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LEAF AND POD SPOT OF COWPEA CAUSED BY *TALAROMYCES TRACHYSPERMUS* IN EL-MINYA GOVERNORATE OF EGYPT, AND THE PATHOGEN SURVIVAL ON CROP RESIDUES.

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ABSTRACT

Cowpea is subject to attacks by a wide range of plant pathogens including bacteria, fungi, viruses, and nematodes. In this study, a field survey conducted among cowpea growing regions of El-Minya governorate of Egypt including Minya, and Maghagha districts for leaf and pod spot disease. Our results revealed that the disease was present in all surveyed fields with the highest disease incidence and severity found in Minya District. The causal agent determined to be *Talaromyces trachyspermus* based on morphology of the fungal isolates, internal transcribed spacer sequence homology to a *T. trachyspermus* strain, as well as fulfillment of Koch's postulates. Our study showed that detached pods of soybean, cowpea and plant foliage of thyme were the most susceptible to *T. trachyspermus*, presenting between 97.77 –77.78% DI% and 38.78- 61.66 DS%, whereas moderate infection was recorded on Chill pepper, Colored bell pepper, bean, faba been, Black seed, Green (sweet) pepper showed the lowest infection caused by this pathogen, while okra, sage and rosemary were the most resistant showed no infection. In addition, survival of *T. trachyspermus* was studied on non-buried and soil buried plant residues for 30 months. Data showed that colony forming units (CFUs) of the pathogen were recovered up to 30 months under both conditions, with a significant decrease in CFUs at each time point. However, the causal pathogen remained capable of producing diseases in cowpea plants. These results highlight the ability of this pathogen to survive on plant residues for long time with the capacity to infect plants.

Keywords: Cowpea, leaf and pod spots, *Talaromyces trachyspermus*, Plant residues

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walpers) is a climbing annual crop, belonging to the family *Fabaceae*, originates from Africa, and grown for its edible seeds and pods. It is an important multipurpose legume crop in semi-arid tropical and subtropical regions, Due to its high protein content (20–25%), carbohydrates (63.3%), iron (48.69 mg kg-1), zinc (29.9–41.8 mg kg-1), fibre (6.3%), fat (1.9%), and essential amino acid lysine (Hafiz and Damarany, 2006 and FAO, 2016). Fresh pods and leaves offer an inexpensive source of vitamins and minerals. In addition, it is planted for its nourishing livestock fodder, green manure crop, hay crop, mulch crop, intercrop, and potential use as a trap crop. Cowpea is characterized by its quick vegetative growth, high ecological diversity, ability to grow in a variety of soil textures, and its tolerate to high temperatures, which makes it an effective cover crop, and soil fertility enhancer (Elawad, 2000; Hector and Jody, 2002; Hall, 2012; Oyewale and Bamaiyi, 2013; Giridhar *et al*., 2020). According to FAOSTAT (2021), the total area under cultivated of cowpea in Egypt was estimated at 1968 hectares with a production of 7216.29 tons of dry seeds.

Many plant pathogens attacks cowpeas including fungi, bacteria, viruses, and nematodes, and causes a severe loss in its stages of growth and development. Leaf spots and blight caused by fungal pathogens such as *Colletotrichum capsici* or *C. truncatum*, *Cercospora canscens*, *Pseudocercospora cruenta*, and *Asochyta phaseolorum*, as well as other foliar diseases such as rust and powdery mildew are the most distractive diseases that causes a great damage to cowpeas crop (Summerfield and Roberts, 1985)

The genus *Talaromyces* is described as the sexual state of *Penicillium* species (Benjamin, 1955), which it is a genus of fungi that produces asci in chains. According to Benjamin (1955), *Talaromyces* is the species that was transferred from the unsatisfactory genus *Penicillium*. However, the morphological, ecological, and molecular characteristics have implied the common intrinsic properties of *Penicillium* subgenus *Biverticillium* and *Talaromyces* from those in subgen. *Aspergilloides*, *Furcatum*, and *Penicillium*, thus *Talaromyces* was regarded as the valid genus for these species irrespective of teleomorphs or anamorphs (McNeill *et al*., 2012). *Talaromyces* species have been isolated from various resources including soil samples (Adhikari *et al*., 2015; and Fang and Shi, 2016), food samples (Tranquillini *et al*., 2017), marine sponges of coral reefs (Dethoup *et al*., 2015), coastal plant roots (Kim *et al*. 2014), and as plant endophytes (Qadri *et al*., 2013; Romão-Dumaresq *et al*., 2016). According to Saikkonen *et al*. (1998) and Schulz and Boyle (2005).

In this regard, many studies reported the isolation of *Talaromyces* species as endophytic fungi with many plant species and found to play a role as plant growth promoter and as a biocontrol agent (Farhat *et al*., 2021). For instance, *Talaromyces trachyspermus* (Shear) Stolk and Samson isolated from leaves of the medicinal plant *Withania somnifera* and was reported as a plant

