Effect of different inoculum level of *Meloidogyne incognita* on chlorophyll content of *Cajanuscajan*(L.) Millsp

AbstractREDO AGAIN ACCORDING TO THE JOURNAL GUIDANCE

METHODOLOGY:The pot culture experiment was conducted during *Kharif* season in the year 2021- 2022 at Greenpolyhouse, Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, (India).in order to study effect of different inoculum level of *M. incognita* on chlorophyll content of pigeonpea(Cajanuscajan (L.) Millsp.).The experiment was laid out in Complete randomized design (CRD) with 5 treatmentsi.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). RESULTSCWith increase in inoculum level there was decrease in chlorophyll content. Highest reduction in chlorophyll in treatment T_4 then T_3,T_2 and then T_1 over control T_5.CONCLUSIONS: 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 of the Pigeonpea plants.?????

Key words

ENLARGE THIS ABSTRACT BECAUSE IT MAY HAVE 300 WORDS AND IT ONLY HAS 144 WORDS

Introduction

Pigeon pea *Cajanuscajan L.* is considered as one of the most important pulse crop 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 [13]. This crop is highly vulnerable to many plant parasitic nematodes, which cause an annual yield loss of over 13% worldwide [12].Root knot nematode, *Meloidogyne incognita*, is the most important nematode species with worldwide distribution in tropical and subtropical climate. The pathogenic effect of root-knot nematodes on growth parameters, yield and nutrient uptake of leguminous crops have been reported by several workers and it is documented as potential threat to various leguminous

Comment [1]: Meloidogyne incognita, Meloidogyne javanica e Meloidogyne enterolobii.

Comment [2]: Binomial name
Meloidogyne incognita
(Kofoid& White, 1919)

FULL SCIENTIFIC NAME: [Cajanuscajan (L.) Millsp.]

Comment [3]: WHERE IS THE RESEARCH OBJECTIVE?????INTRODUCTION ?????

Comment [4]: DOES NOT COMPLY WITH THE JOURNAL GUIDELINES: INTRODUCTION;OBJETIVS; METHODOLOGY. RESULTS -DISCISION AND CONCLUSIONS

Comment [5]: COUNTRY OF ORIGIN OF THE RESEARCH?????

Comment [6]: NO KEYWORDS??? WHERE IS IT????

Comment [7]: Keywords
Immediately after the abstract, about 4-8 keywords should be given. Use of abbreviations should be avoided, only standard abbreviations, well known in the established area may be used, if appropriate. These keywords will be used for indexing.

Abbreviations
Non-standard abbreviations should be listed and full form of each abbreviation should be given in parentheses at first use in the text.

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plants.Reduction in chlorophyll content of infected plant has been reported. [9][10][11][15]AND [14] ??????

Keywords: Pigeon pea(*Cajanuscajan)*, Root knot nematode (*M. incognita*), chlorophyll

Material and Methods

MATERIAL:??????

-stereoscopicmicroscope???????

-colorimeter?????????

-in 50 ml of 80 % acetone

METHODS???? AUTHOR?????

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 stereoscopicmicroscope ?????and released into the holes @ 500J2, 1000 J2, 1500 J2, 2000 J2 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 were removed from the pot soil carefully and the chlorophyll compositions were estimate

DETERMINATION OF CHLOROPHYLLS CONTENT, AUTHOR??????

One hundred fifty mg leaf portion of each treatment were cut from thecomposite 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

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Comment [I8]: DO NOT DEFINE WORK OBJECTIVES

Comment [I9]: RARE THING: THE BIBLIOGRAPHIC REFERENCES BEGIN WITH THE LARGER NUMBERS OF THE REFERENCES. EX. total area [13]. ; LOGO, yield loss of over 13% worldwide [12]. ; LOGO , Reduction in chlorophyll content of infected plant has been reported. [9][10][11][15]. ONLY IN THE INTRODUCTION (HALF PAGE).

Comment [I10]: THE INTRODUCTION MUST NOT HAVE KEY WORDS, BUT THE ABSTRACT.

Comment [I11]: WHERE ARE THE MATERIALS USED IN THE RESEARCH?????

Comment [I12]: BRAND; MODEL, VOLTAGE, ETC.?????

Comment [I13]: BRAND; MODEL, VOLTAGE, ETC.?????

