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# *IN VITRO* ANTIFUNGAL POTENTIAL OF VANILLIC ACID AGAINST *SCLEROTIUM ROLFSII*

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## ABSTRACT

The worldwide demand for making agriculture greener, safer, and more efficient can be met aptly by the application of biopesticides. Vanillic acid is a naturally occurring versatile phenolic molecule with promising antifungal activity, however, there have been no studies of the possible use of vanillic acid for its antifungal activity against a serious soil-borne fungal plant pathogen namely *Sclerotium rolfsii*. This study was performed to assess the antifungal potential of vanillic acid by analyzing growth, morphological, and biochemical changes in *S. rolfsii* under laboratory conditions. The results revealed that vanillic acid (0.003-0.10 %) significantly reduced fungal growth, distorted fungal morphology (hyphae, and sclerotia), altered activity of enzymes (catalase, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase). However, 0.05 and 0.10 % concentrations caused complete inhibition in the fungal growth. The results explained in this work serve as a basis for further research to formulate fungicides using vanillic acid.

**Keywords:** Vanillic acid, growth inhibition, MIC, soil-borne fungus, necrotrophic fungus.

## INTRODUCTION

*Sclerotium rolfsii*, is a polyphagous soil-borne necrotrophic fungus of over 500 host plants, present all around the globe and causing vast losses by inciting crown blight, stem canker, damping-off of seedlings and, fruit rots in the plants (Rafi et al., 2017; Shoaib et al., 2019). *S. rolfsii* can survive several years in soil debris without host plants due to its hard sclerotial structures, which have protective layers of viable hyphae. Sclerotia germinate leading to robust infections at high temperatures (27 to 35 °C) and moist environments (Shoaib et al., 2019). Effective management of robust plant pathogens remains a significant challenge. So far, currently, available disease management options are based on chemical fungicides (Captan, Ridomil, Bayleton, Cupravit, Daconil etc.), and their excessive and irrational use is deteriorating environments (Shoaib et al., 2022). Lately, phytochemical compounds have been receiving special attention as potent antifungal agents (Javed et al., 2021).

Among the different compounds, vanillic acid (VA) is a phenolic molecule (oxidized molecule of vanillin) found in edible plants and fruits, and is used as a flavoring agent, preservative, and food additive as well as in the production of polymers (e.g., epoxy resins) (Stanely et al., 2011; Lubbers et al., 2021). It also holds certain pharmacological properties (e.g., antioxidant, anti-inflammatory, immuno-stimulating, and antimicrobial) and exerts considerable bioactivity against neurodegenerative, cardiovascular, diabetic, cancerous, and hepatic diseases by inhibiting the associated molecular pathways (Jung et al., 2017; Kaur et al., 2022).

Over and above, the bioactive potentials of VA are evidently proven in the activation of defense mechanisms against stress, regulation of growth, and biochemical process in plants (Quan et al., 2016). Exogenous application of VA has been documented to prompt drought tolerance (Quan et al., 2016; Quan and Xuan, 2022), and submergence stress tolerance in rice by increasing photosynthetic and anti-oxidation properties (Parvin et al., 2020). Being an excellent antioxidant, VA at low concentrations stimulated the germination and growth of tomatoes (Ghareib et al., 2010), and quantitatively increased in water-stressed berry skins and fungus-attacked tomato epicarp (Ruelas et al., 2006). The concentrations of VA with some other phenolics were also roused in cotton and rice after the pathogen infection (Rehman et al., 2012). It was revealed that some species of *Fusarium* spp., such as *F. culmorum* and *F. graminearum* (Lanoue et al., 2010) were sensitive to VA, while other pathogenic species were able to degrade it (Michielse et al., 2012). Moreover, the exogenous application of VA rose the vanillin accumulation in the soil after continuous monocropping of cucumber and changed the whole fungal community structure (Zhou et al., 2018). However, the work regarding the antifungal activity of VA is scanty. The current study aimed to access the anti-mycotic potential of VA against *S. rolfsii* by observing growth, morphological, and biochemical changes in the fungus.

