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Eco-friendly synthesis and antifungal properties of *N*-salicylhydrazones derivatives

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ABSTRACT: The growing demand for cleaner and more sustainable chemicals has positioned Green Synthesis as an important cornerstone of modern Organic Synthesis. In this context, *N*-salicylhydrazone derivatives, compounds with a broad range of pharmacological potential, were synthesized using four distinct green methodologies: magnetic stirring at room temperature, microwave-assisted reactions, ultrasound irradiation, and mechanochemistry. The synthetic routes avoided the use of toxic solvents, minimized purification steps and afforded high to excellent yields (56.64-94.07%). The chemical structures were elucidated by FT-IR and NMR spectroscopy. The antifungal activity of the derivatives was evaluated against *Fusarium* sp. and *Corynespora cassiicola*, two phytopathogens of significant agricultural relevance. Nine compounds exhibited inhibitory effects, with the 3-nitro-substituted derivative (compound 2) showing the highest % growth inhibition (33.67% for *Fusarium* sp. and 30.77% for *C. cassiicola*). The results demonstrate the potential of *N*-salicylhydrazones as promising chemical structures for the development of alternative antifungal agents. Overall, this study highlights the relevance of clean synthetic methodologies for the advancement of sustainable chemistry and the discovery of bioactive molecules with promising applications in crop protection.
Keywords: salicylhydrazones; *Fusarium* sp.; *Corynespora cassiicola*; green synthesis.

Síntese verde e propriedades antifúngicas de derivados *N*-salicilhidrazônicos

RESUMO: A crescente demanda por processos químicos mais limpos e sustentáveis posiciona a Síntese Orgânica Verde como um pilar importante da Síntese Orgânica moderna. Nesse contexto, derivados *N*-salicilhidrazônicos, compostos com amplo potencial farmacológico, foram sintetizados por meio de quatro metodologias verdes distintas: agitação magnética à temperatura ambiente, reações assistidas por micro-ondas, irradiação por ultrassom e mecanoquímica. As rotas sintéticas evitaram o uso de solventes tóxicos, minimizaram as etapas de purificação e proporcionaram rendimentos de alto a excelente (56,64–94,07%). Os compostos tiveram suas estruturas químicas elucidadas por meio de análises de FT-IR e de RMN. A atividade antifúngica dos derivados foi avaliada contra *Fusarium* sp. e *Corynespora cassiicola*, dois fitopatógenos de grande relevância agrícola. Nove compostos exibiram efeitos inibitórios, com o derivado 3-nitro-substituído (composto 2) apresentando a maior % de inibição de crescimento (33,67% para *Fusarium* sp. e 30,77% para *C. cassiicola*). Os resultados demonstram o potencial das *N*-salicilhidrazonas como estruturas químicas promissoras para o desenvolvimento de agentes antifúngicos alternativos. De modo geral, este estudo destaca a relevância das metodologias sintéticas limpas para o avanço da química sustentável e para a descoberta de moléculas bioativas com aplicações promissoras na proteção de culturas agrícolas.
Palavras-chave: salicilhidrazonas; *Fusarium* sp.; *Corynespora cassiicola*; síntese verde.

1. INTRODUCTION

The increasing environmental concerns and the need for sustainable practices in organic synthesis have led to the rise of green chemistry as a guiding principle for developing safer, more efficient, and environmentally friendly chemical processes. Organic green synthesis is a fundamental component of this movement, focusing on minimizing the use of hazardous reagents and solvents, reducing waste, and enhancing energy efficiency during chemical reactions. This approach not only aims to lower the ecological footprint of chemical production but also seeks to improve the overall safety and cost-effectiveness of manufacturing processes (ANASTAS; WARNER, 2000; GANESH et al., 2021).

The concept of green synthesis has found significant application in various fields, including pharmaceuticals, materials science, and nanotechnology. In particular, green synthesis methods offer a promising alternative to traditional chemical processes, often involving toxic reagents, high energy consumption, and harsh reaction conditions (KHARISSOVA et al., 2019; ABUZEID et al., 2023). Additionally, the integration of sustainable energy sources, such as microwave, ultrasound-assisted reactions, or

mechanochemical techniques, further supports the principles of green synthesis by reducing energy consumption and enhancing reaction efficiency (XIAO et al., 2017; MACHADO et al., 2021; FANTOZZI et al., 2023).

