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RESEARCH PAPER

Ecofriendly approaches for the management of rose powdery mildew (Podosphaera pannosa var. rosae)

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A B S T R A C T:
Rose and miniature rose powdery mildew caused by *Podosphaera pannosa* is the most important disease in greenhouses and outdoor plantations. The use of fungicides, as a control method, is detrimental to nature and paid public interest. This work was aimed to utilize ecofriendly methods represented with plant extracts and antagonistic fungi. Among 13 plant extracts tested *in vitro*, garlic (*Allium sativum*), ginger (*Zingiber officinale*), and rosemary (*Rosmarinus officinalis*) were the most effective bioagents that resulted in a significant reduction of conidia germination. This result is supported by the greenhouse assessment of the selected extracts, in which the powdery mildew disease was reduced by up to 88.1%. The use of the commercial product of *T. harzianum* in the greenhouse was effective as plant extracts.

KEY WORDS: *Podosphaera pannosa* var. *rosae*, Rosa, plant extracts, biological control, *Trichoderma harzianum*.DOI: DOI: http://dx.doi.org/10.21271/ZJPAS.33.4.10
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1.INTRODUCTION :

Roses (*Rosa spp*.) are the most important ornamental horticultural crops grown throughout the world for their variety of uses in floral decoration, cut flower, medicinal, cosmetic, and industrial sectors. (Hummer and Janick, 2009).

Rose powdery mildew caused by the biotrophic ascomycete fungus *Podosphaera pannosa* var. *rosae*, is a worldwide important disease on indoor and outdoor roses. The disease reduces both plant quality and vigor; therefore, causes serious losses and becomes a limiting factor for rose producers. Distortion, curling, and premature defoliation are all severe damages caused by the pathogen (Phillips and Rix, 1988, Bélanger et al., 1994, Sangani et al., 2018).

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Despite not killing the plants, the disease impairs plant growth and reduces flower production, and weakens the plants (Agrios, 2005). On the other hand, under greenhouse conditions, powdery mildew is the most important and devastating disease (Félix-Gastélum et al., 2014). The disease is favored by conditions like cool, moderate temperature, and reduced light or shade (Kumar and Chandel, 2018a, Rex and Deepika, 2020).

Several methods are practiced to control rose powdery mildew such as the traditional method which is foliar fungicide application that demonstrated to be a very effective method and could extensively reduce the impact of plant diseases. However, the chemical method which normally includes Sulphur compounds encountered public perception and it is undesired due to several negative points accompanying the method. In addition, the method is costly, needs frequent applications, causing detrimental effects to the environment and other beneficial organisms, and the emergence of fungicide resistance (Wheeler, 1978, Brent and Hollomon, 1995, Ribes et al., 2018). Dedication of other microorganisms antagonist to the pathogen could be an option to reduce the losses and can serve as an alternative to synthetic fungicides. In this regard, Ng et al. (1997) explored the efficacy of a naturally occurring ballistospore-forming yeast, *Tilletiopsis pallescens*, isolated from mildew-infected leaves as a bioagent against rose powdery mildew pathogen. The results from the study demonstrated the potential ability of the bioagent for biocontrol of the disease. Commercial products of *Trichoderma harzianum* have been used against grapevine foliar pathogen, *Botrytis cinerea* (O'neill et al., 1996), and in commercial greenhouses for the control of tomato and cucumber diseases (Elad and Shtienberg, 2000). In addition, potential *Trichoderma* isolates used for control of grape powdery mildew disease reduced the disease by 53.4 % (Sawant et al., 2017), squash powdery mildew (Hafez et al., 2018), and mulberry powdery mildew (Manjunatha et al., 2020).

Exploring and adopting other rational strategies as alternatives to chemicals are of imperative demand. Plant extracts may play as another alternative to synthetic chemicals and could be useful in avoiding unwanted effects of fungicides. Furthermore, plant-based compounds are natural in origin, have minimum adverse effects on the physiological processes of plants, and are easily convertible into common eco-friendly organic materials (Gnanamanickam, 2002). The antimicrobial efficacy of plant extracts, both *in vitro* and *in planta*, are as promising antifungal compounds against plant diseases (Saleem et al., 2012, Varo et al., 2017). Plants are considered the main sources of secondary metabolites, contain substances that have important antifungal bioactivities which include essential oils (EOs), phenolic compounds, flavonoids, and alkaloids among others (Fawzi et al., 2009, Mar et al., 2010, Romanazzi et al., 2012, Zaker, 2016).

