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ANTIFUNGAL ACTIVITY OF BOTANICALS ON CAUSAL AGENTS OF TOMATO FUNGAL DISEASES

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ABSTRACT

Tomatoes *(Lycopersicum esculentum)* diseases are among the most constraining factors of tomato production in Côte d'Ivoire. The objective of this study was to evaluate the antifungal activity of *Carica papaya* and *Hydrocotyle bonariensis* leaf aqueous extracts, for preventive efficacy against tomatoes diseases agents *Alternaria* sp*., Fusarium* sp1 and *Fusarium* sp2. A greenhouse study was conducted using three different concentrations of leaf extracts (12.5, 25 and 37.5 mg ml^{-1}) obtained by diluting 25, 50 and 75%, respectively, of the crude extract (50 mg ml^{-1}) on the three tomato pathogenic fungi. The concentrations of each of the two extracts with the best fungistatic activities on fungal isolates were selected for *in vivo* tests. All aqueous extracts inhibited fungal mycelial growth by more than 70%, at the highest concentration (37.5 mg ml^{-1}); compared to the other two concentrations. At 37.5 mg ml^{-1}, the extracts reduced the severity of fungal diseases on tomato plants compared to the control. *Hydrocotyle bonariensis* was more effective. A more refined formulation of these extracts could increase their effectiveness and serve for sustainable control of tomato fungal diseases.

Key Words: *Alternaria* sp*., Fusarium* sp., *Lycopersicum esculentum*

RÉSUMÉ

Les maladies de la tomate (*Lycopersicum esculentum*) sont parmi les facteurs les plus contraignants de la production de tomate en Côte d'Ivoire. L'objectif de cette étude était d'évaluer l'activité antifongique des extraits aqueux de feuilles de *Carica papaya* et *d'Hydrocotyle bonariensis,* pour une efficacité préventive contre les agents pathogènes de la tomate *Alternaria* sp., *Fusarium* sp1 et *Fusarium* sp2. Une étude en serre a été menée en utilisant trois concentrations différentes d'extraits de feuilles (12,5, 25 et 37,5 mg ml^{-1}) obtenues en diluant respectivement 25, 50 et 75% de l'extrait brut (50 mg ml^{-1}) sur les trois champignons pathogènes de la tomate. Les concentrations de chacun des deux extraits ayant les meilleures activités fongistatiques sur les isolats fongiques ont été sélectionnées pour des tests in vivo. Tous les extraits aqueux ont inhibé la croissance mycélienne des champignons de plus de 70%, à la concentration la plus élevée (37,5 mg ml^{-1}); par rapport aux deux autres

concentrations. À 37,5 mg ml^{-1}, les extraits ont réduit la gravité des maladies fongiques sur les plants de tomates par rapport au témoin. *Hydrocotyle bonariensis* s'est avéré plus efficace. Une formulation plus raffinée de ces extraits pourrait augmenter leur efficacité et servir à la lutte durable contre les maladies fongiques de la tomate.

Mots Clés: *Alternaria* sp., *Fusarium* sp., *Lycopersicum esculentum*

INTRODUCTION

Tomatoes *(Lycopersicum esculentum)* diseases are among the most constraining factors of tomato production globally. Moreover, tomato ranks the second fresh or processed vegetable, after potato; which is widely cultivated and consumed worldwide (INRA, 2010). However, its production is characterised by a number of parasitic constraints, including fungal diseases, which cause untold yield losses (Panno *et al.,* 2021; Jha and Lamichhane, 2023). Fungal diseases, including *Alternaria* and *Fusarium,* are by far renowned to be the most devastating bio-stresses of tomato, at different growth cycles, ranging from nursery up to near consumption (Soro, 2014).

Alternaria solani, the cause of early blight, is a serious leaf disease of tomatoes (Faheed *et al*., 2005). It manifests as early leaf scorching, exposing the fruit to sunburn. This disease appears through partial or generalised necrosis at the seedling stage; and by leaf spots during cultivation (Damicone and Brandenberger, 2016). Tomato fruits can also be attacked at all stages of their formation (Sawadogo, 2013). Yellowing, followed by wilting of the entire plant, are also manifestations of these diseases (Ren *et al*., 2010).

Fusarium wilt is also one of the major destructive diseases of tomatoes; which may start on one side of the plant, with yellowing of the lower leaves. The affected leaves then wither and die. The disease progresses rapidly, leading to death of the entire plant (Damicone and Brandenberger, 2016).

