Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 9, Issue. 2, pp: 266-272, 2023
URL: https://arpgweb.com/journal/journal/14
DOI: https://doi.org/10.32861/jac.92.266.272

Academic Research Publishing Group

Original Research **Open Access**

Testing the Effect of Some Materials to Control *Pythium Aphanidermatum* Isolated from Cucumber

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Article History
Received: 6 December, 2022
Revised: 16 February, 2023
Accepted: 30 March, 2023
Published: 9 April, 2023

Abstract

The main objective of this study was to identify the effectiveness of household materials that are used to sterilize vegetables from the fungus *Pythium aphanidermatum (P. aphanidermatum)*. Samples were taken from the damaged cucumber in the local markets of Babil Governorate, Iraq. These samples were kept in sterile cool containers until they reached the Microbiology lab. of the Department of Community Health, Technical Institute of Babylon. The samples were cultured laboratory in the Sabouraud Dextrose Agar (SDA), then a microscopic examination was carried out to identify the fungus using the lacto phenol cotton blue dye. Use in this study most common household materials to sterilize vegetables before keeping them in the refrigerator or before eat are Potassium Permanganate (KMnO4), Sodium Hypochloride (NaOCl) and table salt (NaCl). Five concentrations were used for each sterile material, and five replicates of culture dishes were used for each concentration (A mixture of sterile material with the SDA) after which the fungus was cultured. By using the minimum inhibitory concentration assay, the radial growth inhibition assay and the microscopic fungal examination, the efficacy of sterilizers was identified. The final results showed the presence of antifungal efficacy for all sterilizers used, with different concentrations. This study concluded that these materials can be used by housewives to sterilize vegetables before keeping them in the refrigerator or eating them.

Keywords: Materials; *Pythium aphanidermatum*; Cucumber; Contamination.

How to Cite: Ibtisam Mohammed Hussein, Abbas Razzaq Abed, 2023. "Testing the Effect of Some Materials to Control *Pythium Aphanidermatum* Isolated from Cucumber." *Journal of Agriculture and Crops*, vol. 9, pp. 266-272.

1. Introduction

Plant fungi are considered one of the most harmful agricultural pests that cause *damage to field crops, as well as the fruits of plants in the home, such as fruits and even* vegetables, and this increases the rate of their risk, especially when their toxic metabolic contents are excreted in them and thus will become harmful to human health if they eat them [1] . Secondary metabolic secretions, which are excreted by fungi in vegetable food after their injury, cause very dangerous health problems to human health when ingested, including cancers , genetic mutations and organ diseases such as kidney or liver failure, cellular tissue damage, neurological damage, etc. [2] Pythium is a genus of parasitic oomycetes. They were formerly classified as fungi. Most species are plant parasites [3, 4] . P. aphanidermatum is a soil borne ,which are also called water molds . Oomycetes are not true fungi , cell walls of P. aphanidermatum are made of cellulose instead of chitin, they are diploid in their vegetative state, and they contain co-enocytic hyphae (lacking crosswalls), called a protest [5] . In this study, researchers dealt with the effect of one of the most common fungi (Pythium aphanidermatum) that infects cucumbers and causes damage to it, which leads to economic losses as a result of its damage as a result of this fungus, so the current study aimed to use simple household materials to eliminate the fungus before it grows on the cucumber.

2. Materials and Methods

2.1. P. Aphanidermatum Isolates

P. aphanidermatum were isolate from infected cucumber taken from the local market in Babil Governorate, Iraq. Infected samples of cucumber were kept in a cool container till trans to Microbiology lab / Technical institute of Babylon. Samples were taken from the affected cucumber and cultivated in the SDA for 7 days at 27ºC±1.

2.2. Mycelial of P. Aphanidermatum Microscopic Examination

A small portion were taken from SDA media was contained *P. aphanidermatum* colony by mycological loop and it is placed on clean surface of glass slide and mixed with one drop of Lacto phenol-cotton blue dye. The

mycelium of *P. aphanidermatum* was spread very well on the glass slide. A cover glass slip was gently applied with little pressure to expel air bubbles. The slide was then mounted and observed with X10 and X40 objective lenses respectively [6].

