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Research Article

EFFECTS OF BLACKBERRY AND BLACKTHORN FRUIT EXTRACTS ON PROTEIN AND PEROXIDASE ACTIVITY IN PEPPER

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ABSTRACT

*In this research, blackthorn (*Prunus spinosa* L.) and blackberry (*Rubus sanctus* Schreb.) fruits have been extracted with water, ethanol, ethanol/water solvents. Fruit extracts were sprayed on the leaves of grossum and conoides varieties of *Capsicum annuum* L. 24 and 48 hours after extract applications, healthy leaf of ten weeks old seedlings were harvested for protein and peroxidase analyses. Changing in total protein levels and peroxidase activity were measured spectrophotometrically. The highest total protein level increase was in the application of ethanolic extract of *R. sanctus* to grossum variety when we compare with control group. This increasing 24 and 48 hours after application were determined as 154% and 144% respectively. After the applications of *R. sanctus* fruit extract to the grossum and conoides varieties, peroxidase (POX) [EC 1.11.1.7] activity changing have been found better than *P. spinosa* extract application. After the both fruit extract applications, increasing in the POX activity of conoides variety have been found better than the grossum variety. 24 and 48 hours after application of ethanolic *R. sanctus* extract to conoides variety, POX activity increasing have been found respectively as 76% and 94%. In conclusion, it was shown that *P. spinosa* and *R. sanctus* fruit extracts have been stimulated the plant defense system in grossum and conoides varieties of *C. annuum* at different levels within the scope of total protein amount and peroxidase activity according to the control group.*

Keywords: *Prunus spinosa, Rubus sanctus, Total protein, Peroxidase, Capsicum annuum.*

1. INTRODUCTION

Turkey has a rich natural biodiversity which has a great amount of medicinal and aromatic plant species. Nowadays, their usage aren't limited with medicine, food, beverage, cosmetic industry and veterinary medicine. Organic farming and in phytoremediation as bioaccumulator are the potential usage fields of these plants (Bağdat, 2006). In addition, there are studies that use the extracts prepared from plants as a stimulant for plant defense. For example, the studies of Goupil et al. (2012), was shown that both leaf infiltration and a foliar spray of the red grape extract on tobacco leaves induced defence gene expression: The PR1 and PR2 target genes were upregulated locally and systemically in tobacco plants following grape marc extract treatment. The grape extract elicited an array of plant defence responses making this natural compound a potential phytosanitary product with a challenging issue. Kulaksız (2016), determined that different concentrations and exposure time of *Aloe vera* gels has been stimulated the plant defense system in different ratios. In this study, it has been found that low concentration *Aloe vera* gel increases the peroxidase activity in grossum varieties of *Capsicum annuum*. In the study of Dinç and Akı (2015), shown that the application of ethanolic and methanolic extracts of *Echinacea angustifolia* to *Solanum lycopersicon* seedlings decreased the amount of total protein in the *Solanum lycopersicum* seedlings and increased peroxidase activity. It was determined these effects vary depending on the extraction concentration and time. In the study of Yıldız and Akı (2018), it was shown that *Prunus spinosa* and *Rubus sanctus* leaf raw extracts were able to stimulate plant defense system at different levels.

2. MATERIAL AND METHODS

2.1. Material

In this research, the *C. annuum* seeds have been used as a plant material. Seeds were purchased from Küçük Çiftlik Company. Wild Blackberry (*R. sanctus*) fruits were collected from Terzioğlu Campus of Çanakkale Onsekiz Mart University. Blackthorn (*P. spinosa*) fruits were collected from Sarıcaeli Village of Çanakkale.

2.2. Methods

2.2.1. Growing of Pepper Seedlings

The seeds of *Capsicum annuum* varieties (conoides and grossum) were germinated in violes (capacity 45) containing a mixture of 1/3 perlite-peat under controlled conditions. The seedlings were grown in controlled plant growth chamber at 24±2°C and 60% humidity, under 16/8 hours long day photoperiod. All of the trials were replicated three times. 10 weeks old seedlings have been used for the experiments.

