Eliminating of Pathogenic Soil Borne *Pythium* Species Spreading Across the Marine Port of Duba

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ABSTRACT

Pythium species are considered the most important soil born fungi. They are facultative heterotrophic microorganisms. Under proper conditions, it become highly virulent to plants (depending on the species), causing many diseases, especially in the first stages of growth. These fungi abound in heavy agricultural soil and Egypt is an important source for such fungi that can be transmitted to another country. This research aims to identify the method of transporting *Pythium* contaminated soil by from a famous agricultural country like Egypt to Saudi Arabia via the vehicle tires. A method to disinfect those cars and eliminate contaminants before they enter Saudi territory was introduced.

Two hundreds and forty-six isolates belonging to seven species of *Pythium* were isolated and identified by morphological and molecular criteria. The species belong to *Pythium aphanidermatum, Pythium deliense, Pythium diclinum, Pythium irregulare, Pythium oligandrum*, *Pythium spinosum* and *Pythium ultimum* var. *ultimum.* These were isolated from the soil situated in folds of tires of 50 cars in Duba port that came from the Egyptian port of Hurghada, on September 7th, 2011. *P. aphanidermatum*, *P. deliense* and *P. ultimum* var. *ultimum* proved highly pathogenic to cucumber seeds germination causing 100% damping-off. *P. irregulare*, *P. diclinum* and *P. spinosum* var. *spinosum* were moderately pathogenic causing 74, 70 and 65% damping-off, respectively. On the other hand, P. *oligandrum* showed avirulent behavior towards cucumber germinating seeds with 0% damping-off. The anti-oospores efficacy of sodium hypochlorite (NaOCl) on transmission of *Pythium* spp. via cars' tires was studied. Mean reduction of oospore viability due to treatment with NaOCl was proved. Total destruction of viability was achieved within 30 min exposure when oospores of all tested pythia were treated with 0.42% NaOCl. Elimination of oospores viability was achieved after 30 min exposure with treatment of 0.240% NaOCl in 6 out of the 7 species. This is the first report on transmission of *Pythium* species to Saudi Arabian via seaports.

Key Words: Egypt, NaOCl, Oospores, Port, *Pythium* spp, Saudi Arabia.

INTRODUCTION

Pythium species occur in the soil, especially cultivated ones around the roots of plants, although they may be found in the soil of forests and pastures with minimal presence in desert (Elnaghy *et al*., 2010; Elnaghy

et al., 2014[a]).

These fungi cause serious diseases to crop plants, causing heavy losses in agricultural production, and have been found to cause dangerous diseases to animal and human (Bosco *et al*., 2005; Mendoza *et al*., 2003 and Elnaghy *et al*., 2014b).

Pythium spp. are not air born fungi, their units are transmitted by means of soil and water. Some of these fungi are type locality in definite places and certain countries (Abdelzaher *et al*., 1994c). However, if they meet the opportunity to move from one place to another or from one country to another, they become endemic to the new place and begin a series of problems.

Many investigators paid attention to the distribution of fungi over wide areas and transmitted agencies that permit their spread were evaluated (Bisby, 1943). Fungi are postulated to have appeared through the globe a couple of hundred million years ago. Many species now utilize more than one means of dissemination, such as ascospores by air, conidia by contact (Bisby, 1943). Animals, insects, birds and water act as the transmitted routes of. Transport agencies of the man have disseminated many fungi over the world. The dispersal of parasitic fungi can be frequently traced to transmission by means of seeds or transplanted seedlings (Bisby, 1943 and Plaats-Niterink, 1981). Fungi spread within countries that share water basins, rivers, ponds, streams and spattering rain. Zoospores producing fungi develop and propel themselves in water. Floating plant litters may transport fungi in water current (Moustafa *et al*., 2009).

Little information was known on distribution of most fungi that arrived in any country through ports.

A country like Saudi Arabia, not shared with any other country in the water basins or rivers, can be excluded from contamination via water carried zoospores. *Pythium* species, produced thick walled oospores, can be transmitted to Saudi Arabia only by means of soil that comes mostly traveling with man and his transportation equipments from countries infested by these fungi.

The entering, colonization and succession of *Pythium* spp. in the Zuidelijk Flevoland polder (Netherlands), reclaimed in 1966, were studied during the first six years of its cultivation (Plaats-Niterink, 1975). The number of species per soil sample increased from 0 in 1966 to 13 in 1972 (Plaats-Niterink, 1981).

Subsequently, one of routes that *Pythium* spp. can be accessed to Saudi Arabia from Egypt is the seaport Duba, which is located in the city of Duba,

Saudi Arabia. It is one of the oldest ports on the Red Sea coast. The port was established in 1995 to be a link between the northwestern region of the Kingdom of Saudi Arabia and the global economy. Duba is the nearest port to Egyptian ports on the Red Sea. The distance between Doba and the port of Sharm El-Sheikh is 62 nautical miles, the distance between Doba and the Egyptian port of Hurghada is 96 nautical miles and the port of Safaga 105 nautical miles as it is closer Saudi ports of the Suez Canal, where the distance between them is 253 nautical miles. Traveled ships to Suez Canal take about 17-hour average speed of 12 knots and is therefore the closest to the basin countries of the Mediterranean and a gate in the region on the lines of international trade.

Total received people amounted to 4 million passengers, according to statistics in 2004 as the port received 5,500 ships and 222 thousand cars (webpage: http://www.ports.gov.sa/).