N-salicylhydrazones are scaffolds for the design of novel bioactive molecules that represent a class of azometine compounds with a broad range of pharmacological properties, including anti-inflammatory, antidiabetic, trypanocidal, anticancer, antitubercular, and antifungal properties (BACKES et al., 2014; GLINMA et al., 2015; ALAM et al., 2017; SZKLARZEWICZ et al., 2021; IEQUE et al., 2024). One of their key chemical and pharmacological properties can be attributed to their ability to form stable metal complexes and lead to enhanced biological activities due to the chelation of metal ions, which can improve the stability and bioavailability of the compounds (MATHEW et al., 2011). These compounds can be synthesized by condensation of *N*-salicylhydrazides and carbonyl derivatives (aldehydes or ketones) or salicylaldehyde with different acylhydrazides or their derivatives (MENG et al., 2016).

In terms of antifungal properties, there are a few reports correlating *N*-salicylhydrazones with antifungal activity against *Fusarium* and *Corynespora* species. He et al. (2003) demonstrated that salicylaldehyde salicylhydrazone derivative and its metal complex had a great inhibiting effect on *Fusarium oxysporum vasinfectum* and *Phytophthora capsici* (HE et al., 2003; DI et al., 2019). Cui et al. (2014) synthesized a series of salicyl glycoconjugate hydrazones containing 5-phenyl-2-furan moiety with excellent activity against fungi as *Colletotrichum orbiculare, Fusarium oxysporum, Rhizoctonia solanii,* and *Phytophthora capsici* (CUI et al., 2014).

Considering the resistance to fungicides for microorganisms and the biological properties of some organic substances, the main objective of this study was to synthesize eleven *N*-salicylhydrazones using four different eco-friendly methods and evaluate their antifungal activity against *Fusarium sp.* and *Corynespora cassiicola.* This work aims to contribute to agriculture for more safety and sustainability, decreasing toxicological and ecological risks.

2. MATERIAL AND METHODS

2.1. Instrumental

All chemicals were purchased from the commercial supplier Sigma-Aldrich, Synth, Neon and Dinamica and used as such without further purification. The synthesis was monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 plates, and TLC plates were visualized by exposure to iodine vapors or using potassium permanganate/sodium carbonate stain. The FT-IR spectrum of the compound was recorded in FT-ATR mode on a PerkinElmer spectrometer, UATR Two model. The ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer operating at 400 and 100 MHz, respectively, using TMS as an internal reference.

The compounds were synthesized in a Cristofoli ultrasound equipment operating with a frequency of 42 MHz, in which the reaction flask was placed in an ultrasonic bath containing distilled water. The purities were established by TLC analysis and spectroscopic methods.

2.2. Synthesis of methyl salicylate (MS)

In a 250 mL double-round-bottomed flask were added 6.97 g (50.5 mmol) of salicylic acid (SA), 62 mL of methanol and 2 mL of 98% concentrated sulphuric acid. The reaction was carried out under reflux at 60-70 ºC for 10 hours. The progress of the reaction was monitored by TLC, using hexane (70%) and ethyl acetate (30%) as eluent. After completion of the reaction, the excess of methanol was evaporated, and the resulting mixture was transferred to a separating funnel and extracted with 50 mL of dichloromethane and 50 mL of distilled water. The organic layer was separated and neutralized with 50 mL of a 5% sodium bicarbonate solution and then washed with 100 mL of distilled water. The organic phase was then separated, dried with anhydrous calcium chloride, and then filtered. The excess of solvent was removed using a rotary evaporator, resulting in methyl salicylate (MS) as an oil with a slightly yellow color and a minty aroma. Yield: 65.76% (5.05 g).

2.3. Synthesis of *N*-salicylhydrazide (SH)

In a 100 mL round-bottomed flask, 5.05 g (33.2 mmol) of methyl salicylate and 2 mL of 80% hydrazine hydrate were added, along with enough methanol to solubilize the methyl salicylate (20 mL). The reaction was heated under reflux at approximately 60-70 ºC for 6 hours. The progress of the reaction was monitored by TLC, using ethyl acetate (80%) and methanol (20%) as eluent. After the reaction time had elapsed, the excess solvent was removed by evaporation, and the reaction mixture was transferred to a beaker to crystallize the salicylhydrazide (SH). The SH was obtained as a white solid after washing with methanol/water 1:1. Yield: 76.50% (3.86 g; 25.4 mmol).

