

Research Article

Utilizing Gardenia leaves extracts to control *Fusarium solani* and *Rhizoctonia solani* in vitro and in vivo

Abeer Ahmed Mahmood*

Department of Biology, College of Sciences, University of Mosul, Iraq

Fadiyya Nooruldeen Saeed

Department of Biology, College of Sciences, University of Mosul, Iraq

Sara Nazar Ghanem

Medical Research Center, University of Mosul, Iraq

Fulla Kaydar mohmad Salih

Department of Biology, College of Sciences, University of Mosul, Iraq

*Corresponding author. E-mail: abeerahmed@uomosul.edu.iq

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Abstract

Vigna angularis (red bean), an important legume crop in Iraq and many Asian countries, suffers major yield losses due to fungal pathogens causing root rot, wilting, and damping-off diseases. The impact of *Gardenia jasminoides* leaf aqueous and alcoholic extracts was investigated in this study against the fungi that cause damping off of Adzuki beans, namely *Fusarium solani* and *Rhizoctonia solani*, using concentrations of 10, 15, 20, and 25 mg/ml. *In vitro* study indicated that the alcoholic extract successfully inhibited both fungi by 100% at a concentration of 25 mg/ml and by 85.67% at a concentration of 20 mg/ml against *R. solani*. The aqueous extract exhibited significant activity against *F. solani*, with inhibition reaching 88.9% and 78.91% at concentrations of 25 and 20 mg/mL, respectively. The *in vivo* greenhouse experiments demonstrated the effectiveness of alcoholic and aqueous extracts, as well as leaf powder, in reducing the severity of disease caused by *R. solani*. The disease severity was 1.17, and it decreased to 0.47, 0.49, and 0.65 when the seeds were treated with leaf powder, aqueous extract, and alcoholic extract, respectively. Additionally, the leaf powder demonstrated significant efficacy in treating the soil with both fungi, as disease severity decreased from 0.87 to 0.38. The phytochemical screening of aqueous extracts of *G. jasminoides* showed bioactive compounds such as terpenoids, tannins, saponins, and glycosides. The leaf powder also showed a clear superiority over the alcoholic and aqueous extracts when estimating the activity of the antioxidant enzyme catalase (CAT).

Keywords: Alcoholic extracts, Aqueous extracts, Disease severity, Damping off, Leaf powder

INTRODUCTION

Vigna angularis or red bean as called in Iraq is an important crop belonging to Fabaceae family widely grown in tropical and subtropical regions, including Iraq (Eaglesham *et al.*, 1992) and called Adzuki bean in Korea, P. R., China, India, and Japan (Wang *et al.*, 2000). However, its productivity sometimes decreases due to being infected with a number of agricultural pests, the most important of which are the causative fungi for plant death, root rot, wilting and damping off which lead to economic losses in all types of Iraqi soils (Hussein, 2019). Numerous distinct species of fungus, such as *Sclerotium rolfsii*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora sp.*, and *Fusarium solani*, are responsible of damping-

off and stem rot. (Almosoy, 2012; Alamri *et al.*, 2019; Singh and Rachie, 1985; Aveling and Adandonon, 2000). *R. solani* and *F. solani* were the most frequent fungi in samples of infected Adzuki seedlings that were collected from fields in the center of Iraq (Almosoy, 2012). The widespread soilborne pathogens, species of *Fusarium* and *Rhizoctonia* possess a broad range of hosts. They are able to persist by creating resting structures either integrated or for free in plant waste (Sneh *et al.*, 1995; Sarma and Singh, 2002; Shlevin *et al.*, 2003). *Gardenia jasminoides* species is the most important and most wide spread, commonly name Gardenia in Iraq is a flowering plant belonging to Rubiaceae family that is raised indoors or outside the house depending on the quality of the prevailing atmosphere in the area (Teuton, 2004). In Iraq, Gardenia breeding

