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Factors affecting the infection *by Fusarium oxysporum* and *F. proliferatum* and the progress of garlic clove rot disease during storage

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Abstract

This study investigated factors affecting the infection by *Fusarium oxysporum* and *F. proliferatum* causing cloves rot (CR) of garlic and developing CR disease during storage. Results showed that the Balady cultivar was the most susceptible to CR disease caused by *F. oxysporum* and *F. proliferatum*, which exhibited the lowest cloves germination and disease severity index (DSI) after 21 of planting, followed by Chinese *cv*. In contrast, Seds 40 *cv*. was the least susceptible to the CR disease, followed by American *cv*. During storage of garlic bulbs under room conditions, Balady *cv*. has exhibited the highest disease progress of CR caused by *F. oxysporum* and *F. proliferatum* after 30, 60, and 90 days, followed by Chinese *cv*. On the other hand, Seds 40 *cv*. has exhibited the lowest disease progress of CR, followed by American cv. Also, 30 °C was the most affecting the CR disease progress of garlic caused by both fungi and gradually increased the DSI of CR of stored bulbs after 90 of storage, followed by 25 °C. In contrast, 20 °C was the least affecting the progress of CR disease during storage.

Keywords:
Stored bulbs, rot, fungi, cultivars, temperature.

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the main vegetable plants cultivated in Egypt, with 333,543 tonnes resulting from an area harvested of 15,719 Ha (FAOSTAT, 2020). In Egypt and worldwide, many pests and diseases are responsible for the little crop yield of cultured garlic. However, diseases caused by fungi are the greatest destructive ones (Schwartz and Mohan, 2008). Pathogenic fungi belonging to *Fusarium* spp., the main causes of garlic clove/bulb rot disease, are the most associated virulent pathogens in the field and storage, causing severe yield loss and bulb loss during storage worldwide (Palmero *et al.*, 2010 and 2012; Xu-shuang *et al.*, 2012; Mishra *et al.*, 2013; Moharam *et al.*, 2013; Oh and Kim, 2016; Ignjatov *et al.*, 2018; Arifin *et al.*, 2021; Chrétien *et al.*, 2021; Mondani *et al.*, 2020 and 2021; Gálvez and Palmero, 2021 and 2022). Various fungi associated with garlic clove/bulb rot in the field and storage have been reported in Egypt. In this regard, *F. oxysporum* has been previously reported as the most significant pathogenic fungi causing rots of garlic bulbs during storage (Abdel-Al *et al.*, 1991). However, the fungus *F. proliferatum* (Matsush) Nirenberg has been recently reported as the main causal pathogen of clove rot disease of garlic in the field and storage (Moharam *et al.*, 2013; Elshahawy *et al.*, 2017). *Fusarium proliferatum* is a worldwide fungal pathogen of various plants. In Germany, this fungus in the past few years has been identified as a garlic dry rot agent (Seefelder *et al.*, 2002), and later, the fungus has been reported in many countries as the main causal organism of clove rot (CR) disease of stored garlic bulbs (SGB) worldwide (Stepien *et al.*, 2011; Tonti *et al.*, 2012; Fuentes *et al.*, 2013; Moharam *et al.*, 2013; Elshahawy *et al.*, 2017; Horákova et al., 2020; Chrétien *et al.*, 2021; Anisimova, Olga *et al.*, 2021). This fungus, as a pathogen, may contaminate seed cloves, infects and colonizes plant roots during garlic growth in the field, and causes later cloves rot disease of SGB (Gálvez and Palmero, 2022). Traditional approaches for controlling CR disease of SGB by fungicide applications in the field and storage are dangerous to humans and animals and very harmful to the environment. Therefore, searching for safe, eco-friendly alternative methods to controlling this disease has become necessary. This study was intended to study some factors affecting the infection by *F. oxysporum* and *F. proliferatum* and the development of cloves rot disease, such as garlic cultivars and the storage temperature of bulbs depending on the garlic cultivar stored.