2.2.2. Preparing of Fruit Extracts

For the extraction process, fresh fruits of *R. sanctus* and *P. spinosa*, were used. The fruits were shredded with hand blender. Very small pieces of *P. spinosa* and *R. sanctus* fruits were weighed 25 g each and final volume of 50 mL of water, ethanol/water and ethanol solvents were prepared. The prepared solutions have been shake at 130 rpm for 6 hours in the room temperature. Then all of the solvents were evaporated in the water bath at 60°C for 5 hours. The resulted powders of the fruit extracts were weighed 2 g each and stored at -20°C until the application.

2.2.3. Dissolution of Extracts with DMSO

2 g of fruit extracts were taken and stock solution was prepared with 20 mL of dimethyl sulfoxide (DMSO). The stock solution was diluted with distilled water and an application concentration of 10 mg/mL was prepared.

2.2.4. Application of the Fruit Extracts to *C. annuum* varieties

The stock solutions were diluted with distilled water as 0.01 g/mL (10mL of stock/90mL of distilled water) for application. Diluted stock solutions were sprayed on leaves of 10-week-old "conoides" and "grossum" varieties of *C. annuum* seedlings. Control groups were sprayed with distilled water.

2.2.6. Analysis Procedure

2.2.6.1. Homogenization of Harvested Leaves

After of the application 24 and 48 hours, the healthy leaves of pepper seedlings were harvested. These leaves were homogenized with 5mL 0.05M cold sodium acetate buffer (pH: 6.5) for one minute. Then these homogenants were centrifuged 13000 rpm at +4°C for 15 minutes. After the centrifugation, the supernatant were used for determination of total protein analysis and peroxidase (POX) activity.

2.2.6.2. Total Protein Level and Peroxidase Activity Analyses

The total protein amount in the homogenates was determined by spectrophotometric analysis according to Bradford (1976)'s method using bovine serum albumin (BSA) as a standard. Peroxidase (POX) activity changes in homogenants were determined by spectrophotometric analysis according to the method of Kanner and Kinsella (1983).

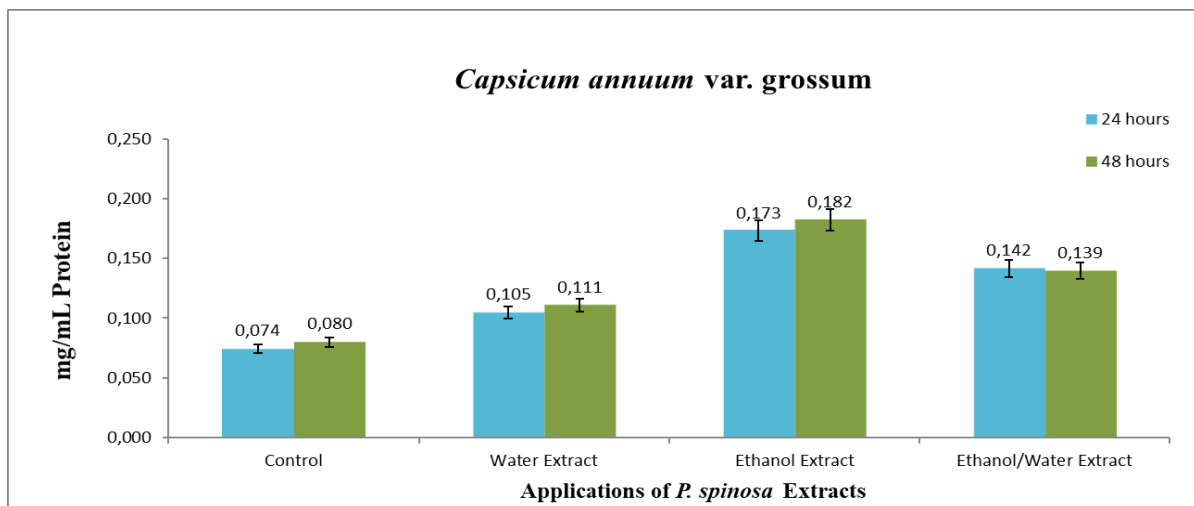
3. RESULTS AND DISCUSSION

3.1. Protein Results

When compared with the control group, it was determined that all plant extracts of this research caused an increasing in the total amount of protein. The highest increase in the total protein amount have been found in both varieties of *C. annuum* ethanolic extract application of both fruits.