2.4. Green synthesis of *N*-salicylhydrazones 1-11

Method A: Synthesis with magnetic stirring at room temperature:

In a 25 mL round-bottomed flask, 1 mmol of salicylhydrazide (152.2 mg) and 1 mmol of the appropriate aldehyde (2-hydroxy-benzaldehyde, 3-nitro-benzaldehyde, benzaldehyde, 4-chloro-benzaldehyde, 4-tert-butyl-benzaldehyde, 2-thiophenecarboxaldehyde, furfural, cinammaldehyde, 4-dimethylamino-benzaldehyde, 4-nitro-benzaldehyde or 4-fluoro-benzaldehyde) were added, along with 5 mL of ethanol and 5 mL of water. The mixture was then stirred magnetically at room temperature for 20 minutes. The reaction mixture was then filtered, and the precipitate was washed with distilled water and dried at room temperature. Yield: 71.84 - 94.07%

Method B: Microwave-Assisted Synthesis:

A mixture of 1 mmol of salicylhydrazide (152 mg), 1 mmol of the corresponding aldehyde, and 1 mL of distilled water was added to a sealed tube. The reactions were irradiated for 1-2 minutes at 700W (80°C), and the solution was poured into ice, and the *N*-salicylhydrazone precipitates were filtered off and dried at room temperature. Yield: 64.60 - 93.03%

Method C: Ultrasound irradiation Synthesis:

A mixture of 1 mmol of salicylhydrazide (152.2 mg), 1 mmol of an appropriate aldehyde, and 20 mL of an ethanol/water mixture (50:50) was added to a 50 mL round-bottom flask. The reaction mixture was irradiated in an ultrasonic bath at 25 °C for 8 minutes until the precipitate formed. The reaction system was then taken to an ice bath to finalize the precipitation of the product. The solid obtained was filtered, washed with distilled water, and dried at room temperature. Yield: 74.33 - 93.82%

Method D: Mechanochemistry with L-proline catalyst: In a mortar, 1 mmol of salicylhydrazide (152 mg), 1 mmol of the corresponding aldehyde (0.5 mL of water was used for solid aldehydes) and approximately 30 mg of *L*-proline were added. The reagents were ground to a paste using a pestle. After 30-40 minutes, the resulting paste was converted into a fine solid. The solid is then removed, washed with water, and the resulting solid is filtered and dried at room temperature. Yield: 56.19 - 86.85%. ^{1}H and ^{13}C NMR spectra data for all derivatives were recently reported in Ieque et al. (2024).

2.5. Antifungal activity

The filamentous phytopathogenic fungi *Fusarium* sp. 9839 and *C. cassiicola* CMES 1802 were kindly donated by Dra. C.D.S. Seixas of the Plant Pathology Area at Embrapa Soybeans and Dr. S.M.T.P.G. Carneiro of Paraná Rural Development Institute (IDR-Paraná), Agronomic Institute of Paraná, IAPAR, Londrina, Brazil, respectively. The fungi were preserved in potato dextrose agar (PDA) at 4°C and subcultured on PDA plates (Difco). The *in vitro* antifungal efficacy of N-salicylhydrazones was evaluated by measuring the radial mycelial growth inhibition of *Fusarium* sp. (9839) and *C. cassiicola* on solid media. The compounds were tested at concentrations of 50 and 100 mg mL^{-1} following the method outlined by Quiroga, Sampietro and Vattuone (2001).

The samples were dissolved in a solution of dimethyl sulfoxide (DMSO, 10%, v/v) and Tween 80 (0.5%, w/v). Aliquots of 0.8 mL of each compound's solution were incorporated into molten PDA medium, which was then dispensed into Petri dishes.

Agar disks (8 mm) containing fungal cultures from both pathogenic strains were placed at the center of the plates and incubated at 28°C. Growth experiments were conducted in triplicate for *Fusarium* sp. (9839) and *C. cassiicola*. For comparison, a commercial fungicide, Frowncide 500 SC® (containing 500 g of active ingredient Fluazinam per liter), was used as a positive control at 0.01 µg mL^{-1}. A negative control was prepared using the solvent mixture (DMSO and Tween 80) without samples or fungicide. After 5 days of incubation, when mycelial growth in the control plates reached the edge of the Petri dish, the radial growth (cm) of fungal colonies was measured. The percentage of growth inhibition (GI) was calculated using Equation 1:

$$GI\ (\%) = MGC - (MGPE/MGC) \times 100 \qquad (01)$$

where: MGC is the mycelial growth in the negative control, and MGPE is the mycelial growth in the presence of *N*-salicylhydrazones.

2.6. Statistical analysis

The antifungal activity data were presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using analysis of variance (ANOVA) to assess the significance of the observed differences. Multiple comparisons between treatments were conducted using Tukey's test.