MATERIALS AND METHODS

Factors affecting the infection by *F. oxysporum* and *F. proliferatum* and the development of garlic cloves rot disease during storage

1. Garlic cultivars (Susceptibility of garlic cultivars to infection by *F. oxysporum* and *F. proliferatum*)

The effect of garlic cultivars on cloves rot disease caused by *F. oxysporum* and *F. proliferatum* was studied under open greenhouse conditions. Also, the progress of cloves rot disease during storage of garlic bulbs after harvesting was studied. The experiments were conducted in the Experimental Farm, Faculty of Agriculture, Sohag University, during the 2020/2021 and 2021/2022 growing seasons. The sowing date of both seasons was the 1st of October. Highly virulent isolates of *F. oxysporum* (No. 13) and *F. proliferatum* (No. 11) causing garlic cloves rot, kindly obtained from fungal stock culture collection of the Plant Pathology Department, Faculty of Agriculture, Sohag University (Moharam *et al.*, 2013), were used in these experiments. Garlic seed cloves of four cultivars, namely; American, Balady, Chinese, and Seds 40, were kindly obtained from Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Cloves were surface disinfected by dipping in 0.1% sodium hypochlorite solution for 3 min and washed three times with sterilized distilled water, and then left at room conditions for drying. The inocula of *F. oxysporum* and *F. proliferatum* were prepared by placing two equal agar disks (0.5 cm) obtained from the 7-day-old cultures into flasks containing 200 ml autoclaved liquid potato dextrose agar medium. Then the inoculated flasks were checked by placing them on a rotary checker at 3.000 rpm and 28±1 °C for 2 weeks. The fungal growth was collected by filtering the growth through sterilized filter paper, washed several times with sterilized

distilled water, and blended in 200 ml of distilled water for 30 sec with a sterilized blender. The fungal spore suspension of each inoculum was then adjusted to 10^6 spores ml^{-1} by a hemocytometer. Then each spore suspension was immediately supplied with 50 mg of streptomycin sulfate. Cloves were soaked in the spore suspension of each fungus for 24 h before planting in 30 cm formalin-sterilized plastic pots filled with formalin-sterilized clay loam soil. Cloves treated with sterilized distilled water served as control. The experiment used a completely randomized block design with 8 pots (replicates) of each tested cultivar. Five cloves were planted in each pot, and the pots were irrigated every other day. Three pots were used to assess cloves germination and clove rot disease within 21 days of planting, and the rest were left to obtain mature bulbs. Clove rot symptoms were visually examined and graded on five scales according to Stankovic *et al*. (2007) as follows: 1 = no rot symptoms; 2 = < 10% rotted cloves; 3 = 10–50% rotted cloves; 4 = > 50% rotted cloves; 5 = completely rotted cloves.The disease severity index (DSI) of each tested fungal isolate in each pot (replicate) was then calculated by the formula:

$$\mathbf{DSI = (\Sigma S_i \times N_i) / (5 \times N_t) \times 100}$$

S_i is the severity rating 0-5, N_i is the number of cloves in each rating, and N_t is the total number of rated cloves. At the end of the trial, garlic bulbs were immediately harvested, left in the open drying shed, and then stored under open room conditions. After 30, 60, and 90 days of storage, the bulbs were visually examined for cloves rot symptoms, and the DSI of stored bulbs was calculated as described above. The means over both growing seasons were calculated and then statically analyzed.

2. Storage temperature

The progress of cloves rot disease during storage of the harvested bulbs depends on the temperature, and the stored garlic cultivar (Balady and Seds 40, the highest and least susceptible *cvs.*, respectively) was studied. The experiments were conducted in the open greenhouse at the Experimental Farm, Faculty of Agriculture, Sohag University, during the 2020/2021 and 2021/2022 growing seasons. The sowing date of both seasons was the 1st of October. As mentioned above, the garlic seed cloves of Balady and Seds 40 cultivars were inoculated with *F. oxysporum* and *F. proliferatum* and sowed in 30 cm plastic pots. Cloves treated with sterilized distilled water served as control. The trials were carried out using a completely randomized block design with 18 pots of each cultivar. Five cloves were planted in each pot, and the pots were irrigated every other day. At the end of the trial, garlic bulbs were harvested, left in the drying shed, and then stored in rooms at 20, 25, and 30 °C. After 30, 60, and 90 days of storage, bulbs were visually examined for CR symptoms, and the DSI was calculated as described before. This trial was done in a split split plot design, and 3 replicates for each treatment were used. The means over both growing seasons were calculated and then statically analyzed.

Statistical analysis

Data obtained were statistically analyzed by the MSTAT-C program version 2.10. Duncan's multiple range tests for means comparing and the least significant difference (L.S.D.) at the p= 0.05 level of probability was used as described by Gomez and Gomez (1984). Values represented in the figures are the means, and the bars show the standard error.

