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Evaluation the Effectiveness of Different Concentrations Phenols, Alkaloids and Terpenes Extracted from *Pimpinella anisum* against *Phytophthora* Fungi.

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Abstract

This study did the isolation, purification, and identification of the fungus *Phytophthora cinnamomi* of some infected plants, including Chili pepper, cucumber, and eggplant**.** The green parts of *Pimpinella anisum* plant were grounded to a semi-powdered state. Phenols, alkaloids and terpenes were extracted from this plant, then the anti-fungal activity was evaluated at different concentrations of 5% and 10%. The percentage of radial growth inhibition of fungi with plant extracts was measured after seven days of incubation. The results showed that the terpene extract was the most effective against fungi and the alkaloid extract had the least antifungal activity. the percentage of radial growth inhibition was 97%-65%, respectively.

Keywords: Phenols, Alkloids, Terpenes, *Phytophthora cinnamomi*, Anti-fungal activity.

1. Introduction

Phytophthora is one of the soil-borne pathogens that cause disease in many plant families.[1] *Pimpinella anisum.* It is an important medicinal plant belonging to the family Apiaceae. This family is known for its distinctive flavors due to its content of essential oils, and this plant has attracted more attention for its effect on human health as an antimicrobial, and anti-fungal. [2] Plants contain many different and well-known chemical compounds [3]. Plants produce these chemicals Terpenoids, alkaloids, and phenols to protect themselves as antibacterial and antifungal [4]. these chemical compounds in the plant are a biological defense function that reduces the risk of infection with fungi and other pathogens [5]. Secondary metabolites include saponins, phenols, flavonoids, lignans, alkaloids, terpenes, plant steroids, curcuminoids, flavonoids, and glucosides [6]. These compounds possess biological properties such as detoxifying [7]. The absence of these compounds does not lead to instant death, but rather to the weakening of the organism [8]. Most secondary metabolites are classified on biosynthetic origin as terpenes, phenolic compounds, and alkaloids [9]. These alkaloids are basic compounds synthesized by organisms containing heterocyclic nitrogen atoms derived from amino acids [10]. In addition, it is a natural product that contains heterocyclic nitrogen atoms, and the name alkaloid is derived from "alkali"

and has been used to describe any nitrogen-containing base. [11] Alkaloids are also known as a large family consisting of more than 15,000 secondary nitrogen-containing metabolites [12]. It has a high solubility in the aqueous media of ethane and generally occurs as salts or nitrogen oxides in plants [13]. Finally, alkaloids are famous for their amazing medicinal effect [14].

2. Materials and Methods

Isolation and Identification of the Fungus

Fifteen samples of infected plants By five samples from each plant, which included a plant chili pepper, five cucumber samples, and five eggplant samples. then infected plants were transferred to the laboratory to isolate the pathogen *P. Cinnamomum* from the infected plant and identify it. The infected plants were placed under running tap water for an hour to clean them from dust. The roots and crown area were then cut into 5 mm pieces the surface was sterilized by soaking them in sodium hypochlorite (1% chlorine-free), washed with sterile distilled water several times, and then placed in PDA plates and incubated for four days at 27 °C [15]. All fungal isolates were cultured on sterile PDA with 100 mg chloramphenicol and incubated for seven days at 27 ^{0}c prepare a tilted culture and store [16]

Pimpinella anisum collection

The leaves and stems of *P.anisum* were purchased locally and ground into semi-powder with a grinder, then stored in containers until used.

Preparation of the Plant Extract

Phenols

Extraction was done according to [17]. Divide 200 g of the dried Materials into two equal quantities, then mix one with 300 ml of distilled water, The other weight was mixed with 300 ml of 1% hydrochloric acid. The samples were placed on an electric shaker for 5 minutes to homogenize and then into a centrifuge at 1 100 rpm for 24 hours. Then all supernatants were mixed with an equal amount of butanol and saturated with an amount of NaCl in a separating funnel.

The two layers in a lower and upper separating funnel were concentrated and dried in an electric oven at 40°C. The dry matter of both layers was kept until use

Alkaloids

According to [17], 100 g of the dried material was placed in an electric shaker with 350 mL of (1:4) ethanol: distilled water to homogenize. then filtered with gauze and then placed in a Buckner funnel.

the filter was concentrated to a quarter of the original volume (100 ml) and acidified by 2% drops H_2SO4 until it is between 1 and 2, and using chloroform three times in a separating funnel the alkaloids are precipitated by adding drops of concentrated NH_4OH until the pH ranged between 9 and 10 and then (chloroform-methanol 1:3) twice as soon as two layers appear. The substrate is dried in an electric oven at 40°C. The remaining layer containing weak alkaloids is dried. in an electric oven at 40°C. and kept until use.