## METHODOLOGY

The stock solution of vanillic acid (VA: Sigma-Aldrich) was prepared by dissolving 0.5 g VA in 100 mL dimethyl sulfoxide, and working concentrations of 0.003, 0.006, 0.012, 0.025, 0.050, and 0.10 % were prepared from the stock solution. Malt extract agar [2 % MEA (pH: 6.5)] was prepared, and after autoclaving, it was amended with six different concentrations of VA. Later, VA-laden-MEA was poured into a 9-cm Petri dish. After solidification of the medium, the disc of 2 mm of *S. rolfsii* was taken from the 7-day-old pure culture and inoculated in the center of the Petri dish. The inoculated plates were kept for 10 days at 27 °C for observing changes in the growth and morphology of the fungus. The morphological characteristics of the hyphae were observed under a compound microscope at 10 X and 40. The minimum inhibitory concentration (MIC is the lowest concentration of VA that will inhibit the visible growth of a fungus after incubation period) of the VA was also recorded. The control treatments were devoid of VA. Each concentration was prepared in sets of triplicates, and the whole experiment was repeated thrice.

Another set of treatments was prepared by adding different concentrations of VA i.e., 0.003, 0.006, 0.012, 0.025, 0.050, and 0.10 % in 2 % ME broth (100 mL) in 250 mL conical flasks following the same protocol as described above. After inoculation of the fungal disc, fungal biomass was harvested after 10 days. The fresh fungal biomass was used for assessing the changes in total protein content and activities of catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO), and phenyl-ammonia lyase (PAL) following the protocols of Shoaib et al., (2021, 2022). For measuring dry biomass, the fresh biomass was kept for drying in an oven (60 °C), and percentage inhibition in fungal growth (GI %) and tolerance indices (TI) were calculated.

The concentrations of VA inhibiting fungal growth in 50 % (effective concentration $EC_{50}$) were measured by the probit method using SAS 9.1. Moreover, data of growth, biomass, and biochemical assays for the fungus was analyzed statistically by applying the LSD test (least significant difference) after one-way ANOVA.

## RESULTS

### *Effect on Growth*

The antifungal potential of different concentrations (0.003, 0.006, 0.012, 0.025, 0.050, and 0.1 %) of VA was checked against *S. rolfsii* both on agar and broth medium (Figure 1) and the inhibition in the mycelial growth (%) was variable for each concentration (Figure 2). On agar medium, the lower concentration of 0.003-0.012% did not affect the fungal growth significantly, while the higher concentration of 0.025 % significantly reduced it by 40 %, and the growth was complexly halted at 0.050 and 0.1 % concentrations (Figure 2 A). The MIC value of VA was recorded at 0.050 %, where there was no visible fungal growth, and $EC_{50}$ was found at 0.025 % as per probit analysis (Figure 2 B).

Results of fungal biomass harvested from the broth medium showed that the mycelial dry weight of the *S. rolfsii* was insignificantly altered at 0.003 to 0.012 % concentrations, while significantly reduced by 50 % at 0.025 % concentration when compared with the control (Figure 2 C). The mycelial dry weight of the pathogen was unable to measure when treated with 0.05 or 0.1% of VA, and $EC_{50}$ was found at 0.020 % of VA in broth medium (Figure 2 D).

### *Effect on Morphology*

The hypha of the fungus altered due to the addition of VA in the growth medium as compared to the control (Figure 3). Therefore, the normal, straight and obtuse hyphae turned thin, contorted, and pointed at 0.012 % concentration, which further became atrophied and twisted hyphae with fragmentation and perforations at 0.025 % concentration though no growth of the fungus was observed at 0.05 and 0.1 % of VA (Figure 3).

