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Histopathological and biochemical variations in resistant and susceptible black pepper cultivars infected with Meloidogyne incognita

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Abstract

Plant-parasitic nematodes cause yield losses ranging from 30 to 65% in black pepper. Root-knot nematodes are a bottleneck in production of black pepper in Kerala, India. Identifying resistant cultivars have gained attention, as they provide an eco-friendly alternative to chemical nematicides. This study aimed to identify a root-knot nematode-resistant black pepper cultivar and determine the biochemical and histopathological changes caused by the nematode. Twenty-six black pepper cultivars (10 KAU released, 2 wild and 14 local) were screened for nematode resistance under pot culture conditions. The local cultivar Koshinadan exhibited resistance to *Meloidogyne incognita* (gall index 2), while the hybrid variety Panniyur 1 showed high susceptibility (gall index 5). Analysis of the biochemical basis of resistance revealed enhanced level of phenol content and activity of peroxidase, phenylalanine ammonia-lyase, and polyphenol oxidase in leaves and roots of Koshinadan compared to susceptible cultivar, Panniyur 1. Histopathological studies of the most resistant (Koshinadan) and susceptible (Panniyur 1) cultivars revealed that giant cells formed near the vascular region at different inoculum levels (0, 100, 500, 1000, 5000, 10000 J_2 pot^{-1}). The number and size of giant cells were lower in Koshinadan compared to Panniyur 1 and complete disorganization of xylem and phloem vessels was observed in the susceptible Panniyur 1. The resistant cultivar identified can serve as rootstock for grafting high-yielding varieties, resulting in nematode free seedlings.

Keywords

biochemical changes; black pepper; histopathology; pot culture; resistant cultivar; root-knot nematode

Introduction

Black pepper, *Piper nigrum* L. is one of the world's highly valued spices, and Kerala is the leading producer of black pepper in India. India cultivates more than 75 black pepper varieties across an area of 278050 ha, with a production of 64000 tonnes per annum (1). The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood being a serious constraint limiting production of black pepper has spread length and breadth of cultivated area of black pepper in India. Its polyphagous nature poses a significant challenge in effectively controlling its population buildup in the rhizosphere of black pepper plants. The juveniles (J_2s) of *M. incognita* infect the plants along at the root tip leading to the formation of giant cells that serve as a nutritive source. These giant cells

exhibit elevated levels of DNA, RNA and photosynthates, particularly 3 to 4 weeks post-infection. As the infestation progresses, the root system exhibits severe galling and adult females carrying egg masses are found embedded in the roots. This process disrupts water and nutrient absorption, resulting in yellowed leaves, shedding of spikes, retardation of growth and top-to-bottom drying of plants leading to a substantial reduction in yield (2). These nematodes also predispose plants to fungal and bacterial pathogens, leading to the formation of disease complexes (3).

Efficient control of these endoparasites is crucial for increasing black pepper production and reducing yield losses. The application of nematicides is limited in black pepper, as this spice crop is a significant export product. There is also a concern regarding environmental and food safety issues. Finding host resistance to *M. incognita* in domesticated or wild black pepper species has received considerable attention, as nematode-resistant cultivars offer an effective and environmentally friendly alternative to chemical nematicides. Resistance to nematodes equips a plant to inhibit nematode multiplication compared to a plant that lacks resistance (4). Identification and integration of resistant varieties in the farming system is the most economical and easy approach to manage the population build-up of the nematode. Nematode resistant wild varieties can be used as rootstock for grafting high yielding varieties which is one of the best methods to raise nematode- free seedlings.

Nematode infestation also causes significant biochemical alterations in the infected cells and the plant systems. The resistance or susceptibility of the host to nematode infection depends on the biochemical changes induced by nematodes. Information on varietal screening of black pepper cultivars, as well as biochemical and histopathological changes in resistant and susceptible cultivars at different *M. incognita* population densities, is important for the effective pathogen management. In this context, the present study aimed to screen black pepper cultivars for resistance to *M. incognita* and to assess the biochemical and histopathological changes in tolerant and susceptible cultivars due to *M. incognita* infestation.

Materials and Methods

Maintenance of nematode inoculum

The pure culture of *M. incognita* was maintained on tomato plants (variety- Vellayani Vijay) in a glass house at the Department of Nematology, College of Agriculture, Vellayani. The conditions were temperatures of 25 °C-28 °C (day) and 18 °C-20 °C (night), 60-75% humidity and 12000-18000 lux light at coordinates 8.4316° N latitude and 76.9860° E longitude. The perineal pattern of female nematode was prepared to identify *M. incognita* (5). Plants infected with *M. incognita* were uprooted and gently rinsed to remove adhering soil particles. Egg masses attached to the root surface were removed with a needle and transferred to a beaker containing distilled water. The second-stage juveniles (J_2s) were collected and counted using a stereo zoom microscope (ZEISS Stemi 305, Germany) after 3 to 5 days. Sterile water was added to adjust the juvenile concentration to the required number per mL of suspension. Fresh hatched juveniles were inoculated to the root zone of two-week-old tomato plants by making small holes at the base. The plants were maintained in pots containing 5 kg of potting mixture of sterile soil, sand and well decomposed farmyard manure in 2:1:1 ratio. The plants were maintained with adequate soil moisture and used as the source of inoculum for screening experiments. Subculturing was performed periodically by inoculating infective J_2 to ensure the availability of sufficient nematode inoculum.

Screening of black pepper cultivars for nematode resistance

Twenty-six black pepper cultivars were screened for relative tolerance to *M. incognita*. The screened cultivars included ten KAU-released varieties (Panniyur 1 to 10), 2 wild varieties and fourteen local cultivars collected from Farming System Research Station, Sadanandapram (Karimunda, Vellamundi, Aimpiriyan, Perumkodi, Palakodi, Kottinadan, Kumbhanadan, Arikkilazhi, Amichakkari, Murithothan, Nithyakalyani, Adukkanazhi and Cholakodi). The trial was carried out in the glass house , Koshinadan of the Department of Nematology, College of Agriculture, Vellayani, using completely randomized design with 3 replications per cultivar. Rooted black pepper cuttings of these varieties were transplanted to pots (diameter 22.5 cm) filled with 5 kg denematized potting mixture containing sterile soil, sand and well decomposed farmyard manure in 2:1:1 ratio. Fifteen days after planting, *M. incognita* juveniles maintained in tomato plants as mentioned above were inoculated into the rhizosphere of black pepper plants at 2J_2/g soil (2000 J_2s/kg soil) by making small holes around the base of the plants (6). Plants were watered regularly to ensure adequate moisture, and all agricultural practices were conducted as per the recommendations of Package of Practices of Kerala Agricultural University (KAU, 2016).

Observations on nematode population characteristics

The plants were uprooted 3 months after inoculation and observations of nematode population characteristics were recorded. The characteristics *viz.,* soil and root nematode population, number of galls, females, egg masses, eggs/egg mass, were assessed. The biometric growth parameters *viz.,* plant height (cm), number of leaves and root and shoot weights (g), were also recorded. To assess the nematode population, soil (200 cc) and root (5 g) samples were collected from the rhizosphere of black pepper plants. The nematodes were extracted using Cobb's sieving and decanting method (7) and modified Baermann funnel method (8). The extracted nematodes were counted using a counting dish under a stereo zoom microscope. The number of galls present in 5 g root was counted and root-knot indexing was performed in 0-5 scale by Taylor and Sasser (9). The number of females in root (5 g) was estimated by differential staining technique using the acid fuchsin-lactophenol method (10). The rate of nematode multiplication expressed as the reproduction factor, was calculated using Oostenbrink's formula (RF=Pf/Pi), which is the ratio of final to initial population (11). The number of egg masses in the root (5 g) was estimated with the method of Southey (12). The root was immersed in Phloxine B solution (0.15 g Phloxine B in 1 L water) for 15 min

to stain the egg masses. The stained egg masses obtained from root bits (5 g) were carefully handpicked using sterilized forceps. Subsequently, the egg mass was placed in distilled water and 20 mL of 5.45% NaOCl was added. The suspension was centrifuged at 1600 rpm for 10 min to dissolve the matrix and achieve uniform dispersal of suspended eggs (13). The suspended eggs were counted using a stereo zoom microscope.

