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The antifungal effect of aqueous extract of *Lawsonia inermis* L tested against some fungal diseases on tomatoes crop

Keltoum Benaissa and Mohamed Belhamra

Abstract

The aim of this study is to determine the effects of an antifungal component from natural products on fungal diseases of tomatoes cultivated under greenhouses.

The aqueous extract of three ecotypes of *Lawsonia inermis* L were evaluate and compare its effect with a fungicide: Fenamidone + Fosthyl-Aluminium, tested against damping-off of seedling (caused by a number of fungi), *in vivo* conditions and against the blight (caused by (*Alternaria solani*), under laboratory conditions by the method of diffusion on solid medium.

The TA treatment, was able to inhibit moderately the growth of fungus of damping-off of seedling with 15mg/L *in vivo* conditions, the same treatment have an antifungal activity against *Alternaria solani*, by presenting the same percentage of growth inhibition of fungus given by the Fanamidone + fosethyl aluminum *in vitro* conditions; the effect of the others treatments were moderate with TZ (less than 60%) and no effect with TF (contaminated plates).

So the aqueous extract of *Lawsonia inermis* L, TA have the same effect of the fungicide

Fenamidone + Fosthyl-Aluminium, this plant can be considered as antifungal agent which will be useful in development of fungicides used against tomatoes diseases..

Keywords: *Lawsonia inermis* L, fungal diseases, tomatoes crop, fungicide

1. Introduction

The fungal diseases are very common on vegetable crops specially on tomatoes plant cultivated under green houses, and the first mode of defending against those pathogens is the use of fungicides which are both toxic for human health and it causes environmental pollution, also they are expensive for the farmers. Other ways the farmers need bio fungicides because the no well applied fungicide may become ineffective us a result of development of fungi resistance.

The alternative choice therefore would be the use of botanical fungicides, which are advocated to be largely non-phytotoxic, systematic and easily biodegradable in nature (Fawcett et Spender, 1970 and Beye, 1978 in Begun *et al*, 2007) [3].

Lawsonia inermis syn. *Lawsonia alba* commonly referred to as Henna, belongs to the Lythraceae family and is the sole species in the genus (Sesti, 1962 in Arun *et al*, 2010 [10] and Semwal *et al*, 2014) [13]. Henna has been found to exhibit antibacterial, antiviral, antifungal and dermatological properties and is found to be practically non-toxic (Lemordant et Foresteier, 1983) [9].

Pandey (1982) in Arun *et al* (2010) [10], showed that the gallic acid and lawsone as found to be the most potential antimicrobial agents. The chemical review of *Lawsonia inermis* L. presented by Chaudhary *et al* showed that the principal coloring matter of henna is lawsone (2-hydroxy-1,4 naphthaquinone C₁₀H₆O₃), besides lawsone there are other constituents present from them the gallic acid.

The semi arid environment from which the henna plant originates possibly favored evolutionary pathways geared towards the production of plant protection metabolites (Semwal *al*, 2014) [13].

Vonderheyden (1934) [14], Chattaoui (1970) and Kowlik (1951), Latour (1957) and Tripathi et Srivastava (1978) in Lemordant et Forestier (1983) [9], showed that in traditional agriculture, the farmers used henna to prevent the plants, the trees and the seeds against fungus parasites by using the henna as fungicide.

The aim of this study is to evaluate the antifungal activity of three ecotypes of *Lawsonia inermis* L collected from Biskra department which is based on the arid bioclimatic region (at the south eastern region of Algeria), using its aqueous extracts against *Petium* and *Phytophthora sp* (and others genus) causal agents of damping-off of seedling and *Alternaria solani* causal agent of blight, to reduce the fungicide application.

2. Materials and Methods

2.1. Plant material

Dried leaves of three ecotypes of *Lawsonia inermis* L were collected from three different regions of Biskra department on the most productive regions during summer of 2013 (from Zribet hamed with the first harvest of product called commonly 79 Henna Aarous TA, from Zribet El Oued TZ and from El Feidh TF).

