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EFFECT OF SALICYLIC ACID ON THE INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN COFFEE PLANTS (*Coffea arabica* L. Var. TIPICA AND GEISHA) TO *Hemileia vastatrix* Berkeley & Broome.

SUMMARY

This study aimed to identify the optimal dose at which Salicylic Acid (SA) induces systemic acquired resistance in coffee plants (*Coffea arabica* L. varieties Tipica and Geisha) against infection caused by Hemileia vastatrix Berkeley & Berkeley and Broome. A completely randomized design was established, with five blocks containing ten plants per variety. Three leaves from each plant were chosen at three different times to assess the severity of the disease. The treatments included various SA concentrations (0.5, 1, and 1.5 mM), water as a negative control (C-), and copper oxychloride as a positive control (C+), which was only applied on day 21 before pathogen inoculation. The plants were sprayed at intervals of 21, 14, and 7 days, and on day 0, the fungus was inoculated at a concentration of 0.5 mgL^{-1}. Subsequently, SA applications were made on 7, 14, and 21 days. For the statistical analysis of the data, an ordinal logistic regression with a cumulative logit link was used to quantify the association between variables using odds ratios and to estimate the probability of disease occurrence according to the levels of rust severity shown. The results showed that in both varieties, the treatment with 1.5 mM SA preserved 27 healthy leaves out of the initial 60, suggesting that SA at this concentration activated the coffee plant's defense mechanism, protecting it from disease attack.

Keywords: Salicylic acid; *Coffea arabica*; Hemileia vastatrix, severity

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INTRODUCTION

Coffee rust, the most devastating disease affecting coffee crops worldwide, causes annual production losses exceeding US$1 billion (Kahn 2019; Secretaría de Agricultura y Desarrollo Rural 2018). In Mexico, after its first report in 1981, the disease became a widespread epidemic in 2013 and 2014, resulting in losses of up to 50% of production (Food and Agriculture Organization of the United Nations, FAO 2015).

In response to this threat, various disease control programs have been implemented, including the renewal of coffee plantations with resistant varieties (Ejea 2009). However, it has been noted that some of the introduced cultivars have been losing this trait over time, because of the pathogen's adaptation breaking down the resistance barrier.

To monitor coffee rust and provide early warning systems and damage severity assessments, fixed and mobile plots have also been established (Organización de las Naciones Unidas para la Alimentación y la Agricultura, FAO 2015; Secretaría de Agricultura, Ganadería Desarrollo Rural Pesca y Alimentación 2013). The use of agrochemicals has been another tactic. Notwithstanding the dangers this poses (Couttolenc-Brenis *et al.,* 2021), it is crucial to stress that coffee plantations offer significant environmental services, including the production of oxygen, mitigation of climate change, reduction of erosion, and conservation of biodiversity, supporting a diverse range of species (Nava and Hernández 2017). Despite their efficacy, excessive use of fungicides can damage soil and vegetation (Loland and Singh 2004) and even cause pathogen resistance (Organización de las Naciones Unidas para la Alimentación y la Agricultura, FAO 2015), jeopardizing the sustainability of production and ecosystem health.

Salicylic acid (SA) is a potent phytohormone and signaling molecule that plays a key role in plant immune response to biotic stress, inducing systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Loland and Singh 2004; Mishra *et al.,* 2024). As a result, it is becoming a viable substitute and has proven effective in protecting various crops from biotrophic and hemibiotrophic pathogens (Ullah *et al*., 2023; Sillero *et al.,* 2012). Its exogenous application has triggered defense mechanisms in plants like wheat against septoria (Zymoseptoria tritici) (Ramos Cabrera *et al.,* 2020); in bananas against Colletotrichum sp. in banana fruits after harvest (Berumen-Varela *et al*., 2015).