2.3. Spores Count

After P. aphanidermatum colony was cultured on the SDA medium at 27ºC for 7 days, spores were collected and suspended in five ml of sterile normal saline. The spores concentration in the suspension was determined by using a haemocytometer method which include one drop of the suspension was added into hemocytometer chamber, spores were calculated under high power 40X of light microscope using the following equation [7]. :-

$$\text{Spore number/ml} = \frac{Zx4x10^6}{N}$$

Where:-Z= total number of counted spores (Spores number in 5 small square of RBCs count). N= total No. of small squares (Five small square of RBCs count x 25 small square in each small square of RBCs count =80).

Final spore suspension should be obtained to equal to 10^7 spore /ml.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

MIC of Materials used (KMnO4 , NaOCl and NaCl) were determined by tube dilution Method [8] , Ten test tubes with 8 ml of Sabouraud Dextrose Broth (SDB) in each were taken and autoclaved (temperature 121ºC , For 15 mints at 15 Ibs). Two ml of each anti-fungal agents (Stock solution) was added and serial double fold dilution was done up to the 10 tube and from the 10 tube, 2 ml of the mixture was discarded. To each tube *P. aphanidermatum* colony spores ($1x10^7$ spore/ml) were added and mixed well. The tubes were incubated for 7 days at 27 ºC for *P. aphanidermatum* colony. The least concentration of Materials used capable of inhibiting the fungal growth was considered MIC.

2.5. Radial Growth Inhibition Assay

All Materials were used in this study were purchased from the Scientific offices in Babylon, Iraq and mixed each one with SDA media after sterilization (Autoclave temperature 121ºC , For 15 mints at 15 Ibs) to obtain on (KMnO4[0.5% , 1% ,1.5% ,2% and 2.5%] , NaOCl[0.25% , 0.5% , 0.75% , 1% and1.25%] and NaCl [10% , 20%, 30%,40% and 50%) , then five mm diameter disc of *P. aphanidermatum* colony mycelia were cut by sterilized cork borer from the periphery of five day old culture and transferred aseptically in the center of SDA media contains different materials used according to a pre-prepared concentrations. All petri plate including control and experimental were incubated at 27°C for 7 days. After 7 days of incubation, observations were recorded and measurement of radial growth of *P. aphanidermatum*colony [9] .

2.6. Statistical Analysis

SPSS (Statistical Package from the Social Sciences, Inc, Chicago, Il, USA) version 13.0 for windows were used to data analysis . Statistical analysis was performed by F-Test -one-way ANOVA analysis of variance. Variances were considered significant if $p < 0.05$ [10] .

3. Result

To prove the effectiveness of KMnO4 , NaOCl and NaCl in its antifungal activity, several tests were used, including minimal inhibitory concentration assay, Radial growth inhibition assay and the microscopic examination of the fungal mycelia.

Minimal inhibitor concentration

The results of the current study showed a discrepancy in the minimal inhibitory concentration of *P. aphanidermatum* for the materials used (KPO4, NaOCl and NaCl) , where NaOCl showed the highest inhibitory activity for the fungus with the minimal inhibitory concentration (0.00384mg/ml) compared with KMnO4 and NaCl , as shown in table No.1.

Table-1. Minimal inhibitor concentration of KMnO4 , NaOCl and NaCl against *P. aphanidermatum*.

