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PATHOGENICITY, LIFE CYCLE AND MORPHOMETRICS OF VARIOUS DEVELOPMENTAL STAGES OF *HETERODERA CAJANI* ON SUSCEPTIBLE AND RESISTANT CULTIVAR OF COWPEA

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Studies were undertaken for the pathogenicity test, life cycle and morphometric of pigeon pea cyst nematode *Heterodera cajani,* in susceptible (RC-19) and resistant cultivar (FTC-27) of cowpea. To ascertain the influence of population levels viz. 0, 10, 100,1000, 10,000 of *H. cajani* juveniles on plant growth, nodulation and cyst nematode multiplication on cowpea susceptible variety; marginal damaging threshold level of *Heterodera cajani* was recorded at 100 juveniles/Kg of soil. A negative correlation was found between the reproduction factor and inoculum density *i.e.* as inoculum level increased the reproduction factor decreased. In penetration studies active J_2S penetrated within 24 hrs and 32 hrs, respectively in RC-19 (susceptible) and FTC -27 (resistant) cultivar. Life cycle studies showed that the time taken for completion of one generation of nematode was 18 and 24 days in RC-19 and FTC-27 varieties, respectively at 29 ± 1°C. Delayed moulting of various developmental stages was observed in FTC-27 variety. Morphometric measurement of different developmental stages showed a comparative reduced size of various stages of nematodes in resistant cultivar compared to susceptible one.

Keywords : *Heterodera cajani;* Nodulation Population level; Pigeonpea cyst nematode.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important pulse crop grown widely in poorly rainfed area of Rajasthan state, India. Plants are used as feed for livestock and also valued as cover or green manure crop. Cowpea leaves are high in protein content and rich in calcium, iron, phosphorus, zinc, carotene, ascorbic acid and folic acid[1]. Cowpea is usually raised as dry land summer crop and can also be grown as pre monsoon and late monsoon crop.. The pigeon pea cyst nematode *Heterodera cajani* is a serious menace to cowpea in the state of Rajasthan, India. Farmers experience heavy economic losses due to this nematode every year.

Several workers have carried out inoculum level studies of various pathogenic nematodes on various crops to express an exact economic threshold level at or beyond which crop suffers heavy economic losses[2-4]. A considerable amount of work has been done on various factors affecting the life cycle duration of *Heterodera* spp. on various plant hosts[5-8]. Literature survey revealed that basic studies regarding pathogenicity test, life cycle etc are lacking on cowpea plant; which suffers heavy losses due to *H. cajani.* The objective of the present investigation was to study the pathogenicity and initial inoculum level of *H. cajani* on susceptible cowpea cultivar and also to study the life cycle and morphometrics of various developmental stages of *H. cajani* on susceptible and resistant cultivar of cowpea.

Material and Methods

(a) *Effect of inoculum level on pathogenicity* -One week old seedlings of cowpea susceptible variety RC-19 were grown in 15 cm diameter earthen pots containing 1 kg autoclaved soil. The seedlings were inoculated in a log series of 0, 10, 100, 1000, 10,000 active second stage juveniles (J_2S) of pigeon pea cyst nematode *Heterodera cajani.* Each treatment was replicated five times. Pots were set in randomised block design to eliminate the effect of sun and shade after proper tagging.

Observations were recorded after 60 days of inoculation. The effect of treatments on vegetative growth of plants was recorded in terms of fresh and dry weight and effect on nodulation. Similarly the effect of different inocula on nematode multiplication was estimated by counting the number of cysts per plant. For counting the number of eggs/ cyst, cyst were crushed in water to get suspension. Total number of eggs present in suspension were counted by using counting dish under stereomicroscope.

Final cyst population per kg soil was calculated by extracting and counting the cysts by simple floatation and visual technique followed by hand picking by brush.

