# Antifungal activity of plant extracts against tomato's fungal diseases

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**Abstract:** Fungal diseases have always been a major problem for tomato crops. Growers generally use chemical fungicides to treat this type of diseases. However, these products are toxic to the environment and the consumer, especially if the pre-harvest interval is not respected. The present study aims to find non-polluting alternatives. Five plant extracts (*Peganum harmala, Ocimum basilicum, Caralluma europaea, Nerium oleander and Eucalyptus globulus*) are tested for their in vitro efficiency against four pathogenic fungi: *Alternaria solani, Botrytis cinerea, Botrytis cinere*a taken from fruit, *Phytophthora infestans and Oïdium oxysporum*. The obtained results reveal that the extract of *Ocimum basilicum* is the most effective on the studied fungi. Indeed, at a concentration of 0.4%, it inhibited at 80% the development of *Botrytis cinerea* and at 81% *Oïdium oxysporum* at a concentration of 0.2%. Followed by *Peganum harmala* and *Nerium oleander*, which also showed an antifungal effect (*Peganum harmala* inhibited up to 73% of the growth of *Alternaria solani* at a dose of 4%). The extracts of *Caralluma europaea* and *Eucalyptus globulus* proved similar antifungal activity, which exceeded 30%. The study of the fungal/fungistatic effect revealed that all the studied extracts have a fungal effect against the treated fungi. The phytochemical screening showed that the plants extracts are rich in polyphenols especially *Ocimum basilicum*, *Peganum harmala* and *Nerium oleander*. This leads us to deduce that the antifungal activity may be due to this. ***Keywords***: Plant extract, fungal diseases, antifungal activity, tomato.

## 1 Introduction

The human being has always drawn on biodiversity. In the past, pesticides have been widely and inappropriately used, such as spraying by air.

These practices have caused many cases of acute or chronic toxicity in humans, contamination of the environment, increasing resistance in the target plant and creating more harmful species. Because of these issues, authorities start defending human, animal and environmental health from the risks associated with pesticides so they can be used properly [1]. When Pesticides are of natural origin or based on living organisms [2] they are called biopesticides. They are receiving increased attention and interest in the development of safe and environmentally friendly integrated crop management (ICM). The global trends today are for decreasing the use of chemical pesticides and emphasizing biopesticides.

According to the Food and Agriculture Organization of the United Nations (FAO), the tomato is one of the world's eighth most valuable agricultural products. In Morocco, tomatoes are one of the main fresh agricultural products exported and one of the country's main income sources. Nevertheless, the tomato production cycle is subject to several fluctuations and stresses, including those caused by fungal diseases. Many plant products can reduce and control cryptogamic diseases. Especially by the natural substances that they contain. Plant extracts are alternatives and means to provide integrated control of foliar pathogens. They can respect the environment [3]. Fungal pathogens cause about 10,000 plant diseases that affect growth, fertility, productivity, etc. [4]. Several fungicides are effective against pathogens affecting tomato. However, to date, environmental and food safety

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issues are considered when choosing a control method. This study was conducted to evaluate the effect of plant extracts of *Peganum harmala, Ocimum basilicum, Caralluma europaea, Nerium oleander* and *Eucalyptus globulus* on *Alternaria solani, Botrytis cinerea, Botrytis cinerea* taken from fruit, *Phytophthora infestans* and *Oïdium oxysporum* in vitro and to analyze their chemical composition.

## 2 Materials and methods

### 2.1 Biological materials

#### *2.1.1. Pathogen:*

Tomato fruit infected by *Botrytis cinerea* and leaves infected by *Phytophthora infestans Oïdium oxysporum*, *Botrytis cinerea* and Alternaria were collected from Skhirat Temara region. After being washed with 2% bleach for 2 to 3min followed by sterile distilled water twice; fragments of 2mm were cut and placed in Petri dishes containing PDA (Potato Dextrose Agar: it allows the development of fungus) and incubated at 28±2°C for 7 days [5]. The identification of the pathogen was made by a microscope observation. Colonies representing the desired pathogen were transferred to another Petri dish and incubated for another 7 days. The operation was repeated until a pure strain was obtained.

#### *2.1.2. Aromatic and medicinal plants:*

Five plants parts were collected: *Peganum harmala* seeds, *Ocimum basilicum* leaves and *Caralluma europaea* leaves were purchased at the market. *Nerium oleander* leaves and *Eucalyptus globulus* leaves were obtained in Zaër region of Morocco.The plants different parts were rinsed with water to eliminate dust particles; they were put at room temperature until dry and then grounded into a fine powder using an electric grinder. The extraction was carried out in the Laboratory of Natural Resources and Sustainable Development of the Faculty of Sciences of Ibn Tofail University.