Biochemical analysis

The biochemical basis of resistance in the identified resistant and susceptible black pepper cultivars was assessed by estimating total phenols, protein content, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase after inoculation with various inoculum levels of *M. incognita.* Rooted cuttings of both tolerant and susceptible cultivars of black pepper were planted in pots filled with denematized potting mixture. Fifteen days after planting, the pots were inoculated with freshly hatched juveniles at 100, 500, 1000, 5000 and 10000 J_2/pot. Uninoculated set of plants was maintained as a control. The experiment was laid out in a Completely Randomized Design with 3 replications. The recommendations of the package of practices suggested by Kerala Agricultural University were followed for plant maintenance (KAU, 2016). Forty-five days post-inoculation, the biochemical assays were carried out using standard protocols.

Estimation of total phenol

The estimation of phenol content in the roots and leaves of resistant and susceptible black pepper varieties was carried out following the protocol of Bray and Thorpe (14). Samples were homogenized in 80% ethanol in the ratio 1:10 and were centrifuged for 20 min at 10000 rpm. The supernatant was collected, and the residue was subjected to further phenol extraction using ethanol and centrifugaion. The supernatant obtained through centrifugations were collected and was evaporated to dryness. The resulting residue was dissolved in 5 mLof double distilled water. To this solution 500 µL of Folin-Ciocalteau reagent was added and 2 mL of 20% sodium carbonate solution was added after 3 min. The solution was thoroughly mixed and heated for 1 min in a water bath. The mixture was cooled and the absorbance was measured at 650 nm in a spectrophotometer (BIORAD- BioSpectrometer) against a reagent blank. Catechol was taken as standard for phenol and the phenol content in the samples were expressed as catechol equivalents (mg catecholg g tissue^{-1}).

Estimation of total protein content

The estimation of total protein content in the roots and leaves of resistant and susceptible black pepper varieties was carried out following the procedure outlined by Bradford (15). Samples were homogenized in 0.1 M sodium acetate buffer (pH 4.7) in the ratio 1:10. The extract was centrifuged at 10,000 rpm for 15 min in a refrigerated centrifuge at a temperature of 4°C. The reaction mixture includes 500 µl of enzyme extract, 500µl of distilled water and 5 ml of coomassie brilliant dye solution. The absorbance was measured at 595 nm against a reagent blank in a spectrophotometer (BIORAD-BioSpectrometer). Bovine serum albumin (BSA) was used as the standard and the protein content in the samples was expressed as equivalents of BSA produced min^{-1} g^{-1} of fresh weight.

Estimation of peroxidase (PO) and polyphenol oxidase (PPO)

The enzyme extract was prepared from root and leaf samples of resistant and susceptible black pepper cultivars. Samples were homogenized in 0.1 M sodium phosphate buffer (pH 6.5) in the ratio 1:5. After homogenization the mixture was centrifuged at 5000 rpm for 15 min at 4 °C. This extract was used for the estimation of PO and PPO.

Peroxidase activity was assayed following the protocol described by Srivastava (16). Peroxidase activity was determined by preparing a reaction mixture consisting of 1 mL of 0.05 µL pyrogallol and 50 µL of enzyme extract. The reaction was initiated by the addition of 1 mL of 1% hydrogen peroxide (H_2O_2) and the absorbance was measured at 420 nm in a spectrophotometer (BIORAD- BioSpectrometer) and the changes in absorbance were recorded every 30 sec intervals. The PO activity was expressed as changes in absorbance min^{-1}g^{-1} fresh weight of tissue.

Polyphenol oxidase activity was determined following the procedure (17). The reaction mixture included 1 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µL of enzyme extract. The reaction was initiated after adding 1 mL of 0.01 M catechol. The absorbance was recorded at 495 nm at 30 sec intervals. PPO activity was expressed as a change in the absorbance min^{-1}g^{-1} of leaf tissue on fresh weight basis.

Estimation of phenylalanine ammonia lyase (PAL)

PAL activity was estimated following the procedure (18). The enzyme extracts from root and leaf samples were prepared using 0.1 M borate buffer (pH 8.8) in the ratio 1:5. After centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was used for the assay. The reaction mixture included 3 mL of 0.1 M sodium borate buffer (pH 8.8), 0.2 mL enzyme extract and 0.1 mL of 12 mM L-phenylalanine prepared in the same buffer. A blank was prepared without L-phenylalanine. Both the reaction mixture and blank were incubated at 40 °C for 30 min and the reaction was read at 290 nm using a spectrophotometer (BIORAD- BioSpectrometer). PAL activity was expressed as µg of cinnamic acid min^{-1}g^{-1} of leaf tissue on a fresh weight basis.

Histopathological changes

The roots of resistant and susceptible black pepper cultivars inoculated with different inoculum levels of *M. incognita* (0, 100, 500, 1000, 5000 and 10000 juveniles/pot) were used for histopathological studies. Plants were uprooted 45 days post-inoculation for histopathological analysis. The roots were collected, washed and selected root bits were cut into small pieces (1 to 2 cm length) and transferred into distilled water. Galled root segments were selected, excised and fixed in formalin-aceto-alcohol solution (FAA: 50 mL 95% ethanol, 35 mL distilled water, 10 mL 40% formaldehyde solution, and 5 mL glacial acetic acid). The root segments were dehydrated in different concentrations of ethanol (35, 50, 75 and 100% for 1 hr), infiltrated and embedded in paraffin wax (50-55 °C). Thin root sections were taken and the sections were carefully transferred to glass slides and left for 2 min to dry (19). Sections stained with safranin and toludine blue were viewed

under a light microscope (ZEISS Axioskop 2 Plus, Germany) at 40X magnification.

Statistical analysis

The data generated were subjected to analysis of variance (ANOVA) and the means were compared by Duncan's New Multiple Range Test (DMRT) at 5% level of significance using the statistical software KAU GRAPES 1.0.0. Variables which did not satisfy the basic assumption of ANOVA were subjected to square root transformation.

Results

Screening of black pepper cultivars for nematode resistance

Twenty-six black pepper cultivars were screened for nematode resistance against *M. incognita* under artificial inoculation. Nematode resistance was evaluated based on nematode population characteristics and growth parameters. Among the cultivars screened, the local cultivar Koshinadan recorded the lowest mean nematode population (353.33 *M. incognita* J_2/200cc soil), while the hybrid variety Panniyur I was found to be highly susceptible, with a nematode population of 1786.67 *M. incognita* J_2 200 cc/soil. Regarding the nematode population in roots (5 g) also Koshinadan recorded the lowest mean number of *M. incognita* juveniles (11.67) compared to Panniyur 1 (82.00) (Table 1). Observation on number of females also showed a similar trend. The maximum number of females (175.50) and vermiform juveniles observed in the rhizosphere of Panniyur I, indicate its highest susceptibility to *M. incognita,* while in Koshinadan it was lowest indicating its resistance. Koshinadan recorded the lowest number of galls (7.67/5g root) and a gall index of 2 indicating resistance to *M. incognita*. Conversely, Panniyur 1 recorded the highest number of galls (126.33/5 g root), showing higher susceptibility to nematode infestation, with a gall index of 5. Other varieties viz., Panniyur 2, Panniyur 3, Panniyur 5, Panniyur 6, Panniyur 7, Panniyur 8, Panniyur 9, Wild 1, Wild 2, Karimunda, Vellamundi, Aimpiriyan, Perumkodi, Palakodi, Kottinadan, Kumbhanadan, Arikkilazhi, Amichakkari, Murithothan, Nithyakalyani, Adukkanazhi, Cholakodi found susceptible to *M. incognita* with gall index of 4. The percentage reduction in number of galls in Koshinadan compared to the susceptible variety, Panniyur 1 was 93.93. This is the first report on the resistance reaction of Koshinadan to *M. incognita*. The number of egg masses (5 g root) was lowest in Koshinadan (6.00) and the highest number was recorded in the susceptible variety, Panniyur 1 (75.00). A similar trend was observed with number of eggs in egg mass. The resistant variety, Koshinadan recorded 82.67 eggs in egg mass, while in the susceptible variety, Panniyur 1 it was 221.33.

