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STUDY OF SELECTED BIOCHEMICAL AND PHYSIOLOGICAL CHANGES AMONG THE INFECTED BY ROOT KNOT NEMATODE AND HEALTHY PLANTS OF BITTER GOURD IN FOUR LOCALITIES AT MUZAFFARPUR

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ABSTRACT

Plants have developed certain mechanisms to protect themselves from the pathogens. This pathogen after infection destroys or disturbs the synthesis of such chemicals. Among the resistant species these are not affected but in sensitive they are influenced by this pathogen. Present study was aimed to analyse the chlorophyll contents of the leaves, the sugars of the roots & stem, the phenylalanine ammonia lysate and peroxidase activities among the healthy and infected by root knot disease causing nematode, *Meloidogyne incognita*. It was noted that both chlorophyll-a and b, were influenced in the infected plants. Further maximum percentage of decrease in chlorophyll-a was found among the infected *plants* collected from Krishnapuri area, while maximum chlorophyll-b decreased among the *plants* collected from Professor Colony of the town. It was further observed that the sugar contents increased it he roots, while it decreased among the shoots. Maximum decrease -60.77 was noted among the plants collected from Krishnapuri area, while it was minimum among the plants collected form Professor Colony (-48.45). Activity of phenylalanine ammonia lyaste revealed maximum gain 48.95 among the *plants* collected form Nayatola area, where as it was the minimum 21.83 among the plants collected from Krishnapuri. Peroxidase enzyme activity was also studied. Here maximum increase 45.41% was noted among the infected plants collected form Krishnapuri, followed by 43.23% among the plants collected from Professor Colony.

KEYWORDS: Root Knot, Bitter Gourd, *Meloidogyne incognita*, Phenylalanine Ammonia Lysate, Peroxidase

Bitter gourd (*Momordica charantia* L.) of family *Cucurbitaceae*, is an economically important crop, being widely cultivated in different states of India as well as different other countries of the world. It is being used as vegetable as well as medicinal plant. *Momordica charantia* is cultivated largely due to its nutritional and medicinal properties (Satkar *et al*., 2013). The fruit of bitter gourd has higher content of folate and vitamin C and Vitamin A. It has medicinal value for the treatment of infectious diseases and specially diabetes as hypohlycemic agent (Grover and Yadav, 2004). The crop is being attacked by different pathogens like bacteria, fungi, nematodes and viruses which cause great loss to its production. Among them fusarium wilt and root knot nematode have been reported as major limiting factors affecting cultivation of bitter gourd (Lin *et al*., 1998). Root knot nematode, belonging to genus *Meloidogyne* has been reported to be predominantly associated with bitter gourd. Root knot nematodes are obligate, sedentary parasites of vascular tissues of plant roots. After entry into the host, they form root galls or knot which hamper the uptake of water and nutrients by the roots, resulting poor growth and yield. Infected plants reveal, poor root systems with fewer feeder roots (Anwar and Javid, 2010). Based on the above ground and underground system the infected plants can be recognized easily.

Root knot disease of Cucurbits, including *Momordica charantia* has been studied by different workers. In addition to its survey and control measures biochemical changes in the infected *plants* have been studied by different workers. Dasgupta *et al*., (1981), Tayal and Agarwal (1982), Ganguly and Dasgupta (1983), Swain & Prasad (1988), Mohanty and Pathak (1990), Nagesh *et al*., (1998), Chakrabory and Mishra (2002), Devrajan and Rajendra (2002), Mohammad and Hasab (2005), Mishra and Mohanty (2007), Rani *et al*., (2008), Nayak and Mohanty (2010), Chaudhary *et al*., (2013), Gautam and Poddar (2014), Nikoo *et al*., (2014), Ramchandra *et al*., (2015), Nayak and Pandey (2016), Nayak *et al*., (2016), Mahapatra and Nayak (2019). Similarly, loss in yield and population density have been studied by Jan *et al*., (2007), Chandra *et al*., (2010), Khan *et al*., (2012), Singh *et al*., (2012), Anwar *et al*., (2013), Singh and Kumar (2013), Gautam *et al*., (2014) and Pungalendhi and Thiruvengadan (2016). In the present study selected biochemical changes in the infected and healthy plants of bitter gourd have been studied.

MATERIALS AND METHODS

Based on the above ground symptoms, chlorotic patches on the leaves, deformed leaves, fruits, reduced fruit setting, poor growth of the branches and underground symptoms such as galls on the roots, decaying root tips, the disease caused by root knot nematode *Meloidogyne incognita* was located in the field.

