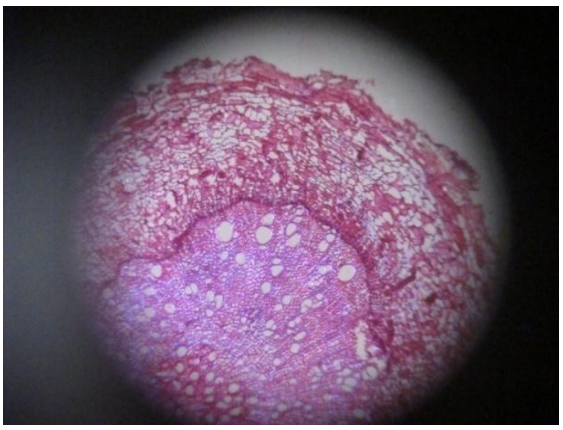


Figure (1)



Figure(2)

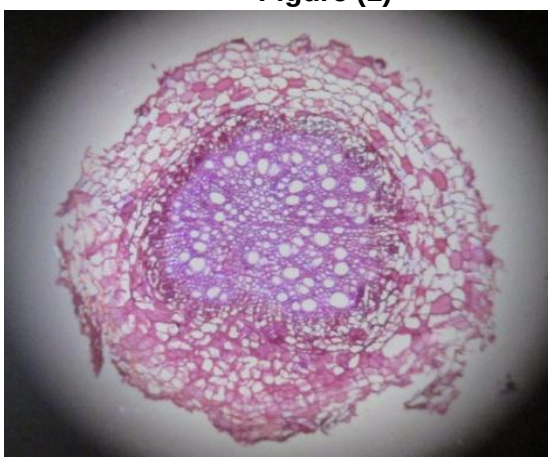


Figure (3)

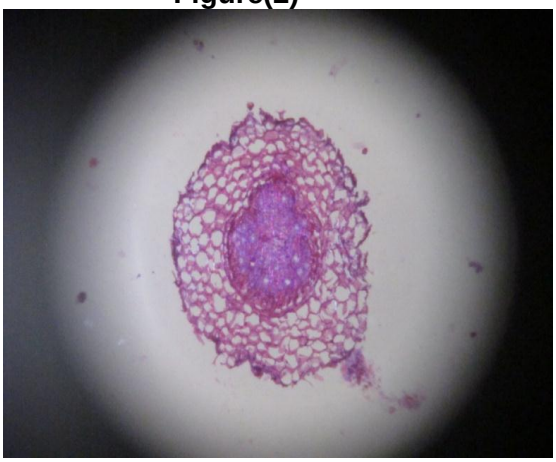


Figure (4)

Figure (1-4): Effect of the most effective control treatments on the internal tissues in the infected pepper plants which Figure (1) = Calcium oxide(CaO), Figure (2) = Hot pepper extract, Figure (3) = Clove oil and Figure (4) = Control.

REFERENCE

Abd-Elhakam, A. Sh. (2017). Studies on white mould disease of beans. Ph.D. Theesis, Faculty of Agriculture, Menoufia University, p.92.

Agrios, G.N. (1978). Subsidiary of Plant pathology. Acdemic Press. pp. (1-703) a Harcourt Brace. Javanvich, Publisher.

Al-Reza, S.M., R. Atiqur, A. Yunus and S.C. Kang (2010). Inhibition of plant pathogens *in vitro* and *in vivo* with essential oils and organic extracts of *Castrum conturnum* L. Pesticide Bio-chemistry and Physiology .

Fokkema, N.J. (1973). The role of saprophytic fungi in antagonism against *Helmithosporium sativum* on agar plats and on rye leaves with pollen. Plant Pathology 3: 195-205.

Guillemond, B. O., M. Cherif, M. Simard and L. Bernier (2005) Histopathology of Fusarium wilt of staghorn sumac (Rhus typhina) caused by *Fusarium oxysporum* f. sp. *callistephi* race 3. I. Modes of tissue colonization and pathogen peculiarities.

Jamiokowska, A. (2008). Pathogenicity of some isolates of colletotrichum coccodes and fusarium spp. To sweet pepper (capsicum annum) seedlings. Pytopathologia Polonica (49): 65-71.

Jeeny, J. (2002). Essential oils a new idea for postharvest disease control. Good and Vegetable magazine, Melbourne, Australia, 11 (3): 50.

Morsy, S.M., E.A. Dargham and G. M. Mohamed (2009). Effect of garlic and onion extracts or their intercropping, on suppressing damping-off and powdery mildew diseases and growth characteristics of cucumber. Egyptian Journal of Phytopathology, 37 (1): 35-46.

Moustafa, B., A.P. Luque, D. Rubiales and N. Rispail (2018). Physical and chemical barriers in root tissue contribute to quantities resistance to *Fusarium oxysporum f. sp. Pisi* in pea, Frontiers in plant science.

