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ORIGINAL PAPER

Viability, Growth and Virulence of *Harpophora maydis* Isolates Preserved Under Different Storage Methods

El-Naggar, A.A.A. and Yassin, M.A.*

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ABSTRACT

Two isolates of *Harpophora maydis*, the causal agent of maize late wilt, were evaluated for viability, morphological characters, radial growth and virulence using twenty storage methods for 3, 12 and 24 months. Colonized discs preserved in sterile distilled water at room temperature (RT) from 25 to 32°C (SDW.RT) was the best treatment since the two isolates were recovered from all samples during the storage periods, with 90, 90 and 82.5%, respectively with no negative effect on their radial growth, morphological characters and virulence compared with the original isolates. Also, storage in 30% glycerol at room temperature (G30%. RT) exhibited a good degree of survivability (from 60 to 90%) and the examined characters were not altered. Unfortunately, fungal isolates were not able to recover after three months of storage when preserved by the methods of potato dextrose yeast agar with paraffin oil at RT, G30%. 4°C, G30%. -20°C and silica gel at -20°C. Based on the statistical analysis of the obtained results, it could be concluded that SDW.RT is simple, reliable and economic preservation method for *H. maydis*, moreover preservation in G30%.RT could be used along with SDW.RT or as alternative method for the tested fungus.

Keywords: Maize, *Zea mays*, *Harpophora maydis*, Late wilt, Viability, Preservation methods, virulence.

***Correspondence: Yassin, M.A.**
mohamdyassin@gmail.com
Abdallah A.A. El-Naggar
https://orcid.org/0000-0001-7200-1182
Mohamed A. Yassin
https://orcid.org/0000-0002-3009-5413
Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

INTRODUCTION

Late wilt of maize (*Zea mays*) caused by the fungus *Harpophora maydis* is one of the most common diseases in Egypt (Samra *et al.*, 1962; El-Naggar *et al.*, 2015; El-Naggar, 2019 and Agag *et al.*, 2021) and acts as a principle limiting factor in maize production. To facilitate the study of this fungus, reliable preservation method of cultures is needed. Preservation can be done usually by continuous subculture method and storage at 4°C (Kitamoto *et al.*, 2002 and Tung *et al.*, 2018), unfortunately fungal culture and medium are dried after short period and then new sub culturing is needed. This method of preservation is time consuming and labor-intensive job. On the other hand, physiological and molecular characteristics may be change as well as the chances of contamination may also be increase (Elliott, 1995; Hornby *et al.*, 1998 and Kitamoto *et al.*, 2002). Storage methods with lyophilization or cryopreservation in liquid nitrogen or a mechanical deep freeze at -70°C acts as the preferred methods for a long-term preservation (Bosmans, 1979; Miguens, 1985; Smith and Onions, 1994 and Ryan *et al.*, 2000). Meanwhile, these methods require special equipment or electric continuous supply as well as liquid nitrogen must be replenished routinely. Fungal cultures can be preserved for up to several years by economical techniques such as agar slants often under oil to prevent dehydration, water, silica gel and even dried soil under temperature ranged from 24°C to - 20°C (Bakerspigel, 1953; Castellani, 1963; Little and Gordon, 1967; Tuite, 1969; Gentles and Scott, 1979; Smith and Onions, 1994 and Palacio *et al.*, 2014). However, most of the previous studies on preservation methods have been related to viability (Carmichael, 1962; Boesewinkle, 1976; de Capriles *et al.*, 1989; Carter and English, 1994 and Sharma and Smith, 1999). Little or few researchers have examined the effect of storage methods for long period on pathogenicity or virulence of fungal isolates (Butler, 1980; Windels *et al.*, 1993; Burdsall and Dorworth, 1994 and Hajek *et al.*, 1995). The goal of this study was to determine the simplest, reliable, and cost-effective method for keeping the *H. maydis* viability, unique characteristics, growth and virulence for 24 months of storage.

MATERIALS AND METHODS

Fungal isolates and their characteristics:

Two single spore cultures of *H. maydis* (Hm-1 and Hm-2) with unique characteristics (rhizoid

in form, flat in elevation, filiform in margin, powdery in texture and white in color) were selected from the culture collection of Maize and Sugar Crops Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt; for using in this study. Morphological characters were assessed for 5d-old cultures grown on potato dextrose agar + 0.2% yeast extract (PDYA) at 27°C (Sabet *et al.*, 1966).

Virulence of the original isolates:

A clean sorghum grains (100g) were soaked in water overnight in 500 ml glucose glass bottle, then the excess water was decanted, and the bottles were autoclaved for an hour. One piece, 2 cm^2, from 7d-old *H. maydis* culture (grown on PDYA at 27°C) was transferred to each bottle and incubated at room temperature (27°C±2) for 15 days, afterward the bottles were used for greenhouse soil infestation (Sabet *et al.*, 1966). Eventually, 100 g of *H. maydis* – colonized sorghum grains of the two selected isolates were added individually to the loamy soil of 10 Kg capacity pot and mixed (5 pots/ isolate). Eight grains of the susceptible hybrid PIONEER SC3062 were planted per pot on May 5, 2017, at 2 cm depth. Five maize plants were left in each pot at 15 days after seedling emergence followed by application of the recommended cultural practices. The experiment was carried out in a completely randomized design. Disease reaction was recorded as a percent of infection after 90 days from seeding date. Data were analyzed by ANOVA using Duncan's multiple range test with significant level of 5% after they transformed to Arcsine. ANOVA was performed using COSTAT version 3.03 software (Steel and Torrie, 1980).

