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VIRULENCE OF WILT PATHOGENS AGAINST PEPPER CULTIVARS IN EGYPT

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Abstract-Pepper is considered one of the most important vegetable crops in Egypt. Wilt is a serious disease attacking pepper plants causing considerable loss of pepper productivity. One hundred and thirty-nine isolates related to 4 genera and 7 species were recovered from 40 wilted pepper plant samples (from each of the root and stem) collected from 15 localities in Assiut and Behera Governorates in Egypt. These species were encountered in the following order of number of isolates: Fusarium solani > F. oxysporum > Alternaria alternata > Macrophomina phaseolina > F. verticillioides > F. subglutinans > Acremonium strictum. Sixty isolates were tested for their pathogenicity and aggressiveness under greenhouse conditions. The highest pathogenic strains were related to Fusarium oxysporum, Macrophomina phaseolina and F. solani. The pathogenicity of these 3 species was tested against 4 cultivars of pepper (sweat balady, hot balady, sweat hybrid and hot hybrid) and the results revealed that Fusarium oxysporum (AUMC11424) showed the highest severity for all cultivars tested. On the other hand, sweat balady was the most susceptible cultivar to the pathogens while hot hybrid was the most resistant.

Keywords: Pathogenicity, Severity, Pepper cultivars, Fusarium oxysporum

1. INTRODUCTION

The main vegetables cultivated in Egypt organically are potato, garlic, onion, beans, sweet pepper, hot pepper, cucumber, cantaloupe, strawberry, tomato, cherry tomato, squash, carrot, and pea [1, 2]

Pepper, Capsicum spp. is a member of the solanaceous family and commonly divided into two groups, pungent and non-pungent, which are also called hot and sweet pepper [3, 4].

Pepper is an important group of vegetables cultivated extensively in Egypt and also widely cultivated in almost every country of the world. Egypt is the second largest producer of pepper after Nigeria [5].

Pepper has high nutritional value comprising biochemical compounds such as antioxidant phenolic compounds, volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins (A, C, E), potassium, folic acid, protein, fiber and mineral elements [6, 7, 8].

In Egypt, the total cultivated area with sweet pepper in the open field during 2013 growing season reached about 65240 faddans (faddan = 0.4 hectare) with a total production of 387964 tons (Food Legume Statistics Department, Field Crops Research Institute, ARC., 2014). In 2007 sweet pepper and hot pepper grown under plastic-houses which were about 4983 in number with an area of 2164081m^2, yielded 22188 tons [9,10] .

Fusarium wilt is considered the major devastative and destructive disease affecting crop production of pepper [11,12]. The pathogen is extremely adaptable, variable and capable of long persistence in the soil in the form of chlamydospores so the fungus survives for several years in soil. It is responsible for considerable plant mortality and consequently high losses in the yield and quality in many parts of the world [13, 14, 15, 16, 17, 18].

In Solanaceae, F. oxysporum was reported as the causal agent in tomato [19, 20], potato [21, 22], eggplant [23] and chili [24, 25, 26]and paprika (*Capsicum annuum* var. *grossum)* [27], sweet peppers (Capsicum annum) [28,29]. Studies of Fusarium wilt in chili were done morphologically [30], biologically [31], and enzymatically[32, 33] .

According to the data collected by [34] it was found that wilt is the main disease in chili crops and F. oxysporum is one of the causal agents of wilting in chili-producing countries of Asia, including India, China, Indonesia and Thailand and in Central Java [26]. The pathogens viz., Fusarium oxysporum, Rhizoctonia solani, Phytophthora capsici and Sclerotium rolfsii were isolated from bell pepper plants (Capsicum annuum L.) [35, 36, 37].

Fusarium wilt first appears as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves [38]. At the seedling stage, plants infected may wilt and die soon after symptoms appear. In older plants, leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant. Browning of the vascular tissue is a strong evidence of Fusarium wilt in sweet pepper [39].

The fungus can invade the plant roots with its sporangial germ tube or mycelium. The roots can be infected directly through the root tips, wounds in the roots caused by hail, wind,mechanical damage or insect feeding

[13, 40]. Once inside the plant, the mycelium grows through the root cortex intercellulary. When the mycelium reaches the xylem, it invades the vessels through the xylem pits [13]. Due to the growth of the fungus within the plant vascular tissue, the plant water supply is greatly affected. This lack of water induces the leave stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly. The resulting spores which on their turn act as new inoculum for further spread of the fungus [13].

