GAU Research Journal 46(4) : 77-82 (October 2021)

Effect of different carbon and nitrogen sources on growth and pathogenicity of *Rhizoctonia solani* and *Meloidygyne incognita* disease complex in tomato c.v.Pusa Ruby.

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Received: 24 May, 2022 **Accepted:**16 July, 2022

Abstract

Studies were made to study the effect of different carbon and nitrogen sources on the pathogenicity of disease complex formed *Rhizoctonia solani* and root knot nematode *Meloidogyne incognita* on tomato cv Pusa Ruby. For this *R. solani* was cultured on different carbon and nitrogen sources. Selected carbon sources were sucrose, arabinose, glucose, fructose, rhamnose, xylose and starch. Selected nitrogen sources were KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, Asparagine, Glycine, Lysine, Methionine and Urea**.** Seedlings of tomato were inoculated with *R. solani* and RKN separately and in combined inoculations. Plant growth, nematode multiplication and phenol contents of plants were observed for development of disease complex. Different sources show varied degrees of pathogenicity. Reduction in growth of plants was highest with fructose in case of carbon sources both in separate and combined inoculations. For nitrogen sources lysine has shown the most adverse effect on growth of plants in mono-pathogenic inoculations while urea and glycine in bi-pathogenic inoculations. Nematode multiplication was highest in glucose, glycine and KNO_3 for carbon and nitrogen sources respectively. A positive correlation was observed in disease complex development and phenol concentration.

Key Words. Carbon Sources, Nitrogen Sources , Growth, Pathogenicity*, Rhizoctonia solani, Meloidogyne incognita*

INTRODUCTION:

Tomato occupies a unique position among fruits! vegetables with its large number of uses. It is grown in almost all parts of the country occupying approximately 2 lakh hectares. The yield is 25 tonnes per hectare (Horticulture Statistics at a Glance, 2018) The yield is affected by losses to crop by various pests and diseases (Singh, 1987). In India, tomato productivity loss has been estimated to range from 10 to 80% (Sharma and Sharma 2005), Large number of diseases due to fungi have been reported (Kalloo, 1986). *Rhizoctonia solani* Kuhn is a widespread fungal pathogen causing pre and post emergence damping -off, root rot and stem rot in tomato. Root knot nematode, *Meloidogyne incognita* (Kofoid and White,1919), Chitwood,1949 is another pathogen causing great losses in tomato (Reddy, 1985). Both are soil borne pathogens and occupy the same niche forming a disease complex. The losses are compounded many times in combined presence of these than the two pathogens caused in mono-pathogenic conditions. It was reported that, the root rot pathogen along with a nematode could cause 38.64–56.74 percent yield loss in tomato (Kumar, D and Singh, K.P. 2006a)

Pathogenicity of soil borne pathogen is affected by their nutritional status.Carbon and nitrogen sources have been reported to alter the pathogenicity of certain pathogenic fungi (Tripathi and Grover, 1983).Hrushovetz (1957), suggested that pathogenicity of some heterokaryotic fungi is influenced by amino acid present in external medium. Keeping this in view *Rhizoctonia solani* has been grown in medium containing different carbon and nitrogen sources in separate studies and its pathogenicity has been determined in disease

complex with root knot nematode *Meloidogyne incognita.*

Material and Methods: Raising inoculum of *Rhizoctonia solani*. Isolated from the rhizosphere of tomato c.v. Pusa Ruby in a previous study (Arya and Saxena 2009). Pure culture of fungi was raised in 250 ml Erlenmeyer flasks on Richard's solution containing different carbon sources separately (Sucrose, arabinose, fructose, glucose, rhamnose, xylose and starch) keeping the amount of carbon equivalent to 50 g of sucrose. Nitrogen sources selected were (KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, Asparagine, Glycine, Lysine, Methionine, and urea keeping the amount of nitrogen equivalent to 10 g of KNO_3.The flasks were sterilized at 15 lbs pressure for 15 minutes in an autoclave. After cooling these were inoculated with test fungi under aseptic conditions. The flasks were incubated at 28^0 C. After 15 days the mycelial mat was filtered through what man filter paper no. 1 and washed with sterilized distilled water. It was later blended in a blender for obtaining homogeneous suspension in sterilized distilled water in such a way so as to obtain 1g of mycelium/10 ml.