The reproduction factor of *M. incognita* was lowest in Koshinadan (0.94), while in other varieties it ranged from 1.21 to 5.11. Koshinadan had the lowest reproduction factor compared to Panniyur 1 and was significantly better than all other cultivars in reducing nematode population characteristics. If the reproduction factor of a plant-parasitic nematode on a specific host plant is below one, it indicates that the nematode failed to multiply on that host. Conversely, when the reproduction factor is greater than one, the nematode can complete its life stages and lay eggs on that host plant (20). Plants can resist nematode infestation through pre-infection resistance, where toxic chemicals prevent nematodes from entering the roots, or post-infection resistance, where nematodes penetrate but cannot develop. The metabolites produced in the root zone of plants also play a crucial role in repelling nematodes (21). Resistant varieties effectively hinder the reproduction of root-knot nematodes and significantly reduce the population (22). Koshinadan was found to be least preferred host of *M. incognita,* recording a significant reduction in the population of nematodes in soil and root system, adult females, galled roots and eggs compared to the highly susceptible KAU-released variety, Panniyur 1 (80.22 to 94.11%). The multiplication ability of *M. incognita* in different black pepper cultivars depends on the suitability of the host for completing its life cycle. The number of egg masses and eggs produced depends on the number of juveniles that entered and their ability to develop into mature adult females. The findings of the present study corroborate with another study(23). They reported the least number of egg masses (2.00) in rootstocks of Sakthi and Thevam the cultivars released by IISR, followed by *Piper colubrinum* Link (4.00). *Piper argyrophyllum* Miquel rootstock recorded the highest number of egg masses (33.00), followed by the Panniyur 1 (27.00) 45 DAI (23).

Koshinadan exhibited the highest root weight (19.1 g) and shoot weight (115.00 g) whereas in Panniyur 1 it was significantly lower (root weight 3.27 g; shoot weight 47.00 g) (Table 2). The results obtained in this study are in accordance with Koshy and Sundararaju (24). The potential of Koshinadan to resist the attack of *M. incognita* could be traced out from its enhanced vigour and growth based on the observations of shoot and root weight. Regarding root and shoot weight, the tolerant cultivar Koshinadan outperformed the other cultivars and the lowest root weight was observed in Panniyur 1. Based on the nematode population characteristics and biometric characters, Koshinadan and Panniyur 1 were identified as resistant and susceptible black pepper cultivars to *M. incognita.* This finding was supported by several workers (24, 25).

Biochemical changes

The lowest preference of *M. incognita* for Koshinadan observed in the study may be attributed to the nematode-resistant traits in the resistant cultivar. The biochemical basis of nematode resistance in Koshinadan and Panniyur 1 was analyzed by estimating phenols, protein and defense enzymes levels at different inoculum levels of *M. incognita*. Both susceptible and resistant cultivars showed a progressive increase in phenol content and defense enzymes (PO, PPO and PAL) in the samples of leaves and root after 45 days of nematode inoculation with an increase in inoculum levels (Table 3 and 4). The phenol content in leaves and roots of both resistant and susceptible cultivars of black pepper increased proportionally with the inoculation of *M. incognita*. The highest phenol content in the leaves and roots of Koshinadan (0.89 and 4.78 mg g^{-1} tissue) and Panniyur 1 (0.50 and 1.84 mg g^{-1} tissue) was observed in plants inoculated with 10000 J_2 pot^{-1}. The uninoculated plants of both tolerant and susceptible cultivars showed reduction in phenol content compared to the inoculated plants, both in leaves and roots. The percentage

Table 1. Response of black pepper cultivars to *M. incognita* infestation based on nematode population characteristics

