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# Unraveling the Role of Phenolic and Flavonoid Compounds in Chilli Resistance to Root Knot Nematodes

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**ABSTRACT: Root knot nematodes (RKN) pose a significant threat to chilli production, leading to huge yield and economic loss worldwide. The management of root-knot nematodes is still a serious concern due to their wide diversity of species and host range. Host plant resistance is the most viable and eco-friendly strategy. By considering all these aspect the present experiment was conducted to investigate the phenolic and flavonoid profiles of resistant chilli lines in response to RKN infection. Liquid chromatography–mass spectrometry analysis (LCMS) was performed to quantify and compare the levels of phenolic and flavonoid compounds in resistant breeding lines (ACRIL 70 and ACRIL 90) and susceptible varieties (Arka Mohini and Arka Suphal). The results revealed that the resistant lines exhibited higher production of phenolic and flavonoid compounds compared to the susceptible varieties. ACRIL 70 displayed the highest levels of salicylic acid, caffeic acid, gallic acid, protocatechuic acid, trans-cinnamic acid, and paracoumaric acid. Furthermore, epicatechin and catechin were found to be the predominant flavonoids in ACRIL 70. These findings suggested that phenolic and flavonoid-based biochemical compounds play a crucial role in conferring resistance to RKN in chilli lines. In nutshell, these resistance lines with traits associated with RKN resistance can be used for developing resistance chilli varieties by using breeding programmes. Understanding these defense mechanisms can contribute to the development of novel strategies for enhancing nematode resistance in chilli cultivation.**

**Keywords:** Chilli, flavonoids, gallic acid, LCMS analysis, root knot nematode, phenolic compounds.

## INTRODUCTION

Chilli (*Capsicum annuum* L.) is a versatile plant cultivated worldwide as a vegetable and spice crop, and it has gained significant popularity as an essential ingredient in various cuisines. Its fruits contain valuable biochemical substances such as carotenoids and capsaicinoids, which has been found to be used in the food, medicinal, and pharmaceutical industries (FAOSTAT, 2019). However, the growth, yield, and quality of chilli plants are often hindered by both biotic and abiotic stresses, as highlighted by Naresh *et al.* (2019). Root-knot nematodes (*Meloidogyne incognita*) pose a significant threat to chilli cultivation worldwide, leading to substantial yield losses and economic implications for farmers (Sasser *et al*., 1987). These microscopic roundworms have a parasitic relationship with the roots of chilli plants, causing characteristic root galls or knots that impair nutrient uptake, water absorption, and overall plant vigor. The damage inflicted by root-knot nematodes is a major concern for chilli growers, as it not only reduces crop productivity but also weakens the plants, making them more susceptible to other pests and diseases (Jones *et al.*, 2013; Morris *et al*., 2016).

Root-knot nematodes are among the most damaging plant parasitic nematodes and are widely distributed in temperate and tropical regions. They have a complex

life cycle, with the juvenile stage penetrating the root tissue, inducing the formation of specialized feeding sites called giant cells (Asghar *et al.*, 2020; Anwar *et al*., 2002). These giant cells provide nourishment for the developing nematodes, leading to the proliferation of nematode populations and subsequent damage to the root system. As the infestation progresses, the affected roots become swollen and distorted, resulting in reduced root development and compromised plant health (Shannon *et al*., 1966).

One of the significant challenges faced by chilli farmers in India and globally is the infestation of root-knot nematodes, particularly the *Meloidogyne spp*. These pests adversely affect commercial crops, leading to qualitative and quantitative losses. Sitaramaiah (1984) identified *Meloidogyne* spp. as the most common nematode pest attacking chilli crops, with various species found in different Indian states. Traditionally, farmers have relied on pesticide strategies to combat nematode infestation. However, the ineffectiveness of chemical control methods and the associated implications have driven the need for alternative techniques. Integrated pest and disease management approaches have been implemented, resulting in a reduction in nematode populations in chilli fields (Nusbaum and Ferris 1973).

Previous studies have shed light on the involvement of phenolic compounds, particularly chlorogenic acid, in the resistance of chilli cultivars, as discovered by Pegard *et al.* (2005) and Skerget *et al.* (2005) using high-performance liquid chromatography. The response to infection varied between nematode species, with resistant lines inhibiting nematode penetration and feeding compared to susceptible cultivars. Further research by Kirwa *et al.* (2018) explored the responses of second-stage juveniles (J2s) of M. incognita to non-volatile components of tomato root exudates. Through chemical analysis and experiments involving stylet thrusting and chemotaxis, they identified specific root exudate chemicals that attract or repel J2s. Phytohormone zeatin (cytokinin), flavonoids quercetin and luteolin, and alkaloids solasodine and tomatidine were among the bioactive compounds detected (Abad *et al.*, 2008). These substances induced concentration-dependent responses in J2s, highlighting their potential role in nematode resistance.