### 2.2 Preparation of the extracts

We opted for aqueous extraction by infusion. The concentrations chosen are 4%, 2%, 0.4% and 0.2%.

### 2.3 Antifungal activity of plant extracts

13.5ml of liquid PDA and 1.5ml of the plant extract were mixed according to the chosen concentrations. After solidification in a Petri dish, a fragment of the pathogen was placed in the center. The effect of the extract is studied by the following formula:

$$\% \text{ Growth inhibition} = \frac{De - Dt}{De} * 100$$

De: Diameter of the fungus in the test
Dt: Diameter of the control fungus **[6]**.

### 2.4 Fungal/fungistatic effect

A plant extract can have a fungal or fungistatic effect on the pathogen. A fragment of fungus is placed on 13.5ml of PDA; a sterile filter paper is soaked with 1.5ml of the extract and placed on the lid of the same box. After 7 days of incubation, the diameter of the fungus is measured and the filter paper is removed for a second incubation for 7 days. If the fungus continues growing after the filter paper's removal, it is a fungal effect. If not, it is a fungistatic effect [7].

### 2.5 Phytochemical tests

The extracts used were subjected to qualitative and quantitative analysis of some secondary metabolites.

#### *2.5.1 Qualitative analysis:*

The qualitative analysis chosen is tube characterization. It consists on the development of insoluble complexes by precipitation reactions.

##### *2.5.1.1 Phenolic compounds characterization*

###### *2.5.1.1.1 Tannins characterization*

A mix of 1ml of the aqueous extract and 1ml of the aqueous solution of $FeCl_3$ at 1% was prepared. The presence of tannins is indicated by the development of a greenish or blackish blue coloration.

The Stiasny reaction is used to differentiate between gallic and catechic tannins:
A mix of 1ml of Stiasny reagent and 2ml of the extract was heated in a water bath at 90° for 15min. The presence of a precipitate indicates the presence of catechic tannins. The filtrate is saturated with sodium acetate with a few drops of 1% FeCl3. Gallic tannins are characterized by the development of a blue-black hue [8].

###### *2.5.1.1.2 Flavonoïds characterization*

- ***Anthocyanins and leucoanthocyane***

1ml of the extract with 1ml of hydrochloric alcohol and 1ml of isoamyl alcohol as well as some magnesium cups were mixed. The appearance of a red coloration at the isoamyl alcohol layer indicates

the presence of flavones and flavonols. The same mix without the magnesium cups was heated for 15 min in a water bath. The leucoanthocyanins are characterized by a cherry red or purplish coloration. The catechols are indicated by the red-brown hue [8].

*2.5.1.1.3 Saponins characterization*

1ml of aqueous extract is diluted to half and shaken for 15 seconds. The saponins are indicated by the foam if it persists for at least 15 min [9].

*2.5.1.1.4 Mucilage characterization:*

5ml ethanol with 1ml of the extract gives a flaky precipitate in the presence of mucilages [10].

*2.5.1.1.5 Characterization of lipoïds*

1g of plant powder is mixed for 30 min with 7.5ml of petroleum ether. Then, it is evaporated on a hot plate. 3 drops of $H_2So_4$ were added to the oily residue. The lipoïdes are indicated by the strong green purple coloration [11].

*2.5.1.1.6 Characterization of iridoïds:*

1ml of the plant extract mixed with 1ml of HCl gives after heating a black precipitate that indicates the presence of iridoïdes [12].

*2.5.1.1.7 Highlighting of alkaloïds:*

1g of plant powder is mixed with 5ml of $H_2SO_4$ diluted to 1/10. After being stirred and macerated for 24h at laboratory temperature it was filtrated. A mix of a few drops of Wagner's reagent was prepared by mixing 1.27g of I2 solubilized in 100ml of distilled water and 2g of KI, it was added to the filtrate to obtain a brown precipitate [9].

*2.5.1.1.8 Characterization of reducing sugars:*

5ml of aqueous decoction was evaporated in a water bath until dry. 1ml of Fehling's reagent was added to the residue. The presence of reducing sugars is indicated by a brick red precipitate [13].

*2.5.1.2 Quantitative analysis*

- ***Determination of total polyphenols***

A mix of 2.5ml of Folin's reagent diluted 10 times and 0.5ml of plant extract was prepared and shaken before adding 1ml of 7.5% (w/v) sodium carbonate solution. The absorbance is read at 765nm. Blanks were prepared by replacing the extract with methanol [14].