Applying SA exogenously can increase a plant's resistance to bacterial, viral, and fungal diseases (Tannuri *et al.,* 2021). By modifying immune-related genes, this substance also enhances plant growth and development (Singh 2023). Given the magnitude of economic losses and the risks associated with conventional control methods for coffee rust, it is essential to explore alternatives such as the use of SA. The objective of this research is to determine the optimal concentration of SA as a bioregulator to strengthen the systemic resistance of coffee plants to coffee rust. By investigating its effectiveness in key commercial varieties such as Tipica and Geisha, we seek to develop more environmentally friendly disease management strategies, contributing to the long-term sustainability of coffee production.

MATERIAL AND METHODS

Location

The study was conducted from March to April 2023 at the Faculty of Agronomy of the Universidad Veracruzana, located in Xalapa, Veracruz, at coordinates 19° 30' 96"N and 96° 55' 06" W. The average temperature at the 1,365-meter-high research site is 18°C.

Fifty plants of the "Tipica" variety that were five months old after germination were chosen for this study. We also selected an equal number of four-month-old "Geisha" variety plants. The "Alarcón" nursery, located at 19° 25' 29.70" N and 96° 6' 58.90" W, in the "Ejido San Marcos de León," Veracruz, Mexico, at an elevation of 1,115 meters above sea level, provided the plant materials. The selection criteria ensured that the plants were robust and healthy, free of rust disease symptoms, and untreated with fungicides.

Determination of the AS effect on systemic acquired resistance of coffee plants during rust infection

The fully randomized block design consisted of ten plants of the rust-prone "Tipica" variety and an equal number of plants of the rust-resistant "Geisha" variety (Tannuri *et al.*, 2021). To track them and assess the severity of the disease on three different dates, each plant had three leaves marked (Table 1). Before the pathogen inoculation, the initial assessment was completed at the start of the experiment. Twenty-six days after infection, the second assessment was conducted, and fifty days after inoculation, the third. Weeds were controlled by hand during the study, and irrigation was used as needed.

Three SA treatments (0.5, 1, and 1.5 mM) were used. On the day of spraying, the SA solutions were made in distilled water, beginning with a concentration of 2.0 mM SA. On days 21, 14, and 7, SA was sprayed prior to the pathogen inoculation. A hand sprayer was used to inoculate the pathogen on day 0 (Ramos Cabrera *et al.*, 2020; Sillero *et al.*, 2012). SA sprays were created at 7-, 14-, and 21-days following pathogen infection to disrupt the pathogen cycle (Cerna-Chávez *et al.*, 2019). Water was used as a negative control to prevent drift contamination, and the recommended dose of 3.7 gL^{-1} copper oxychloride (García *et al.,* 2021) was applied on day 21 before pathogen inoculation (Organización de las Naciones Unidas para la Alimentación y la Agricultura, FAO 2001).

When the plants were sprayed, they were covered with plastic. For inoculum preparation, leaves with abundant rust pustules were collected in the "Ejido San Marcos de León". A total of 70 to 100 rust-infected leaves were collected for inoculum preparation. Using a camel brush, the rust pustules were collected in sterile tubes. The inoculum was then diluted to 0.5 $\mu g\ mL^{-1}$ at the time of inoculation (Pereira *et al.*, 2012) and kept in microtubes at -20°C for a maximum of 48 hours (Pale-Ezquivel *et al.*, 2024). This was then applied to each of the treatment blocks. The spraying was carried out so that the drops reached the underside of the leaf.

Table 1. Modified severity scale to establish rust damage based on the technique of the "Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria (2016)

Category	Severity (%)	Reference image
0	0%	
1	1% Chlorotic spot visualization	
2	From 2 to 6%.	
3	From 7 to 19%	
4	From 20 to 44%.	
5	From 45% to 69	
6	From 70 to 84%	
7	From 85% to defoliation	

Evaluation of rust severity

A modified seven-grade scale was used based on the methodology proposed by the "Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria" (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria, 2016). A seventh category corresponding to the defoliation effect was included (Table 1).

This scale measures the progress of the disease by considering the area of the leaf's underside and assessing the percentage of disease present. The first category is 0, which indicates a healthy leaf with no visible signs of disease. The subsequent categories (1, 2, 3, and 4) correspond to the development of typical disease symptoms.