Mechanisms of resistance to Fusarium wilts include strengthening cell walls through callose deposition at sites of infection, lignification by phenolic infusion in cell walls or suberization of cells, and the blocking of infected vessels and compartmentalizing of root decay through production of vessel gels and tyloses [41, 42, 43, 44, 45, 46]. Carnation (Dianthus caryophyllus L.) roots resistant to Fusarium wilt also exhibit hyperplasia of parenchyma cells adjacent to blocked xylem cells to regenerate vascular tissue [42]. Susceptible cultivars either lack these mechanisms or do not express them quickly enough to prevent the spread of the pathogen [47].

In Egypt, wilt is the main disease in pepper plants and F. oxysporum is one of the causal agents of wilting in pepper [17, 48, 49, 50]. Information on wilt disease of pepper regarding the causal agents and the degree of resistance of the different cultivars is still limited.

This Study is Aimed to:

- Isolation and identification of fungal species recovered from roots of infected plants collected from 15 localities in Assiut and Behera Governorates in Egypt.
- Evaluation of virulence potential of 60 different isolates of Fusarium solani (34), F. oxysporum (8), Alternaria alternata (7), F. verticillioides (5), Macrophomina phaseolina (4), F. subglutinans (2) and Acremonium strictum (1) using pathogenicity test under greenhouse conditions.
- Evaluation of virulence potential of 3 isolates of Fusarium oxysporum, F. solani and Macrophomina phaseolina against 4 cultivars of pepper (sweat balady, hot balady, sweat hybrid and hot hybrid).

2. MATERIALS AND METHODS

2.1 Plant Materials

Plant showing the symptoms of wilt (Figure1) were collected from pepper plantations in different localities in Assiut Governorate (36 samples) in 2012 and Behera Governorate (4 samples) in 2013 as shown in Table (1). Each sample is composite of at least 4 sites in each locality.

2.2 Isolation of Fungi Associated With Stems and Roots of Wilted Balady Pepper

The stems and roots of wilted plants were cut into equal segments (five mm slices) and were subjected to washing with 70% ethanol for one minute, rinsed in sterile water and dried with sterilized filter papers, and three of them were inserted on the surface of each of three Potato Dextrose Agar (PDA) plates. The cultures were incubated at 25 °C for 3 days. Arising colonies were transferred and streaked out into PDA agar plates and incubated for 5 days to get pure cultures of the isolates. The pure fungal isolates were characterized based on the morphology and microscopic features of colonies.

2.3 Identification

The following references were used for the identification of fungal genera and species based on macroscopic and microscopic features: [51, 52, 53] for Fusarium species and [54] and [55] for fungi in general. Identification was checked by Assiut University Mycological Centre (AUMC) specialists. One hundred and thirty nine isolates of fungi were identified and collected.

2.4 Pathogenicity Test and Disease Assessment

Sixty isolates were randomly selected and screened for their pathogenicity to determine the virulence potential under greenhouse and these isolates were related to Fusarium solani (34), F. oxysporum (8), Alternaria alternata (7), F. verticillioides (5), Macrophomina phaseolina (4), F. subglutinans (2) and Acremonium strictum (1) Table 2. The experiment was carried out during the season of 2013 under greenhouse conditions at Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Inocula of the tested isolates (15-day-old) were prepared by growing isolates on barley sand mixture (150 g barley grains + 50 g clean sand + 4 g glucose + 0.2 g yeast extract + 20 ml water) in 500-ml flasks and incubated at 25°C for 15 days. Pots (25 cm in diameter) were sterilized by immersing in 5% formalin solution for 15 minutes then left to dry. Pots were filled with sterilized soil and sand mixture at the rate of 1:2 w/w and thoroughly mixed with the inoculum of the barley-sand mixture bearing the fungus at the rate of 3% w/w and incubated for 15 days. Soil provided with the amount of soil and sand mixture and free fungal inoculum was used as control. Two pepper seedlings of sweet pepper of 4 weeks old age were transplanted into the soil of each pot. Three replicate pots were used for each isolate tested. Disease severity of wilt was determined after 60 days from planting according to [33].

$$DSI = \sum \frac{\text{(Disease severity scale x number of plants in each scale)}}{\text{The highest numerical scale index x total number of plants}}$$

Severity of wilt was determined for each plant using the following scale
0= no disease; 1= minor symptoms on a few leaves; 3= significant dwarfing, yellowing, wilt, and defoliation; 4 = some shoots with severity and some shoots dead, and 5= the whole plant dead
Plants which developed the same identical symptoms as those exhibited in the field during field survey were subjected to re- isolation and identification process to fulfill the Koch's postulate [56].