*2.5.1.2.1 Determination of total flavonoïds*

1ml of the extract with 1ml of AlCL3 solution (2% in methanol). Blanks are prepared by replacing the extract with methanol. The absorbance is read at 430nm after 10 min of reaction [15].

*2.5.1.2.2 Determination of condensed tannins*

2.5ml of vanillin reagent was prepared by mixing an equal volume of 8% HCl in methanol and 1% vanillin in methanol, to be mixed with 0.5ml of plants extracts.

The blanks are prepared by replacing the reagent with the 4% methanol-acid mixture. The absorbance is read at 500nm after 20min at 30°C [16].

## 3 Results and discussion

### 3.1 In vitro effect of plant extracts

All the aqueous extracts inhibited the studied fungus at different degrees and with dose dependent effects. Indeed, for *Alternaria solani* we reached an inhibition rate of 73% by *Peganum harmala* and an inhibition of 50% by *Ocimum basilicum* at a concentration of 4% against an inhibition of 77% by Asoxystrobin (figure 1).
Significant inhibition was also noticed for the rest of the concentrations at respectively increasing degrees (0.2%; 0.4% and 2%).

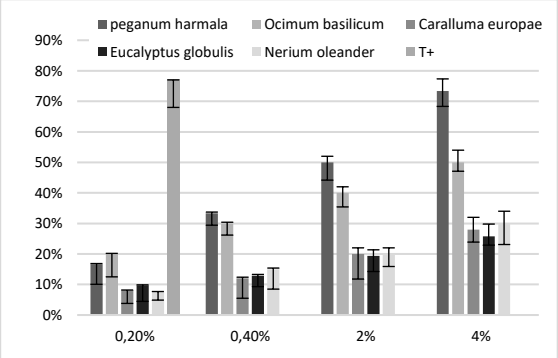


**Fig. 1.** Effect of different extracts on *Alternaria solani*

A concentration of 0.40% of *Ocimum basilicum* inhibited up to 80% *Botrytis cinerea* and reached 87.5% at a concentration of 4% of the same extract. Against 92% for Azoxystrobin as a positive control. *Peganum harmala* inhibited this pathogen at 57% with a dose of 4%. *Eucalyptus globulus* inhibited it with a percentage of 55% at the same dose and *Nerium oleander* with 40% (figure 3).

For *Botrytis cinerea* taken from fruit, the highest rate was at 4% of *Ocimum basilicum* with 54% (figure 2).

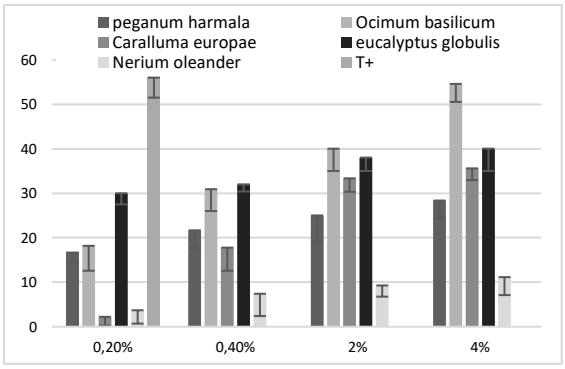


**Fig. 2:** Effect of different plant extracts on *botrytis cineria* in fruit

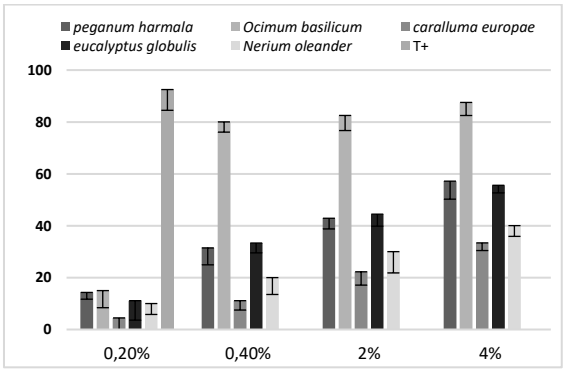


**Fig. 3.** effect of different plant extracts on *Botrytis cineria*

Similarly, *Oïdium oxysporum* was inhibited at 81% with a concentration of 0.2% of *Ocimum basilicum*, at 88% with a concentration of 0.4%, at 94% with a concentration of 2% and 96% with a concentration of 4% of the same extract. This fungus was inhibited at 45% with 4% *Peganum harmala* (figure 4).