RESULTS

Factors affecting the infection by *F. oxysporum* and *F. proliferatum* and the development of garlic cloves rot disease during storage

1. Garlic cultivars (Susceptibility of garlic cultivars to infection by *F. oxysporum* and *F. proliferatum*)

Data in Table 1 shows that out of the four garlic cultivars tested for infection by *F. oxysporum* and *F. proliferatum* during the 2020/2021 and 2021/2022 growing seasons in the open greenhouse, Balady *cv*. was the highest susceptible (HS) one to cloves rot disease caused by *F. oxysporum* and *F. proliferatum*, where it exhibited (51.25 and 37.25%, respectively) and (43.75 and 66.75%, respectively) of cloves germination and DSI, respectively after 21 of planting, followed by Chinese *cv*. with (66.25 and

30.75%, respectively) and (56.25 and 58.25%, respectively) of cloves germination and DSI, respectively. In contrast, Seds 40 *cv*. was the least susceptible (LS) to the cloves rot disease caused by *F. oxysporum* and *F. proliferatum*, which exhibited (76.25 and 17.25%, respectively) and (68.75 and 49.75%, respectively) of cloves germination and DSI, respectively after 21 of planting, followed by American *cv*. with (71.25 and 25.50%, respectively) and (51.25 and 53.75%, respectively) of cloves germination and DSI, respectively. Table 1 and Fig. 1 a and b also show that during storage of the harvested garlic bulbs of both growing seasons under room conditions, Balady *cv*. has exhibited the highest disease progress of cloves rot caused by *F. oxysporum* and *F. proliferatum* after 30, 60, and 90 days, where it showed (23.25, 37.50, and 49.25%, respectively) and (34.25, 47.50, and 68.75%, respectively), followed by Chinese *cv*. with (18.25, 26.75, and 39.25%, respectively) and (30.25, 42.75, and 63.25%, respectively). In contrast, Seds 40 *cv*. has exhibited the lowest disease progress of cloves rot caused by *F. oxysporum* and *F. proliferatum* in stored garlic bulbs after 30, 60, and 90 days of storage, where it exhibited (14.75, 21.25, and 33.75%, respectively) and (25.50, 38.25, and 57.75%, respectively), followed by American *cv*. with (16.25, 24.25, and 36.25%, respectively) and (28.25, 49.75, and 60.25%, respectively).

2. Storage temperature

Data in Table 2 and Fig. 2 a and b show that out of the three storage temperatures affecting the progress of cloves rot disease caused by *F. oxysporum* and *F. proliferatum* in stored bulbs of both garlic Balady (the highest susceptible) and Seds 40 (the least susceptible) cultivars after 30, 60, and 90 days of storage, 30 °C was the most affecting ones. It highly increased DSI of cloves rot disease caused by both fungi in stored bulbs of both garlic cultivars to (50.25 and 34.25%, respectively) and (69.5 and 58.5%, respectively) after 90 of storage, followed by 25 °C with (38.75 and 25.25%, respectively) and (48.25 and 39.75%, respectively) of both fungi, respectively. In contrast, 20 °C was the least affecting the development of cloves rot disease caused by both fungi in both tested garlic cultivars. It slightly increased the DSI of cloves rot disease caused by both fungi in stored bulbs of both garlic cultivars to (22.25 and 13.5%, respectively) and (27.75 and 17.75%, respectively) after 90 of storage.

Table 1: Susceptibility of garlic cultivars to infection with *F. oxysporum* and *F. proliferatum* under greenhouse conditions during the 2020/2021 and 2021/2022 growing seasons and development of cloves rot disease of garlic bulbs after 30, 60, and 90 days of storage under room conditions.

Garlic cultivars	*F. oxysporum*					*F. proliferatum*				
	Cloves germination (%)	DSI*	DSI** of stored bulbs after			Cloves germination (%)	DSI*	DSI** of stored bulbs after		
			30 days	60 days	90 days			30 days	60 days	90 days
American	71.25***	25.50	16.25	24.25	36.25	51.25	53.75	28.25	40.75	60.25
Balady	51.25	37.25	23.25	37.50	49.25	43.75	66.75	34.25	47.50	68.75
Chinese	66.25	30.75	18.25	26.75	39.25	56.25	58.25	30.25	42.75	63.25
Seds 40	76.25	17.25	14.75	21.25	33.75	68.75	49.75	25.50	38.25	57.75
L.S.D. at 5%	2.00	1.91	1.91	1.91	0.99	1.99	0.99	0.99	1.15	1.91

* DSI of rotted cloves after 21 days of planting.