Preservation methods:

Twenty preservation methods with Hm-1 and Hm-2 isolates of *H. maydis* were evaluated for fungal survivability, radial growth and morphological characteristics after 3, 12 and 24 months. Virulence of both isolates was examined also after 12 and 24 months from the storage. The details of each method are provided below and summarized (Table 1). Fungal isolates were grown 7days on PDYA in Petri dishes or slants at 27°C to study the storage treatments using colonized agar plug or suspension of fungal propagules. Plugs were cut with a sterile 5mm cork borer and the suspension was prepared by sterile glass rod.

Table (1): The methods used for storage of *H. maydis* isolates.

Storage methods	Abbreviation
Potato dextrose yeast extract agar slant stored at room temperature.	PDYA.RT
Potato dextrose yeast extract agar slant stored at 4°C.	PDYA.4°C
Potato dextrose yeast extract agar slant covered with sterile Parafin oil and stored at room temperature.	PDYA.PO. RT
Potato dextrose yeast extract agar slant covered with sterile Parafin oil and stored at 4°C.	PDYA.PO.4°C
Fungal propagules in 10% glycerol stored at room temperature.	G10%. RT
Fungal propagules in 10% glycerol stored at 4°C.	G10%.4°C
Fungal propagules in 10% glycerol stored at -20°C.	G10%. -20°C
Fungal propagules in 30% glycerol stored at room temperature.	G30%. RT
Fungal propagules in 30% glycerol stored at 4°C.	G30%.4°C
Fungal propagules in 30% glycerol stored at -20°C.	G30%. -20°C
Colonized agar plugs in sterile distilled water stored at room temperature.	SDW.RT
Colonized agar plugs in sterile distilled water stored at 4°C.	SDW.4°C
Sterile soil saturated with the suspension of fungal propagules and stored at room temperature.	SS. RT
Sterile soil saturated with the suspension of fungal propagules and stored at 4°C.	SS.4°C
Colonized filter paper pieces stored at room temperature.	FPP.RT
Colonized filter paper pieces stored at 4°C.	FPP.4°C
Colonized sorghum grains stored at room temperature.	CSG.RT
Colonized sorghum grains stored at 4°C.	CSG.4°C
Silica gel crystals covered with fungal propagules in skim milk stored at 4°C.	SG.4°C
Silica gel crystals covered with fungal propagules in skim milk stored at -20°C.	SG. -20°C

PDYA slant with or without paraffin oil:

A glass tube (10×1.5cm) sealed with cotton plug was used as a storage unit. Each tube was filled with 5ml of PDYA, autoclaved and solidified at a slant (Sabet *et al.*, 1966). Afterward, each tube was inoculated with 5mm plug of 7 days old *H. maydis* culture, with 60 tubes for each isolate, incubated at 27°C for 7 days then divided into two groups (30 tubes for each group). The first group (PDYA slant only)

was divided again into two subgroups (15 slant / subgroup) then one subgroup was stored at 4°C and its counterpart at room temperature (RT). In the case of the second group, PDYA slant with paraffin oil (PDYA.PO), sterile paraffin oil (autoclaved for two consecutive days at 121°C for an hour) was added to each tube until the agar was covered (2-3cm in depth). Then it was stored either at 4°C or at RT as two subgroups with 15 slants for each. All slants were sealed with Parafilm prior to storage (Krizkova and Balan, 1975 and Tariq *et al.,* 2015).

Preservation in 10 and 30% glycerol:

In these treatments, cryogenic 2.0 mL polypropylene vials with screw cap were used as a storage units. Two ml of sterile 10 and 30% glycerol solution were used for re-suspending the *H. maydis* spores and mycelia individually. After that, 1.8 mL of the suspension with parts of colonized agar of each concentration were added individually in each unit (15 vials for each treatment). Then vials of each concentration of each isolate were stored at RT, 4°C and -20°C for 3, 12 and 24 months (Paul *et al.*, 2015).

Preservation in distilled water:

Six agar discs of each isolate (5mm) were removed aseptically from the developing edge of 7days *H. maydis* old culture and transferred to sterile cryogenic 2.0 mL polypropylene vials filled with 1.5 mL sterile distilled water. Caps of the vials were screwed tightly and stored either at RT or 4°C, with 15 vials for each (Castellani, 1967; De Capriles *et al.,* 1989 and Tariq *et al.*, 2015).

Preservation in soil:

As reported by Atkinson (1954) and Tariq *et al.* (2015), sterile distilled water (6mL) was added to the slant of 7d-old *H. maydis* culture. Surface of colony was harvested gently to produce mycelia and spore suspension. Five ml of the suspension were added drop by drop with the help of sterile pasture pipette to each storage unit (glass tube, 10x1.5 cm with cotton plug) which one-third filled with sterile loamy soil (autoclaved for two consecutive days at 121°C for an hour). Afterward, incubation was done at 27°C for 10 days then stored at RT and at 4°C (15 tubes/each temperature).

