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EFFECT OF SOME MEDICINAL PLANT EXTRACTS AGAINST *SCLEROTIUM ROLFSII* CAUSING BASAL ROT OF CHINESE FOXGLOVE (*REHMANNIA GLUTINOSA*)

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Summary

Effect of some Medicinal Plant Extracts Against *Sclerotium rolfsii* Causing Basal Rot of Chinese Foxglove (*Rehmannia glutinosa*)

Four medicinal herbs (*Artemisia annua*, *Croton tonkinensis*, *Cinnamomum camphora*, and *Datura metel*) were extracted with 96% ethanol in a rate 1:10 (w/v). These dry extracts can be used at various concentrations (from 0.01% to 3.0%) *in vitro* in the PDA medium to inhibit the growth of *Sclerotium rolfsii* Sacc., which causes the basal rot of Chinese Foxglove *(Rehmannia glutinosa* Libosh.) in Bac Giang province. The results demonstrated that the extracts of *C.camphora* and *A. annua* at a concentration of 1.5% inhibited the development of *S. rolfsii*. Similarly, the extract from the *D. metal at* a concentration of 3% on the PDA medium, totally inhibited the *S. rolfsii* after 15 days of inoculation. Finally, a 3% concentration of the *C. tonkinensis* extract significantly inhibited the development of *S. rolfsii* in the PDA medium with the percentage of inhibition reaching over 71%; however 10 days after inoculation, the fungus stopped growing.

Keywords: *Extract, Artemisia annua, Croton tonkinensis, Cinnamomum camphora, Datura metel, Inhibition*

1. Introduction

Rehmannia glutinosa Libosh. is common medicinal plant which planted in some northern areas of Vietnam such as Bac Giang, Vinh Phuc, Phu Tho, and so on. *Rehmannia glutinosa* suffered from some severe diseases such as stem rot (*Sclerotium rolfsii*), root rot *(Rhizoctonia solani),* and leaf blight *(Phomopsis* sp.), in which stem rot was the most common disease in Bac Giang province [1].

Worldwide, *Sclerotium rolfsii* Sacc. is a serious soil-borne pathogenic fungus and causes severe damaged to many economically valuable crops in most of the tropical and subtropical regions [2]. *S. rolfsii* has a wide host range and has been referred to as an almost antipathogenic organism. In addition, *S. rolfsii* can maintain its continuity of generation under adverse conditions by forming sclerotia [3]. Hence, it is very difficult to control this disease even with the use of chemical fungicides.

There are numerous research using plant extracts to control *S. rolfsii* all over the world. Plants extract such as garlic, onion, ginger, neem leaf, and Allamanda leaf were tested for antifungal activities against *S. rolfsii*. The result indicated that the above plant extracts (the ratio of distilled water to plant parts was 200ml to 100g) was effectively produce percent mycelial growth that inhibits the growth of *S. rolfsii* in

culture media [4]. Another research illustrated that the highest percentage of inhibition (25.56%) was recorded using garlic extract, followed by neem extract (22.22%) at 4 days after inoculation. Another study also reported the antifungal effect of *S. rolfsii* of 13 plant extracts in which the highest antifungal effect was reported at 74.07% of the extract of *Prosopis julifora,* followed by the extract of *Prosopis julifora*, meanwhile, the lowest effect belonging to extracts of *Agave americana* (74.0%) and *Nerium indicum* (54.0%) [5]

The antifungal activities against *S.rolfsii* of some medicinal herb extracts (*Allium sativum*, *Artemisia annua*, and *Cinnamomum camphora*) at 100 ml to 20 g of distilled water to 20g powder of herb. These extracts against the complete development of mycelial growth of *S.rolfsii* in PDA media after 72 hours [6].

However, the published extraction methods using distilled water, might difficult to maintain the efficacy of the extracts in long-term storage. In addition, there is no information about the effect of dry plant extracts on the inhibiting growth of soil-born pathogens such as *S. rolfsii* [6].

This study reports the effect of some dry extracts which stem from medicinal material with ethanol 96% on the growth of *S. rolfsii* mycelia in the laboratory.