inside the house is the ideal and appropriate place for the high temperature than required (Kassab Bashi, 2013). This herb has been identified to contain chemical compounds, including yellow pigment and iridoid glycosides, which are typically regarded as the key bioactive and distinctive components (Edward, 1990). Several studies have demonstrated that *G. jasminoides* has protective benefits on the nervous system, as well as antidepressant, anti-inflammatory, and beneficial effects on the gastrointestinal and cardiovascular systems (Abdul Wahab and Awad, 2015). The dried leaves of *G. jasminoides* were used to extract the iridoids glycoside (gardenoside) with a crude extract yield of 6.32% and 4.91% (Adandonon *et al.*, 2004). During the 2011 growing season, azuki bean plants showing the characteristic indications of stem rot were collected from farms in Beijing and Hebei Province, China. *R. solani* was the responsible pathogen acquired by tissue-isolating it from diseased stems (Misawa and Komatsu, 2011). Numerous different diseases can be brought on by *R. solani* AG 4 HGI in a variety of hosts. Therefore, crop rotation strategies should be given more thought to decrease Rhizoctonia stem rot on adzuki beans (Sun *et al.*, 2015). *Fusarium oxysporum f. sp. adzucicola* is the culprit behind the *Vigna angularis* wilt. When the fungal population is high and the soil and weather are ideal, *Fusarium solani* and *Rhizoctonia solani* attack bean plants. These fungi frequently cause damping off in Minnesota edible beans (Meronuck, and Hardman, 1981). *Fusarium spp* produce pink clumps of fungus spores near the base of the stem. The roots appear normal from the outside, but longitudinal sections will reveal discoloured vascular tissue, ranging from yellow to orange or dark red, which may extend well into the stem from the base up. Leaves wilt and turn yellow. Similar to *Fusarium*, *Rhizoctonia* symptoms typically appear earlier in the season when soil temperatures are still low (Ritchot and McNeil, 2015). This study was carried out with the intention of trying to control these pathogens with Gardenia plant extract agents in light of the significance of this pathogen in Iraq and the endeavor to control damping off disease in *Vigna angularis*.

MATERIALS AND METHODS

Preparation of aqueous and alcoholic extracts from the leaves of *Gardenia jasminoides* (*In vitro*)

Plants were pulled from the garden during March, thoroughly cleaned to remove dirt and muck, and then dried in the shade. 40g were crushed with 160ml in the case of an alcoholic extract, 70% ethyl alcohol or sterile distilled water in aqueous extract 1:4 (w/v) by a homogenizer for 30 minutes and then left in the refrigerator at 4°C. For the purpose of soaking for 24 hours, it was then filtered through several layers of gauze, then to increase purity, use a centrifuge at a speed of 35 00rpm

for a quarter of an hour in, and dried to obtain the dry powder (Rios *et al.*, 1987; Mahmood, 2011). After that, To create a 200 mg/lm standard solution, 1 gram of the dry powder was diluted in 5 mL of either distilled water in an aqueous extract or DMSO in an alcohol extract, 65°C water bath sterilization for ten minutes (Grand *et al.*, 1988., Jaafer *et al.*, 2020).

Experimental design

Concentrations of 10, 15, 20, and 25 mg/mL were prepared from the standard solution and poured into Petri dishes at a rate of 3 replicates/concentration/fungi after being added to 100 mL of PDA medium. The plates were inoculated by taking 5 mm samples from the colony edge of each pathogenic fungus with a cork borer under aseptic conditions, placing them in the center of the plate, and then incubating at 25 °C. Concerning the control dishes, they included the medium without addition (Sulaiman and Abdulhafedh, 2013). The results were obtained after a week for *F. solani* and ten days for *R. solani* by calculating the average of the two perpendicular diameters of the diagonal growth of each fungus and determining the percentage of inhibition using the following formula (Abdul Baki and Anderson, 1973; Abo-Elyousr *et al.*, 2022).

$$\% \text{Inhibition} = \frac{\text{Average expansion in control} - \text{general expansion in treated}}{\text{average expansion in control}} \times 100$$

Eq.1

In vivo effect of extract treatment on the ratio of incidence of seedling death and its severity

Three replicators were used in a factorial experiment that used a randomized complete block design using pots or anvils with a capacity of 1 kilo containing sterile soil, sterilized by autoclave (121°C, 15 psi) for one hour, then contaminated with fungi (pre-grown in petri dishes containing PDA media) at a rate of half a petri dish of each fungus per pot after cutting it and mixing it with an appropriate amount of sterile distilled water. The pots were watered for five days (Mohamed, 2001), then planted with *V. angularis* seeds after surface cleaning for one to three minutes with (1%) sodium hypochlorite, washed with sterilized distilled water, and dried on filter paper (10 seeds per pot). Experience included the following transactions:

Uninoculated, sterilized soil as healthy plants.