In an attempt to manage these diseases, producers resort to synthetic fungicides mostly renowned for strong persistence and extremely costly (Lisan, 2012). Several of them are now the subject of numerous criticisms, due to their harmful effects on the environment, as well as on the health of consumers (Gerbore, 2013). In the quest for alternative control methods to chemical control, the use of plant extracts (botanicals) has taken a centre stage in research and crop production fora (Séka *et al*., 2017; Yao *et al*., 2017; Assiri *et al*., 2018).

Among the botanical candidates are extracts of *Carica papaya* and *Hydrocotyle bonariensis*, which are widely used not only in traditional medicine in Africa, Asia and America (Masoumian *et al.,* 2011 ; Romasi *et al.,* 2011; Kaboua *et al.,* 2021), but also for crop protection (Assiri *et al*., 2018 ; Afolabi and Kareem, 2018). Phytochemical analysis of *Carica papaya* leaf extract revealed the presence of Flavonoids; Alkaloids, Proteins, Glycosides, Phenols, Tannins, Saponins, Quinines, oxalates and anthocyanins (Nandini *et al*., 2020; Nilofer and Chenthamarai, 2020). On the other hand, *Hydrocotyle bonariensis* leaf extract showed the presence of Flavonoids, Saponins, Phenols, Tannins, Terpenoids, Sterols and Alkaloids (Obaseki *et al*., 2016; Kaboua *et al*., 2021). These plants are consumed as nutritional supplements or administered orally in the form of medication; and are without toxicity or mortality effects (Monyn *et al.,* 2016; Kaboua *et al.,* 2021).

The objective of this study was to assess the effectiveness of aqueous extracts obtained from *Carica papaya* and *Hydrocotyle bonariensis* leaves to control *Alternaria* and *Fusarium,* the causal agents of tomato diseases in Côte d'Ivoire.

MATERIALS AND METHODS

A greenhose study was conducted during 42 days, at the experimental farm in Nangui Abrogoua University, Côte d'Ivoire. Conditions in the greenhouse were *viz.*, temperature 27 ± 2 °C, relative humidity around 80% and photo-radiation 12 hours of light and 12 hours of darkness.

Plant material. The plant materials from which the foliar extracts were sourced included *Carica papaya* and *Hydrocotyle bonariensis* leaves; and 28-day-old tomato seedlings. The leaves of *Carica papaya* and *Hydrocotyle bonariensis* were used for preparing the aqueous extracts; while the tomato variety Cobra 26 seedlings were used to test for the *in vivo* effectiveness of the extracts.

Fungal material. Three fungal isolates, including two *Fusarium* (*Fusarium* sp1 and *Fusarium* sp2) and one *Alternaria* isolate, associated with symptoms of yellowing, necrosis and wilting of tomato plants (Cobra 26, Prodma and UC Burkina varieties), were used in this study. These fungi were isolated and preserved in the mycological collection of the Plant Pathology laboratory, at the Plant Health Unit of the University NANGUI ABROGOUA.

Preparation of aqueous extracts. Fresh leaves of *Carica papaya* and *Hydrocotyle bonariensis* were washed three times in tap water; and then oven-dried at 30 °C, for 4 days. They were then separately ground into powder; wherefter 50 g of powder from each plant sample were dissolved in 1 L of sterile distilled water and subjected to maceration for 72 hours. They were protected from light and shelved at laboratory room temperature (27 ± 2°C). The resulting mixtures were screened using a CAFEC Abaca Filter Paper, before being sterilised by filtration on 10-cm thick sterile hydrophilic cotton (Yao *et al.,* 2017). For each botanical extract obtained, 50 mg ml^{-1} concentration was diluted to 75, 50 and 25% with super cooled PDA medium, in order to obtain concentrations of 12.5, 25 and 37.5 mg ml^{-1}, of PDA medium amended with botanicals, espectively.

***In vitro* effectiveness of aqueous extracts.** The antifungal activity of *C. papaya* and *H. bonariensis* aqueous extracts were tested on fungal mycelial growth of *Fusarium (Fusarium* sp1 and *Fusarium* sp2*)* and *Alternaria* isolates, at three different concentrations (12.5, 25 and 37.5 mg ml^{-1}). Potato dextrose agar (PDA) media, amended with aqueous extracts, was homogenised and distributed in 90-mm diameter Petri dishes, at a rate of 20 ml per dish. Mycelial discs of 7 mm in diameter, were then taken from 7-day-old pure fungal cultures and placed in the centre of the Petri dishes (point of intersection of two perpendicular lines drawn on the reverse), containing the extract-PDA mixture. The cultures were then incubated in the laboratory at room temperature (27±2 °C). For each extract concentration, three Petri dishes were used as replications.

