ARTICLE / INVESTIGACIÓN

Evaluation of the fungicidal activity of the aqueous extracts of some medicinal plants against *Fusarium* spp

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Abstract: To assess the performance of the aqueous extracts of Rhus coriaria, Boswellia carterii, Nigella sativa and Aloe vera. Three concentrations (5%, 10%, and 15%) for each extract were tested in vitro for their activity against three isolates of Fusarium spp. All extracts have a high inhibitory capacity against tested isolates. An inhibition percentage for selected isolates was increased with concentrations (5%, 10%, 15%). The concentration of Rhus coriaria extract 15 % resulted in a significant increase in inhibition percentage of mycelial growth of Fusarium sp.1 (63.17%), Fusarium sp2 (61.69%) and Fusarium sp3 (59.35%) compared to other concentrations, the concentration of Boswellia carterii extract 10% led to a significant increase in inhibition percentage of mycelial growth of Fusarium sp2 (82.45 %) compared to concentration 5% (73.67 %), the concentration of Nigella sativa extract 15% was recorded high inhibition percentage in Fusarium sp2 (66.15 %) compared to concentration 5% (22.21%). In contrast, concentrations 5% and 10% were recorded highest inhibition percentages in Fusarium sp3, reaching 31.73 % and 22.02%, respectively, compared to concentration 15% (4.90%).

Key words: *Rhus coriaria*, *Boswellia carterii*, *Nigella sativa*, Aloe vera and *Fusarium* spp.

Introduction

Plant diseases cause considerable damage during growth of plants, resulting in a reduction in yield and produce quality[1]. Fusarium species exist in the soil both in the tropical and temperate regions. They survive in the soil as chlamydospores and mycelia; they attack the roots and settle in the xylem, leading to stunting, wilting, and plant death, thus leading to severe damage[2,3]. The Wilting via Fusarium is a significant disease that damages sesame plants worldwide. Excessive use of chemical fertilizers causes environmental pollution besides affecting the quality of fruit[3]. There is a critical need to reduce the use of chemical pesticides to limit environmental pollution and improve plant growth; it has become indispensable to choose methods that are not harmful to the environment to enhance the health and validity of crops, so botanical extracts and some microorganisms are the best alternatives to these hazardous chemicals. *Rhus coriaria* L., the sumac plant, belongs to the family Anacardiacea. Aqueous sumac extract includes anthocyanins, hydrolyzable tannins, flavonoids, isoflavonoids, terpenoids, butein, iridoid and derivatives of coumarin[5]. Boswellia carteri, commonly known as olibanum tree or frankincense, is a deciduous middle-sized tree belonging to the order Sapindales and the family Burseraceae[6,7]. Black cumin (Nigella sativa) belongs to the family Ranunculaceae and is commonly known as black seed. N. sativa contains tannins, phloba tannins, flavonoids, transanethole, terpenoids, apiole and thymoquinone[8]. Aloe vera is from the Lilaceae family that contains 200 active compounds, including minerals, enzymes, vitamins, amino acids, salicylic acid, saponin, lignin, sugar and anthraquinones, besides over 75 component nutrients[9]. Bio-control already exists in nature, and it is an eco-friendly approach and effective against various pathogens and pests. Besides, it can be successfully exploited in integrated disease and pest management[2]. Our aim of the trial was to test the effect of aqueous plant extracts of viz. *Rhus coriaria, Boswellia carterii, Nigella sativa* and Aloe vera against three isolations of *Fusarium* sp.

Materials and methods

Plant collection and fungus isolation

A laboratory experiment was conducted at the plant pathology Lab in the Diyala Agriculture Directorate in 2020; the plant materials such as *R. coriaria*, *B. carterii*, and *N. sativa* were collected from the local market of Baqubah, Diyala, the A. vera plant was obtained from a house garden. The pathogenic fungi Fusarium sp1, Fusarium sp2 and Fusarium sp3 were isolated from sesame plant roots from three regions viz Salah Elden, Baghdad and Diyala, respectively.

Preparation of aqueous extracts

Each powder of *R. coriaria*, *B. carterii*, and N. sativa at a rate of 50 g was soaked in 200 ml distilled water for 24 hours; each of them and 50 g of A. vera gel were blended in the blender for homogeneity and mixing, then filtered through two layers of clothes, then placed in the centrifuge at a speed of 3000 RPM for 3-10 minutes and the supernatants were preserved in the refrigerator until use.