A mixed-effects model was used to evaluate the effects of treatment (PINHEIRO; BATES, 2000). Random effects were defined as the compounds C1 through C9, while fixed effects included fungal species (*Fusarium* sp. and *C. cassiicola*), concentration levels (50 and 100 mg mL^{-1}), and their interaction. The response variable was the percentage of mycelial growth. The model used for this analysis is described by Equation (2):

$$y_{ijk} = \mu + u_k + \beta_1 F_i + \beta_2 C_j + \beta_{12}(F \times C)_{ij} + \varepsilon_{ijk} \qquad (02)$$

where: y_{ijk} represents the response variable for the *ijk*-th observation, μ is the overall mean, F_i $(i = 1,2)$ represents the fixed effect of the *i*-th fungus, C_j $(j = 1,2)$ represents the fixed effect of the *j*-th concentration, $(F \times C)_{ij}$ is the effect of the interaction between the *i*-th fungus and the *j*-th concentration, u_k $(k = 1, \ldots, 9)$ is the random effect associated with the *k*-th compounds, $\beta's$ are unknown regression coefficients and ε_{ijk} is the error term of the *ijk*-th observation

The model was fitted using the *nlme* package in R, with parameter estimation based on restricted maximum likelihood (REML). The fixed effects were tested at a 5% significance level ($p < 0.05$). All analyses were performed in R (R Core Team, 2013).

3. RESULTS

N-salicylhydrazones were obtained from salicylic acid (SA) in a three-step synthetic procedure. According to our synthesis protocol (Figure 1), Methyl salicylate (MS) was initially synthesized by a traditional Fisher esterification reaction using SA, methanol and concentrated sulfuric acid as a catalyst. Subsequently, salicylhydrazide (SH) was obtained from the condensation reaction of MS with hydrazine hydrate. Finally, the *N*-salicylhydrazones (1-11) were synthesized using four different eco-friendly synthetic methods: stirring at room temperature, microwave-assisted synthesis, ultrasound irradiation synthesis and mechanochemistry. Only non-toxic solvents were used in all synthesis procedures, and the products were obtained in good to optimal yields in short-time reactions. In all the methods, it was not necessary to purify the obtained products.

Their chemical structures were confirmed by spectroscopic data (FT-IR, ^{1}H and ^{13}C NMR. Below we reported the data for derivative 2-hydroxy (1), 3-nitro (2), 4-chloro (4) and thiophenyl (6). The other *N*-salicylhydrazones derivatives are reported in Ieque et al. (2024).

- Compound 1: (*E*)-2-hydroxy-N'-(2-hydroxybenzylidene)benzohydrazide; White solid FT-IR (ATR): υ (cm^{-1}) = 3185 (O-H); 1662 (C=O); 1606 (C=N); 1374 (C_{ar}-O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 12.04 (s, O–**H**, 1H); 11.79 (s, O–**H**, 1H); 11.22 (s, N–**H**, 1H); 8.69 (s, **H**C=N, 1H); 7.90(d, J=8.0 Hz, C-**H**, 1H); 7.57 (d, J=8.0 Hz, 1H); 7.48~7.44 (t, 1H); 7.34~7.30 (t, 1H); 7.01~6.92 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) = 164.99 (**C**=O); 159.47 (**C**-O); 157-.99(**C**-O); 149.48 (**C**=N); 134.46; 132.11; 129.96; 129.06; 119.88; 119.51; 119.09; 117.77; 116.93; 116.10.
- Compound 2 : (*E*)-2-hydroxy-N'-(3-nitrobenzylidene)benzohydrazide White solid FT-IR (ATR): υ (cm^{-1}) = 3245 (O-H); 1633 (C=O); 1607 (C=N); 1524 (NO2); 1345 (C-O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 12.04 (sl, N–**H**, 1H); 8.57 (s, **H**C=N, 1H); 8.56 (s, 1H); 8.28 (d, J=4 Hz, 1H); 8.18 (d, J=8 Hz, 1H); 7.90 (d, J=8 Hz, 1H); 7.79~7.75 (t, 1H); 7.47~7.44 (t, 1H); 7.01~6.95 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) =165.22 (**C**=O); 159.22 (**C**-O); 148.70 (**C**-NO_2); 146.51 (**C**=N); 136.52; 134.36; 133.91; 130.97; 129.41; 124.87; 121.55; 119.46; 117.74; 116.74.