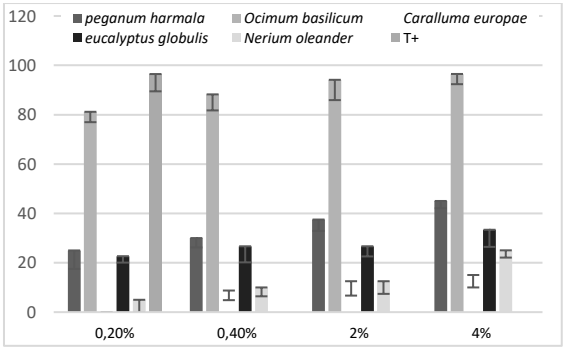


**Fig.4.** effect of different plant extracts on *oidium oxysporum*

All extracts inhibited the growth of *Phytophthora infestans* to moderate degrees. *Ocimum basilicum* was the highest with 48% at 4% followed by *Peganum harmala* at 47%, *Nerium oleande*r at 45% and 40% for *Caralluma europeae*, all at the same percentage of 4%. Against 56% for Asoxystrobin (figure 5).

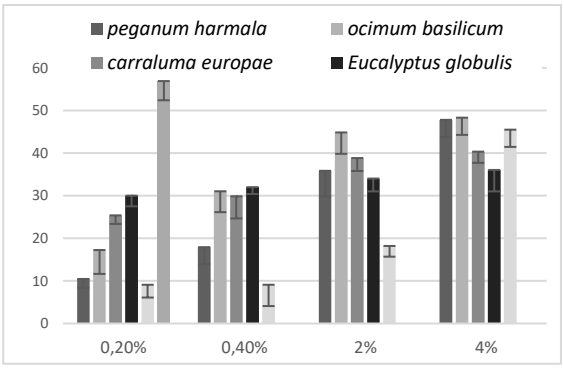


**Fig. 5.** Effect of different plant extracts on *Phytophtora infestans*

Based on the in vitro results, the five aqueous extracts studied *(Peganum harmala, Nerium oleander, Caralluma europae, Ocimum basilicum* and *Eucalyptus globulus*) showed an antifungal effect in a dose-dependent manner and from one extract to another.

According to Azhar et al. [17], as the concentration of the extract increases, the inhibition rate increases, which were noticed during this study as well. According to the same source, the inhibition rate of *Peganum harmala* reached 36.61% against *Alternaria solani*.

A percentage that recalls the results of the study. Indeed, at a concentration of 0.40% of the extract an inhibition of 33% was reached against the same fungus. Similarly, for *Nerium oleander against Alternaria solani* according to Muralidhar Mysore and Koteshwara Anandarao [18] the inhibition diameter reached 33.11±1.20 cm and according to Yanar et al. [19] the inhibition percentage of the same extract on the same fungus reached 49.75%.

At a concentration of 5% the inhibition percentage was 32.2% according to Nashwa and Abo-Elyousr, [3]. Results that are in perfect agreement with what we found. At a dose of 4% the percentage of inhibition of *Nerium oleander* against *Alternaria solani* reached 30%.

### 3.2 Fungal/fungistatic effect

All the extracts presented a fungal effect in favor of the studied fungi. Indeed, a growth resumption of *Phytophthora infestans, Alternaria solani, Botrytis cinerea* and *Oïdium oxysporum* fungi was observed after stopping exposure to aqueous extracts of *Peganum harmala, Caralluma europaea, Eucalyptus globulus, Ocimum basilicum* and *Nerium oleander*.

*Picture 1: Fungal/fungistatic effect of Nerium oleander on Phytophthora infestans* during the first week.

As presented on picture 1, *Nerium oleande*r inhibited *Phytophthora infestans.* After the removal of the filter paper, the fungus continues its growth.

### 3.3 Phytochemical screening

Table 1 shows the phytochemical screening of the plant aqueous extracts. Tannins are present in all the extracts except *Peganum harmala*. Indeed, *Eucalyptus globulus* contains tannins at a medium dose and catechic and gallic tannins at a high concentration.

*Caralluma europaea* contains catechic tannins in high concentration, as well as *Ocimum basilicum*. *Nerium oleander* is characterized by gallic tannins at a high concentration as well.