Inoculum of root knot nematode. Roots of 6-12 week old infected tomato roots were cut into 1-2 cm segments and shaken vigorously in 200 ml solution of 0.5% solution of sodium hypochlorite. (Barker, 1985) for 2 minutes. The solution was quickly transferred to 200 mesh sieve placed on 500 mesh sieve to collect freed eggs. The 80 mesh sieve containing eggs was placed under tap water and rinsed to remove residual sodium hypochlorite. The remaining portion of roots were also rinsed to collect the rest of the eggs. The eggs were transferred to 100 mesh sieves of 3 cm diameter placed in 5 cm Petri dishes containing water and incubated at 25^0C. The larvae emerged out each day, were collected and used for inoculation. A suspension of larvae was made in sterile distilled water in such a way so as to contain 1000 larvae/ml. The 1 ml suspension was poured in the four holes around the roots zone with the help of pipettes. The holes were covered with soil

Raising of Seedlings. Seeds of tomato c.v. Pusa Ruby after having been sterilized with 0.1% sodium hypochlorite and rinsed three times with sterilized distilled water. Seeds were sown in 10cm pots. The soil of pots was first sterilized in an autoclave and then infested with 5g /kg soil *of Rhizoctonia solani* and 1000 j2 larvae of *Meloidogyne incognita* separately and in combination. Un- inoculated seeds served as a check. 25 days old seedlings were transplanted in 15 cm pots containing autoclaved sterilized soil. The soil was infested with *R. solani* and root knot nematode separately and in combination.

Recording of observations. After inoculation of plants with test organisms the plants were kept in a greenhouse in a randomized manner. There were ten replicates of each treatment.

After 45 days of inoculation the plants were uprooted, and the root system was thoroughly washed with running water. Length of roots and shoots was measured. The plants were kept between blotting papers to remove excess of water before determining the fresh weight of roots and shoots. After cooling, the dry weight of plants was determined. Roots were examined for the intensity of root-knot development rated as follows.

Nematode population estimation. The soil from each pot was mixed thoroughly and a sub sample of 250 g soil was processed through a set of sieves of different meshes by following Cobb's sifting and gravity method followed by Baermann funnel technique. (Southey, 1970.)

The suspension was collected in a beaker and volume was made up to 100 ml. After stirring, 1 ml of the suspension was drawn by a pipette and transferred to a counting dish. The number of juvenile larvae was counted under stereoscopic microscope. Ten such counts were made , meaning all such counts were taken for the final population of nematodes/kg soil.

Resistance assessment*:* For all assessment methods, tomato plants were uprooted and washed clean of soil 45 days after inoculation on the basis of gall index Gall index 1 was on a scale of 0 to 5 (Taylor and Sasser, 1978).

Category	Number of galls.	Reaction
0	0 galls / root system	Immune
1	01-02 galls/ root system	Resistant
2	03-10 galls/ root system	Moderately resistant
3	11-30 galls/ root system	Moderately susceptible
4	31-100 galls/ root system	Susceptible
5	101 and above galls/ root system	Highly susceptible

Phenols estimation. Total phenols and ortho dihydroxy phenols contents of culture filtrate were also estimated. Phenols were extracted by the methods of Biehn *et al.* (1968) and estimated by using Folin Ciocalteu reagent (1954). Ortho dihydroxy phenols were estimated by the method of Johnson and Shaal(1952) using Arnow's reagent. P Cresol and catechol were used to prepare the standard curves.

Result. **a**. **Growth of plants.** Results shown in table 1 and 2 and 3 show that the growth and phenolic contents of tomato were greatly influenced as a result of inoculation with *R. solani* grown in medium containing different carbon and nitrogen sources.