Soil contaminated with pathogenic fungi, each individually and together as a control.

Aqueous and alcoholic Gardenia extract, where 2% xanthan gum was added as an adhesive, and the seeds were steeped in a solution containing 25 mg/ml of each extract for five hours. (Sulaiman and Abdulhafedh, 2013). Then, they were dried for half an hour in air and planted in soil contaminated with both pathogenic fungi separately and together.

Gardenia leaves powder, the seeds were soaked in a

solution of Xanthan sugar (2%) for 10 minutes and treated with leaf powder at a concentration of 8 g/kg seeds before planting (El-Shaer,2002)

The results were taken by calculating the percentage of seedlings dying before emergence after one week of sowing seeds, as well as the final results of seedling death after emergence and the severity of disease two weeks after transplantation, based on the Wheeler (1970) modified method, which consists of 4 degrees, which are:

0 = Healthy seedling, 1 = live seedling but infected with root rot, 2 = seedling died before appearing. 3 = seedling died after appearing. The severity of the infection was calculated using the formula shown below:

$$\text{Disease severity} = (\text{Sumetion number of seedling category} * \text{Its degree}) / (\text{Total number of seedling} * \text{the highest degree})$$

Eq.2

Phytochemical screening

Determination of secondary metabolites in aqueous extract

Alkaloids by dragendroff reagent, tannins by ferric chloride with D.W and glycosides by acetic acid plus ferric chloride (Ndam et al., 2014), phenols by ferric chloride only, terpenoids by chloroform and resins as in (Shahatha,2020), steroids and saponins (EL-Gali and Hapa, 2018), phlobatannines and amino acid by ninhydrine test (lfy et al., 2021).

Measuring the activity of the enzyme catalase

Five seedlings were harvested from each treatment after ten days of sowing, two grams of leaves were taken, and the leaves were crushed with a phosphate buffer of pH 7 in a ceramic mortar at a ratio of 2:1. The mixture of leaves and phosphate buffer solution was then transferred to 10 mL test tubes. The tubes underwent a rapid evaporation process at 4000 rpm for 15 minutes. Keep the filtrate at 0°C until the catalase activity test is performed. The activity of the enzyme catalase was measured using a spectrophotometer (EMCLAB) by mixing 0.5 ml of hydrogen peroxide at a concentration of 65 mmol/L with 0.5 ml of phosphate buffer solution at

a concentration of 60 mmol/L (pH 4.7) and 0.2 ml of filtrate leaf mixture in test tubes, which were left for 4 minutes at 25 °C. The response came to an end when 1 ml of ammonium molybdate at a concentration of 32.4 mmol was added, followed by noting a change in colour at a wavelength of 450 nm, using an average to compare the significant variations in sample rates (Antar, 2010).

Analysis of the data

A statistical evaluation of the experiment was performed, and Duncan's novel multiple range tetrahedrons was used to compare the significant variations in sample rates. (DMRT) Antar, 2010).

RESULTS

Table 1 findings demonstrate *in vitro* results of *Gardenia jasminoides* alcohol extract, at all concentrations (10, 15, 20, and 25 mg/mL), tested against *R. solani*, suppressed its growth to varying degrees. The highest inhibition was 100% at 25 mg/ml and 85.76% at 20 mg/ml, respectively, and then it began to decline with lower Concentrations (Fig. 1A). Also, a concentration of 25mg/ml showed 100% inhibition against *F. solani*, followed by concentrations of 20, 15, and 10 mg/ml with inhibition rates of 80.96%, 79.26%, and 77.82%, respectively (Fig. 1B). The aqueous extract had a weaker effect on *F. solani* than the alcoholic extract, with the highest inhibition of 88.9% at 25 mg/ml. The inhibition gradually decreased with decreasing concentration, reaching a minimum of 74.24% at concentrations of 10 –15 mg/mL. Concerning *R. solani's* response to the aqueous extract, the concentration of 10mg/ml achieved the highest inhibition of 69.7%.