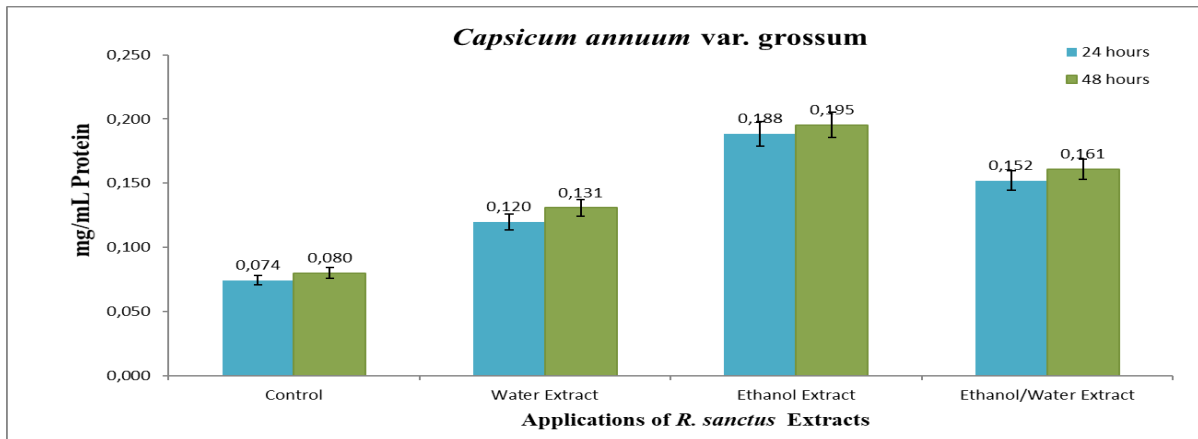
24 and 48 hours after application of ethanolic *P. spinosa* extract to grossum variety, total protein levels increasing have been found respectively as 134% and 127,5%.

Fig. 1 Effects of *P. spinosa* Extracts on Total Protein Amount in *C. annuum* var. grossum



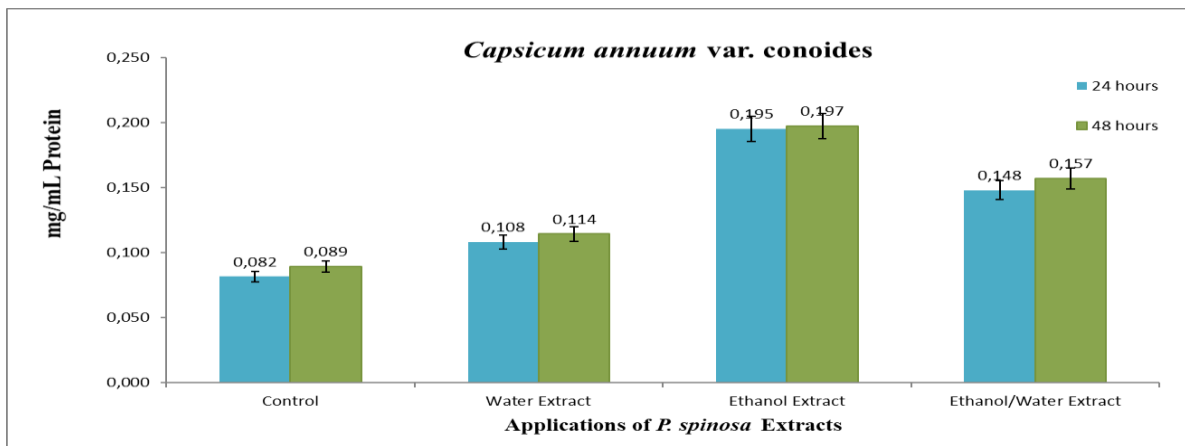
The highest total protein level increase was in the application of ethanolic extract of *R. sanctus* to grossum variety when we compare with control group. This increasing 24 and 48 hours after application were determined as 154% and 144% respectively.