This study was designed to determine the possibility of transmission of the plant parasite *Pythium* spp. from an agricultural country (Egypt) to Saudi Arabia through passenger car tires and entering the borders of the Kingdom of Saudi Arabia. Ways to eliminate these fungal spores to enter Saudi Arabia were also tested. This is the first report of such study in Saudi Arabia.

MATERIALS AND METHODS

Geography of Duba port:

Samples were collected from Duba port (27º20´57.3´´N, 35º41´46.2´´E), a marine port on the Red sea, Saudi Arabia (Figure 1).

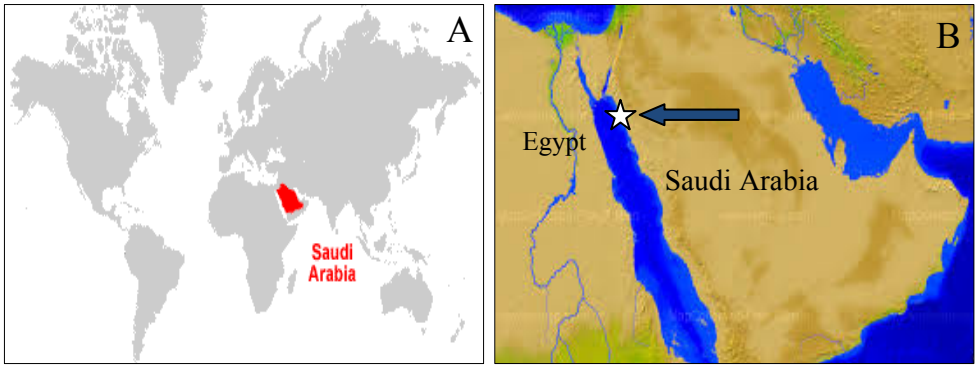


Figure (1, A-B): (A) World map showing the location of Saudi Arabia. (B) Map of Saudi Arabia showing the location of Duba port (☆).

Collection of soil samples from folds of tires of the cars:

1. Soil samples were collected from 50 cars coming from Egypt on September 7th, 2011 to Duba Port.
2. Of each car, soil samples were collected from 4 Tires (approximately 100 g of soil), and then placed in a clean sterilized plastic bags, and kept until the return to the laboratory.

Isolation of *Pythium* spp.:

Soil from folds of tires of the cars from Duba port were subjected for isolation of *Pythium* species using the following methods.

1. Baiting technique from Soil:

This method was proved to be suitable for the isolation of the virulent *Pythium* spp. (Abdelzaher *et al*., 1995). Soil (5 g), from each 100 g collected from one car (sometimes, soil of 100 g could not be collected, therefore, rest of 100 g was taken from the soil adhered around the tires) , was placed in sterilized 9 cm diam petri-dishes. Ten ml of sterile distilled water was added to enable the baits to float on the surface (Abdelzaher *et al*., 1997). Autoclaved maize leaf discs were used as baits. After 5 days of incubation at 25°C, baits were removed and placed on a petri-dish containing a selective medium (NARM). This medium was described by Morita and Tojo (2007) and modified by Senda *et al*. (2009) for isolation of *Pythium* spp., selectively [Nystatin (10 mg L^{-1}), Ampicillin (250 mgL^{-1}), Rifampicin (10 mg L^{-1}) and Miconazole (1 mg L^{-1}) in corn meal agar (CMA)]. The plated baits were incubated at 20°C for 3 days until the presence of fungal growth over the baits. Hyphal tips of the fungal colony on the NARM medium were transferred to water 2.5-3% agar agar (WA) to obtain a colony of approximately 1 cm diameter. Bacterial contamination were removed following the method of (Abdelzaher, *et al*., 1994a) in which the whole agar medium, containing growth of *Pythium*, in the same plate was then turned upside down with flamed forceps and then incubated until the growth reached before the edge of the dish wall. Meanwhile, the mycelia free of bacteria penetrated the agar medium to the top. Using a sharp needle, small pieces of the agar containing only one hyphal tip of emerging *Pythium* were removed from the periphery of the colony and then moved to corn meal agar (CMA) slants for maintain the fungus and to CMA plates supplemented with 500 µg/ml wheat germ oil to check the formation of fungal sexual structures (Abdelzaher *et al*., 1995).

2. Inoculating soil on the plates surfaces:

The method of Abdelzaher *et al*. (1995) was used.

Identification of Pythium spp.:

Morphological identification

Position, shape and size of sporangia, zoospores production, and position, shape and size of antheridia, oogonia and oospores, wall thickness of oospores were determined, microscopically (Waterhouse, 1967 and Plaats-Niterink, 1981). Morphological identification was done microscopically using the keys of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990), as well as the original description of each isolated *Pythium* species.

Molecular identification:

DNA extraction

Mycelia were grown in V8 agar medium at 25 ºC for 7 days or until adequate growth was observed. To extract the total genomic DNA, the method of Senda *et al*., (2009) was followed.

DNA amplification and sequencing

The nuclear rDNA region of the internal transcribed spacer (ITS), including the 5.8S rDNA, was amplified using two universal primers namely, the primer ITS4 (5′TCCTCCGCTTATTGATATGC3′) and ITS5 (5′GGAAGTAAAAGTCGTAACAAGG3′). Depending on the experiment, sometimes, primers of ITS1 (5′ TCCGTAGGTGAACCTGCGG3′) and ITS2 (5′GCTGCGTTCTTCATCGATGC 3′) were used as described by White *et al*., (1990) and Matsumoto *et al*. (1999). The amplicons were 700-900 bp long.

