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Survey, Pathogenecity and Biology of *Heterodera cajani* on Pigeonpea and Reaction of Some Pigeonpea Varieties Against Pigeonpea Cyst Nematode

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Abstract : Studies were undertaken for the survey, pathogenecity test, life-cycle and reaction of some pigeonpea cultivars against *Heterodera cajani*. An intensive survey of pigeonpea field around Alwar district revealed the 100% occurrence of *H. cajani* and it causes a serious threat to this crop. Pathogenicity test showed that 100 J2S/plant is an economic threshold point of pigeonpea cyst nematode and this inoculum level causes considerable loss to crop. This nematode completed its life-cycle in 34 days upon pigeonpea plant. In screening reaction none of the varieties were found resistant out of eleven varieties.

Key words : Pathogenecity, *Heterodera cajani*, Pigeonpea, Cyst Nematode.

Introduction

Pigeonpea (*Cajanus cajan* L.) is major pulse crop of India providing for much of the protein supplement to vegetarian population ((Siidiqui *et al*., 1998).The pigeonpea cyst nematode, *Heterodera cajani*koshy is widely prevalent in all major pigeonpea growing region of India. This nematode species causes significant loss in crop yields (Gupta and Edward, 1974; Devi and Gupta, 1991). An attempt was made to study the survey, host parasitic relationship (pathogenecity test, life-cycle) and attempt was also made to screen available pigeonpea varieties/lines locating source of resistance, if any, against *H. cajani*.

For sustainable production of pigeonpea pulse crops which are primarily grown with low inputs, growing of crop cultivars resistant to nematode in infested area provides ideal and environmentally safe method for maintaining the nematode densities below the damaging levels (Cook and Evans, 1987).

Materials and Methods

An intensive survey of plant parasitic nematodes associated with pigeonpea was conducted in Alwar district during the kharif season (July-Sept) in the year, 2005. A total of 67 samples from twelve different localities of Alwar after examination of above ground symptoms of nematode injury. In some areas where mixed cropping with other legumes and millets were practiced, soil samples were collected from the rhizosphere of pigeonpea plants only. Samples were processed further for cysts extraction by Cobb's sieving decantion method followed by Baermann's funnel technique (Thorne, 1961) or Oostenbrink elutriator method. The assessment of loss due to nematode was calculated as per formula given below:

$$\text{Per cent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants observed}} \times 100$$

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$$\% \text{ Loss in yield} = \frac{\text{Yield of healthy plants - Yield of diseased plants}}{\text{Yield of healthy plants}} \times 100$$

Pathogenecity Test

Seeds of pigeonpea variety cv. UPAAS-120 were sown in 15cm sized earthen pots filled with autoclaved sandy loam soil and replicated thrice. At two leaf stage, the seedlings were inoculated with larvae of *H. cajani* in a logarithmic series i.e. 0.10, 100, 1000 and 10,000 nematodes/plant. The experiment was terminated after 60 days of inoculation. Observations were recorded on plant growth characters viz. shoot-root length and weight, number of nodules and nematode reproduction (number of cysts per plant, number of eggs per egg sac).

Biology of *Heterodera cajani* on pigeonpea

Seeds of pigeonpea cv. UPAAS-120 were surface sterilized with 0.1% mercuric chloride for one minute and then washed in distilled water. The seeds were then sown in pots of 1kg capacity containing autoclaved soil at the rate of two seeds/pot and 45 such pots were maintained. Seven days after germination, plants were inoculated with freshly hatched J2 @ 1000/pot. From the day of inoculation, the plants were carefully removed with root intact @ one plant/pot/day, washed, stained with acid fuchsin lactophenol and observed for juvenile penetration and developmental stages under a stereo-binocular microscope. The daily minimum and maximum temperature were recorded throughout the experimental period.

Varietal Screening Test

Seeds of eleven varieties of pigeonpea, ASJ-112, ASJ -118, ASJ-102, ASJ-117, ASJ-127, ASJ-114, ASJ-121, ASJ-113, ASJ-124 and ASJ-122 obtained from Durgapura Agriculture Research station, Jaipur were sown in pots containing approximately 1kg sterilized soil. Seven days after germination, the seedling was inoculated with freshly hatched J2 @ 1000/ pot. The juvenile's suspension used for inoculation was obtained by crushing cysts collected from culture of *H. cajani* maintained on cowpea cv. RC-19. Three replicates were kept for each variety along with un-inoculated check plants. After 60 days of inoculation, roots and soil per pot were examined and cysts were counted under stereoscopic microscope. The varietal reactions were recorded by using the scale given by Bhatti and Jain (1994), which is as follows:

Numbr of cysts	Resistance rating
0	HR (Highly Resistant)
1-5	R (Resistant)
6-25	MR (Moderately Resistant)
26-50	S (Susceptible)
51-more	HS (Highly Susceptible)

Results and Discussion

Survey: An intensive faunistic survey of pigeonpea fields of Alwar district revealed the occurrence of two important genera *H. cajani* and *M. incognita* associated with rhizosphere of pigeonpea. *H. cajani* was observed with 100% occurrence in Thanagazi, Lalpura, Amra ka Bass, Dhaipedi, Keshroli, Beejwa, Naugawa, Jajore, Tuleda and Chikanni. However, in Ramgarh and Nadka there were 75% and 56%, respectively (Table-1). In the present survey, the infected plants collected from pigeonpea infected fields showed above ground and under ground symptoms such as chlorosis, stunting and deformed leaves. White glistening cysts were seen early in the season and black cysts late in season in case of *H. cajani*. Similar report was given by Shukla and

Table 1 : Data on different parameters of fields of Pigeonpea crop surveyed in and around Alwar

S. no.	Name of locality	No. of samples	Area under cultivation	Condition of crop	Disease incidence (% PDI)	Percent loss in yield	Occurrence of nematodes
1	Thanagazi	25	10 Bigha	Boundary crop	80	92	*H. cajani*
2	*Amra Ka Bass*	16	8 Bigha	Single crop	64	60	*H. cajani*
3	*Lal Pura*	12	4 Bigha	Single crop	75	65	*H. cajani*
4	*DhaiPedi*	8	12 Bigha	Boundary crop	55	40	*H. cajani*
5	*Jajore*	6	5 Bigha	Inter-crop with guar & bajra	20	30	*H. cajani*
6	*Keshroli*	14	15 Bigha	Single crop	78	70	*H. cajani*
7	*Naadka*	20	2 Bigha	Boundary crop	70	62	*H.cajani+M. incognita*
8	*Ram garh*	9	7 Bigha	Boundary crop	65	74	*H.cajani+M. incognita*
9	*Beejwa*	10	6 Bigha	Boundary crop	76	85	*H. cajani*
10	*Tuleda*	14	8 Bigha	Single crop	60	76	*H. cajani*
11	*Chikanni*	7	10 Bigha	Inter-crop with guar & bajra	48	40	*H. cajani*
12	*Naugawa*	5	6 Bigha	Inter-crop with guar & bajra	40	45	*H. cajani*

Hasseb (2002) who conducted survey of farmer's fields, growing pigeonpea at different localities in Agra, Aligarh, Bulandshahar, Hathras and Mathura district.

Per cent loss in yield was maximum in Thanagazi (92%) followed by Beejwa (85%), Tuleda (76%) and minimum with 30% in Jajore. The cyst nematode feeds on other leguminous crops in mixed cropping and availability of wide variety of collateral host makes them predominant and results in increase in highest cyst population and attributes to multiplying the pathogenic threshold by manifolds and caused heavy losses to the crops. In Rajasthan, Arya (1957) reported a root-knot species *Meloidogyne* from Jodhpur district. Yadav *et al*, (1971) reported infection of *H. cajani* on sesame in Rajasthan. Saxena and Reddy (1987) noted crop losses in pigeonpea and mungbean by *H. cajani* and found greater loss in mungbean (67%) than the pigeonpea (30%) under similar cyst population. Climatic factors like sandy loam soil, high pH level (7-9.2); poor soil fertility etc. might be responsible for enhancement of *H. cajani* infection (Filder and Beaven, 1963; Williams and Beane, 1972). Wallace (1966) observed that better aeration and available oxygen were important factors, affecting *Heterodera schactii* population in soil. In the present study, the agroclimatic conditions and soil texture of Alwar region was favorable for both, the crop as well as parasitizing nematodes. Swarup and Sethi (1977) and Nama and Tikyani (1977) also reported various phytonematodes viz. *Tylenchorhynchus*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne* and *Xiphinema* and other from Rajasthan and found them associated with various trees, leguminous, graminacious and solanaceous crops. Pandey (2001) observed the occurrence of cyst nematode (*Heterodera* 60.28%), root knot nematode (*Meloidogyne* 69.8%), spiral nematode (*Helicotylenchus* 62.6%), lance nematode (*Hoplolaimus* 55.5%), root lesion nematode (*Pratylenchus* 68.5%), dagger nematode (*Xiphinema* 71.8%) reniform nematode (*Rotylenchus* 42%) and saprozoic nematode encountered were 100% in and around Jaipur region. Survey of phytonema populations associated with rhisosphere of sesame crop (Sharma and Trivedi, 1994) and Pandey *et al*, (2003) on cowpea, Jaipur, Rajasthan, India supported our findings. Baqri (2000) reported about 142 species of plant and soil nematodes from Rajasthan including 21 species as new records from the state Rajasthan. In conclusion, it could be said that phytoparasitic nematodes on pigeonpea constitute a very important and significant part of the soil fauna of Rajasthan and cause a serious problem to pigeonpea fields and other leguminous crops in Alwar region.