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The galls containing roots and rhizospheric soil were used for detection and confirmation of the disease.

Infected plants of bitter gourd were located in four different localities. Similarly, healthy plants were also located in the same field. Now samples such as leaves, roots and stems were collected from healthy as well as infected plants for the estimation of chlorophylls, Sugar, and enzyme activities among the infected as well as the healthy plants.

Estimation of Chlorophyll

Both healthy as well as leaves of infected plants were collected from different localities and brought in the laboratory in the separate polybags. These leaves were washed properly and extra water was blotted with filter paper. Now the midribs of the leaves were removed with the help of a sharp blade. 150 mg leaf was taken separately from the healthy as well as the infected plants. These leaves were chopped and kept in a conical flask containing 50 ml, of 80% acetone. Above flasks were stored in the dark of 24 hours, for the extraction of chlorophylls. Next day the extracts were filtered through Whatman No.-1 filter paper. The filtrate was used for the estimation of chlorophyll-a and chlorophyll-b. The absorbance of the extract was taken at 645 nm and 663 nm using Spectrophotometer. 80% Acetone was used as the blank.

The amount of chlorophyll-a and chlorophyll-b was calculated by using the following formula:

Chlorophyll-a (Mg/g fresh weight of leaf) =

$$12.7X(D-663)-2.69X(D-645)X\frac{V}{1000XW}$$

Chlorophyll-b (Mg/g fresh weight of leaf) =

$$22.9X(D-645)-4.68X(D-663)X\frac{V}{1000XW}$$

Total Chlorophyll (Mg/g fresh weight of leaf) =

$$20.2X(D-645)+8.02X(D-663)X\frac{V}{1000XW}$$

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 g of different leaves, taken form healthy ad infected plants.

Estimation of Sugar Contents in Roots & Shoots of Infected and Healthy Plants of Bitter Gourd

Roots and shoots of healthy as well as infected plants were collected from different localities. They were brought in the laboratory and washed properly with tap water, followed by distilled water. Fresh root as prepared above was chopped into pieces and was dried at room temperature. Above dried roots and shots were grounds to powder separately. 100 mg of powder of ground roots were taken in 15 ml centrifuge tube containing 10 ml of 80% ethanol. The mouth of the centrifuge tube was covered with polythene paper and it was kept in water bath at 80-85^{0}C for 30 minutes. The tubes were allowed to cool and were centrifuged for 15 minutes at 2000 rpm. The supernatant was carefully taken in a 25 ml volumetric flask with the help of pipette. Above method was repeated and supernatant was collected in 25 ml volumetric flask. The volume was made 25 ml by adding sterilized distilled water. It was filtered through Whatman No.-1filter paper. The extract taken from healthy as well as infected roots and shoots were stored and labeled for the same and the localities and were used for the estimation of sugar.

Estimation of Sugar

2 ml of the above extract was taken in 50 ml volumetric flask and volume was made 50 ml by adding distilled water. 5 ml of this was taken in 25 ml volumetric flask. In the mean time standard of 0 ml, 1ml, 1.5ml and 2 ml of 100 mg/l glucose solution were taken in 25 ml volumetric flasks. The volume of these standards solution was made up to 5 ml by adding distilled water and 2 drops of 80 percent ethanol. Above flask containing samples and standard were placed on ice-bath. To each volumetric flask 10 ml of anthrone (2 g anthrone in one liter of 95% H_2So_4) was added. The flasks were shaken slowly and then thoroughly. Above flask were kept in boiling water bath for exactly 7.5 minutes. Flasks were allowed to cool on ice-bath. The absorbance was taken at 630 nm. The sugar content was calculated with the help of standard curve.

Estimation of Enzyme Activity

Phenylalanine Ammonia Lysate (PAL)

Above powdered roots prepared from the roots of infected as well as healthy roots were used for the above experiments.1g of powered was homogenized in prechilled 10 ml acetone at 15^{0}C. The homogenate was filtered through Whatman filter paper No.-1. It was again rinsed with 10 ml of acetone and filtered. The filtrate was

dried at room temperature and stored in freeze. Above residue was suspended in 0.1 m cold borate buffer (pH 8.8). Here 6 ml buffer was used for 1 g of sample. The suspension was incubated at 0^0C for 1 hour. It was filtered through cheese cloth. The filtrate was centrifuged at 5000 rpm at 4^0c for 10 minutes. The aliquot was sued as the source of enzyme.