2. Material and Methods

The experiments were conducted at the Laboratory, Department of Cultivation and Plant Pathology, Hanoi Research Centre of Medicinal Plants, National Institute of Medicinal Materials. Extracts were extracted at the Department of Extraction Technology, National Institute of Medicinal Materials from January to December 2022.

Collection of diseased specimens

Disease stem samples with the stem–rot symptoms of *Rehmannia glutinosa* were collected from the fields of *Rehmannia glutinosa* in Dinh Tri village, Hiep Hoa district, Bac Giang province in 2022. Collected samples were placed in paper bags, then preserved about 1-2 days at 4°C for isolation.

Laboratory diagnosis of the pathogen

The pathogens associated with the basal rot disease of *Rehmannia glutinosa* were isolated following the tissue planting method. At first, the diseased plant parts (stems) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into small pieces (5 mm) from the advancing end of the lesions. The cut portion was surface sterilized with 1% ethanol 70% for 1 minute and rinsed with sterilized water 3 times. Surface sterilized plant pieces were plated on PDA media petri dishes (90 mm) and incubated at room temperature of 25-27°C for 7-10 days and examined daily for any fungal growth. The pathogenicity of the identified pathogens was confirmed by artificial inoculation on healthy Chinese Foxglove plants following Koch's postulates [7].

Preparation of raw materials

Four types of botanicals were collected from different places. The leaf and fruit of *Datura metel*, the leaf of *Artemisia annua* in Vinh Phuc, the leaf of *Croton tonkinensis* in Hung Yen, and the leaf of *Cinnamomum camphora* in Hanoi in 2021 (Species correctness of medicinal plants studied was identified by Master Tran Van Loc - *National Research Center for Medicinal Plant Germplasm and Breeding, NIMM)*. The above plant materials were washed under clean water 3 times and placed under the shadow to dry. After that, the dried materials were ground. Finally, the powder was used for extraction.

Preparation of plants extracts

The dried materials were extracted with ethanol (EtOH) 96% at the ratio of 1:10 at ambient temperature, then the extract was filtered through a membrane filter (5 μm – 100 μm) and freeze-dried (Harvestright HR-3) at 20-30°C for 28 hours. The moisture content of the dry product was 3-5% (Moisture Analyzers Ohaus MB25). Finally, the dry extracts were ground and then the powder was used experiment.

Evaluation of antifungal activities of the plant extracts

To evaluate the antifungal activities of the extracts, desired concentrations from 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, and 3.0% were prepared by adding the appropriate amount of standard basic powder of medicinal extracts to PDA in Petri plates. Each treatment was replicated three times, and 5 petri plates for each replication. PDA without medicinal extracts served as control. Each plate was inoculated with a 6mm diameter mycelial disc taken from 4 days-old culture of *S.rolfsii* grown on PDA. The

inoculated plates were incubated at 25-27°C and radial mycelial growth was recorded after from 1 day to 15 days, respectively. The percent inhibition of mycelial growth over control was calculated [8].

$$PI\ (\%) = \frac{DC - DT}{DC} \times 100\%$$

PI (%): Percentage of colony inhibition
DC (mm): Diameter of control colony
DT (mm): Diameter of treated colony

Statistical analysis

Data of experiments were analyzed by CropStat 7.2 and Excel 2010 software, and the treatment were delineated bt applying LSD test ($P \leq 0.05$).

3. Results and discussion

3.1. Identification of the pathogen causing basal rot disease of Chinese Foxglove (Remannia glutinosa)

The symptoms of the basal rot disease appearred initially as small lesions of discoloration that later turned into discrete lesions with white mycelium around the lesions. The later developed symptoms involved rotting of the lower part of the stem causing the dieback, and mustard seed-like brown sclerotia could be formed (Fig. 1a). When the mycelium of *S. rolfsii* covers full the plate on the PDA medium, the young sclerotia are formed. Sclerotia began as small tufts of white mycelium that formed spherical sclerotias (Fig. 1b) after that sclerotias were darken as they were mature, and became tan to dark brown (Fig. 1c)