growth promoting traits and biocontrol, which may enhance the medicinal value of the plant (Sahu *et al*., 2019). As a biocontrol agent, *Talaromyces trachyspermus* was reported in many studies to control pathogens such as *Verticillium dahliae, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotinia rolfsii* which consider important soil borne pathogens in several crops including aubergine, beans, cotton, potato, and tomato (Dutta, 1981; Madi *et al*., 1997; Tjamos and Fravel, 1997; Menendez and Godeas, 1998; Naraghi *et al*., 2012). However, many species of *Talaromyces* including *T. trachyspermus, T. bacilisporus, T. helicus, T. macrosporus,* and *T. stipitatus* were found to be a food borne pathogens producing a heat-resistant ascospores and distinctive mycotoxins that can cause cancer in the liver and kidneys, including cyclochlorotine, islanditoxin, erythroskyrine, and luteoskyrin (Pitt and Hocking, 2009: Yilmaz *et al.*, 2014;; Schafhauser *et al*., 2016). As a plant pathogen, species of the genus *Talaromyces* were found to be the causal agents of postharvest diseases, *i.e., T. albobiverticillius* on pomegranate (Mincuzzi *et al*., 2017), *T. rugulosus* on grapes (Yang *et al.*, 2017), *T. minioluteus* on onion bulbs and quince, tomato and orange fruit (Stošić *et al*, 2020), and the latter two species on pears as well (Stošić *et al*., 2021). In addition, *Talaromyces trachyspermus* causes necrosis, blight, wilt, and soft rot symptoms on the bases of broomrape (*Orobanche* spp.) plants in the North-west of Iran (Hemmati and Ghlolizadeh, 2019). Moreover, In 2020, Youa *et al*., isolated *T. halophytorum* sp. nov. from the decayed roots of *Limonium tetragonum* in Korea. It is worth mentioning here that Climate change, especially global warming, affects the geographical distribution of species on earth, and is a vital factor of new emergency of plant diseases.

More than 19,000 fungi are known to cause plant diseases. These pathogens may survive on both living and dead plant tissues while dormant until conditions are right for their multiplication, while some fungi can grow inside the tissues of the host plant. Wind, water, soil, insects, and other invertebrates helps fungus spores to easily disseminate (Lazarovits *et al*., 2014). It is generally known that crop residues act as a source of inoculum for plant diseases, and studies demonstrated that residues of diseased hosts should be considered in plant disease management systems. Of course, when crop residues are preserved in the field after harvest using reduced- or no-tillage techniques, soil organic carbon is created, soil structure is improved, erosion is prevented, water is filtered and retained, and evaporation is decreased (Govaerts *et al*. 2007: Derpsch *et al*. 2010). However, plant residue was reported to have negative contribution by several collaborators as source of the primary inoculum (Awada *et al*. 2014; De Freitas and Landers 2014; Kertesz and Madar' asz 2014), which increases the risk of "residue-borne" or "stubble-borne" disease epidemics (Bailey, 1996; Bockus and Shroyer, 1998: Bailey and Lazarovits, 2003;). In fact, it is known that several pathogens that infect plants including legumes, and cereal crops can live on agricultural leftovers (residue)

between cropping seasons (Cook *et al.,* 1978: Bockus and Shroyer, 1998).

This study aimed to isolate and identify the pathogen associated with cowpea leaf spot symptoms which were observed in El-Minya Governorate of Egypt during summer of 2019, determine its host range, and to study the survival of the pathogen in the cowpea residue.

MATERIALS AND METHODS

Sampling and isolation of the associated pathogen(s)

Field survey was conducted during the summer of 2019, among cowpea growing fields of Maghagha, Beni Mazar, and Minya cities belongs to El-Minya governorate. Symptomatic cowpea leaves, stems, and pods were collected and carried to the laboratory for further analysis and fungal isolation. Plant organs were first cleaned under running tap water, surface sterilized by dipping in sodium hypochlorite solution (NaOCl, 2%) for 1–2 minutes, rinsed three times in sterilized tap water, and dried between two layers of sterilized filter paper. Samples were chopped into small pieces (0.5 cm long) using as sterilized sharp scalpel and placed on potato dextrose agar (PDA) medium supplemented with Amoxiallin (15mg/L). Plates were then incubated at 25°C in dark and monitored every day for five to seven days. Hyphal tip and single spore procedures was used to purify the appeared fungal colonies before being sub-cultured on PDA media (Noman *et al.* 2018). Stock cultures of the isolated fungus was preserved inoculated test tubes with slants of PDA media at 5^{o}C for further studies.

Identification of the isolated pathogen(s):

Isolated fungi were identified based on morphological and molecular characterization. For morphological identification, the microscopic featured of mycelium and conidia spores were observed from 10-15 old PDA cultures according to the methods of Yilmaz *et al*. (2014) and Sun *et al* 2020). Then, the conserved ribosomal interna**l** transcribed spacer (ITS) region was sequenced and compared for molecular identification (White *et al*. 1990). Pure cultures of the fungal isolates grown on PDA medium was sent to Bio Tech Research Lab (Cairo, Egypt), Sigma Scientific Service for DNA extraction using the Patho Gene-spinTM DNA/RNA extraction kit. PCR amplification was used for generation of ITS region using a forward primer (ITS1: 5′- TCCGTAGGTGAACCTGCGG-3′) and a reverse primer (ITS4:5′ TCCTCCGCTTATT GATATGC- 3′) (Kumar *et al*. 2013). PCR reaction was carried out in a 20 µl volume containing 1.5 µl DNA, 5 µl Taq PCR PreMix (iNtRON Biotechnology Company, Korea), and 1µl of each primer (10 pmol). PCR conditions were 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 52°C for 1 min and 72 °C for 1 min with a final extension at 72 °C for 7 min (Wu *et al*. 2019). The PCR products were separated by 1% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after red staining (iNtRON Biotechnology Company, Korea). Then, PCR products was sequenced, and the sequences were homology searched using NCBI's online Basic Local

Alignment Search Tool (http:// www.ncbi.nlm. nih.gov).

Pathogenicity tests

In planta assays were carried out at the green house of the Plant Pathology Department greenhouse of Faculty of Agriculture, Minia University. In brief, Cowpea (Cream 7 cv.) seeds received from Minia University's Department of Horticulture were surface sterilized by dipping in 2% NaClO for 2 minutes followed by washing twice with sterilized water. Ten seeds were planted in sterilized clay pots (30 cm in diameter) containing autoclaved Nile clay (about 4 Kg/pot) soil. Plants were thinned to five plants/ pot, two weeks after emergence. Before sowing, seeds were treated with symbiotic bacteria (*Rhizobium* spp., $2x10^6$ CFU/g) obtained from Institute of Soil and Water Research, ARC, Ministry of Agriculture, Giza), Fertilizers were also added at recommended rates, and pots containing seeds/plants were watered when necessary.