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Poisoned food test

To assess the performance of extracts against the tested fungi, the technique of poisoned food using three concentrations of each extract 5, 10, and 15 % were prepared with PDA medium (Potato Dextrose Agar), then poured separately into petri plates (9 cm), the control treatment included only PDA medium, after the medium became solid, agar discs (6 mm) of F. sp1, F.sp2 and F. sp3 from the seven-day-old culture were transferred to a petri plates, then incubated at 25±2 Co for seven days. The tests were performed in three replications for each concentration against all of the fungi, and the diameters mean of fungus growth was measured. The inhibition ratio was calculated according to the following formula.

% Inhibition = (1- control colony/ treatment colony) X 100.

Statistical analysis

The Statistical Analysis System (SAS (2012) program was used to detect the effect of different factors on study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between means in this study[10] significantly.

Results

The recorded results in Table (1) and Fig (1) revealed that R. coriaria concentrations led to a significant increase in the inhibition percentage of mycelial growth, where a concentration of 15 % was significantly superior in the inhibition percentage of F.sp1, F.sp2 and F.sp3, which reached 63.17%, 61.69% and 59.35%) respectively. In comparison, a concentration of 10 % led to a significant increase in the inhibition percentage of mycelial growth of F.sp1 (46.41%) compared to a concentration of 5 % (32.45%), whereas no significant differences between concentrations 5 and 10% for both F. sp2 and F. sp3.

Table (2) and Fig (2) shows that a concentration of 10% of B. carterii resulted in a significant increase in the inhibition percentage of mycelial growth of F.sp2 (82.45 %) compared to the concentration of 5% (73.67 %). In contrast, there were no significant differences between all concentrations in inhibition percentage of mycelial growth of F.sp1 and F.sp3.

From Table (3) and Fig (3), N. sativa concentrations showed an increment in the inhibition percentage of mycelial growth of all fungi; a concentration 15% was recorded with a high inhibition percentage in F.sp2 reached 66.15 %) compared to a concentration 5% (22.21%). In comparison, concentrations 5% and 10% were recorded as the highest inhibition percentage in F.sp3, reaching 31.73 % and 22.02%, respectively, compared to concentration 15% (4.90%), whereas no significant differences between all concentrations in F.sp1.

A. vera concentrations showed an increment in inhibition percentage of mycelial growth of all fungi, with no significant differences between all concentrations (Table 4 and Fig 4).

Cost Components

It was determined that Ni 4190 flower buds are required during plant material introduction, and N0 9429 staminodes must be processed to induce callogenesis to produce a batch of 100,000 plantlets (Sheet 2 S2: Stages of production). For the multiplication stage, N1 + N2 + N3, a total of 27,827 embryos were processed. 101,189 embryos were processed in the N4 maturation stage, and 506,803 plantlets were processed in germination stages N5, N6, and N7 (Table 3).

Using this estimation methodology, the total cost of US $72,986 was obtained for a production batch with 100,000 plants, with a cost per plantlet of US $0.73/unit.

Monte Carlo Simulation Analysis (MCS)

After structuring costs, the most influential cost component was direct labor, representing 53% of the total cost. The cost of culture media was 12% of the total, IMC represented 5%, and operating expenses, including administrative expenses and infrastructure, were 30% (Figure 2).

Plants propagated by SE. Figure 3 shows that the cost of plants per unit can be inferred between USD $0.6835 and USD $0.7786, with an average USD $0.7290 (Sheet 1 S2: Total cost), due to the production process's cost structure. If strict control is maintained over the variables while executing the productive lot as es-tablished in this study, the average price per plantlet has been proposed to be USD $0.7290, with a 95% reliability. However, as one may observe, there is a certain asymmetry towards the right, which indicates that the process could increase in cost. That is to say, the cost may have deviated over the average or over USD $0.7290 after moving the lot.