### *Effect on Activities of Enzymes*

The activity of enzymes (CAT, POX, PPO, and PAL) increased significantly up to 50 % at 0.003 and 0.006 %, respectively, in contrast to the control, whereas at concentrations of 0.012 and 0.025 % a less significant increase was observed as compared to control.

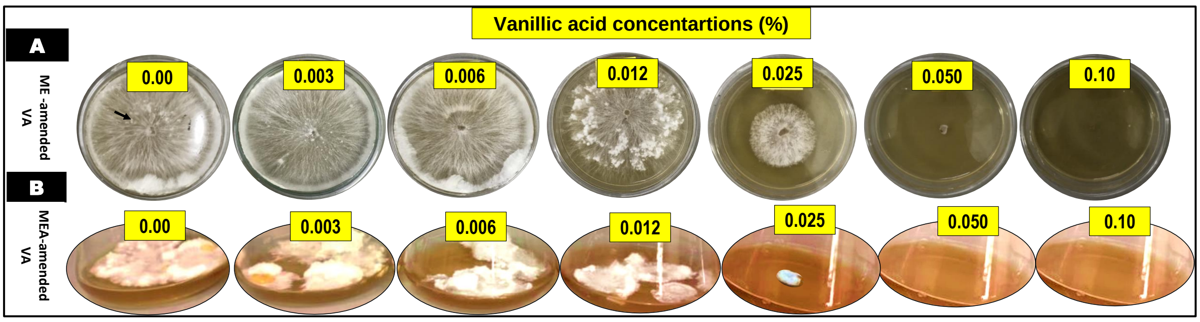


**Figure 1: (A & B): Growth of *Sclerotium rolfsii* with different concentrations of vanillic acid (VA) after 10 days of incubation at 28 °C on 2 % malt extract agar (A) and malt extract broth (B).**

