

Original Research Paper

Root exudates mediated resistance mechanism in chilli (*Capsicum annuum* L.) against root knot nematode (*Meloidogyne incognita*)

Santhosh G.^{1,2}, Umamaheswari R.^{2*}, Shivashankara K.S.³, Naresh P.⁴, Lakshmana Reddy D.C.³, Shalem Raju R.⁵, Nayak D.K.¹, Devindrappa M.², Narayana Swamy G.^{2,6} and Anjali T.¹

¹Department of Nematology, ⁵Department of Plant Physiology, Odisha University of Agricultural Technology, Bhubaneswar - 751 003, India

²Division of Crop Protection, ³Division of Basic Sciences, ⁴Division of Vegetable Science, ICAR-Indian Institute of Horticultural Research, Bengaluru - 560 089, India

⁶Department of Horticulture, Agricultural College (Acharya N. G. Ranga Agricultural University), Pulivendula - 516 391, India

*Corresponding author Email: umanema369@gmail.com

ABSTRACT

Chilli is an economically important vegetable and spice crop in India, but its productivity is severely constrained by root-knot nematodes, particularly *Meloidogyne incognita*. In this study, root exudates of chilli were analyzed using gas chromatography–mass spectrometry (GC-MS) to elucidate resistance mechanisms. Ninety-nine recombinant inbred lines (RILs) were previously screened, and two resistant RILs (ACRIL 70 and ACRIL 90) along with two susceptible varieties (Arka Mohini and Arka Suphal) were selected. Resistant RILs inoculated with *M. incognita* exhibited higher proportions of nematicidal compounds such as 2,6-di-tert-butylbenzoquinone (12.42% and 6.75%), hexadecanoic acid (15.12% and 6.78%), (7,7-dimethyl-1,4-dioxo-2,3,4,5,6,7-hexahydro-1H-inden-2-yl) acetic acid (4.62% and 5.05%), and heptadecane (0.70% and 0.38%) in ACRIL 70 and ACRIL 90, respectively. Notably, hexadecanal (10.72%) was detected only in inoculated ACRIL 90. In addition, 2,6-di-tert-butylbenzoquinone was present in uninoculated resistant RILs (6.12% and 5.33%). In contrast, these compounds were present at much lower levels or absent in susceptible varieties. Resistant rootstocks also showed higher levels of antimicrobial and phenolic compounds both before and after inoculation, whereas susceptible lines exhibited significantly lower responses. These findings highlight the biochemical basis of nematode resistance in chilli and provide a foundation for breeding nematode-resistant cultivars, contributing to sustainable and eco-friendly nematode management.

Keywords: Chilli, GCMS analysis, Resistant RILs, root exudates, root-knot nematode

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a versatile crop that is cultivated both as a vegetable and spice. The biochemical compounds from the pepper fruits possess numerous industrial applications such as the carotenoids are used as natural colorants and capsaicinoids have a wide range of applications in the food, medicine and pharmaceutical industries. Globally, chillies and peppers are cultivated extensively, with world production reaching about 36.97 million tonnes in 2022 and rising further to nearly 40.2 million tonnes in 2023 according to recent FAO-based estimates FAOSTAT database, accessed 2024/2025. The yield, growth and quality of the fruits are highly impacted by many biotic and abiotic factors (Naresh et al., 2019). Nearly every crop in the world is attacked by root-knot nematodes (RKN), making

them the most commercially significant group of plant parasitic nematodes (Sasser & Freckman, 1987). *M. incognita* infection severely damages the root system and causes huge economic loss (10-50%) in pepper (Thies et al., 1998). Managing RKN through host plant resistance is a cost effective, farmer friendly and eco-friendly approach. Various host plant resistance mechanisms come in defence against RKN infection that include hypersensitive reaction (HR), a rapid and localized cell death, reactive oxygen species (ROS), antioxidant enzymes including peroxidase (PO), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), super oxide dismutase (SOD) and catalase (CAT). The proteins produced by the plants in response to pathogen attack known as the pathogenesis-related (PR) proteins and systemic acquired resistance (SAR) are associated with disease



resistance mechanisms (Chandrawat et al., 2020). Similarly, numerous compounds are associated with resistance and the genes responsible for providing various defense mechanisms encode the protein sequence of them. The central dogma of life reveals the transcription and translation of certain amino acids that lead to the formation of the respective compounds which ultimately results in a compatible or non-compatible relation between the host and the pathogen. Hence, identification of the compounds and the rate/number of compounds produced in both resistant and the susceptible reactions through GCMS will provide a clear picture of the nature of the reaction.

