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Plant extracts and commercial products for controlling black spot and inducing resistance in rose bushes

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Abstract

Rose is one of the most appreciated flowers in the world; however, fungal diseases can affect its production, decreasing its commercial value. Thus, the objective of this work was to assess the potential of extracts of medicinal plants and commercial products based on plant extracts, plant oils, and potassium phosphite to control black spot (*Diplocarpon rosae*) in roses by evaluating the activation of plant defense mechanisms. The treatments consisted of using crude aqueous extract (CAE) of *Rosmarinus officinalis* leaves; CAE of *Equisetum arvense* stems; CAE of *Moringa oleifera* seeds; commercial product (CP) based on fermented plant extracts; CP based on potassium phosphite; CP based on plant oils; CP based on citrus mass; and fungicide. Severity of black spot disease, and yield, biometry, chlorophyll contents, and enzyme activity of peroxidase, catalase, and polyphenoloxidase in rose plants were evaluated. The treatments presented no difference from the control (water) for disease incidence and severity and yield. However, the longest stem was found in rose plants in the treatment with potassium phosphite-based commercial product, whereas the thickest stems were found in the three treatments with commercial products. The highest enzyme specific activities of peroxidase, catalase, and polyphenoloxidase were found in plants treated with potassium phosphite, CAE of *M. oleifera,* and CAE of *R. officinalis*. The results found showed that the tested natural products did not control black spot in rose bushes, despite their positive effect on plant physiology.

Keywords: alternative management, *Diplocarpon sp*., enzyme activity, yield

Introduction

Rose is one of the most appreciated flowers in the world. The rose market in Brazil is approximately R$ (BRL) 496 million, with approximately 285 million stems produced per year. The largest rose producing states are Minas Gerais, Ceara, and Rio Grande do Sul, with 97% of the total planted area and 94% of the national production (Carvalho et al., 2019).

Obtaining maximum quantity and quality of rose buds depends on management of several factors, and the occurrence of diseases is one of the most important factors. Rose black spot stands out among these diseases; it is caused by the phytopathogen *Diplocarpon rosae* (Wolf), which is *Marssomia rosae* (Lib) when it is at the anamorphic stage, and is the more important disease for cultivation of roses. Its symptoms are spots on the leaf surface that become dark purple-green with fringed edges, causing yellowing of the leaf blade with subsequent leaf drop. The main control method used for this disease is application of fungicides combined with cultural methods, such as removing and eliminating parts of affected plants and fallen leaves on the ground and avoiding sprinkler irrigation (Alexandre et al., 2016; Marolleau et al., 2020).

An alternative control method for prioritizing safety for humans and environment is the activation of latent resistance mechanisms in plants, i.e., induction of plant resistance. It is characterized by application of inducing agents that promotes the activation of signaling pathways in the plant. These mechanisms can be structural and biochemical responses, such as accumulation of phytoalexins and proteins related to the pathogenesis, as peroxidases, catalases, and polyphenoloxidases (Gonçalves-Trevisoli et al., 2017; Pascholati et al., 2018). (Simon et al., 2016) found that aqueous extracts of *Equisetum arvenense, Rosmarinus*

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officinalis, and *Moringa oliefera* were efficient in reducing sporulation and increasing synthesis of protein and peroxidases in the mycelium of *D. rosae,* respectively.

Few studies on ornamental plants are focused on the control of diseases using alternatives products under field conditions. (Shalini et al., 2019) found that the plant extracts at 20% of *Allium sativum, Leucas martinicensis, and Zingiber officinale* presented significant antifungal activity against *D. rosae* under field conditions.

In this sense, the objective of this work was to assess the potential of extracts of medicinal plants (*Rosmarinus officinalis*, *Equisetum arvense*, and *Moringa oleifera*) and commercial products based on plant extracts, plant oils, and potassium phosphite to control black spot (*Diplocarpon rosae*) in roses by evaluating the activation of plant defense mechanisms.

Material and Methods

The plant materials used for obtaining crude aqueous extracts were leaves of *Rosmarinus officinalis* L., stems of *Equisetum arvense* L., and seeds of *Moringa oleifera* L., collected in the upper third of the plants in the morning, in the autumn, in an experimental area in Maringa, state of Parana, Brazil. Crude aqueous extracts (CAE) were obtained, using 10 g of *R. officinalis* leaves, which were crushed with 100 mL of distilled water in a blender for 2 minutes and, then, filtered through gauze. The same procedure was carried out for obtaining CAE of *E. arvense* L. stems and CAE of seeds removed from dry capsules of *Moringa oleifera* L.