On the other hand, a regression was performed on the correlation coefficients of each of the cost model's variables to identify which ones influence the variable response, which is to say cost the most. This analysis showed that the most significant variables are in the productive process' last stages, corresponding to ger-mination and acclimation, followed by the maturation stage. It was specifically found that the plantlet growth (-0.69) and plantlet development (-0.60) phases, as well as the plantlet conversion phase (0.35) had more significant effects on cost, with 95% reliability. Therefore, when these variables, which are expressed as the percentage of explants' response, rise above 60%,

Concentrations of *R. coriaria*	Isolates		
	Fusarium sp1	*Fusarium* sp2	*Fusarium* sp3
5%	32.45	32.77	32.90
10%	46.41	38.36	32.31
15%	63.17	61.69	59.35
LSD 0.05	11.53	9.908	13.16

Table 1. Effect of various concentrations of *R. coriaria in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

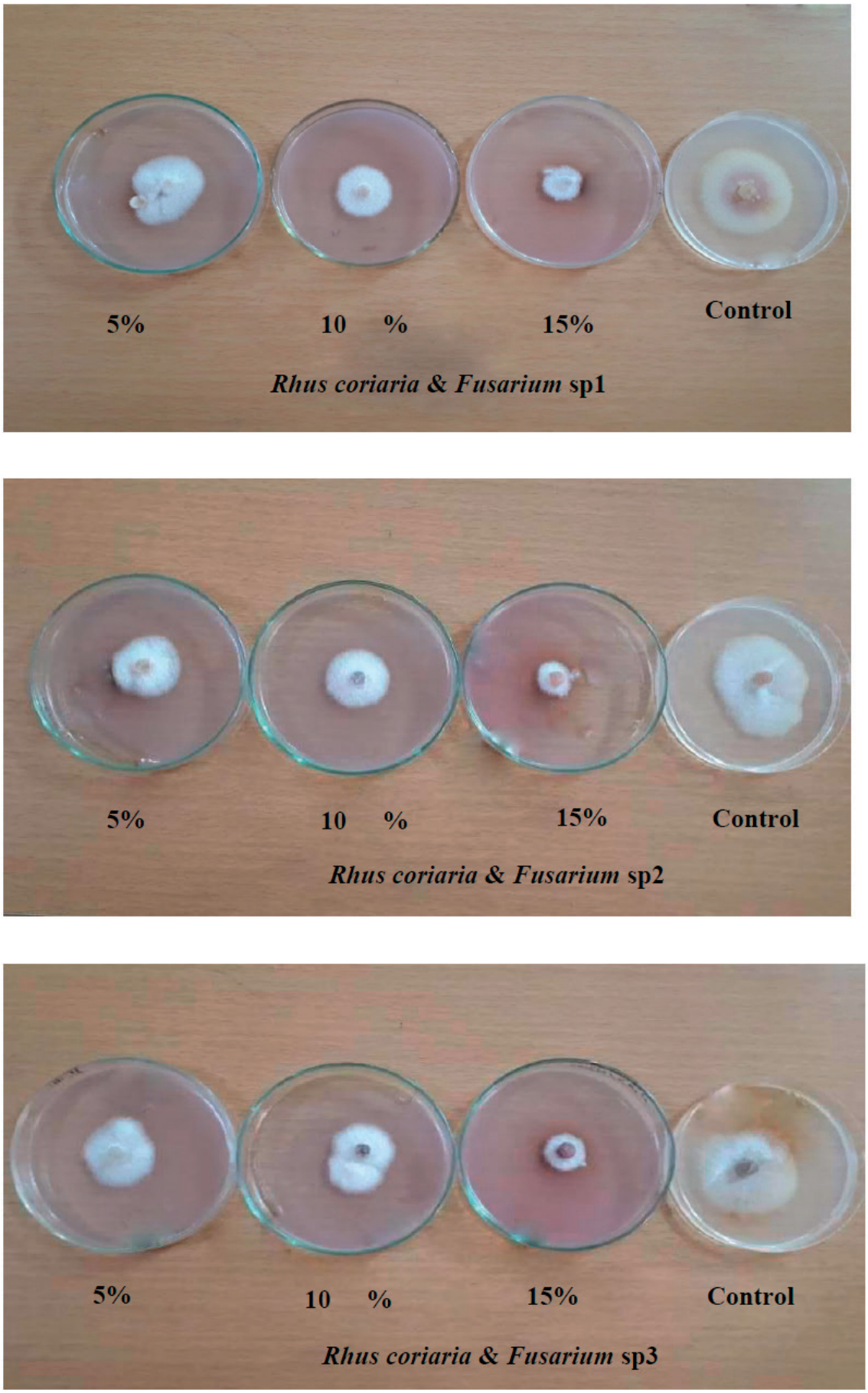

Figure 1. Effect of various concentrations of *R. coriaria in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3