Since chilli plants are vulnerable to RKN damage, identifying the resistant lines and understanding their mechanism of action helps in efficient management of RKN. As root exudates play a major role in nematode hatching, host recognition and penetration, an attempt was made through this study to assess the anti-nematode compounds in RKN resistant RILs together with susceptible checks when infected by nematodes.

MATERIALS AND METHODS

Planting material

The present study was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru, India during 2021 to 2022 on ninety-nine recombinant inbred lines (RILs) derived from the crosses Anugraha × CM334 and IIHR-B-HP 130 × CM334 through the single seed descent method, were screened for resistance against *Meloidogyne incognita*. From these, two resistant lines (ACRIL 90 and ACRIL 70) and two susceptible varieties (Arka Mohini and Arka Suphal) were selected for further studies to elucidate the underlying resistance mechanism. Seedlings (28 days old) of the selected plants were transplanted at 1 plant per bag in black polythene bags (1 kg capacity) filled with sterilized potting mixture (1:1:1; sand, soil, FYM). The poly bags were arranged in completely randomized block design with five replications and five plants per replication. Another set of uninoculated plants were also maintained for root exudates analysis.

Preparation of nematode (*M. incognita*) inoculum

Egg masses of *Meloidogyne incognita* race 2 were collected from tomato plants (var. Arka Rakshak) maintained as culture hosts in the Nematology Glasshouse, Division of Crop Protection, ICAR-Indian

Institute of Horticultural Research, Bengaluru, India. Second stage juveniles (J2) hatching out from the eggs were harvested every day, and only J2 not less than 5 days old were utilized for inoculation at 1000 J2 per plant in polybags containing the test seedlings. Plants were uprooted after 15 and 30 days for analysis of root exudates and also scored for gall index after 30 days.

Host reaction to nematodes

The set of genotypes uprooted at 30 days were counted for number of galls in roots using a Motic SMZ 168 series stereoscopic zoom binocular microscope. The screened genotypes were categorized according to the 0-5 gall index provided in Table 1 (Taylor & Sasser, 1978).

Table 1 : Gall index scoring of RILs based on root knot index

| Scoring | No. of root galls/egg masses/plant | Reaction |
|---------|------------------------------------|---------------------------|
| 0 | 0 | Immune (I) |
| 1 | 1 to 2 | Highly resistant (HR) |
| 2 | 3 to 10 | Resistant (R) |
| 3 | 11 to 30 | Moderately resistant (MR) |
| 4 | 31 to 100 | Susceptible (S) |
| 5 | 101 and above | Highly susceptible (HS) |

Collection and analysis of root exudates

Root exudates were extracted from the resistant RIL's and susceptible varieties (Fig. 1) at 15 and 30 days after nematode inoculation and analyzed using GC-MS technique, varian-3000 Gas Chromatograph coupled with varian 4000 GC-MS-MS ion trap mass selective detector (Facundo et al., 2012). The composition of volatile organic compounds in the double plates was evaluated by solid phase micro-extraction (SPME) through GC- MS. Based on retention time and relative peak, an area of volatile organic compounds (VOCs) was identified as available in the library.



Fig. 1 : Root galls in highly susceptible lines
a. Arka Mohini, b. Arka Suphal

For GC-MS analysis, four genotypes with two being resistant (ACRIL-70 and ACRIL 90) and two being susceptible (Arka Mohini and Arka Suphal) to RKN were analysed in nematode inoculated and uninoculated conditions. The sample size used for analysis was 5 grams. Based on retention time and relative peak an area of VOCs was identified as available in the library.

Identification and quantification

In GC equipped with FID (flame ionization detector)/MSD (mass selective detector) detectors, the peak for the identification of the individual compound their retention index or Kovats index and the mass spectra was compared with the standard compounds as available in the library.