Category 1 is characterized by the development of chlorotic spots on the leaves, which turn yellow. In category 2, there is evidence of disease progress ranging from 2 to 6%; small pustules are visible. In category 3, disease progress is observed in the range of 7 to 19%; a yellowish powder corresponding to the disease spores can be seen. In the later categories (5, 6, and 7), the leaves exhibit different stages of disease progression. In category 7, leaf senescence occurs due to nutrient deficiency and weakening of the leaves.

Statistical analysis

Descriptive analyses of the data were performed using tables and stacked bar charts. The probability of rust severity reaching or surpassing a particular level was then estimated by fitting an ordinal logistic regression model with cumulative logit link (Angarita-Fonseca 2015; Bombelli *et al.*, 2013), taking into account the effects of treatments and coffee varieties. Strong evidence of the relationship between the explanatory variables (varieties and treatments) and the likelihood of observing greater severity was provided by the odds ratios. The analysis was performed in R statistical software (version 2023.03.0 Build 386) with the support of the MASS, VGAM, and Brant libraries.

RESULTS AND DISCUSSION

The results of the degree of progress and the variability in the frequency of rust severity are shown in Table 2. It was observed that, from the second evaluation, a reduction in the number of healthy leaves was observed in all treatments due to the progress of the disease, placing them in the different severity categories, referring to the damage caused to the leaf area of the coffee leaves (Table 2; Fig. 1 and 2).

It draws attention to the significant increase in the number of leaves affected for the same category and treatment across the different evaluation dates. The negative control (water) and 1.0 mM SA treatments had the most affected leaves in the most recent evaluation, with 10 and 16, respectively. However, 30 and 27 leaves remained healthy in the 1.5 mM SA treatment and the positive control (fungicide), respectively.

Table 2. Progress in the frequency distribution of severity observed in coffee leaves

No. Assessment	Category: % severity	Treatments				
		Water (C-)	0.5 mM SA	1.0 mM SA	1.5 mM SA	Fungicide (C+)
1 2 3	0: 0% no damage	60 19 10	60 32 22	60 26 16	60 43 28	60 45 30
2 3	1: 1 chlorotic spot	11 10	7 9	11 8	5 11	3 1
2 3	2: from 2 and up to 6%	15 10	6 5	8 7	6 6	3 7
2 3	3: From 7 and up to 19%	10 12	9 7	9 9	3 8	6 6
2 3	4: From 20 to 44%.	2 11	5 8	5 13	3 2	3 11
2 3	5: From 45% to 83%.	3 0	1 5	1 3	0 4	0 3
2 3	6: From 70 to 84%	0 2	0 3	0 0	0 0	0 2
2 3	7: From 85% to defoliation	0 5	0 1	0 4	0 2	0 0

It's interesting to note that the 1.5 mM SA treatment preserved 27 of the 60 leaves evaluated in the prior reading. Furthermore, 11 leaves in category 1 were impacted, suggesting that the rust damage in the leaves treated with this method was not particularly severe. The leaves from this treatment were categorized using a higher severity progression (Table 2). The control treatment (C-) water presented ten affected leaves in the last reading in category 1; this was the highest value observed; likewise, in categories 2 and 3 there were 10 and 12 leaves, respectively. In Category 2, the 0.5 mM SA treatment showed 5 affected leaves, while the C+ (fungicide) treatment showed 7 leaves (Table 2).

Similarly, in category 3, (C-) had twelve damaged leaves, (C+) had six, and the 1.0 mM SA treatment had nine leaves. The 1.0 mM SA treatment had 13 damaged leaves in category 4, which was comparable to the (C+) treatment's 11 damaged leaves. With two affected leaves, the 1.5 mM SA treatment produced the lowest value (Table 2). Five leaves in category 5 were found to be affected by the 0.5 mM SA treatment in the most recent reading, whereas the control group (C-) showed no signs of leaf damage. On the last observation date in category 6, it was observed that the 0.5 mM SA treatment had three diseased leaves, while the 1.0 mM and 1.5 mM SA treatments showed no diseased leaves (Table 2). (C-) with five leaves and 1.0 mM of SA with four leaves were the treatments that had the greatest effect in category 7, which corresponds to leaf loss; the (C+) treatment did not exhibit any damage. At the conclusion of the study, it was found that (C+) had the greatest number of healthy leaves (30), while 1.5 mM SA had the closest record (27); in both situations, a variable efficacy is indicated, being effective in reducing some severities but not in others (Table 2).