Many natural products of plants have been reported to have antifungal efficacy in controlling fungal plant pathogens, for instance, extracts of neem, eucalyptus, datura, garlic, oleander, and many other plants had been used (Ćosić et al., 2010, NAShwA and Abo-ElyouSr, 2013). Grapefruit extract was investigated to control rose powdery mildew in which promising results were obtained (Wojdyła, 2001). Volatile compounds of garlic (*Allium sativum*) cloves, onion (*Allium cepa*) bulbs, and ginger (*Zingiber officinale*) rhizome volatile found to show complete inhibition of conidia germination of *Erysiphe polygoni* (Singh, 1981, Khunt et al., 2017). Compounds derived from garlic (*Allium sativum*), protected plants against *Sphaerotheca pannosa* var. rosae and *S. fuliginea*, the cause of powdery mildew of rose and cucumber, respectively (Kumar and Chandel, 2018b, Abd Elwahed et al., 2019). Phytoextracts of garlic cloves tested *in vitro* also showed more than 50% inhibitory effect on conidia germination of *Erysiphe cichoracearum*, the cause of okra powdery mildew (Jadav and Kadvani, 2019).

Keeping in view these adverse consequences of fungicides and increasing international demand to reduce the use of toxic pesticides, because of human health and environmental hazards, the present study has been undertaken to investigate the potential efficiency of plant extracts and exploiting the potential of biological control agents as eco-friendly and sound methods for the management of rose powdery mildew.

2.MATERIALS AND METHODS

2.1Preparation of plant extracts

Extracts of 13 plant species listed in table 1 were prepared following the method used by Narayana et al. (1994). Aqueous plant extracts were prepared by taking 200g fresh parts (leaves, peel, bulb, stem, root) of each plant material, washed with sterilized distilled water, and then dried by using an electric oven at 45^{o}C for one week. The dried plants ground into a fine powder using a stainless-steel grinder, placed in Erlenmeyer flasks contained 200 mL of sterilized distilled water and were then placed in a shaker for 24 hours. The material was homogenized for 5 min by a magnetic stirrer and then filtered through double-layered muslin cloth then followed by further filtration using Whatman's filter paper No.1. The extracts were concentrated by evaporation using a rotary vacuum evaporator at 40^{o} C by utilizing a water bath until dense powder-like material was prepared. The dried extract of the total plant extracts contents, which was considered as standard stock, was placed in a refrigerator for later use.

2.2In vitro assessment

Conidia germination of *P. pannosa* was evaluated following the method described by Sadasiva and Ellingboe (1962). According to the procedure, visible powdery mildew conidia were harvested from young leaves of rose plants. To avoid the presence of old conidia, lesions were gently shaken first by a glass rod to discard any old conidia presented on such young leaves. The young conidia, which formed on conidiophores after four to six hours, were spread on dry clean glass slides previously received 0.1 ml of each one of the previously prepared plant extracts (Table 1) with the concentration of 20 % (w/v). To choose the suitable concentration for *in vitro* assays, preliminary tests were conducted using a 10-fold serial dilution constitutes 1, 10, 20, and 30 mg mL^{-1}. Glass slides prepared with sterilized distilled water only, were served as control treatment. The conidia were examined microscopically to determine the uniformity of distribution and the number of spores that had germinated in Situ. This percentage was used as a correction factor to determine the actual conidial germination. Each slide was placed on a U-shaped glass rod in a moist chamber made up of a sterile Petri plate lined with filter paper saturated with sterile distilled water. Petri plates were incubated at 25^{o}C for 24 hours after which the conidia germination on slides was examined at 400×. Five slides were used as replicates for each particular treatment. The percentage of germination and inhibition was calculated according to the following formula:

$$\text{Germination (\%)} = \frac{\text{GC}}{\text{TC}} * 100$$

Where: GC = germinated conidia, and TC = total number of measured conidia

Germination inhibition (%) = 100 – Percentage of germination

The experiment was a complete randomized design (CRD) with 5 replicates.

Table 1: Plant extracts screened for the *in vitro* efficacy against conidia growth of *P. pannosa*.