2.2. Preparation of fungicide

The fungicide solution Feramidone+ Fosthyl d'Aluminium was prepared by weighting 0,2 g added to 100 ml of distilled water (suggested dose for this fungicide is 2 kg /ha).

2.3. Microorganism and media preparation

2.3.1. Damping off diseases

The plant of tomatoes cultivated in container (1/2 weight of sand and 1/2 weight of peat) under green houses on November 2014, were irrigated en excess to stimulate the fungal diseases, which appear couple of weeks after.

2.3.2. *Alternaria solani*

The fungi of *Alteraria solani* 1st inoculums was obtained from infected tomatoes, and then purified on potato dextrose agar (PDA) medium on Petri plates. The medium was prepared according to Larpent (1997) [8] protocol.

Three cut stems diseased plants were excised and surface-sterilized in a 0.5% sodium hypochlorite solution for 2 min and rinsed twice on distilled water then placed on P.D.A. medium and incubated for 7 days on a stove at 25 °C.

2.4. Preparation of aqueous extracts

The aqueous extracts were prepared using Denis (2007) [5] protocol, in which 15 g of each samples was soaked in 1L of distilled water, put to ebullition 20 mn, then the decoctions were kept overnight.

The mothers' solutions were filtered and used to prepare the different treatments at the concentration of 10% (15 mg/ L).

2.5. Antifungal test under *in vivo* conditions

This test was to determine the inhibition of *Petium* and *Phytophthora* growth on tomatoes seedling cultivated under greenhouses conditions; the three treatments TA, TZ and TF were applied on soil and seedling (2 plants by container) by pouring the solution on the container until saturation point, with three repetitions.

2.6. Antifungal test under laboratory conditions:

To test the effect of aqueous extract of *L. inermis* on *Alternaria solani* we used a disk from the purified mycelium developed from the 1st inoculums, cultivated on Petri plates on P.D.A. medium, we used 5 treatments:

- TA, TZ and TF aqueous extracts of *L. inermis*
- T Fongicide
- T control

We compared the diameter of mycelium growth on the control plates and the other plates and we calculate the growth

inhibition using the following formula:

$$\text{Growth inhibition ntag} = \left(\frac{CD}{CD - TD} \right) \times 100$$

CD: control diameter extension

TD: treatment diameter extension

3. Results and Discussions

The results of the effect of the three aqueous extracts of *L. inermis* tested against *Phytophthora*, *Petium* and *Alternaria* after a week of treatments applications were not the same, Habbal *et al* (2005) [7] showed that the antimicrobial activity of henna leaves was not thesame for all the microorganisms tested (14 strains of bacteria and one fungi strain). Begum *et al* showed that the ethanolic extract of henna tested for antifungal activity against phytopathogenic fungi, reacted differently, from 8 Percent radial mycelial growth inhibition in *Cuvularia lunata* Petri plates to 31% radial mycelial growth inhibition in *Fusarium equiseti*

The figure 01, shows the results of the three aqueous extracts of henna on damping-off diseases, only the TA affect moderately on *Phytophthora sp* and *Petium* growth.

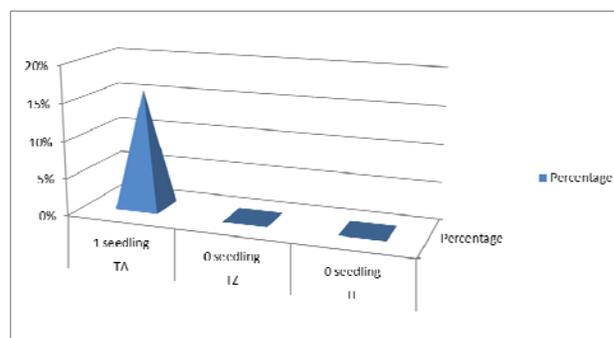


Fig 1: The effect of aqueous extract of henna on *Petium* and *Phytophthora sp*.