The commercial products used were based on fermented plant extracts (0.01%) + adjuvant (lecithin and propionic acid) (0.0025%); potassium phosphite (0.01%) + adjuvant (0.0025%); plant oils (0.01%) + adjuvant (0.0025%); citrus mass (0.01%); fungicide methyl thiophanate (0.007%); and adjuvant (0.0025%). The control treatment consisted of water.

The experiment was carried out in a greenhouse (15 m × 7 m) at the Inga Faculty campus and in the Laboratory of Alternative Control and Resistance Induction of of the Center for Agricultural Sciences of the State University of Maringa. The climate of the region is Cfa, humid subtropical mesothermal (Köppen-Geiger, 1948). The experiment period was from November 2014 to April 2015. The means of rainfall depths and temperatures during the six months of experiment were 206.21 mm and 25.9 °C, respectively. A drip irrigation system was used during the experiment, with daily irrigation for one hour at flow of 1 L^{-1} $hour^{-1}$ $plant^{-1}$. A randomized block experimental design was used, with 10 treatments, 8 blocks, and two plants per plot.

Rose seedlings from grafting of the rose variety Grand Gala (red color) were obtained from a grower in the region of Marialva, PR, 50 days after grafting. They were selected and transplanted into the soil with spacing of 0.25 ×1.40 m in a greenhouse (area of 105 m^2). Soil fertilizer based on caprine manure was applied after transplanting, using approximately 500 g per plant. Pruning and trellising were carried out during the experiment period.

Four applications of the treatments were carried out during the experiment, with a 10-day interval, at the end of the afternoon, through leaf application by aspersion. The variables evaluated were: incidence and severity of disease (black spot; *Diplocarpon rosae*), and yield, biometry, chlorophyll contents, and enzyme quantification in rose plants.

Disease incidence was determined by presence and absence of leaves with disease symptoms, considering the natural occurrence of the disease without need for inoculation; the following formula was used: % Incidence = (number of infected plants / total number of plants) × 100.

The severity of black spot was visually evaluated through observations of the first disease symptoms in the rose plants, attributing severity scores, using a using a diagrammatic scale adapted from the soybean leaf spot scale (Soares et al., 2009). Disease severity was evaluated every 5 days after the first application of the treatments, on two leaves in the middle third of each plant in the plot, which were identified with plastic ropes, totaling 8 evaluations. Disease severity data were used to determine the area under the disease progress curve (AUDPC) through the equation proposed by (Campbell & Madden, 1990): $AUDPC = \Sigma (yi + yi+1)/2 * (ti+1 – ti)$, where: n = number of evaluations; y = severity of disease (%); t = time (days). Eight evaluations with a five-day interval were carried out.

Yield was evaluated by counting the flower buds per plant, using two plants per replication, during two cycles after the beginning of the experiment: at 40 (first harvest) and 80 (second harvest) days.

The following variables were evaluated for biometric classification: stem length and diameter of stem and bud length, using a ruler tape and digital caliper. Two flower stems were used per replication. Stem length was measured from the stem base to the bud tip; stem diameter was measured at the half the stem length; and bud length was measured from the petal base to the bud tip. Stems were classified according to the standard created by Veiling Holambra (Cooperative, 2018): flower

bud length varying from 3.5 to 4.5 cm, flower stem length from 25 to 80 cm, and minimum stem diameter from 3 to 5 mm.

Two 2-mm diameter discs were removed from leaves 25 and 40 days after the first application of treatments for determining chlorophyll contents. The methodology used was adapted from (Arnon, 1949). The plant tissue samples were placed in amber glass vials containing 5 mL of 80% acetone for 7 days in the dark at 25 ˚C. Subsequently, chlorophyll *a* and chlorophyll *b* were evaluated through readings in a spectrophotometer at 663 nm and 645 nm, respectively. Chlorophyll *a* and *b* contents were determined using the formulas, respectively, (0. 0127.A663) -(0. 00269.A645) and (0. 0229. A645) -(0. 00468.A663). The total chlorophyll content was obtained by summing the chlorophyll *a* and *b* contents, and the result was expressed as mg g^{-1} fresh tissue.