Common name	Scientific name	Family	Plant part
Rosemary	*Rosmarinus officinalis*	Lamiaceae	Leaf
Mint	*Mentha asiatica*	Lamiaceae	Leaf
Aloevera	*Aloe vera*	Asphodelaceae	Leaf
Whitetop	*Lepidium draba* L.	Brassicaceae	Stem
Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaf
Pomegranate (sour)	*Punica granatum*	Lythraceae	Peel
Burdock	Arctium lappa	Asteraceae	Stem
Turmeric	*Curcuma longa* L.	Zingiberacea	Roots
Mandarin	*Citrus reticulata*	Rutaceae	Peel
Dodonia	*Dodonaea viscosa*	Sapindaceae	Leaf
Datura	*Datura stramonium*	Solanaceae	Leaf
Garlic	*Allium sativum*	Amaryllidaceae	Bulb
Ginger	*Zingiber officinale*	Zingiberaceae	Tuber

2.3Greenhouse experiments

Based on the results of *in vitro* assay, Rosemary, Ginger, and Garlic extracts (10 mg mL^{-1}) which showed the highest efficiency were selected for *in planta* assessment in a greenhouse. The commercial product of *Tricoderma harzianum*, Biocont-T (Dr. Rajan Laboratories, Tamil Nadu, India) at a rate of 4g L^{-1} to give a final concentration of 10^7 spore mL^{-1} (Carillo et al., 2020) and Nazole, a triazole fungicide (Propiconazole 25 EC, Syngenta, Switzerland), in the rate of 20 mL 100 mL^{-1}, were also included in the assay for comparison purposes. Two sets of one-year-old plants (roses and miniature roses), grown in pots, were spray inoculated with conidia suspension, prepared instantly from infected rose plants with powdery mildew, adjusted to 1×10^5 conidia mL^{-1} using Haemocytometer (improved Neubauer chamber, Germany). The negative controls received sterilized water only. Three plants of dog rose (*Rosa canina*), commonly known as Nasrin, were also received the inoculum and placed with other treatments as an additional control for comparisons. After the first initiation of mildew symptoms, the curative application of the treatments was applied by spraying the inoculated plants until run off with the aqueous suspension of the selected plant extracts, the spore suspension of the bioagent, *T. harzianum*, and Nazole fungicide. The plants were then placed in a plastic house provided with 90 – 100 % relative humidity (RH). After 8 days, the powdery mildew disease was assessed by measuring the disease severity on 5-6 randomly selected leaves of each plant. The disease severity index (DSI), based on white powder area on leaves, was used as criteria for disease assessment depending on the disease scale classes of 0 - 5 (Biswas et al., 1992). Where: 0 = no powdery mildew observed, 1 = 1 - 20 % of the leaf area infected, 2 = 21 - 40 % of the leaf area infected, 3 = 41 – 60 % of the leaf area infected, 4 = 61 - 80 % of the leaf area infected, 5 = 81 - 100 % of the leaf area infected.

The efficiency of the treatments was calculated according to the following formula:

$$\text{Efficiency (\%)} = \frac{\text{DC}}{\text{DT}} * 100$$

Where: DC = disease of untreated control, and DT = disease at each treatment.

2.4 Data analysis

All data were analyzed using general analysis of variance (ANOVA) and for comparisons, multiple range tests using the least significant difference (P=0.05) were made using StatGraphics Centurion software.