Table 3. Rust symptoms following inoculation in the varieties "Típica" and "Geisha," corresponding to treatment 1.5 mM SA.

Evaluation dates	**Varieties**	
	TIPICA	**GEISHA**
1: day 0 start of the experimentation		
2: day 26 after inoculation		
3: day 50 post-inoculation		

It should be mentioned that each of the two varieties, “Tipica” and “Geisha,” had 150 healthy leaves (experimental units) at the start of the study, which corresponded to the total of all the treatments; these were distributed in accordance with the severity scale used in this study after infection and as the rust progressed. The damage inflicted by the 1.5 mM SA treatment on the two study varieties during the three evaluation dates is displayed in Table 2.

At the end of the last evaluation, the “Geisha” variety retained 76 healthy leaves in total, in contrast to the “Tipica” variety, which retained only 29 leaves. In both cases, the highest number of diseased plants were located between categories 1 and 4 (Fig. 2, Table 3).

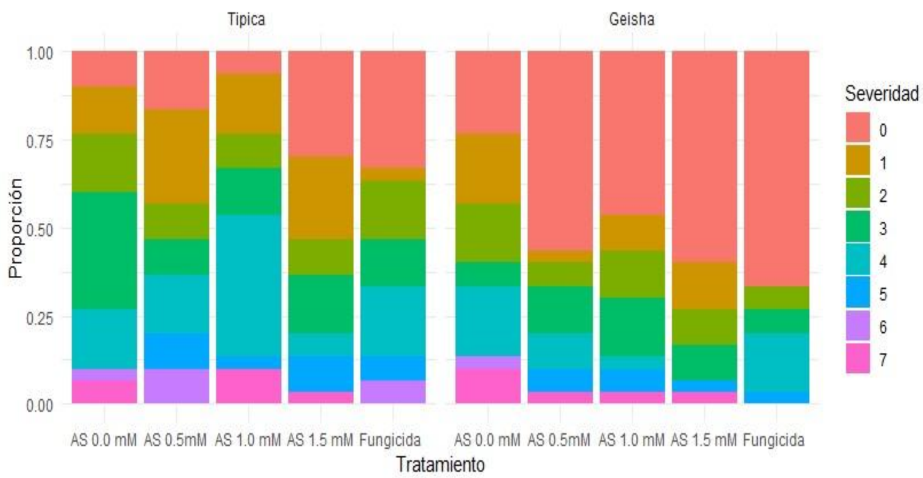

Fig. 2. Distribution of rust severity by variety and SA treatments

The number of diseased leaves observed in the last evaluation for the "Tipica" variety was 121 while for the "Geisha" variety, there were around 74 affected leaves. It should be noted that more than 50% of the affected leaves were distributed among categories 1, 2, 3, and 4 (Fig. 2, Table 4).

Table 4. Frequency distribution by variety, rust severity and date of assessment

No. Assesment	Category: % severity	Varieties Típica	Geisha
1	0: 0% no damage	**150**	**150**
2		56	109
3		29	76
2	1: 1 chlorotic spot	31	6
3		25	14
2	2: from 2 and up to 6%	17	21
3		19	16
2	3: from 7 and up to 19%	32	5
3		26	16
2	4: from 20 to 44%.	12	6
3		30	15
2	5: from 45% to 83%.	2	3
3		9	6
2	6: from 70 to 84%	0	0
3		6	1
2	7: from 85% to defoliation	0	0
3		6	6

In the first categories, from 1 to 4, the highest number of diseased plants was distributed for both coffee varieties.