Concentrations of *B. carterii*	Isolates		
	Fusarium sp1	*Fusarium* sp2	*Fusarium* sp3
5%	79.96	73.67	76.54
10%	84.02	82.45	78.39
15%	84.35	80.11	81.96
LSD 0.05	NS	6.585	NS

Table 2. Effect of various concentrations of *B. carterii in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

Concentrations of *N. sativa*	Isolates		
	Fusarium sp1	*Fusarium* sp2	*Fusarium* sp3
5%	56.53	22.21	31.73
10%	51.19	35.77	22.02
15%	50.66	66.15	4.90
LSD 0.05	NS	34.14	14.73

Table 3. Effect of various concentrations of *N. sativa in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

Concentrations of *A. vera*	Isolates		
	Fusarium sp1	*Fusarium* sp2	*Fusarium* sp3
5%	59.03	55.33	60.41
10%	54.42	58.25	58.26
15%	59.96	55.32	58.95
LSD 0.05	NS	NS	NS

Table 4. Effect of various concentrations of *A. vera in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

50%, and 50%, respectively, the variable of cost per plantlet decreases. During the embryos' development phase, both the multiplication coefficient (-0.07) and percentage of explants' response (-0.07) tend to decrease cost per plantlet as their prices increase. They are currently at 10% and 70%, respectively. Besides, it was observed that the RMR (0.15) has a positive effect on cost. Cost per plantlet will increase as RMR increases (Figure 4).

Discussion

The methanolic extract of *Rhus coriaria* showed the highest inhibition in the fungi *Aspergillus flavus*, *Candida albicans* and *Penicillium citrinin*[11].(12) reported that *Rhus coriaria* extract inhibited the growth of *Penicillium citrinum*, *Aspergillus flavus* and *Candida albican*s. (13) stated the superior inhibitory activity of *Rhus Mueller* against *Fusarium oxysporum* f. sp. lycopersici. The antimicrobial activity of *R. coriaria* extract against food pathogens has been proved in numerous of vitro studies[14]. *Rhus coriaria* extract has more effective antifungal activity than the standard antifungal Nystatin[15]. The presence of phenolic and flavonoid constituents in *R. coriaria* suppresses enzyme actions and cellular membrane functions of pathogenic fungi[16].

The *B. carteri* extract demonstrated effectiveness against *Fusarium solani*, *Helminthosporium rostratum* and Alternaria alternate *in vitro*, where the scanning electron microscopy proved the existence of ultra-structural variations in a treated mycelia with extract of *B. carterii* comparison with mycelia which untreated[17]. An oil of Boswellia serrata led to inhibition of the following fungi such as *Fusarium verticillioides, Fusarium equiseti, F. oxysporum, Fusarium udum, Fusarium lateritium, Penicillium citrinum, Aspergillus ochraceous, Aspergillus geophila, Aspergillus brassicola, Aspergillus fumigatus, Aspergillus terreus, Curvularia tetramera, Penicillium expansum* and *Penicillium citrinum*[18]. The antifungal activity of the essential oil of *B. carter* against *Candida albicans, Aspergillus niger* and *Cryptococcus neoformansi* was the result of the presence of a-copaene, p-cymene, d-cadinene, b-caryophyllene and limonene with at various ratios[19].

It can use *N. sativa* as an eco-friendly fungi-toxicant against Macrophomina phaseolina and Fusarium oxysporum because it has considerable potential as fungicidal[20]. Antifungal activity of *N. sativa* oil was determined against eight fungi viz., *Fusarium oxysporum, Fusarium moniliforme, Fusarium semitectum, Fusarium nivale, Alternaria alternata, Aspergillus niger, Aspergillus flavus, Drechslera hawiensis*[21]. Proteins of *N. sativa* have a significant effect