On the other hand, 563 bp of the *cox II* gene was amplified in certain *Pythium* with the primer pair FM66 (5′TAGGATTTCAAGATCCTGC3′) and FM58 (5′CCACAAATTTCACTACATTGA 3′) (Martin, 2000). Amplification of the sequencing template was carried out with DNA Thermal Cycler 2700 (Applied Bio-systems) with a cycling profile of pre-PCR at 94ºC for 5 min, followed by denaturation at 94ºC for I min, I min primer annealing at 55ºC for ITS, 52ºC for *cox* II and elongation at 72ºC, 2 min for 40 cycles, with a 7 min extension at 72ºC after the final cycle. To check the presence of PCR products, 5 µl of the PCR reaction mixture was loaded in 2% L03 (Takara Bio) agarose gel, electrophoresed at 100 V, 20-30 min, and stained with ethidium bromide. The sequencing templates were purified with GenElute PCR Clean-up kit (Sigma Chemical Co., St Louis,

Missouri, USA) following the manufacturer's instructions. Sequencing was performed with BigDye Terminator v3.1 cycle sequencing reaction kit (Applied Bio-systems) using the same primers in the initial PCR step. After purifying the sequencing reaction mixture through ethanol precipitation it was run on ABI 3100 DNA Sequencer (Applied Bio-systems).

Pathogenicity test:

In agar bottles

Pathogenicity was evaluated in WA as a medium for seed germination. One hundred ml of WA (2%) was poured each in 250 ml Erlenmeyer flasks and then sterilized by autoclaving. Cucumber seeds which proved to be highly susceptible to damping-off disease by the pathogenic *Pythium* species (Abdelzaher *et al*., 1994d) were sterilized by surface disinfection using sodium hypochlorite 2% for 3 min and then washing three times by sterilized distilled water followed by 1 min in 70% ethyl alcohol and finally three times using sterilized distilled water. Seeds were germinated to form radicles and plumules for 2 days at 25ºC and selected viable ones were used, thereafter. In each Erlenmeyer flask, 4 surface sterilized cucumber seeds were planted for damping-off test. Three discs of the tested *Pythium* species were taken from actively growing margin of *Pythium* colonies grown on CMA medium were added to each flask containing sterilized cucumber seeds under aseptic conditions. All inoculated flasks were then incubated in a growth cabinet at 25ºC with 12 h photoperiod (91 µmol $m^{-2}S^{-1}$). Damping-off was determined as the difference between seedlings emergence in non-inoculated controls and inoculated ones.

In pots experiment

Inoculum preparation of each tested *Pythium* species was performed as described by Al-Sheikh and Abdelzaher (2012). Emerged seedlings were counted at regular intervals until the development of 2 true leaves in the control. Damping-off was determined as number in emergence seeds to the total number of sowed seeds.

Effect of sodium hypochlorite (NaOCl) on oospore germination of seven Pythium spp.:

Oospore production of the selected Pythium spp.

Each *Pythium* species were cultured to produce oospores at 28ºC for three weeks in 100 ml Erlenmeyer flasks containing 10 ml of clarified V-8 juice medium containing 20% V8 vegetable juice, V/V, and 0.25% $CaCo_3$ and was clarified by centrifugation at 13,200 g for 30 min. (V-8) (Lumsden

and Ayers, 1975; Abdelzaher *et al*., 1994b). Oospores suspensions of each of *Pythium aphanidermatum* (Edson) Fitzp., *Pythium deliense* Meurs, *Pythium diclinum* Tokunaga, *Pythium irregular* Buisman, *Pythium oligandrum* Drechsler, *Pythium spinosum* Sawada, *Pythium ultimum* Trow var. *ultimum* were then obtained by mincing mycelial mats in a blender for 3 minutes. The resulting suspension was filtered through a sieve (15 µm pore diameter) in order to produce a suspension of oospores reasonably free of hyphal fragments. The number of mature (vital contents and intact walls) oospores were counted and related to the examined criteria.

Test concentrations of NaOCl

Survival of *Pythium* oospores was determined after exposure to 1.0, 5.7 and 10% v/v a commercial bleach (i.e., 0.042, 0.2240 and 0.420% sodium hypochlorite, respectively) over a period of 30 min. The pH of each bleach solution was 10.41, 11.21 and 11.41.

Exposure times

To determine the minimum amount of time required for complete destruction of oospores, Series of exposure times was selected for each test concentration of sodium hypochlorite. A pre-test showed that oospore destruction occurred within minutes of exposure. Contact times chosen were 1, 2, 4, 6, 8, 10, 20 and 30 min.

Treatment of oospores with NaOCl

Aliquots of 10 µl of oospores (contained 25 viable oospores and prepared from the original oospore suspension in distilled water) of each tested *Pythium* spp. were re-suspended to complete 1ml using sodium hypochlorite test solution. The used concentrations were 0.042, 0.2240 and 0.420% that are equal to 1.0, 5.7 and 10.0% dilutions (v/v) of a commercial bleach sold in the market. After mixing, it was incubated at room temperature (23ºC) for the required duration of exposure (i.e., 1-30 min). Oospores were then washed immediately with distilled water to remove residual quantities of sodium hypochlorite, then pulse centrifuged. The resulting oospores (25 ones) were re-suspended to 1ml distilled water. Positive controls were treated similarly, with the exceptions that distilled water replaced sodium hypochlorite and there was no incubation period. Five replicates of each sample was performed. Mean of the treated and positive controls were counted and related to each measurement.

