PATHOLOGIC REACTION OF GARDEN PEA (*Pisum sativum* L.) CULTIVARS/ADVANCED BREEDING LINES TO ROOT-KNOT NEMATODE, *Meloidogyne incognita* (Kofoid and White)Chitwood [1]

J.R.P.Dimaguiba [2] ***and L. M. Villanueva*** [3]

[1] A portion of the Ph.D. dissertation of the senior author

[2] Part time faculty member and Ph.D. Biology student, Saint Louis University, Baguio City and [3]Chair , Graduate Advisory Committee and Professor VI, Benguet State University, La Trinidad, Benguet

ABSTRACT

Pot experiments were carried out under greenhouse conditions to evaluate the reactionsof eight garden pea cultivars/ advanced breeding lines to *Meloidogyne incognita*. Seeds were sown in pots containing heat-sterilized soil.After three weeks when the root system has been fully established, the seedlings were inoculated with 1,500 freshly hatched *M. incognita* juveniles (J2s) per pot. Each garden pea cultivar/ advanced breeding line was replicated four times and arranged in Completely Randomized Design. The effect of nematode inoculation on plant height, fresh and dry weights of shoots and roots and number and weight of pods were assessed sixty days after planting. Likewise, the roots were also examined for the presence of galls and egg masses including the number of developmental stages of the nematode.

Based on the root knot gall and egg mass indices, no cultivar/ advanced breeding linewas found to exhibit resistance to *M. incognita*. Advanced breeding lines Chinese Green Pea (CGP) 59, CGP 11, CGP 13, and Chinese Light Green (CLG) were highly susceptible while CGP 110, CGP 34, CGP 154, and Betag were susceptible to the root knot nematode.

Keywords: resistance, inoculation, root galls, eggmasses, plant parameters

INTRODUCTION

Garden pea (*Pisum sativum* L.) is one of the most important legume vegetables in Benguet and Mt. Province. Like other legumes, garden pea is high in protein and carbohaydrates. It is also the most profitable crop to grow. However, garden pea is very susceptible to root knot nematodes, *Meloidogyne incognita*, one of the most economically damaging genera of plant parasitic nematodes attacking horticultural and field crops (Davide, 1988). They are distributed worldwide and are obligate parasites of the roots of thousands of plant species including monocotyledonous and dicotyledonous herbaceous and woody plants. *M. incognita* is more serious on vegetable crops such as tomato, okra, and celery and on fiber crops such as ramie. The nematode has been reported to cause severe yield losses of up to 20-33% (Upadhyay and Dwivedi, 1987). *M. incognita* larva infects plant roots causing the development of root galls that drain the plant of nutrients. Nematode infection in young plants may be lethal while infection of mature plants causes decrease in yield.

The use of resistant crops is one of the most effective management tools that improves crop yield in the presence of nematode population densities that exceed the damage threshold. Because resistance to nematodes is usually developed by selection of plants with reducedrates of nematode reproduction, nematode population densities are typically lower following a resistant cultivar than a susceptible cultivar. However, this is not always the case if the crop has only partial resistance (Verma and Gupta , 1993).

The crop cultivars showing high degree of resistance with all susceptible agronomic features are commonly recommended for nematode infested fields either as a routine crop or in a rotational sequence of the crops. Therefore, the growing of resistant varieties against the target nematode species demands correct identity of nematodes with prevailing races existing in the area (Khan, 2008).

Niblack et al. (1986) demonstrated that at moderate to high initial population densities, *M. incognita* reach their maximum levels at about 90 days after planting on a susceptible soybean cultivar presumably due to extensive damage to the host, whereas on partially resistant cultivarsthat were less severely damaged by the nematodes, the population densities were still increasing at 120 days after planting.

Although resistance to root knot nematodes is available in several crop species including *P. sativum* (Verma and Gupta, 1993), new sources of resistance are needed to improve the level of root knot resistance.

Over the years, efforts have been exerted to evaluate crops for their resistance to *Meloidogyne* in controlled and field experiments (Cardwell and (Ingham, 1997, Agdebiteet al. 2005;Rehman et al.; 2006,Dong et al. 2007;Khan, 2009)The study was undertaken to evaluate the resistance of eight garden pea cultivars/ advanced breeding lines to root-knot nematode, *M. incognita* under greenhouse conditions.

MATERIALS AND METHODS

Plant Materials

Eight garden pea cultivars/advanced breeding lines were used in the experiment. Two cultivars: Betag and Chinese Light-green (CLG) and six advanced breeding lines, Chinese Garden Pea (CGP) 59, CGP 11, CGP 110, CGP 34, CGP 154 and CGP 13were included. Garden pea seeds were provided by the Highland Crops Research Station, Institute of Plant Breeding, Benguet State University, La Trinidad, Benguet.