Solid phase micro extraction (SPME) method

Solid phase micro extraction was based on the adsorption of analytes onto the coated phase of fused silica fibre and the partitioning of analytes between the stationary phase of the fibre and the extraction medium as air. It consists of a 1-2 cm long fused silica fibre, coated with a stationary phase such as poly dimethyl siloxane (PDMS), divinyl benzene (DVB), carboxen (CAR) or the mixture of all the three and bonded to a stainless- steel plunger and holder. A hole was made in the double petri dish inoculated with the sample covered with the parafilm and then SPME fibre (DVB/CAR/PDMS) was inserted into the plates through the hole and was allowed to adsorb the head-space volatiles for 2 h. These fibres were to be first conditioned at 250°C for 21 h in the injector port of GC with the continued flow of helium gas. Later fibre was removed and injected into a GC-MS for separation and identification of compounds.

GC-MS analysis

Subsequently, the fibre was allowed to remain in the injector port in the inlet during the run. The MS column was fused-silica capillary column of 30 mm x 0.25 mm id, 0.25 mm film thickness for the analysis. The injector temperature was set at 250°C and all injections were split-less mode for 0.2 min., detector temperature was 270°C and the temperature programmes for column was as follows: 40°C for 3 min at an increment 3°C/min to 19°C, hold for 1 min, then 5°C/min to 220°C and maintained the constant temperature for 5 min. The mass spectrometer was in the external electron ionization mode with the

carrier gas helium 1 mL/min., injector temperature, 250°C; trap temperature 190°C (EI), ion source-heating a 190°C, transfer line temperature 260°C, EI-mode at 70 eV, with full scan-range 50-350 amu. The compounds were identified by comparing the retention index which was determined by using homologous series of n-alkanes (C5 to C32) as standard and compared the spectra using two spectral libraries available as Wiley and NIST-2007 (Facundo et al., 2012).

Sample preparation

Extracted ethyl acetate fractions of root exudates were separated through TLC (thin layer chromatography) and concentrated in a rotary flash vacuum evaporator. This concentrated extract was used for GC-MS analysis.

RESULTS AND DISCUSSION

Host reaction to nematodes

Gall index was scored on a 0 to 5 scale to the selected plants based on the infection caused by the RKN after 30 days (Fig. 2). The results revealed that ACRIL 70, ACRIL 90 recorded an average number of galls to be <1 and a gall index of 1 upon RKN inoculation. These were considered to be highly resistant lines. While susceptible check varieties like Arka Mohini, Arka Suphal had recorded >100 galls and a gall index of 5, thus considered as highly susceptible to RKN.

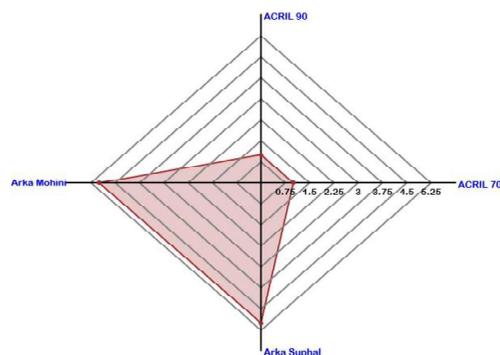


Fig. 2 : Radar chart representing the phenotypic reaction of RILs towards gall formation upon RKN inoculation

GCMS

The GCMS peaks were identified based on the RI and mass spectrum (Table 2 & 3). The metabolites identified from the tested lines are presented in Fig. 3 & 4. A total of 21 metabolites were identified by the analysis of the GCMS peaks.

Table 2 : GC-MS analysis anti-microbial compounds (%) in RKN susceptible checks and resistant RILs