Terpene

According to the method [17],10 g of dried material is successively extracted in a soxhlet extractor for 24 hours. with 200 mL of chloroform and then remove the solvent using an electric oven at 40 °C; then is kept until use.

Indicators used to detect plant extracts

Terpenoid indications

Acetic Anhydride Detector

According to [17], one ml of the extract is added to it two drops of chloroform, then one drop of anhydrous acetic acid, then one drop of H_2SO_4 turns brown indicating the presence of terpenes.

Alkaloid indications

tannic acid reagent

This is used to precipitate alkaloids according to [17] where 1% tannic acid is prepared and 1-2 ml of this reagent is added to 5 ml of the extract. White turbidity indicates the presence of alkaloids.

phenol indicators

Ferric Chloride Test

Dissolve (30-50) mg of the dried material in (1 ml) of water. three drops of 2% add to the aqueous solution of ferric chloride. It is observed that a blue or purple precipitate formed, indicating the presence of phenols [17].

Evaluation of plant extracts as a phytopathogenic fungicide

According to [18], the medium poisoning technique was used to determine the inhibitory concentration of plant extracts on *Phytophthora cinnamomi*. Different values of terpenes, Alkaloids, and phenols were prepared and mixed with 100 ml of (Potato Dextrose Agar) to prepare the required concentrations of these extracts (0, 5, 10%). The mixture of both extract and PDA was shaken well poured into Petri dishes and left to solidify in sterile conditions. A 5 mm piece of fungal growth of the five-day-old culture is deposited into the center of each plate. Inoculated plates are incubated at 27 C˚ for seven days. Three replicates are made for each treatment. Fungal growth diameters are measured after seven days, then the antifungal activity is calculated for each concentration By measuring growth inhibition using the following formula.

$$\text{Growth inhibition\%} = \frac{[\text{Control growth} - \text{Treatment growth}]}{\text{Control growth}} \text{ x } 100$$

Statistical Analysis

Three replicates are performed for each treatment using a complete randomized design (CRD) and all data are analyzed using analysis of variance (ANOVA) and separate tests and alpha values of 0.05 (by a computer program called Genstat v. 7.2, third version, and SPSS software).

3. Results and Discussion

After isolating *Phytophthora cinnamomi* from infected plants, it is identified after purification and collection according to morphological and microscopic characteristics.. [19] Early detection and diagnosis of *Phytophthora cinnamomi* are necessary to develop an effective disease control strategy. Phenols, terpenes, alkaloids and are extracted from the leaves of *Pimpinella anisum.* Then the plant extracts are evaluated as antifungals against *P.cinnamomi* fungi. The results showed that the percentage of growth inhibition varies according to the concentrations of the extract used in this study. The phenols, alkaloids, and terpenes from the plant extract show an inhibitory effect against *Phytophthora Cinnamomum* at all concentrations used in this study **Figure1.**

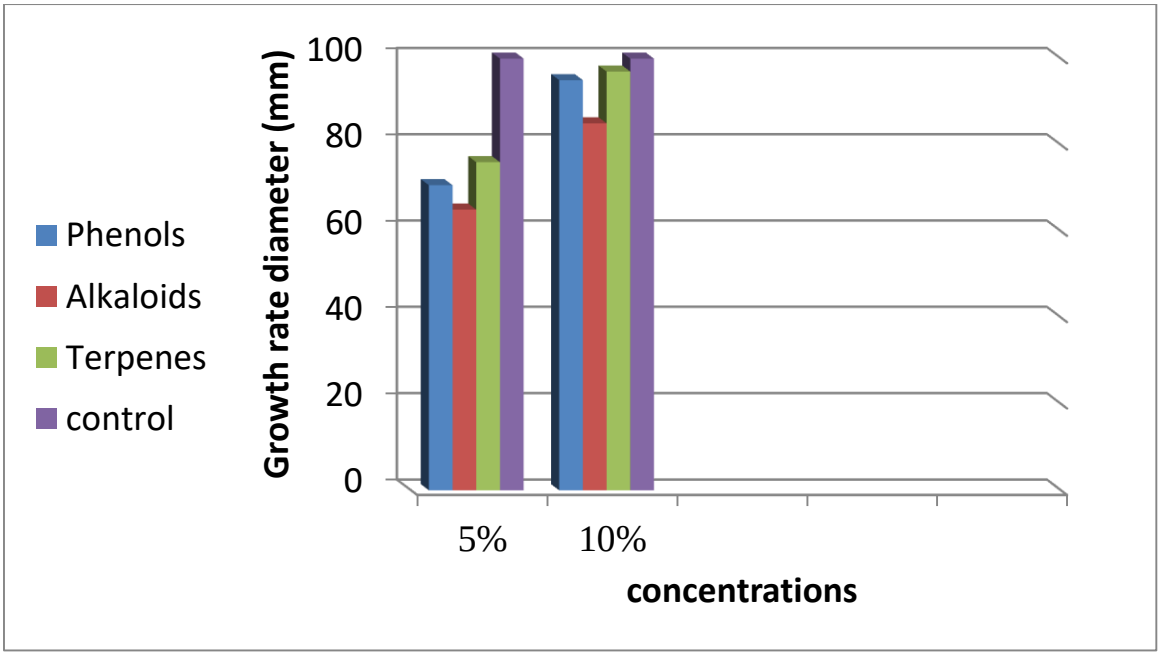


Figure 1: Percentage of growth Inhibiting *Phytophthora* cinnamoni by using terpenes phenols and alkaloids extracted from *Pimpinella anisum* at a concentration of 5% and 10% compared with control

