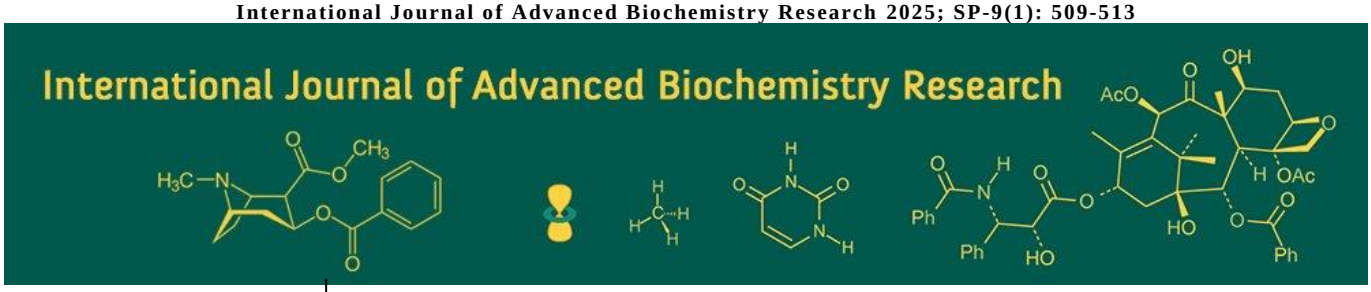

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Physiological and morphological response of turmeric (*Curcuma longa* L.) to root-knot nematode infestation: Estimation of avoidable yield loss

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Abstract
Turmeric (*Curcuma longa*) is a crucial spice crop in India, recognized for its culinary, medicinal, and cosmetic benefits. However, challenges such as pest infestations, particularly from root-knot nematodes, soil erosion, and fluctuating market prices hinder the yield and profitability of turmeric farming. To investigate the impact of root-knot nematodes, replicated pathogenicity and yield loss trials were conducted for two consecutive years (2020-2022) at the College of Agriculture, Chiplima, focusing on the turmeric variety Rajendra Sonia. Pathogenicity tests involved inoculating earthen pots with varying densities of *Meloidogyne incognita* (0, 10, 100, 1000, and 10,000 J2/kg soil). Results showed a progressive decline in relative water content, leaf water potential, and proline content of leaves as the inoculum density increased, while relative water deficit increased. Statistical analysis indicated significant differences among treatments, with pooled analysis showing highly significant effects. Histopathological examinations of infected roots revealed disorganization of xylem vessels and other cellular changes due to nematode invasion, impacting water uptake and reducing plant health. Yield loss trials in infested fields included treatments with carbofuran (2.0 kg a.i/ha) compared to untreated plots. Carbofuran-treated plots exhibited significant increases in plant height, leaf dimensions, shoot and root dry weight, and rhizome yield, alongside reductions in root-knot index and nematode populations by 45.76% in soil and 35.51% in roots. The avoidable loss in rhizome yield was estimated at 23.66% in the first year and 20.00% in the second, with reductions in yield attributing characters such as plant height, number of leaves, length, and breadth of leaf, number of tillers, shoot, and root dry weight irrespective of the year of investigation.

Keywords: Turmeric, root-knot nematode, histopathology, physiology, yield loss

Introduction
Odisha is one of the leading turmeric-producing state in India, where turmeric holds significant cultural and economic importance, being a staple in local diets, religious rituals and traditional medicine. The state's favourable tropical climate with adequate rainfall mostly favours the turmeric cultivation. The productivity of this crop is gradual declining due to different biotic (Nematodes, pests and diseases) and abiotic stress (environmental hazards) Despite of these challenges, turmeric remains a key cash crop for smallholder farmers in the state, contributing significantly to the rural economy. Root-knot nematode, *Meloidogyne incognita* has been found to be frequently associated with turmeric in almost all the states cultivated with this crop (Ayyar 1926; Mani *et al*. 1987) [1, 8]. This biotrophic heterotrophic parasite induces symptoms of chlorosis, incipient wilting, stunting and galling in the plants. Due to its increased dependency on the host, key plant functions are manipulated resulting in altered plant metabolism along with formation of specialized neoplastic cellular changes in adaptive manner. Therefore, the present study was undertaken to investigate the changes in several physiological and biochemical parameters along with altered cellular changes by *M. incognita* and assessment of yield loss in turmeric.