Plant inoculation and diseases assessments.

Cowpea plants of 31-day old and contains 5-7 leaves were inoculated according to the methods of Mahadevakumar and Janardhana (2014), with some modifications. In brief, Inoculum of isolated fungus (9 isolates) was grown under sterilized conditions in a 500 ml Erlenmeyer flasks containing 200 ml of potato dextrose broth (PD) medium at 25^oC for two weeks.Tween-20 (250 µl/ml) was added while the fungus growth and medium were homologized, and plants were then sprayed using a hand atomizer with the fungal inoculum ($3x10^5$ conidia/ml) until a visible mist covers the foliage. Instead of spore suspensions, control plants were sprayed with sterile water.

Inoculated plants were grown for 48 hours in polyethylene-covered cages with high humidity (relative humidity of 80-100%), at a temperature ranging from 20 to 35 °C, before being moved onto an open field. Three replicates/treatment were used, each consisting of three pots with five plants / pot. Plants were daily observed for 35 days, to determine the percentage of disease incidence (DI) and severity (DS) of cowpea leaf and pod spot disease according to the following formula:

DI (%)=(Number of infected plants / Total numbers of plants) x 100 , DS was determined using a Disease severity index of 0 to 4 (Vicent *et al*. 2012), where 0 = healthy (no visible disease symptoms), 1 = ≤ 25% of the leaves or pods with spot symptoms, 2 = > 25% but ≤50% of the leaves or pods with spot symptoms, 3 = > 50% but ≤ 75% of the leaves or pods with spot symptoms, and 4 = > 75% of the leaves or pods with spot symptoms. DS was calculated using the formula:

$DS = [\Sigma (n \times DI)/4N] \times 100$, where n is the number of plants in each DI category and N is the total number of plants in each survey or treatment.

Susceptibility of some plants to *Talaromyces trachyspermus*

To determine the host range of *Talaromyces trachyspermus* detached organs from various plants were carefully washed with tap water, sterilized by soaking for 2 min in sodium hypochlorite, followed by rinsing in sterilized water. Those parts includes

pods of bean (*Phaseolus vulgaris*), broad bean (*Vicia faba*), soybean (*Glycine max*), and cowpea (*Vigna unguiculata*); fruits of Chili Pepper 'Super Chili' (*Capsicum annuum*), Green (Sweet) pepper (*Capsicum annuum*), Colored bell pepper (*Capsicum annuum*), and okra (*Abelmoschus esculentus*); foliar parts of rosemary (*Salvia rosmarinu*, family *Lamiaceae*, sage (*Salvia officinalis*, family *Lamiaceae*), thyme (*Thymus vulgaris* family *Lamiaceae*); and capsules of Black seed (*Nigella sativa*, family *Ranunculaceae*), Sesame seed capsule (*Sesamum indicum*, family *Pedaliaceae*, to the cowpea leaf spot pathogen (*Talaromyces trachyspermus*). The detached plant organs were arranged in disinfected plastic boxes (30x12x5 cm) containing a double layer of wetted and sterilized filter papers. Plant organs were slightly injured using a sterile needle and sprayed with fungus suspension (10^5 CFU/ml) using a hand sprayer until the plant organs were wet, incubated at 25^{o}C for 5-7 days with daily observation. Three replicates were used, each replicate containing 3 boxes and each box contained 5 plant organs. The percentages of DI and DS were recorded.

***Talaromyces trachyspermus* survival in non-buried crop residues**

To check the survival of the pathogen in buried and non-buried crop residues artificially infected cowpea leaves, stems, and pods from susceptible cv. Cream 7, severely affected by *T. trachyspermus* were collected from non-sprayed fields at Experimental Farm of Plant Pathology Department, Faculty of Agriculture, Minia University, in September 2019. Collected cowpea residues were air dried at room temperature (25 ± 2°C) for 3 days and stored separately in paper bags 0, 10, 20 and 30 months. Each 5 g of dried sample was grounded and suspended in 100 mL sterile distilled water and the mixture was stirred for 1 h. Dilution series were prepared from 10^{-1} to 10^{-5} using sterile distilled water. Aliquots (100 µL) of the dilutions were spread uniformly in triplicate onto PDA Petri dishes and incubated at 25 °C under daily observation for until no further new colonies appeared. The average colony forming units (CFUs) on the three replicate Petri dishes was determined and expressed as CFUs/g dry substrate.

***Talaromyces trachyspermus* survival and viability in soil-buried crop residues**

For buried residues, samples were processed as described by Khan *et al*. (2008) with some modifications. In brief, Residue (waste) samples of 10 symptomatic cowpea plants were placed in nylon bags and sealed in protective plastic mesh sacks. The sacks were pinned with 1-m-long aluminum wire and placed at depths of 10-15 cm in a Nile silty- clay soil (54.7% clay, 35.3% silt, 9.9% sand and 1.7% organic matter, pH 7.97), for 30 months, a period of experiment. Sacks were placed in the field in September 2019 and retrieved after 10, 20, and 30 months to check the fungal survival. At retrieval, and air dried at 25± 2°C for 3days, 5 grams were processed as described above for colony count on PDA petri dishes. To test the pathogen viability and virulence cowpea plants (cv. Cream) was inoculated by

sprinkling 5 grounded and suspended grams of the retrieved *T. trachyspermus* inoculum directly on the leaves. Inoculated plants then were covered with transparent plastic bags for 24 h to maintain high relative humidity (RH) (≥90%) conditions favorable for the pathogen infection. At 21 days post inoculation DI and DS was recorded and the pathogen was reisolated from the leaf spots on PDA medium to confirm the presence of *T. trachyspermus*.