** DSI of rotted cloves of stored garlic bulbs after 30, 60, and 90 days of storage under room conditions.

*** The values are the means over the two growing seasons.

Table 2: Effect of storage temperature on the development of cloves rot disease caused by *F. oxysporum* and *F. proliferatum* in stored bulbs of garlic Balady (HS) and Seds 40 (LS) cultivars after 30, 60, and 90 days of storage.

Storage Temperature (°C)	Garlic cultivar	*F. oxysporum*				*F. proliferatum*			
		30 days	60 days	90 days	**Mean**	30 days	60 days	90 days	**Mean**
20	Balady	14.75*	19.50	22.25	**18.83**	17.75	23.50	27.75	**23.00**
	Seds 40	6.75	10.25	13.50	**10.16**	9.25	14.50	17.75	**13.83**
	Mean	**10.75**	**14.78**	**17.78**	**14.49**	**13.50**	**19.00**	**22.75**	**18.42**
25	Balady	20.25	28.75	38.75	**29.25**	29.25	36.75	48.25	**38.08**
	Seds 40	11.50	19.75	25.25	**18.83**	21.50	27.50	39.75	**29.58**
	Mean	**15.87**	**24.25**	**32.00**	**24.04**	**25.37**	**32.12**	**44.00**	**33.83**
30	Balady	24.75	39.25	50.25	**38.08**	35.75	48.25	69.50	**51.16**
	Seds 40	12.25	22.50	34.25	**23.00**	26.25	39.50	58.50	**41.41**
	Mean	**18.50**	**30.87**	**42.25**	**30.54**	**31.00**	**43.87**	**64.00**	**46.28**
General Average		**15.04**	**23.30**	**30.67**	**23.02**	**23.29**	**31.66**	**43.58**	**32.84**
L.S.D. at 5%									
Storage temperature (A)		1.52	2.23	3.57	2.19	2.28	3.65	3.68	3.76
Cultivars (B)		1.83	2.92	4.89	2.82	2.96	4.94	4.95	4.99
A × B		0.71	0.97	1.66	0.93	0.99	1.69	1.76	1.74

* The values of DSI of cloves rot of stored bulbs are the means over the 2020/2021 and 2021/2022 harvest seasons.

DISCUSSION

In this study, some factors affecting the infection by *F. oxysporum* and *F. proliferatum* in the open greenhouse and the development of clove rot disease during storage of bulbs, such as garlic cultivars and storage temperature, were also studied. In this concern, the susceptibility of certain garlic cultivars to infection by *F. oxysporum* and *F. proliferatum* under open greenhouse conditions and the progress of cloves' rot disease during storage under room conditions were evaluated. Results showed that out of the garlic cultivars tested, Balady *cv*. was the most susceptible to cloves rot disease caused by *F. oxysporum* and *F. proliferatum*, which exhibited the lowest cloves germination and disease severity index of cloves rot after 21 days of planting, followed by Chinese *CV*. In contrast, Seds 40 *cv*. was the least susceptible to the cloves rot disease, which exhibited the lowest cloves germination and highest disease severity index of cloves rot 21 after plantings, followed by American *cv*. Also, during storage of garlic bulbs under room conditions after harvesting both growing seasons, Balady *cv*. has exhibited the highest disease progress of cloves rot caused by *F. oxysporum* and *F. proliferatum* after 30, 60, and 90 days, followed by Chinese *cv*. In contrast, Seds 40 *cv*. has exhibited the lowest disease progress of cloves rot of stored bulbs after 30, 60, and 90 days of storage, followed by American *cv*. These findings could be interpreted in light of similar other results previously reported by Shalaby *et al.* (2012), Moharam *et al.* (2013), El-Marzoky, Hanan and Shaban (2014), Elshahawy *et al.* (2017), and Ahmed, Naglaa *et al.* (2022). In addition, several investigators worldwide have reported other resistant and susceptible garlic cultivars and varieties to clove/bulb rot disease caused by *F. oxysporum* and *F. proliferatum* and/or other garlic diseases caused by pathogenic fungi (Shalaby *et al.*, 2012; Palmero *et al.*, 2013; Ignjatov *et al.*, 2018; Jannatun *et al.*, 2020; Filyushin *et al.*, 2021; Anisimova, Olga *et al.*, 2021). Thus, a closely established relationship exists between the presence of the pathogen *F. oxysporum* and *F. proliferatum* in the garlic bulb at harvest and the incidence and progress of cloves rot disease in storage. Differences in susceptibility of