Preservation on filter paper:

Two preservation treatments using filter paper (Wattman No.1) were conducted. Stripes of filter paper 2×6 cm were sterilized by autoclave for one hour then oven dried. *H. maydis* mycelium was grown across a sterile filter paper placed on PDYA in 9cm Petri plate for 2 weeks at 27°C. After that, the colonized stripes were removed from the agar plates and transferred into new sterilized plates without any culture medium. Then the Petri dishes were incubated again for 15-20 days at 27°C until the paper and the fungus were completely dried. With a sterile blade under aseptic conditions, filter paper stripe was cut into approximately 1 cm square pieces. After that, 7 pieces were placed in sterile 2 ml cryogenic vial and immediately screwed tightly by the cap (Elliott, 2005). Half of the vials were stored at 4°C and the other at -20°C (15 vials for each).

Preservation on sorghum grains:

According to the methods reported by Tareq *et al.* (2015) with a slight modification, sorghum grains (5g) were immersed in water overnight in clean glass tube storage unit (10×1.5cm). Excess water was decanted off and grains were sterilized by autoclave at 121°C for an hour. One disc, 1cm^2, from the margin of actively growing culture was transferred and placed between the grains. Then, the tubes were incubated for 10 days at 27°C. After that, the tubes were separated into two storage temperatures, RT and 4°C, with 15 tubes for each.

Preservation on silica gel:

Screw-cap glass tubes (7×1.5cm) were filled one-third with anhydrous silica gel crystals (6-12 mesh, grade 40, uncolored). The tubes were sterilized in an oven at 180°C for 4 h and chilled before use for at least 20min. Fungal spores and mycelial suspension (7days old culture) was prepared in cold sterilized skim milk (5% w/v) using a sterile glass rod. Afterward, 2 mL of the suspension were added using sterile Pasture pipette drop by drop in each tube and incubated at 27°C for 10 days with the cap slightly loose until the silica gel was crystallized. Then, caps of the tubes were screwed tightly and stored at 4°C and -20°C with 15 tubes for each (Perkins, 1962).

Assays:

After 3, 12 and 24 months of preservation treatment, *H. maydis* isolates of each treatment were tested for viability, morphological characteristics and radial growth. On the other hand, virulence of the tested isolates was evaluated after 12 and 24 months from the storage.

Viability test for fungal isolates:

Samples of stored isolates were plated onto fresh PDYA in Petri plates to confirm fungal viability. The Petri dishes were incubated at 27°C and examined daily for 10 days.

The experiment was carried out in quadruplicates (five fungal segments for each

replicate). If there was no recovery of any of the four replicates of a storage treatment, the isolate was transferred again to ensure that it was not viable in that replicate of storage treatment of that sampling date. Therefore, assays for isolate survival were stopped when no fungal growth had been obtained for all replicates of a storage treatment in one sampling date. The data were recorded as percentages of *H. maydis* viability.

Radial growth on PDYA:

At the time of survival tests, the radial growth of each isolate under any storage treatment was evaluated for the comparison with the radial growth of the original isolate before storage. Plugs (5mm) taken from *H. maydis*-colonized PDYA plate of one replicate of each storage treatment were transferred to the center of fresh PDYA plates with 4 plates/ storage treatment. Radial growth was measured after 5 days at 27°C (Pottinger *et al*., 2008).

Virulence of viable *H. maydis* isolates:

Growth of the survived isolates of one replicate of each storage treatment was used after 12 and 24 months from storage to test their virulence. The inoculum was prepared as mentioned before in pathological characteristics of the original isolates. Five grains of the susceptible hybrid PIONEER SC3062 were planted (June 5, 2018, and June 10, 2019) in infested pots (100g of inoculum / 10Kg capacity pot) at 2 cm depth. The experiment was carried out in a complete randomized design with three replicates. Disease assessment and data analysis were done as mentioned before.

RESULTS

In this study, twenty storage methods were used to test viability of two *H. maydis* isolates after 3, 12 and 24 months from storage date as well as their effects not only on fungal morphology but also radial growth and virulence. Concerning survivability after 3 months of storage, twelve treatments (PDYA.RT, PDYA.4°C, G10%.RT, G30%.RT, SDW.RT, SS.RT, SS.4°C, FPP.4°C, FPP.-20°C, CSG.RT, CSG.4°C and SG.4°C) exhibited the best results for viability since fungal recovery was 90% (Table 2), but the recovery was 26.3 and 30 % with SDW.4°C and PDYA.PO.4°C treatments, respectively. Whereas, PDYA.PO.RT, G10%.4°C, G10%.-20°C, G30%.4°C, G30%.-20°C and SG. -20°C treatments were the worst storage methods since the fungus was not able to recover from any replicate of any treatment.

Moreover, the best treatments for survivability after 12 months of storage were G10%. RT, SDW.RT, SS.RT, SS.4°C, G30%.RT, PDYA.RT and *H. maydis* colonized filter paper piece (FPP.4°C) since the recoverability ranged from 67.5 to 90 %.

After 24 months of storage, eight treatments exhibited positive recovery of the fungal isolates with variation in degree of significance (Table 2). The best three treatments for survivability were glycerol 10% at room temperature (G10%.RT), *H. maydis* colonized agar plugs preserved in sterile distilled water (SDW.RT) and sterilized soil saturated with the fungal spore suspension and mycelium (SS.RT) since the fungal recoverability ranged from 67.5 to 82.5% (Table 2). However, SS.4°C, G30%. RT and FPP.4°C treatments exhibited acceptable survivability ranged from 56 to 67.5% for the two isolates. Also, PDYA.RT treatment showed the lowest degree of recovery with Hm-1 and Hm-2 isolates (37.5 and 41% respectively). It should be noted that after 24 months from the storage date, all replicates of PDYA slants without paraffin oil and CSG treatments were dried out at RT or 4°C.