Statistical analysis

Data of all treatments were arranged and presented as mean from three replicates. The experimental designs of all experiments were completely randomized. Data were statistically analyzed for significance in Statistix (8^{th} edition, Analytical Software, USA, Steel *et al*., 1997) using analysis of variance (ANOVA). Significance between means was compared by Duncan's multiple range test at $p<0.05$ probability according to the method of Gomez and Gomez (1994).

RESULTS

Isolation and Identification of the pathogen(s) associated with leaf spot of cowpea.

Symptomatic Cowpea (Cream cv.) leaves, stems, and pods displayed a variety of symptoms, including irregular semicircular patches with a yellow halo and the ability to shatter through the middle and a light tan to grey center bordered by a dark reddish edge (Figure 1). Spots frequently appear on older leaves first, and in case of severe infection, spots are merged and formed a large necrotic region on leaves. On pods and stems, necrotic dark reddish to brown lesion, which eventually enlarged to cover the whole pod or about 1.5 -2 cm on the stem. We found that the estimated disease severity index (DSI) ranged between 6.38 - 48.25%, whereas the estimated disease incidence was 14.0-75.5%. The months of July and August saw the highest incidence of disease incidence and severity, most probably because of the favorable meteorological conditions during those two months (temperature, 25–38 °C; and humidity, 55–90%).

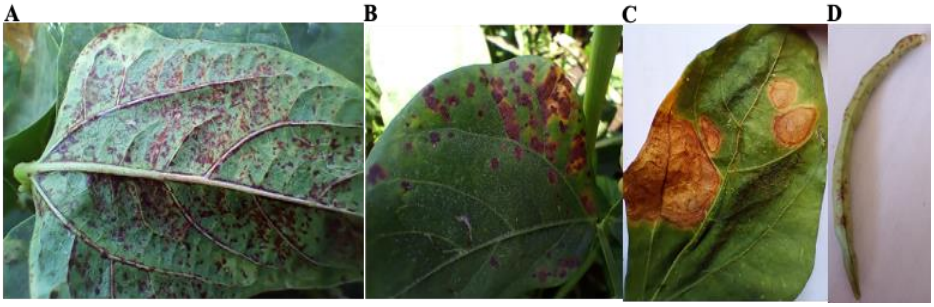

Figure 1: Leaf spot symptoms on upper (A) and lower (B) leaf surfaces. When developed, spots are merged together and formed a large necrotic region on leaves (C), as well as on pods (D) of cowpea

Nine isolates of fungi (Table 1) were obtained from cowpea naturally infected samples Including 5 isolates from Maghagha district, 4 isolates from El-

Minya district. After purification the isolates were further processed for morphological and molecular identification. After 7 days at 25^{o}C, colonies on PDA expanded modestly, reaching a diameter of 90 mm. Both the front and rear faces of the colony were white. The texture was floccose, sporulation ranged from moderately dense to dense, and conidia were abundant. Conidiophores were mono-overticillate, lacking subterminal branches, and ranged in length from 10 to 35 µm. The outside of the stipes was smooth. Phialides were lanceolate, 8–18 x 2.1–2.3 µm in size, and 2–5 in a verticil. Small verticils of 2- 3 range 10 - 12 x 1.4 - 2.3 µm in size contain metulae. Conidia had smooth walls, ellipsoidal to ovoidal shapes, with diameters ranging from 5.80 to 7.80 µm. Based on morphological features, the fungal isolates were identified as *Talaromyces* spp. (*Talaromyces* sections *Talaromyces* and *Islandici*). For further confirmation and to determine the species of the fungal isolates, the ITS region of T1 isolate (Table 1) was amplified by PCR and the sequence of the 522-bp PCR product was searched in GenBank by BLASTn and phylogenetic analysis was performed (Figure 2). The sequence has a high nucleotide similarity of 95-100% with the ITS region of *Talaromyces trachyspermus* (accession number: MT528783.1) in GenBank. The ITS sequence of T1 was deposited at the GenBank with the accession No.: OR486265.

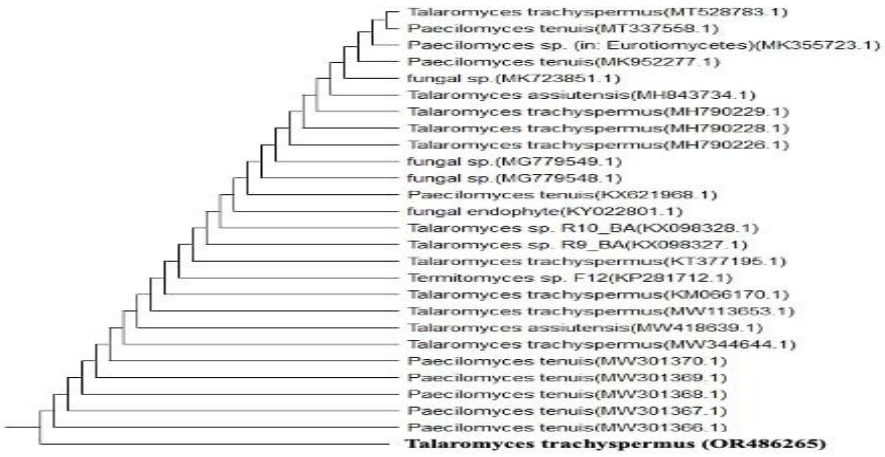

Figure 2: Phylogenetic tree based on the ITS region sequences showing the position of Isolated fungi (T1 isolate), among related species. The tree was constructed by neighbor-joining algorithm using maximum composite likelihood model. Bootstrap percentages from 100 replicates are shown. Sequences determined in this study have their accession numbers in boldface.