3.RESULTS AND DISCUSSIONS

3.1In vitro assessment

The discrimination test of thirteen plant extracts (PE) was conducted and assessed *in vitro*. The results in Figure 1 exhibits significant (P < 0.05) differences between the treatments. Both garlic and ginger were the most efficient PE that showed significant differences with other treatments in which the least conidia growth was noticed (14.6 and 15.5 %, respectively), followed by rosemary and datura, respectively. Other PEs were showed variable influences on conidia germination. Garlic and ginger extracts were also showed the highest inhibitory effects on conidia germination (Figure 2). The discrimination test was to select the most effective plant extracts and use them against rose and miniature rose powdery mildew *in planta*. The plant extracts with the highest inhibitory effects were chosen and decided to select them for *in planta* assessment. Garlic, ginger, and rosemary were reported to be effective against other plant pathogens. For instance, Obagwu and Korsten (2003) found that water and ethanol extracts of garlic at 0.1% v/v were most effective in inhibiting *Penicillium digitatum* and *P. italicum*, the cause of citrus green and blue mold of citrus. The broad-spectrum activity of garlic was also revealed by Curtis et al. (2004) who assessed the extracts, both *in vitro* and *in planta*. They found its efficacy against a range of pathogenic bacteria and several plant pathogenic fungi like *Alternaria brassisicola*, *Botrytis cinerea*, *Plectosphaerella cucumerina*, *Magnaporthe grisea*, and the Oomycete *Phytophthora infestans*. The inhibitory activity of garlic is referred to as the essential oil compounds in which diallyl disulfide, diallyltrisulfide, allicin, ajoene, allylmethyltrisulfide, considered the major components working against plant pathogens (Wang et al., 2019, Fufa, 2019). Ginger extracts were found to be effective against spoilage pathogens such as *Aspergillus niger*, *Fusarium sambucinum*, *Pythium sulcatum*, and *Rhizopus stolonifera* (Mvuemba et al., 2009), anthracnose of bell pepper fruits (Alves et al., 2015), and several other important plant pathogenic fungi on tomatoes like *Alternaria solani*, and *Pythium ultimum* (Muthomi et al., 2017). It has been proved that the potential activity of ginger extract is from essential oils that are composed of monoterpenes (transcaryophyllene, camphene, geranial, eucalyptol, and neral) and sesquiterpene hydrocarbons (*α*-zingiberene, ar-curcumene, *β*-bisabolene, and *β*-sesquiphellandrene). The mode of action of these essential oils that affects both the pathogen cell's external envelope and internal structures, made them have a wide range of activities against many phytopathogens (Abdullahi et al., 2020). The antifungal activity of rosemary against plant pathogenic fungi was also evaluated by other researchers. Ribeiro et al. (2016) assessed the potential of the rosemary extract for controlling charcoal rot, *Macrophomina phaseolina*, in soybean and found to reduce the fungal growth on solid and liquid media by 44% and 74%, respectively, and the number of microsclerotia by 61%. There was a disease inhibition of 53% and 56% in the area under the disease progress curve in the first and in second assay, respectively.

It seems that the essential oils (EOs), extracted from *Allium sativum* (garlic) and *Rosmarinus officinalis* (rosemary), the most active components of the extracts. In contact and vapor assays (two *in vitro* tests that measure the minimal inhibitory concentration), Hosseini et al. (2020) found that both *A. sativum* and *R. officinalis* EOs significantly inhibited the mycelial growth and conidial germination of *Colletotrichum nymphaeae*, the cause of strawberry anthracnose. Furthermore, the EC_{50} assay indicated that garlic EO was more effective than rosemary EO against the pathogen.

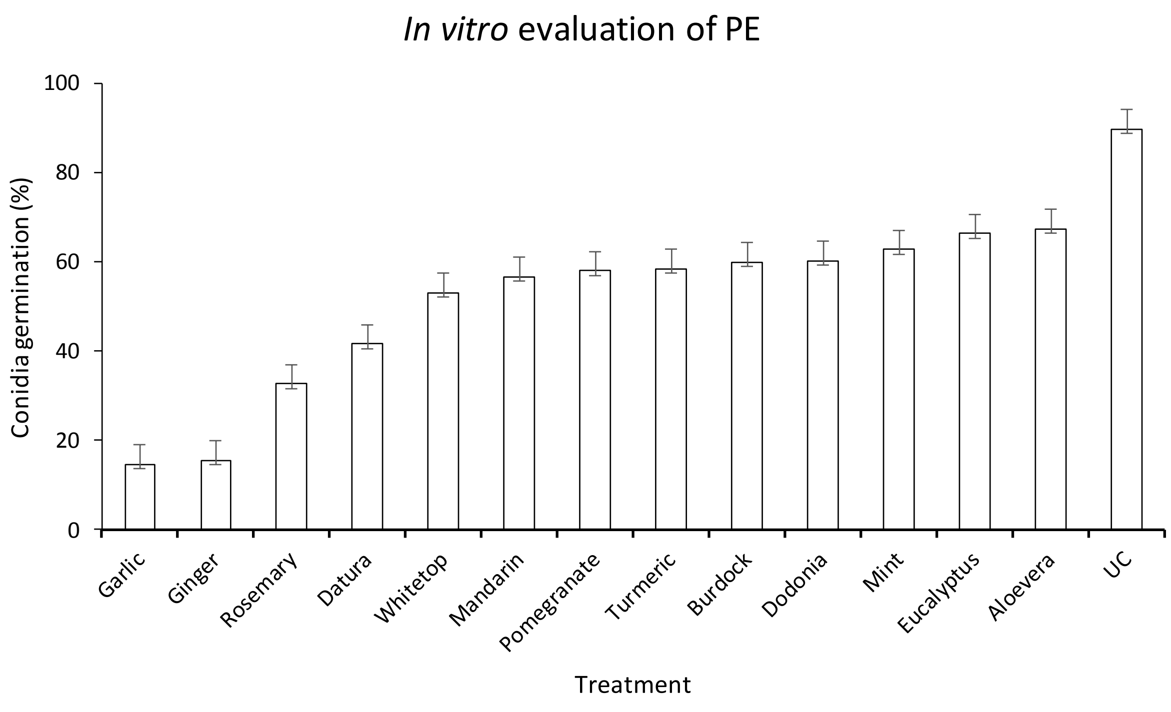

Figure 1: *in vitro* assessment of the efficacy of 13 PEs on conidia germination of *P. pannosa*.