Leaves were collected from the middle third of the rose bushes 24 hours after each treatment application, totaling four collections, for the biochemical analyses. These leaves were weighed and stored in a freezer at - 20 ˚C.

The leaves enzyme extract was obtained by crushing the leaves in a mortar, using liquid nitrogen. Subsequently, the material was homogenized in 4 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, with addition of 1% polyvinylpyrrolidone (PVP). The extract obtained was centrifuged at 14500 rpm for 30 minutes at 4 ˚C, and the supernatant was considered enzyme extract and stored in a freezer at -20 ˚C for determining the total protein content and the specific activity of peroxidase, catalase, and polyphenoloxidase enzymes.

Total proteins were quantified using the methodology of (Bradford, 1976). An aliquot of 50 µL of enzyme extract was added to 2.5 mL of Bradford reagent under shaking and incubated for five minutes. Subsequently, reading was carried out in a spectrophotometer at 595 nm. The blank used consisted of 50 µL of distilled water with 2.5 mL of Bradford. The standard curve was determined using bovine serum albumin (BSA).

Specific activity of peroxidase (EC 1.11.1.7) was determined by measuring the conversion of guaiacol into tetraguaiacol at 470 nm (Lusso & Pascholati, 1999). An aliquot of 100 µL of enzyme extract was mixed with 2.9 mL of substrate [250 µL of guaiacol and 306 µL of hydrogen peroxide in 100 mL of 0.01M phosphate buffer (pH 6.0)] and maintained at 30 ˚C. A cuvette containing 3 mL of the solution with guaiacol and hydrogen peroxide in phosphate buffer was used as reference. Specific activity of peroxidase was determined over a period of two minutes and results were expressed as units of absorbance min^{-1} mg^{-1} protein.

Specific activity of catalase (EC 1.11.1.6) was obtained according to the methodology of (Tomanková et al., 2006), through the stable complex formed by ammonium molybdate with hydrogen peroxide. An aliquot of 100 µL of enzyme extract was incubated in 500 µL of reaction mixture containing 60 mM hydrogen peroxide in 60 mM potassium phosphate buffer (pH 7.4) at 38 ˚C for 4 minutes. Subsequently, 500 µL of 32.4 mM ammonium molybdate was added. Reading was carried out in a spectrophotometer at wavelength of 405 nm for determining the consumption of hydrogen peroxide by the catalase in the extract. A blank was prepared for each sample by adding ammonium molybdate to the reaction mixture, disregarding the incubation period. The difference between the absorbances of the blank and the incubated sample indicated the amount of hydrogen peroxide used by the enzyme. H_2O_2 concentration was determined using the extinction coefficient ϵ = 0.0655 mM^{-1} cm^{-1}, and results were expressed as µmol min^{-1} mg^{-1} protein.

Specific activity of polyphenoloxidase (EC 1.14.18.1) was determined according to the methodology of (Duangmal & Apenten, 1999). Oxidation of catechol converted into quinone was quantified, as this reaction is mediated by the enzyme polyphenoloxidase. A substrate composed of catechol at concentration of 0.02 mM was dissolved in 0.1 M potassium phosphate buffer (pH 6.8) and maintained at 30 ˚C for 30 minutes. Then, 900 µL of this substrate was mixed with 100 µL of enzyme extract for reading in a spectrophotometer at 420 nm for 2 minutes. The results were expressed as absorbance min^{-1} mg^{-1} protein.

The data obtained were subjected to normality and Shapiro-Wilk tests ($p<0.05$). The variables that did not met the assumptions of normality, such as disease severity and enzyme activity, were transformed by the square root. Subsequently, the data were subjected to analysis of variance and compared by the Scott-Knott test at 5% probability, using the statistical program SISVAR (Ferreira, 2011).

Results and Discussion

No significant difference was found between the treatments for incidence and the severity of black spot disease in rose bushes. The mean of the treatments at 41 days after the first application of treatments was 98% and 1.4 for disease incidence and area under the disease

progress curve (AUDPC), respectively. Despite the four applications of the treatments, the environmental conditions, such as high rainfall depths (206 mm) and a mild temperature (25.9 °C) contributed to the progress of the disease in rose bushes, i.e., the occurrence of the disease depends on susceptible host, virulent pathogen, and favorable environmental conditions for the pathogen development (Alexandre et. al., 2016).