Chilli cultivation faces challenges posed by nematode infestation, particularly root-knot nematodes of the Meloidogyne species. Researchers are studying resistant and susceptible lines to unravel the mechanisms underlying nematode resistance in chilli plants (Escobar *et al.*, 2015; Lu *et al.*, 2020). By analyzing biochemical changes, including phenols and defense enzymes, as well as the composition of root exudates, they aim to identify specific compounds associated with nematode resistance. These findings can contribute to the development of alternative management strategies for nematode control in chilli crops, reducing reliance on pesticides and enhancing sustainable cultivation practices. In order to gain insights into the underlying mechanisms of nematode resistance in chilli plants, the present study was conducted to investigate the biochemical changes and defense compounds derived from the root exudates associated with the resistance to root knot nematode by using Liquid chromatography–mass spectrometry (LC–MS) at 30 days after nematode inoculation.

## MATERIALS AND METHODS

**Nematode collection and inoculation.** Egg masses were obtained from tomato plants (cv. Arka Rakshak) cultivated in the Nematology glasshouse at the Division of Crop Protection, ICAR – Indian Institute of Horticultural Research (IIHR) in Bengaluru. The collection of egg masses was carried out as part of the experimental procedure. Subsequently, the second-stage juveniles (J2) that emerged from these eggs were carefully harvested on a daily basis. Only J2 nematodes that were at least 5 days old were selected for inoculation purposes in the subsequent experiments. In a completely randomized design with six replications, two resistant lines of plants were grown in polybags in a net house. The plants were subjected to both inoculated (nematode-infested) and uninoculated (control) conditions. After 15 and 30 days of inoculation, the plants were uprooted for analysis. Biochemical changes, including total phenols, lignin content, protein content, and defense enzyme activities, were assessed in the resistant lines. These parameters provide insights into the defense mechanisms and responses of the plants against nematode infestation.

**Liquid chromatography–mass spectrometry (LC–MS) analysis**

**Extraction procedure.** The extraction procedure utilized a modified method based on Wallis *et al.* (2008); Wallis and Chen (2021). Root samples with galls, were homogenized using liquid nitrogen and divided into three 0.10 g aliquots, centrifugation at 12,000 rpm for 10 minutes, the resulting pellets were subjected to overnight extraction at 4 °C using methanol. Additional extraction was performed on the remaining pellets using 0.5 mL of the same solvent, and the second extract was combined with the initial 1.0 mL extract. High-performance liquid chromatography (HPLC) with a Shimadzu LC20AD pump, Supelco Ascentis RP-18 column, and Shimadzu PDA-20 photodiode array detector was employed to analyze the methanol extracts. Piceatannol, resveratrol, and ε-viniferin provided by Sigma-Aldrich served as reference compounds for identification, while other compounds were identified using liquid chromatography-mass spectrometry (LC-MS) by comparing molecular weight information and retention times with previous reports. Phenolic quantification

relied on resveratrol-based standard curves. The analysis yielded valuable insights into the composition and concentration of phenolic compounds within the root samples.

**Liquid Chromatography-Mass Spectrometry LC-MS.** Liquid chromatography was performed using an Acuity UPLC-H class system from Waters Inc. (USA), which consisted of a quaternary pump, online degasser, auto-sampler, and temperature control column compartment. The analytical column used was a 2.1 × 50 mm UPLC BEH C18 column with 1.7 µm particle size, while the guard column temperature was maintained at 25°C. The LC gradient program spanned 15 minutes with a flow rate of 0.2 mL/min. The initial gradient comprised 85% solvent A and 15% solvent B, held for 1 minute. At 12 minutes, the gradient shifted to 15% solvent A and 85% solvent B, maintained for 1 minute, followed by a linear gradient to 85% solvent A and 15% solvent B, held for 14 minutes. The system returned to the initial conditions at 15 minutes, followed by a 1-minute equilibration before the next injection. Hormone identification and quantification were achieved using a TQD-MS/MS system (Waters, USA) optimized for hormone analysis.