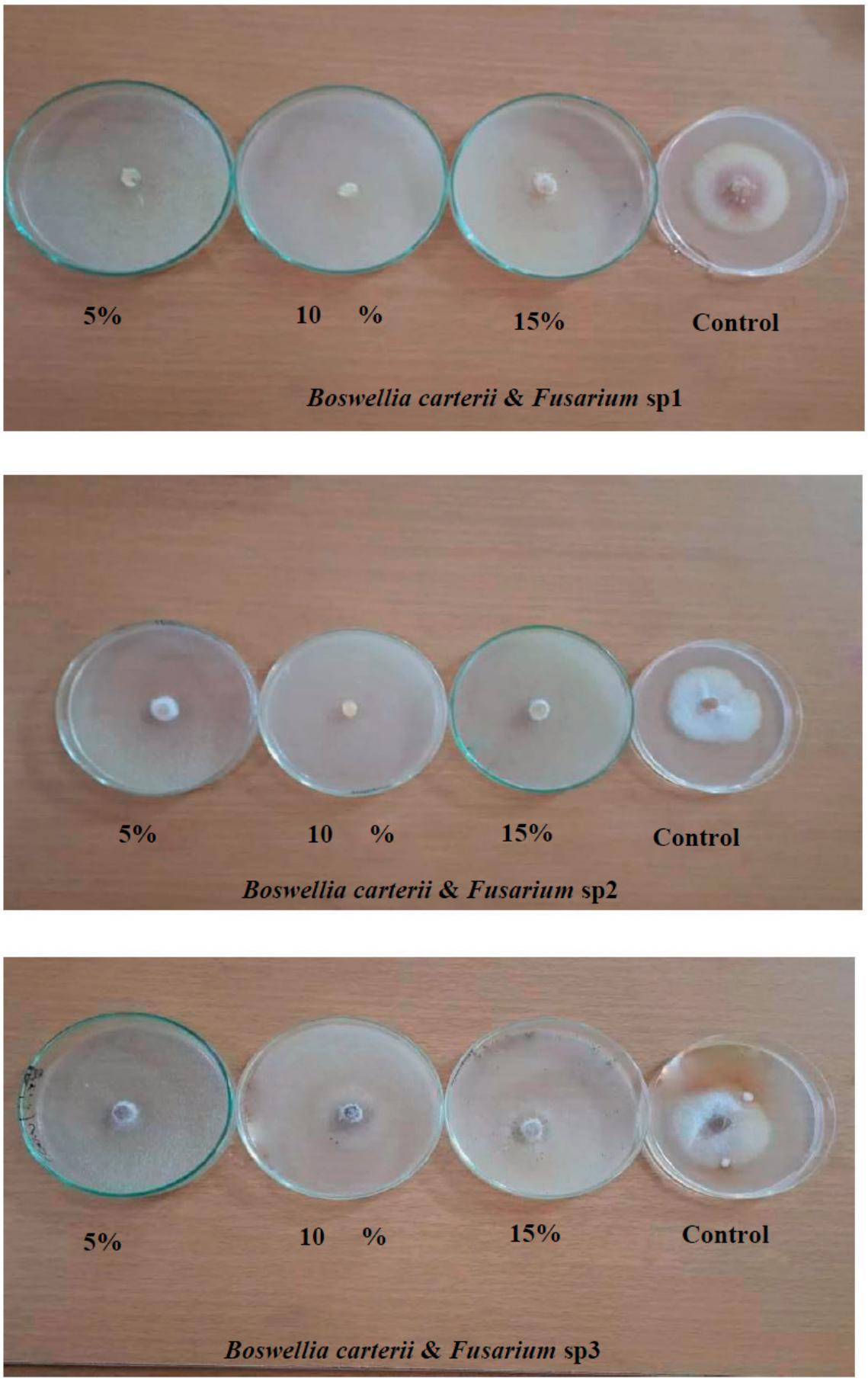

Figure 2. Effect of various concentrations of *B. carterii in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