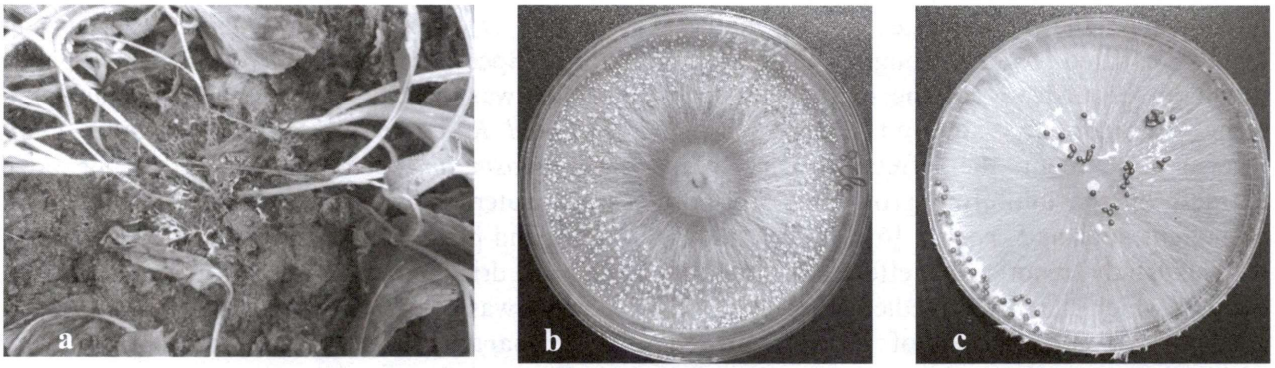

Fig. 1. Isolation of causal organism; (a) symptom of basal rot disease on *R. glutinosa*, (b) the young sclerotia formation of the pathogen, (c) the old sclerotia formation of the pathogen

3.2. In vitro efficacy of medicinal extracts inhibition of mycelial growth of S.rolfsii

3.2.1. The effect of Datura metel extract on the growth of Sclerotium rolfsii:

D. metel material extracted with EtOH 96% was studied at concentrations of 0.01%, 0.05%; 0.1%, 1.0%; 1.5%; 2.0%, and 3.0% to evaluate the ability to inhibit fungus *S. rolfsii* on PDA medium, and the results are shown in Table 1 and Fig. 2.

The Table 1 and Fig. 2 (a-i) indicated that the *D. metel* extract has a significant inhibitory effect on the growth of mycelia *S. rolfsii* at a concentration above 0.5% after four days compared to the control. Specifically, the extract of *D. metel* at 3.0% completely inhibited the mycelial growth (inhibitory efficiency over 90%) on PDA media, followed by concentrations of 2.0%; 1.5%; 1.0%, and 0.5% with the inhibition reach 87.8%; 75.3%; 60.4% and 52.7%, respectively. In addition, the growth of *S. rolfsii* mycelium at concentrations higher than 1.0% showed an abnormality compared with the control, and the growth of the mycelium was heterogeneous and round like that in the control (Fig. 2i).

The remaining concentration (0.1%, 0.05%, and 0.01%) all had a low inhibitory effect, of which the lowest concentration was 0.01% with a 0.0% inhibitory effect compared to the control. Thus, the extract of *D. metel* was effective in inhibiting the *S. rolfsii* fungus completely on PDA media after 15 days at a concentration of 3.0%.

Table 1. Effect of *D. metel* extract on the inhibition of mycelial growth of *S. rolfsii in vitro* condition