Preparation of *M. incognita* Inocula

M. incognita from garden pea was cultured in tomato (*Lycopersicon esculentum*). Eggs were extracted from tomato plant roots by agitating in 1% NaOCl solution for 4 minutes (Hussey and Barker, 1973). The eggs were collected and rinsed with tap water on nested 325 and 500µm-pore sieves. The egg suspension was collected and poured over blue sieves lined with tissue paper. The *M. incognita* J2s were collected after 2 days of incubation. One thousand five hundred freshly-hatched *Mi* J2s were inoculated in each potted plant three weeks after the establishment of the root system.

Evaluation of Resistance in Garden Pea Cultivars/ Advanced Breeding Lines against M. incognita

Seeds of the different varieties/advanced breeding lines of garden pea were sown in 7-inch diameter polyvinyl chloride (PVC) plastic pots containing 2kg heat-sterilizedsandy loam soil. Each pot was placed above a plastic plate to contain the inoculated nematodes and to prevent contamination of the neighboring test plants. The plants were arranged randomly in the greenhouse using the Completely Randomized Design with four replications. Uninoculated pot for each cultivar served as control for that particular cultivar/ breeding line. The pots were watered daily. Ninety days after inoculation the plants were carefully uprooted and the following data were recorded: plant height, fresh and dry weights of shoots and roots, and the number and weight of pods.

A root gall scoring scale of 1-5 was used to measure resistance (Coyne and Claudius-Cole, 2007), where 1- no galling (0 galls/ egg masses)= highly resistant, 2- slight galling (1-10 galls/ egg masses) = resistant, 3- mild galling (11-30 galls/ egg masses) moderately resistant, 4- moderate galling (31-100 galls/ egg masses)- susceptible, 5- severe galling (above 100 galls/ egg masses)-highly susceptible.

Nematode reproduction was also taken by determining the reproductive factor (Rf) based on the criteria of Robinson (1980) using the formula Rf=Pf/ Pi, where: Rf=reproductive factor, Pi= initial nematode population and Pf= final nematode population. Non- hosts or non-efficient host plants had a reproductive value less than 1 while Rf value of more than 1 is considered as efficient host of root-knot nematode.Roots were collected and the number of root galls and egg masses were counted using a hand tally counter. The same roots (1 g/ plant) were stained using the McCormic Red Food Color (RFC) staining method (Theiset al., 2002). About 200 grams soil samples per plant were also assessed for the presence of *M.incognita* by using the modified Baermann tray technique. The number of developing stages of the nematode (J2, J3-J4 and adult females) in the roots was counted under a stereoscopic microscope.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and multiple comparison using Bonferroni and Duncan's Multiple Range Test (DMRT) where the differences among treatments were separated at 1% and 5% level of significance. Data on nematode population estimates were transformed to normalize variances: square root or log transformationfor variables measured using nematode counts while arcsin transformation was used for data expressed in percentage.

RESULTS AND DISCUSSION

Effect of *M. incognita* Inoculation on Plant Growth

Regardless of garden pea cultivars/advanced breeding lines tested, inoculation of *M.incognita* resulted in significant reduction in plant height as well as on fresh and dry weights of shoots and roots (Figure 1). On the other hand, nematode inoculation significantly increased the pod weight. Significant reduction in plant height was noted in CGP 11 (Table 1). Although not significant, inoculated CGP 110, CGP 154, and Betag were much taller than the uninoculated ones. In terms of fresh shoot weights, significant reduction was recorded in inoculated CGP 59, CGP 11, CGP 34, Betag and CLG. Likewise, reduction in the dry shoot weight of CGP 59, CGP 11, and CGP 34 was also noted. However, inoculation of *M. incognita* resulted in significant increase in dry shoot weight in cultivar Betag. On the other hand, plants inoculated with root-knot nematode gave significantly lower fresh root weight in CGP 59, CGP 110, and CLG with percentage reduction of 42.0, 42.7, and 54.3, respectively (Fig.3). In terms of dry root weight, only CGP 154 gave significant reduction with 61.9%. No significant effect was noted in nematode-inoculated and uninoculated plants on the number of pods of the different cultivars/ advanced breeding lines of garden pea. Interestingly, inoculation of root-knot nematode in CGP 11, CGP 110, CGP 154, CGP 13, and cultivar Betag significantly increased the pod weight. On the other hand, significant reduction in pod weight was observed in garden pea cultivar CLG.