Materials used	MIC mg/ml
KMnO4	2.5mg/ml
NaOCl	0.00384mg/ml
NaCl	600 mg/ml

3.1. Redial Inhibitor Growth Assay

3.1.1. Potassium Permanganate

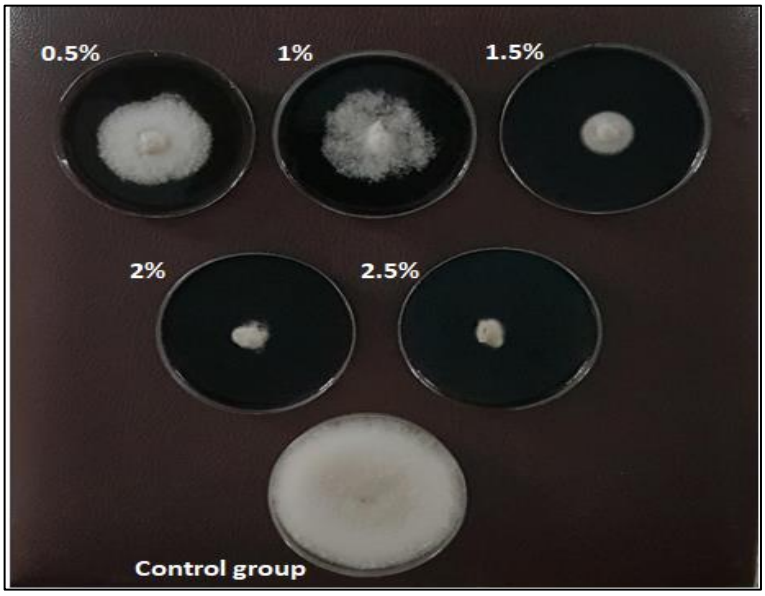
The increase in the concentrations used for KMnO4 mixed with the culture medium was inversely proportional to the growth of the *P. aphanidermatum* disc in SDA , as shown in table No. 2 and figure 1.

Table-2. Inhibiting the radial growth of *P. aphanidermatum* on the surface of SDA mixed with different percentages of KMnO4

Group (%) KMnO4	M±SE Radial growth inhibition (mm)
0.5	47.20±0.58 A
1	43.40±0.92 B
1.5	22.60±0.87 C
2	12.60±0.87 D
2.5	10.40±0.50 E
Control group	82.20±0.37 F

LSD=1.74
Different capital letters denote significant results (P<0.05) between different concentration.

Figure 1. Inhibiting the growth diameter of *P. aphanidermatum* growing on the surface of SDA mixed with different percentages of KMnO4 compared with the control group.



3.1.2. Sodium Hypochlorite

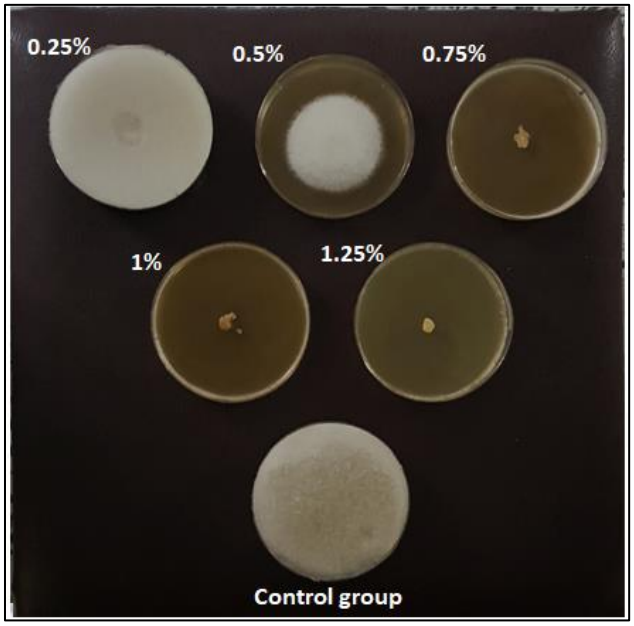
The low concentration (0.25 %) of NaOCl did not show an effect on inhibiting the growth of *P. aphanidermatum* on the SDA , while the rest of the concentrations showed a "significant" inhibition in the radial fungal growth when compared with the control group, as shown in table No. 3 and figure No. 2.