Plating and quantification

To facilitate the determination of the number of viable oospores

remaining after treatment, 25 viable oospores per ml distilled water were taken from each exposure criterion. Each ml containing 25 oospores were added to a sterile disposable Petri plate and then 10 ml NARM medium (45ºC) were then added and the mixture was gently swirled to evenly distribute the oospores. Plates were sealed and maintained at 28ºC in the dark. Two negative controls were prepared for each replicate to evaluate the sterility of procedures and materials, one consisting of NARM only and the other consisting of NARM inoculated with 10 µl of the same sodium hypochlorite of water that was used for preparing oospore suspensions and dilutions of sodium hypochlorite.

Cumulative counts of the number of *Pythium* colonies observed on plates derived from each exposure time were determined 3, 4, 5 and 6 days post inoculation. Only those plates with no fungal growth were examined again on day 10 to ensure that slow developing oospores were not missed. Plates were viewed under 10x magnification and were quantified.

Mean counts were determined from 5 (positive) Petri plates of each exposure time.

Treatment of soils infested with oospores with NaOCl

This experiment was designed to test the effect of NaOCl on oospores in soil (as the natural dissemination rout of *Pythium* spp.). Soils from folds of tires of the cars were kept in a freezer (-20ºC) for 2 days. During freezing, hyphae, hyphal swellings, and other asexual units of the fungus ruptured and died). Previous investigations proved that oospores can only withdrawn freezing status (Abdelzaher *et al*., 1994b). Serial dilution method described by Hanlin and Ulloa, (1979) was then used. Five grams of the pre-freezed soil was added to 45 ml of sterile distilled water and shaken for 5 minutes to get a stock solution. One ml of the stock was pipetted into 9 ml of sterile distilled water in a test tube to make a serial dilution of 10^{-2}. 1ml of 10^{-2} serial dilution was pipetted into 9 ml of distilled water in a test tube to give 10^{-3} serial dilution. Similar method was carried out to give final concentrations of 10^{-4} and 10^{-5}. 1ml of dilutions: 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} for each of soil sample distributed on the surface of sterile Petri-dishes containing the selective NARM medium. Plates were incubated at 25°C for 5 days during which cultures were daily examined microscopically until final counts of oospores were known. One ml of a suitable dilution was added to 9 ml of the desired concentration of NaOCl to give final counts of 25 oospores per 10 µl with a definite concentration of NaOCl. Procedures was followed as described above in pure oospore suspension.

Treatment means were separated using Waller-Duncan *K*-ratio t-test

(Waller and Duncan, 1969). All of the experiments were repeated twice and data of one of set were subjected to the above-mentioned statistical calculation.

RESULTS

Occurrence and identification of Pythium spp. in folds of tires of the cars in Duba port:

Two hundreds and forty-six isolates belonging to seven species were identified as species belong of *Pythium aphanidermatum, Pythium deliense, Pythium diclinum, Pythium irregulare, Pythium oligandrum*, *Pythium spinosum, Pythium ultimum* var. *ultimum.* These were isolated from the soil situated in folds of tires of 50 cars in Duba port , came from the Egyptian port of Hurghada, on September 7th, 2011 (Table 1).

Table (1)
Frequency of isolates of *Pythium* spp. from soils adhered folds of tires of cars in Duba port, Saudia Arabia on September 7th, 2011.

No.	*Pythium* spp.	Number of species isolates	Frequency of isolates of *Pythium* sp., (%)
1	*P. aphanidermatum*	78	31.7
2	*P. deliense*	58	23.6
3	*P. diclinum*	19	7.7
4	*P. irregular*	42	17
5	*P. oligandrum*	9	3.7
6	*P. spinosum*	29	11.8
7	*P. ultimum* var. *ultimum*	11	4.5
Total number of isolates & frequency (%)		246	100

Identification of *Pythium* spp.:

Morphological Identification of Pythium spp.

Pythium species were subjected to identification using morphological characteristics and were named as follows:
Pythium aphanidermatum (Edson) Fitzp. (JU166)
P. deliense Meurs (JU266)
Pythium diclinum Tokunaga (JU366)
Pythium irregulare Buisman (JU466)
P. oligandrum Drechsler (JU566)
Pythium spinosum Sawada var. *spinosum* (JU666)
P. ultimum Trow var. *ultimum*. (JU766)

Oospores of the identified species were characterized as follows (Fig. 2):