The plants treated with 1.5 mM SA were characterized by the distribution of affected leaves. More than 50% of the experimental units fell into the initial categories of the severity scale, indicating a significant reduction in rust progression. As a result, the severity of symptoms observed in this treatment was lower than in the other treatments. Additionally, many of the affected leaves exhibited rust at the infected sites, which further limited the spread of the disease (Fig. 3). This effect can be attributed to the activation of reactive oxygen species (ROS) due to the accumulation of SA, which triggered a restriction in the area affected by the infection.

Fig. 3. Oxidation effect observed in the rust-affected sites, which may have contributed to slowing the progression of the disease

The reference categories for the ordinal logistic regression (OLR) model were the fungicide-corresponding positive control treatment (C+) and the "Tipica" variety. Plants treated with 0.0 mM SA significantly increased the probability of finding affected leaves in high-severity categories compared to plants treated with SA and fungicide. With an odds ratio (exponent) of 2.375 and an ordinal regression coefficient (ORC) of 0.8650 ($p=0.00908$) as a positive and significant result, it was clear that this treatment increased the probability of severity by 1.37 times when compared to the applied fungicide (Table 4). Despite being 0.16 times more susceptible than the fungicide, the 1.5 mM SA treatment showed a (ORC) of -0.1748, a non-significant result ($p=0.60530$), indicating that this treatment significantly protected the plant from rust, which is why it does not present a high severity index (Table 5).

The “Geisha” variety's ORC was -1.0835 ($p=4.40e-07$), which is highly significant and negative. This indicates that the “Geisha” variety is less prone to rust, meaning that it has a much lower chance of falling into a higher severity category than the "Tipica" variety (Table 5; Figs. 1 and 2). In comparison to the "Tipica" variety, the “Geisha” variety decreased severity by 66.2%, according to the odds ratio of 0.338 (Table 5; Figs. 1 and 2).

The main conclusions of this study indicate that spraying a 1.5 mM solution of salicylic acid (SA) on the "Tipica" and "Geisha" coffee varieties every seven days resulted in effects that were statistically comparable to those obtained with copper oxychloride. A common contact, preventive, and protective fungicide used to lessen rust damage to coffee crops is copper oxychloride.

Table 5. Rust severity in SA treatments compared to that observed with the fungicide (C+)

SA Treatments (mM)	OCR*	P (≤0.05)	Odds ratio (exponent)	Severity compared to (C+)
0.0	0.8650	0.00908	2.375	1.37 times more susceptible
0.5	0.3305	0.31998	1.392	0.39 times more susceptible
1.0	0.7119	0.03165	2.038	1.03 times more susceptible
1.5	-0.1748	0.60530	0.840	0.16 times more susceptible

*ORC (Ordinal Regression Coefficient)

Plants treated with 1.5 mM SA were 0.16 times more susceptible to the disease than those treated with the fungicide, indicating that this treatment offers protection that is very similar to that of copper oxychloride, with the added benefit of avoiding environmental contamination.

The ability of salicylic acid (SA) to trigger different defensive responses against pathogens in a variety of crops has attracted a lot of attention in recent years. It is acknowledged as a crucial signaling molecule in plant defense responses to these pathogens. According to research, SA inhibits the catalase enzyme and, via a variety of mechanisms, increases systemic and local disease resistance. Because of this, SA and its derivatives have been used extensively to create systemic acquired resistance to various pathogens. Similar to our findings, Dieryckx *et al*., (2015) discovered that there was inhibition of Botrytis cinerea mycelial growth; however, methyl salicylic acid was the most effective in preventing fungal growth, followed by acetylsalicylic acid and salicylic acid. They conclude that there is circumstantial evidence for the role of SA as a signaling molecule to induce resistance.

Tannuri *et al*., (2021) analyzed the response of seven-year-old coffee plants (in the production stage) of the “Topazio” variety treated with SA at a concentration of $0.15gL^{-1}$, with the foliar application once a month for five months, and found that the incidence and severity of rust were reduced from 36.3 to 54.7% when compared with untreated plants, results similar to those obtained in our study.