Fig. 2 Effects of *R. sanctus* Extracts on Total Protein Amount in *C. annuum* var. grossum



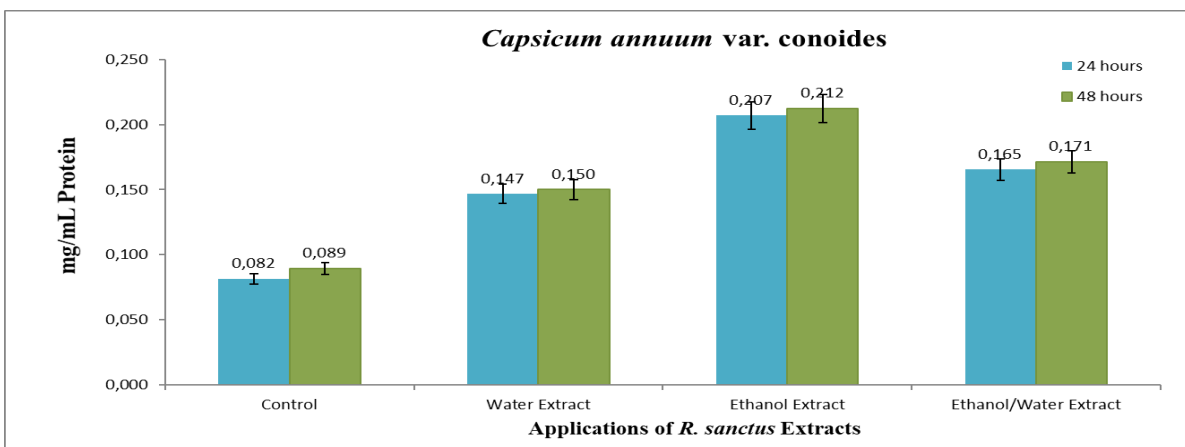
24 and 48 hours after application of ethanolic *P. spinosa* extract to conoides variety, total protein levels increasing have been found respectively as 138% and 121%.

Fig. 3 Effects of *P. spinosa* Extracts on Total Protein Amount in *C. annuum* var. conoides



24 and 48 hours after application of ethanolic *R. sanctus* extract to conoides variety, total protein levels increasing have been found respectively as 152% and 138%.

Fig. 4 Effects of *R. sanctus* Extracts on Total Protein Amount in *C. annuum* var. conoides



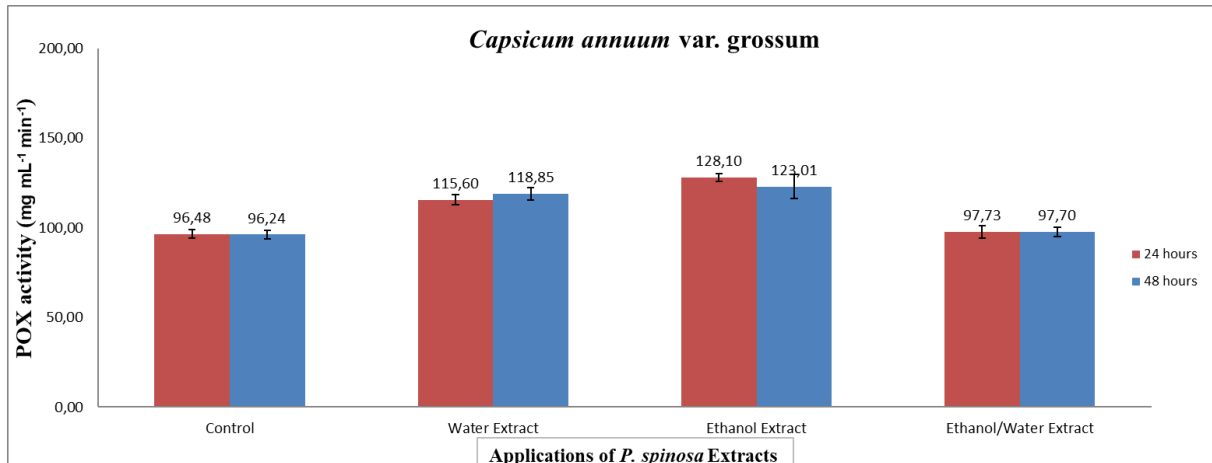
As a result, application of *Rubus sanctus* fruit extracts increased the total protein amount of *Capsicum annuum* seedlings compared to the application of *Prunus spinosa* fruit extract. There weren't significant difference between the varieties in terms of a total protein of the seedlings.

3.2. Peroxidase Results

All extracts were found to increase peroxidase (POX) [EC 1.11.1.7] activity in pepper seedlings. The extract that increased the maximum POX activity of the pepper seedlings was ethanol extracts.