Treatments	Final nematode population*			Reproduction factor* Rf=Pf/Pi)	Number of egg* masses (5 g root)	Number of eggs* in egg mass	Number of galls* (5 g root)	Gall index	Reaction
	Soil (200 cc)	Number of juveniles (5 g root)	Number of females (5 g root)						
Panniyur 1	1786.67 (42.24 ± 1.82) ^{n}	82.00 (9.05 ± 0.39) ^{i}	175.50 (13.45 ± 0.63) ^{l}	5.11 (2.26 ± 0.08) ^{o}	75.00 (8.66 ± 0.21) ^{o}	221.33 (14.88 ± 0.39) ^{m}	126.33 (11.24 ± 0.30) ^{n}	5	HS
Panniyur 2	1346.67 (36.65 ± 2.26) ^{klm}	60.33 (7.76 ± 0.52) ^{efgh}	123.67 (11.10 ± 0.97) ^{jk}	3.83 (1.96 ± 0.12) ^{lmn}	64.67 (8.02 ± 0.66) ^{lmno}	153.00 (12.37 ± 0.32) ^{fghij}	95.00 (9.75 ± 0.19) ^{lm}	4	S
Panniyur 3	1260.00 (35.49 ± 1.13) ^{jkl}	57.67 (7.59 ± 0.37) ^{efgh}	89.33 (9.44 ± 0.45) ^{fgh}	3.52 (1.88 ± 0.05) ^{klm}	56.33 (7.50 ± 0.39) ^{jklm}	160.67 (12.68 ± 0.08) ^{ijk}	65.33 (8.06 ± 0.69) ^{fghij}	4	S
Panniyur 4	1466.67 (38.28 ± 1.52) ^{m}	67.67 (8.21 ± 0.58) ^{hi}	143.33 (11.93 ± 1.21) ^{k}	4.19 (2.05 ± 0.09) ^{n}	69.33 (8.33 ± 0.13) ^{mno}	191.33 (13.83 ± 0.38) ^{l}	103.00 (10.14 ± 0.22) ^{m}	5	HS
Panniyur 5	1046.67 (32.33 ± 1.28) ^{efgh}	49.67 (7.03 ± 0.57) ^{cde}	98.33 (9.91 ± 0.36) ^{ghi}	2.99 (1.73 ± 0.06) ^{ghij}	50.67 (7.10 ± 0.61) ^{ghijk}	141.33 (11.88 ± 0.55) ^{defg}	77.33 (8.79 ± 0.38) ^{ijk}	4	S
Panniyur 6	1286.67 (35.81 ± 2.56) ^{jklm}	55.67 (7.45 ± 0.53) ^{efgh}	92.00 (9.58 ± 0.66) ^{fgh}	3.59 (1.89 ± 0.12) ^{klm}	41.67 (6.44 ± 0.59) ^{efgh}	154.67 (12.43 ± 0.59) ^{ghij}	76.00 (8.71 ± 0.59) ^{hijk}	4	S
Panniyur 7	1273.33 (35.68 ± 0.43) ^{jklm}	63.67 (7.97 ± 0.54) ^{fgh}	85.00 (9.21 ± 0.43) ^{efg}	3.56 (1.89 ± 0.02) ^{klm}	44.33 (6.65 ± 0.31) ^{fghi}	166.00 (12.88 ± 0.34) ^{jk}	67.67 (8.22 ± 0.37) ^{ghij}	4	S
Panniyur 8	1173.33 (34.21 ± 2.20) ^{hijk}	63.00 (7.93 ± 0.44) ^{fgh}	79.00 (8.89 ± 0.35) ^{efg}	3.29 (1.81 ± 0.11) ^{ijk}	43.00 (6.55 ± 0.28) ^{efghi}	142.33 (11.93 ± 0.33) ^{defgh}	66.00 (8.11 ± 0.54) ^{ghij}	4	S
Panniyur 9	1120.00 (33.44 ± 1.56) ^{fghij}	58.67 (7.65 ± 0.43) ^{efgh}	90.67 (9.51 ± 0.61) ^{fgh}	3.17 (1.78 ± 0.09) ^{hijk}	52.67 (7.24 ± 0.60) ^{hijkl}	171.00 (13.07 ± 0.35) ^{k}	78.33 (8.82 ± 0.85) ^{jk}	4	S
Panniyur 10	1440.00 (37.92 ± 1.72) ^{lm}	60.00 (7.72 ± 0.82) ^{efgh}	115.67 (10.75 ± 0.29) ^{ij}	4.04 (2.01 ± 0.07) ^{mn}	71.00 (8.43 ± 0.26) ^{no}	187.67 (13.70 ± 0.15) ^{l}	102.33 (10.11 ± 0.22) ^{m}	5	HS
Wild 1	1066.67 (32.63 ± 1.69) ^{efghi}	52.33 (7.23 ± 0.38) ^{efg}	87.33 (9.34 ± 0.35) ^{fg}	3.02 (1.74 ± 0.08) ^{ghij}	34.33 (5.79 ± 1.06) ^{cde}	109.00 (10.43 ± 0.48) ^{b}	74.33 (8.62 ± 0.35) ^{hijk}	4	S
Wild 2	746.67 (27.30 ± 1.49) ^{c}	49.00 (6.97 ± 0.84) ^{cde}	51.67 (7.16 ± 0.75) ^{cd}	2.12 (1.46 ± 0.06) ^{cd}	37.00 (6.02 ± 1.08) ^{def}	131.67 (11.47 ± 0.44) ^{cd}	37.00 (6.06 ± 0.71) ^{bc}	4	S
Karimunda	853.33 (29.19 ± 1.38) ^{cd}	40.67 (6.35 ± 0.72) ^{cd}	56.00 (7.47 ± 0.64) ^{cd}	2.38 (1.54 ± 0.07) ^{de}	29.00 (5.33 ± 0.89) ^{bcd}	164.67 (12.83 ± 0.24) ^{jk}	36.33 (6.00 ± 0.69) ^{bc}	4	S
Vellamundi	1240.00 (35.21 ± 0.76) ^{ijkl}	65.33 (8.07 ± 0.53) ^{gh}	66.67 (8.13 ± 0.87) ^{de}	3.43 (1.85 ± 0.03) ^{jkl}	36.33 (6.02 ± 0.31) ^{def}	132.00 (11.48 ± 0.48) ^{cd}	52.67 (7.24 ± 0.60) ^{def}	4	S
Aimpiriyan	1286.67 (35.85 ± 1.56) ^{jklm}	51.67 (7.18 ± 0.31) ^{def}	79.67 (8.92 ± 0.40) ^{efg}	3.55 (1.88 ± 0.07) ^{klm}	39.33 (6.27 ± 0.20) ^{efg}	148.67 (12.19 ± 0.19) ^{efghi}	56.67 (7.52 ± 0.36) ^{efg}	4	S
Perumkodi	1000.00 (31.61 ± 0.84) ^{defgh}	48.67 (6.97 ± 0.33) ^{cde}	86.00 (9.26 ± 0.48) ^{fg}	2.84 (1.68 ± 0.04) ^{fghi}	42.67 (6.52 ± 0.36) ^{efghi}	122.67 (11.07 ± 0.30) ^{c}	74.33 (8.61 ± 0.46) ^{hijk}	4	S
Palakodi	1140.00 (33.75 ± 1.35) ^{ghij}	49.67 (7.05 ± 0.18) ^{cde}	75.67 (8.70 ± 0.33) ^{ef}	3.16 (1.78 ± 0.06) ^{ghijk}	42.33 (6.50 ± 0.27) ^{efgh}	139.67 (11.82 ± 0.13) ^{def}	59.67 (7.72 ± 0.46) ^{fg}	4	S
Kottinadan	1026.67 (32.03 ± 0.96) ^{efgh}	51.67 (7.16 ± 0.73) ^{def}	78.00 (8.83 ± 0.21) ^{efg}	2.89 (1.70 ± 0.05) ^{fghi}	39.00 (6.23 ± 0.52) ^{ef}	134.67 (11.60 ± 0.26) ^{cde}	63.33 (7.96 ± 0.28) ^{fgh}	4	S
Kumbanadan	1146.67 (33.85 ± 1.22) ^{ghij}	63.00 (7.93 ± 0.57) ^{fgh}	111.33 (10.54 ± 0.61) ^{hij}	3.30 (1.82 ± 0.06) ^{ijkl}	60.67 (7.79 ± 0.16) ^{klmn}	155.67 (12.48 ± 0.24) ^{hij}	95.33 (9.76 ± 0.40) ^{lm}	4	S
Arikilazhi	700.00 (26.44 ± 1.35) ^{c}	29.00 (5.37 ± 0.52) ^{b}	49.33 (6.97 ± 1.02) ^{c}	1.95 (1.39 ± 0.08) ^{c}	23.00 (4.78 ± 0.45) ^{b}	88.00 (9.38 ± 0.61) ^{b}	33.67 (5.78 ± 0.69) ^{b}	4	S
Amichakkari	513.33 (22.54 ± 2.77) ^{b}	38.67 (6.21 ± 0.20) ^{bc}	42.33 (6.50 ± 0.31) ^{bc}	1.49 (1.21 ± 0.13) ^{b}	54.33 (7.36 ± 0.41) ^{ijkl}	99.67 (9.97 ± 0.24) ^{b}	35.33 (5.93 ± 0.34) ^{bc}	4	S
Murithothan	973.33 (31.16 ± 1.77) ^{defg}	51.67 (7.18 ± 0.35) ^{def}	87.33 (9.32 ±0.84) ^{fg}	2.78 (1.66 ± 0.09) ^{efgh}	46.67 (6.83 ± 0.29) ^{fghij}	132.33 (11.50 ± 0.18) ^{cd}	84.67 (9.18 ± 0.71) ^{kl}	4	S
Nithyakalyani	946.67 (30.75 ± 1.14) ^{def}	52.67 (7.25 ± 0.45) ^{efg}	83.00 (9.10 ± 0.58) ^{efg}	2.71 (1.64 ± 0.06) ^{efg}	46.67 (6.83 ± 0.29) ^{fghij}	154.67 (12.44 ± 0.21) ^{ghij}	63.67 (7.97 ± 0.51) ^{fghi}	4	S
Adukkanazhi	1173.33 (34.24 ± 1.10) ^{hijk}	48.00 (6.91 ± 0.59) ^{cde}	52.67 (7.20 ± 1.16) ^{cd}	3.19 (1.78 ± 0.05) ^{hijk}	33.00 (5.74 ± 0.37) ^{cde}	156.33 (12.50 ± 0.28) ^{hijk}	45.67 (6.74 ± 0.56) ^{cde}	4	S
Cholakodi	913.33 (30.13 ± 2.89) ^{de}	50.00 (7.06 ± 0.57) ^{cde}	34.33 (5.80 ± 0.99) ^{b}	2.49 (1.58 ± 0.12) ^{def}	25.67 (5.05 ± 0.56) ^{bc}	132.33 (11.50 ± 0.43) ^{cd}	43.33 (6.57 ± 0.46) ^{bcd}	4	S
Koshinadan	353.33 (17.98 ± 2.18) ^{a}	11.67 (2.99 ± 0.34) ^{a}	10.33 (3.20 ± 0.33) ^{a}	0.94 (0.97 ± 0.17) ^{a}	6.00 (2.43 ± 0.42) ^{a}	82.67 (9.08 ± 0.47) ^{a}	7.67 (2.75 ± 0.37) ^{a}	2	R
CD (0.05)	(2.759)	(0.85)	(1.10)	(0.137)	(0.84)	(0.591)	(0.826)	-	-
CV (%)	5.171	7.247	7.581	4.845	7.822	3.007	6.349	-	-
SE(m)	0.972	0.3	0.388	0.048	0.296	0.208	0.291	-	-

(*Mean ± SD of three replications), Figures in the parentheses are square root transformed values

Pf- Final nematode population Pi- Initial nematode population HS - Highly susceptible, S - Susceptible, R - Resistant

CD: Critical difference; CV: Coefficient of Variation; SE(m): Standard Error of mean

Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range (DMRT) Test at P = 0.05

Table 2. Growth parameters of different black pepper cultivars due to *M. incognita* infestation