| Antimicrobial activity and combined compounds | RT (min) | Arka Mohini | | ACRIL70 | | Arka Suphal | | ACRIL90 | |
|--|----------|-------------|------|---------|-------|-------------|-------|---------|-------|
| | | UN | I | UN | I | UN | I | UN | I |
| 3 - Pentanol, 2, 4 - dimethyl | 4.53 | - | - | - | 52 | - | - | - | 3.39 |
| 3 - Hexanol, 3, 5 - dimethyl | 6.14 | - | 1.3 | - | - | - | 0.11 | - | - |
| 1, 2 - Benzenedicarboxylic acid, butyl 2 - methylpropyl ester | 24.05 | - | 4.34 | 5.41 | 9.39 | - | 4.76 | - | 6.7 |
| 3 - Pentanol, 2, 4 - dimethyl | 4.52 | - | - | - | 3.74 | - | - | - | - |
| Z - 2 - Tetradecen - 1 - ol acetate | 22.26 | - | - | - | - | - | 3.73 | - | - |
| Nonadecane, 2 - methyl | 24.67 | - | 3.82 | - | 90.81 | - | - | - | - |
| Butanoic acid, 3 - methyl | 5.07 | - | - | - | - | - | - | - | 2.7 |
| Benzenepropanoic acid, 4 - hydroxy | 20.14 | - | - | - | - | - | - | - | 7.934 |
| 5 - Hydroxy- 2, 3, 3 - trimethyl - 2 - (3 - methyl - buta-1,3- dienyl) - cyclohexanone | 20.7 | - | - | - | - | - | - | - | 0.288 |
| Nonadecane | 24.58 | - | 3.19 | 30.4 | 42.51 | - | 8.417 | 29.81 | 36.03 |

*RT: Retention time, %: values based on the peak area percentage

Table 3 : GC-MS analysis nematicidal compounds (%) in RKN susceptible checks and resistant RILs

| Compound | RT (min) | Arka Mohini | | ACRIL70 | | Arka Suphal | | ACRIL90 | |
|--|----------|-------------|------|---------|-------|-------------|------|---------|-------|
| | | UN | I | UN | I | UN | I | UN | I |
| NEMATICIDAL | | | | | | | | | |
| 2, 6 - Di - tert - butylbenzoquinone | 17 | - | 1.67 | 6.12 | 12.42 | - | 2.8 | 5.33 | 6.75 |
| Hexadecanal | 22.31 | - | 3.47 | - | - | - | - | - | 10.72 |
| Heptadecane | 20.84 | - | - | - | 0.7 | - | - | - | 0.38 |
| (7, 7 - Dimethyl - 1, 4 - dioxo - 2, 3, 4, 5, 6, 7 - hexahydro - 1 H - inden - 2 - yl) acetic acid | 24.05 | - | - | - | 4.62 | - | 1.96 | - | 5.05 |
| Hexadecanoic acid | 24.51 | - | 0.89 | - | 15.12 | - | 2.76 | - | 6.775 |
| Ethyl hexadecanoate | 24.78 | - | - | - | - | 3.97 | - | - | - |
| ANTI BACTERIAL | | UN | I | UN | I | UN | I | UN | I |
| 1, 2 - Benzenediol | 13.67 | - | - | - | - | 10.43 | - | - | - |
| Hydroquinone | 13.86 | 2 | - | - | 2.98 | - | - | - | 6.485 |
| Oxacyclotetradecan - 2 - one, 14 - methyl | 20.38 | - | - | - | 9.548 | - | - | - | - |
| Pentadecanoic acid | 21.82 | - | - | - | - | - | - | - | 4.155 |
| Geranyl isovalerate | 19.84 | - | - | - | 4.29 | - | - | - | - |
| ANTI FUNGAL | | - | - | - | - | - | - | - | - |
| p - Benzoquinone | 6.21 | - | - | - | - | - | - | - | - |
| Pentadecanoic acid | 21.82 | - | - | - | - | - | - | - | 4.155 |
| OTHER COMPOUNDS | | - | - | - | - | - | - | - | - |
| 3 - Hexanol, 3, 5 - dimethyl | 6.14 | - | - | - | - | - | - | - | - |
| Butanoic acid | 5.07 | 24.087 | - | - | - | - | - | - | - |