Pathogenicity tests:

Pathogenicity tests of the 9 isolates (Table 1), was carried out under greenhouse conditions using the susceptible cowpea cultivar Cream 7. Results in (Table 1, Figure 3) indicated that all tested isolates of *T. trachyspermus* infected cowpea plants causing spot and blotch symptoms on cowpea leaves and pods typical of that shown in surveyed fields. Isolates T1, T2, T3, T4 and T6 of *T. trachyspermus* caused DIs 68.9-80.0% and 60.0 -75.5% and DSs of 27.8-46.63% and 26.7 - 36.7% on leaves and pods, respectively. Isolate T7 caused DI of more than 50%, and DS of 20-22% on leaves and pods respectively. Where T5 isolate form Maghagha showed DIs 40.0 -46.7% and the lowest DSs of 15.0-20.5% on leaves and pods. Among the 9 isolates T1 showed to be the most aggressive isolate and was chosen for molecular identification of the ITS region using PCR techniques.

Susceptibility of some plants to *Talaromyces trachyspermus*

Detached fruits, pods or plant foliage of bean, black seeds, colored bell pepper broad bean, Chili pepper, cowpea, green (sweet) pepper, okra, rosemary, sage, sesame seed capsule, soybean, and thyme were used to evaluate their susceptibility to *T. trachyspermus* in laboratory. Data in (Figure 4) show that pods of cowpea, soybean and plant foliage of thyme were the most susceptible to the pathogen, presenting 97.77 –77.78% DI% and 38.88- 61.66% DS%. Where infection of sesame seed capsule, chili paper fruits, broad bean pods, bell pepper fruit, bean pods, black seed, colored bell pepper, bean, broad bean, and green (sweet) pepper with *T. trachyspermus* showed a moderate response of 73.33 - 28.89 % of DI, and 15.00– 53.33% of DS. Green pepper fruits showed the lowest response to infection by *T. trachyspermus*, giving a DI of 28.89 % and DS of 15.0%. Interestingly, Okra fruit, and plant foliage of sage and rosemary were resistant for the pathogen showed no infection.

Survival of *Talaromyces trachyspermus* in non-buried crop residues

Data in (Figure 5), shows the fungus population recovered from plants residue stored at room temperature for 10, 20, and 30 months. *Talaromyces trachyspermus* was recovered from all samples until 30 months, although the CFUs decreased significantly at each time point (Figure 5). Results indicates that the population of the fungus show a 31.93% reduction from 0 to 10 months, 34.57% reduction from 11 to 20 months, and more gradual decrease from 21 to 30 months giving a population reduction of 54.72%.

***Talaromyces trachyspermus* survival and viability in soil-buried crop residues**

Crop residues, and the decaying parts of the crop plant are sometimes considered as the main source of infection. Thus, studying the survival of *Talaromyces trachyspermus* survival on cowpea residues buried in soil is crucial for understating the epidemiology of this emerging diseases. We found that similarly to the room temperature samples the pathogen was recovered up to 30 months, but with significant decrease in CFUs at each time point (Figure 6A). interestingly, results shows that the reduction rate of pathogen

population was faster in case of buried restudies (Figure 6A), than in case of non-buried samples (Figure 5), showing a 39.67% reduction from 0 to 10 months, 65.99 % reduction from 11 to 20 months, and more gradual decrease from 21 to 30 months giving a population reduction of 85.03%.

Table 1 Pathogenicity tests of *T. trachyspermus* isolates on cowpea cultivar Cream7.

Isolate	Source	On leaves		On pods	
		DI%	DS%	DI%	DS%
T1	Minya	80.0*a**	46.63a	75.5a	36.7a
T2		73.3ab	41.11ab	71.1a	36.7a
T3		68.9a-c	35bc	68.9ab	29.4b
T4		75.6ab	33.3b-d	68.9ab	29.44b
T5	Maghagha	46.7e	20.5f	40.0e	15.0e
T6		68.9a-c	27.8c-f	60.0bc	26.7bc
T7		53.3de	20.55f	51.0cd	22.2cd
T8		66.7bc	30.0c-e	46.7de	20.6de
T9		60.0cd	25.0d-f	46.7de	15.44e
Mean		65.9	30.97	42.5	28.9
LSD 5%		12.61	8.93	9.38	5.84

DI = Disease incidence, and DS = Disease severity

*Data presented the average of three replicates each containing 5 plants.

**Values followed by the same letter (s) within each column do not differ significantly.

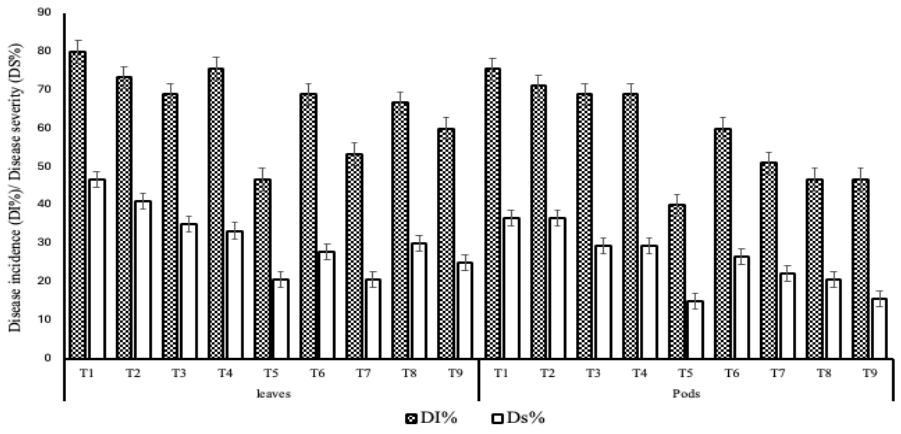

Figure 3: Disease incidence and disease severity percentages observed on leaves and pods of cowpea cultivar Cream 7 treated with T. *trachyspermus* compared with uninfected plants.