Materials and Methods
A replicated pot culture experiment and a field trial was conducted in the Collage of Agriculture, Chiplima, Sambalpur. Twenty numbers of earthen pots (12″dia) were surface sterilized, filled up to the brim with steam sterilized soil (9 kg/pot) and compost mixture,

planted with healthy turmeric rhizomes (cv. Rajendra Sonia) of 15-20 g with at least two buds during April followed by mulching. The turmeric plants in pots were inoculated with second stage juveniles of root-knot nematode (*Meloidogyne incognita*) at 5 different concentrations as 5 treatments namely T_1- 0 J2/kg soil (check), T_2- 10 J2/kg soil, T_3- 100 J2/kg soil, T_4- 1000 J2/kg soil and T_5- 10,000 J2/kg soil in 4 replications at 40 days after planting. Observations on relative water content (RWC), relative water deficit (RWD), leaf water potential and proline content were recorded 90 days after inoculation. The second leaf of turmeric plant of each treatment from top was carefully cut and the fresh weight (FW) was recorded. All the leaves were soaked in water for 2 h and then the turgid weight (TW) was recorded. Finally, those leaves were sundried for one week followed by hot air oven drying for 48 h at 80 °C to record leaf dry weight (DW). The relative water content of leaf was calculated by using the formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

The relative water deficit of all the leaves was obtained by subtracting the relative water content of the respective leaf from hundred.

$$RWD = 100 - RWC$$

The leaf water potential of fresh leaves of the treatments was determined by Dewpoint Potentia meter. Proline content was estimated as per the protocol described by Sadasivam and Manickam (1992) [14]. Healthy as well as *Meloidogyne incognita* infected roots of turmeric were collected, fixed in F.A.A and processed for histopathological investigations. Such roots were processed for dehydration, infiltration and embedding. Transverse and longitudinal sections of 12µ thickness were cut in a Spencer rotary microtome and mounted on glass slides coated with egg albumin mixture. The slides were further processed through xylene series, stained in safranin, counter stained in malachite green and mounted by placing a drop of DPX mountant and putting the cover slip. The permanent slides were examined for histopathological observations by using a research microscope and photomicrographs were taken in MIPS.

For estimation of avoidable yield loss, healthy turmeric rhizomes of 15 - 20 g each with at least two buds were planted and mulched in 4 m ×1 m × 0.15 m raised beds with five replications each following pared plot design. A set of beds was treated with carbofuran 3G (2.0 kg a.i/ha) and the other was left untreated. soil nematode population was estimated prior to application of treatment by Cobb's sieving and decantation method. All the recommended fertilizers and cultural operations were practised as per the specification. Observations on different growth parameters *viz*; plant height, number of leaves per plant, length and breadth of leaf and number of tillers were recorded 150 days after planting. Shoot dry weight, root dry weight, rhizome yield, root-knot index (on the basis of 1-5 scale), soil and root nematode population were recorded after harvesting. The data thus generated from the experiment were converted to percentage loss by using paired plot design as well as used for test of significance through paired 't' test.

This experiment was conducted for two consecutive years (2020-2022) and pooled analysis was performed to know the significant difference of treatments and years.

