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Effect of Different Inoculum Level of *Meloidogyne incognita* on Chlorophyll Content of Pigeon Pea, *Cajanus cajan* (L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A pot culture experiment was conducted during Kharif season in the year 2021- 2022 at Green polyhouse, Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha in order to study effect of different inoculum level of *M. incognita* on chlorophyll content of pigeon pea. The experiment was laid out in Completely randomized design (CRD) with 5 treatments i.e T_1 (500 J_2/plant), T_2(1000J_2 / Plant), T_3(1500J_2/plant), T_4(2000J_2/plant), T_5(Control)and 4 varieties were UPAS-120(R), IPA-15-1 (MR), IPA 14-7(S), CO-6(HS). To find out the effect of increase in inoculum level of nematode on chlorophyll content of pigeon pea leaves showed that highest reduction in chlorophyll in treatment T_4 then T_3, T_2 and then T_1 over control T_5. The results have demonstrated that nematode infestation leads to highest decreased by 41.75% total chlorophyll content (a+b) in UPAS -120(R)) in the leaves and highest decrease found in case of high inoculum level inoculation of nematodes in the pigeon pea plant.

Keywords: Pigeon pea (Cajanus cajan); root knot nematode (M. incognita); chlorophyll.

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1. INTRODUCTION

"Pigeon pea, *Cajanus cajan L.* is considered as one of the most important pulse grown in Indian subcontinent. It is the second most important pulse crop after gram. It is a good source of protein (20-23%), dietary fiber, and various vitamins: thiamin, magnesium, phosphorus, potassium, copper, and manganese. India ranked first in the world with 79.65% and 67.28% of world's acreage and production respectively. Globally it is cultivated on 4.9 m ha of which India alone occupies 3.5 m ha i. e. 72% of the total area" [1]. This crop is highly vulnerable to many plant parasitic nematodes, which cause an annual yield loss of over 13% worldwide [2]. "Root knot nematode, *Meloidogyne incognita*, is the most important nematode species with worldwide distribution in tropical and subtropical climate. Among the various pests, *M. incognita* poses a potential threat to the cultivation of pulse crops by infecting upon severe yield losses" [3, 4, 5]. "Physiological and biochemical activities of the host plant are drastically affected on nematode infection" [6]. "Leaf pigment composition is sensitive to plant stress and nematode infection causes either a loss of photosynthetic pigments (e.g. chlorophyll) or higher levels of photoprotective pigments, such as zeaxanthin or β-carotene" [7]. "Various forms of abiotic and biotic stresses damage plant leaf tissue and the chloroplasts" [8]. "The chlorophyll released from damaged chloroplasts has to be degraded rapidly to avoid cellular damage owing to its high reactivity" [9]. "Failure to degrade the chlorophyll may cause an accumulation of reactive oxygen species (ROS) that can easily damage the cellular organelles" [10, 11]. So our objective of study is to know the effect of different inoculum of Root knot nematode on chlorophyll content of pigeon pea leaves.

2. MATERIALS AND METHODS

In order to understand the basics of resistance to nematode (*Meloidogyne incognita*) inoculated four varieties UPAS-120 (Resistant), IPA-15-1 (Moderately resistant), IPA-14-7(Susceptible) and CO-6 (Highly susceptible) were grown in pots filled with aerated sterilized soil (autoclaved at 1.1kg/cm^2 pressure for one hour daily for two consecutive days) mixed with sand and FYM in the ratio of 2:1:1 following Complete Randomized Design (CRD) with five treatments. The water used for irrigation had a five hundred mesh screen before use. Two weeks after seedling emergence agenized nematodes were counted under a stereoscopic microscope and released into the holes @ 500 J_2, 1000 J_2, 1500 J_2, 2000 J_2 per seedling and one control. For chemical analysis three sets of plants were maintained. Each set was arranged on separate platform in the green house in order to avoid cross infection. At 30 days after inoculation, inoculated plants.

Estimation of chlorophyll: One hundred fifty mg leaf portion of each treatment were cut from the composite leaves and were immersed in 50 ml of 80 % acetone in a conical flask and kept in dark for 24 hours for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were filtered through Whatman No.1 filter paper. Absorbance of the chlorophyll extract was measured at 645 nm and 663 nm using a colorimeter.