In the control of septoria, or wheat leaf spot, Ramos-Cabrera *et al.,* (2020) observed that plants treated with 1 mM SA for two years reduced the necrotic area, even with lower values than the control in the first four leaves; however, in terms of pycnidia cover, the results were not significant compared to the control. Similarly, SA at 0.5 mM sprayed on detached rice leaves inhibited Rhizoctonia solani fungal biomass growth and lesion formation (Sillero *et al.,* 2012), according to Kouzai *et al.,* (2018) found that broad bean plants treated preventively with 5 mM SA had less Uromyces viciae-fabae rust infection than the control.

In order to confirm the effect of SA in inducing systemic resistance in *C. arabica* against the coffee cherry disease Colletotrichum kahawae (Alemu *et al.*, 2019) found that applying 10 mM SA reduced the disease by 50–65% in high and moderately susceptible coffee hypocotyls. Our results, which demonstrated that plants treated with 1.5 mM SA had less severe rust, are corroborated by these studies. There are two phases to systemic acquired resistance (SAR). The first step is the identification of an invasive pathogen, which sets off signaling cascades that cause local defense reactions. Salicylic acid (SA) builds up inside cells because of this process. Reactive oxygen species (ROS) levels rise as SA builds up, and genes encoding pathogenesis-related proteins (PR) and the development of the hypersensitive response (HR) are expressed. A type of programmed cell death known as the hypersensitive response takes place to limit the pathogen's area of infection. Chen *et al*., (1993) proposed a mode of action of SA on the basis that it binds to and inhibits the enzyme catalase Loake and Grant (2007). Catalase inhibition activates other reactive oxygen species that seem to be involved in the signaling cascade for the expression of defense genes and may increase the concentration of hydrogen peroxide ($H2O_2$) molecules that may have antibiotic activity against pathogens, according to multiple studies (Rangel-Sánchez *et al.,* 2010).

Breeding programs involving coffee varieties have been developed because H. vastratrix rust poses significant challenges. However, it can take years of research and be very expensive to obtain a cultivar resistant to this disease. It is now recognized that the coexistence of the rust fungus and the coffee plant results in mutual co-evolution, as evidenced by the fungus's virulence towards the plant and the wide genetic diversity of plant resistance to the fungus (Avelino and Rivas 2014). More than 55 rust races have been identified in recent years (Ingelbrecht *et al*., 2023), demonstrating the difficulty of obtaining genetic materials with the resistance genes required for total immunity to this disease.

In coffee production, rust-resistance breeding has been an important tactic. Renovating coffee plantations with rust-resistant cultivars of two species-*Coffea arabica* (60%) and Coffea canephora (40%)—has been one strategy. *C. arabica* is especially prone to rust, and some of its popular varieties, like "Typica," "Bourbon," and "Geisha," are highly valued for their outstanding cup quality. Studying the various cultivars of this species is therefore of great interest. In the early stages of rust infection (germination, appressoria, and haustoria formation), Couttolenc *et al., (*2021) assessed the local response of two cultivars, "Oro Azteca" and "Garnica." They discovered that resistance in "Oro Azteca" was significantly higher than in "Garnica," with 8.2% and 53.3%, respectively, because of increased expression of the pathogen recognition gene CaNDR1 and those linked to SA, such as CaNPR1, CaPR1, and CaPR5.

The "Tipica" variety was more vulnerable to rust, as evidenced by the increased signs and progression of the disease compared to the "Geisha" variety, which is in line with our study's findings. Further research on the expression of these genes in the "Tipica" and "Geisha" varieties would be intriguing.

CONCLUSIONS

The results obtained help confirm the effectiveness of salicylic acid (SA), which, according to previous research, activates the defense mechanisms of coffee plants affected by coffee rust. The study observed a significant reduction in the pathogen's infection rate. More than 50% of the affected leaves were classified in the lowest severity categories, indicating a significant decrease in the spread of coffee rust. Furthermore, the research found that using 1.5 mM of SA can help reduce reliance on agrochemicals for rust control. These chemicals are expensive and can irreparably damage crop health.

Conflict of Interest

The authors declare that they have no financial interests or personal relationships that could have influenced the work presented in this document.

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