Data on yield (number of flower buds) and biometry (flower bud length and stem length and diameter) of rose plants at 40 days (first harvest) showed no significant difference between treatments. The means found for yield, flower bud length, stem length, and stem diameter were, respectively, four flower buds per plant, 4.2 cm, 35 cm, and 3.5 mm. These results are within the quality standards established by (Veiling-Holambra Cooperative, 2018).

No significant difference was found between treatments for number of flower buds per plant in the second harvest, at 80 days (**Table 1**). However, the number of flower buds per plant in the treatment with CAE of *R. officinalis* (T1) was maintained (four), whereas it decreased by 50% (two) in the treatment with CAE of *M. oleifera* (T3) and to three in the others treatments. No significant difference was found between treatments for flower bud length.

The treatment with commercial product based on potassium phosphite (T5) resulted in a stem length 31% higher than the control, followed by T1 (15%), and T4 (commercial product based on fermented plant extracts) and T6 (commercial product based on plant oil), both with 12%. Regarding stem diameter, the treatments T4, T5, and T6, promoted increases of 20%, 29%, and 20%, respectively, when compared to the control.

Flower bud length and stem length and diameter were within the standards established by the classification criteria for cut roses. Using of potassium phosphite resulted in increases in rose stem length and diameter. This is probably due to optimization of potassium in the plants, which is advantageous, as K has many functions in the plant, mainly those involved in the synthesis of compounds of high molecular weight (proteins, cellulose, and starch) and in the activation of several enzymatic systems, such as those involved in antioxidant processes (Almeida et al., 2020).

The treatments evaluated in the present work had no effects on photosynthetic efficiency, as no significant difference was found for total chlorophyll and chlorophyll *a* and *b* contents in rose leaves. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in rose leaves were 0.06, 0.03, and 0.09 mg^{-1} fresh tissue, respectively, at 40 days after the first application. However, greener leaves, due to higher chlorophyll contents, can increase the attractiveness of plants, improving its marketing (Carvalho-Zanão et.al., 2018).

The analysis of specific activity of peroxidase (**Table 2**) showed that the first and second applications of the treatments T3 (CAE of *M. oleifera*), T6 (plant oils), T7 (citrus mass), T8 (thiophanate methyl fungicide), and T9 (adjuvant) increased the enzyme activity. Regarding the treatment T4 (fermented plant extracts), the highest enzyme activity in plants was found with the first application. No significant difference was found between the other treatments for peroxidase activity, in the four applications carried out.

Only the treatment with potassium phosphite differed from the control in the first application, resulting in an increased peroxidase activity. Studies indicated that phosphite can increase the activity of several defense enzymes, such as ascorbate peroxidase, catalase, glutathione reductase, and superoxide dismutase (Silva et al. 2022).

Table 1: Yield (number of flower buds per plant) and biometry (flower bud length, and stem length and diameter) of rose plants in the second harvest (80 days after the first application of treatments)

Treatments	Number of flower buds	Bud length (cm)	Stem length (cm)	Stem diameter (mm)
T1: CAE of *R. officinalis* (1%)	4.00	5.73	48.75 b*	3.34 b
T2: CAE of *E. arvense (*1%)	3.00	4.47	40.77 c	3.52 b
T3: CAE *of M. oleífera* (1%)	2.00	6.07	40.47 c	3.51 b
T4: CP - fermented plant extracts (0.01%)	3.00	5.75	47.43 b	3.86 a
T5: CP - potassium phosphite (0.01%)	3.00	6.15	55.40 a	4.17 a
T6: CP - plant oils (0.01%)	3.00	6.06	47.31 b	3.87 a
T7: CP - citrus mass (0.01%)	3.00	5.75	45.84 b	3.63 b
T8: thiophanate methyl fungicide (0.007%)	3.00	5.69	37.45 c	3.50 b
T9: Adjuvant 0.0025%	3.00	5.81	45.12 b	3.54 b
T10: Control (water)	3.00	5.75	42.25 c	3.21 b
CV (%)	41.42	17.91	16.39	12.17

CAE = crude aqueous extract. [2]Commercial product. *Means followed by same uppercase letter in the column and lowercase letter in the row are not statistically different by the Scott-Knott test ($p < 0.05$).