## RESULTS AND DISCUSSIONS

The results of our study revealed that the resistant RILs, ACRIL 70 and ACRIL 90, exhibited significantly higher production of phenolic and flavonoid compounds compared to the susceptible varieties, Arka Mohini and Arka Suphal (Table 1). These findings align with previous research that has demonstrated a positive correlation between phenolic/flavonoid content and resistance to parasitic nematodes (Chin *et al*., 2018). Interestingly, ACRIL 70 displayed the highest production of the tested compounds among the resistant RILs. This suggests that ACRIL 70 possesses unique genetic traits that contribute to enhanced phenolic and flavonoid synthesis, making it more effective in combating nematode infestation. In contrast, ACRIL 90 exhibited a slightly lower level of phenolic and flavonoid production compared to ACRIL 70, indicating variations in the resistance mechanisms within the resistant RILs (Wu *et al*., 2018).

In the susceptible varieties, the production of phenolics and flavonoids was lower in the inoculated plants compared to the uninoculated ones. This observation is consistent with previous studies that have reported reduced phenolic and flavonoid synthesis in susceptible plants upon nematode infection (Chin *et al*., 2018). Our LCMS analysis identified several noteworthy phenolic compounds in the resistant RILs, including salicylic acid, caffeic acid, gallic acid, protocatechuic acid, trans-cinnamic acid, and paracoumaric acid (Table 2). Among these, salicylic acid stood out as the most abundant compound in ACRIL 70, which is in agreement with previous studies highlighting the crucial role of salicylic acid in plant defense against biotic stressors (Ryals *et al*., 1996; Chandrawat *et al*., 2020).

Furthermore, our findings indicated that flavonoids, particularly epicatechin and catechin, were present in higher quantities in the inoculated ACRIL 70. Flavonoids have been associated with various defense mechanisms in plants, such as altering feeding site development and influencing chemotactic interactions in the rhizosphere (Chin *et al*., 2018). The significant alterations in phenolic and flavonoid activity observed in the resistant chilli lines provide strong evidence for the involvement of these biochemical compounds in conferring resistance against root knot nematodes. The higher production of salicylic acid, in particular, supports the notion that its synthesis is closely linked to the resistant phenotype and acts as a potent defense molecule against invading pathogens (Chawla *et al*., 2013; Ryals *et al*., 1996).

In conclusion, our comprehensive LCMS analysis highlighted the superior phenolic and flavonoid content of the resistant RILs, particularly ACRIL 70, compared to the susceptible varieties. These findings underscore the importance of phenolic and flavonoid-based biochemical activity as major contributors to the resistance of chilli plants against root knot nematodes. The identification of salicylic acid as the most abundant compound further reinforces its significance in the defense response. Overall, this research provides valuable insights into the mechanisms underlying resistance in chilli plants and lays the foundation for future studies aimed at developing nematode-resistant crop varieties through targeted manipulation of phenolic and flavonoid synthesis pathways.

In conclusion, our research on the phenolic and flavonoid content of resistant RILs (ACRIL 70 and ACRIL 90) and susceptible varieties (*Arka Mohini* and *Arka Suphal*) has shed light on the role of these compounds in conferring resistance against root knot nematodes in chilli plants. The results demonstrated that the resistant RILs exhibited significantly higher production of phenolic and flavonoid compounds compared to the susceptible varieties. Among the resistant RILs, ACRIL 70 showed the highest production of tested compounds, indicating its superior resistance mechanisms. The LCMS analysis identified salicylic acid as the most abundant compound in the inoculated roots of ACRIL 70, emphasizing its crucial role in plant defense against biotic stressors. Additionally, several other phenolic compounds, such as caffeic acid, gallic acid, protocatechuic acid, trans-cinnamic acid, and paracoumaric acid, were found to contribute to the resistance of chilli plants against root knot nematodes (Lu *et al.*, 2020).

Furthermore, our findings highlighted the significance of flavonoids, particularly epicatechin and catechin, in the defense response of ACRIL 70. These compounds may influence feeding site development and play a role

in chemotactic interactions in the rhizosphere. The observed reductions in phenolic and flavonoid production in the susceptible varieties upon nematode infection further support the association between lower levels of these compounds and susceptibility to nematode infestation.