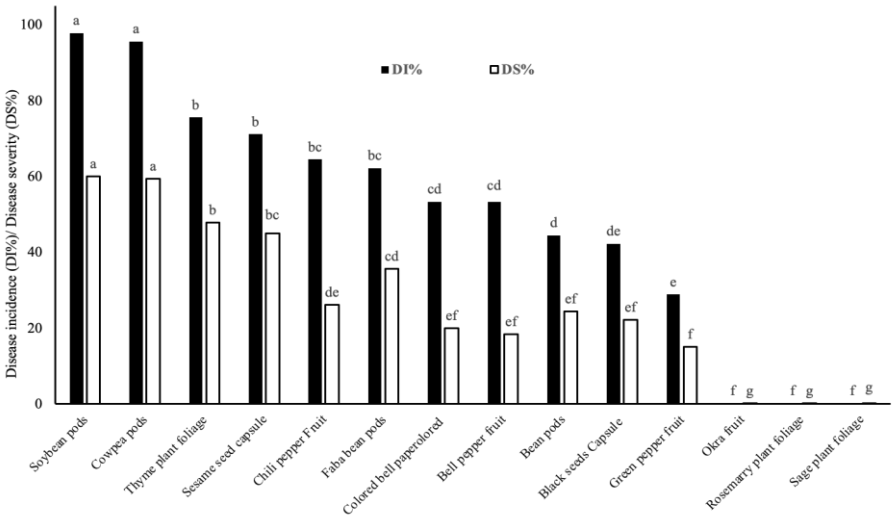

Figure 4: Response of different hosts to infection with T. *trachyspermus* in laboratory, using detached pods, fruits, or foliar parts, 3 -5 days after inoculation.

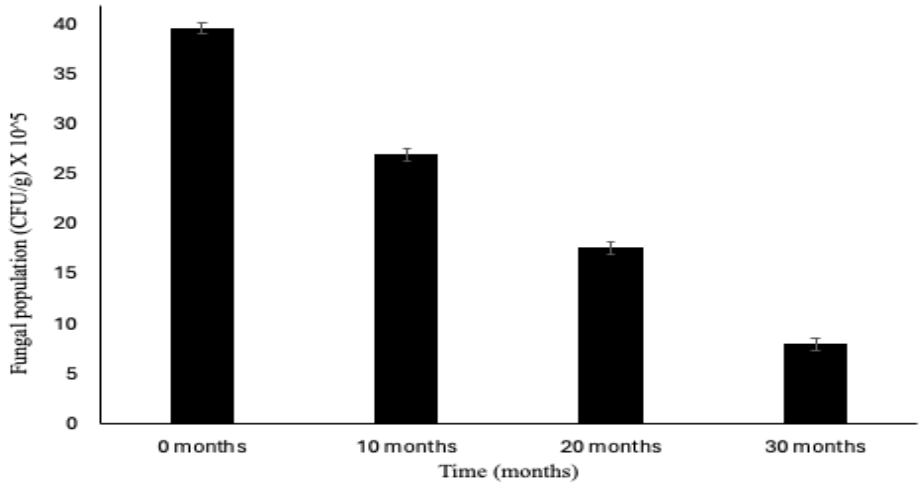

Figure 5: Survival of *Talaromyces trachyspermus* on air dried residuals of cowpea at 0, 10, 20, and 30 months after store in room temperature. Recovery of the fungus de creased significantly at each time point with more considerable decreases at 30 months.

However, the inoculum recovered from each time point were still pathogenic on cowpea plants. Data in (Figure 6B) showed the recorded DI and DS of cowpea plants inoculated with grounded residuals buried for 0, 10,20, and 30 months. The percentages of reduction of *T. trachyspermus* viability were 11.53% for DI after 10 and 20 months and 26.55% after 30 months. Meanwhile, the DS shows a reduction of 3.51% after 10 months, 21.05 % after 20, and 28.08% at 30 months (Figure 6B).

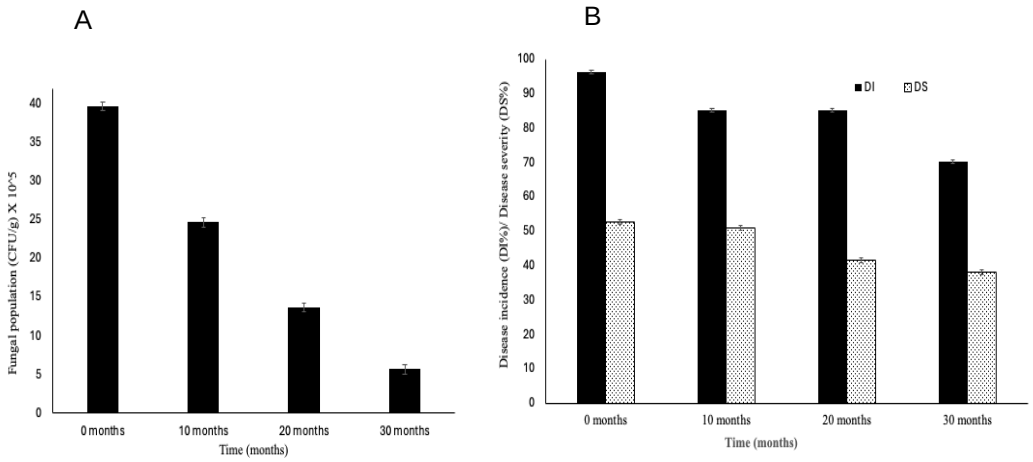

Figure 6: Survival of *Talaromyces trachyspermus* on buried residuals of cowpea at 0, 10, 20, and 30 months (A), and Disease incidence and disease severity percentages observed on leaves and pods of cowpea cultivar Cream 7 inoculated with inoculum recovered from each time point

DISCUSSION

Cowpea (*Vigna uguiculata, Fabaceae*) is an annual herbaceous legume cultivated for seed and fodder. Leaf and pod spot is a serious disease and causes severe yield loss in cowpea. A survey conducted in summer of 2019 found that 14.0–75.5% of cowpea plants in the commercial fields belonging to El-Minya governorate had indications of leaf spot. Leaf spots started as small, round, wet lesions near the leaf's core and margins. Over time, they grew larger and occasionally merged. Uneven semicircular patches with a dark reddish edge, a pale tan to grey center, and a yellow halo that can break through the middle. Pale brown and necrotic tissue resulted from the infection.