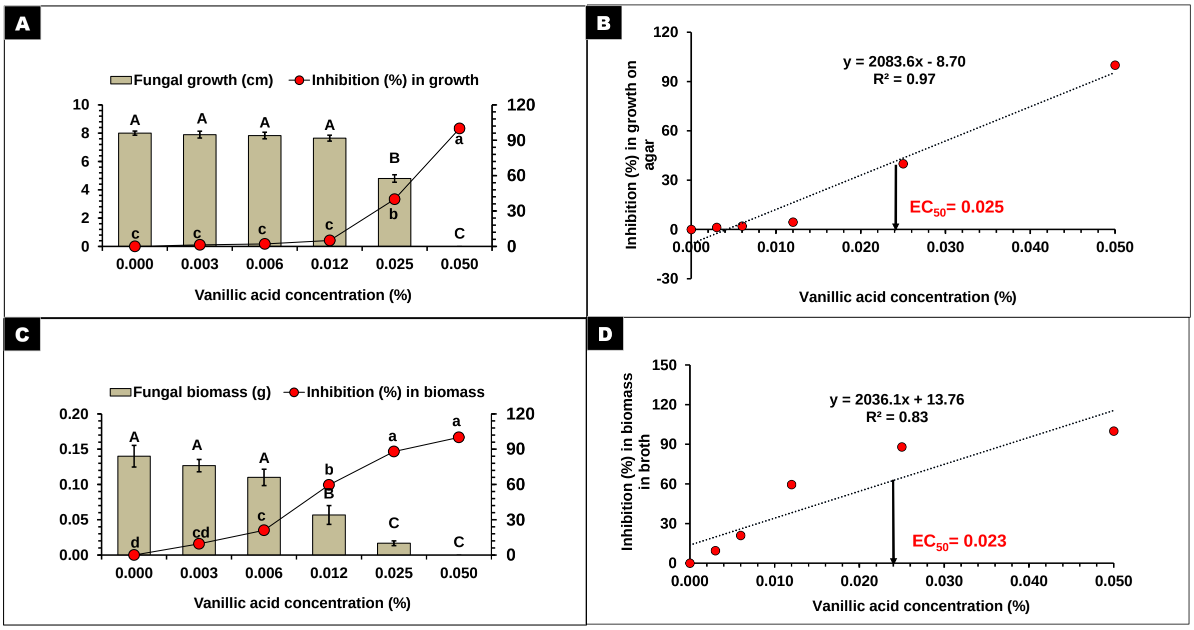


**Figure 2: (A-D): Effect of different concentrations of vanillic acid (VA) on the growth of *Sclerotium rolfsii* after incubation for 10 days at 28 ℃. A: Growth on 2 % malt extract agar; B: $EC_{50}$ (effective concentration) of VA on agar; C: Dried biomass on 2 % malt extract broth; D: $EC_{50}$ (effective concentration) of VA on broth. Vertical bars show the standard error of the mean of three independent biological replicates (n=3) of two independent experiments. Values with a different letter at their top show a significant difference ($P < 0.05$) as determined by the LSD test.**

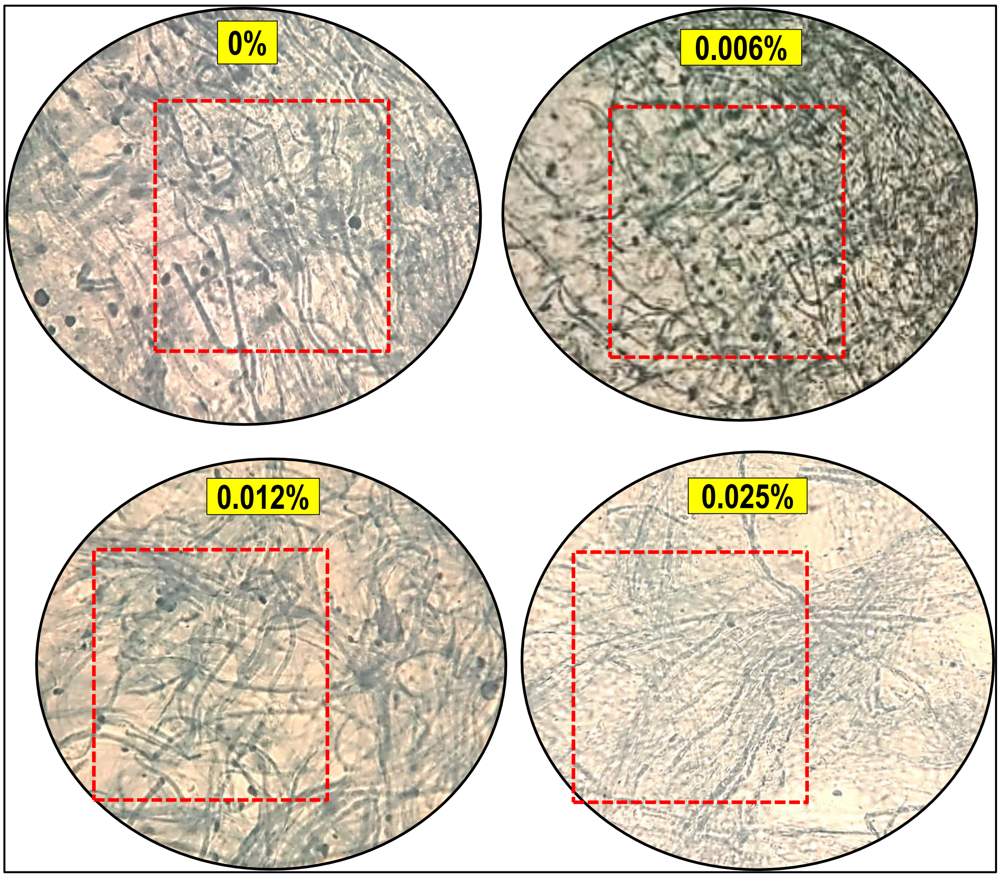


**Figure 3: (A-D): Morphological characteristics of the hyphae of *Sclerotium rolfsii* on 2 % malt extract agar (MEA) supplemented with vanillic acid (VA) by light microscopy at 40× magnification.**