Treatments	*Plant height (cm)	*Number of leaves	*Number of branches	*Weight of root (g)	*Weight of shoot (g)
Panniyur 1	68.33 ± 8.51^{m}	14.67 ± 2.08^{j}	1.33 ± 0.58^{gh}	3.27 ± 0.45^{m}	47.00 ± 5.57^{k}
Panniyur 2	100.67 ± 15.04^{ij}	38.67 ± 9.50^{cd}	2.67 ± 0.58^{cd}	11.03 ± 1.60^{c}	115.33 ± 12.22^{a}
Panniyur 3	109.00 ± 7.00^{fjhij}	21.33 ± 3.22^{fghij}	1.00 ± 0.00^{h}	7.07 ± 0.80^{ghij}	78.00 ± 9.00^{defghi}
Panniyur 4	74.67 ± 2.52^{m}	17.00 ± 4.36^{hij}	1.00 ± 0.00^{h}	5.17 ± 0.64^{m}	51.33 ± 8.96^{jk}
Panniyur 5	152.00 ± 29.46^{ab}	22.67 ± 1.53^{fghi}	1.00 ± 0.00^{h}	6.23 ± 0.31^{ijkl}	63.67 ± 5.51^{ij}
Panniyur 6	119.00 ± 3.61^{defgh}	37.67 ± 4.51^{cd}	2.33 ± 0.58^{deW}	7.37 ± 0.40^{efghi}	113.00 ± 15.40^{a}
Panniyur 7	126.00 ± 7.21^{def}	37.67 ± 6.11^{cd}	3.00 ± 0.00^{c}	7.77 ± 0.51^{defgh}	120.00 ± 12.49^{a}
Panniyur 8	107.67 ± 11.06^{ghij}	21.33 ± 3.22^{fghij}	2.00 ± 0.00^{ef}	6.97 ± 0.70^{ghij}	80.00 ± 7.94^{defgh}
Panniyur 9	103.33 ± 10.97^{hij}	24.00 ± 6.25^{fgh}	1.33 ± 0.58^{gh}	8.17 ± 0.42^{defg}	87.00 ± 15.13^{cdef}
Panniyur 10	114.33 ± 6.51^{efghij}	35.00 ± 5.57^{de}	1.67 ± 0.58^{fg}	7.23 ± 0.42^{fghij}	117.67 ± 16.65^{a}
Wild 1	115.67 ± 5.51^{efghi}	61.33 ± 7.02^{a}	5.33 ± 0.58^{a}	5.60 ± 1.14^{kl}	96.00 ± 7.55^{bc}
Wild 2	107.33 ± 9.71^{ghij}	43.00 ± 5.57^{c}	4.33 ± 0.58^{b}	14.87 ± 1.02^{b}	118.33 ± 10.07^{a}
Karimunda	126.00 ± 12.53^{def}	51.67 ± 5.69^{b}	2.00 ± 0.00^{ef}	15.27 ± 1.37^{b}	107.00 ± 8.19^{ab}
Vellamundi	127.00 ± 7.55^{de}	22.33 ± 2.31^{fghi}	1.00 ± 0.00^{h}	8.53 ± 1.07^{de}	97.00 ± 10.44^{bc}
Aimpiriyan	122.00 ± 2.00^{defg}	22.67 ± 1.16^{fghi}	1.00 ± 0.00^{h}	6.23 ± 0.35^{ijkl}	64.67 ± 2.08^{hij}
Perumkodi	157.67 ± 2.52^{a}	21.00 ± 1.00^{fghij}	1.00 ± 0.00^{h}	6.60 ± 0.66^{hijk}	69.00 ± 9.17^{ghi}
Palakodi	134.67 ± 14.05^{bcd}	20.67 ± 2.52^{ghij}	1.00 ± 0.00^{h}	7.07 ± 0.68^{ghij}	71.67 ± 5.51^{fghi}
Kottinadan	110.67 ± 7.51^{efghij}	24.33 ± 3.51^{fg}	2.00 ± 0.00^{ef}	6.60 ± 0.66^{hijk}	73.00 ± 4.00^{efghi}
Kumbanadan	97.33 ± 11.02^{jk}	20.00 ± 2.00^{ghij}	1.67 ± 0.58^{fg}	5.27 ± 0.35^{l}	65.00 ± 7.21^{hij}
Arikilazhi	79.33 ± 6.66^{lm}	28.00 ± 3.61^{ef}	1.33 ± 0.58^{gh}	8.43 ± 0.57^{def}	91.33 ± 8.02^{cd}
Amichakkari	146.00 ± 7.21^{abc}	19.67 ± 3.06^{ghij}	1.33 ± 0.58^{gh}	8.83 ± 0.78^{d}	88.33 ± 5.03^{cde}
Murithothan	97.00 ± 5.00^{jkl}	19.00 ± 1.00^{ghij}	2.33 ± 0.58^{de}	6.07 ± 0.35^{jkl}	71.67 ± 8.08^{fghi}
Nithyakalyani	105.67 ± 6.51^{ghij}	20.00 ± 2.65^{ghij}	1.00 ± 0.00^{h}	6.53 ± 0.97^{hijk}	64.00 ± 7.81^{ij}
Adukkanazhi	79.67 ± 20.84^{klm}	16.33 ± 2.08^{ij}	1.33 ± 0.58^{gh}	6.30 ± 0.56^{ijkl}	74.67 ± 10.21^{efghi}
Cholakodi	146.67 ± 9.87^{abc}	18.00 ± 1.73^{ghij}	2.00 ± 0.00^{ef}	6.57 ± 0.25^{hijk}	83.33 ± 4.04^{cdefg}
Koshinadan	133.67 ± 10.12^{cd}	31.67 ± 5.51^{de}	2.00 ± 0.00^{ef}	19.10 ± 0.72^{a}	115.00 ± 10.58^{a}
CD 0.05	17.872	7.005	0.643	1.243	15.423
CV (%)	9.577	15.665	21.246	9.476	11.01
SE(m)	6.298	2.469	0.226	0.438	5.435

*Mean ± SD of three replications

CD: Critical difference; CV: Coefficient of Variation; SE(m): Standard Error of mean

Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range (DMRT) Test at P = 0.05

Table 3. Effect of different inoculum levels of *M. incognita* on phenol, protein and defense enzyme activity in leaf and root of Koshinadan

Treatments (No. of J_2/ pot)	*Phenol content (mg catechol g tissue^{-1})		*Protein (µg of BSA g^{-1} fresh weight)		*Peroxidase (PO) (min^{-1} g^{-1} fresh weight)		*Phenylalanine ammonia lyase (PAL) (µg of cinnamic acid g^{-1} fresh weight)		*Polyphenol oxidase (PPO) (min^{-1} g^{-1} fresh weight)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Uninoculated	0.29 ± 0.04^{d}	0.30 ± 0.03^{f}	4.12 ± 0.13^{c}	4.28 ± 0.16^{b}	3.98 ± 0.29^{e}	9.71 ± 0.36^{e}	13.70 ± 0.44^{f}	13.90 ± 0.20^{f}	4.05 ± 0.25^{e}	4.08 ± 0.24^{e}
100	0.43 ± 0.05^{c}	0.65 ± 0.04^{e}	4.26 ± 0.06^{bc}	4.46 ± 0.09^{b}	6.54 ± 0.39^{d}	13.34 ± 0.78^{d}	15.55 ± 0.38^{e}	17.81 ± 0.21^{e}	6.34 ± 0.35^{d}	7.73 ± 0.22^{d}
500	0.51 ± 0.02^{c}	0.86 ± 0.05^{d}	4.41 ± 0.04^{bc}	4.51 ± 0.16^{b}	9.20 ± 0.40^{c}	19.06 ± 0.42^{c}	17.06 ± 0.31^{d}	20.65 ± 0.31^{d}	8.68 ± 0.33^{c}	8.71 ± 0.34^{c}
1000	0.60 ± 0.07^{b}	1.83 ± 0.08^{c}	4.76 ± 0.08^{b}	4.53 ± 0.17^{b}	10.60 ± 0.33^{b}	24.68 ± 0.37^{b}	19.31 ± 0.27^{c}	24.20 ± 0.31^{c}	10.38 ± 0.32^{b}	11.28 ± 0.23^{b}
5000	0.81 ± 0.06^{a}	3.79 ± 0.13^{b}	5.55 ± 0.15^{a}	5.49 ± 0.03^{a}	11.66 ± 0.27^{a}	32.48 ± 0.45^{a}	23.73 ± 0.24^{b}	27.29 ± 0.28^{b}	11.57 ± 0.39^{a}	12.66 ± 0.35^{a}
10000	0.89 ± 0.06^{a}	4.78 ± 0.10^{a}	5.78 ± 0.18^{a}	5.62 ± 0.32^{a}	12.08 ± 0.31^{a}	32.97 ± 0.85^{a}	24.37 ± 0.39^{a}	28.93 ± 0.39^{a}	12.10 ± 0.25^{a}	13.16 ± 0.29^{a}
CD 0.05	0.089	0.145	0.609	0.453	0.601	1.020	0.613	0.518	0.568	0.502
CV (%)	8.507	4.008	7.114	5.288	3.752	2.601	1.819	1.315	3.606	2.94
SE(m)	0.029	0.047	0.198	0.147	0.195	0.331	0.199	0.168	0.184	0.163

* Mean ± SD of three replications

CD: Critical difference; CV: Coefficient of Variation; SE(m): Standard Error of mean

Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range (DMRT) Test at P = 0.05

Table 4. Effect of different inoculum levels of *M. incognita* on phenol, protein and defense enzyme activity in leaf and root of Panniyur 1