2.5 Evaluation of the Susceptibility of 4 Pepper Cultivars to Wilt Fungal Pathogens Under Green House Conditions

The three highly virulent strains of each of Fusarium oxysporum, Macrophomina phaseolina and F. solani previously tested were selected to assess their pathogenicity toward the seedlings of the 4 cultivars of Capsicum plant (sweat balady, hot balady, sweat hybrid and hot hybrid).

RESULTS

A. Identification of Wilt Causing Fungal Pathogens

Results of 40 wilted plant samples collected from different localities in Assiut and Behera Governorates and cultured on PDA medium contributed 139 fungal isolates Fig.1.1 Table1.1. The fungi isolated were identified based on their colony characteristics and counted in the following descending order: Fusarium solani (70 isolates from root and 37 from stem), Fusarium oxysporum (10 from stem and 2 from root), Alternaria alternata (7 from stem), Macrophomina phaseolina (5 from root), Fusarium verticillioides (5 from stem), F. subglutinans (2 from stem) and Acremonium strictum (1 isolate from stem only).

Fig. a. Showing Stages of Wilt Symptoms in Pepper Plants

B. Pathogenicity Test

The results of Table 1.2 (Fig. a) showed that some tested isolates of F. oxysporum (5 isolates from stem), F. solani (2 isolates from root and stem) and Macrophomina phaseolina (2 from root) have high pathogenic ability toward pepper plants and caused symptoms typical of wilting. The highly pathogenic isolates showed percentages ranging in case of Fusarium oxysporum from 57-73% severity, of M. phaseolina from 51.2-62.2 and of F. solani (51.1-60.1%) compared to the control (2.2%). The moderately pathogenic isolates were related to F. solani (5 isolates, 31.1-48.9% severity), F. oxysporum (3 isolates, 33.3-35.5% severity) and M. phaseolina (2 isolates, 40-44.4% severity). The low pathogenic isolates were related to F. solani (17 isolates, 4.4- 26.7% severity), F. verticillioides (3 isolates, 3.7-11.1% severity), Alternaria alternata (1 isolate, 8.1%), F. subglutinans (1 isolate, 5.9%) and Acremonium strictum (1 isolate, 17.8%). The remaining isolates of F. solani (9 isolates), Alternaria alternata (6) and F. verticillioides (2) showed no infection.

Table-1.1 Incidence of Fungi Recovered from Wilted Plants of Pepper in Different Localities in Assiut (Season of 2012) and Behera Governorates Season of 2013), Presented as the Number of Segments from Which the Fungal Isolates were Recovered from Nine Segments in each Sample of Root and Stem

Assiut	Localities	No. of samples (*)	Acremonium strictum		F. solani		F. oxysporum		F. subglutinans		F. verticillioides		Macrophomina phaseolina		Alternaria alternata		No. of isolates	Mean per sample
			root	stem	root	stem	root	stem	root	stem	root	stem	root	stem	root	stem		
	Dirout	1			2	2											4	0.1
	El-Koussia	3			5	3											8	0.2
	Manfalout, El-Hawatka	6			12	5		1									18	0.45
	Manfalout, Bani-sand	8			15	7		1									23	0.5
	Manqabad	7			12	8		1		1		1					23	0.5
	Alghanaim	1			5	3				1							9	0.2
	El-Fath	1			3	2						1					6	0.1
	Abnoub	5			4	2						2					8	0.2
	shdb	1			3	1											4	0.1
	Abo-teeg	2			4	1						1					6	0.1
	Reefa	1			1	1											2	0.05
Behera	Kom Hamada	2		1	2	1	1	4					2			3	14	0.35
	Badr	2			2	1	1	3					3			4	14	0.35
Total		40	0	1	70	37	2	10	0	2	0	5	5	0	0	7	139	3.4

(*) samples of roots and stems are composite of at least four sites in each locality.