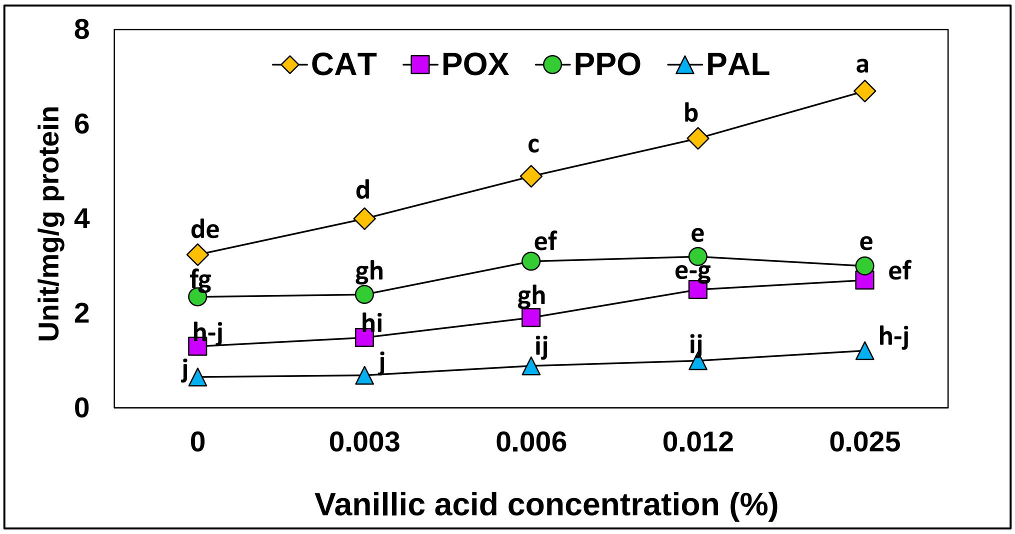


**Figure 4: Effect of different concentrations of vanillic acid (VA) on the biochemistry of *Sclerotium rolfsii* after incubation for 10 days at 28 ℃. CAT: catalase; POX: peroxidase; PPO: polyphenol oxidase; PAL: phenylalanine ammonia-lyase. Vertical bars show the standard error of the mean of three independent biological replicates (n=3). Values with a different letter at their top show a significant difference (*P* < 0.05) as determined by the LSD test.**

As the rest of the two concentrations (0.05 and 0.1%) completely halted the fungal growth, hence no biochemical parameters were taken at these concentrations (Figure 4).

## DISCUSSION

Vanillic acid (VA) is a phenolic compound (4-hydroxy-3-methoxy benzoic acid), produced by many plants as secondary metabolites and exhibits antimicrobial properties among other outstanding properties (Tijerina-Ramírez et al., 2014). This study aimed to explore the antifungal potential of VA against a phytopathogenic fungus namely *S. rolfsii*, which is responsible for huge losses in many economically important crops (Sana *et al.*, 2017).

The results of the present investigation demonstrated a significant inhibitory effect of VA on the growth, morphology, and biochemical attributes of *S. rolfsii*. As the concentration of VA increased (0.003, 0.006, 0.012, 0.025, 0.050, and 0.1 %) the growth of *S. rolfsii* became less feathery and less dense, whereby the fungal growth was completely constrained at 0.025 %. The minimum inhibitory concentration was found at 0.05 % VA concentration. Moreover, growth alterations were accompanied by an enhancement in the activity of CAT, POX, PPO, and PAL till 0.025 % VA concentration followed by a decline in the enzyme activity at latter concentrations, and $EC_{50}$ was also found at the 0.025 % VA concentration. Vargas et al. (2014) findings ascribed the presence of aldehyde moiety and side-group position on the benzene could be responsible for the antimycotic potential of VA against many fungal species (Sanchez-Medina et al., 2010; Tapwal et al., 2011). Ramírez et al. (2004) described a reduction in the growth and metabolism of *Fusarium graminearum* due to alteration in the physiology of the organism, which may lead to higher oxidative stress and cell injury (Shoaib et al., 2022). Tijerina-Ramírez et al. (2014) stated that the failure of the fungus to sustain a positive tannin pressure in the mycelium could be responsible for the interruption in the water availability in the presence of VA in the growth medium. Therefore, the study might be suggested that adsorption/penetration of VA into the hyphal cell surface altered cellular metabolism, which altered fungal growth and adversity leading to fragmentation and perforations to complete inhibition in the fungal hyphal growth at 0.05 % VA concentration due to oxidative injury.