*RT: Retention time, %: values based on the peak area percentage

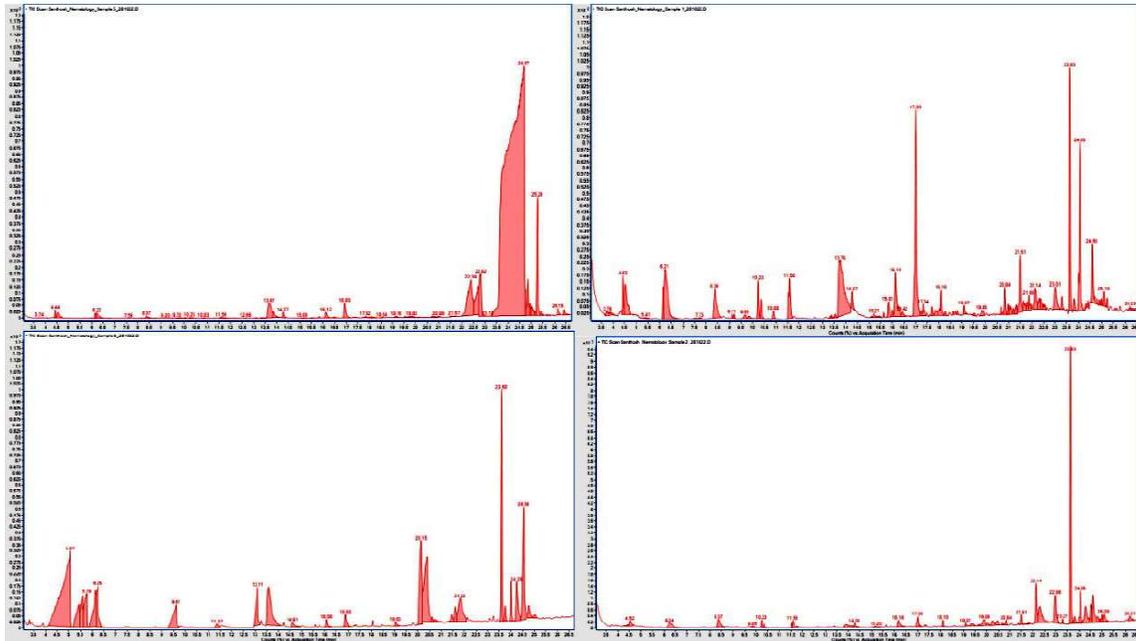


Fig. 3 : Production of volatile compounds from the GCMS analysis of resistant RILs (higher content)

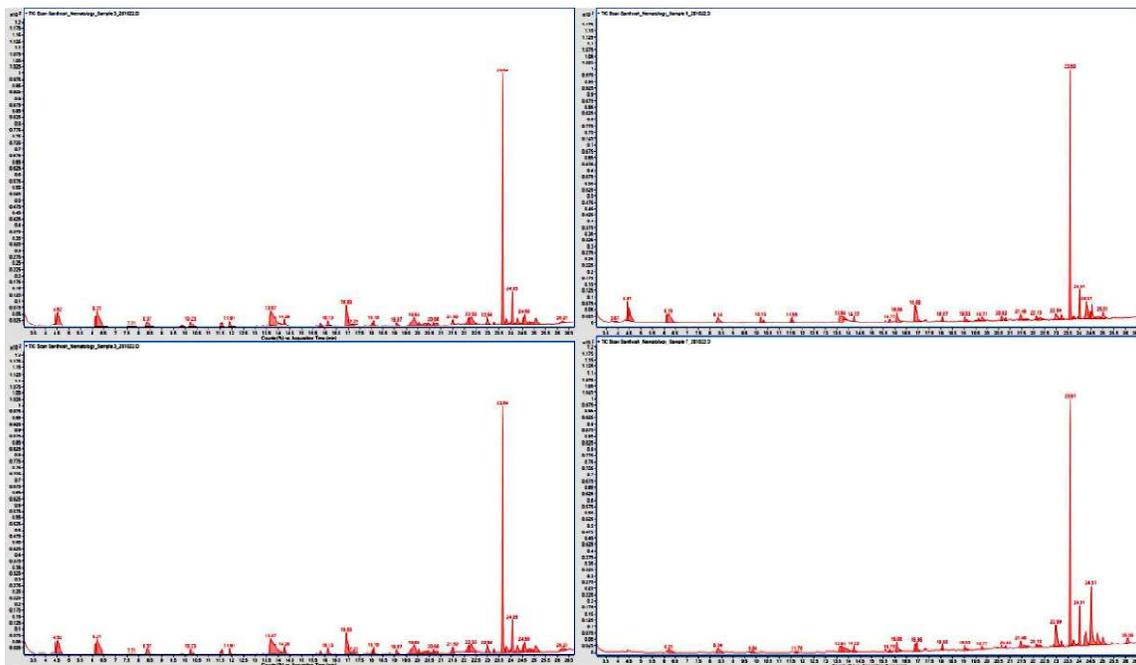


Fig. 4 : Production of volatile compounds from the GCMS analysis of susceptible varieties (lower content)