**Table 1: LCMS phenol content of selected resistant RILs and susceptible varieties.**

| Sr. No. | Compound name | Std value | S1 ng/g | S2 ng/g | S3 ng/g | S4 ng/g | S5 ng/g | S6 ng/g | S7 ng/g | S8 ng/g |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Benzoic acid | 0.00544 | 54,58 | 44.61 | 63.47 | 48.60 | 64.37 | 101 | 63.65 | 89.58 |
| 2. | Para hydroxybenzoic | 0.00063 | 17.62 | 12.96 | 18.12 | 15.41 | 25.70 | 68.02 | 25.14 | 29.27 |
| 3. | Salicylic acid | 0.07501 | 1400.72 | 712.60 | 1592.71 | 827.61 | 2527.84 | 7636.02 | 1862.75 | 3130.42 |
| 4. | 3-hydroxy benzoic | 0.00072 | 23.38 | 12.89 | 26.04 | 18.55 | 34.97 | 61.85 | 26.59 | 46.44 |
| 5. | Trans cinnamic acid | 0.17182 | 108.82 | 57.27 | 126 | 85.91 | 131.73 | 624.28 | 126.00 | 189.00 |
| 6. | 2,4-Dihydroxy benzoic | 0.06000 | 94 | 62 | 118 | 66 | 196 | 414 | 166 | 202 |
| 7. | Gentisic acid | 0.20686 | 227.55 | 144.8 | 262.02 | 158.59 | 393.03 | 448.2 | 310.29 | 434.41 |
| 8. | Protocatechuic acid | 0.21835 | 196.52 | 72.78 | 298.41 | 116.45 | 531.32 | 931.63 | 320.25 | 880.68 |
| 9. | Para-coumaric acid | 0.11931 | 155.1 | 99.43 | 194.87 | 115.33 | 218.74 | 544.85 | 202.83 | 532.92 |
| 10. | o-Coumaric acid | 0.08106 | 99.97 | 78.36 | 110.78 | 81.06 | 126.99 | 251.29 | 121.59 | 126.99 |
| 11. | Vanillic acid | 0.01749 | 114.68 | 47.81 | 127.68 | 106.11 | 146.33 | 246.03 | 130.01 | 205.22 |
| 12. | Gallic acid | 0.13236 | 286.78 | 180.89 | 476.5 | 269.13 | 648.56 | 997.11 | 480.91 | 913.28 |
| 13. | Caffeic acid | 0.17769 | 396.84 | 254.69 | 781.84 | 325.77 | 882.53 | 1990.1 | 787.76 | 1729.5 |
| 14. | Ferulic acid | 0.15315 | 2685.2 | 2481 | 2996.6 | 2659.7 | 3532.7 | 4058.5 | 3476.5 | 3609.2 |
| 15. | Syringic acid | 0.00077 | 7.24 | 5.72 | 8.14 | 6.78 | 12.01 | 13.14 | 8.26 | 12.32 |
| 16. | Sinapic acid | 0.03941 | 28.9 | 13.14 | 32.84 | 26.27 | 57.8 | 97.21 | 49.92 | 76.19 |
| 17. | Ellagic acid | 0.01439 | 14.87 | 4.8 | 15.83 | 13.91 | 22.54 | 28.3 | 20.63 | 25.42 |
| 18. | Chlorogenic acid | 0.00087 | 0.7 | 0 | 6 | 0.01 | 12.53 | 24 | 6.24 | 14.38 |