Natural infected leaves and pods of cowpea, Cream 7 cv., were collected and used for isolation of the associated pathogen(s). Nine isolates of fungi were obtained from cowpea naturally infected samples collected from Maghagha district (5 isolates) and El-

Minya district (4 isolates), then were purified and used for pathogenicity tests. All tested isolates showed ability to infect cowpea plants inducing spot and blotch symptoms on cowpea leaves and pods similar to that shown in the naturally infected plants with different degrees.

Isolates T1, T2, T3, T4 and T6 were the most aggressive ones, respectively, whereas isolate T7 caused moderate symptoms. The lowest disease infection and severity was caused by isolate T5, which isolated from Maghagha. Isolate T1, which induced the highest disease incidence and severity was sent to confirm identification applying PCR techniques.

To determine the evolutionary relationship between isolate T1 and previously described *Talaromyces* species, ITS rDNA sequences were examined. The isolate was most closely related to *T. trachyspermus* and belonged to a monophyletic group with bootstrap values of 100% . The phylogenetic analysis indicates that the isolate used in the study is *T. trachyspermus* (Shear) Stolk & Samson, 1972) and it was deposited in GenBank (accession number: OR486265). The comparison with GenBank revealed a 95–100% match between the sequences.

The genus *Talaromyces* belongs to fungal species producing asci in chains. In 1955, Benjamin designated a group of *Penicillium* species that may induce a sexual state under the name *Talaromyces*. The genus was distinguished by its ability to produce soft ascocarps with cleistothecial walls that typically contain yellow ascomata with oblong to globose asci and spiny ascospores and several layers of interwoven hyphae (Benjamin, 1955; Pitt; 1979, and Yilmaz *et al*., 2014). Samson *et al.* (2011) reassigned the bulk of recognized species of *Penicillium* subg. *Biverticillium* to *Talaromyces* based on phenotypic, extradite, phylogenetic, and the idea of one fungus, one name. A total of 88 species were added to the genus' monograph in 2014. These species were divided into seven clearly defined divisions, including *Islandici*, *Talaromyces*, *Bacillispori*, *Helici*, *Purpurei*, *Trachyspermi*, and *Subinflati* (Yilmaz *et al.*, 2014). In 2020, Sun *et al*., reported that more than 150 *Talaromyces* species have been described worldwide.

Many previous studies have reported that *T. trachyspermus* is capable to use as antifungal agent for controlling some aggressive soil borne pathogens such as *Rhizoctonia solani, Sclerotinia sclerotioru, Sclerotinia rolfsii, Verticillium dahliae,* and in several crops including cotton, potato, tomato, aubergine and beans (Dutta, 1981; Madi *et al.*, 1997; Tjamos and Fravel, 1997; Menendez and Godeas, 1998; Naraghi *et al* 2012). As a plant pathogenic, recent reports of these fungi was found as a postharvest pathogens concern *T. albobiverticillius* on pomegranate (Mincuzzi et al., 2017 and Nargund *et al*., 2012), *T. rugulosus* on grapes (Jayalakshmi *et al*., 2013 and Yang *et al.*, 2017), *T. minioluteus* on onion bulbs and quince, orange, and tomato fruit (Rahimlou *et al*., 2014 and Stošić et al, 2020), and both of the latter two species on pears (Joshi *et al*., 2014 and Stošić et al., 2021) and *Talaromyces pinophilus* on sugar beet (Haque and Parvin, 2020). From the leaves of the *Withania somnifera*, Sahu *et al*. (2019)

isolated *Talaromyces trachyspermus*, and reported that a fungal species with significant agricultural importance. Hemmati and Gholizadeh (2019) reported that *T. trachyspermus* caused a significant reduction in the number of tubercles and number and fresh weight of broom rape shoots, in addition to causing rot symptoms in the stalks of inoculated plant shoots.

Secondary metabolites from *Talaromyces* are alkaloid (Chu *et al*., 2010), terpene (Li *et al*., 2011) and polyketide classes (Guo *et al*., 2016 and Li *et al*., 2011) identified talaperoxides from an endophytic fungus *T. flavus* from medicinal plants showed potent cytotoxic activity against cancer cell lines. Farhat *et al*. (2021) reported also that GC-MS profiling of n- hexane fraction of *T. trachyspermus* yielded several new compounds from this source. The presence of these compounds may play a vital role in antimicrobial activity of *T. trachyspermus*. Funicone-like compounds are a homogeneous group of polyketides that, so far, have only been reported as fungal secondary metabolites. *Given that Talaromyces spp., the main producers of funicones, are widely distributed endophytes (*Vinale *et al*., 2017 and Nicoletti *et al*., 2018*).* It is possible that additional *Talaromyces* spp. makers of these substances may damage fruit and other crop products, as has been reported for pineapple, even though none of these species are known to create funicones. The identification of *T. funiculosus* as a cause of peach fruit core rot (Mukhtar *et al*., 2019) lends credence to this view. Hemmati and Ghlolizadeh (2019) mentioned that *T. trachyspermus* causing rot in broom rape shoots and leave spots on several plants, but no infect tomato plant.