Comment [I14]: BRAND; MODEL, VOLTAGE, ETC.?????

Comment [I15]:

Comment [I16]: WHO WAS RESPONSIBLE FOR IDENTIFYING (Meloidogyne incognita)?????? THERE ARE OTHER PARASITES OF THIS GENDER. MEANING OF (UPAS-120 (Resistant), IPA-15-1 (Moderately resistant), IPA-14-7(Susceptible) and CO-6 (Highly susceptible)

Comment [I17]: OUT OF PLACE THESE OBJECTIVES. THE OBJECTIVES MUST BE DEFINED IN THE INTRODUCTION OF THE ARTICLE AND THEN THEY ARE WRITTEN AGAIN IN THE ABSTRACT OF THE ARTICLE.

Comment [I18]: THIS LOOKS LIKE A CHLOROPHYLL EXTRACTION METHOD, BUT THE AUTHOR IS NOT INDICATED. ACCORDING TO WORK EXPERIENCE, THERE ARE MORE EFFICIENT EXTRACTION METHODS THAN SHOWN IN THE ARTICLE.
THE METHOD INDICATED IN THE ARTICLE BY OPINION AND BY MACERATION EXTRACTION.

WITH THE LABORATORY LIGHTS OFF
IN THE LITERATURE IT IS PREFERREDLY INDICATED TO MAKE A MACERATION USING CALCIUM CARBONATE AND WASHED SAND IN AMAFORIZ AND GRIP THE LEAVES, WITHOUT THE CENTRAL RIBS, WITH 80% ACETONE, UNTIL TOTAL DEGRADATION OF THE VEGETABLE MATERIAL. LOGO FILTER DIRECTLY INTO A 10 ML VOLUMETRIC BALLOON AND COMPLETE THE VOLUME WITH 80% ACETONE

Comment [I19]: .
HOW WERE THE LEAVES CUT? WERE CRUNCHED BEFORE WEIGHING???
HOW WEIGHT ???/ APPLIANCE, SCALE????

VERY FRACTION METHOD OF CHLOROPHYLL EXTRACTION.
"MACERATION FOR 24 HOURS WITH 50 ML ACETONE 80% "

AUTHOR??????

... [1]

colorimeter. ????The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations (Anon, 1949).DOES NOT APPEAR IN THE BIBLIOGRAPHIC REFERENCES OF THE ARTICLE- WRONG NAME (IT'S ARNOM, 1949)

i) Chlorophyll -a (mg/g fresh weight of leaf) = 12.7 x (D-663) – 2.69 x (D-645) $\times \frac{V}{1000 \times W}$

ii) Chlorophyll-b (mg/g fresh weight of leaf) = 22.9 x (D-645) – 4.68 x (D-663) $\times \frac{V}{1000 \times W}$

iii) Total chlorophyll (mg/g fresh wt. of leaf) = 20.2 x (D-645) + 8.02 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 correspondingamount of fresh leaves used in the extraction of chlorophyll.

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 thephotosynthates.

It is clear from data presented in the Table -1 in variety UPAS-120(R)that chlorophyll-a was found 1.34,1.17,1.03,0.86mg/g against 1.47mg/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.

LOCATION FOR TABLE 1. PLACE HERE.

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 J2 /plant respectively over control.

LOCATION FOR TABLE 2. PLACE HERE.

It is clear from data presented in the Table -3 in variety IPA-14-7(S)that chlorophyll-a was found

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(The traditional method for determining the chlorophyll content in leaves is carried out via maceration with acetone or another organic solvent and subsequent reading in a spectrophotometer (Arnon, 1949). However, this method results in the destructive collection of plant material and is time consuming.)

Comment [I22]: Tables & Figures
Tables & figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. It is suggested that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text.

Comment [I23]: IT SEEMS THAT IT REPEATS IN THE TABLES THE SAME PARAGRAPH DATES AND DOES NOT DISCUSS ITS RESULTS

Comment [I24]: IT SEEMS THAT IT REPEATS IN THE TABLES THE SAME PARAGRAPH DATES AND DOES NOT DISCUSS ITS RESULTS

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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.

LOCATION FOR TABLE 3 PLACE HERE.

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.