Under uninoculated conditions, two compounds *viz.*, nonadecane and 2, 6 - Di-tert- butylbenzoquinone were identified in both the resistant RIL's, while, 1, 2 - Benzenedicarboxylic acid, butyl 2-methylpropyl ester were detected only in ACRIL 70. At the same time, none of the identified compounds were common for the two susceptible varieties tested under uninoculated

conditions. Here, the compound hydroquinone was identified from the susceptible Arka Mohini while, ethyl hexadecanoate; 1, 2 - benzenediol; and 3 - Hexanol, 3, 5 - dimethyl in Arka Suphal Under inoculated conditions, eight compounds *viz.*, 3 - Pentanol, 2, 4 - dimethyl; 1, 2 - Benzenedicarboxylic acid, butyl 2 - methylpropyl

ester; nonadecane; 2, 6 - Di-tert-butylbenzoquinone; heptadecane; (7, 7 - Dimethyl - 1, 4 - dioxo - 2, 3, 4, 5, 6, 7 - hexahydro - 1 H - inden-2-yl) acetic acid; hexadecanoic acid; and hydroquinone were common in both the resistant RILs. The compounds, 3 - Pentanol, 2, 4 - dimethyl; nonadecane, 2 - methyl; oxacyclotetradecan-2-one, 14 - methyl; and geranyl isovalerate were unique to ACRIL 70; whereas, butanoic acid, 3-methyl; benzenepropanoic acid, 4 - hydroxy; 5 - Hydroxy - 2, 3, 3 - trimethyl - 2 - (3 - methyl-buta-1, 3 - dienyl) - cyclohexanone; hexadecanal; and pentadecanoic acid were only detected in ACRIL 90. Similarly, in case of inoculated susceptible varieties, the compounds that were common in both the varieties included 3 - Hexanol, 3, 5 - dimethyl; 1, 2 - Benzenedicarboxylic acid, butyl 2 - methylpropyl ester; nonadecane; 2, 6 - Di-tert-butylbenzoquinone; and hexadecanoic acid. In Arka Mohini, nonadecane, 2 - methyl; and hexadecanal were unique, while, Z-2-Tetradecen-1-ol acetate; and (7, 7 - Dimethyl - 1, 4 - dioxo - 2, 3, 4, 5, 6, 7 - hexahydro - 1H - inden-2-yl) acetic acid were unique in Arka Suphal. It was observed that a higher number and concentration of chemical compounds were detected from the resistant RILs than in the susceptible varieties. The compound with the highest concentration was nonadecane, 2-methyl (90 ng/g fresh weight) followed by 3-Pentanol, 2, 4-dimethyl (52 ng/g fresh weight) was detected in the inoculated ACRIL 70.

The present study revealed a higher number and concentration of chemical compounds in the resistant RILs compared to the susceptible varieties, indicating the involvement of those excess observed compounds in the plant defence mechanisms against RKN as the phenomenon was commonly observed in resistant plant types when exposed to biotic stress (nematode infection). Among the detected compounds, Nonadecane, 2-methyl, followed by 3-Pentanol, 2, 4-dimethyl, exhibited the highest concentrations in the inoculated ACRIL 70. Additionally, hexadecanoic acid was exclusively detected in the inoculated plants, with the highest concentration recorded in the resistant RILs, particularly ACRIL 70 and ACRIL 90.

Nonadecane has been reported to possess antibacterial properties (Kumari et al., 2019), while, recent evidence suggests that 3-pentanol exhibits nematocidal properties against *M. incognita* by immobilizing and killing its J2 stage. Wang et al. (2023) observed that root-knot nematode resistant cultivars had higher

concentrations of heptadecane compared to the susceptible plants in toato, which aligns with our findings where heptadecane was exclusively detected in the two nematode inoculated resistant RILs and not found in uninoculated or susceptible varieties. Furthermore, hexadecanoic acid, isolated from Pistia and Eichornia (Tyagi & Agarwal, 2017) as well as *B. amyloliquefaciens* (Tadigiri et al., 2020), has been reported to possess nematocidal and antimicrobial properties. Similarly, nematocidal compounds like Octadecadienoic acid was identified in bacterium-based resources (Kanagarajan et al., 2016).