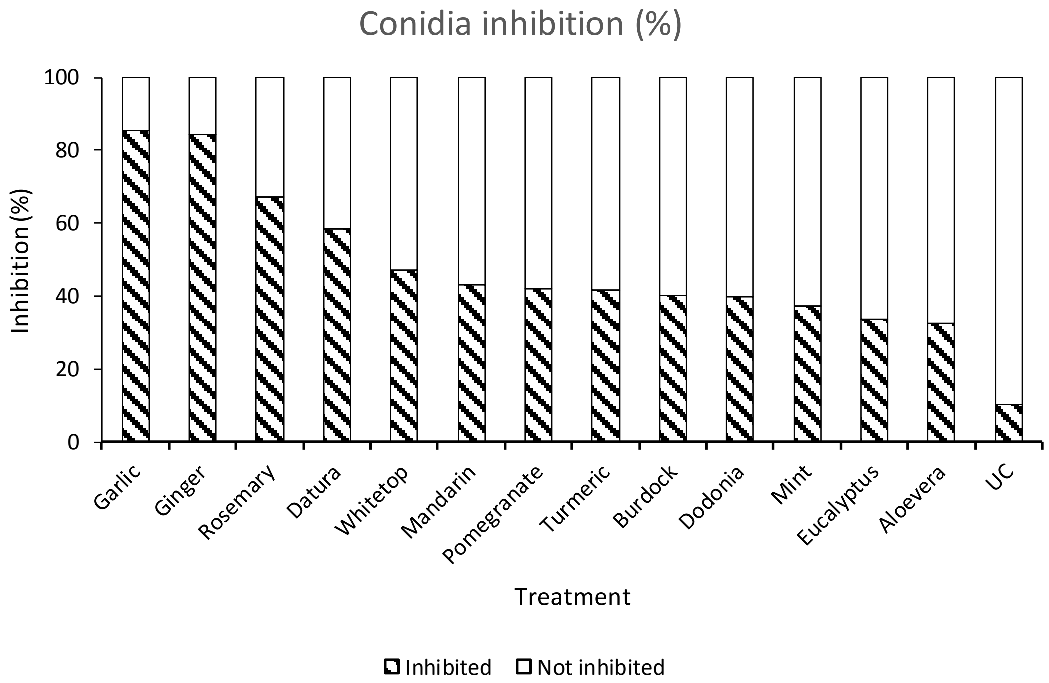

Figure 2: *in vitro* assessment of 13 PEs on % of conidia growth inhibition of *P. pannosa*.

3.2Greenhouse experiments

The results of the application of the selected plant extracts, the bioagent (*Trichoderma harzianum*) and the fungicide (Nazole), used in greenhouses, showed significant differences in DSI on roses ($P < 0.05$) and miniature roses. The results revealed that garlic, ginger, and Biocont were as effective as the Nazole fungicide, in which they retained the lowest DSI with no significant differences between them (Figure 5). However, in terms of efficiency, after the fungicide, garlic was the most efficient plant extract in reducing powdery mildew on miniature roses, followed by the bioagent (Biocont) and then ginger extract which have the efficiencies of 88.1, 86.8, and 86.5 %, respectively. Rosemary, however, didn't exhibit such good effectiveness in inhibiting the disease (Figure 6). On Roses, both garlic and ginger were the most efficient plant extracts with DSI of 7.3 and 7.5 %, respectively, in which they have not differed significantly with Nazole fungicide (6.7%). However, Rosemary extract showed less effect with DSI of 8.8% (Figure 3) and efficiency of 76.1% (Figure 4). Garlic and ginger also showed a significant efficiency in disease reduction by 86.2 and 84.2%, respectively with no significant differences with Nazole fungicide. Therefore, Nazole as a synthetic fungicide was outperformed in the efficiency which inhibited the disease by 89 %, followed by garlic and ginger with the efficiencies of 86 and 84 %, respectively (Figure 4). Nasrin plants, which were placed as an additional control for comparison, compared to roses were, showed comparatively low DSI (12%) and disease efficiency of 11.2% (Figure 3 and 4). On miniature roses, similar results were obtained in which garlic and ginger outperformed other plant extracts in both DSI and efficiency (Figures 5 and 6).