Ortiz, E., M. Cruz, L.M. Melgarejo, X. Marquínez and L. H. Carvajal (2014). Histopathological features of infections caused by *Fusarium oxysporum* and *F. solani* in purple passion fruit plants (Pass flora edulis Sims). Summa Phytopathologica, v.40, n.2, p.134-140.

Ponce, A.G., C.D. Valle and S.I. Roura (2004). Shelf life of leafy vegetables treated with natural essential oils. Journal of Food Sci., 69 (2): FMS50-FMS56.

Sawai, J. and T. Yoshikaw (2003). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. Journal of Applied Microbiology, 96, 803–809.

Viuda, M. M., Y. Ruiz-Navajas, J. Fernadez-lopez and J. A. Perez-alvarez (2006). Antifungal activites of thyme, clove and oregano essential oils, Journal of food safety 91-101.

Williams, H.J., T.D. Philliips, E.P. Jolly, K.J. Stiles, M.C. Jolly and D. Aggrawal (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure,

potential health consequences and interventions, Am. J. Clin. Nutr. 80.

Xue, A.G. and J. X. Zhang (2014). Biocontrol of *sclerotinia* stem rot (*Sclerotinia sclerotiorum*) of soybean using novel *Bacillus subtilis* strain SB24 under control conditions. Plant Pathology, 59(2): 382-391.

تقييم بعض بدائل المبيدات الكيميائية لمكافحة مرض الذبول في الفلفل المتسبب عن فطر *Fusarium oxysporum* f. sp. *Capsica*

محمد محمد بيومى عمار ، السعيد زكى خليفه، سعيد محمد حسن كامل،

ساره عبدالسلام محمد خضر

(1) قسم النبات الزراعى – كلية الزراعة جامعة المنوفية

(2) معهد بحوث أمراض النبات مركز البحوث الزراعية

الملخص العربي

يعتبر محصول الفلفل (*Capsicum annum* L.) من اهم محاصيل العائلة الباذنجانية (Family: *Solanacae*) وهو احد المحاصيل الاقتصادية الهامة في مصر وكل دول العالم لما يحتويه من نسبة عالية من فيتامينات ومضادات الاكسدة.

ويصاب محصول الفلفل بالعديد من الامراض الفطرية التي قد تتسبب في دماره والقضاء عليه، من اهم هذه الامراض مرض الذبول الفيوزاريومى المتسبب عن الفطر *Fusarium oxysporum* f. sp. *Capsici*

ويمكن تلخيص نتائج البحث في النقاط التالية:-

1- تم جمع بعض نباتات الفلفل التي ظهرت عليها أعراض الاصابة من ذبول عام على النباتات واصفرار على الاوراق وتغير في لون الجذور من اماكن مختلفة داخل المحافظات الثلاثة (المنوفية و كفر الشيخ و البحيرة).

وكانت اعلى نسبة اصابة في محافظة المنوفية بالمقارنة بمحافظتي كفر الشيخ والبحيرة

2- تم اجراء اختبار القدرة المرضية للعزلات المرضية التي تم الحصول عليها من نباتات الفلفل المصابة في صوبة قسم أمراض نباتات الخضر بمعهد بحوث أمراض النبات بمركز البحوث الزراعية بالجيزة , حيث تمت عدوى التربة بالعزلات الممرضة والري لمدة اسبوع ثم زراعة شتلات الفلفل في التربة المعداه صناعيا.

3- في هذه الدراسة تم استخدام اكثر من طريقة لمكافحة الفطر المسبب للمرض تتمثل في:-

1- استخدام بعض المستخلصات النباتية مثل (مستخلص الفلفل الحار ومستخلص الفلفل الحلو ومستخلص الثوم و مستخلص النعناع) .

2- استخدام بعض الزيوت النباتية مثل (القرنفل و النعناع و الخردل و الزعتر) .

3- استخدام بعض مواد النانوتكنولوجى مثل Calcium oxide (CaO) و Zinc oxide (ZnO) .

تم استخدام طرق المكافحة هذه في المعمل وفى الصوبة ومقارنتها بأحد المبيدات الكيماوية الفطرية (Mon-cut 25%) .

- أكثر طرق المكافحة فعالية ضد الفطر الممرض :-

1- Calcium oxide (CaO) 2- مستخلص الفلفل الحلو 3- زيت القرنفل

لذلك تم عمل قطاعات عرضية في الجذور على شرائح ميكرو مترية لدراسة تأثير الفطر المسبب على الاوعية الداخلية وتأثير الفطر ايضا على جدر الاوعية وسمكها ونصف قطر أوعية الخشب .

السادة المحكمين

أ.د/ ناجى يسين عبدالغفار كلية الزراعة – جامعة عين شمس

أ.د/ الشوادفى منصور موسى كلية الزراعة – جامعة المنوفية