Results and Discussion

Since *M. incognita* has become a major constraint in turmeric production globally, the present investigation was undertaken to assess the cascade of activities leading to physiological and histopathological alterations induced by this nematode at varying inoculums level in turmeric. Data on different parameters (Table 1) revealed a progressive decrease in the RWC of leaf (95.18 to 91.88%), leaf water potential (-1.76 to -3.27 Mpa) and proline (49.23 to 7.03 µg g^{-1}Fw) over check with increase in the initial inoculums density of *M. incognita* irrespective of year of study. On the contrary RWD (4.82 to 8.12%) increased with increase in initial inoculums density. On the contrary leaf water potential and proline was significantly more in 1st year. In the 1st year of the experiment all the treatments show no significant difference between the treatments in RWC%. But in case of 2nd year and pooled data the treatments are significantly different from each other at 5% & 1% level of significance respectively. In case of RWD%, all the treatments show significance at 5% & 1% level of significance in 1st and 2nd year respectively with high levels of significance in pooled data. The treatments in leaf water potential show 1% level of significance in 1st year and non-significance in 2nd year with a high level of significance in the pooled mean data. The treatment effect in proline content shows high level of significance in 1st year, 2nd year, and pooled mean data. Polona *et al*. 2012 have reported reduced hydraulic conductivity of the root system as the major cause resulting in reduced leaf water potential, decreased stomatal conductivity, transpiration, and photosynthesis in nematode-infested tomato plants. Relative water content is the most reliable measure of plant water status in terms of physiological consequences of cellular water deficit. Water potential an estimate of energy status is useful in carrying out water transport in the soil-plant continuum. Photosynthesis and other hydrolysis reactions are dependent upon water which supplies the electrons and protons for light reaction. Hence, the biotic stress threshold level of *M. incognita* for disturbing homeostasis in the physiological and biochemical parameters in turmeric plants was estimated to be 1000 J2/kg soil.

Healthy root section (Plate I) of turmeric exhibited intact undisturbed plant tissue comprising of distinct epidermis, hypodermis/cortex, endodermis, pericycle, 8 numbers of radially arranged xylem and phloem bundles radially alternating to each other along with a small pith. In the infected root tissue, second-stage juveniles of *M. incognita* after penetration in to the host tissue migrated in the intercellular spaces and remained parallel to the longitudinal axis of the plant in the apoplast of the cortex and inserted it's head into the xylem parenchymatous tissue opposite to the protoxylem pole adjoining to the pericycle cells. It induced 4-7 giant cells (Plate II) in the xylem parenchyma. Giant cells were round or oval with irregular shapes and the volume of the giant cell increased to several folds. Giant cell walls were uneven along with number of wall ingrowths. Polynucleated giant cells (Plate III) through continuous mitosis in the absence of cytokinesis with intense cytoplasmic activities were demonstrated at early stages. The giant cell nuclei as well as nucleoli were enlarged and

distinct (Plate IV). Cells surrounding the nematode head in the pericycle were hyperplastic and cortical cells hypertrophied which was in confirmation with the findings of Das *et al*. (2018) [2]. The multinucleated giant cells due to neoplastic adaptive cellular changes served as the metabolic sink for the developing nematode. With the senescence of the plants, metabolic activities of the cell declined resulting in reduced cytoplasmic activities, withdrawal of the cytoplasm with shrinkage of the giant cells. The results of the present investigation indicating the site of giant cell formation near xylem pole and hyperplasia of the pericycle cells with eukaryotic division of nuclei and thickened wall ingrowths are in confirmity with the work of Surega and Ramakrishnan, (2018) [20]. Enlarged nuclei and nucleoli as observed in the present investigation were also reported by Molina and Neelson (1983) [9] and Sharma and Tyagi (1989) [16]. Mahapatra and Swain (2006) [7] also reported multinucleated giant cells by *M. incognita* in black gram roots along with amoeboid nuclei and dense cytoplasmic activity in corroboration to the present study. Susceptible cultivars of cowpea exhibiting polynucleated giant cells having 12-34 nuclei inside each giant cell by Singh *et al*. (2007) [18] conform with the present study.