(b) *Penetration, life cycle and morphometric study* - Penetration study was carried out in 10 cm plastic cups containing autoclaved soil + sand (3:1) mixture. Seeds of

two cowpea varieties RC-19 (susceptible) and FTC-27 (resistant) were sown separately in these plastic cups. Two different sets were replicated thrice. One week old seedlings of cowpea were thinned one plant per cup and was inoculated with 100 freshly hatched juveniles of *Heterodera cajani*. Plants were uprooted carefully upto emergence of 2nd stage juveniles of next generation. The time of penetration of larvae in roots of two cultivar was noted and number of juveniles penetrated in the root system were counted for calculating the per-cent penetration.

The life cycle of nematode was studied in 15 cm diameter earthen pots. Seeds of two cowpea varieties were surface sterilized in 0.1% mercuric chloride and were sown in autoclaved soil+sand (3:1) mixture in pots. At two leaf stage seedlings were inoculated with five hundred freshly hatched juveniles of *H. cajani* per pot. Observations were taken initially on every day up to seven days and finally after 2 days interval. Root system of three plants from each variety were examined after staining with 0.1 % acid fuchsin in lactophenol under stereobinocular microscope. The number and day of moulting of various stages of juveniles up to ovipositing white female or adult male was noted. The atmospheric temperature ranged between 29±1°C during the study. Morphometric measurements of the different stages of the pigeon pea cyst nematode were recorded. For staining the nematode infected root tissue method[9] was followed.

Results and Discussion

Effect of inoculum level on pathogenicity-Pathogenicity 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 1). These results are in agreement with those reported on pigeon pea and moth bean and on pea infected by *Meloidogyne incognita*[10,11]. On contrary, a significant damage at 10 and 100 larvae/plant[12], whereas 5000 larvae/plant in guar was found to cause drastic damage[13].

Table 1 indicates a slight increase in plant growth at 10 larvae/kg soil as compared to uninoculated plant which might be due to sensitisation 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 cowpea (Table 1). This is supported by the work on urad infected with *Rotylenchulus reniformis* and on black gram by *R. reniformis*[14,15].

Observation on nematode multiplication from Table 1 showed a maximum nematode multiplication at highest inoculum level and minimum at lowest inoculum level. These results are in agreement with on barley and maize infected by *Heterodera* spp.[16,17].

The rate of population increase (R.P.I) or the number of eggs/cyst was negatively correlated with initial population densities. As the initial inoculum increased RPI decreased (Table 1) 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[18,19].

Penetration, lifecycle and morphometric study- The penetration and life cycle study of Heterodera cajani as from Table 2 showed that active second stage juveniles J_2S penetrated the roots of susceptible (RC-19) variety within 24 hours of inoculation while in roots of resistant variety FTC-27 they took 32 hours to penetrate (Table 2). Reduction in per cent penetration (10-20%) was observed in resistant variety, FTC-27 as compared to susceptible, RC-19 variety where it was 55-60%. Similar findings in the difference in penetration percentage of *Heterodera avenae* larvae between the susceptible and resistant variety of barley was reported[19,20]. Poor penetration in resistant variety might be due to lack of attractant in the root exudates of FTC-27 variety as compared to susceptible one. Root exudates of resistant variety might be antagonistic to the juveniles of *Heterodera cajani* making them inactive.

Observation from Table 2 revealed that total time required by *H. cajani* to complete one generation at an average soil temperature 29 ± 1°C was eighteen and twenty four days in RC-19 and FTC-27 varieties, respectively. Similar findings were observed where *H. cajani* life cycle was completed in 16 days on pigeon pea[5].

The average number of cysts, their size and cyst content were also found to be higher in case of susceptible variety as compared to the resistant one. A possible mechanism of disease resistance of cowpea to *H. cajani* may be due to hypersensitivity reaction where a rapid and localized necrosis of plant cell at the site of infection limit the multiplication and spread of invading organism. This reaction has been supported[21].

Morphometric studies from Table 3 revealed reduction in size of different stages of life cycle in resistant variety compared to susceptible one, which also supports the resistance nature of variety.