The results are agreed with the results of [20] that growth inhibition increases with increasing concentrations of plant extracts where the inhibitory percentage for all extracts ranged between 65 -76 at 5 % concentration, while the growth inhibition percentage at 10% concentration ranged between 85% - 97% on Phytophthora cinnamomi. The results also show that the percentage of inhibition differs according to the extract, the terpene extract record the highest inhibition rate of 97% compared to the alkaloid extract which records 65%. **Table 1.**

Table (1): Percentage of growth inhibition on *phytophthora cinnamomi* by using alkaloids, terpenes and phenols extracted from *pimpinella anisum* at a 5% concentration

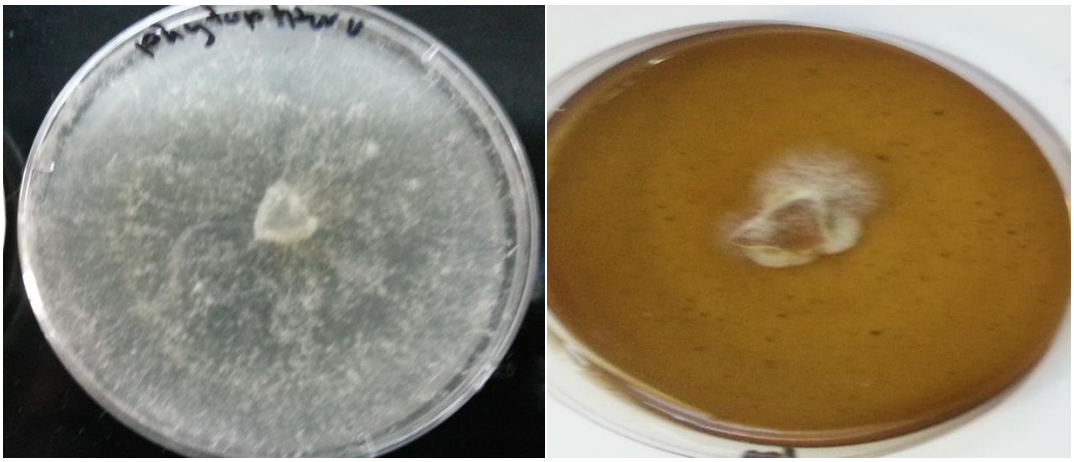
Fungus	% of growth inhibition		
	Pimpinella anisum		
Phytophthora cinnamomi	Phenol	Alkaloids	Terpenes
	70.66	65	76

Results of growth inhibition percentage of fungi by plant extracts ranged between 85 - 97 % at 10 % con. *Pimpinella anisum* terpenes significantly inhibited radial growth of *Phytophthora cinnamomi* more than other plant extracts which recorded 97 %, while phenol inhibited radial growth of *Phytophthora cinnamomi* which record 95 %.**Table2**

Table (2): Percentage of growth inhibition on *Phytophthora cinnamomi* by using alkaloids, terpenes and phenols extracted from *pimpinella anisum* at a 10% concentration

Fungus	% of growth inhibition		
	Pimpinella anisum		
Phytophthora cinnamomi	Phenol	Alkaloids	Terpenes
	95	85	97

Results show that *Pimpinella anisum* alkloids was a significant percentage of lower inhibition of *Phytophthora cinnamomi* which record 85 % while terpenes are the highest percentage inhibition of *Phytophthora cinnamomi* growth which record 97 %, **Figure 2.**



A **B**

Figure 2 . Effect of plant extract terpene on *Phytophthora cinnamomi* at 10% con.

A - control

B - *Phytophthora cinnamomi* treated with terpen

Terpenes affect membrane enzymes and interfere with respiratory passages. Terpenes also degrade fungal hyphae and the hyphae appear to be devoid of cytoplasmic content. [21]. at low concentrations, phenols affect the enzymatic activity of fungi, while at high concentrations they cause protein denaturation [22]**.**

4. Conclusion

The research results show that terpenes extracted from *P.anisum* have a high anti-fungal activity compared to the alkaloid and phenolic extract, so they can be used as a pesticide for plant diseases resulting from *Phytophthora.*

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