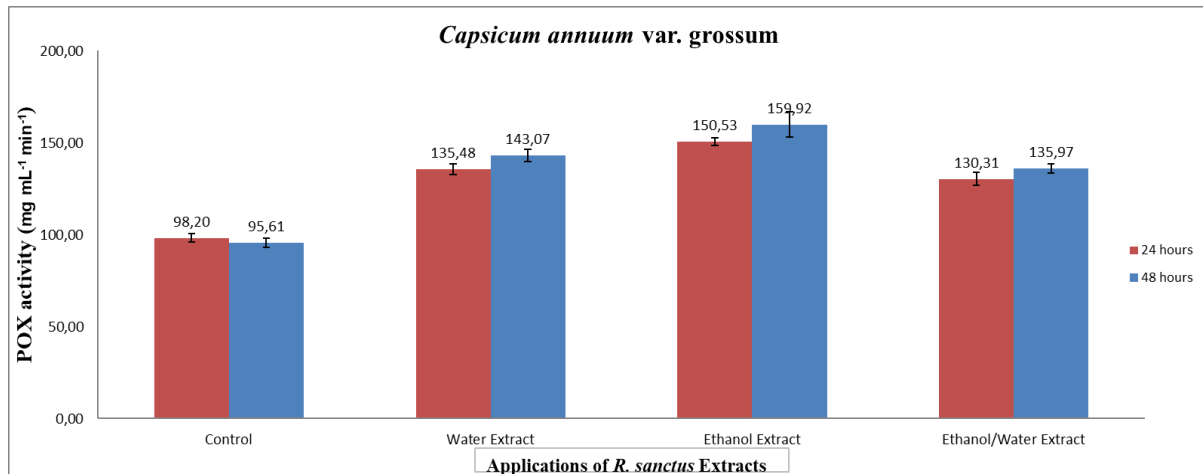
24 and 48 hours after application of ethanolic *P. spinosa* extract to grossum variety, POX activity increasing have been found respectively as 33% and 28%.

Fig. 5 Effects of *P. spinosa* Extracts on POX activities in *C. annuum* var. grossum



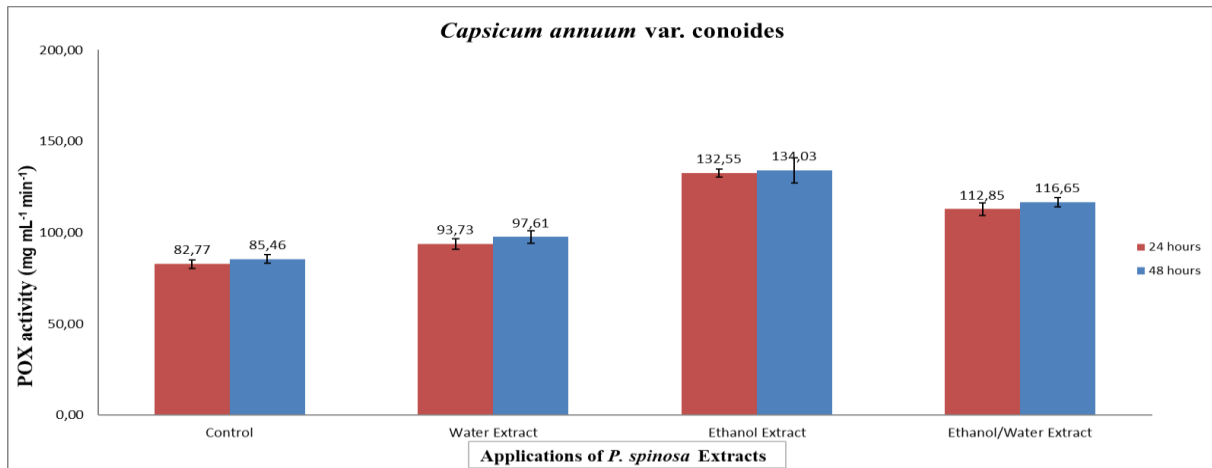
24 and 48 hours after application of ethanolic *R. sanctus* extract to grossum variety, POX activity increasing have been found respectively as 53% and 67%.