Calculation: The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations [Anon, 1949].

a) Chlorophyll -a (mg/g fresh weight of leaf)

$$= 12.7 \text{ x (D-663)} – 2.69 \text{ x (D-645)} \times \frac{V}{1000 \times W}$$

b) Chlorophyll-b (mg/g fresh weight of leaf)

$$= 22.9 \text{ x (D-645)} – 4.68 \text{ x (D-663)} \times \frac{V}{1000 \times W}$$

c) Total chlorophyll (mg/g fresh wt. of leaf)

$$= 20.2 \text{ x (D-645)} + 8.02 \text{ x (D-663) x} \times \frac{V}{1000 \times W}$$

Where,

D –645 = Optical density at 645 nm
D-663 = Optical density at 663 nm
V = Final volume of 80 % acetone chlorophyll extract in ml
W = Fresh weight in gram of corresponding amount of fresh leaves used in the extraction of chlorophyll.

3. RESULT AND DISCUSSION

Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of the plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll content of plants by disrupting its nutrient uptake and partitioning of the photosynthates.

It is clear from data presented in the Table 1 in variety UPAS-120(R)that chlorophyll-a was found 1.34,1.17,1.03,0.86 mg/g against 1.47 mg/g; chlorophyll-b was found 1.35, 1.19, 1.06, 0.88mg/g against 1.51 mg/g; total chlorophyll was found 2.65, 2.35, 2.06, 1.73 mg/g against 2.97 mg/g in the experiment plant inoculated with 500, 1000, 1500, 2000 J_2 /plant respectively. The total chlorophyll content was reduced by 10.89, 20.88, 30.64, 41.75% in 500, 1000, 1500, 2000 J2 /plant respectively over control.

It is clear from data presented in the Table 2 in variety IPA -15-1(MR) that chlorophyll-a was found 1.41, 1.25, 1.12, 0.96 mg/g against 1.53 mg/g; chlorophyll-b was found 1.42, 1.31, 1.24,1.05 mg/g against 1.58 mg/g; total chlorophyll was found 2.82, 2.63, 2.24, 1.77 mg/g against 3.10 mg/g in the experiment plant inoculated with 500, 1000, 1500, 2000 J_2 /plant respectively. The total chlorophyll content was reduced by 9.13, 15.15, 27.93, 43.07% in 500, 1000, 1500, 2000 J_2 /plant respectively over control.

It is clear from data presented in the Table 3 in variety IPA-14-7(S)that chlorophyll-a was found 1.50, 1.37, 1.24, 1.10 mg/g against 1.61 mg/g; chlorophyll-b was found 1.61,1.49, 1.27,1.15 mg/g against 1.72 mg/g; total chlorophyll was found 3.11, 2.86, 2.50, 2.24 mg/g against 3.31 mg/g in the experiment plant inoculated with 500, 1000, 1500, 2000 J_2 /plant respectively. The total chlorophyll content was reduced by 6.04, 13.70, 24.37, 32.23% in 500, 1000, 1500, 2000 J2 /plant respectively over control.

It is clear from data presented in the Table 4 in variety CO-6(HS)that chlorophyll-a was found 1.59, 1.46, 1.34, 1.21 mg/g against 1.69 mg/g; chlorophyll-b was found 1.62, 1.59, 1.37, 1.26 mg/g against 1.79 mg/g; total chlorophyll was found 3.20, 3.01, 2.70, 2.47mg/g against 3.47 mg/g in the experiment plant inoculated with 500, 1000, 1500, 2000 J_2 /plant respectively. The total chlorophyll content was reduced by -7.77, 13.34, 22.17, 28.79% in 500, 1000, 1500, 2000 J2 /plant respectively over control.