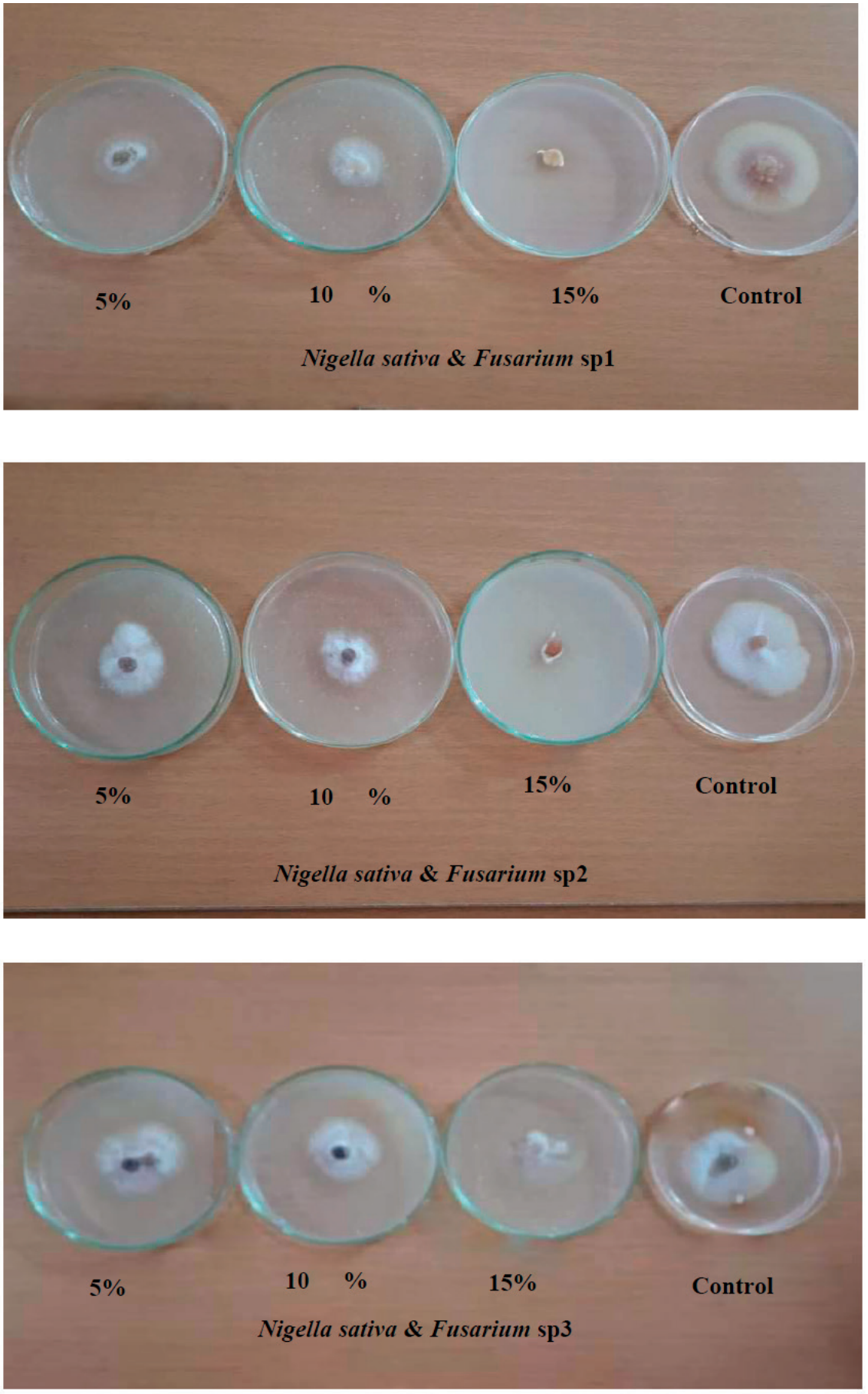

Figure 3. Effect of various concentrations of *N. sativa in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