The growth of plants was most adversely affected when plants were inoculated with the fungus grown on medium containing fructose as carbon source followed by medium containing sucrose, rhamnose, starch, ribose, glucose, arabinose, and xylose as carbon sources. However when plants were inoculated with the nematode together with fungus, the reduction in plant growth was highest when *R. solani* used in inoculum was grown in medium containing fructose and ribose followed by that grown in medium containing starch, sucrose, xylose, rhamnose, arabinose and glucose. Reduction in growth of plants is more when fungus was grown in a medium containing fructose, irrespective of the fact that plants were inoculated with the fungus alone or together with nematode. But the difference lies in the order of reduction in growth of plants in inoculation made with fungus alone or in combination with root knot nematodes.

Nitrogen sources used in the medium to grow fungus, influenced the pathogenicity of *R.solani.* The growth of plants was most adversely affected when the fungus was grown in the medium containing lysine and least affected when the fungus was grown with methionine as a nitrogen source. Among the plants inoculated with nematodes together with the fungus, the most adversely affected plants were those inoculated with fungus grown on media containing urea/ glycine and least where the nitrogen source was methionine.

Nematode multiplication. Nematode population was lesser in the plants treated with fungus and nematode together than nematode alone.

The nematode number was least affected when the fungus component was grown in the medium containing glucose and most affected when fungus was grown with arabinose. On the other hand gall formation was less in concurrent inoculation (nematode and *R. tsolani)* where the fungus was grown in the medium containing sucrose and highest number of galls was observed with glucose as carbon source.

In nitrogen sources the least number of nematodes was observed when methionine was used as nitrogen source to grow *R. solani*, while highest number with glycine. Highest number of galls was observed in plants treated with fungus grown with KNO_3 and lowest with methionine.

Phenolic contents of plants. There has been increase in phenols and ortho dihydroxy phenols contents of plants as a result of inoculation with the two pathogens separately and together. The increase in phenols was more when two pathogens present together. However, the carbon and nitrogen sources in which the fungus was grown influenced the phenolic contents of plants. For carbon sources, the phenolic contents was highest when fungus was grown in xylose and least for fructose for separate inoculations. For combined inoculations highest phenols were observed when glucose was used as carbon source and least in ribose and fructose.

For nitrogen sources highest phenolic content was observed in the plants inoculated with the

fungus containing methionine and lowest in lysine used as nitrogen sources. When plants were inoculated with two pathogens together, the phenolic content was highest with the fungus grown in methionine and least in glycine.

Discussion: The disease development due to *R.solani*either alone or with root knot nematode, has been highest when the fungus has been grown in a medium containing fructose. It is interesting to note that fructose as carbon source not only support better growth.

The pathogenicity, besides other factors , also depends on the metabolites of fungus which in turn is influenced by content of culture media (Toussoun et al , 1960).. Therefore in the present studies the changed pathogenicity could be in part due to change in metabolites of fungus brought about by different carbon and nitrogen sources. This is in conformity with Hrushovetz (1957) wherein it has been reported that amino acid in the external medium influenced the pathogenicity. The mycelium of R. solani is somewhat heterokaryotic (Ogoshi, 1987) , which could explain its behavior. These results are also in agreement with those of Weinhold et al (1969), who reported that nutritional status of the inoculum of *R. solani* plays an important role in the pathogenicity. Gangopadhyay and Wylie (1979) also reported the damage to the plants by *Macrophomina phaseoli* (Maubl) Ashby, besides inoculum level , is determined by the carbon nitrogen ratio of the medium in which it was grown. It appears that some mechanism of preference on the part of nuclei as suggested by Hrushovetz (1957) might be operating in the present case.

There appears to be a positive correlation between the damage of plants as a result of infection with root knot nematode and *R.solani* (separate or combined) and the phenol contents of plants. Phenols seem to play some role. They are already known to interfere with the respiratory mec.