Leaf chlorophyll content provides a measure of photosynthetic capacity and is related to the nitrogen concentration in the plant [4]which *M. incognita* can influence by interfering with water and nutrient transport[3][6][8]. Therefore, because chlorophyll content is affected by nitrogen concentration, it can be an indicator of the damage caused to the plant by *M. incognita*. Previous studies have shown that infection of plants by *M. incognita* can result in reduced chlorophyll content and photosynthesis [5][7]. Decrease in chlorophyll content with increase in inoculum level [1][2].

LOCATION FOR TABLE 4. PLACE HERE.

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)±	**0.17**		**0.09**		**0.12**	
CD(0.05)	**0.05**		**0.03**		**0.04**	

Comment [I25]: IT SEEMS THAT IT REPEATS IN THE TABLES THE SAME PARAGRAPH DATES AND DOES NOT DISCUSS ITS RESULTS

Comment [I26]: IT SEEMS THAT IT REPEATS IN THE TABLES THE SAME PARAGRAPH DATES AND DOES NOT DISCUSS ITS RESULTS

Comment [I27]: DOES THIS REFER TO THE DISCUSSION OF WORK????? TOO WEAK

BROWSE SPECIALIZED LITERATURE FOR MORE REFERENCES ON THE SUBJECT UNDER STUDY.

Comment [I28]: ON PAGE 4 OF THE ARTICLE, REFERENCES 1, 2, 3, 4, 5, 6, 7 AND 8 appear in the same paragraph.

NOTHING IS DISCUSSED ABOUT THE RESULTS OBTAINED IN THE RESEARCH

Comment [I29]: MOVE TO LOCATION TO TABLE 1. PLACE THERE.

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Table-2 Effect of different inoculum level of *M. incognita* on chlorophyll content (var.IPA-15-1)

Comment [I30]: MOVE TO LOCATION TO TABLE 2. PLACE THERE.

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)±	**0.16**		**0.06**		**0.18**	
CD(0.05)	**0.05**		**0.02**		**0.06**	

Table-3 Effect of different inoculum level of *M. incognita* on chlorophyll content (var. IPA-14-7)

Comment [I31]: MOVE TO LOCATION TO TABLE 3. PLACE THERE.

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)±	**0.16**		**0.07**		**0.16**	
CD(0.05)	**0.05**		**0.02**		**0.05**	

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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)±	**0.15**		**0.09**		**0.12**	
CD(0.05)	**0.05**		**0.03**		**0.04**	

Comment [I32]: MOVE TO LOCATION TO TABLE 4. PLACE THERE.

Conclusion

In conclusion, the present study suggested that clearly indicated that *Meloidogyne incognita* played key role in altering the normal physiology of the tested host plant. Further, Basic studies relating to physiology mechanism of resistance in pigeonpea to the Root knot nematode made elucidate the physiology basis of resistance to host to the nematode observation were made in the changes in physiology parameters. Chlorophyll a, b and total chlorophyll content decreases in leaves of infected plants as compared to control.

Comment [I33]: .
SCIENTIFIC NAME OF BEANS ????

Comment [I34]: .
THESE CONCLUSIONS COULD BE SAID WITHOUT THE NEED TO CARRY OUT THIS STUDY

DEFINE THE OBJECTIVES OF YOUR RESEARCH WORK FIRST. SO, ANALYZE YOUR RESULTS AND COMPARE WITH THE SPECIELIZED LITERATURE TO REACH YOUR CONCLUSIONS SOON:

References

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Comment [I35]: THE REFERENCES MATCH IN NUMBER WITH THOSE REPORTED IN THE ARTICLE. THE REFERENCE [14] DOES NOT APPEAR IN THE ARTICLE OR IT HAS NOT BEEN LOCATED.

Comment [I36]: Reference style
References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

DOES NOT COMPLY WITH THE JOURNAL'S GUIDELINES FOR LISTING BIBLIOGRAPHIC REFERENCES.

IT IS NOT IN ALPHABETICAL ORDER, BUT IN ORDER OF APPEARANCE IN THE ARTICLE.

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nitrogen, phosphorus and calcium in soybean infected with *Meloidogyne incognita and M. javanica*. *FitopatologiaBrasileira*. 2002; 27:141–150.

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Comment [137]: THE REFERENCE [14] DOES NOT APPEAR IN THE ARTICLE OR IT HAS NOT BEEN LOCATED.

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