The experiment was laid out in a random design, replicated and repeated three times. Dishes containing PDA medium without aqueous extract made under the same culture conditions served as the control.

The radial growth of the mycelial colonies was measured daily, following the two parallel lines drawn on the reverse, until the surface of the culture medium was completely covered, in the control. The inhibition rate (Ic) of mycelial growth was then calculated according to the formula of Kumar *et al*. (2007), as follows:

$$Ic\ (\%) = \frac{1}{3} \Sigma \frac{(Dt-De)}{Dt} \times 100$$

Where:

Ic = mycelial growth inhibition rate (%); Dt (mm) = diameter of the mycelial colony of the control and De (mm) = diameter of the mycelial colony of the trial.

Mycelial growth inhibition rates were used to classify fungal isolates according to their reactions to aqueous extracts, following the scale proposed by Kumar *et al*. (2007) as follows: 1 = highly sensitive (> 90% inhibition); 2 = sensitive (> 75 - 90% inhibition); 3 = moderately resistant (> 60 - 75% inhibition); 4 = resistant (> 40 - 60% inhibition); and 5 = highly resistant (< 40% inhibition).

***In vivo* effectiveness of aqueous extracts.** Sixty four 28-day-old tomato seedlings, free from fungal disease symptoms, were treated by spraying with 25 µl of each *C. papaya* and *H. bonariensis* aqueous extracts, at the highest *in vitro* inhibitory concentration. Thus, 32 tomato seedlings were used to assess the ability of each extract to reduce the level of fungal diseases on tomatoes. Seven days after initiation of these treatments, the same seedlings were treated separately with the fungi *Fusarium* sp1, *Fusarium* sp2 and *Alternaria* inoculants, at a rate of seven seedlings per fungus.

Twenty-one other seedlings inoculated separately with the three fungi, but without the spray of the aqueous extracts, served as controls. All seedlings were watered at two days intervals with 250 ml of water, in order to maintain soil humidity.

The capacity of the aqueous extracts to protect tomato plants from fungal attacks was determined at 42 days after inoculation, through symptom prevalence (P) and severity (S) according to the formulas used by Yao (2019):

$$\text{Disease prevalence (P)} = \frac{\text{Number of symptomatic plants}}{\text{Total number of inoculated plants}} \times 100$$

$$\text{Symptom severity (S)} = \frac{\text{Number of plants with the same severity score x score value}}{\text{Total number of inoculated plants}}$$

Fungal disease symptoms severity score was determined according to the insect damage index scale proposed by Soro and Hgaza (2014), with a slight modification involving the replacement of damage by disease symptoms. The damage index scale was as follows, *viz*. 1 = 0 to 25% of seedlings showing symptoms; 2 = 25 to 50% of seedlings showing symptoms; 3 = 50 to 75% of seedlings showing symptoms 4 = 75 to 100% of seedlings showing symptoms.

Statistical analysis. The data collected were analysed using Statistica 7.1 software. The mean inhibition diameters of fungi mycelial colonies, prevalence and symptom severity indices were subjected to homogeneity test, followed by one-way analysis of variance (ANOVA 1). Significantly different means were separated using the Fisher LSD test at 5% threshold.

RESULTS

***In vitro* effectiveness of plant aqueous extracts.** The mycelial growth inhibition rates of the three fungal isolates varied depending on the types of aqueous extracts used (Fig. 1). It was higher with *H. bonariensis* aqueous extract (from 40.91 to 50.29%) than with *C. papaya* aqueous extract (from 20.68 to 45.23%). Thus, *H. bonariensis* botanical extract caused the highest inhibitions on fungus *Fusarium* sp1; this was followed by *Alternaria* sp and finally *Fusarium* sp2. On the other hand, with *C. papaya* botanical extract, the mycelial growth inhibition was higher for the fungus *Fusarium* sp1, then *Fusarium* sp2 and *Alternaria* sp. Analysis of variance showed a significant differences between the inhibition rates of fungi mycelial growth ($P < 0.05$).

