

The effect of different extracts from *Zingiber officinale* to control *Alternaria solani* and pepper seeds germination, emergence

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Abstract: The researches focus on medicinal plants that are rich with beneficial secondary components having biological activity. Ginger rhizomes (*Zingiber officinale*) content of various chemical constituents gives therapeutic benefits antibacterial and antifungal. This research aims to use extractions of ginger rhizomes by different solvents: petroleum ether, chloroform and methanol. Chloroform gave the highest yield (8.2 %), followed by methanol and petroleum ether (6.3 and 5.8 %), respectively. Phytochemical analysis revealed that flavonoids, alkaloids, triterpenoid, steroids and tannins are abundance in chloroform and methanolic extracts. However, saponins were absent in all extracts. Petroleum ether extract contained triterpenoid, steroids and tannins. Quantitative analysis showed that the chloroform extract contains the highest amount of phenolic and flavonoids, reaching 98.4 mg as pyrogallol/g and 48.6 mg quercetine/g dry weight, respectively. The fungitoxicity was evaluated in the form of chloroform extract (82.4%), methanolic extract (74.5%) and petroleum ether extract (65.3%) at concentration 4 mg/ml against *A. solani* *in vitro*. Evaluation treatment of pepper seeds with ginger extracts at median effective concentration (EC_{50}) in prevent and control *A. solani* and germination and emergence. The results followed both the germination and emergence of the seeds, as well as the development of pathogen *A. solani*. So, use of ginger extracts as fungicide from natural extracts against pathogens *A. solani*, as *in vivo* assessment is critical, before recommendation for substances of synthetic fungicides.

Keywords: Pepper seeds, ginger rhizome extracts, phytochemicals, antifungal activity, *Alternaria solani*.

1.Introduction

The medicinal plants are used in traditional medicine for thousands of years either in the pure form or crude extracts (Parekh and Chanda, 2007). In recent years, the researches focus on medicinal plants because of their antimicrobial, insect, nematodes and vertebrates traits. Plants are rich with beneficial secondary metabolites (Alinezhad *et al.*, 2011). Biological active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a healthy source of potential pathogens control agents (Behiry *et al.*, 2020). Every part of the plant, including the leaves, stem, bark, roots, flowers, fruits and seeds, can provide active components. Ginger (*Zingiber officinale*) was used as an herbal medicine having a wide range of medicinal properties (Ko *et al.*, 2019). The ginger rhizome content of various chemical constituents gives its therapeutic benefits and pharmacological uses, including anti-

inflammatory, anti-diabetic, antibacterial, antifungal, anti-oxidant, anti-tumor, anti-cancer, anti-proliferative, and anti-platelet effects (Wan-Nadilah *et al.*, 2019). The qualitative analysis involves the preliminary phytochemical screening of the ginger rhizome extract to detect the presence of various secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds (Karishma and Laxmikant, 2023). The fungitoxicity of *Z. officinale* rhizome was evaluated in the form of powdered (20%), boiled (20%), crude (20%) and ethanol (2%) extracts against ten fungal pathogens viz., *Rhizoctonia solani*, *R. bataticola*, *Phoma sorghina*, *Colletotrichum gloeosporioides*, *Fusarium pallidorosem*, *F. oxysporum* f. sp. *ciceri*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Alternaria solani* and *A. alternata* under *in vitro* condition (Manvendra *et al.*, 2022). Fungal, *Alternaria* sp. causes severe family Solanaceae yield and quality losses in world (Shoaib *et al.*, 2019). Uses of ginger extract as fungicides from natural extracts against pathogens *A. alternata*, as well

as the reduction of synthetic fungicides using alternative non-polluting method (Cătălin and Nicoleta, 2022).

The present study aimed to extraction using different solvents, qualitative and quantitative analysis of each extracts component and evaluation of their potency to prevent and control the *A. solani in vitro* trial and effects on pepper seeds germination and emergence.

2. Materials and Methods

2.1. Plant material use

The ginger rhizome (*Zingiber officinale*) was obtained from a farm in Luxor Governorate, Egypt.