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Table 1 - Effect of inoculation of seedlings of tomato cv Pusa Ruby with Rhizoctonia solani (grown in medium containing different Carbon and Nitrogen Sources separately) alone and together with root knot nematode on phenolic content of plants.

	Carbon Treatment/ Carbon Source in which fungus grown	Phenols (mg/ml)	Ortho dihydroxy phenol (mg/ml)		Nitrogen Source	Phenols (mg/ml	Ortho dihydroxy phenol (mg/ml)
Uninoculated		0.393	0.161	Uninoculated		0.357	0.123
Inoculated with nematode		0.675	0.168	Inoculated with nematode		0.413	0.131
R	Sucrose	0.431	o.171	R	KNO_3	0.524	0.172
R+N		0.712	0.211	R+N		0.673	0.231
R	Arabinose	0.607	0.189	R	NH_4NO_3	0.493	0.161
R+N		0.769	0.225	R+N		0.618	0.198
R	Fructose	0.399	0.169	R	$(NH4)_2SO_4$	0.435	0.152
R+N		0.693	0.195	R+N		0.495	0.168
R	Glucose	0.524	0.178	R	Asparagine	0.522	0.168
R+N		0.778	0.257	R+N		0.595	0.188
R	Rhamnose	0.482	0.172	R	Glycine	0.396	0.418
R+N		0.751	0.219	R+N		0.471	0.159
R	Ribose	0.524	0.178	R	Lysine	0.371	0.146
R+N		0.693	0.198	R+N		0.671	0.228
R	Xylose	0.694	0.199	R	Methionine	0.573	0.197
R+N		0.741	0.213	R+N		0.756	0.261
R	Starch	0.482	0.172	R	Urea	0.431	0.152
R+N		0.694	0.214	R+N		0.488	0.168
C.D.at 5%		0.033	0.016	C.D.at 5%		0.0365	0.0116

Each figure is mean of 10 replicates in each treatment,

R= Rhizoctonia solani,

N= Nematode (Meloidogyne incognita)

R+N= R.solani + Nematode

Table 2. Effect of inoculation of seedlings of tomato cv ,"Pusa Ruby" with Rhizoctonia solani (grown in the medium containing different carbon and nitrogen sources)

Treatment /Carbon sources in which R. solani grown		Length (cm)	Fresh wt. (g)	Dry wt. (g)	No of galls /root sys.	RKI	No of larvae/ root sys	No of ♀/ root sys	No. of larvae / kg soil	Total nematode population
Uninoculated		69.88	26.63	4.65						
Inoculated with nematode		65.13	20.95	3.83	73	4		69	1365.0	3524
R	Sucrose	51.25	24.48	3.13			2090.0	33		
R+N		44.00	14.19	2.54	39	4			880.0	2253.0
R	Arabinose	68.38	24.35	4.11			1340.0	43		
R+N		66.13	21.75	3.17	48	4			880.0	2108.5
R	Fructose	39.38	12.05	1.96			1134.5	51		
R+N		41.75	11.64	1.91	53	4			931.0	2811.0
R	Glucose	59.93	22.50	3.88			1730.0	56		
R+N		44.33	21.50	3.71	60	4			1345.0	3221.0
R	R h a m - nose	66.63	24.38	3.39			1820.0	42		
R+N		44.75	17.88	2.99	48	4			794.0	2148.0
R	Ribose	65.75	25.00	3.80			1312.0	48		
R+N		38.75	14.18	1.91	54	4			970.0	2629.5
R	Xylose	66.63	24.75	4.19			1611.5	42		
R+N		51.00	17.75	2.97	45	4			805.0	2147.5
R	Starch	51.80	23.28	3.44			1300.5	53		
R+N		47.00	15.91	1.94	56	4	1730.0		970.0	2753.0
C.D. at 5%		02.091	01.063	0.220	3.442		20.82	03.65	30.77	

S= Standard (100 percent) Each figure is a mean of ten replicates.

S/10= 010 percent Each Petri-plate containing ten egg masses for hatching

S/100= 001 Percent Each Petri-plate containing 100 larvae for mortality