Concentration (%)	Days after inoculation							
	1 day		2 days		3 days		4 days	
	RMG (mm)	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*
Control	32.2^{a}	0.0^{e}	63.3^{a}	0.0^{g}	90.0^{a}	0.0^{f}	90.0^{a}	0.0^{g}
0.01	28.6^{b}	11.2^{d}	51.8^{b}	18.2^{f}	90.0^{a}	0.0^{f}	90.0^{a}	0.0^{g}
0.05	23.1^{c}	28.3^{c}	42.2^{c}	33.3^{e}	61.4^{b}	31.8^{e}	80.0^{b}	11.1^{f}
0.1	10.5^{d}	67.4^{b}	23.3^{d}	63.2^{d}	38.2^{c}	57.6^{e}	51.5^{c}	42.8^{f}
0.5	10.1^{d}	68.6^{b}	18.0^{e}	71.6^{c}	30.4^{d}	66.2^{d}	42.6^{d}	52.7^{e}
1.0	6.8^{e}	78.9^{a}	10.6^{f}	83.3^{b}	16.9^{e}	81.2^{c}	27.5^{e}	69.4^{d}
1.5	6.0^{e}	81.4^{a}	7.3^{fg}	88.5^{ab}	10.4^{f}	88.4^{b}	22.2^{f}	75.3^{c}
2.0	6.0^{e}	81.4^{a}	6.0^{g}	90.5^{a}	8.6^{f}	90.4^{a}	11.0^{g}	87.8^{b}
3.0	6.0^{e}	81.4^{a}	6.0^{g}	90.5^{a}	6.0^{f}	93.3^{a}	6.0^{h}	93.3^{a}
LSD (5%)	*1.9*	*4.3*	*4.3*	*5.9*	*4.4*	*5.1*	*4.0*	*3.4*
CV (%)	*7.7*	*6.9*	*9.7*	*8.4*	*6.5*	*7.6*	*5.0*	*6.2*

Note: RMG: Radial mycelial growth; PI: Percentage of colony inhibition.
Value with different letters at the top of data shows a significant difference (P≤0.05) as determined by LSD test.

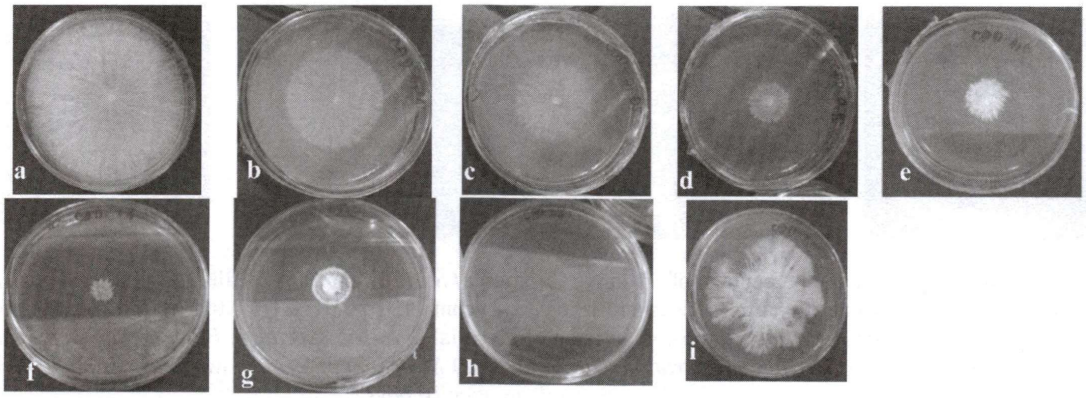

Fig. 2. Radical mycelial growth *of S. rolfsii* on PDA medium adding different *D. metel* extract concentration after 3 days: (a) control (0%), (b) 0.01%; (c) 0.05%; (d) 0.1%; (e) 1.0%; (f) 1.5%; (g) 2.0%; (h) 3.0%; (i) the abnormalities of mycelial *S. rolfsii spread on PDA medium supplemented with D. metel extract at 1.0% after 7 days*

3.2.3. Effect of *Croton tonkinensis extract on the growth of Sclerotium rolfsii:*

In order to assess their capacity to control the fungus S. *rolfsii* on PDA media, *C. tonkinensis* material extracted with EtOH was investigated at doses of 0.01%, 0.05%; 0.1%, 1.0%; 1.5%; 2.0%, and 3.0%. The findings are provided in Table 2 and Fig. 3.