In general, the best storage methods for survivability in all sampling dates were G10%.RT, SDW.RT and SS.RT followed by G30%. RT, SS.4°C and FPP.4°C. On the other hand, the worst treatments for survivability after 12 and 24 months were PDYA slants with paraffin oil at room temperature or at 4°C; glycerol 10% at 4°C or at -20°C; glycerol 30% at 4°C or at -20°C, SDW.4°C and silica gel at -20°C, since the two isolates of the fungus were not recovered from all replicates. Finally, survivability percentage was decreased during the entire 24-month period examined (Table 2).

In respect to the effect of storage methods on morphological characters of *H. maydis* isolates, the two isolates maintained their unique morphological characteristics and did not influence in all sampling dates (Fig.1). When Hm-1 and Hm-2 isolates were recovered, they were white in color, rhizoid in margin, powdery in texture and flat in surface reflecting the same fungal characteristics before storage (Fig. 2).

Concerning the effect of storage methods on *H. maydis* radial growth, recovered isolates from G30%.RT and SDW.RT after 3, 12 and 24 months of storage were not significantly affected in growth when compared (after 5 d of incubation at 27°C) with the radial growth of the original isolates at the initial date of storage (Table 3). However, the growth of the recovered isolates obtained from the remaining methods

was influenced and was not stable in all sampling dates (Table 3).

Likewise, the virulence of the two isolates was not significantly affected by the treatments of SDW.RT and G30%.RT in all sampling dates when compared with the virulence of original isolates before storage (Table 4), but it was influenced (decreased or increased) by the other storage methods (Table 4).

Table (2): Survival of *H. maydis* isolates after 3, 12 and 24 months of storage using twenty preservation methods.

Preservation methods	Survival (%)					
	Hm-1 isolate			Hm-2 isolate		
	3 mo.	12 mo.	24 mo.	3 mo.	12 mo.	24 mo.
PDYA.RT	90 a	75 ab	37.5 c	90 a	82.5 a	41.3 c
PDYA.4C	90 a	56.3 b	0.0 d	90 a	52.5 bcd	0.0 d
PDYA.PO.RT	0.0 c	-*	-	0.0	-	-
PDYA.PO.4C	30 b	0.0 c	-	30 b	0 e	-
G10%.RT	90 a	90 a	67.5 ab	90 a	90 a	67.5 ab
G10%.4C	0.0 c	-	-	30 b	0.0 e	-
G10%. -20C	0.0 c	-	-	30 b	0.0 e	-
G30%.RT	90 a	82.5 ab	60 b	90 a	75 ab	67.5 ab
G30% 4C	0.0 c	-	-	0.0 c	-	-
G30%. -20C	0.0 c	-	-	0.0 c	-	-
SDW. RT	90 a	90 a	82.5 a	90 a	90 a	82.5 a
SDW. 4C	26.3 b	0.0 c	-	26.3 b	0.0 e	-
SS.RT	90 a	90 a	82.5 a	90 a	90 a	82.5 a
SS.4C	90 a	90 a	56.3 b	90 a	90 a	60 b
FPP.4C	90 a	67.5 ab	67.5 ab	90 a	67.5 abc	63.8 b
FPP. -20C	90 a	26.3 c	0.0 d	90 a	30 d	0.0 d
GSS.RT	90 a	22.5 c	0.0 d	90 a	26.3 de	0.0 d
GSS. 4C	90 a	67.5 ab	0.0 d	90 a	45 cd	7.5 d
SG. 4C	90 a	22.5 c	7.5 d	90 a	45 cd	0.0 d
SG. -20C	0.0 c	-	-	0.0 c	-	-

*= treatment was not used for fungal recovery since the survivability was 0 in all replicates of previous sampling date.