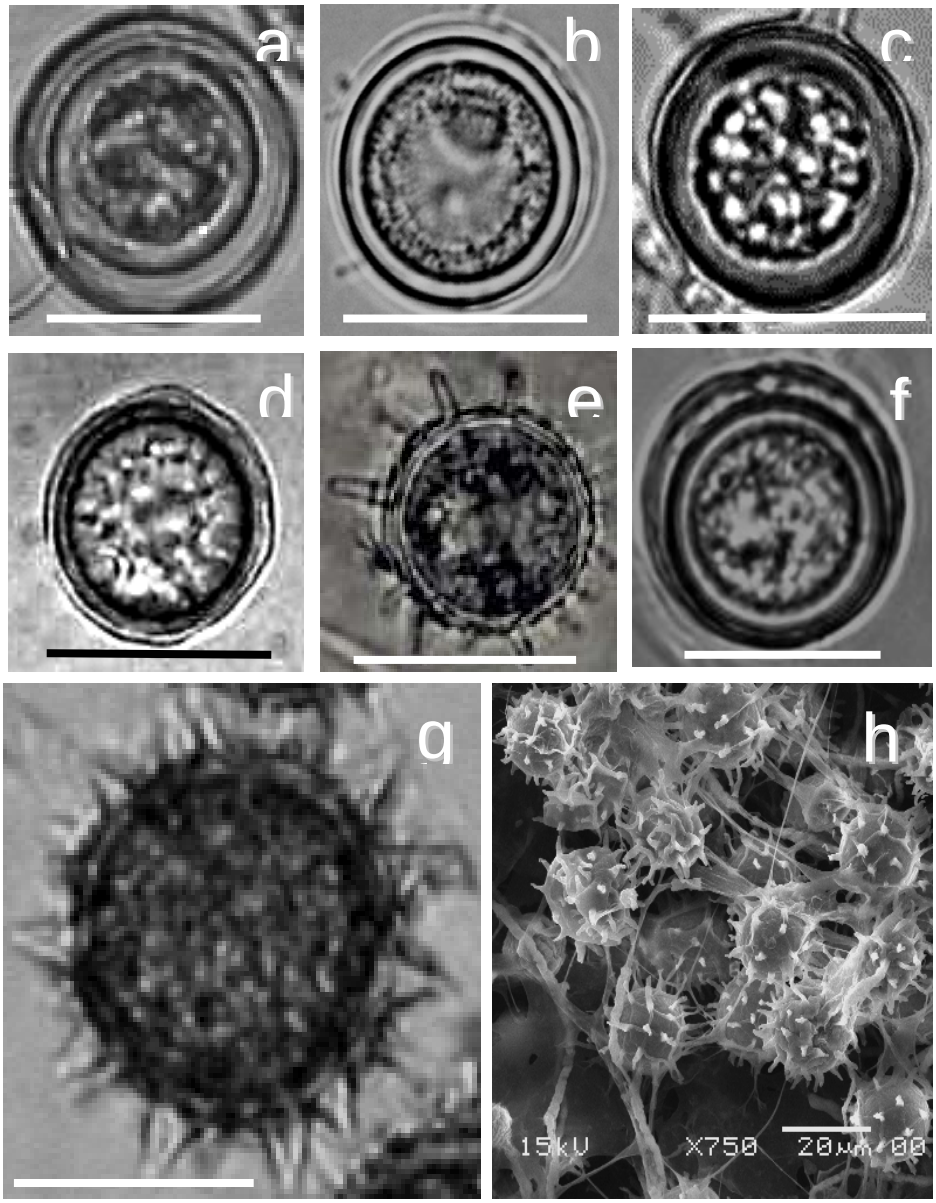


Fig. 2. Morphology of oospores of the isolated *Pythium* spp. a. *P. aphanidermatum*, b. *P. deliense*, c. *P. diclinum*, d. *P. irregular*, e. *P. spinosum*, f. *P. ultimum* var. *ultimum*, g. *P. oligandrum*, h. Scanned electron photograph of *P. oligandrum*. Bar on each photo equal 20 µm.

Molecular identification

Sequencing of rDNA-ITS including the 5.8 SrDNA were analyzed for the *Pythium* spp. and tested by the method of Kageyama *et al*., (2003) to confirm identification of the species. The sequence of the isolate (JU166) was closely related with that of *P. aphanidermatum* (GenBankaccession number, AB274404.1) with 100% similarity. The sequence of (JU266) was closely related with that of *P. deliense* (GenBankaccession number, AY598689.1) with 100% similarity. The sequence of (JU366) was closely related with that of *P. diclinum* (GenBankaccession number, AY598689.1) with 99% similarity. The sequence of (JU466) was closely related with that of *P. irregulare* (GenBankaccession number, AF452142.1) with 100% similarity. The sequence of (JU566) was closely related with that of *P. oligandrum* (GenBankaccession number, AY986954.1) with 100% similarity. The sequence of (JU666) was closely related with that of *P. spinosum* (GenBankaccession number, AY598701.1) with 100% similarity. The sequence of (JU766) was closely related with that of *P. ultimum* var. *ultimum* (GenBank accession number, AY598657.1) with 100% similarity.

Pathogenicity test of the 7 isolated *Pythium* spp. to cucumber germinating seeds:

In agar bottles

Pathogenicity (pre-emergence damping-off) of 7 isolated pythia was tested on cucumber seeds. *P. aphanidermatum*, *P. deliense* and *P. ultimum* var. *ultimum* proved to be highly pathogenic to cucumber seeds causing 100% damping-off. *P. irregular*, *P. diclinum* and *P. spinosum* var. *spinosum* were moderately pathogenic causing 74, 70 and 65% damping-off, respectively. On the other hand, *P. oligandrum* showed avirulent behavior towards cucumber germinating seeds with 0% damping-off in agar bottles (Fig. 3).

In pots

Usually, pathogenicity was less frequently in soil than in agar media. In pots, *P. aphanidermatum*, *P. deliense* and *P. ultimum* var. *ultimum* were also proved highly pathogenic to cucumber seeds causing 100% damping-off. *P. irregulare*, *P. diclinum* and *P. spinosum* var. *spinosum* were moderately pathogenic causing 65, 62 and 60% damping-off, respectively. *P. oligandrum* showed also avirulent behavior towards cucumber germinating seeds with 0% damping-off (Fig. 3).