Table 2. Effect of *C. tonkinensis* extract on the inhibition of mycelial growth of *S. rolfsii in vitro* condition

Concentration (%)	Days after inoculation							
	1 day		2 days		3 days		4 days	
	RMG (mm)	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*
Control	32.2^{a}	0.0	63.3^{a}	0.0	$90,0^{a}$	0.0	90.0^{a}	0.0^{e}
0.01	27.6^{b}	14.3	54.8^{b}	13.4	$90,0^{a}$	0.0	90.0^{a}	0.0^{e}
0.05	19.0^{c}	41.0	41.2^{c}	34.9	90.0^{a}	0.0	90.0^{a}	0.0^{e}
0.1	18.2^{c}	43.5	38.6^{c}	39.0	90.0^{a}	0.0	90.0^{a}	0.0^{e}
0.5	15.9^{d}	50.6	32.6^{d}	48.5	67.9^{b}	24.6	90.0^{a}	0.0^{e}
1.0	11.3^{e}	64.9	24.9^{e}	60.7	40.5^{c}	55.0	62.1^{b}	31.0^{d}
1.5	6.7^{f}	79.2	8.6^{f}	86.4	24.0^{d}	73.3	37.3^{c}	58.6^{c}
2.0	6.6^{f}	79.5	7.6^{f}	88.0	18.8^{e}	79.1	31.3^{d}	65.2^{b}
3.0	6.6^{f}	79.5	7.4^{f}	88.3	13.5^{e}	85.0	25.5^{e}	71.7^{a}
LSD (5%)	*1.8*	*3.1*	*4.1*	*4.2*	*4.8*	*3.8*	*3.8*	*3.7*
CV (%)	*6.0*	*5.7*	*7.5*	*7.2*	*4.8*	*9.9*	*3.3*	*13.7*

Note: RMG: Radial mycelial growth; PI : Percentage of colony inhibition.
Value with different letters at the top of data shows a significant difference (P≤0.05) as determined by LSD test.