## CONCLUSION

The results showed that vanillic acid (0.003-0.025 %) altered fungal morphology by fragmentation and distortion of hyphae, which caused alteration in enzymatic activity and reduction in the fungal growth with increasing concentration. The fungal growth was completely inhibited at 0.05 % concentration. Therefore, vanillic acid could be used in fungicide formulation to manage diseases caused by *S. rolfsii* in plants.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## AUTHOR’S CONTRIBUTION

Amna Shoaib: Conceptualization, supervision, data statistical analyses and drafted the manuscript; Muhammad Yousaf: Experiment; Qudsia Fatima: Microscopy and formatted manuscript; Shanila Bukhari: Formatted manuscript; Numan Ali: Edited manuscript; Uswa Fatima: Experiments

## REFERENCES

Ghareib HR, Abdelhamed MS, Ibrahim OH (2010). Antioxidative effects of the acetone fraction and vanillic acid from Chenopodium murale on tomato plants. Weed Biol Manag., 10(1): 64-72.

Javed S, Mahmood Z, Khan KM, Sarker SD, Javaid A, Khan IH, Shoaib A (2021). Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold Macrophomina phaseolina. Sci Rep., 11, Article number: 8417.

Jung Y, Park J, Kim HL, Youn DH, Kang J, Lim S, Jeong MY, Sethi G, Park SJ, Ahn KS, Um JY (2017). Vanillic acid attenuates testosterone-induced benign prostatic hyperplasia in rats and inhibits proliferation of prostatic epithelial cells. Oncotarget, 8(50): 87194-87208.

Kaur J, Gulati M, Singh SK, Kuppusamy G, Kapoor B, Mishra V, Gupta S, Arshad MF, Porwal O, Jha NK, Chaitanya MV (2022). Discovering multifaceted role of vanillic acid beyond flavours: Nutraceutical and therapeutic potential. Trends Food Sci Technol., 122: 187-200.

Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse US (2010). De novo biosynthesis of defense root exudates in response to Fusarium attack in barley. New Phyt., 185(2): 577-588.

Lubbers RJ, Dilokpimol A, Nousiainen PA, Cioc RC, Visser J, Bruijnincx PC, de Vries RP (2021). Vanillic acid and methoxy hydroquinone production from guaiacyl units and related aromatic compounds using Aspergillus niger cell factories. Microb Cell Fact., 20(1): Article ID: 151.

Michielse CB, Reijnen L, Olivain C, Alabouvette C, Rep M (2012).

Degradation of aromatic compounds through the β-ketoadipate pathway is required for pathogenicity of the tomato wilt pathogen Fusarium oxysporum f. sp. lycopersici. Mol Plant Pathol., 13(9): 1089-1100.

Sana N, Javaid A, Shoaib A (2017). Effect of NPK fertilizers and commercial biofertilizers on southern blight disease and plant growth in chili. Bang J Bot., 46: 659-666.

Parvin K, Nahar K, Hasanuzzaman M, Bhuyan MB, Mohsin SM, Fujita M (2020). Exogenous vanillic acid enhances salt tolerance of tomato: Insight into plant antioxidant defense and glyoxalase systems. Plant Physiol Biochem., 150: 109-120.