The antifungal activity of both aqueous extracts varied significantly, depending on their concentrations (Fig. 2). Growth of the mycelial colonies of the three fungi increased along with

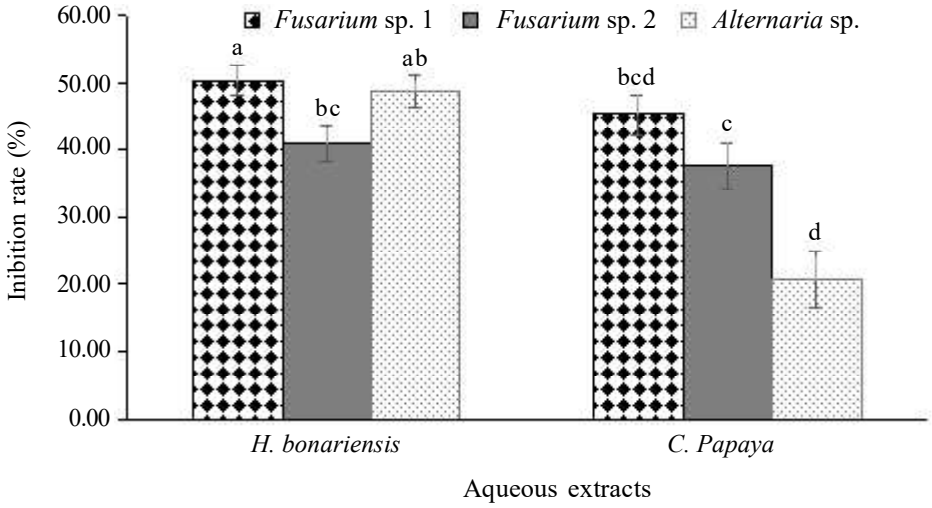

Figure 1. Inhibition rate of fungi mycelial growth depending on aqueous extracts.

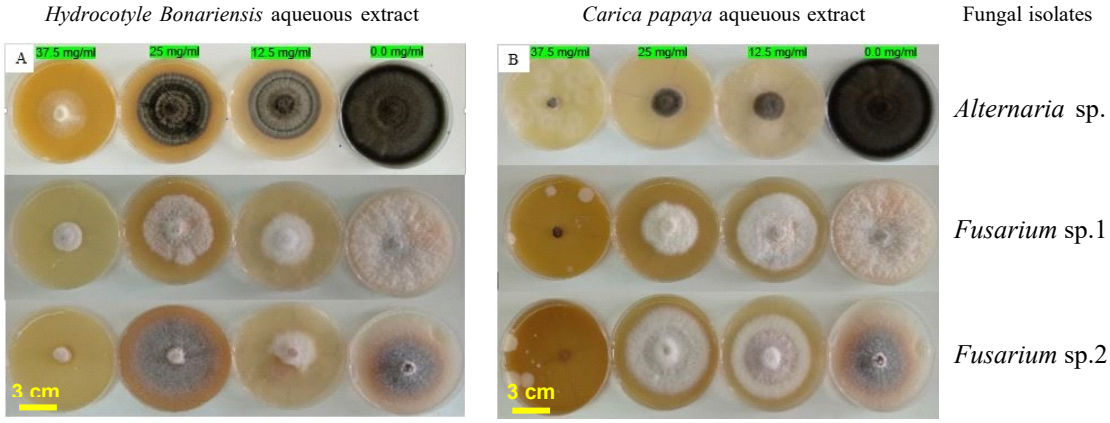

Figure 2. Mycelial colonies of fungal isolates on PDA medium amended with aqueous extract at different concentrations.

decreasing concentrations of the aqueous extracts. The inhibition rates of the three fungal isolates were then higher at 37.5 mg ml^{-1}. At this highest concentration, the inhibition rates oscillated from 63 to 78% (Fig. 3). A difference was noted between the behaviors of the fungal isolates towards the extracts; whereby *Fusarium* sp2 and *Alternaria* sp. isolates were moderately resistant to *H. bonariensis* and *C. papaya* aqueous extracts, respectively. In contrast, *Fusarium* sp1 and *Alternaria* sp. were sensitive to *H. bonariensis* aqueous extract, as were both *Fusarium* isolates to *C. papaya* aqueous extract. Significant differences were also observed between inhibition rates with respect to extract concentrations ($P < 0.05$).