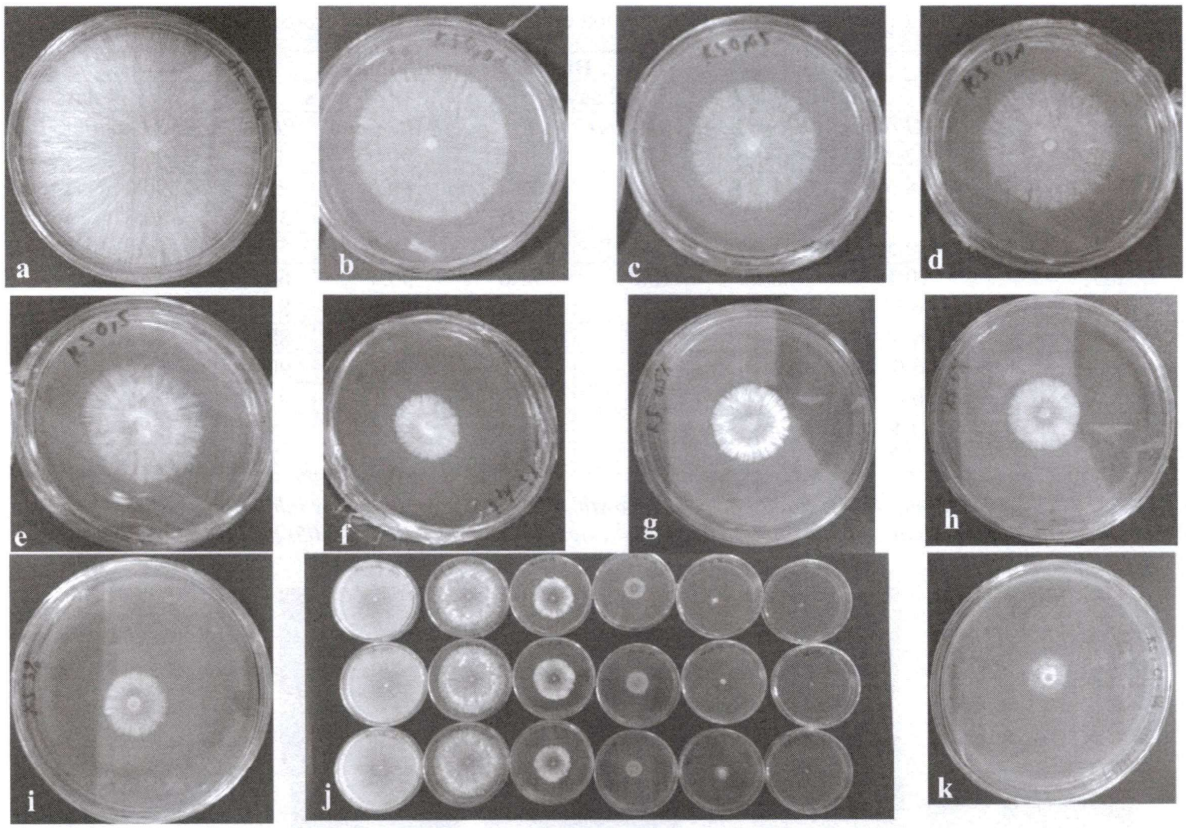

Fig. 3. Mycelial growth and percentage of conlony inhibition *of S. rolfsii* on PDA medium with *C. tonkinensis extract at* different concentration after 3 days and 10 days: (a) control, (b) 0.01%; (c) 0.05%; (d) 0.1%; (e) 0.5%; (f) 1.0%; (g) 1.5%; (h) 2.0% (i) 3.0%; (j) the comparision of mycelial *S. rolfsii spread on PDA medium supplemented with C. tonkinensis extract at different concentration with control; (k) Mycelium of S.rolfsii was underveloped on 3.0% C. tonkinenesis medium after 10 days*

It is showed that the *C. tonkinensis* extracts have a significant inhibitory effect on the growth of mycelia of *S. rolfsii* at a concentration above 1.5% after four days compared to the control. Specifically, the extract of *C.tonkinensis* at 3.0% has the greatest inhibition (74.7%) against the mycelial growth on PDA media, followed by concentrations of 2.0%; 1.5% with inhibition of 62.5 and 58.5% respectively. The inhibitory effect of concetration extract at 1.0% was subtaintially lower, at 31%. Additionally, the growth of *S.rolfsii* mycelium at 3.0% showed underdevelopment compared to the control after ten days (Fig. 3k). In constrast, PDA medium with *C. tonkinensis* extract at 0.5%, 0.1%, 0.05%, and 0.01% did not effectively inhibit the mycelial growth of *S.rolfsii* compared to the control after four days of follow-up.

3.2.3. Effect of *Cinnamomum camphora extract on the growth of Sclerotium rolfsii:*

C. camphora extract was used in five concentrations to test *S. rolfsii* inhibition on PDA medium. The result is shown in Table 3 and Fig. 4.

Table 3. Effect of *C. camphora* extract on the inhibition of mycelial growth of *S. rolfsii in vitro* condition

Concentration (%)	Days after inoculation							
	1 day		2 day		3 days		7 days	
	RMG (mm)	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*
Control	32.2^{a}	0.0^{c}	63.3^{a}	0.0^{b}	90.0^{a}	0.0^{c}	90.0^{a}	0.0^{e}
0.5	7.0^{b}	78.3^{b}	9.5^{b}	85.0^{a}	16.8^{b}	81.3^{b}	90.0^{a}	0.0^{e}
1.0	6.3^{b}	80.4^{a}	6.9^{bc}	89.1^{a}	7.5^{c}	91.7^{a}	46.2^{b}	48.7^{d}
1.5	6.0^{b}	81.4^{a}	6.0^{c}	90.5^{a}	6.0^{c}	93.3^{a}	15.5^{c}	82.8^{c}
2.0	6.0^{b}	81.4^{a}	6.0^{c}	90.5^{a}	6.0^{c}	93.3^{a}	6.0^{c}	93.3^{a}
3.0	6.0^{b}	81.4^{a}	6.0^{c}	90.5^{a}	6.0^{c}	93.3^{a}	6.0^{c}	93.3^{a}

Concentration (%)	Days after inoculation							
	1 day		2 day		3 days		7 days	
	RMG (mm)	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*
LSD (5%)	*1.6*	*4.5*	*2.8*	*5.3*	*2.2*	*6.7*	*10.6*	*9.5*
CV (%)	*8.4*	*9.7*	*9.3*	*10.1*	*5.6*	*6.8*	*13.7*	*11.1*

Note: RMG: Radial mycelial growth; PI : Percentage of colony inhibition.
Value with different letters at the top of data shows a significant difference (P≤0.05) as determined by LSD test.