- Compound 4 : (*E*)-N'-(4-chlorobenzylidene)-2-hydroxybenzohydrazide; White solid FT-IR (ATR): υ (cm^{-1}) = 3250 (O-H); 1630 (C=O); 1601 (C=N); 1378 (C-O); 747 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 11.90 (s, N–**H**, 1H); 11.79 (sl, O–**H**, 1H); 8.46 (s, **H**C=N, 1H); 7.90~7.88 (dd, J_{ortho}=6,2 Hz, J_{meta}=1,76 Hz, 1H); 7.78 (d, J=8 Hz, 2H); 7.54 (d, J=8 Hz, 2H); 7.47~7.43 (m, 1H); 7.00~6.95 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) = 165.20 (**C**=O); 159.33 (**C**-O); 147.80 (**C**=N); 135.21; 134.33 (**C**-Cl); 133.55; 129.45; 129.34; 129.14; 119.49; 117.73; 116.50.
- Compound 6 : (*E*)-2-hydroxy-N'-(thiophen-2-ylmethylene)benzohydrazide; White solid FT-IR (ATR): υ (cm^{-1}) = 3248 (O-H); 1625 (C=O); 1606 (C=N); 1325 (C-O); ^{1}H NMR (DMSO-d6, 400 MHz): δ (ppm) = 11.81 (s, N–**H**, 1H); 8.63 (s, **H**C=N, 1H); 7.84 (d, J=4 Hz, 1H); 7.66 (d, J=4 Hz, 1H); 7.46 (s, 1H); 7.42 (s, 1H); 7.14 (t, J=4 Hz, 1H); 6.98~6.93 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) = 165.04 (**C**=O); 159.11 (**C**-O); 144.34 (**C**=N); 139.10; 134.35; 131.94; 131.94; 129.81; 129.02; 128.48; 119.63; 117.69; 116.44.

Table 1. Yields of eco-friendly synthesis for *N*-salicylhydrazones derivatives 1-11.
Tabela 1. Rendimentos obtidos para síntese verde dos derivados *N*-salicilidrazonas 1-11.

Method Compound	A	B	C	D
1	87.57	87.53	80.45	57.52
2	91.96	87.96	74.33	79.52
3	86.86	80.04	76.58	60.22
4	75.71	77.97	85.15	72.06
5	94.07	91.29	83.52	58.06
6	72.62	64.60	56.64	56.19
7	89.31	93.03	89.76	76.42
8	85.05	74.26	93.82	86.85
9	80.28	78.93	76.87	60.24
10	78.49	85.37	93.44	77.65
11	71.84	91.49	75.11	65.53
Means	83.07	79.32	80.52	64.39

A: magnetic agitation-assistant synthesis; B: Microwave irradiation-assistant synthesis; C: Ultrasound irradiation-assistant synthesis; D: Mechanochemistry.
A: Síntese assistida por agitação magnética; B: Síntese assistida por irradiação de micro-ondas; C: Síntese assistida por irradiação ultrassônica; D: Mecanoquímica.

According to Table 1, the most significant yield was obtained for Methods A and C with media yield values of 83.07% and 83.27%, respectively. For method A, the values range from 71.84% for compound 11 to 91.96% for derivative 2, whereas for method C, the values range from 74.33% for compound 2 to 93.82% for compound 8. Method A yields ranged from 71.84% (compound 11) to 94.07% (compound 5), highlighting the robustness of this procedure under mild conditions. Similarly, Method C exhibited excellent performance for several derivatives, reaching up to 93.82% for compound 8. In contrast, Method D exhibited significant variability, with yields as low as 56.19% (compound 6), although in some cases, such as compound 8 (86.85%), satisfactory results were achieved.

According to Table 2, nine compounds showed some antifungal activity against *C. cassiicola* and *Fusarium* sp. Among them, compound 2 (3-nitro) was the most effective with % GI growth of 26.53% (50 μg mL^{-1}) and 33.67% (100 μg mL^{-1}) for *Fusarium* sp., and of 24.62 % (50 μg mL^{-1}) and 30.77% (100 μg mL^{-1}) for *C. cassiicola*. Compounds 10 and 11 did not have activity against the strains tested.