Table 1. Effect of Different Inoculum Level of *M. incognita* on Chlorophyll Content (Var. UPAS-120)

Treatments	Chlorophyll-a	% Change Over Control	Chlorophyll -b	% Change Over Control	Total Chlorophyll	% Change Over Control
T_1 500J_2	1.34	-8.78	1.35	-10.60	2.65	-10.89
T_2 1000J_2	1.17	-20.15	1.19	-21.19	2.35	-20.88
T_3 1500J_2	1.03	-29.71	1.06	-29.80	2.06	-30.64
T_4 2000J_2	0.86	-41.31	0.88	-41.94	1.73	-41.75
T_5(Control)	1.47		1.51		2.97	
SE(m)±	0.05		0.03		0.04	
CD (0.05)	0.16		0.09		0.12	

Table 2. Effect of Different Inoculum Level of *M. incognita* on chlorophyll content (Var. IPA-15-1)

Treatments	Chlorophyll-a	% Change Over Control	Chlorophyll-b	% Change Over Control	Total Chlorophyll	% Change Over Control
T_1 500J_2	1.41	-8.06	1.42	-10.34	2.82	-9.13
T_2 1000J_2	1.25	-18.30	1.31	-17.09	2.63	-15.15
T_3 1500J_2	1.12	-27.02	1.24	-21.73	2.24	-27.93
T_4 2000J_2	0.96	-37.25	1.05	-33.54	1.77	-43.07
T_5(Control)	1.53		1.58		3.10	
SE(m)±	0.05		0.02		0.06	
CD (0.05)	0.15		0.06		0.12	

Table 3. Effect of Different Inoculum Level of *M. incognita* on Chlorophyll Content (Var. IPA-14-7)

Treatments	Chlorophyll-a	% Change Over Control	Chlorophyll-b	% Change Over Control	Total Chlorophyll	% Change Over Control
T_1 500J_2	1.50	-7.02	1.61	-6.58	3.11	-6.04
T_2 1000J_2	1.37	-15.29	1.49	-13.35	2.86	-13.70
T_3 1500J_2	1.24	-23.14	1.27	-26.11	2.50	-24.37
T_4 2000J_2	1.10	-31.61	1.15	-33.08	2.24	-32.23
T_5 (Control)	1.61		1.72		3.31	
SE(m)±	0.05		0.02		0.05	
CD(0.05)	0.15		0.06		0.15	

Table 4. Effect of different inoculum level of *M. incognita* on Chlorophyll Content (Var. CO-6)

Treatments	Chlorophyll-a	% Change Over Control	Chlorophyll-b	% Change Over Control	Total Chlorophyll	% Change Over Control
T_1 500J_2	1.59	-5.53	1.62	-9.14	3.20	-7.77
T_2 1000J_2	1.46	-13.24	1.59	-10.82	3.01	-13.34
T_3 1500J_2	1.34	-20.55	1.37	-23.32	2.70	-22.17
T_4 2000J_2	1.21	-28.06	1.26	-29.29	2.47	-28.79
T_5 (Control)	1.69		1.79		3.47	
SE(m)±	0.17		0.09		0.14	
CD(0.05)	0.05		0.03		0.04	

4. DISCUSSION

Maximum reduction in chlorophyll content found is in case of highest inoculum level of nematode inoculated plant due to reduction in water and nutrient transport [12,13,14,15]. "It provides a measure of photosynthetic capacity and is related to the nitrogen concentration in the plant" [16]. "Chlorophyll molecule has mg2+ at center which makes it ionic and hydrophilic and the ring hydrophobic in nature. Results are in similar with the earlier findings" [17,18]. "Chlorophyll content is affected by nitrogen concentration, it can be an indicator of the damage caused to the plant by *M. incognita*. Abiotic and biotic stresses damaged the plant leaf tissues, which was rapidly degrade the chloroplast and decreased the phosphorus, potassium, nitrogen, magnesium levels. Previous studies have shown that infection of plants by *M. incognita* with increase in inoculum level can result in reduced chlorophyll content and photosynthesis" [19,20,21].

5. CONCLUSION

The current investigation amply demonstrated that *Meloidogyne incognita* significantly altered the host plant's normal physiology. Furthermore, basic studies on the physiology mechanism of resistance in pigeon pea to the Root knot nematode were conducted in order to elucidate the physiology basis of resistance to host to the nematode observation was made in the changes in physiology parameters. Chlorophyll a, b, and total chlorophyll content reduces in infected plant leaves when compared to controls.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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