Because one concentration was tested, we can considerate that the effect of the aqueous extract of *Lawsonia inermis* could affect the fungi growth with more concentrate treatment *in vivo* conditions.

Al juraifani (2013) [1], shows that the aqueous extract of henna were able to inhibit the growth of *Trichophton rubrum* when the concentrations increased from 2 mg/l (0% no effect) to 5 mg/l (96% of growth inhibition).

Yusef *et al* show that the henna extract was found to be more effective in fungal growth inhibition when applied alone (85–96% fungal reduction). Effective inhibition of *Candida glabrata* required high concentration of henna leaves extract. An inhibition rate of more than 95% was obtained when 20% henna extract was applied on un-mordanted wool.

During the antifungal screening of higher plants, the leaves of *Lawsonia inermis* were found to exhibit strong fungitoxicity where naphthoquinones were found to be the active factor (Tripathi and al 1978 in Dinesh Babu and Subhasree, 2009) [6].

Fungi purification

The purification of the fungi on Petri plates gives a pictures as that described by Blancard *et al* (2009) [4] which is a confirmation that the symptoms of the blight disease is caused by *Alternaria solani* (Fig 2).



Fig 2: *Alternaria solani* under microscope (x 40)

The results of the effect of aqueous extract of henna on the mycelia growth of *Alternaria solani* on Petri plates is represented in the table 01.

Table 1: The effect of the three aqueous extracts of *L. inermis* L on *Alternaria solani*

| Treatments | TA | TZ | TF | Fungicide |
|---------------------------|------|-----|----|-----------|
| Mycelia growth inhibition | +++ | ++ | - | +++ |
| Percentage of inhibition | 100% | 54% | 0% | 100% |

+++ = >31-40 mm zone of inhibition

++ = >11-30 mm zone of inhibition

+ = > 10 mm zone of inhibition

= > 0 mm (no inhibitory activity detected)

The aqueous extract of *L. inermis* TA gives considerable antifungal activity at 15 mg/l, the mycelia growth of *Alternaria solani* was inhibited 100%, the similar effect done with the fungicide. Both of the treatments TZ and TF give moderate effect (54%) and no effect (0%) on mycelia growth inhibition (fig 3)



Fig 3: The effect of aqueous extract of henna on the mycelia growth of *A. solani*

The extract of leaves of *L. inermis* has been shown to possess antimicrobial activity

Bhuvanewari *et al* (2002) in Rahmou *et al* (2013) [11, 12]. Ethanolic extract of the whole plant was found to have antifungal activity (Rahmoun *et al*, 2013) [11, 12].

Al-Hamadani et Al-Mehna (2009) [2], showed that the prepared extracts gave a statistically significant inhibitory effect on growth of the skin fungi (*Trichophyton mentagrophytes*) and the alcoholic extract was the most

effective formula in an inhibitory effect on growth of tested fungus, where it showed in three concentration (15,20,25 mg/ml) an equal inhibition to antifungal (clotrimazole) which was 100%.

4. Conclusion

Medicinal plants have been used for ages in the treatment of diseases. In recent years, herbal medicines have increasingly been used to treat infections difficult to manage, but their use as plant preservative was rarely studied. Although, the antibacterial and the antifungal activities of *Lawsonia inermis* has been investigated against human diseases, but the screening of the aqueous extracts against fungal strains responsible for the tomatoes diseases has not been elucidated. This investigation has provided that the aqueous extract of *Lawsonia inermis* has an antifungal activity against damping-off disease (*Phytophthora sp*, *Rhizoctonia*, *Pythium* and other genus of fungus caused this disease) and against *Alternaria solani*. This study demonstrates that the origin and the ecotypes of the henna leaves has a difference on the efficiency of the biofungide.

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