In addition, our study showed that detached pods of soybean, cowpea and plant foliage of thyme were the most susceptible to *T. trachyspermus*, presenting between 97.56 –75.56% DI% and 47.78- 60.0 DS%. Whereas moderate infection with the pathogen was recorded on Chill pepper, Colored bell pepper, bean, faba been, and black seed. Green (sweet) pepper showed the lowest infection caused by this pathogen, whereas okra, sage and rosemary were the most resistant for the pathogen showed no infection.

These results agree with that obtained by Hemmati and Ghlolizadeh (2019) and Sahu *et al*. (2019), who found that *Talaromyces trachyspermus* causing blight or soft rot symptoms on broomrape (*Orobanche* spp.) plants and infected leaves of the medicinal plant *Withania somnifera*. Also, many species of the genus *Talaromyces were* recorded as plant pathogenic fungi causing postharvest diseases, *i.e.*, *T. albobiverticillius* on pomegranate (*Punica granatum* L.*)* in Southern Italy (Mincuzzi et al., 2017), *T. rugulosus* on grapes (Yang et al., 2017), *T. minioluteus* on onion bulbs, quince (*Cydonia oblonga* Mill.), orange, and tomato fruit (Stošić et al, 2020), and *T. rugulosus* and *T. minioluteus* on pears (Stošić et al., 2021). *Talaromyce funiculosus* was recorded for causing fruit core rot of peach (*Prunus persica* L. Batsch) in Fuzhou, China (Mukhtar *et al*., 2019).

It is widely known that a number of soil-borne phytopathogenic bacteria and fungi can persist in crop wastes

(Conway, 1996). To eradicate the presence of this kind of microorganism in this plant material, several strategies like biofumigation and solarization have been tried (Bello *et al.,* 2000, and Stapleton *et al*., 2000). After harvesting, crop residue must be managed in the field to reduce negative environmental consequences like bad smells or the emergence of plant diseases, especially if the pathogens involved can infect succeeding crops.

Talaromyces trachyspermus population was recovered from all samples stored at room temperature until 30 months, although the CFUs decreased significantly at each time point. The fungal population was reduced by 31.93% when stored to 10 months, 34.57 % reduction from 10 to 20 months, and 54.72% decrease from 21 to 30 months. The present data revealed also that the pathogen was recovered up to 30 months, but with significant decrease in CFUs at each time point, when the cowpea residues was buried in soil. Our results shows that the reduction rate of pathogen population was faster in case of buried restudies than in case of non-buried samples, showing a 37.67% reduction of fungal population from 0 to 10 months, 65.99 % reduction from 10 to 20 months, and 85.03% reduction was showed in samples poured for 30 months. The inoculum recovered from each time point were still pathogenic on cowpea plants

The *T. trachyspermus* viability was reduced by 11.53% for DI after 10 and 20 months and 26.55% after 30 months. Meanwhile, the DS shows a reduction of 3.51% after 10 months, 21.05 % after 20, and 28.08% at 30 months. Pool and McKay (1916) reported that *Cercospora beticola* survives in sugar beet residue more than 8 months, whereas Nagel (1938) showed that *C. beticola* survived in naturally infested soil for 20 months. *Cercospora beticola* was found, also, survives much longer than 22 months if the inoculum was placed on soil surface compared with inoculum placed at depths of 10 and 20 cm, but only after 10 months when placed at depths of 10 and 20 cm (Khan *et al*., 2008).

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الملخص العربي

تبقع الأوراق والقرون فى اللوبيا المتسبب عن الفطر *Talaromyces trachyspermus* فى محافظة المنيـا – مصر، وفترة بقاء الفطر الممرض على مخلفات المحصول

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تتعرض اللوبيا لهجمات مجموعة واسعة من مسببات الأمراض النباتية بما في ذلك البكتيريا والفطريات والفيروسات والنيماتودا. في هذه الدراسة، تم إجراء حصر لمدى انتشار مرض تبقع الأوراق والقرون فى اللوبيا فى مناطق زراعتها بمركزي المنيا ومغاغة بمحافظة المنيا، مصر. وقد بينت الدراسة أن المرض منتشر في جميع الحقول التي شملتها الدراسة، وكانت أعلى معدلات حدوث المرض وشدته موجودة في مركز المنيا. وقد تم تعريف الفطر المسبب للمرض على أنه *Talaromyces trachyspermus* بناءً على الصفات المورفولوجية للعزلات الفطرية، واختبار ال PCR. وقد أظهرت الدراسة أن القرون المنفصلة من فول الصويا واللوبيا وأوراق نبات الزعتر كانت الأكثر عرضة للإصابة بـالفطر الممرض *T. trachyspermus*، حيث تراوحت بين 97.77 – 77.78% DI% و38.78 – 61.66 DS%، في حين كانت الإصابة متوسطة على كل من الفلفل البارد، الفلفل الملون والفاصوليا والفول البلدى والحبة السوداء والفلفل الأخضر (الحلو) حيث أظهرت أقل نسبة إصابة بهذا الممرض، في حين كانت البامية والمريمية وإكليل الجبل الأكثر مقاومة ولم تظهرعليها أي إصابة. بالإضافة إلى ذلك، تمت دراسة بقاء الفطر *T. trachyspermus* على بقايا النباتات غير المدفونة والمدفونة في التربة لمدة 30 شهرًا. وأظهرت النتائج أنه تم نمو المستعمرات الفطرية للفطر الممرض بعد 30 شهرًا في كلتا الحالتين، مع انخفاض كبير في عدد وحدات المستعمرات في كل فترة زمنية مدروسة (10 و20 و30 شهر). ومع ذلك، ظل العامل الممرض قادرا على إنتاج الأمراض في نباتات اللوبيا. تسلط هذه النتائج الضوء على قدرة هذا العامل الممرض على البقاء على مخلفات النباتات لفترة طويلة مع القدرة على إصابتها.