Treatments (No. of J_2/ pot)	*Phenol content (mg catechol g tissue^{-1})		*Protein (µg of BSA g^{-1} fresh weight)		*Peroxidase (PO) (min^{-1} g^{-1} fresh weight)		*Phenylalanine ammonia lyase (PAL) (µg of cinnamic acid g^{-1} fresh weight)		*Polyphenol oxidase (PPO) (min^{-1} g^{-1} fresh weight)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Uninoculated	0.19 ± 0.03^{d}	0.20 ± 0.02^{f}	3.43 ± 0.19^{a}	3.61 ± 0.12^{a}	3.39 ± 0.60^{e}	9.57 ± 0.34^{d}	13.67 ± 0.31^{e}	13.83 ± 0.31^{f}	4.00 ± 0.21^{e}	3.91 ± 0.17^{e}
100	0.23 ± 0.02^{cd}	0.34 ± 0.05^{e}	3.26 ± 0.11^{ab}	3.50 ± 0.14^{ab}	4.39 ± 0.33^{d}	11.90 ± 0.61^{c}	14.24 ± 0.30^{d}	15.34 ± 0.18^{e}	4.77 ± 0.18^{d}	4.88 ± 0.05^{d}
500	0.27 ± 0.03^{c}	0.52 ± 0.05^{d}	3.18 ± 0.10^{abc}	3.48 ± 0.04^{ab}	5.56 ± 0.37^{c}	12.42 ± 0.60^{c}	15.18 ± 0.24^{c}	16.86 ± 0.27^{d}	5.30 ± 0.26^{c}	5.69 ± 0.15^{c}
1000	0.33 ± 0.03^{b}	0.68 ± 0.07^{c}	3.14 ± 0.12^{bc}	3.38 ± 0.11^{bc}	6.59 ± 0.46^{b}	15.15 ± 0.82^{b}	15.69 ± 0.26^{b}	17.40 ± 0.22^{c}	5.84 ± 0.09^{b}	6.32 ± 0.20^{b}
5000	0.46 ± 0.04^{a}	1.53 ± 0.04^{b}	2.98 ± 0.18^{cd}	3.31 ± 0.10^{bc}	7.34 ± 0.27^{a}	16.37 ± 0.31^{a}	16.48 ± 0.27^{a}	18.08 ± 0.28^{b}	6.28 ± 0.28^{ab}	6.68 ± 0.27^{b}
10000	0.50 ± 0.04^{a}	1.84 ± 0.09^{a}	2.85 ± 0.17^{d}	3.24 ± 0.08^{c}	7.95 ± 0.42^{a}	16.65 ± 0.25^{a}	16.54 ± 0.30^{a}	19.91 ± 0.29^{a}	6.41 ± 0.42^{a}	7.17 ± 0.32^{a}
CD 0.05	0.056	0.102	0.266	0.192	0.748	0.94	0.500	0.464	0.464	0.375
CV (%)	9.609	6.722	4.763	3.161	7.163	3.864	1.835	1.543	4.796	3.653
SE(m)	0.018	0.033	0.086	0.062	0.243	0.305	0.162	0.151	0.150	0.122

*Mean ± SD of three replications

CD: Critical difference; CV: Coefficient of Variation; SE(m): Standard Error of mean

Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range (DMRT) Test at P = 0.05

increase in phenol content in leaf samples of uninoculated Koshinadan plants was higher (0.29) compared to Panniyur 1 (0.19) and a similar trend was observed in root samples. The percentage increase in phenol content over Panniyur 1 in root samples of Koshinadan was 50.00% in uninoculated plants (Fig. 1). Phenol content in the leaf samples of tolerant cultivar (Koshinadan) inoculated with different inoculum levels ranged from 0.43 to 0.89 mg/g, while in susceptible variety Panniyur 1 it ranged from 0.23 to 0.50 mg/g. Percentage increase in phenol content in root samples of Koshinadan over susceptible variety Panniyur 1 ranged from 91.18 to 159.78 at different inoculum levels which was found to be higher than leaf samples (Fig. 1). Similar findings were reported in banana (26), rice (27) and tomato (28). The presence of hypersensitive reaction (HR) indicates the involvement of phenols, which play a role in plant defense (29).

The protein content in leaves and roots samples of the tolerant cultivar showed a progressive increase at different inoculum levels (100, 500, 1000, 5000 and 10000) of *M. incognita* at 45 DAI. However, the protein content in the leaves and roots of the susceptible cultivar, Panniyur 1 decreased with an increase in nematode inoculum levels. The highest protein content in the leaf and root of Koshinadan (5.78 and 5.62 µg of BSA g^{-1} fresh weight) was found in 10000 J_2 pot^{-1} inoculated plants. The protein content in the leaf and root of Panniyur 1 was higher in uninoculated plants (3.43 and 3.61 µg of BSA g^{-1} fresh weight) and the lowest protein content was noticed in treatment where 10000 J_2 was introduced in the rhizosphere of plant pot^{-1}. The percentage increase in protein content in leaf samples of uninoculated Koshinadan plants was higher (4.12) compared to Panniyur 1 (3.43). A similar trend was found in root samples also. In uninoculated plants, the percentage increase in protein content over Panniyur 1 in root samples of Koshinadan was 18.56. The percentage increase in the protein content of the leaf of Koshinadan at different inoculum levels ranged from 30.67 to 102.81 and in root it ranged from 27.43 to 73.46 compared to Panniyur 1 (Fig. 2). The protein content of Koshinadan increased with an increase in nematode inoculum level, while in Panniyur 1 it decreased. In a study by Gautam and Poddar (30), it was found that plants initiate an early primary resistance mechanism against nematode attacks, by increasing protein synthesis during nematode invasion.

The activity of the enzymes, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) in the samples of leaves and roots of Koshinadan and Panniyur 1 increased with higher inoculum levels of root knot nematode at 45 DAI (Table 3 and4). The uninoculated plants of both tolerant and susceptible cultivars showed the lowest enzyme activity compared to the inoculated plants, both in leaf and root samples. The defense enzymes exhibited higher activity in the resistant cultivar compared to the susceptible cultivar. The PO activity in Koshinadan plants inoculated with different inoculum levels of *M. incognita* (100, 500, 1000, 5000 and 10000) increased by 48.97 to 51.95% in leaf samples and 12.10 to 98.02% in root samples compared to Panniyur 1 (Fig.

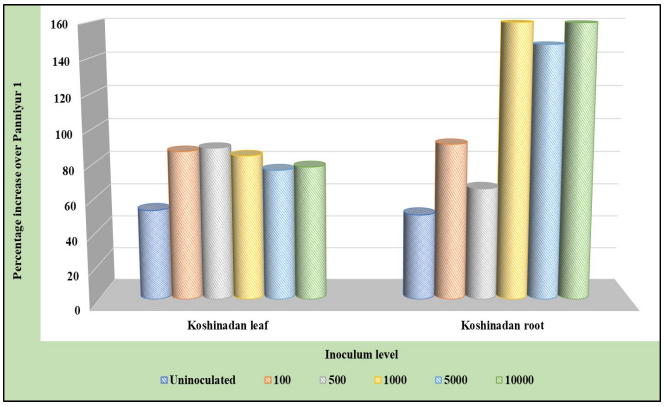

Fig. 1. Effect of different inoculum levels of *M. incognita* in phenol content (mg catechol g tissue^{-1}) in leaf and root of Koshinadan in comparison with Panniyur 1

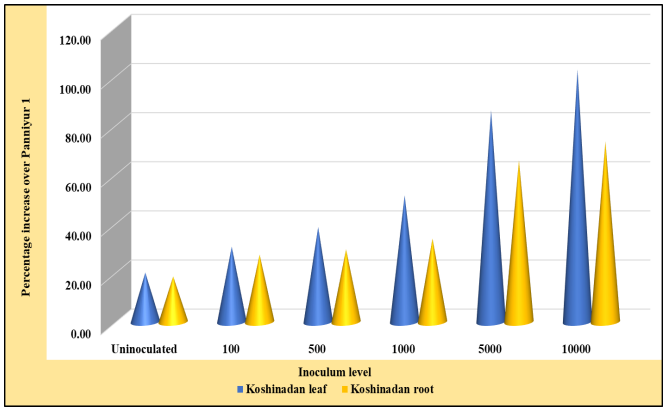

Fig. 2. Effect of different inoculum levels of *M. incognita* in protein content (µg of BSA g^{-1} fresh weight) in leaf and root of Koshinadan in comparison with Panniyur1

3). A similar trend was observed in PAL and PPO activity in leaf and root samples of both cultivars. The PAL activity showed an increase of 9.20 to 47.34% in the leaf and 16.10 to 45.30% increase in the root of Koshinadan at different inoculum levels of *M. incognita*, compared to the susceptible cultivar Panniyur 1 (Fig. 4). The increase in the PPO activity in leaves ranged from 32.64 to 88.77% and roots ranged from 58.40 to 83.54% respectively, in plants inoculated with 100, 500, 1000, 5000 and 10000 J_2 pot^{-1} compared to Panniyur 1 (Fig. 5).