Previous studies also showed the efficacy of both garlic and ginger on *Colletotrichum nymphaeae*, the cause of strawberry anthracnose under greenhouse conditions; in which they significantly reduced the development of fruit decay and anthracnose disease incidence and severity (Hosseini et al., 2020). In an accordance with our results, Tahir et al. (2018) noticed a significant reduction of bitter melon (*Momordica charantia*), a cucurbit plant, powdery mildew

(*Sphaerntheca fuliginea*) incidence by garlic extracts, used at 5%, 10%, and 15% concentrations. Ginger was also outperformed plant extracts in the reduction of mulberry powdery mildew (*Phyllactinia corylea*) in which it reduced the disease to 60.31% (Manjunatha et al., 2020).

The antagonistic fungus, *Trichoderma harzianum* retained significant control capability *in planta* and was successful in reducing DSI and increases in disease inhibition percentage. On Roses, *Trichoderma* showed a significant disease reduction expressed as DSI (8.87%) and efficiency (75.7%) which has not differed significantly from that of ginger and rosemary plant extracts (Figure 3 and 4). On miniature rose, the bioagent, *Trichoderma*, not differed significantly in both DSI (7.3%) and disease inhibition (86.8%) with Nazole fungicide and the foremost plant extracts, ginger and garlic (Figure 5 and 6). Species of *Trichoderma* have been used against a wide range of plant pathogenic fungi including powdery mildew fungi. Several previous studies have revealed the capability of *Trichoderma* species in reducing powdery mildew disease on roses and other different plant species. On greenhouse cucumber, powdery mildew (*Podosphaera fuliginea* also known as *Podosphaera xanthii*)) reduction up to 97 % was achieved on young leaves by the commercial product of *T. harzianum*, TRICHODEX (Elad et al., 1998). Similar to our finding, Picton and Hummer (2003) stated that the incidence and the severity of gooseberry powdery mildew on young leaves and stems were reduced significantly by *T. harzianum* combined with mineral oils when applied to plants in 2-week intervals in potted plants in the greenhouse. Additionally, its ability in disease reduction was as good as Thiophanate fungicide used in the trial, enabling it to be a commercially acceptable product. The commercial formulations of the bioagent, either in the organic or inorganic carriers, have been used for seed treatment, seed bio-priming, seedling dip, soil application, and foliar spray for the management of plant diseases.

The mechanism of mycoparasitism of *T. harzianum* involves nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes (Chet et al., 1997, Ghorbanpour et al., 2018).

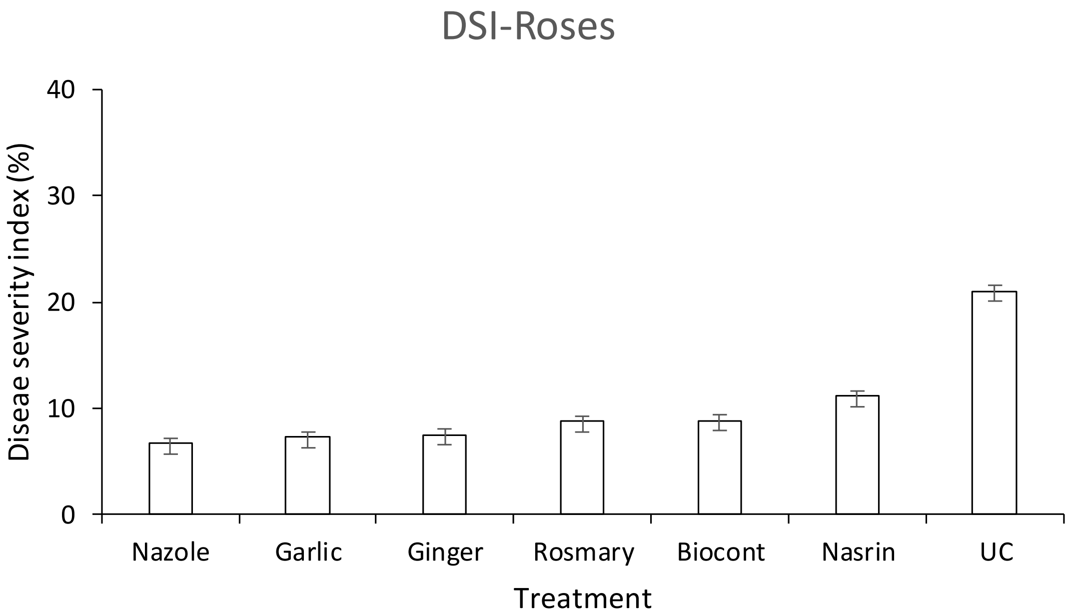

Figure 3: *in planta* activity of 3 selected plant extracts, one bioagent, and a synthetic fungicide on DSI of rose powdery mildew.