Pepper seeds (*Capsicum annuum* cultivar Panorama hybrid) were obtained from Unit of Vegetable Crops, Horticulture Research Institute, Agricultural Research Centre (ARC), Giza, Egypt.

2.2. Chemicals

Petroleum ether, chloroform, methanol and dimethyl sulfoxide (DMSO) were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.3. Preparation of crude plant extracts

The fresh ginger rhizomes were cutting into slices and dries at room temperature. Then, they were grind into partial fine and extraction with different solvents in sequence as follows: petroleum ether (40-60), chloroform and 80% methanol alcohol by maceration method according to Handa *et al.* (2008). 100g of powder was macerated three time in 500 ml of petroleum ether for 3 days, then filtered and evaporated by rotary evaporator at 40 °C. The defatted part were extracted using method with 500 ml of chloroform for the above time- point, then filtered and evaporated as described above. The same plant well macerated with 500 ml of 80% methanol alcohol for the same time, filtered, evaporated to dryness using rotary at 40 °C and stored at 4°C until use (Ahmad *et al.*, 2013). The percentage yield for all extracts was calculated as follows:

$$\% \text{Yield} = (\text{Weight of extract} / \text{Weight of plant}) \times 100$$

2.4. Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for all extracts as per standard methods described by Harborne (1998)

2.4.1. Test for Flavonoids

Few drops of dilute sodium hydroxide were added to one ml of extracts. An intense yellow color indicates the presence of flavonoid compounds.

2.4.2. Tests for Alkaloids

Dragendorff's reagent was prepared by mixing a solution of 0.8 g bismuth nitrate pentahydrate with 40 ml distilled water and 10 ml glacial acetic acid with solution of 8.0 g potassium iodide in 20 ml distilled water. Then, 1ml dilute HCl (1%) were added to 5 ml of extracts and 1ml dragendorffs reagent. An orange or red precipitate produced immediately indicated the presence of alkaloids.

2.4.3. Test for Triterpenoids

Ten mg of extracts were added to 1 ml of acetic anhydride and 2 ml of concentrated H₂SO₄. Formation of reddish violet color indicated the presence of triterpenoids.

2.4.5. Test for Steroids

One ml of the extracts was mixed with 10 ml of chloroform, and then equal volume of concentrated H₂SO₄ at sides of the test tube. The upper layer turns red and acid layer showed a yellow with green fluorescence. This indicated the presence of steroids.

2.4.6. Test for Saponins

The extracts were diluted with 20 ml distilled water and agitated in a graduated cylinder for 15 min. The formation of 1cm layer of foam showed the presence of saponins.

2.4.7. Test for Tannins

Few drops of (1%) lead acetate were added to 5 ml of the extracts. A yellow precipitate was formed indicating the presence of tannins.

2.5. Quantitative phytochemical analysis

2.5.1. Determination of total phenolic content

The colorimetrically method of folin-ciocalteu described by Jonathan *et al.* (2012) was used 100 µl of extract were diluted to 3 ml with bidistilled water and well mixed in a dry test tube, then 0.5 ml of folin-ciocalteu reagent was added. After 3 min, 2 ml of saturated sodium carbonate solution (20%) were added to the mixture and kept in the dark for 60 min. The absorbance was measured at 650 nm by UV-vis spectrophotometer (Milton Roy, Model 601) against a blank sample (100 µl methanol 80% and reagent only). Phenolic contents were calculated from the standard solution prepared from pure pyrogallol (10 mg/100 ml bidistilled water) and the content was expressed as mg/g dry weight.

2.5.2. Determination of total flavonoid

The colorimetrically method described by Quettier *et al.* (2000) was used one ml of extract or standard

solutions of quercetin (QE) was added to 4 ml double distilled water, and 0.3 ml (5% NaNO₂). After 5 min, 0.3 ml of (10%) aluminum chloride (AlCl₃) was added. After 6 min 2 ml of 1 M sodium hydroxide (NaOH) solution was added and the total volume was made up to 10 ml with bidistilled water. Quercetin; (0.1 g was dissolved in 100 ml ethanol 98%) and determined by reading the developed red color at 510 nm by UV. vis spectrophotometer (Milton Roy, Model 601) against a blank sample; (1.0 ml methanol 80% and reagent only). Total flavonoid contents were expressed as mg/g dry weight.