The increased enzyme activity in resistant varieties was linked to a decrease in aromatic amino acids including phenylalanine and tyrosine and they play a significant role in the lignification process in plants (31). Increased resistance to the root-knot nematode may be due to the comparative increase in defense-related enzymes, protein and phenol content in the resistant cultivar, Koshinadan, over the susceptible cultivar, Panniyur 1. Higher activity of phenol, protein and defense enzyme was seen in roots than in leaves of both cultivars. The increase in phenol content due to nematode infection as evidenced in this study may be due to the defense mechanism of the plant. Excess hydrogen peroxide, increased respiration and activation of hexose monophosphate shunt pathway and acetate pathways may cause phenolic buildup, along with the release of bound phenols by hydrolytic enzymes (32).

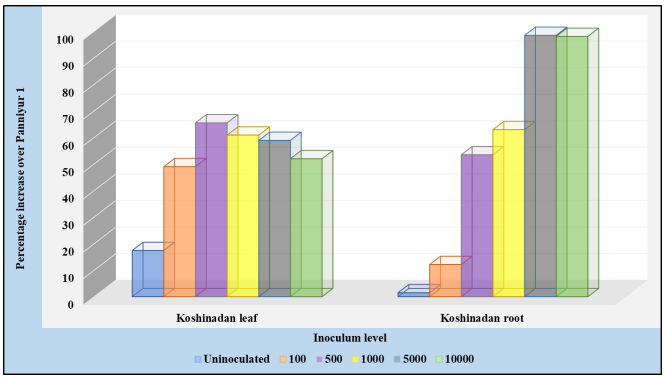

Fig. 3. Effect of different inoculum levels of *M. incognita* in peroxidase (PO) activity (min^{-1} g^{-1} fresh weight) in leaf and root of Koshinadan in comparison with Panniyur 1

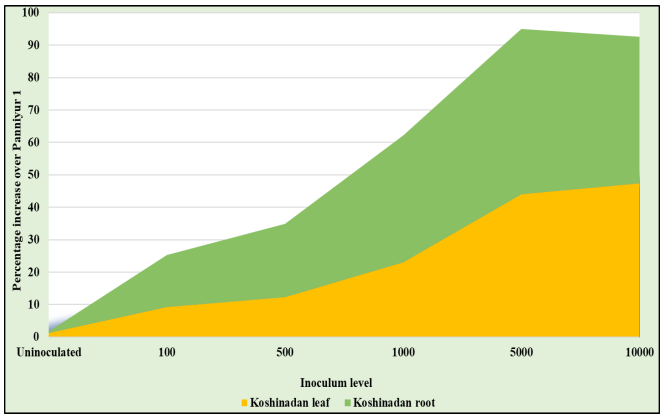

Fig. 4. Effect of different inoculum levels of *M. incognita* in Phenylalanine ammonia lyase (PAL) activity (µg of cinnamic acid g^{-1}fresh weight) in leaf and root of Koshinadan in comparison with Panniyur 1

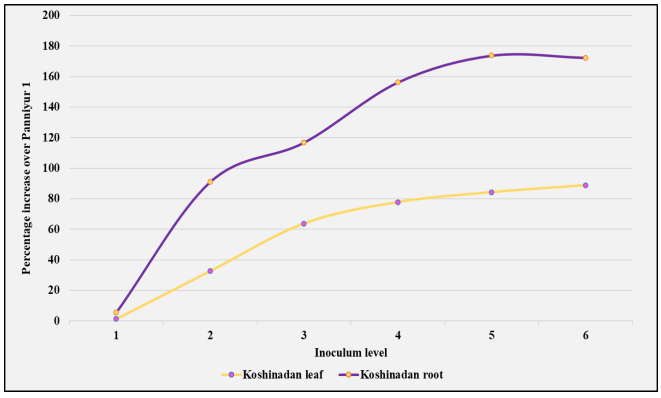

Fig. 5. Effect of different inoculum levels of *M. incognita* in polyphenol oxidase (PPO) activity (min^{-1} g^{-1} fresh weight) in leaf and root of Koshinadan in comparison with Panniyur 1

Histopathological changes

Histological sections of healthy roots of black pepper cultivars Koshinadan (resistant) and Panniyur 1 (susceptible) showed normal cell organization in epidermal, cortical and stellar regions. However, when infected with *M. incognita*, both cultivars exhibited disorganized xylem and phloem cells. Giant cells were observed near the stellar region of the root section. In Panniyur 1, the xylem vessels and parenchyma cells were found disorganized, whereas in Koshinadan, abnormalities in the xylem parenchyma were noted near the giant cell complex only and the remaining xylem and phloem vessels appeared to be completely functional and intact. Panniyur 1 was an efficient host, facilitating nematode development and multiplication. In the infected roots of Panniyur 1, significant variation in cell size was observed, with some cells slightly enlarged and others severely hypertrophied, whereas in Koshinadan, giant cell formation was very rare.

The extensive distortion in vascular tissues and giant cell formation in the susceptible variety, Panniyur 1 due to the parasitism of *M. incognita* observed in the study is in accordance with the cellular changes reported in the roots of rice (33). The swelling of infected root is a primary symptom associated with root-knot nematode infection. These nematodes induce the formation of root galls, disrupting water supply and leading to stunted and chlorotic growth (34). Large and well-developed galls were observed in Panniyur 1 roots, while Koshinadan roots exhibited only small residual swelling around the feeding sites, without the presence of large, well-defined galls. Based on the size of the galls, 1-3 adult females of root knot nematodes were found associated with each transverse section of Panniyur 1 and only 1 female was seen in Koshinadan, both found in the vascular bundle. This is in accordance with histopathological investigations on the roots of bermuda grass where giant cells were formed near the feeding area through penetration of the head in the vascular system by the female nematode (35). In the highly susceptible cultivar Panniyur 1, gall formation disrupted the normal structure of the roots, causing hyperplastic reactions, vascular tissue disruption, cell suberization and the formation of multinucleated large cells.

The size of giant cells ranged from 154.21 to 342.23 μm^2 in Koshinadan (Fig. 6) and 372.232 to 1080.987 μm^2 in Panniyur 1 (Fig. 7). The size of the giant cells in Koshinadan was smaller than in Panniyur 1 due to the collapse of giant cells as a result of the hypersensitive reaction of the host tissues. The length of female in Koshinadan (Fig. 8) was 610.23 µm and in Panniyur 1 (Fig. 9) it was 715.43 µm. Nematodes in Koshinadan roots failed to reach a complete pear-shaped female and the egg mass produced is relatively very small. In