As shown from *in vivo* tests in Table 2, all seed treatments significantly reduced the severity of seedling death caused by *R. solani* compared to the control. The leaf powder and aqueous extract showed a significant advantage in reducing the severity of the disease at rates of 0.47 and 0.49, respectively. The leaf powder treatment led to a significant reduction in the rate of

Table 1. Effectiveness of various crude concentrations of aqueous and alcoholic extracts of *Gardenia jasminoides* leaves in inhibiting the growth of the tested fungus in the laboratory.

Concentration (mg/ml)	Growth inhibition % Aqueous extract		Growth inhibition % Alcoholic extract	
	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>
Control	0.00±0.00b	0.00±0.00b	0.00±0.00c	0.00±0.00c
10	69.7±5.27a	74.24±14.35a	12.41±2.25c	77.82±2.24b
15	4.21±4.29b	74.24±14.35a	77.22±19.75b	79.26±3.72b
20	4.68±1.87b	78.91±6.93a	85.67±12.50ab	80.96±5.64b
25	0.21±0.37b	88.9±10.02a	100±0.00a	100±0.00a

Numbers in the same column that are immediately followed by the same letters do not differ significantly, according to Duncan^s multiple range test with a P-value of 0.05

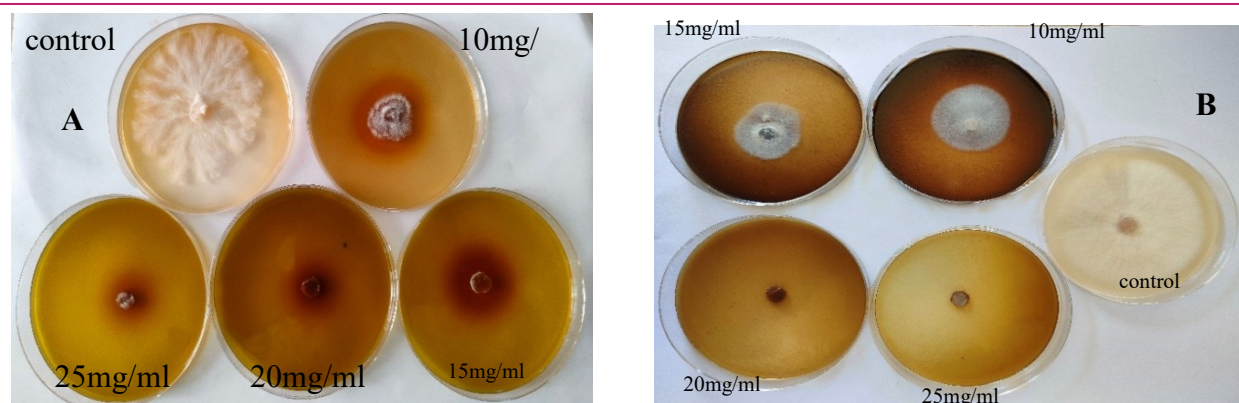


Fig. 1. Effective concentration of alcoholic and aqueous extracts of *Gardenia jasminoides* on the tested fungi – **A.** *Rhizoctonia solani*, **B.** *Fusarium solani* showing inhibition zones in all concentrations.

seedling death after emergence, to 13.33%. Treatments of seeds with alcoholic and aqueous extracts, as well as leaf powder, were weakly effective against *Fusarium* and could not inhibit the fungus, as shown in Table 3. From the same table, it is clear that the severity of the disease was significantly reduced by treating seeds with the leaf powder and alcoholic extract against the two fungi together, from 0.87 in the control to 0.38 and 0.75, respectively. Also, terpenoids, tannins, saponins, and glycosides give positive results according to the phytochemical screening of aqueous extracts of *G. jasminoides*, as shown in Table 3.

The results in Table 4 demonstrate that the various therapies differ significantly from one another, affecting *R. solani* in the concentration of catalase enzyme, as the leaf extract was superior by 5.67 units per gram compared to the untreated seeds by 3.47 units per gram. The leaf powder also showed a significant superiority over all treatments used for *Fusarium* resistance,

where the concentration of catalase reached the highest level of 7 units per gram compared to 3 units per gram for untreated seeds. Regarding the amount of catalase present in seedlings of Adzuka cultivated in soil tainted with *R. solani* and *F. solani*, it was not affected by any treatment, and the effect of alcoholic and aqueous extracts and leaf powder was weak.