Table-3. Inhibiting the radial growth of *P. aphanidermatum* on the surface of SDA mixed with different percentages of NaOCl

Group (%) NaOCl	M±SE Radial growth inhibition (mm)
0.25	81.40±0.50 A
0.5	48.00±0.83 B
0.75	5.00±0.00 C
1	5.00±0.00 C
1.25	5.00±0.00 C
Control group	82.40±0.50 A

LSD=1.09
Different capital letters denote significant results (P<0.05) between different concentration.

Figure-2. Inhibiting the growth diameter of *P. aphanidermatum* growing on the surface of SDA mixed with different percentages of NaOCl compared with the control group



3.1.3. Table Salt (NaCl)

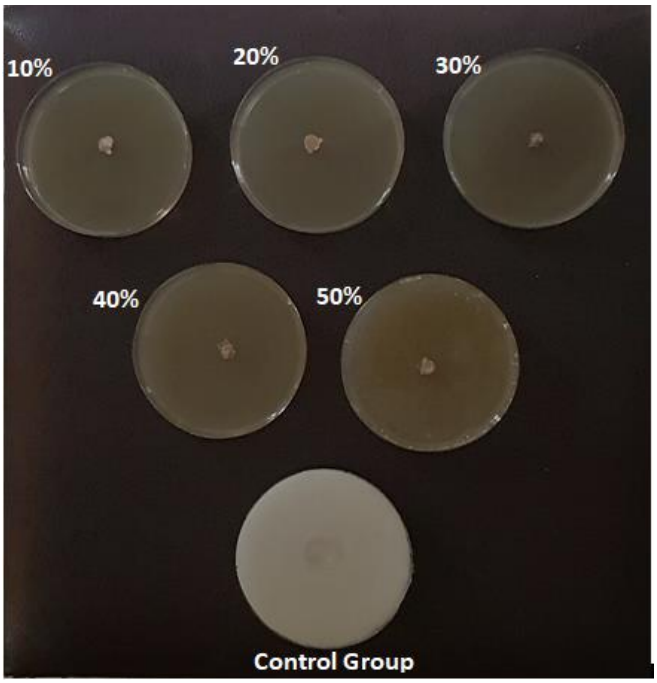
All concentrations used for NaCl showed "complete" inhibition of *P. aphanidermatum* grown on the surface of the SDA without any mycelia growth of this fungus when compared with the control group as shown in table No. 4 and figure 3.

Table-4. Inhibiting the radial growth of *P. aphanidermatum* on the surface of SDA mixed with different percentages of NaCl.

Group (%) NaCl	**M±SE** **Radial growth inhibition (mm)**
10	5±0.00 A
20	5±0.00 A
30	5±0.00 A
40	5±0.00 A
50	5±0.00 A
Control group	81± 0.37 B

LSD= 0.37
Different capital letters denote significant results (P<0.05) between different concentration.

Figure 3. Inhibiting the growth diameter of *P. aphanidermatum* growing on the surface of SDA mixed with different percentages of NaCl compared with the control group



4. Microscopic Examination

Microscopic examination of the fungal mycelia of *P. aphanidermatum* indicated the presence of many abnormalities in the developing tops and fungal hyphae, which greatly affected the growth of this fungus on the surface of the SDA mixed with different proportions of potassium permanganate, Sodium hypochloride and table salt, as shown in figure No. 4.