Fig. 6. Size and number of giant cells in Koshinadan

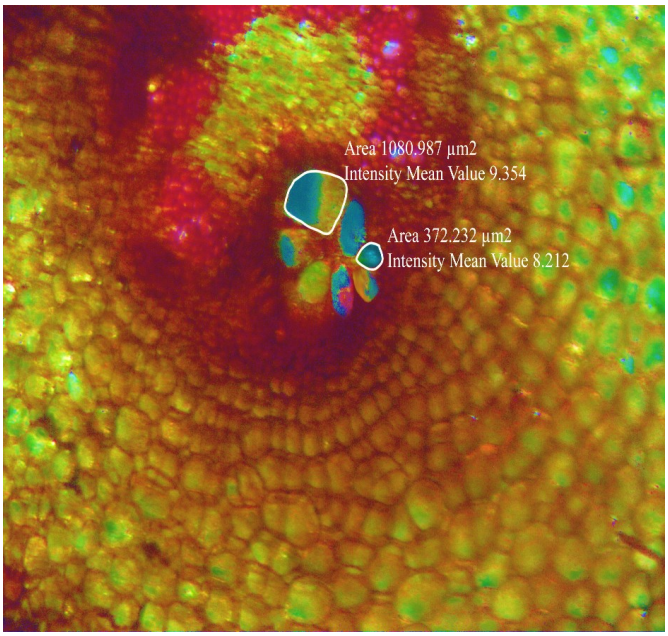

Fig. 7. Size and number of giant cells in Panniyur 1

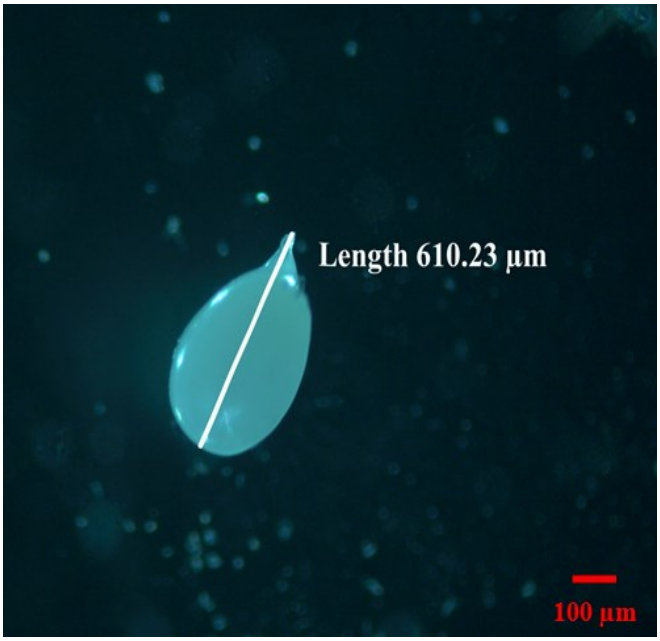

Fig. 8. Adult female in Koshinadan

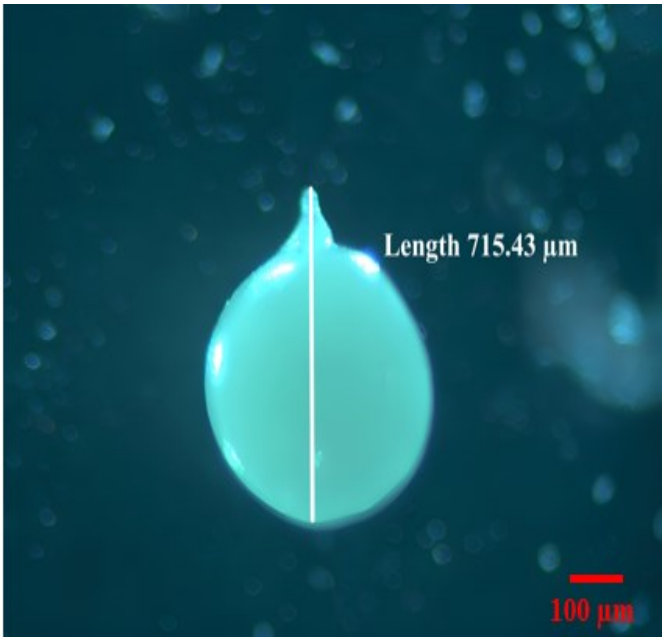

Fig. 9. Adult female in Panniyur 1

contrast, Panniyur 1 exhibited a well-developed adult female, with larger egg masses compared to Koshinadan. Panniyur 1 exhibited well-developed adult females with larger egg masses compared to Koshinadan. In the resistant cultivar Koshinadan, the disorganization of xylem parenchyma was noticed only near the giant cells however, in Panniyur 1 xylem and phloem vessels were completely disorganized. Hypertrophy and hyperplasia were observed in both cultivars as there was giant cell formation. In Koshinadan (Fig. 10), hyperplasia is not very evident in the galled portion but in Panniyur 1 (Fig. 11), hyperplasia was evident in the cells adjoining stelar region and the cortical cells. Due to a hyperplasia of the cells of the cortical parenchyma, Panniyur 1 exhibited a multilayered cortex compared to Koshinadan. There are two reported mechanisms for RKN resistance in plants: plant resistance in the pre-infection stage, in this mechanism nematodes will not enter plant roots due to toxic or antagonistic substances exuded from root tissues and resistance in the post-infection stage, where nematodes can colonize roots but subsequent development is hampered (36,

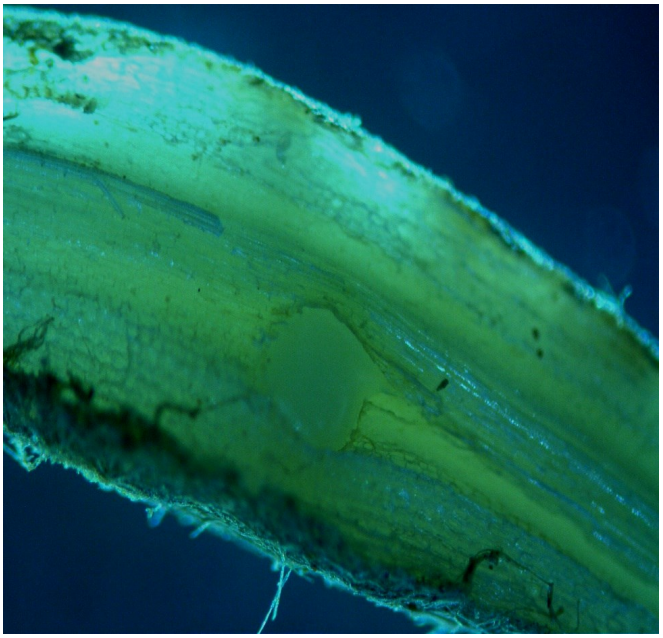

Fig. 10. Hyperplasia due to *M. incognita* in Koshinadan

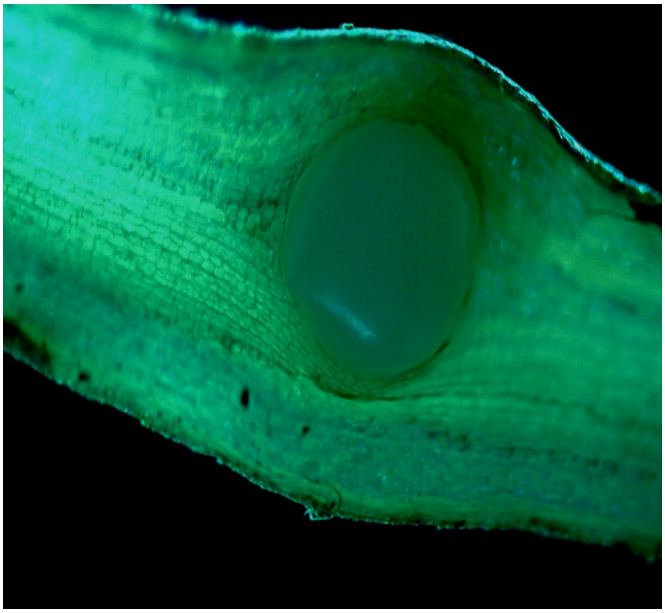

Fig. 11. Hyperplasia due to *M. incognita* in Panniyur 1

37). Resistance in the post-infection stage is frequently due to early Hypersensitive Reaction (HR)-mediated cell death, where the cells around nematode head are dead. This prevents the formation of feeding sites of nematodes and enhancement of resistance. Second-stage juveniles in Koshinadan may be linked to hypersensitive reactions in the vascular cylinder. Due to a hyperplastic reaction, the cells of the cortical parenchyma divided periclinally, resulting in multiple layered cells in the cortex of Panniyur 1 and the cortical cells in Koshinadan seem to be normal. The hyperplasia of tissues surrounding the giant cells contributed to the formation of root galls. The findings of the current investigation indicate that even though Panniyur 1 is a hybrid variety having high yield, it is highly susceptible to *M. incognita* infestation.

Conclusion

The investigations highlighted that Koshinadan, a local cultivar of black pepper, is resistant to *M. incognita* with a gall index of 2, while Panniyur 1, is highly susceptible to *M. incognita* showing a root knot index of 5. The phenol, protein and defense enzyme (PO, PAL and PPO) activities were higher in the roots of Koshinadan compared to those of the susceptible cultivar, Panniyur 1. Koshinadan exhibited smaller giant cells near the vascular region, attributed to a hypersensitive reaction, whereas Panniyur 1 displayed larger and more numerous giant cells, accompanied by complete disorganization of the xylem and phloem vessels. Based on the findings of this study, the resistant cultivar Koshinadan can be utilzed in breeding programs by identifying its nematode resistance traits and transferring them to suitable high-yielding varieties or as rootstock for grafting high-yielding commercial cultivars to achieve nematode management in black pepper nurseries and orchards.

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Authors' contributions

NMS conceived and designed the research work. SRJ carried out the experiments and analyzed the data. NMS wrote the manuscript and NR edited the manuscript. AR participated in biochemical analysis and editing the manuscript. GR edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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