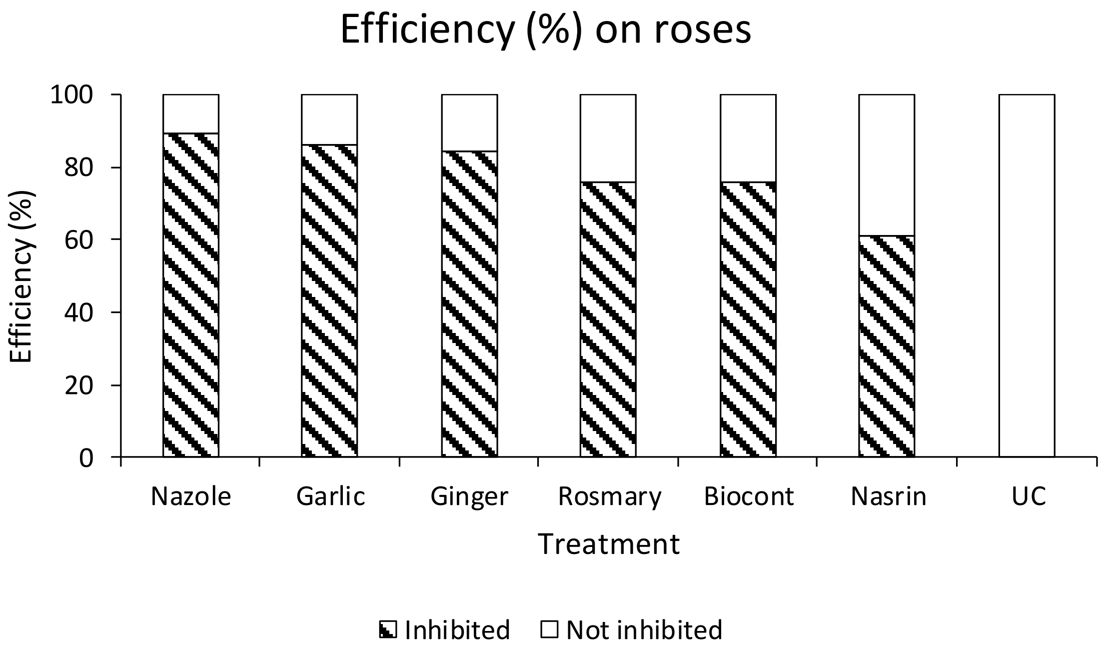

Figure 4: *in planta* efficiency of 3 selected plant extracts, one bioagent, and a synthetic fungicide on rose powdery mildew.