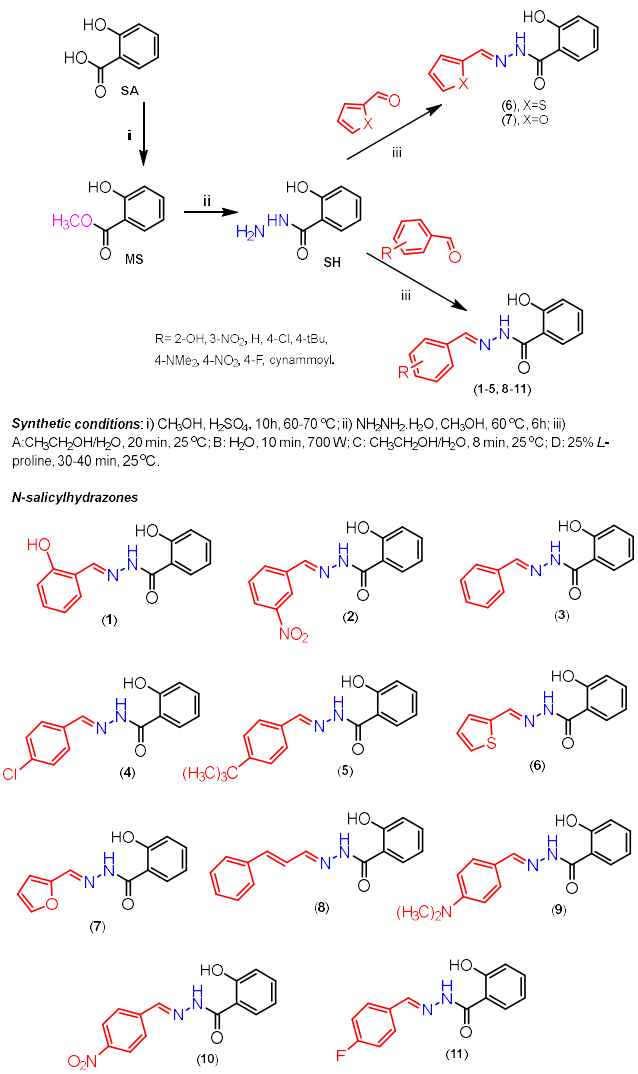

Figure 1. Synthetic route for *N*-salicylhydrazone and its precursor.
Figura 1. Rota sintética para *N*-salicilidrazona e seu precursor.

From Table 3, it was observed that the interaction between concentration and fungal species did not present a significant effect on the percentage of inhibition. Given this result, it was possible to evaluate the main effects independently. In this context, the fungal species showed no significant influence, whereas the compound concentration proved to be relevant. Specifically, increasing the concentration from 50 to 100 mg mL^{-1} resulted in a 7.29% (Table 3) increase in the inhibition percentage.

Regarding the predicted values of the random effects shown in Figure 2, it was found that compounds 2, 4, 7, and 9 (C2, C4, C7, and C9) exhibited positive effects, indicating performance above the estimated overall mean (10.47%), with compound 2 standing out with an effect of 6.77. In contrast, compounds 1, 4, 5, 6, and 8 (C1, C3, C5, C6, and C8) presented negative effects, reflecting performance below the estimated overall mean, with compound C8 showing the most pronounced effect (-6.76).

Table 2. Growth inhibition (GI, %) of *Fusarium* sp. and *Corynespora cassiicola* by *N*-salicylhydrazones.
Tabela 2. Inibição do crescimento (GI, %) de *Fusarium* sp. e *Corynespora cassiicola* por *N*-salicilidrazonas.

Compound	µg/mL	*Fusarium* sp.		*Corynespora cassiicola*	
		Radial mycelial growth (cm)	GI (%)	Radial mycelial growth (cm)	GI (%)
1	50	3.57 a,b,c	27.14	5.47 b,c	15.85
	100	3.52 b,c,d,f,l	28.16	5.2 h,l	20.00
2	50	3.60 a,b,d,e	26.53	4.90 a,d	24.62
	100	3.25o	33.67	4.50	30.77
3	50	4.00	18.37	5.70 e,f	12.31
	100	3.52 b,c,d,f,l	28.16	4.86 a,g,m	25.23
4	50	3.60 b,f,g	26.53	4.80 d,g,h, i	26.15
	100	3.35 o,p	31.63	4.70 h	27.69
5	50	3.81 i,k,m	22.24	5,72 j,k	12.00
	100	3.70 e,g,q	24.49	4,90 a,i,m	24.62
6	50	3.85 h,j,k	21.43	5.53 a,b	14.92
	100	3.80 h,m,q	22.45	5.32 a,l	18.15
7	50	4.48 n	8.57	5.80 a,e,j	10.77
	100	3.42 l,p	30.2	5.56 a,c	14.46
8	50	4.80 h	2.04	6.30	3.08
	100	4.70 h	4.08	6.00	7.69
9	50	4.52 n	7.76	5.80 a,f,k	10.77
	100	3.90 i,j	20.41	5.31 a,h	18.31
FRO		0.90	81.63	1.40	78.46
NC		4.90	-	6.50	-