Based from the present findings it is clear that 100 J_2S/plant is an economic threshold point of pigeon pea cyst nematode, *H. cajani* on cowpea and this inoculum level cause considerable losses to the host plant. Pathogenicity test also gives conclusive evidence of the pathogenic potentiality of *H. cajani* on cowpea. In the life cycle study on resistant and susceptible variety it is clear that *H. cajani* completes many generations in one growing season of cowpea and a delayed life cycle completion was observed in resistant variety and similarly a marked reduction in size of various developmental stages

Table 1. Effect of inoculum levels of *Heterodera cajani* on cowpea growth parameters.

Inoculum level	Plant Growth Characters				Number of nodules	Nematode Reproduction		
	Fresh weight (g)		Dry weight (g)			Number of cyst/root	No. of eggs/cyst	Final cyst population
	Shoot	Root	Shoot	Root				
0	37.8	7.34	5.85	1.62	34	0 (1)	0 (1)	–
10	39.04	7.58	6.23	1.70	31	21.3 (4.68)	81.2 (9.06)	101.8
100	31.62	6.0	4.03	1.42	25	180.6 (13.47)	75 (8.71)	1862.9
1,000	22.8	3.61	3.7	0.51	18	697 (26.41)	61.1 (7.87)	4768
10,000	18.4	2.15	2.53	0.36	11	745 (27.30)	42.1 (6.56)	5420
SEM ±	1.0382	1.1618	0.3829	0.0258	1.3170	2.3476 (0.4427)	1.2293 (0.4281)	-
CD at 5%	2.6692	2.9869	0.9844	0.0663	3.3860	6.0356 (1.1381)	3.1605 (1.1006)	-

Observations are mean of five replicates.

Table 2. Life cycle of *Heterodera cajani* in susceptible and resistant cultivar of cowpea.

Penetration & Development	Days after Inoculation	
	Susceptible cultivar RC-19	Resistant cultivar FTC-27
Penetration time	24 hrs	32 hrs
Per-cent Penetration	55-60%	10-20%
Moulting 2nd stage larvae	3	4
Early third stage	6	7
Third stage male	8	11
Third stage female	9	12
Fourth stage male	10	14
Fourth stage female	12	16
Adult male	13	17
Adult female with posterior egg sac	15	21
Emergence of 2nd stage larvae	18	24
Yellow cyst	29	32
Brown cyst	40	45

of *H. cajani* was observed in resistant FTC- 27 variety as compared to the susceptible RC-19 variety. Conclusively, growing resistant cowpea variety is the most effective, economical and easy method for reducing *H. cajani* population in field.

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Table 3. Morphometrics of various developmental stages of *Heterodera cajani* in susceptible and resistant cultivar of cowpea.

Stages	Susceptible (RC-19) Length × Width (µm)	Resistant (FTC-27) Length × Width (µm)
2nd stage juvenile	422-503 × 15.3-25.0 (415 ×21)	412-510 × 14.2-23 (414.5 × 21)
Early third stage larvae	334-350 × 50-60 (349 × 55.5)	312-334 × 41-57 (323 × 49)
Late third stage male	354-385 × 57-98 (363 × 74.2)	315-337 × 50-65 (325 × 52)
Late third stage female	352-373.5 × 60-85 (353.1 × 66.5)	300-353 × 52-73 (323.5 × 66)
Fourth stage male	315-356 × 63-91.2 (334.5 × 71)	285-341 × 58-73 (314 × 57.2)
Fourth stage female	363-412.3 × 112-155 (387 × 136.5)	299-413 × 101-143.5 (343 × 109.1)
Adult male	945-1293 × 30-32 (1098.5 × 31.5)	840-1091 × 27-30.2 (998.3 × 29.2)
Adult female/cyst	670.1-805 × 463-558.3 (734.0 × 521.6)	435.3-690 × 373-490 (569 × 432.5)
Egg	103.5-135 × 50-67 (118.5 × 59.5)	97-115 × 45-64.7 (105 × 52.3)

Figure in parenthesis are mean of five replicates.

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