A replicated field trial was conducted for 2 consecutive years from 2020 to 2022 with 2 treatments comprising of a treated (carbofuran @ 2.0 kg a.i/ha) and an untreated check. Observations of field experiments in turmeric infected by *Meloidogyne incognita* on various growth parameters like plant height, length and breadth of leaf, number of tillers, shoot and root dry weight, including the rhizome yield, root-knot index and population of nematode in soil and root were recorded in Table 2. The percent decrease in the above parameters is mentioned in Table 3. Among these parameters, there was a significant increase in plant height, length, and breadth of leaf, shoot, and root dry weight including rhizome yield, and a decrease in root-knot index, soil, and root nematode population in carbofuran-treated plots as compared to check. Reddy and Rao (2015) [13] reported 20–25% rhizome yield losses in severely infested fields. Similarly, Pandey *et al*. (2019) [10] found that infestation caused up to 30% reduction in yield, with significant declines in plant height, number of leaves, and rhizome size. Sharma *et al*. (2020) [15] demonstrated that high nematode density (10,000 J2/kg soil) could lead to over 40% reduction in rhizome yield in controlled trials

The present investigation was carried out to estimate the ultimate effects of parasitism of this nematode species on turmeric. In the present study, avoidable loss in rhizome yield was estimated to be 23.66% in 1st year and 20.00% in 2nd year along with a pooled mean yield loss of 21.99%. The differences in the yield loss estimates by *M. incognita* in the present study to that of the above workers are primarily to the initial level of *M. incognita* inoculums as well as to the cultivars of the crops. In the present investigation, a susceptible variety Rajendra Sonia was used with a base level initial soil population of 22493 J_2 of *M. incognita/kg* soil. In the present study, estimation of reduction in yield attributing characters in addition to the rhizome yield was also made. Significant reductions in different yield attributing characters *viz*; plant height, number of leaves, length and breadth of leaf, number of tillers, shoot and root dry weight irrespective of the year of the investigation were 5.35, 8.54, 7.87, 9.81, 42.11, 17.10, and 13.64% respectively. The increase in yield by carbofuran (2.0 kg a.i/ha) treated plots over untreated ones was due to a decline in the population of nematodes in the soil by 45.76%, in the root by 35.51% along with a decrease in the root-knot index by 42.55% which was also confirmed by Singh *et al*. (2021) [17]. The decline in nematode population resulting in a reduction in yield loss was reported by Reddy and Rao (2015) [13] in turmeric, Haidar *et al*. (2009) [4] in pea, Hazarika *et al*. (2006) [5] in jute, Ploeg and Phillips (2001) [11] in melon, and Jonathan and Rajendran (2000) [6] in banana with 20-25, 18.32, 18.30, 65.00 and 30.9% respectively similar to the present study.

Table 1: Effect of initial inoculums level of *M. incognita* on plant water relations and proline content of turmeric

Treatment		RWC (%)			RWD (%)			Leaf water potential (Mpa)			Proline (µg g^{-1} Fw)		
		1st Year	2nd Year	Pooled mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean
T_1		94.15	96.21	95.18	5.85	3.79	4.82 (9.32)	-1.94	-1.58	-1.76 (-4.43)	53.33	45.13	49.23
T_2		94.00	95.96	94.98	6.00	4.04	5.02 (9.78)	-2.41	-1.73	-2.07 (-5.12)	43.01	36.71	39.86
T_3		93.82	94.98	94.40	6.18	5.02	5.6 (11.31)	-2.97	-1.75	-2.36 (-5.63)	21.96	15.56	18.76
T_4		93.76	93.20	93.48	6.24	6.80	6.52 (13.92)	-3.00	-1.84	-2.42 (-5.81)	9.00	5.05	7.03
T_5		92.26	91.50	91.88	7.74	8.50	8.12 (17.36)	-4.43	-2.11	-3.27 (-7.53)	8.96	5.10	7.03
Mean		93.60	94.37	-	6.4 (8.46)	5.63 (16.22)	-	-2.95 (-5.10)	-1.80 (-6.31)	-	27.25	21.51	-
CV		2.18	1.26	1.78	11.82	6.17	8.10	-19.62	-15.84	-17.53	7.98	7.38	7.81
SEM		1.02	0.59	-	0.38	0.17	-	0.29	0.14	-	1.09	0.79	-
	Year	-	-	0.53	-	-	0.32	-	-	0.32	-	-	0.60
	Treatment	-	-	0.84	-	-	0.50	-	-	0.50	-	-	0.95
	Y*T	-	-	1.18	-	-	0.71	-	-	0.71	-	-	1.35
CD		3.15	1.83*	-	1.17*	0.53**	-	0.89**	0.44	-	3.35**	2.44**	-
	Year	-	-	1.09	-	-	0.65**	-	-	0.65**	-	-	1.24**
	Treatment	-	-	1.73**	-	-	1.03**	-	-	1.03**	-	-	1.96**
	Y*T	-	-	2.44	-	-	1.46**	-	-	1.46	-	-	2.78