2.6. Inoculum preparation

Old cultures of *A. solani* growing for 7 days using petri dishes with a diameter of 9 cm containing a potato dextrose agar (PDA) medium at 25 ± 2 °C were used to make the inoculum. The mycelial growth was repeatedly rinsed using 50 ml sterile distilled water and ground using ceramic mortar then filtered, the spore's suspension was collected and the concentration was set at 5×10⁵ spores/ml (Derbalah *et al.*, 2018).

2.7. Effect of different extracts of ginger on *A. solani* in vitro

Use three different extracts (petroleum ether, chloroform and methanolic) of ginger re-dissolved in DMSO, were tested at concentrations of 0.25, 0.5, 1, 2 and 4 mg/ml against *A. solani* by Poisoned Food Technique (Mohanty *et al.*, 2012). All extracts were added separately to get the required concentrations, then mixed with 50ml of sterilized PDA medium, before solidification and transferred equally into three petri dishes. The media was allowed to solidify. Then, seven day old fungal culture disk of 5-mm diameter was taken and inoculated to the center of petri dishes containing extracts in separate manner. Instead of PDA medium without extracts form was served as control. All dishes were incubated at 25±2 °C and radial growth of colony was measured when the mycelia of control had almost filled the petri dishes. Each test was performed in triplicates.

The fungal growth inhibition was calculated due to treatment against control by the following formula (Satya *et al.*, 2014).

$$I \% = \frac{R - r}{R} \times 100$$

Where, R is diameter of the mycelia growth in the control, and r is diameter of the mycelia growth in the treatment.

LDP line program was used to calculate median effective concentration (EC₅₀) and nightly effective concentration (EC₉₀) values according to Finney (1971).

2.8. The experiment blotch assay

In this experiment, an evaluation of the effectiveness of different extracts of ginger treatment such as (petroleum ether, chloroform, methanolic extracts) for each EC₅₀ (mg/ml) in preventing and controlling the *A. solani* in vitro was conducted. The test was carried out in the sterile chamber with laminar flow. We used 50 seeds of pepper for each treatment, where they were disinfected with 70% ethyl alcohol, washed with sterile distilled water and dried between twins sterile filter paper. They were moistened by immersion for 20 min with a mixture of the previous extracts for each EC₅₀ and artificially contaminated by immersion in a calibrated spore suspension (5 × 10⁵ spores/ml) for 1hr according to Constantinescu (1974). The seeds were placed into sterile petri dishes between the folds of blotting paper moistened with sterile distilled water. Three dishes/treatment, 3 dishes infected with *A. solani* were served as infected control and 3 dishes as untreated control. All petri- dishes were incubated at 24±2 °C. Then result observations were recorded after 3, 6 and 9 days of incubated as seeds germination and emergence (Iacomi *et al.*, 2004).

3. Results and discussion

3.1. The effect of different solvents on presented the yield extracts

Data in Table (1) presented the yield of crude extracts using petroleum ether, chloroform and methanol from dry powder of *Z. officinale* where chloroform gave the highest yield (8.2%), followed by methanol and petroleum ether (6.3 and 5.8%), respectively. This finding is logical since petroleum ether extract (non-polar solvent) represents lower percentage compared to chloroform and methanol (more polar). Variations in yield extracts from plant might be attributed to the availability of different extractable components defined by the chemical composition of the plant (Hsu *et al.*, 2006). Solvent extraction is technique involved in soaking the sample in a solvent and allowing them to remain at room temperature for several days with frequent agitation. The type of compound extracted from a sample depends on the solvent used for extraction (Azwanida, 2015).