DISCUSSION

The results from *in vitro* and *in vivo* studies confirm the significant activity of *Gardenia jasminoides*. Leaf extract, especially alcoholic extract, against *R. solani* and *F. solani*. The *G. brighamii* leaf extract in acetone had MIC values of 3.25 mg/mL and MFC values of 6.5 mg/mL against *F. verticillioides*. (Kafuaa et al., 2010). *F. solani* showed a high sensitivity towards both alcoholic and aqueous extracts at all studied concentrations *in vitro*. Based on instrumental analyses, the newly dis-

Table 2. Effect of treatment with aqueous, alcoholic extracts and leaf powder on the percentage of germination and severity of seedling death and Adzuki bean root rot

Soil treatment	Seed treatment	Pre-emergence damping off (%)	Post-emergence damping	Severity disease	Germination rate %
<i>Rhizoctonia solani</i>	control	26.67±20.82de	20±10.00ab	1.17±0.42a	73.33±20.82ab
	Alcoholic E	33.33±5.77 bcde	20±10.00 ab	0.65±0.095bc	66.67±5.77abcd
	Aqueous E	26.67±23.09de	16.67±5.77bc	0.49±0.24bc	73.33±23.09ab
	Leaves powder	30±20.00cde	13.33±15.28bc	0.47±0.30bc	70±20.00abc
<i>Fusarium solani</i>	control	30±0.00cde	16.67±11.55bc	0.68±0.104bc	70±0.00abc
	Alcoholic E	56.67±15.28abcd	20±10.00ab	0.82±0.106abc	43.33±15.28bcde
	Aqueous E	30±17.32 cde	16.67±5.77bc	0.62±0.26bc	70±17.32abc
	Leaves powder	63.33±11.55abc	36.67±5.77 a	1.18±0.208 a	36.67±11.55cde
<i>Rhizoctonia solani + Fusarium solani</i>	control	86.67±5.77a	0.00±0.00c	0.87±0.58ab	13.33±5.77e
	Alcoholic E	70±30.00 a	3.33±5.77bc	0.75±0.23abc	30±30.00 e
	Aqueous E	66.67±25.00ab	10±10.00bc	0.88±0.104ab	33.33±28.87de
	Leaves powder	23.33±23.09 ce	10±17.32 bc	0.38±0.49 cd	76.67±23.09ab
Healthy control		13.33±5.77e	0.00±0.00c	0.00±0.00d	86.67±5.77a

Duncan's multiple range test with a P value of 0.05 found no significant difference between values in the same column that are immediately followed by the same letters

Table 3. Biochemical constituents of aqueous extracts of *Gardenia jasminoides*

Secondary metabolite	Result
Alkaloids	-
Terpenoids	+
phenols	-
Tannins	+
Glycosides	+
Resins	-
Steroids	-
Saponins	+
Phlobatans	-
Amino acid	-

+ refer to positive test, -refer to negative test

covered antifungal substances in *Gardenia jasminoides* against *Fusarium oxysporum* and *Corynespora casicola* were named genipin and geniposide (Lelono et al., 2009). Plant extracts contain large amounts of lipids, phenolics, terpenes, polyacetylenes, tannins, resins, volatile and fixed oils, cyanogenic glycosides, glucosinolates, alkaloids, and other bioactive substances that are kept in specific plant parts, including leaves, bark, and seeds (Gupta et al., 2012; Borges et al., 2018).

According to the results of a previous study, it was possible to isolate and extract antibacterial peptides from the flowers of *Gardenia* and *Arabica* coffee, and to demonstrate their inhibitory effect on important types of pathogenic bacteria (Mahmood and Essa, 2014). The addition of different concentrations of aqueous extracts showed a significant inhibition of mycelium growth in varying proportions; the highest inhibition of all concentrations was 69.7% in concentration 10 mg/ml and began decreasing in other concentrations; this may be attributed to a change in the properties of the growing medium because of the antagonistic effect between the active ingredients against *R. solani* (Abdul Wahab and Awad, 2015). Linalool, alpha-farnesene, and z-3-hexenyl tiglate were the main chemical components of *G. jasminoides*' essential oil (Quhuan et al., 2022).