µg mL^{-1} = concentration; GI = Growth Inhibition; a-p: Means followed by the same letter, in each column, do not differ statistically (Tukey, p > 0.05). FRO = Frowncide 500 SC®: fungicide (5 ppm). NC = Negative control used to calculate inhibition: DMSO 10%, 0.5% of Tween 80. Compounds 10 and 11 did not have antifungal activity against the strains tested.
µg mL^{-1} = concentração; GI = Inibição do Crescimento; a-p: Médias seguidas pela mesma letra, em cada coluna, não diferem estatisticamente (Tukey, p > 0,05). FRO = Frowncide 500 SC®: fungicida (5 ppm). NC = Controle negativo usado para calcular a inibição: DMSO a 10% e Tween 80 a 0,5%. Os compostos 10 e 11 não apresentaram atividade antifúngica contra as cepas testadas.

Table 3. Model-estimated parameters for fungal inhibition by species and concentration (linear random-effects model).
Tabela 3. Parâmetros estimados pelo modelo de inibição fúngica por espécie e concentração (modelo linear de efeitos aleatórios).

Parameter	Estimative	Standard error	*p*-value
Intercept	10.47	2.16	<0.01**
Fungal species	2.43	2.05	0.25^{ns}
Concentration	7.29	2.05	<0.01**
Interaction	-0.39	2.89	0.89^{ns}

ns = indicates a non-significant difference; ** denotes statistically significant differences at the 1% significance level.
ns = indica uma diferença não significativa; ** denota diferenças estatisticamente significativas ao nível de significância de 1%.

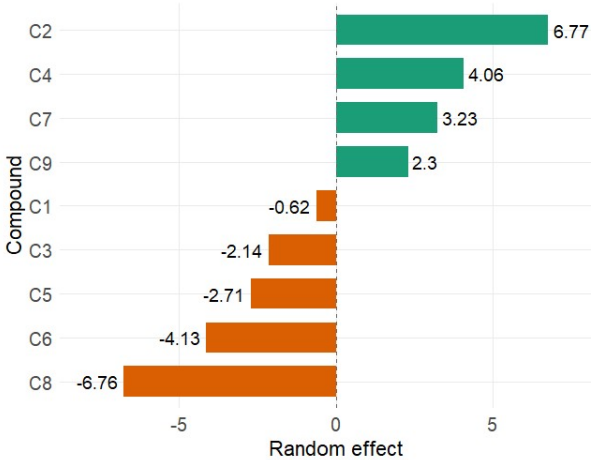

Figure 2. Compounds' influence on average inhibition.
Figura 2. Influência dos compostos na inibição média.

4. DISCUSSION

The methods for obtaining *N*-salicylhydrazones derivatives may be traditionally based on conventional synthesis methods employing toxic solvents, hazardous reagents, long-time reactions, and prolonged heating (BELYAEVA et al., 2022). However, recent advancements in green chemistry have led to the development of eco-friendly conditions for the synthesis of these derivatives to minimize environmental impact and promote sustainability while maintaining high efficiency and selectivity. Recently, several studies have looked at the use of "green" synthetic methodologies to obtain acylhydrazone-based molecules with good yields and short-time reactions (XIAO et al., 2017; ZHAO et al., 2018; SIMIJONOVIĆ et al., 2023). In previous work, our research group has also been able to obtain *N*-acylhydrazone derivatives (3,5-dinitrobenzoylhydrazones and 4-isonicotylpyridineacylhydrazones) using ultrasound irradiation, obtaining good results in terms of yield, reaction time and purity (VALVERDE et al., 2022; SAMPIRON et al., 2023).

Spectroscopic data further confirmed the successful synthesis of the *N*-salicylhydrazones. The FT-IR spectrum of compound 1 exhibited characteristic absorption of bands for the acylhydrazone moiety, including the C=O stretching at 1662 cm^{-1} and the imine C=N vibration at 1606 cm^{-1}. In the ^{1}H NMR spectra, the singlet observed at δ 8.69 ppm was consistent with the signals of the imine proton. The chemical shifts at δ 12.04, 11.79, and 11.22 ppm were attributed to two phenolic and one amide proton, confirming the presence of hydrogen linked to electronegative atoms (NH and OH bonds). The chemical shift of the imine moiety was used to

confirm the synthesis of all *N*-salicylhydrazones and was observed around δ 8.32 to 8.69 ppm in ^{1}H NMR spectra.