CONCLUSION

The analysis of chemical compounds in root exudates revealed a notable contrast between resistant and susceptible genotypes. Resistant chilli RILs exhibited elevated levels of antimicrobial, phenolic, and nematocidal compounds, both before and after *M. incognita* inoculation. In contrast, the check varieties displayed significantly lower or nil production of these compounds upon RKN inoculation. These findings emphasize the potential of antimicrobial and nematocidal compounds in conferring resistance to RKN damage in chilli, offering promising prospects for future crop improvement strategies.

ACKNOWLEDGEMENT

The authors express their appreciation to Dr. P. D. Kamala Jayanthi, National Professor, Division of Crop Protection for providing GC-MS facility and ICAR-Indian Institute of Horticultural Research, Bengaluru, India for providing financial support for research work.

REFERENCES

- Chandrawat, B. S., Siddiqui, A. U., Bhati, S. S., & Saharan, V. (2020). Bio-agents: A source for initiation of defence enzymes in chilli infected with root-knot nematode, *Meloidogyne incognita*. *Journal of Entomology and Zoology Studies*, 8(6), 1684-1688.
- FAOSTAT database, accessed 2024/2025: <http://faostat3.fao.org/home/index.html#download>.
- de Vasconcelos Facundo, H. V., dos Santos Garruti, D., dos Santos Dias, C. T., Cordenunsi, B. R. & Lajolo, F. M. (2012). Influence of different banana cultivars on volatile compounds during ripening in cold storage. *Food Research International*, 49(2), 626-633. <https://doi.org/10.1016/j.foodres.2012.08.013>

- Kanagarajan, M., Devimarudachalam, D., Ponnuraj, S., & Jagathan, D. (2016). Synergistic effect of ethno medicinal plants against biofilm-forming *Streptococcus pyogenes* isolated from upper respiratory tract infection. *International Journal of Phytomedicine*, 8, 208-216.
- Kumari, A., Kumar, P., Kumar, M., & Kumar, J. (2020). Antibacterial activity of *Glycyrrhiza glabra* root extracts against *Staphylococcus* sp. and *Escherichia coli*. *World Journal of Pharmaceutical Research*, 9, 1069-1080. doi: 10.20959/wjpr202015-19285
- Naresh, P., Meenu, K., Acharya, G. C., Reddy, A. C., & Lakshmana, D. (2019). Genetics and molecular markers for resistance to major soil borne pathogens in chilli (*Capsicum annuum* L.). *Research Journal of Biotechnology*, 14, 101-105.
- Sasser, J. N., & Freckman, D. W. (1987). A world perspective on nematology: the role of the society. In: Veech, J.A., and Dickson, D.W. (eds) *Vistas on Nematology. Society of Nematologists*, Hyattsville. 7-14. doi: 10.4236/oalib.1101372
- Tadigiri, S., Das, D., Allen, R., Vishnu, V., Veena, S., & Karthikeyan, S. (2020). Isolation and characterization of chemical constituents from *B. amyloliquefaciens* and their nematicidal activity. *Journal of Entomology and Zoology Studies*.
- Thies, J. A., Mueller, J. D., & Fery, R. L. (1998). Use of a resistant pepper as a rotational crop to manage southern root-knot nematode, *HortScience*, 33, 716–718. doi: 10.21273/HORTSCI.33.4.716
- Taylor, A. L., & Sasser, J. N. (1978). *Biology, identification and control of root-knot nematodes*. International Nematology Project, North Carolina State University, Graphics, Raleigh, 11. doi: 10.4236/as.2019.108082
- Tyagi, T., & Agarwal. (2017). M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 195-206. doi: 10.4236/fns.2022.132013
- Wang, X., Wang, C., Chen, R., Wang, W., Wang, D., & Tian, X. (2023). Plant genotype shapes the soil nematode community in the rhizosphere of tomatoes with different resistance to *Meloidognye incognita*. *Plants*. 12(7), 1528. <https://doi.org/10.3390/plants12071528>

(Received : 30.7.2024; Revised : 11.9.2025; Accepted : 16.9.2025)