Table-1.2 High, Moderate and Low Pathogenic Potential of Fungal Isolates on Sweet Pepper

Fungal taxa	Isolate No.	% of infection
Highly pathogenic		
F. oxysporum	AUMC 11423	66.7±3.8
F. oxysporum	AUMC 11424	73±6.1
F. oxysporum	AUMC 11425	60±3.8
F. oxysporum	4	57±15.7
F. oxysporum	5	67±7.8
F. solani	6	51.1±16.6
F. solani	AUMC 11513	60.1±7.6
Macrophomina phaseolina	AUMC 11512	51.2±1.2
M. phaseolina	9	62.2±2.5
Moderately pathogenic		
Fusarium oxysporum	12	35.5±2.5
F. oxysporum	13	35±1.3
F. oxysporum	14	33.3±2.2
Fusarium solani	15	39.9±10.1
F. solani	16	44.4±6.7

F. solani	17	33.3 ±8
F. solani	18	48.9±3.4
F. solani	19	31.1±1.2
F. solani	20	46.7±3.8
Macrophomina phaseolina	10	40±5.8
M. phaseolina	11	44.4±12.8
Low pathogenic		
Acremonium strictum	21	17.8±3.8
A. alternata	22	8.1±10.5
F. solani	23	4.4±2.5
F. solani	24	4.4±1.2
F. solani	25	8.9±5.1
F. solani	26	8.9±3.4
F. solani	27	15.6±4.6
F. solani	28	13.3±5.8
F. solani	29	15.5 ±1.3
F. solani	30	4.4±2.5
F. solani	31	8.8±2.5
F. solani	32	8.8±2.5
F. solani	33	4.4±2.5
F. solani	34	26.7±3.8
F. solani	35	11.1±3.4
F. solani	36	13.3±3.8
F. solani	37	26.6±11.4
F. solani	38	23.7±2.5
F. solani	39	25.2±2.5
F. subglutinans	40	5.9±1.3
F. verticillioides	41	4.4±3.8
F. verticillioides	42	3.7±6.4
F. verticillioides	43	11.1±7.7
control		2.2

Non - pathogenic (17 isolates): 6 Alternaria alternata (Nos 44, 45, 46, 47, 48, 49), 9 F. solani (Nos 50, 51, 52, 53, 54, 55, 56, 57, 58) and 2 F. verticillioides (Nos 59, 60)