Table 2: Specific activity of peroxidase (absorbance min^{-1} mg^{-1} protein) in rose leaves after application of treatments

Treatments	Before application 0 hours	Application 1 1 day 24 hours	Application 2 10 days 24 hours	Application 3 20 days 24 hours	Application 4 30 days 24 hours
T1: CAE of *R. officinalis (*1%)	0.54 Aa*	0.57 Ba	0.64 Aa	0.52 Aa	0.47 Aa
T2: CAE of *E. arvense (*1%)	0.46 Aa	0.56 Ba	0.50 Aa	0.43 Aa	0.46 Aa
T3: CAE *of M. oleífera* (1%)	0.54 Aa	0.65 Ba	0.67 Aa	0.37 Ab	0.34 Ab
T4: CP - fermented plant extracts (0.01%)	0.60 Aa	0.65 Ba	0.45 Ab	0.40 Ab	0.42 Ab
T5: CP - potassium phosphite (0.01%)	0.48 Ab	1.00 Aa	0.58 Ab	0.45 Ab	0.44 Ab
T6: CP - plant oils (0.01%)	0.60 Aa	0.66 Ba	0.57 Aa	0.45 Ab	0.36 Ab
T7: CP - citrus mass (0.01%)	0.59 Aa	0.65 Ba	0.71 Aa	0.43 Ab	0.45 Ab
T8: thiophanate methyl fungicide (0.007%)	0.62 Aa	0.54 Ba	0.53 Aa	0.32 Ab	0.41 Ab
T9: Adjuvant 0.0025%	0.57 Aa	0.68 Ba	0.55 Aa	0.31 Ab	0.42 Ab
T10: Control (water)	0.58 Aa	0.54 Ba	0.56 Aa	0.39 Aa	0.44 Aa
CV (%)	24.50	29.63	32.19	38.24	34.31

CAE = crude aqueous extract. [2]Commercial product. *Means followed by same uppercase letter in the column and lowercase letter in the row are not statistically different by the Scott-Knott test ($p < 0.05$).

Regarding catalase activity, the highest enzyme activity in the treatments T3 (CAE of *M. oleifera*) and T5 (potassium phosphite) was found with the first application (**Table 3**), whereas that in the treatment T4 (plant extracts) was found with the fourth application. The treatment T8 (fungicide methyl thiophanate) promoted the highest catalase activity with the first, third, and fourth applications. The treatments T3 and T5 promoted higher enzyme activity than the control 24 hours after the first application, which was also found 24 hours after the second application for these treatments, followed by T1 (CAE of *R. officinalis*).

The highest polyphenoloxidase activity in rose plants (**Table 4**) in the treatments T2 (CAE of *E. arvense*), T4 (fermented plant extracts), T5 (potassium phosphite), T6 (plant oils), and T9 (adjuvant) was found with the second application of treatments. The treatment T3 promoted the highest polyphenoloxidase activity with the first application.

The treatments T1, T2, T7, and T8 differed from the control in the first application, presenting lower polyphenoloxidase activity. Regarding the third application, T1 and T3 promoted higher polyphenoloxidase activity than the control. No difference was found between treatments for the second and fourth applications.

The results showed higher levels of catalase than peroxidase, which can be explained by the competition of these enzymes for the same active site—the degradation of hydrogen peroxide. The plants may have been affected by oxidative stress after recognizing the pathogen, which resulted in higher catalase and lower peroxidase levels, as both enzymes convert H_2O_2 into H_2O and O_2 (Abiddem et al., 2020; Safari et al., 2019). The enzyme polyphenoloxidase also can contribute to plant resistance to diseases (Wang et al., 2020).

The treatments evaluated in the present work did not prevent the disease progress; however, the treatment with potassium phosphite (T5) improved the plant physiological quality, promoting increases in stem length. The treatments T5, T1 (CAE of *R. officinalisI)*, T2 (CAE of *E. arvenseI)*, and T3 (CAE *M. oleifera*) increased the enzymatic activity of peroxidase, catalase, and

Table 3: Specific activity of catalase (µmol min^{-1} mg^{-1} protein) in rose leaves after application of treatments