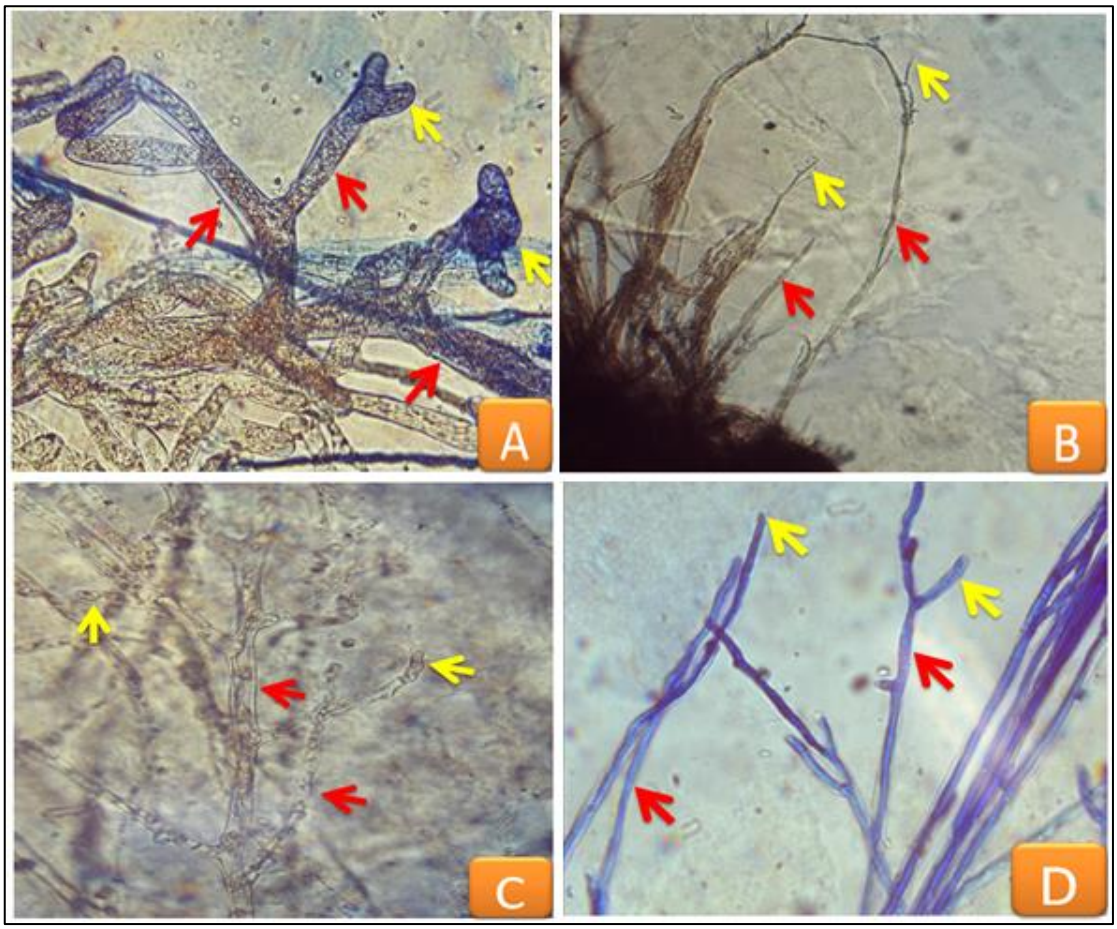


Figure 4.Microscopic examination of *P. aphanidermatum* mycelia. **A=** Effect of KMnO4 mixed with SDA on the growth of fungal mycelia of *P. aphanidermatum* fungus, where the fungal hyphae and their growing tops are characterized by their enlargement, deformation and lack of branching. B=The effect of NaOCl mixed with SDA on the growth of the fungal mycelia of *P. aphanidermatum* fungus, where the fungal hyphae and their growing tops are characterized by their thinness, lack of cytoplasm and lack of branching. C=The effect of NaCl mixed with SDA on the growth of the fungal mycelia of *P. aphanidermatum*, where the fungal hyphae and their developing tops appear transparent, ghostly and devoid of cytoplasm. D=represent the control group. Note:- the yellow arrows represent the developing tops and the red arrows represent the aerial hyphae (X40).

5. Discussion

Post-harvest disinfection of fresh vegetables and fruit an essential first step in post-harvest handling. The lower requirement for disinfection procedures is to keep goods and facilities free from post-harvest fungal pathogens and bacterial human pathogens thus improving food safety [11] .