1.5 ml of above extract was taken in a tube and 1 ml of 0.05M, L-phenylalanine and 2.5 ml of 0.1M borate buffer was added to it. It was incubated for 1 hour at 40^0C and the reaction was stopped after it by adding 0.1 ml of 5 N Cl and 7.5 ml ether. The mixture was evaporated to complete dryness and residue was dissolved in 0.05N, NaoH. The amount of trans cinnamic acid formed above was estimated by measuring the absorbance at 290 nm. Blank was used without the extract. The enzyme activity was expressed as mg of trans-cinnamic acid formed per gram of fresh weight of roots of healthy as well as infected *plants* of bitter gourd.

Estimation of Peroxidase Activity in Roots

Well cleaned roots were taken. Now 1 g of root was grinded with 5 ml of cold 1 M phosphate buffer (pH 7.0) in a pre chilled mortar and pestle. The homogenate was centrifuged at 15000 rpm at 4^0C for 30 minutes the supernatant was used as the source of the enzyme.

1 ml of above extract was mixed with 2 ml of 0.1 M phosphate buffer (pH 7.0) 1 ml of 0.01M pyrogallol, 1 ml of 0.005 M H_2O_2. It was left for 5 minutes at 25^0C and the reaction was stopped by adding 1 ml of 1.5 N H_2So_4. The absorption was measured at 420 nm to measure the amount of purpurogallin formed above. The enzyme activity was expressed in optical density absorbency units.

All the experiments were repeated thrice and the mean of the data was taken for discussion. The data were placed in table 1-4 respectively.

Table 1: Reduction in chlorophyll content (a, b, total) due to infection by *Meloidogyne incognita* the root knot casing plant parasitic nematode

Site	Chlo-a mg/g leaf				Chlo-b mg/g leaf				Total Chlo- mg/g leaf			
	Healthy	Infected	Mean	% loss	Healthy	Infected	Mean	% loss	Healthy	Infected	Mean	% loss
A	2.978	1.982	2.48	33.45	1.484	1.326	1.41	30.65	4.462	3.308	3.885	25.86
B	2.754	1.864	2.310	32.33	1.280	0.946	1.113	26.09	4.034	2.81	3.422	30.03
C	2.886	2.028	2.460	29.73	1.390	0.622	1.06	44.75	4.276	2.65	3.463	44.33
D	2.678	1.926	2.302	28.08	1.242	0.646	0.944	47.98	3.920	1.972	2.945	49.69

A = Krishnapuri, B = Damuchak, C= Naya Tola, D= Professor Colony

Table 2: Percentage increase/decrease of total sugar content in the root and shot of bitter gourd infected by *Meloidogyne incognita*

Site	Healthy		Infected		Mean		% increase/decrease over control	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
A	1.674	11.562	2.784	4.536	2.117	8.049	66.30	-60.77
B	1.878	13.456	2.842	6.528	2.360	9.992	51.33	-51.49
C	1.756	14.562	3.788	7.382	2.772	10.972	115.72	-49.93
D	1.712	14.884	3.826	7.658	2.769	11.271	123.48	-48.45

A = Krishnapuri, B = Damuchak, C= Naya Tola, D= Professor Colony

Table 3: Estimation of phenylalanine ammonia lysate in infected roots by *Meloidogyne incognita* and in the healthy roots of bitter gourd

Site	Activity of phenylalanine ammonia lysate in healthy and infected root of bitter gourd mg/g			
	Healthy	Infected	Mean	% increase over control (Healthy)
A	23.68	28.85	26.265	+21.83
B	28.26	39.74	34	+40.62
C	32.48	48.38	40.43	+48.95
D	27.54	38.44	32.99	+39.58

A = Krishnapuri, B = Damuchak, C= Naya Tola, D= Professor Colony

Table 4: Percentage increase/decrease in the enzyme peroxidase in healthy and infected plants of bitter gourd by root knot nematode (*Meloidogyne incognita*)

Site	Enzyme activity OD units/g fresh weight of roots		% increase (+) or decrease (-)
	Healthy	Infected	
A	1.284	1.867	+45.41
B	1.386	1.938	+39.82
C	1.722	2.376	+37.97
D	1.316	1.885	+43.23