Table (1): The yield of extraction with different solvents (w/w %)

Weight (100 g) powder	% yield		
	Petroleum ether	Chloroform	Methanol
Ginger	5.8	8.2	6.3

3.2. Qualitative screening for phytochemical constituents of different extracts

Regarding to phytochemical screening of *Z. officinale* extracts, data in Table (2) showed the presence of flavonoids, alkaloids, triterpenoid, steroids and tannins in chloroform and methanol extracts. However, saponins

were absent in all extracts. While, petroleum ether of ginger contained triterpenoid, steroids and tannins. Data recorded the absence of flavonoids, alkaloids saponins in petroleum ether extracts. These results are in agreement with those reported by **Riaz *et al.* (2015)** and **Azwanida (2015)**.

Table (2): Phytochemical analysis of different extracts.

Components	extracts		
	Petroleum ether	Chloroform	Methanol
Flavonoid	-	+	+
Alkaloid	-	+	+
Triterpenoid	+	+	+
Steroid	+	+	+
Saponins	-	-	-
Tannins	+	+	+

(+): found; (-): disappear

3.3. Total phenolic and total flavonoid content of different extracts

The effect of different solvent extraction on total phenolic and total flavonoid of *Z. officinale* plant is shown in Table (3). Data demonstrated that chloroform extract of ginger contained the highest amount of phenolic and flavonoids, reaching 98.4 mg as pyrogallol/g and 48.6 mg quercetine/g dry weight, respectively, followed by methanolic extract where total phenolic and flavonoids contents were 73.6 mg as pyrogallol/ g and 48.6 mg quercetine/g dry weight, respectively. While, the lowest amounts of total

phenolic and not total flavonoids contents were detected in petroleum ether extract. These results are in agreement with those obtained by **Womeni *et al.* (2013)** who found that total phenols in methanolic extract of *Z. officinale* are higher than those of *C. zeylanicum* reaching 17.72 and 4.31 g gallic acid/100 g extract, respectively. The methanolic extract showed a total phenolic content of 187.10 mg/g and a total flavonoids content of 171.33 mg/g, as determined by gallic acid equivalents and Rutin equivalents (**Karishma and Laxmikant, 2023**).

Table (3): Quantitative analysis of total phenolic and total flavonoids of different extracts

Extracts	Total Phenolic (mg/g) as Pyrogallol	Total Flavonoids (mg/g) as Quercetine
Petroleum ether	65.8	n.d.
Chloroform	98.4	48.6
Methanolic	73.6	34.2

n.d: no detected

3.4.Evaluation of antifungal activity *in vitro*

The evaluation of efficacy as antifungal activity of ginger using three extracts (petroleum ether, chloroform and methanolic), was conducted, where they prepared in DMSO at concentrations (0.25, 0.5, 1, 2 and 4 mg/ml) against *A. solani* compared to DMSO solvent and control free. The obtained results in Table (4) and (Fig.1) indicated that, all extracts were found to inhibit fungi growth. Chloroform extract displayed the highest mycelial growth inhibition (82.4%) for *A. solani*, followed by methanolic extract (74.5%) at concentration

4 mg/ml. While, petroleum ether extract gave the inhibition percent of (62.3%) at the same concentration. The results indicated that, the *A. solani* showed the response to chloroform and methanol extractions (EC_{50} values; 0.53 and 0.82 mg/ml), respectively, greater than that of petroleum ether extract (EC_{50} value; 1.7 mg/ml). These results are in agreement with Masuduzzaman *et al.* (2008) who observed that ethyl acetate extract showed better performance. This may be due to a fact that some of the essential compounds the abundance in these extracts.

Table (4): Effect of different extracts from ginger against *A. solani*.