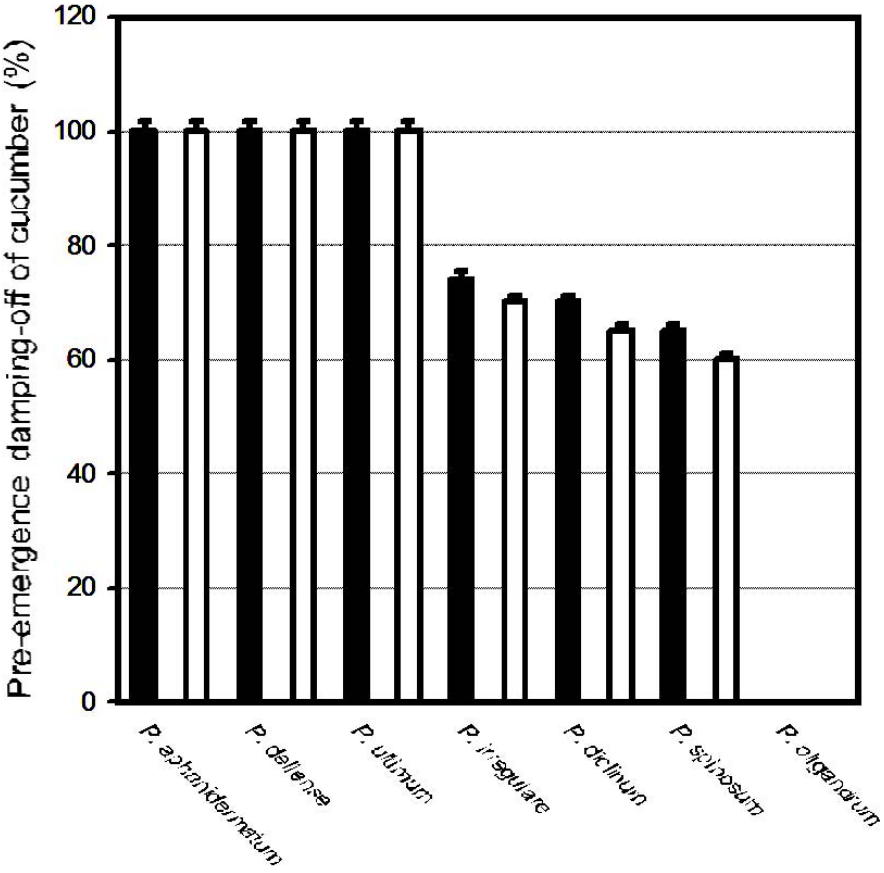


Fig. 3. Pre-emergence damping-off of cucumber seedlings grown in either water agar□ or clay sand soil■ infested with tested *Pythium* spp. Bars indicate standard errors of 25 measurements. All of data represent means and the two-tailed P value is less than 0.001. By conventional criteria, this difference is considered to be very highly significant (t *test*).

The effect of NaOCl on oospore germination of *Pythium* spp.:

Mean reduction of oospore (from oospores preparation) viability due to treatment with NaOCl is shown in Table 2. Total elimination of viability was achieved within 30 min exposure when oospores of all tested pythia were treated with 0.42% NaOCl. Elimination of oospores viability was also done after 30 min exposure with treatment of 0.240% NaOCl in 5 out of 7 species.

Table (2)
Mean reduction (%) of oospores viability in oospores suspension of the tested *Pythium* spp. isolated from soils presented in folds of tires of cars passed through Duba port on September 7th, 2011.

Pythium sp.	NaOCl, %	Contact time, min							
		1	2	4	6	8	10	20	30
P. aphanidermatum	0.00	0	0	0	0	0	0	0	0
	0.042%	20	32	41*	55**	55**	60**	77***	80***
	0.24%	55**	67**	70***	76***	88***	88***	95***	100***
	0.42%	77**	90***	95***	95***	95***	100***	100***	100***
P. deliense	0.00	0	0	0	0	0	0	0	0
	0.042%	18	30	40*	52**	50**	58**	78***	78***
	0.24%	60**	68**	74**	79**	89**	91***	95***	100***
	0.42%	80***	88***	90***	90***	95***	100***	100***	100***
P. diclinum	0.00	0	0	0	0	0	0	0	0
	0.042%	40**	55**	70***	75***	88***	90***	95***	95***
	0.24%	80***	85***	88***	90***	90***	91***	95***	95***
	0.42%	90***	92***	95***	96***	100***	100***	100***	100***
P. irregular	0.00	0	0	0	0	0	0	0	0
	0.042%	35*	48*	66***	69***	78***	85***	100***	100***
	0.24%	83***	88***	89***	88***	90***	92***	100***	100**
	0.42%	81***	89***	91***	96***	96***	100***	100***	100***
P. oligandrum	0.00	0	0	0	0	0	0	0	0
	0.042%	50**	57**	73***	89***	92***	93***	96***	100***
	0.24%	90***	90***	91***	95***	95***	96***	100***	100***
	0.42%	95***	96***	98***	100***	100***	100***	100***	100***
P. spinosum var. *spinosum*	0.00	0	0	0	0	0	0	0	0
	0.042%	23	35*	38*	59**	58***	66***	81***	92***
	0.24%	60*	67***	79***	87***	95***	100***	100***	100***
	0.42%	90***	94***	98***	98***	100***	100***	100***	100***
P. ultimum var. *ultimum*	0.00	0	0	0	0	0	0	0	0
	0.042%	20	31	31	55**	55**	61**	76***	82***
	0.240%	53**	69**	81***	87***	98***	100***	100***	100***
	0.420%	92***	92***	95***	98***	100***	100***	100***	100***

For each row,
Means followed by * are significantly different at 0.05 probability level,
Means followed by ** are highly significantly different at 0.01 probability level,
Means followed by *** are very highly significantly different at 0.001 probability level.