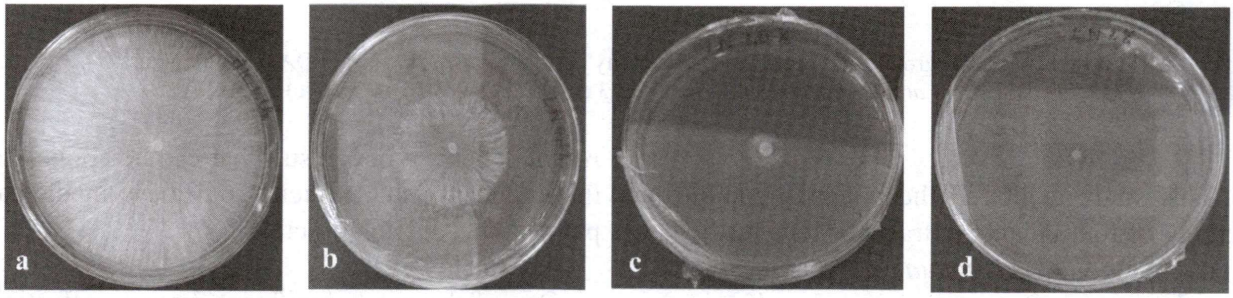

Fig. 4. Mycelial growth and percentage of conlony inhibition *of S. rolfsii* on PDA medium with *C. camphora extract at* different concentration *after* 3 days: (a) control, (b) 0.5%; (c) 1,0%; (d) 2.0%

When compared to the control after three days, it can be noted that the *C.camphora* extract was witnessed completely control the development of *S. rolfsii* mycelia at a concentration over 1% (Fig. 4c and Fig. 4d). After seven days, only two concentrations 2.0% and 3.0% maintained the affection. The inhibitory impact of *C.camphora* extract at 1.0% was much lower, at 48,7% after seven days, and loose the exhibitory after nine days. In addition, the *S. rolfsii* mycelium growth at 1.5% showed underdevelopment in comparison to the control, and the mycelium growth stopped growing after nine days. However, the mycelial growth of *S. rolfsii* was not effectively inhibited on a PDA medium containing extract of *C. camphora* at concentrations of 0.5% compared to the control after four days of follow-up.

3.2.4. Effect of *Artemisia annua extraction on the growth of Sclerotium rolfsii:*

Four concentrations of *A. annua* extract were conducted to analyze *S. rolfsii* inhibition on PDA medium. The statistics are presented in Table 4 and Fig. 5.

Table 4. Effect of *A. annua* extraction on the inhibition of mycelial growth of *S. rolfsii in vitro* condition

Concentration (%)	Days after inoculation							
	1 day		2 days		3 days		4 days	
	RMG (mm)	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*	*RMG (mm)*	*PI (%)*
Control	13.7^{a}	0.0^{d}	28.8^{a}	0.0^{d}	66.3^{a}	0.0^{d}	90.0^{a}	0.0^{d}
0.5	7.4^{b}	46.0^{c}	11.9^{b}	58.7^{c}	17.8^{b}	73.2^{c}	30.5^{b}	66.1^{c}
1.0	6.9^{b}	49.6^{b}	8.8^{c}	69.4^{b}	11.8^{c}	82.2^{b}	18.1^{c}	79.9^{b}
1.5	6.0^{b}	56.2^{a}	6.0^{d}	79.2^{a}	6.0^{d}	91.0^{a}	6.0^{d}	93.3^{a}
2.0	6.0^{b}	56.2^{a}	6.0^{d}	79.2^{a}	6.0^{d}	91.0^{a}	6.0^{d}	93.3^{a}
LSD (5%)	*0.6*	*2.1*	*2.4*	*2.6*	*3.6*	*2.8*	*7.2*	*10.3*
CV (%)	*4.2*	*2.6*	*10.5*	*2.7*	*8.9*	*2.9*	*12.8*	*14.8*

Note: RMG: Radial mycelial growth; PI : Percentage of colony inhibition.
Value with different letters at the top of data shows a significant difference (P≤0.05) as determined by LSD test.