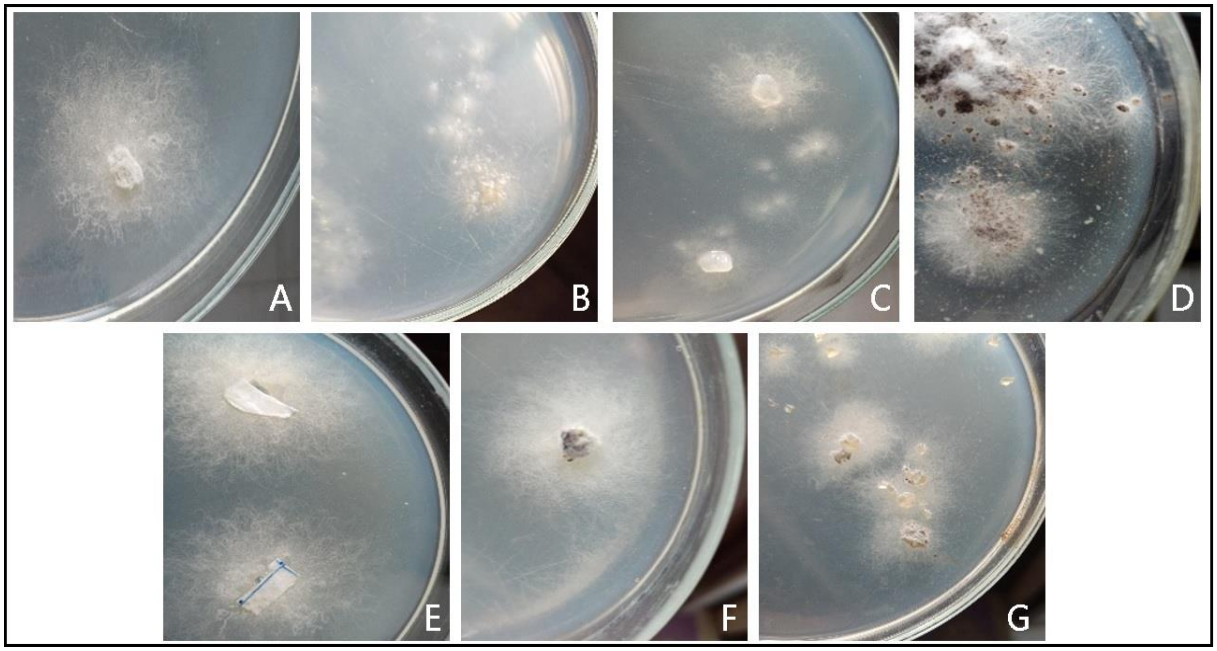

Fig. (1): Cultures of *H. maydis* recovered from: A, PDYA.RT; B, G30%. RT; C, SDW.RT; D, SS.RT; E, FPP.4°C; F, CSG.4°C and G, SG.4°C after 24 months of storage. Cultures incubated for 3 days at 27°C.

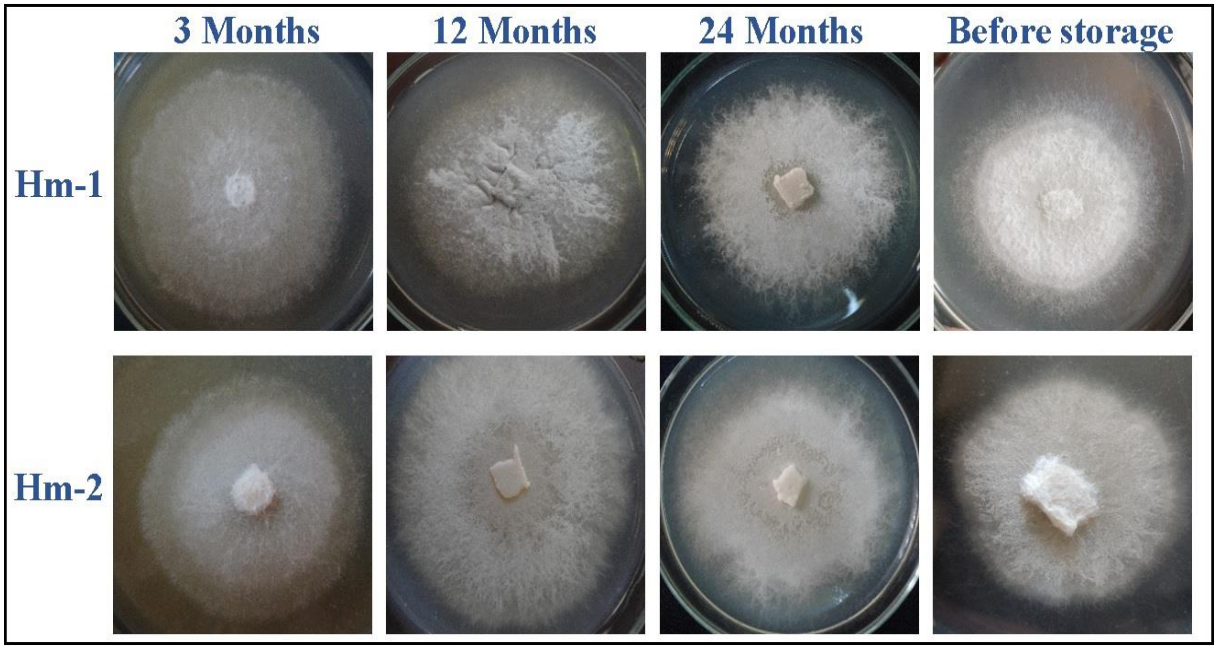

Fig. (2): Cultures of *H. maydis* Hm-1 and Hm-2 isolates after 3, 12 and 24 months of storage in SDW.RT and the original isolates before storage. Cultures incubated for 5 days at 27°C.

Table (3): Radial growth of survived *H. maydis* isolates after 3, 12 and 24 months of storage using twenty preservation methods.