Fig. 6 Effects of *R. sanctus* Extracts on POX activities in *C. annuum* var. grossum



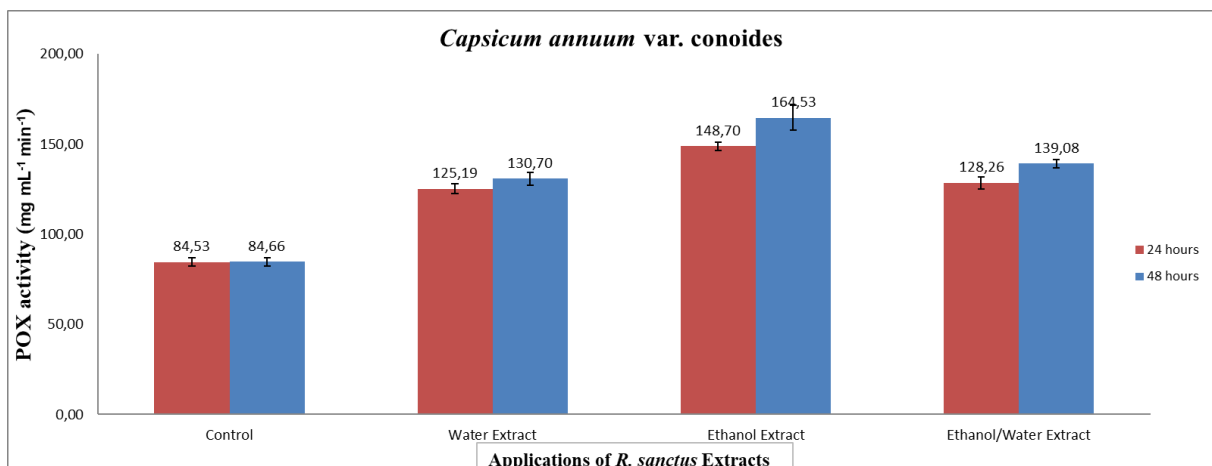
24 and 48 hours after application of ethanolic *P. spinosa* extract to conoides variety, POX activity increasing have been found respectively as 60% and 57%.

Fig. 7 Effects of *P. spinosa* Extracts on POX activities in *C. annuum* var. conoides



After the applications of *R. sanctus* fruit extract to the grossum and conoides varieties, POX activity changing have been found better than *P. spinosa* extract application. After the both fruit extract applications, increasing in the POX activity of conoides variety have been found better than the grossum variety. 24 and 48 hours after application of ethanolic *R. sanctus* extract to conoides variety, POX activity increasing have been found respectively as 76% and 94%.

Fig. 8 Effects of *R. sanctus* extracts on POX activities in *C. annuum* var. conoides



3.3. Discussion

In the research of Demiraslan and Akı (2015), in which some plant activators were applied to *Capsicum annuum* seedlings, plant activators were reported to increase total protein and peroxidase activity in *Capsicum annuum* seedlings.

In our research, *P. spinosa* and *R. sanctus* fruit extracts, like plant activators, were shown that the plant increased both total protein and POX activity at the same time.

Both *Aloe vera* and *Echinacea angustifolia* extracts showed increase in the POX activity in the application plants but the extracts showed decrease in the amounts of total protein in the application plants (Dinç,2016; Kulaksız,2016).

There are a study that was determined effect of *P. spinosa* and *R. sanctus* leaf extracts on the total protein amount and POX activity in *C. annuum* seedlings (Yıldız and Akı, 2018). When we compare this research with fruit application research, it was seen the fruit extracts, increase the total protein amount of *C. annuum* seedlings more than the leaf extracts.

4. CONCLUSION

Pesticides are widely used in agricultural production, which protect plants from diseases, insects or weeds (Kannan et al., 1997). Although pesticides are developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from occupational exposure and from residues in food and drinking water (Damalas and Eleftherohorinos, 2011). Natural plant activators are important because of the potential to reduce or no use of pesticides. Natural plant activators are important in this context. It was determined that fruit extracts which prepared from the *R. sanctus* and *P. spinosa* plants could stimulate the defense system of the pepper plant. The results in this research are consistent with the results of the previous research results that plant extracts can be used as natural plant activators. In this research, promising results have been obtained that *P. spinosa* and *R. sanctus* fruits contents could be used as plant activator developmental studies. However, more scientific research is needed to evaluate that these fruit extracts can be used as plant activators.

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