Overall, all the studied concentrations of the *A. annua* extract have an inhibitory effect on the growth of *S. rolfsii* fungus on PDA medium. At a concentration of more than 1.5%, the *A. annua* extract was seen to entirely restrict the growth of *S. rolfsii* mycelia as compared to the control after three days, at more than 90%. These figures still intact after 15 days. The 1.0% and 0.5% of *A. annua* extract had a dramatically lower inhibitory effect, reaching 79.9% and 66.1% respectively after four days. Furthermore, the *S. rolfsii* mycelium growth also showed underdevelopment at 1% compared to the control and ceased growing after ten days.

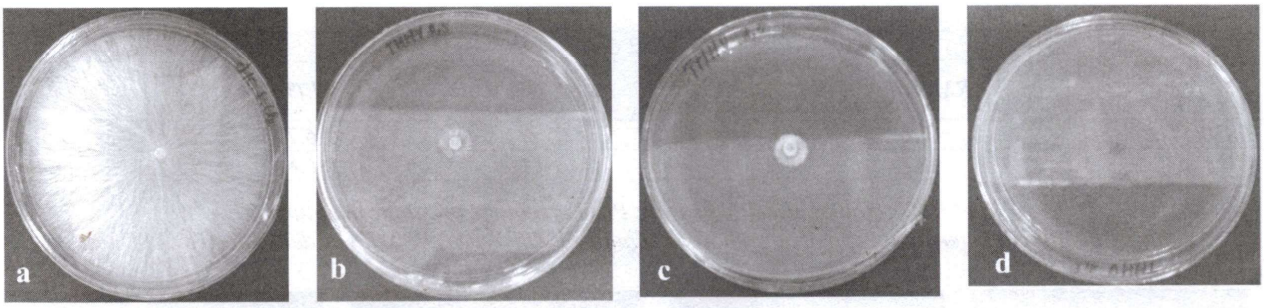

Fig. 5. Mycelial growth and percentage of conlony inhibition *of S. rolfsii* on PDA medium with *A. annua extract at* different concentration *after* 3 days: (a) control, (b) 0.5%; (c) 1,0%; (d) 2%

3.3. Discussion

This study exhibited the potential inhibitory activity of four ethanol extracts of four medicinal plants including *A. annua*, *C. tonkinensis*, *C. camphora*, and *D. metel* against *S. rolfsii* in the PDA medium. *C. camphora* and *A. annua* extracts had the best effect of the four above-mentioned extracts and had an almost complete inhibitory effect on this fungus even at a concentration of 1.5%, completely inhibiting it at concentrations above 2%. After that, the extract from the *D. metel* also completely inhibited the *S. rolfsii* at a concentration of 3%. Finally, the extract from *C. tonkinensis* at a concentration of 3% did not completely inhibit the growth of *S. rolfsii* on the PDA medium, but after 15 days, this fungus did not grow anymore.

Compared with the results reported by My et al., 2018 [6]. When extracting these medicinal herbs with distilled water it is required the concentration to be as high as 20%. This result indicated that it is possible to apply the extraction method with methanol and freeze-drying to reduce the concentration used to control the *S. rolfsii*. This is a new result that can be applied to the application of extracts from these medicinal plants in production practice

4. Conclusion

One of the most significant diseases affecting Chinese foxglove is basal rot, which is caused by *S. rolfsii* and produces brown sclerotia and white mycelium around the lesions on the fields. After 3 or 4 days in the incubator, brown sclerotia might appear on the PDA medium.

Four medicinal plants (*A. annua*, *C. tonkinensis*, *C. camphora*, and *D. metel*) have been extracted with ethanol at various concentrations, which had a different effect on the inhibition of *S.rolfsii*. The highest percentage of inhibition (over 90%) was recorded using *A. annua* and *C. camphora* extracts at a higher concentration of 2%, followed by *D. metel* extract (over 90%) at 3% after 4 days after inoculation. The lowest effect belonged to extracts of *C. tonkinensis* (71.70%) at a concentration of 3%; however, the growth of *S. rolfsii* did not grow anymore on this media after 15 days of inoculation.

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