From the present results, it is evident that using alcoholic extract and leaf powder to treat the two fungi considerably lessened the disease's severity. This may be attributed to the ability of ethanol alcohol to extract polar compounds in general, such as various phenols, for example: flavonoids, anthocyanins, lignin, and other compounds (Harborne, 1973). When all bean varieties were tested for pathogenicity, it was discovered that the disease severity rates for *F. oxysporum*, *F. solani*, and *R. solani* AG-4 varied from 48.3% to 91.7%, 33.3% to 95.0%, and 26.7% to 50%, respectively. The Adzuki seed variety, Spir, Gezin/Elaz-1, and kidney bean cultivars were the most susceptible hosts to the three diseases (Omar et al., 2021). The air-dried *G. jasminoides* *Ellis* flower extract in dichloromethane supplied a new

iridoid natural product as well as a diastereomeric combination of 2a and 2b, and the fruits and leaves of *G. jasminoides* *Ellis* as a stimulant and poultice, respectively (Ragasa et al., 2007). It was also possible for the aqueous extract to lessen the severity of the disease, and this agrees with the results of the first table. The reason for this is the presence of cyclotides. There are approximately 15–60 different peptides of the type of cyclotides in each plant species of the family Cardinal. The isolated cyclotides of the cardinal family play an effective role against various types of pathogenic bacteria, as well as viruses and other pathogens (Gruber, 2012). *R. solani* caused Pre-emergence damping by 26.67%. The A207 amino acid residue endoglucanohydrolase produced by *R. solani* has two functional sections for plant immune function (Guo et al., 2022).

According to Gaur et al. (2020), pathogens in field crops typically cause a variety of symptoms, and disease can spread from seeds to seedlings to adult plants. *Gardenia* compounds have been shown to be positively effective against various pathogens in laboratory and greenhouse studies (Bakkali et al., 2008). From every section of the *Gardenia* plant, many chemicals have been extracted and identified. Flavonoids, iridoid glycosides, monoterpenoids, triterpenoids, carotenoids, organic acids, and their derivatives are among these substances (Ayuni and Andayani, 2022). A previous study (Omura et al., 2021) proved that on the tenth day after sowing, catalase activity increased in Adzuki bean seedlings analyzed for their enzyme activity and protein content (Catalase, peroxidase, and phenylala-

Table 4. Effect of seed treatment at a concentration of 25 mg/ml for alcoholic and aqueous extracts and leaf powder at a rate of 8 mg/kg on the activity of catalase enzyme

Soil parameters	Seed parameters	Catalase activity (unit/g)
<i>Rhizoctonia solani</i>	untreated seeds	3.47±2.64 abcd
	alcohol extract	3.07±0.46 abcd
	aqueous extract	0.67±0.61 cd
	leaves powder	5.67±2.53 ab
<i>Fusarium solani</i>	untreated seeds	3±0.53 abcd
	alcohol extract	1.2±1.31bcd
	aqueous extract	0.87±0.81 cd
<i>Rhizoctonia solani</i> + <i>Fusarium solani</i>	untreated seeds	5.27±6.84 abc
	alcohol extract	0.00±0.00 d
	aqueous extract	2.4±0.69 bcd
<i>Fusarium solani</i>	leaves powder	2.67±1.16 abcd
Healthy control	Healthy control	4.4±2.65 abcd

Duncan's multiple range test with a P value of 0.05 found no significant difference between values in the same column that are immediately followed by the same letters

nine ammonia-lyase) However, not all treatments were able to affect the activity of the catalase enzyme when the soil was treated with both fungi together. The discrepancy in the activity of the enzyme catalase between treatments may be due to the period of rapid growth, the flowering stage, a stage characterized by excessive cell activity, or to genetic causes (Stoilova et al., 2006).

Conclusion

This study revealed that *G. jasminoides* leaf powder exhibited superior antifungal activity compared to its alcoholic and aqueous extracts, significantly reducing the severity of damping-off disease in Adzuki bean seedlings caused by *Rhizoctonia solani*. The treatment decreased disease severity from 1.17 to 0.47 and enhanced catalase activity, indicating improved plant defense responses. These findings suggest that *G. jasminoides* leaf powder could serve as a promising natural antifungal and biostimulant agent. Further studies are recommended to isolate and characterize the active constituents responsible for its inhibitory and defense-inducing effects, as well as to evaluate its potential application in sustainable crop protection strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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