Mean reduction of oospore (from oospores of soil suspension) viability due to treatment with NaOCl is shown in Table 3.

Table (3)
Mean reduction (%) of oospores viability in soils from folds of cars tires of the tested *Pythium* spp.

Pythium sp.	NaOCl, %	Contact time (min)							
		1	2	4	6	8	10	20	30
P. aphanidermatum	0.00	0	0	0	0	0	0	0	0
	0.042%	10***	25***	32***	46***	47***	51***	72***	72***
	0.24%	40**	55***	60***	69***	77***	78***	90***	98***
	0.42%	65***	88***	91***	90***	92***	95***	100***	100***
P. deliense	0.00	0	0	0	0	0	0	0	0
	0.042%	15**	21***	30***	43***	44***	51***	71***	75***
	0.24%	48***	59***	66***	71***	77***	80***	90***	100***
	0.42%	75**	86***	90***	91***	91***	93***	100***	100***
P. diclinum	0.00	0	0	0	0	0	0	0	0
	0.042%	32**	39**	55**	62**	78***	80***	90***	90***
	0.24%	69***	74***	79***	80***	81***	84***	90***	93***
	0.42%	82***	85***	88***	88***	90***	90***	100***	100***
P. irregulare	0.00	0	0	0	0	0	0	0	0
	0.042%	22	38*	50**	60**	67***	72***	85***	93***
	0.24%	70**	77**	80***	80***	85***	90***	98***	100***
	0.42%	79**	84***	90***	89***	92***	98***	100***	100***
P. oligandrum	0.00	0	0	0	0	0	0	0	0
	0.042%	37*	47**	59**	75**	83**	88***	95***	95***
	0.24%	76**	79***	83***	85***	90***	93***	98***	100***
	0.42%	80***	80***	85***	88***	90***	93***	100***	100***
P. spinosum var. *spinosum*	0.00	0	0	0	0	0	0	0	0
	0.042%	20	28	31*	47**	55**	64**	76***	90***
	0.24%	48*	55**	68**	75***	78***	81***	96***	100***
	0.42%	77***	88***	90***	95***	95***	99***	100***	100***
P. ultimum var. *ultimum*	0.00	0	0	0	0	0	0	0	0
	0.042%	14	23	24	39*	45*	50*	70***	77***
	0.240%	36*	53*	74***	77**	88**	92***	100***	100***
	0.420%	90***	92***	93***	99***	100***	100***	100***	100***

For each row,
Means followed by * are significantly different at 0.05 probability level,
Means followed by ** are highly significantly different at 0.01 probability level,
Means followed by *** are very highly significantly different at 0.001 probability level.

DISCUSSION

There is more than one way for moving fungi from one place to another on the surface of the globe (Plaats-Niterink, 1981). Transmission of fungi from one location to another takes place by either air, water, or soil. Tools

used for cultivation of plants are likely to pass on plant pathogens from one place to another. Spores of pathogens in this case are usually passed in the form of bits of plant disease debris lying in the soil (Al-Sheikh and Abdelzaher, 2012). Additionally, soil can be transmitted from one place to another via several outlets including tires of cars traveling from one country to another. Cars coming from some agricultural countries can carry soil rich in many pathogenic fungi causing contamination of agricultural soils to the access country. The fungi that have thick-walled spores, especially sexual ones that tolerate unfavorable conditions, can be transmitted for long distances withdrawing difficult climatic factors (Abdelzaher *et al*., 1994b).

Pythium species are considered to be soil born fungi that transmitted through the soil. These fungi possessed sexual thick-walled oospores that can be transmitted from an agricultural country such as Egypt to Saudi Arabia.

A possible route that the agricultural soil enter Saudi Arabia from Egypt is through conjoined in the tires of cars via seaports, such as the Saudi port of Duba on the Red Sea. Regularly, many cars enter Saudi Arabia, carrying huge amount of the pathogenic fungal spores.

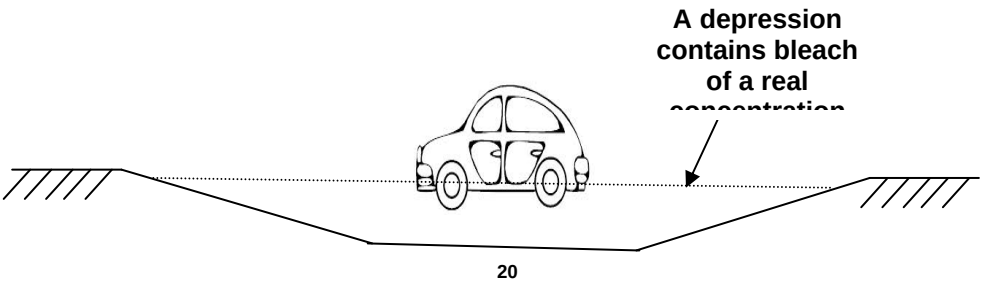
Results here showed that the soil conjoined in tires of 50 cars coming from Egypt on September 7th, 2011, contained seven oospores of *Pythium* species. *P. aphanidermatum*, *P. deliense*, *P. diclinum*, *P. irregulare*, *P. oligandrum*, *P. spinosum* var. *spinosum* and *P. ultimum* var. *ultimum* were isolated from soil adhered to tires of cars and identified according to morphological and molecular criteria. All of these species have previously been isolated from Egyptian soil (Elnaghy *et al*., 2014a and b).