Among the four eco-friendly synthetic methods, magnetic stirring at room temperature (Method A) and ultrasound irradiation (Method C) provided the better results on average yields (83.07% and 80.52%, respectively). These results suggest that mild mixing conditions and ultrasonic energy are particularly efficient in promoting the condensation of SH with different aldehydes. Microwave-assisted synthesis (Method B) also afforded satisfactory yields (79.32%), supporting previous reports that microwave irradiation accelerates reactions by promoting uniform heating and reducing activation energy (BOUBEKRI et al., 2024). In contrast, mechanochemistry (Method D) displayed the lowest overall performance (64.39%), likely due to the limited mobility of reactants in the solid state. However, for some derivatives (e.g., compound 8, 86.85%), good yields were obtained. In this method, *L*-proline was used as an organocatalyst based on the use of this compound as a catalyst in reactions involving imine and enamine group formation. This organocatalyst is commonly used in aldolic, Mannich and Michael-type reactions and is often chosen for its abundance and low cost, differentiating it from other catalysts (RODRIGUES et al., 2022).

Regarding the antifungal assay (Table 2), nine of the synthesized compounds tested showed some antifungal activity against *C. cassiicola* and *Fusarium* sp. Among them, Compound 2 (3-nitro) was the most effective against both tested strains.

C. cassiicola is a widely distributed necrotrophic fungus capable of infecting various crops and causing substantial losses in agricultural production (RONDON; LAWRENCE, 2021; PENG et al., 2025). Controlling this pathogen is challenging due to its resistance to conventional fungicides, making it essential to search for new antifungal compounds. Fungi of the genus *Fusarium* include endophytic fungi capable of producing bioactive metabolites with antifungal and antibacterial activities (SUN et al., 2023). However, some species are also pathogenic, producing mycotoxins that are harmful to crops and human and animal health (O'DONNELL et al., 2013).

Among the compounds tested, the most effective against *C. cassiicola* were compound 2 (100 mg mL^{-1}) and compound 4 (100 mg mL^{-1}), with inhibition of 33.67% ($p = 0.000042$) and 31.63% ($p = 0.000042$), respectively. Both demonstrated significant potential for biocontrol, considerably reducing the radial growth of the fungus compared to the negative control. On the other hand, compound 8 (50 mg mL^{-1}) was the least effective, with only 2.04% inhibition.

The most effective compounds against *Fusarium* sp. were compound 2 (100 mg mL^{-1}), with 30.77% inhibition ($p = 0.000042$), followed by compound 4 (100 mg mL^{-1}), with 27.69% inhibition ($p = 0.000042$). These results suggest that both compounds exhibit significant antifungal activity against this pathogen. However, the inhibition observed was lower than that recorded for *C. cassiicola,* indicating that *Fusarium* sp. is less susceptible to the tested compounds.

In general, it was shown that *C. cassiicola* was more susceptible to treatment than *Fusarium* sp. The effectiveness of the conventional antifungal drug was validated by a positive control, which demonstrated 78.46% inhibition for *Fusarium* sp. and 81.63% inhibition for *C. cassiicola* ($p<0.0001$). The results show that even while the compounds under evaluation showed antifungal efficacy, there is still an opportunity for improvement in the way these chemicals are used to find alternatives, such as the production of inorganic complexes.

5. CONCLUSIONS

In summary, we synthesized eleven *N*-salicylhydrazones using four eco-friendly synthetic procedures. All approaches employed green chemistry principles, resulting in good to excellent yields, short reaction times, and avoiding the need for toxic solvents or extensive purification. Among the methodologies tested, magnetic stirring at room temperature and ultrasound irradiation proved to be the most efficient, affording the highest average yields.

The antifungal screening demonstrated that several *N*-salicylhydrazones exhibited inhibitory activity against *C. cassiicola* and *Fusarium* sp., with compound 2 showing the most promising results, followed by compound 4. The results highlight the potential of these derivatives as alternative antifungal agents.

Overall, the findings reinforce the relevance of *N*-salicylhydrazones as scaffolds for the development of novel bioactive compounds and demonstrate that environmentally benign synthesis methodologies can be effectively applied to obtain pharmacologically and agriculturally relevant molecules. Such approaches may contribute to safer and sustainable substances for managing fungal pathogens in agriculture, aligning with the principles of green chemistry.

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