A = Krishnapuri, B = Damuchak, C= Naya Tola, D= Professor Colony

RESULTS AND DISCUSSION

Chlorophyll content of healthy as well as leaves of plant infected by root knot disease causing nematode was analyzed. In the present experiment, it was noted that chlo-a was reduced to 1.982 mg/g of leaves in the infected plant which were collected form Krishnapur. Similarly, Chlo-b was reduced from 1.484 mg/g to 1.326 mg/g in infected leaves. Leaves of plants collected form Damuchak revealed that chlo-a was reduced to 1.864 mg/g in the infected *plants* from 2.754 mg/g found in healthy leaves. Similarly chlo-b was reduced from 1.280 mg/g to 0.946 mg/g. Chlo-a was reduced to 0.028 mg/g in the infected leaves collected from Nayatola from 2.886 mg/g of the healthy leaves. Here chlo-b was reduced to 0.622 mg/g in infected leaves from 1.390 mg/g of healthy leaves. Chlo-a was reduced to 1.926 mg/g in infected leaves from 2.678 mg/g of healthy leaves collected from Professor Colony at Muzaffarpur. The percentage of reduction due to infection by root knot nematode in Chlo-a varied from 28.08 to 33.45. Here lower percentage for reduction was noted among the *plants* collected form Professor Colony where as the highest one in case of plants collected from Krishnapuri locality. Likewise, percentage of reduction in chlo-b from 26.09 to 47.98. Here the lowest reduction was found among leaves collected from Damuchak while the highest among the plants collected form Professor Colony. Total chlorophyll also revealed decreased concentration among the infected leaves than that of the healthy one. It varied from 25.86 percent to 49.69%. Here again the higher percentage of reduction was among the infected leaves collected form Professor colony than that of the others.

Chlorophyll contents are the most important constituent of the plants because, growth and yield depend on it. Once a plant is infected, its chlorophyll becomes the target and either it is degraded or no synthesis of new chlorophylls takes place. This was also evident in case of a plant infected by *Meloidogyne incognita* the root knot causing nematode. Present findings corroborate with the findings of Nayak *et al*., (2016) and Mahapatra and Nayak (2019).

In the present work percentage increase and decrease of total sugar in roots and shoots of healthy as well as infected *plants* were studied the mean of the data obtained was presented in table 2. From the table it may be noted that while in the roots there was gain of the sugar, in the shoot it was reduced. Maximum percentage of gain was 123.48 which were the highest and the sample was collected form Professor Colony locality. The minimum increase 51.33% was among the sample collected form Damuchak locality. Similarly, highest percentage in decrease sugar in the stem was (-60.77) in the sample collected form Krishnapuri area, followed by (-51.49) in the sample collected form Nayatola.

The activity of phenylalanine ammonia lysate (PAL) was also studied in healthy as well as the infected plants. Mean of the data was presented in the table 3. PAL was measured as mg of trans-cinnamic acid formed per gram of fresh root extract. From the table it may be noted that this was 23.68 mg/g, 28.26 mg/g, 32.48 mg/g and 27.54 mg/g in the healthy roots collected form Krishnapuri, Damuchak, Nayatola nd Professor Colony localities respectively. Whereas among the infected roots collected from the same localities it was 28.85 mg/g, 39.74 mg/g, 48.38 mg/g and 38.44 mg/g respectively. The percentage of increase over the healthy one was therefore, 28.83, 40.62, 48.95 and 39.58 respectively.

Percentage increase and decrease in the enzyme peroxidase in healthy and infected plants of bitter gourd were also studied the mean of the data was presented n table 4. Here highest increased percentage in peroxidase (45.41) was found in the infected plant sample collected form Krishnapuri locality, which was followed in the sample 43.23 collected form Professor colony. It was recorded 39.82 among the sample collected form Damuchak locality. The sample collected form Nayatola locality revealed 37.97% increase only.

Physiological and biochemical in different plants infected by the root knot causing nematode *Meloidogyne incognita* have been reported by Ganguly and Dasgupta (1983) in brinjal, Chakrabory and Mishra (2002) in chick pea, Mohammad & Hasab (2005) in cotton, Mishra and Mohanty (2007), Rani *et al*., (2008), Chakraborty et la., (2013) in tomato. Nikoo et la., (2014) in tomato, and Mahapatra *et al*., (2019) in bitter gourd. Their findings are in agreement with the present observations as they considered the aforesaid parameters among the above crops and reported alteration in chlorophyll contents, sugar contents, phenolics and in enzyme activates. *Plants* have developed biochemical defense and whenever they are attacked, these chemicals are altered due to host parasite interactions. Variations in data for same parameter in the infected plants at different locality may be due to nematode population load and soil conditions.

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