Treatments	Before application 0 hours	Application 1 1 day 24 hours	Application 2 10 days 24 hours	Application 3 20 days 24 hours	Application 4 30 days 24 hours
T1: CAE of *R. officinalis (*1%)	3.45 Aa*	3.59 Ba	2.89 Ba	3.82 Aa	3.85 Aa
T2: CAE of *E. arvense (*1%)	3.38 Aa	3.37 Ba	3.79 Aa	4.32 Aa	4.11 Aa
T3: CAE *of M. oleífera* (1%)	3.83 Ab	5.08 Aa	4.02 Ab	4.33 Ab	4.24 Ab
T4: CP - fermented plant extracts (0.01%)	4.03 Aa	3.75 Bb	3.11 Bb	3.69 Ab	4.57 Aa
T5: CP - potassium phosphite (0.01%)	4.09 Ab	5.36 Aa	4.31 Ab	4.59 Ab	4.09 Ab
T6: CP - plant oils (0.01%)	3.16 Aa	3.34 Ba	3.17 Ba	3.82 Aa	3.31 Aa
T7: CP - citrus mass (0.01%)	3.57 Aa	3.92 Ba	3.63 Ba	4.04 Aa	4.11 Aa
T8: thiophanate methyl fungicide (0.007%)	3.61 Ab	3.94 Ba	3.09 Bb	4.46 Aa	4.16 Aa
T9: Adjuvant 0.0025%	3.37 Aa	3.22 Ba	3.40 Ba	4.16 Aa	3.67 Aa
T10: Control (water)	3.33 Aa	3.49 Ba	3.93 Aa	3.75 Aa	3.90 Aa
CV (%)	15.54	23.93	21.97	23.23	16.30

CAE = crude aqueous extract. [2]Commercial product. *Means followed by same uppercase letter in the column and lowercase letter in the row are not statistically different by the Scott-Knott test ($p < 0.05$).

Table 4: Specific activity of polyphenoloxidase (abs min^{-1} mg^{-1} protein) in rose leaves after application of treatments

Treatments	Before of application 0h	Application 1 1 day 24h	Application 2 10 days 24h	Application 3 20 days 24h	Application 3 20 days 24h
T1: CAE of *R. officinalis (*1%)	0.36 Aa*	0.33 Ba	0.34 Aa	0.37 Aa	0.28 Aa
T2: CAE of *E. arvense (*1%)	0.32 Aa	0.29 Ba	0.28 Aa	0.21 Bb	0.14 Ab
T3: CAE *of M. oleífera* (1%)	0.38 Ab	0.51 Aa	0.33 Ab	0.28 Ac	0.14 Ad
T4: CP - fermented plant extracts (0.01%)	0.33 Aa	0.38 Aa	0.22 Ab	0.19 Bb	0.11 Ab
T5: CP - potassium phosphite (0.01%)	0.37 Ab	0.48 Aa	0.34 Ab	0.24 Bc	0.14 Ac
T6: CP - plant oils (0.01%)	0.41 Aa	0.44 Aa	0.29 Ab	0.24 Bb	0.11 Ac
T7: CP - citrus mass (0.01%)	0.44 Aa	0.27 Bb	0.32 Ab	0.15 Bc	0.19 Ac
T8: thiophanate methyl fungicide (0.007%)	0.29 Aa	0.23 Ba	0.19 Aa	0.20 Ba	0.19 Aa
T9: Adjuvant 0.0025%	0.35 Aa	0.37 Aa	0.29 Aa	0.20 Bb	0.12 Ab
CV (%)	31.85	40.99	33.63	51.62	49.29

CAE = crude aqueous extract. [2]Commercial product. *Means followed by same uppercase letter in the column and lowercase letter in the row are not statistically different by the Scott-Knott test ($p < 0.05$).

polyphenoloxidase; however, they did not show positive correlation with disease control (black spot), probably due to a delay in the induction of enzyme activity or activation of other metabolic pathway, thus denoting the need for further studies on this pathosystem.

Conclusions

Using crude aqueous extracts (CAE) of medicinal plants (*Rosmarinus officinalis, Equisetum arvense*, *and Moringa oleifera*) or commercial products based on plant extracts, plant oils, and potassium phosphite does not control black spot in rose bushes.

Using potassium phosphite-based commercial product promotes the development of longer and thicker rose stems.

Using potassium phosphite-based commercial product, CAE of *R. officinalis,* and CAE of *M. oleifera* induces activity of peroxidase, catalase, and polyphenoloxidase, which are enzymes connected to plant defense mechanisms.

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