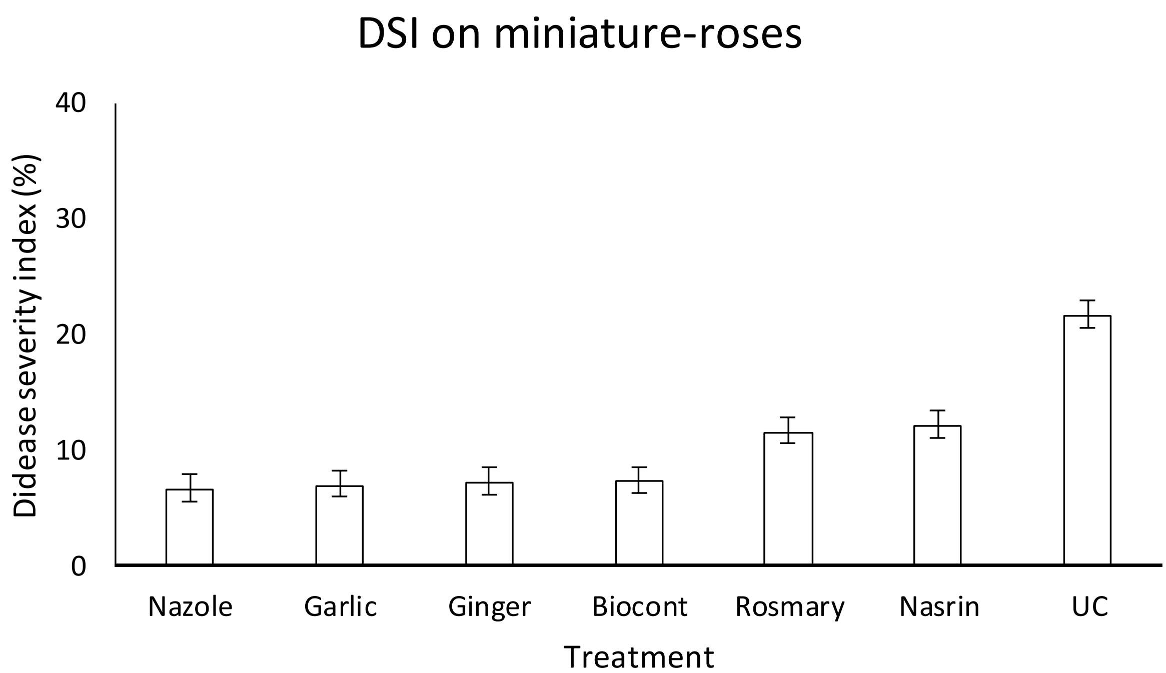

Figure 5: *in planta* activity of 3 selected plant extracts, one bioagent, and a synthetic fungicide on DSI of miniature-rose powdery mildew.

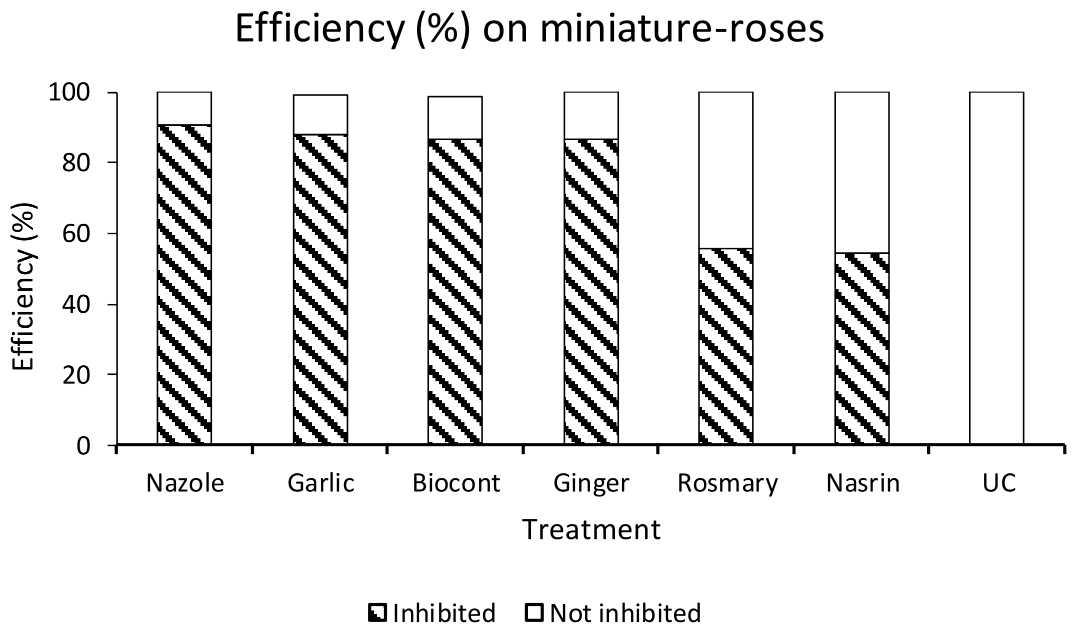

Figure 6: *in planta* efficiency of 3 selected plant extracts, one bioagent, and a synthetic fungicide on miniature-rose powdery mildew.

4.CONCLUSIONS

In conclusion, plant extracts as biofungicides, may work efficiently against a range of fungal pathogens including powdery mildew fungi. This work found that extracts of garlic, ginger, and rosemary are the most efficient plants that contain components that work as an antifungal against rose and miniature rose powdery mildew. The fungal bioagent, *T. harzianum*, on the other hand, also works effectively in disease reduction. Both plant extracts and the bioagent could be used as ecofriendly approaches to combat plant powdery mildew and play as alternatives to the deleterious impacts of synthetic inorganic chemicals. It is also concluded that dog rose (*Rosa canina*), commonly known as Nasrin, were generally less sensitive to rose powdery mildew.

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