The bracketed values are the Aitken's transformed value

* It indicates significant at 5% level of significance

** It indicates significant at 1% level of significance

Table 2: Percent loss in above-ground parameters of turmeric (cv. Rajendra Sonia) infected by *M. incognita*

Treatments	Plant height(cm)			No. of leaves			Length of leaf (cm)			Breadth of leaf (cm)			No. of tillers			Shoot dry weight (g)		
	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean
Treated (Carbofuran @ 2 kg a.i/ha)	163.18	198.51	180.85	9.00	9.25	9.13	116.60	135.00	125.80	19.20	21.16	20.18	2.40	1.40	1.90	41.58	45.10	43.34
Untreated control	157.14	185.21	171.18	8.20	8.50	8.35	109.80	122.00	115.90	17.80	18.60	18.20	1.20	1.00	1.10	32.26	39.60	35.93
% loss	3.70	6.70	5.35	8.89	8.11	8.54	5.83	9.63	7.87	7.29	12.10	9.81	50.00	28.57	42.11	22.41	12.20	17.10
t (0.05)	3.53**	16.65**	10.31**	2.14	4.74**	3.36**	3.89**	10.13**	14.85**	1.25	3.28**	2.77	2.45	1.00	3.14**	5.77**	3.16**	6.09**

**significant at P (0.05)

Table 3: Percent loss in below-ground parameters including the yield of turmeric (cv. Rajendra Sonia) infected by *M. incognita*

Treatments	Root dry weight (g)			Root-knot index			Rhizome yield (kg/m²)			Soil population			Root population		
	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean	1st Year	2nd Year	Pooled Mean
Treated (Carbofuran @ 2 kg a.i/ha)	4.98	4.98	5.28	2.40	3.00	2.70	1.86	1.95	1.91	1356.00	1632.00	1494.00	9937.60	2188.00	6062.80
Untreated control	3.91	3.91	4.56	4.80	4.60	4.70	1.42	1.56	1.49	2284.80	3226.40	2755.60	14437.60	4365.00	9401.30
% loss	21.49	6.81	13.64	50.00	37.21	42.55	23.66	20.00	21.99	40.65	49.42	45.76	31.17	49.87	35.51
t (0.05)	6.72**	2.93**	11.53**	6**	3.14**	6.32**	8.86**	23.22**	16.13**	11.99**	19.38**	18.49**	4.77**	11.43**	7.33**