Preservation methods	Radial growth after 5 days of incubation at 27°C (mm)					
	Hm-1 isolate			Hm-2 isolate		
	3 mo.	12 mo.	24 mo.	3 mo.	12 mo.	24 mo.
PDYA.RT	48 c	43 b	41.3 e	60.3 bcd	46.5 e	53 c
PDYA.4C	50 c	54.5	-	58 cde	48 de	-
PDYA.PO.RT	-*	-	-	-	-	-
PDYA.PO.4C	56.3 b	-	-	58.3 bcde	-	-
G10%.RT	59.8 ab	59.8 a	44.3 cde	57.5 de	51.3 cde	53.3 c
G10%.4C	-	-	-	57.5 de	-	-
G10%. -20C	56 b	-	-	-	-	-
G30%.RT	49 c	43.5 b	50.8 b	63.3 abc	60 ab	61.3 ab
G30% 4C	-	-	-	-	-	-
G30%. -20C	-	-	-	-	-	-
SDW. RT	50 c	42.5 b	43 de	58 cde	60 ab	54.5 bc
SDW. 4C	59.8 ab	-	-	67.3 a	-	-
SS.RT	60 ab	56.8 a	46 a	62.5 abcd	54.5 abcd	60.8 ab
SS.4C	59 ab	55 a	50 b	63.5 ab	53 bcde	66.3 a
FPP.4C	59.8 ab	55 a	63.5 a	63 abc	60 ab	65 a
FPP. -20C	63 a	44 b	-	59.8 bcde	56.5 abc	-
GSS.RT	47.3 c	55.5 a	-	61.8 bcd	48.3 de	-
GSS.4C	59 ab	57.8 a	-	58 cde	55 abcd	55.3 bc
SG.4C	49 c	57.8 a	48 bc	-**	53.8 bcde	-
SG. -20C	-	-	-	-	-	-
Original isolate at storage time	46.5 c	46.5 b	46.5 bcd	61.3 bcd	61.3 a	61.3 ab

*= treatment was not used for radial growth since the survivability was 0 in all replicates.
**= contaminated

Table (4): Virulence of survived *H. maydis* isolates after 12 and 24 months of storage using twenty preservation methods.

Preservation methods	Disease incidence of late wilt (%)			
	Hm-1 isolate		Hm-2 isolate	
	12 mo.	24 mo.	12 mo.	24 mo.
PDYA.RT	17.7 c	35 b	26.6 g	43.1 cde
PDYA.4C	26.6 c	-	39.2 egf	-
PDYA.PO.RT	-*	-	-	-
PDYA.PO.4C	-	-	-	-
G10%.RT	46.9 b	35 b	59.2 bc	30.8 e
G10%.4C	-	-	-	-
G10%. -20C	-	-	-	-
G30%.RT	57 ab	55 a	63.4 bc	55 bc
G30%. 4C	-	-	-	-
G30%. -20C	-	-	-	-
SDW. RT	51.1 b	55 a	55 cd	55 bc
SDW. 4C	-	-	-	-
SS.RT	59.2 ab	51.1 a	63.4 bc	81.1 a
SS.4C	55.3 ab	50.8 a	72.7 b	81.1 a
FPP.4C	72.3 a	55 a	72.3 b	63.4 ab
FPP. -20C	46.9 b	-	59.2 bc	-
GSS.RT	8.9 c	-	30.8 fg	-
GSS.4C	50.8 b	-	90 a	35 de
SG.4C	55 ab	-	43.1 def	-
SG. -20C	-	-	-	-
Original isolate at storage time	46.9 b	46.9 ab	50.2 cde	50.2 bcd

*= treatment was not used for virulence of the fungus since the survivability was 0 in all replicates.

DISCUSSION

According to a decision-based key for determining the most appropriate protocol for fungal preservation reported by Ryan *et al.* (2000), cryopreservation or lyophilization is recommended with fungi that produce asexual spores in cultures, a character associated with *H. maydis*. Unfortunately, these methods require special equipment which are relatively expensive, units of storage should be refrigerated, high initial and continuing costs as well as the risks of continuous access to liquid nitrogen (Elliott, 2005). So, the goal of this study was to determine a cost effective, easy and reliable method for preservation and maintenance of one of the most common important maize pathogenic fungi (*H. maydis*). The obtained results revealed that, colonized agar discs in sterile distilled water stored at room temperature (SDW.RT) was the best preservation method for *H. maydis* survivability, morphological characteristics, radial growth and virulence during the entire 24 months. Similar result has been described by Elliott (2005), who reported that all five *Gaeumannomyces graminis* var. *graminis* strains were recovered from all four replicates for 120 months when used as agar plugs stored in water at ambient temperature. Likewise, Hornby *et al.* (1998) found that *Gaeumannomyces graminis* survived more than 20 years in sterile water at ambient temperature. Also, numerous investigators have been reported high recovery rates for phytopathogenic cultures stored in sterile water (De Capriles *et al.*, 1989; Borman, *et al.*, 2006

and Sakr 2018). Water may be played a role for reducing fungal metabolism to very low levels by preventing desiccation and diminishes gas exchange. Furthermore, the current study revealed that preservation in glycerol 30% at RT (G30%.RT) could be considered as alternative method for *H. maydis* preservation since it exhibited a good degree of stability of the tested characteristics (Paul *et al*., 2015). However, Shearer *et al.* (1974) reported that 24 strains of *Septoria* stored in soil up to 1 year were recovered by 100% with stability of their pathogenicity. In this study, preservation in sterilized soil showed increasing in virulence of the tested isolates after12 and 24 months of storage which significantly different from that of the original isolates. This may be due to mutation affected on its pathogenicity (Windles *et al.,* 1993 and Tariq *et al.* 2015).