garlic cultivars to *F. oxysporum* and *F. proliferatum* mainly due to genetic factors (Shalaby *et al.*, 2012; Palmero *et al.*, 2013; El-Marzoky, Hanan and Shaban, 2014; Elshahawy *et al.*, 2017; Ignjatov *et al.*, 2018; Jannatun *et al.*, 2020; Filyushin *et al.*, 2021; Anisimova, Olga *et al.*, 2021). In this study, out of storage temperatures affecting the development of cloves rot disease of garlic Balady (the highest susceptible) and Seds 40 (the least susceptible) cultivars after 30, 60, and 90 days of storage, 30 °C was the most affecting ones and gradually increased disease severity index of cloves rot caused by both fungi on stored bulbs of both garlic cultivars after 90 days of storage, followed by 25 °C. In contrast, 20 °C was the least affecting the progress of cloves rot disease caused by both fungi on both tested cultivars. These results could be interpreted in light of other similar findings reported by Mishra *et al.* (2013), Palmero *et al.* (2013), El-Marzoky, Hanan and Shaban (2014), and Gálvez and Palmero (2022). Until now, as available information in the literature, a few investigators have discussed storage temperature as a factor affecting the infection with *F. oxysporum* and *F. proliferatum* and the development of cloves rot disease of garlic bulbs during storage depending on the temperature and the garlic cultivar stored. In previous studies, the high temperature (30 °C or above) favored *Fusarium* rot in stored garlic bulbs caused by *F. solani* (Mishra *et al.*, 2013), and clove rot incidence and severity of garlic caused by *F. proliferatum* increased progressively with an increase in storage time at room temperature of 20 and 25 °C (Palmero *et al.*, 2013; Gálvez and Palmero, 2022). In this study, the response of both garlic cultivars to storage temperatures was exceptionally gradual and different from cloves' rot development in all storage periods (30, 60, and 90 days), with the disease severity index being much lower in Seds 40 *cv.* than Balady *cv.*, likely due to different varietal susceptibility to infection by both fungi. In addition, the optimum temperature for mycelial growth of *F. oxysporum* and *F. proliferatum* is 25, and 30 °C (Mishra *et al.*, 2013; Palmero *et al.*, 2013; Elshahawy *et al.*, 2017), which favors *Fusarium* clove rot of garlic during storage and explain the high increase in the disease severity index of cloves rot caused by both fungi when bulbs were stored at 25 and 30 °C for 30, 60, and 90 days.

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العوامل المؤثرة على الإصابة بكل من الفطرين *Fusarium oxysporum* و *F. proliferatun* وتطور مرض عفن الفصوص الثوم أثناء التخزين

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تم دراسة بعض العوامل المؤثرة علي الإصابة بالفطرين المسببين لعفن فصوص الثوم. أظهرت النتائج أن الصنف "بلدي" للثوم كان الأكثر عرضة للإصابة بمرض عفن الفصوص المتسبب عن الفطرين *F. proliferatum* و *F. oxysporum*. في المقابل، فإن الصنف "سدس 40" كان الأقل عرضة للإصابة بمرض عفن الفصوص، يليه الصنف "الأمريكي". أثناء تخزين أبصال الثوم تحت ظروف الغرفة، أظهر الصنف "بلدي" أعلى نسب تطور لمرض عفن الفصوص المتسبب عن الفطرين *F. proliferatum* و *F. oxysporum* بعد 30 و 60 و 90 يومًا من التخزين، يليه الصنف "الصيني". في المقابل، أظهر الصنف "سدس 40" أدنى تطور لمرض عفن الفصوص، يليه الصنف "الأمريكي". أيضًا، كانت درجة الحرارة 30 مئوية هي الأكثر تأثيرًا على تطور مرض عفن الفصوص للثوم المتسبب عن الإصابة بالفطرين حيث زادت تدريجيًا قيم معامل شده المرض لعفن الفصوص للأبصال المخزونة بعد 90، تليها درجة حراره 25 مئوية. في المقابل، كانت درجة الحرارة 20 مئوية هي الأقل تأثيرًا على تطور مرض عفن الفصوص أثناء التخزين.

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