Conc. (mg/ml)	Mycelial growth inhibition (%)		
	Petroleum ether extract	Chloroform extract	Methanolic extract
0.25	23.3	36.4	31.5
0.5	31.9	48.8	41.9
1	41.8	61.4	53.4
2	52.1	72.9	64.5
4	62.3	82.4	74.5
Control	0.0	0.0	0.0
EC_{50}	1.7	0.53	0.82
EC_{90}	52.5	8.6	17.85
Slope	0.87 ± 0.11	1.06 ± 0.12	0.96 ± 0.11

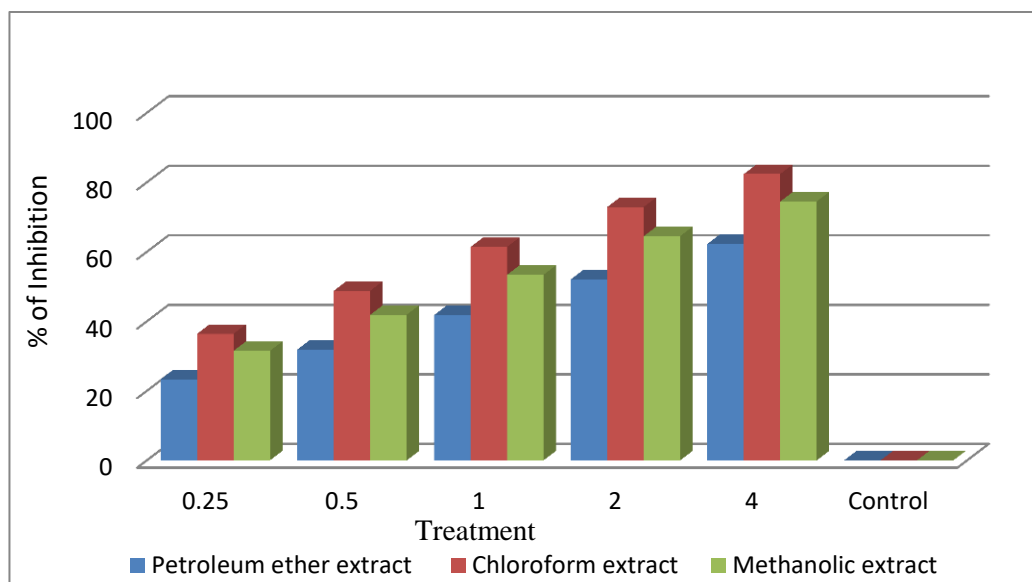


Fig. (1): The mean growth inhibition percent of ginger extracts in tested *A. solani* mycelia exposed to concentrations 0.25, 0.5, 1, 2 and 4 mg/ml.

3.5. Evaluation of ginger extracts treatment that used to median concentration (EC_{50}) in preventing and controlling the *A. solani* and pepper seeds germination, germination

In the group treated with petroleum ether extract (Fig.2A), the concentration (EC_{50}) allowed germination percent (78%) after 9 days and didn't allow the development of *A. solani*. In the group treated with chloroform extract (Fig.2B), the concentration (EC_{50}) allowed had higher germination of pepper seeds (83%) after the above time- point. In the middle of the growth period the root and hypocotyl have developed, the rest presenting the root, hypocotyl and cotyledons and inhibited the development of the *A. solani*. Regarding methanolic extract (Fig.2C), the concentration (EC_{50}) allowed a germination of 80%, and no development of

A. solani. However, the untreated control (Fig.2D), registered (68%) after the same period, where the seeds developed root, hypocotyl and cotyledons, but the seedlings were covered with *Rhizopus* sp. In the group infected control (Fig.2E), germinated in a percentage of 37%, after the above time point showed necrotic roots, hypocotyl and cotyledons, the seedling being covered with a black mycelium *A. solani*. These results are in agreement with **Ahmad and Qureshi (2017)** who proved that ginger extracts have antifungal activity against *A. alternata* and *A. solani* fungi. Also, **Sharma et al. (2013)** observed that essential oil extracted from ginger has a wide range of antifungal activities against *Aspergillus niger* van Tiegh., *Penicillium chrysogenum* Thom., *A. alternata* (Fr.) Keissl. and *F. roseum* Link., whereas minimum inhibition concentration showed results at 1.0 ml/cm^3 of oil with respect to all fungi.