**Table1:** Phytochemical screening of the plant extracts

| | *Peganum harmala* | *Caralluma europaea* | *Ocimum basilicum* | *Nerium oleander* | *Eucalyptus globulus* |
|---|---|---|---|---|---|
| *Tannins* | - | - | + | ++ | ++ |
| *Catechic tannins* | - | +++ | +++ | - | +++ |
| *Gallic tannins* | - | - | + | +++ | +++ |
| *Cyanidins* | - | - | ++ | - | - |
| *Flavones* | - | - | ++ | + | - |
| *Flavonoles* | - | - | ++ | - | - |
| *Anthocyans* | - | - | - | - | - |
| *Leucoanthocyans* | - | ++ | ++ | + | ++ |
| *Alkaloïds* | +++ | +++ | + | +++ | - |
| *Saponins* | - | - | + | - | + |
| *Mucilages* | - | - | - | - | - |
| *Reducing sugars* | - | - | - | - | - |
| *Lipoïds* | - | ++ | - | ++ | - |
| *Iridoïds* | +++ | - | +++ | - | - |

– : negative test ; + : positive test ; ++ : moderately positive test ; +++ : very positive test

Cyanidins are present only in *Ocimum basilicum* at a medium dose with a low dose of flavones for *Nerium oleander*. Leucoanthocyanins are present at medium level for *Caralluma europaea, Ocimum basilicum* and *Eucalyptus globulus* and at low level for *Nerium oleander*. Alkaloids are present in high concentration for *Peganum harmala, Caralluma europaea* and *Nerium oleander,* and at a low level for *Ocimum basilicum* as well as saponins. The iridoïds are strongly present in *Peganum harmala* and *Ocimum basilicum.*

The aqueous extract of *Peganum harmala* and Ocimum *basilicum* are the highest for total flavonoïds as mentioned in table 2. Same for total polyphenols, the highest concentrations are for the aqueous extract of *Peganum harmala, Ocimum basilicum* and *Nerium oleander* as well as condensed tannins.

**Table 2:** Flavonoids, total polyphenols and condensed tannins content

| Plant aqueous extracts | Total flavonoïds content (µg/ml) | Total polyphenols content (mg/ml) | Condensed tannin content (mg/ml) |
|---|---|---|---|
| *Peganum harmala* | *39±2* | *0,204±0.05* | *0,11±0.065* |
| *Ocimum basilicum* | *27±1* | *0,375±0.035* | *0,39±0.025* |
| *Caralluma europae* | *15±5* | *0,16±0.062* | *0* |
| *Nerium oleander* | *16±3* | *0,34±0.036* | *0* |
| *Eucalyptus globulus* | *17±4* | *0,0575±0.048* | *0,15±0.05* |

Several researches showed that Basil has an antifungal activity against a several pathogenic fungi in the fields of medicine and agriculture, such as *Fusarium sp*. and *Sclerotium rolfsii* [6,20,21].

In this study, the percentage of inhibition is 87.5% against *Botrytis cinerea* and 96.47% against *Oidium oxysporum*.

The analysis performed by Senhaji et al., [28] revealed that the extraction by infusion of *Peganum* harmala brought out few flavonoids 8.65±0.35 mg GAE / mg E which may explain why these compounds did not appear in our analyses. This extract is also a great source of alkaloids as confirmed by Lamchouri et al., [22,23,24] .

The appearance of catechic tannins in *Caralluma europae* is confirmed by Amrati et al. [25]. While the appearance of flavonoids is mentioned by the same source and also by Ouassou et al. [26], wich is confirmed by correlation. The variation in extracts composition is linked to several factors such as the extraction methods, plant parts and genotypes, geographical origin and environment conditions, the harvesting period, the degree of drying, and drying conditions [27,28].

The inhibition of fungal diseases by aqueous extracts is due to natural organic compounds. The action is attributed to secondary metabolites such as polyphenols and triterpenoids [29].

*Ocimum basilicum* for example is composed by linalool, methyl-cavicol (estragol), camphor, and eugenol [6,21].

The antifungal activity is due to their phytochemical composition. Polyphenols have demonstrated their antifungal capacity that is associated with the thiol group at the active site of the plant extract [29]. wich is responsible for the inactivation of enzymes [30]. Flavonoïds ensure, through the lipophilic character, a better penetration of the molecules in the fungal membrane [31].

The polyphenols are polar and soluble in water, reason why they are present in all our extracts. In fact, *Ocimum basilicum*, *Peganum harmala* and *Nerium oleander* showed the most fungal efficacy against the studied fungi since the polyphenol content for these extracts is respectively in the order of 0.375±0.035, 0.204±0.05 and 0.34±0.036. The level of flavonoïds is also high 27±1, 39±2 and 16±3 respectively. Otherwise, the efficacy can also be attributed to the synergy of the different elements present in the extract [29].

## 4 Conclusion

As a conclusion, the studied plant extracts have all shown an antifungal effect against the studied fungi at different degrees. Indeed, *Peganum harmala*, *Caralluma europaea*, *Eucalyptus globulus*, *Ocimum basilicum* and *Nerium oleander* had all shown an antifungal activity that can goes up to 81%. Their use remains promising, especially in integrated control because of the nontoxicity effect on the environment.

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