Although, cryopreservation at -20°C is more applicable to fungi that produce asexual spores (Ryan *et al.,* 2000), fungal recovery was not obtained after 12 and 24 months of storage in the current study. Similar difficulties have been described by Elliott (2005) with *Gaeumannomyces graminis* var. *graminis* in 40% glycerol at -20°C and Morris *et al.* (1988) with *Serpula lacrymans* when stored at -196°C. This may be due to formation of ice crystals during cooling resulted in cell damage during freezing and thawing for recovery. Also, the quantity of formed crystals in this method could have been higher since the freezing rate was lower. Storage in silica gel at -20°C was one of the worst preservation methods for viability although it has been successfully used for preserves *Fusarium* species for ten years (Windels *et al.,* 1993). This may be due to the weakness of bond formed between matrix and mycelia (Sharma and Smith, 1999; Ryan, 2001 and Homolka *et al.,* 2007). In this study, less or no fungal recovery were obtained from PDYA with paraffin oil at 4°C and at RT, respectively reflecting non suitability for preservation of this fungus and this may be due to the excess of paraffin oil (2cm depth) used in these methods. Previous investigators revealed that 1-cm layer of oil or more creates relatively anaerobic conditions causing cellular damage (Pumpianskaya, 1964 and Cintia & Katia, 2000).

Although *H. maydis* colonized filter paper piece (FPP.4°C) revealed a good degree of survivability and stability in unique characters, radial growth, and virulence of the tested isolates were significantly affected. This finding reflects unsuitability of this method for *H. maydis* maintenance. This result was consistent with the exceptions reported by Morales (2008) for stability in morphological and pathological characters of fungi recovered from stored colonized filter paper pieces. The most unexpected result from this study was successful of two preservation methods (SDW.RT and G30%.RT) under room conditions with variable temperatures (air condition turned off at night). These results referring to that electrical power is not essential for preserve *H. maydis* cultures by these methods. As a conclusion, *H. maydis* colonized discs in sterile distilled water and stored at room temperature (SDW.RT) was the best preservation and maintenance method for *H. maydis* during the entire 24 months. Also, fungal propagules stored in 30% glycerol (G30%.RT) at room temperature could be used along with or as alternative method of SDW.RT for fungal preservation. The two methods are simple and inexpensive.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Agag, S.H.; Sabry, A.M.; El-Samman, M.G. and Mostafa, M.H. 2021. Pathological and molecular characterization of *Magnaporthiopsis maydis* isolates causing late wilt in maize. Egypt. J. Phytopathol., 49 (2): 1-9.

Atkinson, R. 1954. Quantitative studies on the survival of fungi in five-year-old dried soil cultures. Can. J. Bot. 32(5): 673-678.

Bakerspigel, A. 1953. Soil as a storage medium for fungi. Mycologia, 45: 596-604.

Boesewinkle, H.J. 1976. Storage of fungal cultures in water. Trans. Br. Mycol., Soc., 66: 183-185.

Bosmans, J. 1979. Ten years lyophilization of pathogenic fungi. Mycopath. Mycol. Appl., 3: 13-23.

Borman, A.M.; Szekely, A.; Campbell, C.K. and Johnson, E.M. 2006. Evaluation of the viability of pathogenic filamentous fungi after prolonged storage in sterile water and review of recent published studies on storage methods. Mycopathologia, 161: 361-368.

Burdsall, Jr.H.H. and Dorworth, E.B. 1994. Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water in cryovials. Mycologia, 68: 275-280.

Butler, E.E. 1980. A method for long-time culture storage of *Rhizoctonia solani*. Phytopathology, 70: 820-821.

Carmichael, J.W. 1962. Viability of mold cultures stored at -20°C. Mycologia 54, 432-436.

Carter, M.V. and English, H. 1994. Long-term storage of *Eutypa lata*, the cause of an important dieback disease of apricot and grape. Plant Dis., 78: 925.

Castellani, A. 1963. Further researches on long viability and growth of many pathogenic fungi and some bacteria in sterile distilled water. Mycopathol. Mycol. Appl., 20: 1-6.

Castellani, A. 1967. Maintenance and cultivation of common pathogenic fungi of man in sterile distilled water. Further Researches. J. Trop. Med. Hyg., 70: 181-184.

Cintia, M.B. and Katia, F.R. 2000. Viability and sporulating capability of coelomycetes preserved under a range of different storage regimes. Revista Iberoamericana de Micologia, 17: 142-145.

De Capriles C.H.; Mata, S. and Middelveen, M. 1989. Preservation of fungi in water (Castellani): 20 years. Mycopathogia, 106: 73-79.

Elliot, M.L. 1995. Disease response of bermudagrass to *Gaeumannomyces graminis* var. *graminis*. Plant Dis. 79: 699-702.

Elliot, M.L. 2005. Survival, growth and pathogenicity of *Gaeumannomyces graminis* var. *graminis* with different methods of long-term storage. Mycologia, 97(4): 901-907.

El-Naggar, A.A.A.; Sabry, A.M. and Yassin, M.A.2015. Impact of late wilt disease caused by *Harpophora maydis* on maize yield. J. of Biol. Chem. Environ. Sci., Ain-Shams Univ., 10 (3): 577-595.

El-Naggar, A.A.A. 2019. PCR-based assay for detection of *Harpophora maydis* the causal agent of late wilt disease in maize plant parts. Egypt. J. Phytopathol., 47 (1): 277-295.

Gentles, J.C. and Scott, E. 1979. The preservation of medically important fungi. Sabouraudia, 17: 415-418.