**significant at P (0.05)

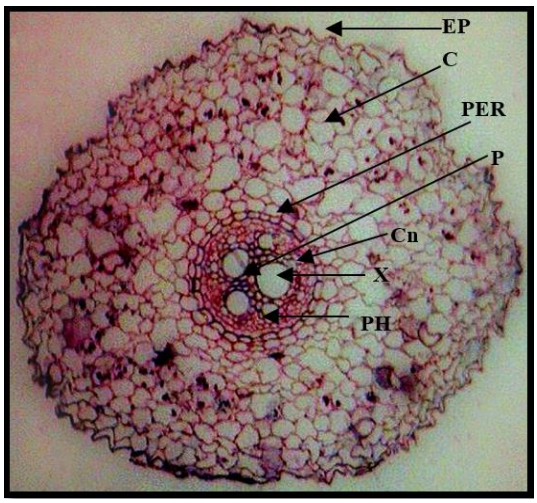

Plate I: TS of healthy turmeric root cross section

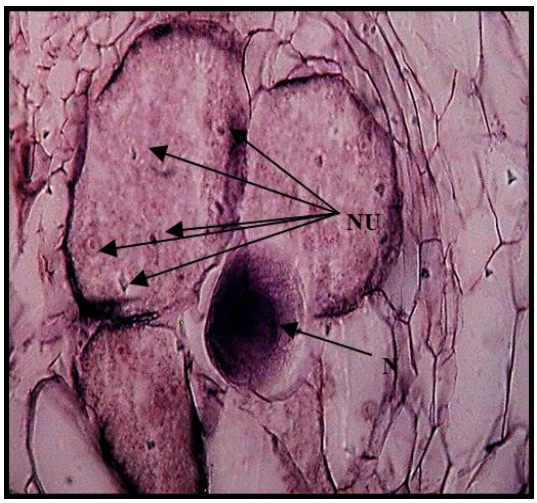

Plate III: Giant cells exhibiting multinucleated condition

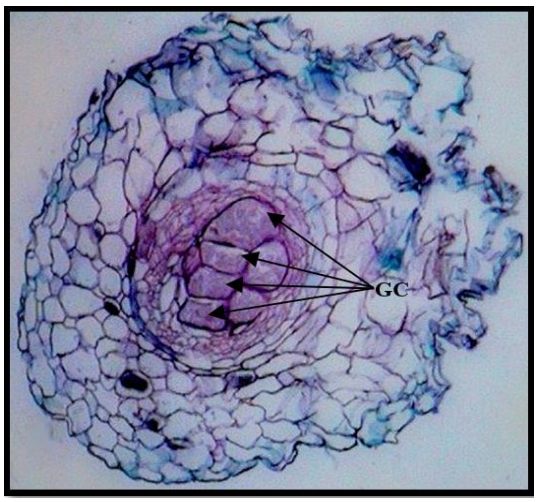

Plate II: TS of *M. incognita* infected turmeric root with giant cells

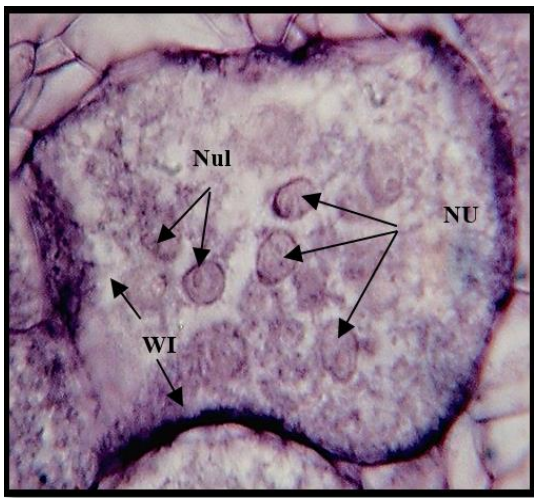

Plate IV: Giant cell exhibiting enlarged amoeboid nuclei and nucleoli with wall ingrowths

* C- Cortex, Cn- Casperian strip, EP-Epidermis, GC- Giant cell, N- Nematode, NU- Nucleus, Nul- Nucleolus, P- Pith, PH- Phloem, WI- Wall ingrowth, X- Xylem

Conclusion

In the pathogenicity trial, the biotic stress threshold level of *M. incognita* for disturbing homeostasis in the physiological and biochemical parameters in turmeric plants was estimated to be 1000 J2/kg soil. Histopathological examinations of infected roots proved that the disorganization of xylem vessels and other cellular changes due to nematode invasion impacted water uptake and reduced plant health. The yield loss trial showed a significant reduction in turmeric rhizome yield with root-knot nematode infection along with a reduction in all yield-attributing characteristics as compared to the treated plots.

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