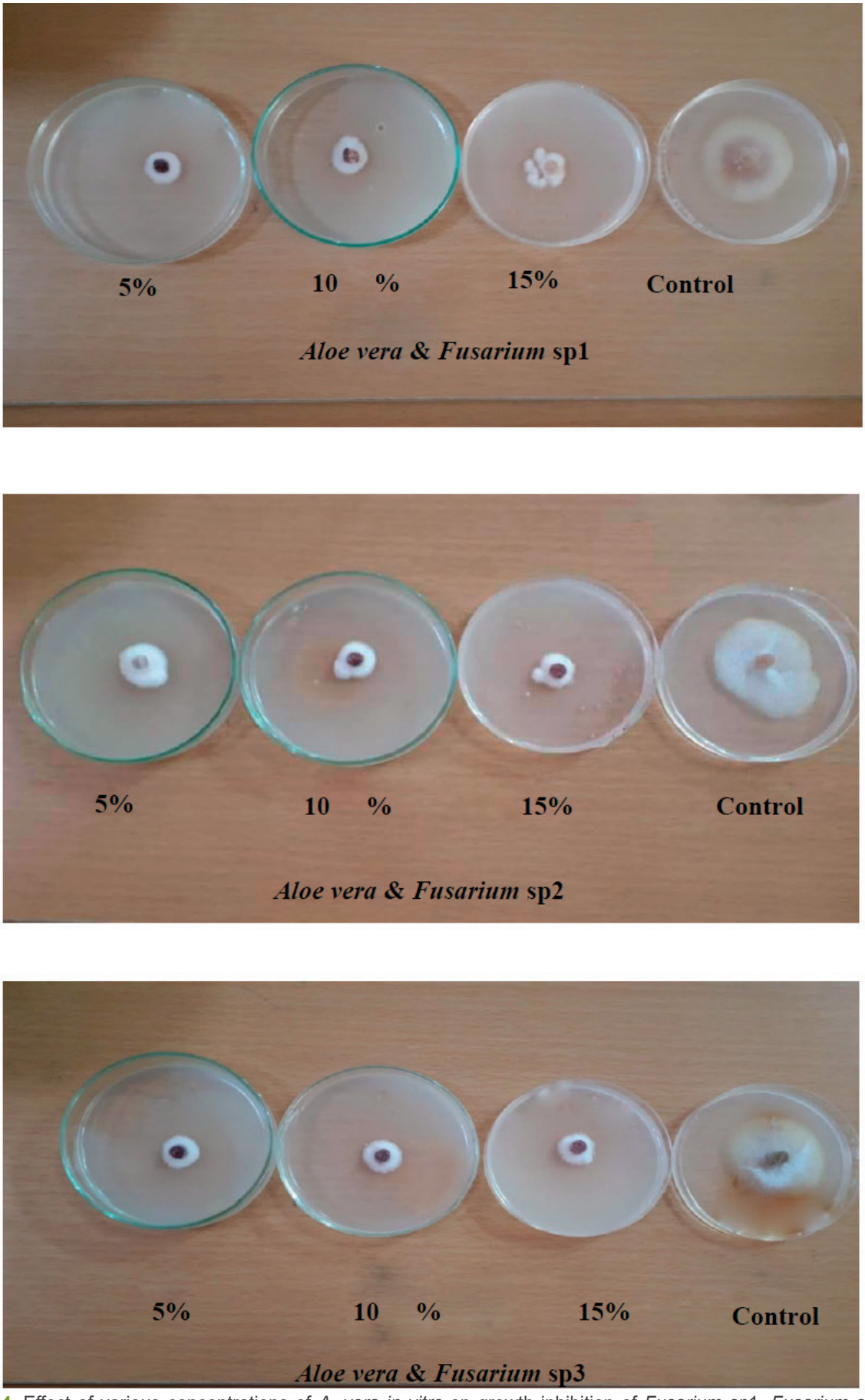

Figure 4. Effect of various concentrations of *A. vera in vitro* on growth inhibition of *Fusarium sp*1, *Fusarium sp*2 and *Fusarium sp*3.

on the fungal cell permeability[22]. *N. sativa* oil showed more efficacies in sporulating and reducing mycelial growth of *Aspergillus flavus*[23].

Aloe vera extract significantly affected the inhibition of mycelial growth of *Fusarium udum*, reaching 55.9%[24]. Aloe vera extract has antifungal activity against *Fusarium oxysporum, Penicillium gladioli, Botrytis gladiolus and Heterosporium prune*[25]. (26) found that the Aloe vera is effective against *Fusarium oxysporum*, *Collectotrichum coccodes* and *Rhizoctonia solani*. Aloe vera extract can inhibit the growth of fungi such as *Alternaria alternata, Alternaria citri* and *Alternaria tenuissima*[27]. Aloe vera extract had an antifungal effect on *Aspergillus glaucus, Aspergillus flavus, Candida albicans, Candida tropicalis, Trichophyton rubrun* and *Trichophyton mentagrophytes*[28]. Aloe vera gel caused a significant reduction in the growth of pathogenic fungi viz., *Penicillium digitatum, Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Drechslera hawaiensis* (Sitara *et al.* 2011).

Conclusions

The present investigation suggests that aqueous extracts such as *R. coriaria, B. carterii, N. sativa* and *A. vera* have great potential as a natural fungicide, but further studies are required.

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