**Table 2 : LCMS flavanoid content of selected resistant RILs and susceptible varieties.**

| Sr. No. | Compound name | Std value | S1 ng/g | S2 ng/g | S3 ng/g | S4 ng/g | S5 ng/g | S6 ng/g | S7 ng/g | S8 ng/g |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Umbelliferone | 0.00128 | 1.19 | 0.43 | 1.28 | 0.55 | 2.18 | 3.07 | 1.96 | 2.47 |
| 2. | Apigenin | 0.01871 | 9.36 | 4.99 | 9.36 | 6.24 | 11.23 | 15.59 | 10.6 | 11.85 |
| 3. | Galangin | 0.00274 | 1.19 | 1 | 1.46 | 1.1 | 2.37 | 2.83 | 1.55 | 2.37 |
| 4. | Naringenin | 0.0952 | 47.6 | 38.08 | 60.29 | 44.43 | 82.51 | 98.37 | 79.33 | 88.85 |
| 5. | Kampherol | 0.00301 | 1.3 | 1.1 | 1.71 | 1.1 | 2.01 | 2.71 | 1.71 | 2.21 |
| 6. | Leutoline | 0.27568 | 119.46 | 91.89 | 137.84 | 91.89 | 165.41 | 294.06 | 147.03 | 192.98 |
| 7. | Fisetin | 0.00016 | 0.07 | 0.05 | 0.08 | 0.07 | 0.15 | 0.22 | 0.14 | 0.19 |
| 8. | Eriodictyol | 0.00011 | 0.09 | 0.06 | 0.1 | 0.09 | 0.13 | 0.15 | 0.12 | 0.14 |
| 9. | Catechin | 0.1605 | 171.2 | 139.1 | 251.45 | 165.85 | 342.4 | 518.95 | 321 | 406.6 |
| 10. | Epicatachin | 0.41324 | 440.79 | 275.49 | 523.44 | 427.01 | 647.41 | 881.58 | 606.09 | 702.51 |
| 11. | Hesperetin | 0.03848 | 15.39 | 11.54 | 19.24 | 15.39 | 23.09 | 51.31 | 20.52 | 24.37 |
| 12. | Quercetin | 0.21998 | 87.99 | 58.66 | 95.32 | 58.66 | 139.32 | 190.65 | 117.32 | 183.32 |
| 13. | Epigallocatechin | 0.19833 | 145.44 | 132.22 | 191.72 | 132.22 | 211.55 | 271.05 | 211.55 | 271.05 |
| 14. | Myricetin | 0.38439 | 269.07 | 179.38 | 294.7 | 217.82 | 448.46 | 499.71 | 320.33 | 461.27 |
| 15. | Rutin | 0.17766 | 112.52 | 82.91 | 165.82 | 112.52 | 201.35 | 284.26 | 183.58 | 213.19 |

*__S1:__ Arka Mohini-Uninoculated, __S2:__ Arka Mohini- Inoculated, __S3:__ Arka Suphal-Unioculated, __S4:__ Arka Suphal-Inoculated
__S5:__ ACRIL-70-Uninoculated, __S6:__ ACRIL-70-Inoculated, __S7:__ ACRIL-90-Unioculated, __S8:__ ACRIL-90-Inoculated

## CONCLUSIONS

In conclusion, our study on the phenolic and flavonoid content in resistant and susceptible chilli varieties exposed to root knot nematode infection provides important insights into the mechanisms of resistance against these destructive pests. The results clearly demonstrate that resistant RILs, particularly ACRIL 70, exhibited significantly higher production of phenolic and flavonoid compounds compared to the susceptible varieties. Salicylic acid emerged as a key player, with ACRIL 70 showing the highest concentrations of this compound in the inoculated roots. Other phenolic compounds, including caffeic acid, gallic acid, protocatechuic acid, trans-cinnamic acid, and paracoumaric acid, were also found to contribute to the resistance. Furthermore, our findings underscore the significance of flavonoids, such as epicatechin and catechin, in the defense response of the resistant RILs. Conversely, the susceptible varieties exhibited reduced phenolic and flavonoid production upon nematode infection. Overall, this research highlights the importance of phenolic and flavonoid-based biochemical activity in conferring resistance against root knot nematodes in chilli plants. The results provide a basis for further exploration of the genetic and molecular mechanisms involved and suggest potential strategies for developing nematode-resistant crop varieties through targeted enhancement of these compounds.

## FUTURE SCOPE

The future scope of this research lies in further exploring the specific mechanisms by which phenolic and flavonoid compounds confer resistance to root knot nematodes. This could involve studying the signaling pathways involved in their synthesis and their interaction with nematode infection. Additionally, the identification of specific genes or genetic markers associated with enhanced phenolic and flavonoid production could pave the way for marker-assisted breeding programs aimed at developing nematode-resistant chilli varieties. Furthermore, the potential application of other plant defense mechanisms, such as induced systemic resistance, could be investigated in combination with phenolic and flavonoid-based strategies for enhanced nematode control. Overall, this study opens up avenues for further investigation and innovation in the field of nematode resistance in crop plants.

**Author contributions.** G. Santhosh and Dr. R. Umamaheswari contributed equally to this work. G Santhosh and Dr. R. Umamaheswari collected the samples and performed the experiments. Dr. K.S. Shivashankara and Dr. Naresh Ponnam involved in physiological work. Tammireddy Anjali involved in data collection. Dr. R. Umamaheswari designed the experiment. G Santhosh and Dr. R. Umamaheswari, drafted the manuscript and all authors revised it. Dr. D C Lakshman Reddy, Dr. D.K. Nayak and Dr. B.K. Dash involved in graphical representation and manuscript editing.

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**Conflict of Interest.** None.

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