Fruits and vegetables require a surface sterilization method that kills disease-causing microorganisms but does not affect the smell or taste of food. Freshly harvested vegetables and fruits are considered to be of high food safety risk [12, 13] . Because it may contain microorganisms flecked at levels between 3-7 logarithmic units, depending on the season, freshness, and region of production [14, 15].

As a result of poor storage of vegetables in preservation devices and the provision of appropriate conditions of temperature and humidity helps the growth of the fungus *P. aphanidermatum* and thus causes a large loss of stored materials, and at the same time this fungus has the ability to adapt and live inside the preservation devices and therefore these devices will be a source of infection. Housewives use several types of sterilizers to get rid of the fungi that infect vegetables before keeping them in the refrigerator, as well as to sterilize contaminated preservation devices, And the study dealt with the most common sterile materials "in the sterilization of vegetables and conservation devices by knowing the concentration of sterile material that kills contaminated fungus and prevent its growth. Household sterilizers (KMnO4, NaOCl, and NaCl) are easy to use, safe, cheap and available in the market, and have no cumulative action when used to sterilize vegetables [16] .Where this study proved the effectiveness of

each of KMnO4, NaOCl and NaCl, they have good efficacy in inhibiting the growth of the fungus *P. aphanidermatum*, and this inhibition came from the mechanism of action of the sterilizer on the fungal cell.

Potassium permanganate is consists of K + and MnO− 4. It is a black-purple crystalline salt that dissolves in H_2O to give intensely purple pink or solutions . Also Potassium permanganate is included in the World Health Organization's Model List of Essential Medicines, the safest and most effective medicines required in a health system [17] . In this study KMnO4 concentrations (1.5% , 2%and 2.5%) proved highly effective in inhibiting the growth of Pythium fungi compared to concentrations (0.5% and 1.5%) and the control group, and the reason for this is that the concentrations used were suitable for cytostatic, and prevent it from growing because Potassium permanganate is a powerful oxidizing agent [18] , that alters the cell walls of pathogenic microorganisms and it interferes with their DNA structure and exerts effective microbial activity on protozoa , fungi ,bacteria and viruses [19] .

Also the results of the current study showed that the concentrations of NaOCl (0.25 % and 1%) had no effect on the inhibition of the fungus *P. aphanidermatum* . While the concentrations (1.25%, 1.5% and 1.75%) showed a high inhibitory activity (there was no growth on the surface of the culture medium) compared with the other concentrations used and the control group. Sodium hypochlorite is comprising a sodium cation (Na+) and a hypochlorite anion (OCl−or ClO−) [20, 21] . NaOCl act as antifungal action by reacting with amino acids and fatty acids , by saponification reaction, it acts as an organic and fat solvent, degrading fatty acids to form glycerol and fatty acids. While Sodium hypochlorite react with amino acids to neutralize them and form salt and water . other mechanism of action include Hypochlorous acids (HOCl-) present in sodium hypochlorite solutions may act as solvents in contact with organic tissue to release chlorine, which forms chloramines when combined with the protein amino group that disrupt cell metabolism [22]**.**

Sodium chloride is an ionic compound with the chemical formula NaCl, consisting of a positive sodium ion and a negative chloride ion and has the ability to dissolve in water [23] . All concentrations of NaCl used in this study led to a complete inhibition of *P. aphanidermatum* growth compared with the control group, and the reason for this is due to the effects of NaCl stress on microbe growth are ion toxicity by sodium or chloride Farooq, *et al.* [24] and Evelin, *et al.* [25] . Increase in Na^+ and Cl^- ions cause structural damage to microbes' cells [26] .

6. Conclusion

Potassium permanganate, sodium hypochloride and table salt showed high efficacy in concentrations that completely slowed down the growth of Pythium fungus. Therefore, these substances are considered materials that can be used to sterilize vegetables before keeping them in the refrigerator.

Acknowledgments

We would like to thank the Department of Microbiology , Community health department . technical institute of Babil.

Conflict of Interest

The authors declares that there is no conflict of interest

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