Pathogenecity Test

In the pathogenecity test study revealed that with an increase in inoculum level of cyst nematode from 100-10,000 there was significant suppression of plant growth attributes like fresh and dry weight of root and shoot and number of nodules (Table-2). Stunted growth, reduced pod yield with yellowing of leaves was marked with increase in inoculum level. The highest inoculum level of 10,000 larvae caused 64.82% reduction in shoot length and 61.98%, 47.25% 30.55% reduction was recorded at 1000, 100, 10 larval levels. The difference between treatments were found statistically significant. Similarly the highest inoculum level of 10,000 larvae caused 67.30% reduction in dry weight of shoot and 61.90%, 42.85%, 33.96% reduction were recorded at 1000, 100, 10 larval levels. These results are in agreement with those reported by Zaki and Bhatti (1986) on pigeonpea and moth bean and by Bhagwati and Phukan (1991) on pea infected by *Meloidogyne incognita*. Contrary to these Thakar and Patel (1984) found significant damage at 10 and 100 larvae/plants, whereas 5000 larvae/plant in guar was found to cause drastic damage by (Walia and Bhatti, 1988).

Results indicates increase in plant growth at 10 larvae/kg soil as compared to uninoculated plant which might be due to sensitization of plant leading to more lateral roots at foci of infections in response to attack of low nematode number which facilitate greater water and nutrient uptake of roots as compared to heavily infected plant roots. A significant reduction in nodules as compared to control were noted at an initial inoculum level of 100 juveniles/plant which is considered to be a damaging threshold level of nodulation on pigeonpea (Table-2). This is supported by the work of Gupta and Yadav (1979) on urad infected with *Rotylenchus reniformis* and Mishra and Guar (1981) on black gram by *R. reniformis*. Observations on nematode

Table 2 : Effect of inoculum level of *H. cajani* on pigeonpea (*Cajanus cajan* L.) after 90 days of inoculation

S. No.	Inoculum levels	Length		Fresh wt.		Dry wt.		Number of nodule	Number of cysts /root	Number of Eggs/cysts
		Shoot	Root	Shoot	Root	Shoot	Root			
1	0	136.8	124	78	54	15.75	10.8	65	0	0
2	100	95	75.3	52	30	10.4	5.75	38.8	75	62.34
3	500	72.15	68.2	45.12	25.1	9	5.15	30.15	100.25	70
4	1000	52	36.5	30.45	10.4	6	2.62	25.54	125.75	85.15
5	5000	48.12	32	25.55	8	5.15	2.02	22.15	256.05	110
CD@1%		6.73	5.35	4.93	3.74	2.51	1.33	3.68	7.45	7.25
CD@5%		4.73	3.76	3.47	2.63	1.76	0.93	2.59	5.24	5.1
CV		3.22	3.08	4.13	5.68	10.49	0.79	3.92	2.58	4.28

multiplication showed dose dependency maximum multiplication at highest inoculum level and minimum at lowest inoculum level. These results are in agreement with findings Shahina and Maqbool (1990) on barley and maize infected by *Heterodera* spp.

The rate of population increase (R.P.I.) or the number of eggs/cyst was inversely correlated with initial population densities; as the initial inoculum increased, RPI decreased (Table-2) which might be due to competition among nematode population for food and space on host plant. Lesser the nematode lesser the competition and greater rate of multiplication or population increase. These findings are corroborated by Haque and Hesling (1958) and Gokte and Swarup (1984).