Quan NT, Anh LH, Khang DT, Tuyen PT, Toan NP, Minh TN, Minh LT, Bach DT, Ha PT, Elzaawely AA, Khanh TD (2016). Involvement of secondary metabolites in response to drought stress of rice (Oryza sativa L.). Agriculture, 6(2): 23.

Quan NT, Xuan TD (2022). Foliar application of vanillic and p-hydroxybenzoic acids enhanced drought tolerance and formation of phytoalexin momilactones in rice. Arch Agron Soil Sci., 64 (13): 1831-1846.

Rafi S, Shoaib A, Awan ZA, Rizvi NB, Shafiq M (2017). Chromium tolerance, oxidative stress response, morphological characteristics, and FTIR studies of phytopathogenic fungus Sclerotium rolfsii. Folia Microbiol., 62(3): 207-219.

Ramírez ML, Chulze SN, Magan N (2004). Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols in Fusarium graminearum. Mycologia, 96: 470–478.

Rehman F, Khan FA, Badruddin SMA (2012). Role of phenolics in plant defense against insect herbivory. (Eds. Khemani LD, Srivastava MM, Srivastava S). In: Chemistry of phytopotentials: health, energy and environmental perspectives. Springer Berlin Heidelberg. pp. 309-313.

Ruelas C, Tiznado- Hernández ME, Sánchez- Estrada A, Robles- Burgueno MR, Troncoso- Rojas R (2006). Changes in phenolic acid content during Alternaria alternata infection in tomato fruit. J Phytopathol., 154(4): 236-244.

Sanchez-Medina A, Peña-Rodríguez LM, May-Pat F, Karagianis G, Waterman PG, Mallet AI, Habtemariam S. (2010). Identification of sakurasosaponin as a cytotoxic principle from Jacquinia flammea. Nat Prod Commun., 5(3): 365-368.

Shoaib A, Abbas S, Nisar Z, Javaid A, Javed S (2022). Zinc highly potentiates the plant defense responses against Macrophomina phaseolina in mungbean. Acta Physiol Plant., 44(2): 1-17.

Shoaib A, Akhtar M, Javaid A, Haider A, Nisar Z and Javed S (2021). Antifungal potential of zinc against leaf spot disease in chili pepper caused by Alternaria alternata. Physiol Mol Biol Plants, 27: 1361-1376.

Shoaib, Shehzad A, Javaid A, Akhtar S, Awan ZA (2019). Evaluation of biocontrol strategies and its synergistic interaction permitting the chickpea plant to trigger the appropriate defense responses against Sclerotium rolfsii. Biologia, 65(2): 329-334.

Stanely, P.; Rajakumar, S.; Dhanasekar, K (2011) Protective effects of vanillic acid on electrocardiogram, lipid peroxidation, antioxidants, proinflammatory markers and histopathology in isoproterenol induced cardiotoxic rats. Eur J Pharmacol., 668: 233–240.

Tapwal A, Nisha Garg S, Gautam N, Kumar R (2011). In vitro antifungal potency pf plant extracts against five phytopathogens. Braz Arch Biol Technol., 54: 1093-1098.

Tijerina-Ramírez N, Lira-Méndez K, Moreno-Medina VR, González-Prieto JM, Mayek-Pérez N (2014). Efecto del estrés osmótico in vitro en el crecimiento, patogenicidad y producción de osmolitos en Macrophomina phaseolina. Rev Mex Micol., 39: 31-39.

Vargas DAA, Gamboa AM, Medina BIL, Pérez BD (2014). Evaluation of native Yucatecan plant extracts against Alternaria chrysanthemi and antifungal activity spectrum of Acalypha gaumeri. Rev Mex Fitopatol., 32: 1-11.

Zhou X, Jia H, Ge X, Wu F (2018). Effects of vanillin on the community structures and abundances of Fusarium and Trichoderma spp. in cucumber seedling rhizosphere. J Plant Interact., 13(1): 45-50.