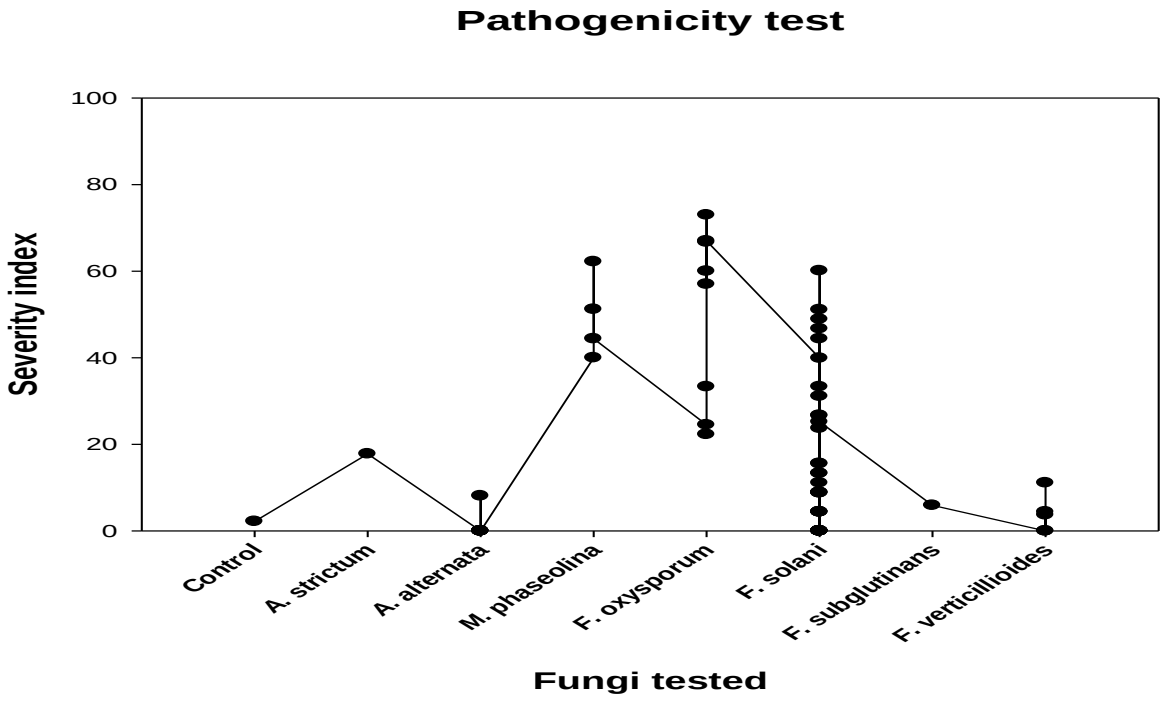

Fig. b. Pathogenic Potential of Fungal Isolates Against Sweet Pepper

C. Evaluation of the Susceptibility of Pepper Cultivars to Fungal Wilt Pathogens Under Green House Conditions

Three isolates Fusarium oxysporum (AUMC11424), Macrophomina phaseolina (AUMC11512) and F. solani (AUMC11513) which showed high pathogenic ability towards sweet pepper plants were selected and tested against 4 pepper cultivars, namely sweat balady, hot balady, sweat and hot hybrid. The isolates were pathogenic to the four cultivars but at different rates Table 1.3.

- Sweat balady was almost the most susceptible cultivar to all fungi while the hot hybrid was almost the most resistant.
- Sweat and hot balady showed higher susceptibility to Macrophomina phaseolina than the other two cultivars.
- Sweat balady (68.90%) was the most susceptible cultivar to F. oxysporum followed by hot balady (46.63), sweet hybrid (37.33%) and hot hybrid (31.13%).
- Sweat balady (44.47) was more susceptible cultivar to F. solani than the other three cultivars.
- F. oxysporum strain (AUMC11424) showed the highest severity among the four cultivars.

Table-1.3 Evaluation of the Susceptibility of The 4 Pepper Cultivars to Fungal Wilt Pathogens Under Green House Conditions, Calculated As Average Percentage Of Three Replicates

Fungi	AUMC	Sweat Balady	Hot Balady	Sweat Hybrid	Hot Hybrid
Fusarium oxysporum	11424	68.90	46.63	37.77	31.13
Macrophomina phaseolina	11512	42.20	43.23	37.77	22.20
Fusarium solani	11513	44.47	26.10	26.67	20.00
control		0.00	0.00	0.00	0.00
Mean		38.89	28.99	25.55	18.33

L.S.D: 0.05, Fungi: 16.6, Cultivars: 16.0

DISCUSSION

Results of the current survey of 40 wilted plant samples (from the root and stem) collected from different localities in Assiut and Behera Governorates revealed the isolation of 139 fungal isolates on PDA medium. These isolates showed the following descending order: Fusarium solani (34 isolates), F. oxysporum (8), Alternaria alternata (7), F. verticillioides (5), Macrophomina phaseolina (4), F. subglutinans (2) and Acremonium strictum (1). In this respect, [50] isolated Alternaria spp., F. oxysporum, Pythium spp., Rhizontonia solani, Sclerotium rolfsii and Trichoderma spp. from the roots of wilted sweet pepper plants in Egypt. [57] Recovered Alternaria alternata, Fusarium oxysporum, F. equiseti, F. solani and Colletotrichum coccodes from stem base and root rot of hot pepper. [58] Isolated from wilted chile pepper (Capsicum annuum) in New Mexico, Verticillium dahliae, Pythium capsici, R. solani, and species of Fusarium, Penicillium, and Aspergillus. [59] Identified from root (P. capsici) and stem (P. capsici and F. oxysporum) of stem rot and wilt diseases of pepper plant in Northern Nigeria.