Hajek, A.E.; Shimaz, M. and Humber, R.A. 1995. Instability in pathogenicity of *Entomophaga maimaiga* after long- term cryopreservation. Mycologia, 87: 483-489.

Hornby, D.; Bateman, G.L.; Gutteridge, R.J.; Lucas, P.; Osboum, A.E. and Yarham, D.J. 1998. Talk-all disease of cereals: a regional perspective. Wallingford, United Kingdom: CAB International. 384pp.

Homolka, L.; Lisa, L. and Nerud, F. 2007. Basidiomycete cultures on perlite survive successfully repeated freezing and thawing in cryovials without subculturing. J. Microbiol. methods, 69(3): 529-532.

Kitamoto, Y.; Suzuki, A.; Shimada, S. and Yamanaka, K. 2002. A new preservation method of fungus stock cultures by deep freezing. Mycoscience, 43(2): 143-149.

Krizkova, L. and Balan, J. 1975. Prolonged preservation of oomyctes and predacious fungi imperfecti under paraffin oil. Folia Microbiol (Praha) 20: 351-353.

Little, G.N. and Gordon, M.A. 1967. Survival of fungus cultures maintained under mineral oil for twelve years. Mycologia, 59: 733-736.

Miguens, M.P. 1985. Methods of maintaining stock cultures. Mykosen, 28: 134-137.

Morales, A. 2008. A simple way to preserve fungal cultures: Cornell mushroom blog. https://blog.mycology.cornell.edu

Morris, G.L.; Smith. D. and Coulson, G.E. 1988. A comparative study of the changes in the morphology of hypha during freezing and viability upon thawing for twenty species of fungi. J. Gen Microbiol., 134(11): 2897-2906.

Palacio. A.; Yessico, G.; Diego R.; Lucia, A. and Paola, Z. 2014. Viability of basidiomycete fungal strains under different conservation methods: cryopreservation vs. freeze-drying processes. Actual Biol., 36(100): 13-21.

Paul, J.S.; Tiwari, K.L. and Jadhav, S.K. 2015. Long term preservation of commercial important fungi in glycerol at 4C. Int. J. Biol. Chem., 9(2): 79-85.

Perkins, D.D. 1962. Preservation of *Neurospora* stock cultures with anhydrous silica gel. Can. J. Microbiol., 8: 591.

Pottinger, B.M.; Ridgway, H.J.; Bourdot, G.W. and Stewart, A. 2008. Influence of storage method on viability, mycelial growth and pathogenicity of *Sclerotinia sclerotiorum* sclerotia. Australas. Plant Pathol., 37: 609-614.

Pumpianskaya, L.V. 1964. Storage of microorganisms under mineral oil. Mikrobiologiya, 33: 1065-1070.

Ryan, M.J.; Smith. D. and Jeffries, P. 2000. A decision-based key to determine the most appropriate protocol for the preservation of fungi. World J. Microbiol. Biotech., 16: 183-186.

Ryan, M.J. 2001. The use of inmobilisation for the preservation of *Serpula lacrymans*. Mycologist, 15(2): 65-67.

Sabet, K.A.; Samra, A.S. and Mansour, I.M. 1966. Late wilt disease of maize and a study of the causal organism in U.A.R. pp 8-45. In: Samra, A.S. and Sabet, K.A., Abdel Rahim, M.F., El Shafey, H.A., Mansour, I.M., Fadl,

F.A., Dawood N.A. and Khalil, I.H.I. Investigations on stalk rot diseases of maize in U.A.R. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.

Sakr, N. 2018. Evaluation of two storage methods for fungal isolates of *Fusarium* sp. and *Cochliobolus sativus*. Acta Phytopathol. Entomol. Hun., 53(1): 11-18.

Samra, A.S.; Sabet, K.A. and Hingorani, M.K. 1962. A new wilt disease of maize in Egypt. Plant Dis. Reptr., 46: 481-483.

Sharma, B. and Smith, D. 1999. Recovery of fungi after storage for over a quarter of a century. Word J. Microbiol. Biotechnol., 15(4): 517-519.

Shearer, B.L.; Zeyen, R.J. and Ooka, J.J. 1974. Storage and behavior in soil of *Septoria* spp. Isolated from cereals. Phytopathology, 64: 163-167.

Smith, D. and Onions, A. 1994. The preservation and maintenance of living fungi. 2nd ed. Wallingford, United Kingdom: CAB International. 122 pp.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of Statistics. McGraw Hill Book Company Inc., New York 481pp.

Tariq, A.; Naz, F.; Rauf, C.A. and Irshad, G. 2015. Long term and least expensive preservation methods for various fungal cultures. Pak. J. Phytopathol., 27(2): 147-151.

Tuite, J. 1969. Plant pathological methods: fungi and bacteria. Minneapolis, Minnesota: Burgess Publishing Co. 239 pp.

Tung, H.J.; Ong, C.E.; Goh, Y.K.; Goh, Y.K and Goh, K.J. 2018. Survival and pathogenicity of monokaryotic and dikaryotic *Ganoderma boninense* following three different preservation methods. Transactions on Science and Technology. 5(1): 46-52.

Windels, C.E.; Burnes, P.M. and Kommendhal, T. 1993. *Fusarium* species stored on silica gel and soil for ten years. Mycologia, 85: 21-23.