In vivo effectiveness of plant aqueous extracts

Symptom prevalence. Prevalence of diseases caused by fungi, *Fusarium* and *Alternaria* in, varied significantly ($P<0.05$, Table 1) Plants inoculated with *H. bonariensis* botanical

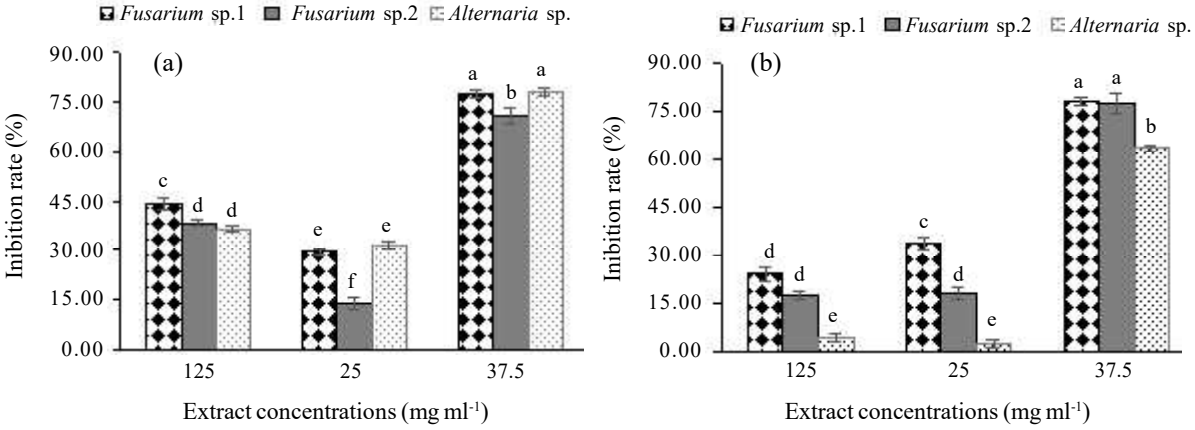

Figure 3. Average inhibition rate of fungi mycelial growth after 10-day incubation on PDA medium amended with aqueous extract.

TABLE 1. Prevalence and severity of symptoms depending on treatments, 42 days after inoculation

Treatments	Average prevalence (%)	Average severity
Control	37.25 ± 8.28^{b}	2.75 ± 0.47^{b}
C.papaya	34.14 ± 8.10^{ab}	1.80 ± 0.30^{ab}
H.bonariensis	15.11 ± 2.90^{a}	0.91 ± 0.14^{a}
Statistics	F = 4.98P = 0.014	F = 6.24P = 0.006

The averages bearing the same letters, in the same column, are statistically identical at 5% threshold according to Fisher's LSD test

extract showed mild symptoms with mean prevalence of 15%; while those treated with *C. papaya* extract showed pronounced symptoms with mean prevalence of 34%. The control plants were the most symptomatic, with over 37% of plants attacked (Fig. 4). A significant difference was noted between treatments in terms of symptom prevalence (Table 1).

Symptom severity. Severity of symptoms caused by fungi, also varied across the treatments, at a concentration of 37.5 mg ml^{-1}. The highest severity (2.75/4) was recorded in the controls and the lowest (0.91/4) in the plants treated with *H. bonariensis* aqueous extract (Table 1). The severity of the plants treated with *C. papaya* aqueous extract was intermediate (1.80/4). There were also significant difference ($P<0.05$) between treatments in terms of symptom severity (Table 1).

DISCUSSION

Aqueous extracts of *H. bonariensis* and *C. papaya* showed good efficacy not only *in vitro*, but also *in vivo*. *In vitro*, these extracts significantly inhibited growth of mycelial colonies of the fungi *Fusarium* spp. and *Alternaria* sp. This inhibitory activity is undoubtedly due to the combined action of secondary metabolites such as saponins, phenols, flavonoids, tannins, terpenoids, sterols

(a) (b) (c)

(a) Plants inoculated with *Alternaria* sp. and treated with *C. papaya* (AP); *H. bonariensis* (AH) and Control (A) aqueous extracts
(b) Plants inoculated with *Fusarium* sp2 and treated with *C. papaya* (VP); *H. bonariensis* (VH) and Control (V) aqueous extracts
(c) Plants inoculated with *Fusarium* sp1 and treated with *C. papaya* (BP); *H. bonariensis* (BH) and Control (B) aqueous extracts

Figure 4. Tomato plants treated with aqueous extracts, 42 days after inoculation.

and alkaloids contained in *H. bonariensis* extract (Obaseki *et al*., 2016; Kaboua *et al*., 2021) and flavonoids; alkaloids, proteins, glycosides, phenols, tannins, saponins, quinines, oxalates and anthocyanins in *C. papaya* extract (Nandini *et al*., 2020; Nilofer and Chenthamarai 2020).