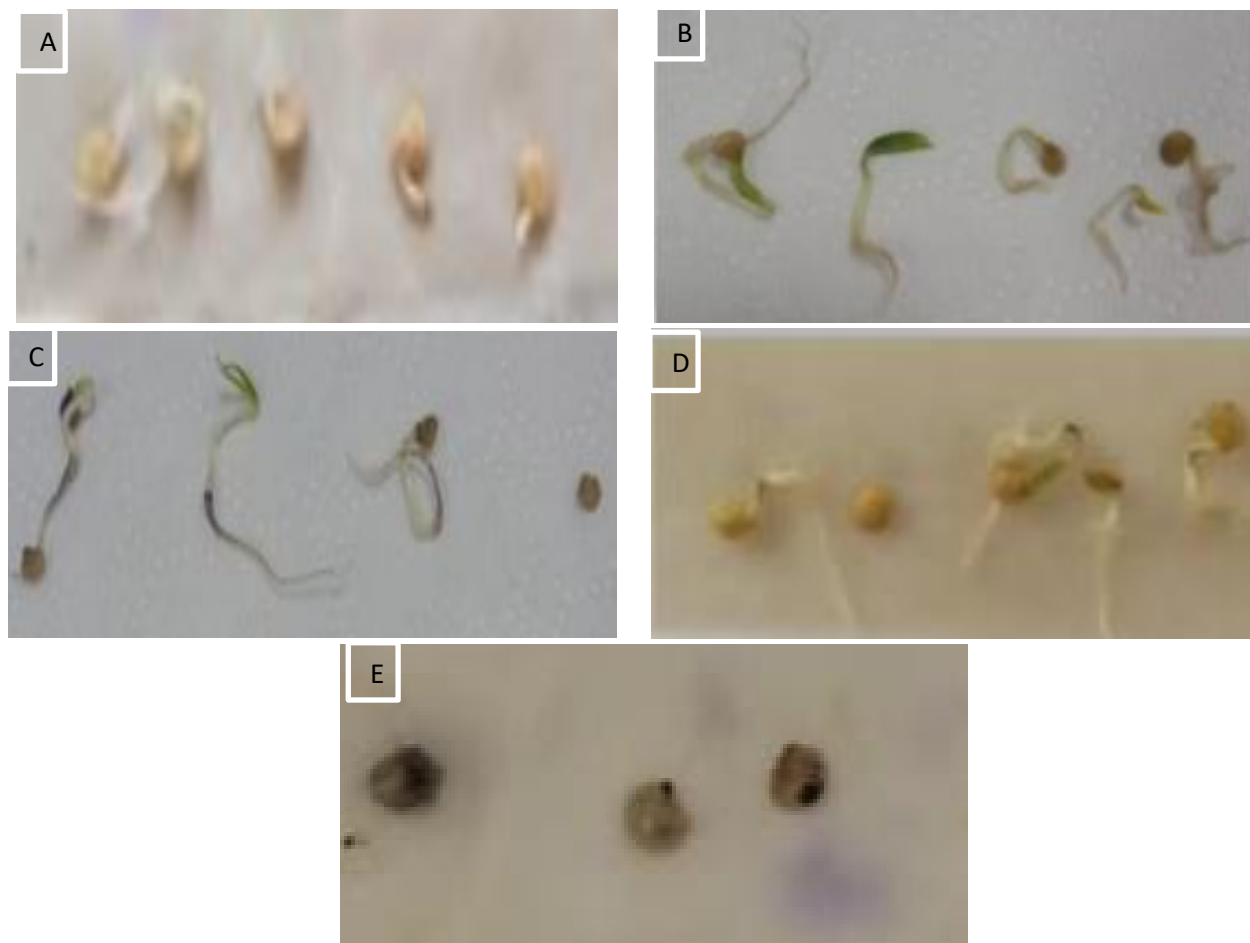


Fig. (2): The effect of treatment with different extracts of ginger in preventing and controlling the *A. solani* and pepper seeds germination, emergence, (A) The effect of curative treatment with petroleum ether extract, (B) The effect of curative treatment with chloroform extract, (C) The effect of curative treatment with methanolic extract, (D) untreated control, (E) Infected control.