Results also showed that 6 out of 7 of the isolated *Pythium* species were able to infect cucumber seedlings. *P. aphanidermatum*, *P. deliense* and *P. ultimum* var. *ultimum* proved to be highly pathogenic species whereas *P. diclinum*, *P. irregulare*, and *P. spinosum* var. *spinosum* were moderately pathogenic to cucumber seedlings in the pathogenicity test. Many previous reports proved the pathogenicity of these isolated fungi to different crop plants (Al-Sheikh , 2010; Al-Sheikh and Abdelzaher, 2010 a and b). For this reason, occurrence of such fungi is dangerous to the crop plants especially when environmental conditions favor disease prevalence.

Since sexual oospores of *Pythium* species tolerate inappropriate factors, many studies examined on the effect of some chemical compounds on the vitality of those oospores. In an attempt to solve the problem of entering those pathogenic fungi to Saudi Arabia via car tires coming from Egypt through the port of Duba sea, the present work have investigated the effect

of NaOCl on the viability of oospores. Previous research confirmed the possibility of using disinfectants including NaOCl to sterilize surfaces and killed some fungi spores (Ebling, 2007; Jiang and Erwin, 1990 and Stanley *et al*., 2009). Our results showed that the concentration of 10% of the commercial bleach, containing actual concentration of 0.420% of NaOCl, inhibited the growth of oospores of 7 *Pythium* species by sowing oospores for 30 minutes in the solution.

In order to eliminate movement of these oospores from Egypt to Saudi Arabia, precaution policy was designed and suggested, including the sterilization of tires of cars coming from Egyptian ports throughout crossing a depression containing NaOCl. Analogy on the same pattern, passengers should passed-over foot mat soaked with NaOCl. For this reason, we can say that it can use the bleaches containing a concentration of at least 0.420 of NaOCl in the sterilization of tires coming through the ports, and the following Fig.4, shows a proposal for the possibility of sterilization of tires in various ports:



20

Fig. 4. Illustration represents how the passage of vehicles on a depression containing bleach to sterilize tires. The car must remain inside the depression which containing bleach for 30 minutes, moving forward and backward every 5 minutes. Spraying the bleach under pressure to wash places around the tire must be accomplished. This procedure can be performed during check-in and passport checks.

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انتشار فطريات البيثيم الممرضة المحمولة على التربة عبر ميناء ضباء البحري مع طريقة محتملة لمنع ذلك الانتشار

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الملخص

تعتبر فطريات البيثيم من أهم الفطريات المتطفلة شديدة الضراوة التي تحمل على التربة. وهي كائنات مسببة الكثير من أمراض النباتات، خاصة في أطوار النمو الأولى. تكثر هذه الفطريات في الأراضي الزراعية السوداء، وتشكل مصر مورداً مهماً لهذه الفطريات المحمولة أساساً على التربة والتي يمكن أن تنتقل من بلد إلى آخر. ويهدف هذا البحث إلى تعرف طريقة مهمة لحمل التربة الملوثة بتلك الفطريات من بلد زراعي شهير مثل مصر إلى المملكة العربية السعودية عبر إطارات المركبات مع كيفية تطهير تلك السيارات والقضاء على الملوثات قبل دخولها الأراضي السعودية.

تم تعريف ودراسة مائتين وست وأربعين عزلة من الفطريات بمعايير الشكل الظاهري والخصائص الجزيئية لهذه الفطريات. حيث تبين أن الأنواع المعزولة تنتمي إلى سبعة أنواع هي *Pythium aphanidermatum, Pythium deliense, Pythium diclinum, Pythium irregulare, Pythium oligandrum, Pythium spinosum, Pythium ultimum var. ultimum.*

تم عزل هذه العينات من التربة الموجودة في طيات إطارات خمسين سيارة متواجدة في ميناء ضباء قادمة من ميناء الغردقة المصري بتاريخ السابع من سبتمبر 2012. بينت الدراسة أن الفطريات *P. aphanidermatum*, *P. deliense* and *P. ultimum var. ultimum* لها مقدرة شديدة على إصابة بذور نبات الخيار حيث كانت نسبة إعاقة نمو البذور هي 100%. في حين بينت الدراسة أن فطريات *P. irregulare, P. diclinum* and *P. spinosum var. spinosum* كانت متوسطة الضراوة وبلغت 74، 70 و 65% على التوالي. من ناحية أخرى أظهرت فطرة P. oligandrumعدم المقدرة على إصابة بذور الخيار بنسبة 0%. تم في هذه الدراسة تقييم مدى فعالية استخدام هيبوكلوريت الصوديوم (NaOCl) في القضاء على فطريات البيثيوم. وقد بينت الدراسة أن الطريقة

المثلى للقضاء على هذه الفطريات هي تعريض الإطارات لمدة 30 دقيقة لمحلول 0.42% NaOCl حيث تم القضاء على ستة أنواع من أصل 7 أنواع معزولة في هذه الدراسة. هذا هو التقرير الأول عن انتقال فطريات Pythium الممرضة إلى المملكة العربية السعودية عبر الموانئ البحرية.

الكلمات المفتاحية: انتقال فطريات البيثيم، جراثيم بيضية، مصر، المملكة العربية السعودية، ميناء، هيبوكلوريت الصوديوم.