Growth reduction of some pathogens by these extracts has been noted by several authors. Thus, the reduction in radial growth of the fungi *Aspergillus niger*, *Botryodiplodia. theobromae* and *Fusarium ventricosum* by the extract of *C. papaya* leaves was recorded by Afolabi and Kareem (2018).

Similarly, an extract from the same plant was used to inhibit growth of the fungus *Rhizopus stolonifera* (Romasi *et al.,* 2011). They attributed this antifungal activity to the phenolic compounds contained in the plant extract. Nilofer and Chenthamarai (2020) also diminished the growth of the fungus *Candida albicans*, the causative agent of human candidiasis. Entrocassi *et al.* (2021) observed strong *in vitro* inhibitory activity of the dichloromethane extract of *H. bonariensis* on the growth of two species of Chlamydia bacteria, responsible for sexually transmitted infections in humans. These authors justify the efficacy of this extract by its antiseptic properties.

During the present study, the highest rates of activity inhibition of these fungal pathogens were observed at highest concentration of the extracts. This would show that the antifungal activity of aqueous extracts was proportional to the dose used. The high concentration of the extract would also be synonymous with a high proportion of these plants' active ingredients in the aqueous solution. Similar results were reported by several researchers, including Afolabi and Kareem (2018) during their *in vitro* test of *Carica papaya* leaf extract on *Fusarium ventricosum*. They reported greatly reduced growth of mycelial colonies of this *Fusarium* species at the highest concentration (1.54 mg ml^{-1}). Péninna *et al.* (2014) also pointed out that the effectiveness of a plant extract would be due to its concentration of active substances. In this sense, *H. bonariensis* extract showed higher

inhibition rates than the *C. papaya* extract. Both fungal genera were sensitive to *H. bonariensis* extract, while only the *Fusarium* genus was sensitive to *C. papaya* extract.

The effectiveness of the extracts would, therefore, depend on the nature of the phytochemical compounds they contain. The secondary metabolites contained in the extracts have variable biological properties, giving each plant extract a very specific nature. Alkaloids have antibiotic and antiparasitic properties; while Flavonoids have anti-inflammatory and anti-carcinogenic activities (Badiaga, 2011). Saponins and tannins have antiviral, antibacterial and antifungal properties, in addition to the antiseptic activity of tannins (Badiaga, 2011; Entrocassi *et al*., 2021).

Nesmi (2010) also pointed out that, in addition to the concentration of the extract, the antimicrobial activity of active substances depends mainly on their chemical nature. *In vivo*, *H. bonariensis* extracts significantly reduced the prevalence and severity of fungal disease symptoms, when treated before crop infection. This reduction in the level of disease as a preventive measure, could be explained by a protective effect of the antimicrobial substances contained in these plants' extracts. These substances could reduce spore germination or germ tube elongation of such pathogens, resulting in a low level of disease development. Similar results were obtained by Yao *et al*. (2017), who showed that the aqueous extract of *Azadirachta indica* seeds inhibited the germination of *Colletotrichum gloeosporioides* conidia and reduced the incidence of yam anthracnose disease, just like the synthetic fungicide taken as a control.

Concerning the period of preventive treatment of extracts, Abo-Elyousr and Asran (2009) showed that the severity index of tomato bacterial wilt, caused by *Ralstonia solanacearum,* was most strongly reduced when plant extracts, including the garlic one, were applied at the same time as the inoculation of tomato plants; this contrasted with the present study whereby the plants were inoculated with fungi, 7 days after treatment.

CONCLUSION

The botanical extracts of *C. papaya* and *H. bonariensis* leaves were efficacious on the control of *Fusarium* and *Aleternaria* fungal diseases of tomato at various botanical extract concentrations. These extracts showed variable antifungal activity against the two fungal genera used. Overall, *C. papaya* extract was more effective against *Fusarium* spp at the concentration of 37.5 mg L^{-1}; while *H. bonariensis* extract was effective against both fungal genera (*Fusarium* and *Alternaria*), at the same concentration.

These extracts evaluated *in vivo* at this concentration, significantly reduced the prevalence and severity of fungal symptoms caused on tomato plants, with *H. bonariensis* extract being most effective. A more refined formulation of *H. bonariensis* could improve the efficacy of these extracts for effective and long-lasting control of tomato fungal diseases. Precise identification of the most active ingredients in each extract and for the different diseases is recommended.

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