Conclusion

This study has revealed the importance of extracts from ginger, *Z. officinale* which used for the control of pathogen *A. solani*. Extraction was done by using different polarity solvents (petroleum ether, chloroform and methanol), qualitative and quantitative analysis of each extracts component. The flavonoids, alkaloids, triterpenoid, steroids and tannins in chloroform and methanol extracts. However, saponins were absent in all extracts. While, petroleum ether of ginger contains triterpenoid, steroids and tannins. The high antagonistic effect was conducted of petroleum ether, chloroform and methanolic extracts against *A. solani* under laboratory conditions. Evaluation treatment of pepper seeds with ginger extracts at EC₅₀ levels in preventing and controlling the *A. solani* and their germination and emergence were conducted. Petroleum ether extract allowed germination registered 78% after 9 days and didn't allow the development of *A. solani*. Chloroform extract allowed had higher germination of pepper seeds (83%), in the middle of the growth period the root and hypocotyl have developed, the rest presenting the root, hypocotyl and cotyledons and inhibited the development of the *A. solani*. Methanolic extract allowed a germination of 80%, and no development of the fungi. So, use of ginger extracts as fungicide from natural extracts against pathogen *A. solani*, is more critical after *in vivo* trail, before decision as eco-friendly fungicide.

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تأثير مستخلصات مختلفة من نبات الزنجبيل في مكافحة *Alternaria solani* وإنبات بذور الفلفل وظهورها

عزة رسمى عمارة

قسم بحوث المبيدات الفطرية والبكتيرية والنيماتودية - المعمل المركزي للمبيدات- مركز البحوث الزراعيه - الدقي - الجيزة - مصر

الملخص العربي

تركز الأبحاث على النباتات الطبية فهي غنية بالمكونات الثانوية المفيدة وذلك لنشاطها البيولوجي. محتوى جذور الزنجبيل (*Zingiber officinale*) من المكونات الكيميائية المختلفة هو ما يعطيها فوائد علاجية كمضاد للبكتيريا والفطريات. يهدف هذا البحث إلى الاستخلاص باستخدام مذيبات ذات قطبية مختلفة (إيثر البترول والكلوروفورم والميثانول)، حيث أعطى الكلوروفورم أعلى عائد (8.2%) يليه الميثانول وإيثر البترول (6.3، 5.8%) على التوالي. أظهر التحليل الكيميائي النباتي وجود الفلافونويدات والقويدات والتربينات والستيرويدات والتانينات في مستخلصات الكلوروفورم والميثانول. ومع ذلك، كانت الصابونينات غائبة في جميع المستخلصات. بينما يحتوي مستخلص إيثر البترول على التربينات والستيرويدات والتانينات. وقد أظهرت التحاليل الكمية أن مستخلص الكلوروفورم يحتوي على أعلى كمية من الفينولات الكلية والفلافونويدات، حيث بلغ 98.4 ملجم بيروجالول/جم. و 48.6 ملجم كوريسيتين/جم وزن جاف على التوالي. وتم تقييم سمية فطريات حيث أعطت مستخلصات الكلوروفورم (82.4%) والميثانول (74.5%) والإيثر البترولي (65.3%) عند تركيز 4 ملجم/مل ضد *A. solani* في المختبر. تم تقييم معاملة بذور الفلفل بمستخلصات الزنجبيل بتركيز متوسط (EC_{50}) في الوقاية والسيطرة على *A. solani* وإنباتها وظهورها. وقد تابعت النتائج كل من إنبات البذور وظهورها، وكذلك تطور المرض *A. solani*. لذا تم استخدام مستخلصات الزنجبيل كمبيدات فطريات من المستخلصات الطبيعية ضد مسببات الأمراض *A. solani* وكذلك تقليل المبيدات الفطرية الاصطناعية باستخدام طريقة بديلة غير ملوثة.