Fungal isolates obtained from diseased pepper plants varied widely in their virulence of induction of wilt disease under greenhouse conditions and can be classified into four groups:(1) highly pathogenic isolates showed percentages ranging in case of F. oxysporum from 57-73% severity, M. phaseolina from 51.2-62.2 and F. solani (51.1-60.1%) compared to control (2.2%), (2) Moderately pathogenic isolates were also related to F. solani (5 isolates, 31.1-48.9% severity), F. oxysporum (3 isolates, 33.3-35.5%) and M. phaseolina (2 isolates, 40-44.4%), (3) Low pathogenic isolates were related to F. solani (17 isolates, 4.4-26.7%), F. verticillioides (3 isolates, 3.7-11.1% severity), Alternaria alternata (1 isolate, 8.1%), F. subglutinans (1 isolate, 5.9%) and Acremonium strictum (1 isolate, 17.8%), and (4) non-pathogenic isolates of F. solani (9 isolates), Alternaria alternata (6) and F. verticillioides (2). [60] Reported that all isolates of Fusarium obtained from diseased pepper plants are not necessarily capable of eliciting pepper disease. This is not surprising because isolates morphologically classified as F. oxysporum likely comprise several biological species based on their genetic differences [60, 61, 62, 63]. Also, [64] showed that not all isolates of Fusarium species cause wilt disease in tomato plants indicating that some isolates were probably not aggressive pathogens, they may be facultative pathogens that require high levels of plant stress to cause disease; this would indicate that both pathogenic and nonpathogenic strains of this species commonly colonize pepper plants.

Many fungi i.e Fusarium, Macrophomina, Rhizoctonia, Pythium, Verticillium and Sclerotinia causing damping-of, root rot and wilt diseases in pepper plants are commonly encountered in the greenhouse,

nurseries and fields [17, 65, 66, 67, 68]. The symptoms of Fusarium wilt observed in pepper plants in the current work were similar to those earlier described by [69]. Pathogens specific for certain plant hosts are known as 'formae speciales' [28, 66, 70, 71, 72, 73, 74]. The more detailed tests confirmed that F. oxysporum f.sp. capsici is specific for pepper plant. Recently, [75, 76, 77] showed that F. oxysporum f. sp. capsici was a reported pathogen to sweet pepper [78] . but the symptoms found are closer to those manifested by the formae speciales that cause root and crown rot of other plants while F. oxysporum f. sp. radicis-capsici f. sp. nov. was host-specific to pepper root and lower stem rot and did not cause damage to tomato, eggplant, cucumber, watermelon and chinese cabbage.

F. oxysporum was also reported as the causal agent of wilt in sweet peppers (Capsicum annum) [24, 25, 26, 28, 29, 32].

In the current work Fusarium solani induced high pathogenic ability (2 strains), moderate (6) or low (17 out of 34). Fusarium solani is extremely common in soils and is a saprohytic fungus, which means it can colonize dead or dying plant tissues. It can produce certain overwintering spores called chlamydospores that may remain viable for years. The fungus can invade pepper stems at the nodes or at the soil line, taking advantage of wounds created by pruning or salt damage [79]. Fusarium solani was reported to cause wilt disease in Indian chili crops [18, 80], in tomato and brinjal crops [81, 82, 83].

The present results are also in agreement with those recorded by [60] that the isolates of F. subglutinans and F. verticillioides tested exhibited either non-pathogenic or low virulent capability to pepper seedlings.

Macrophomina phaseolina induced high pathogenic ability (2 strains) and moderate ability (2). M. phaseolina induces diseases on a range of crops, ranging from seedling blight, root and stem rot, wilt, and pre- to post-emergence damping off, which result in decreased stem height, girth, root and head weight, or death, of affected plants [84]. [85] Isolated M. phaseolina from root rot of pepper, Egypt.

The present results revealed that Fusarium oxysporum strain (AUMC11424) was the most serious to the four cultivars tested comparable to the other two strains tested (M. phaseolina AUMC11512 and F. solani AUMC11513). On the other hand, sweat balady was the most susceptible cultivar to the three pathogens while the resistant one was the hot hybrid. [86] Revealed that all the inbred and hybrid cultivars have high level of resistance to Fusarium wilt in pigeonpea. [49] Reported that under field conditions, balady cultivar of white lupine was highly susceptible towards damping-off and root rot diseases caused by F. solani and M. phaseolina.

CONCLUSION

The present evaluation thus gave clear indication that the isolates of Fusarium oxysporum (AUMC11424) and Macrophomina phaseolina (AUMC11512) and F. solani (AUMC11513) isolated from diseased pepper plants are highly virulent pathogens, against the four cultivars of pepper (sweat balady, hot balady, sweat hybrid and hot hybrid). Sweat balady was the most susceptible cultivar to the pathogens while hot hybrid was the most resistant.

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