Biology of *H. cajani* on pigeonpea

Penetration of *H. cajani* juveniles in pigeonpea roots was observed within 12 hour of inoculation, which increased gradually with the progress of time till 4^{th} day. After penetration, juveniles oriented themselves at first parallel to the stelar region and then by adjusting their heads perpendicular to the stele. Moulting of 2^{nd} stage juveniles was initiated after 4^{th} day of inoculation and on 9^{th} day fourth stage juveniles were formed. Further, swelling in next 3 to 4 days resulted in cavity formation and rupturing of the cortical tissue, enabling the white females to protrude out the root with neck embedded in the stelar region. Adult male was observed on 11^{th} day while adult female was observed on 12^{th} days of inoculation, where it took 5 days to become egg laying female. Further on 28^{th} day light brown cysts were formed which after 6 days forming hard cyst. It was observed that the nematode took 34 days for completion of one life-cycle on pigeonpea at 25.5+0.5°C (Table-3). Similar observations were made by Koshy and Swarup (1971a) on second, third and fourth moult in female on pigeonpea. The life cycle of *H. cajani* on various other hosts has been studied by several workers. It thrives best and multiplies quickly in high temperature. Observations of Koshy and Swarup (1971b) on pigeonpea at a temperature regime of 29°C showed that *H. cajani* completed its life cycle in 16 days where as, it took 45-80 days at 10-25°C. In the present study, the emergence of second stage juveniles from egg on 17^{th} day was supported with the findings of biology of *H. cajani* on blackgram by Senthamizh *et al.* (2005). Gupta and Edward (1973) reported the completion of *H. vigni* life cycle in seventeen days on cowpea. Yadav and Walia (1988) reported that the nematode took 23 days for completion of one life cycle on pigeonpea, daincha, moth bean and sesame, 26 days on green gram and 27 days on black gram and cowpea. These differences in life cycle were probably due to the effect by host and prevailing temperature conditions.

Table 3 : Life cycle of *Hetrodera cajani* in pigeonpea.

S. No.	Penetration & Development	Days after Inoculation
1	Penetration initiated	Within 12 hrs4
2	Moulting 2nd stage larvae	4
3	Moulting third-stage larvae	7-8
4	Fourth stage larvae	9
5	Adult male	11
6	Adult female	12
7	Deposition of eggs in egg-sac	16-17
8	Emergence of second generation larvae	18
9	Brown Yellow cyst	28
10	Brown cyst	34

Table 4 : Screening of some pigeonpea cultivars against *Heterodera cajani*

S. No.	Treatments	Length		Fresh weight		Dry weight		Number of nodules/	Number of cysts/	Number of eggs/cysts	Reaction
		Shoot	Root	Shoot	Root	Shoot	Root				
1	ASJ-118	112	89.7	61.07	38.04	12.2	7.57	52	28.76	150.2	S
2	ASJ-112	110	82.3	60.96	36.2	2.1	7.16	50.22	30.45	152.34	S
3	ASJ-102	100.8	85.3	55.92	32.28	1.16	6.45	48.55	34.74	154.46	S
4	ASJ-117	92	80.3	55.27	28.88	11.03	5.76	46	38.12	158.55	S
5	ASJ-127	90	66	52.14	26.97	10.65	5.42	40.45	40.55	162.76	S
6	ASJ-114	82	80.7	48	22.88	9.75	4.42	38.86	46.84	166.49	S
7	ASJ-121	66	62	36.67	18.19	7.25	3.88	36	50.06	160.28	S
8	ASJ-125	59	33	33.55	14.9	6.78	2.96	31.56	60.36	165.36	HS
9	ASJ-113	50	29	28.68	12.24	5.73	2.44	30.14	80	169	HS
10	ASJ-124	45	16.8	25.79	10	5.15	2	28.65	100.42	170.26	HS
11	ASJ-122	42.44	23.5	23.67	O9.48	4.76	1.9	26.48	120.62	172.04	HS
11	Control	138.1	124	81.75	52.76	16.25	10.4	65	0	0	0

ASJ- = pigeonpea cultivars , Heterodera cajani , S = Susceptible , HS = Highly susceptible

Varietal Screening Test

On the basis of number of cysts the eleven tested varieties were categorized as susceptible and highly susceptible according to resistance rating scale. Maximum number of cysts (120.62) were found in ASJ-122 followed by ASJ-124(100.42), ASJ-113 (80.00), ASJ-125(60.36), ASJ-121(50.06), ASJ-114(46.84), ASJ-127(40.55), ASJ-117(38.12), ASJ-102(34.74) and ASJ-112(30.45) while least number of cysts were produced in ASJ-118 (28.76). None of cysts was formed in control plant. Out of eleven varieties seven varieties recorded as susceptible (26-50 cysts) and rest four was highly susceptible (more than 50 cysts). The results indicate that the accessions of evaluated pigeonpea, was susceptible to varying degree. Hence none of cultivars were immune to *H. cajani*. Our findings were supported with the study of Patel and Patel (1999), wherein screening study none of the cowpea cultivar were resistant to *M. incognita* and *M. javanica*.

Kalairasan *et al*, (2006,2007) studied the pulse crop (pigeonpea, cowpea, blackgram and green gram) genotypes for resistance against *H. cajani* and in case of pigeonpea out of 15 genotypes tested along with Co6 as susceptible check, only the VBN-1 with 25 cysts/plant was found to be moderately resistant while rests were highly susceptible.

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