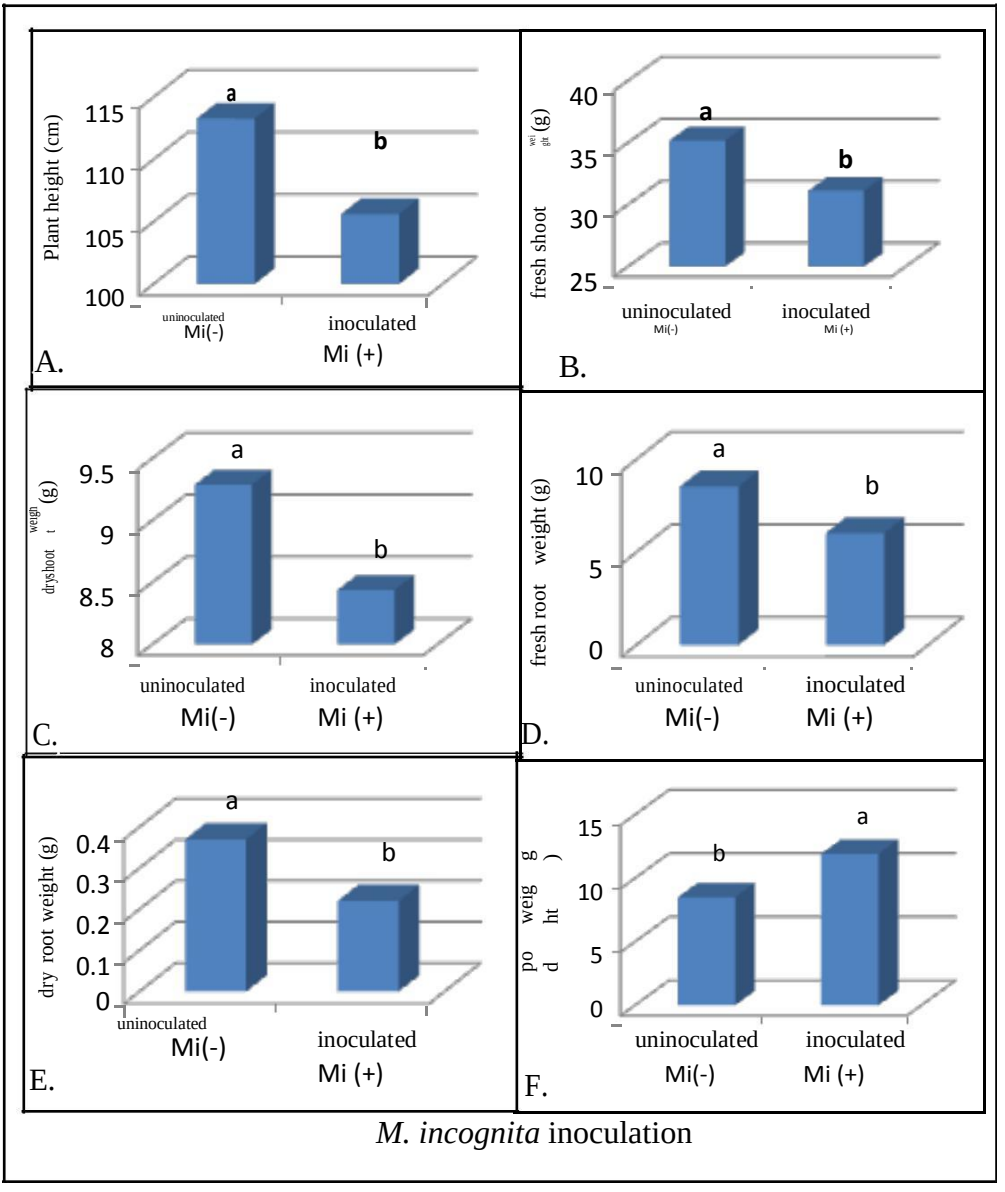

Fig. 1. Main effect of *M. incognita* inoculation on: a) plant height b) fresh shoot weight, c) dry shoot weight d) fresh root weight e) dry root weight and f) weight of pods

Table 1. Summary of the effect of *M. incognita* on plant growth of eight garden pea varieties/advanced breeding lines 60 days after inoculation of 1500 J2/plant

Var/ ABLge	PLANT PARAMETERS: Plant height (cm) Mi-	Plant height (cm) Mi +	Plant height (cm) %change	Fresh shoot weight (g) Mi-	Fresh shoot weight (g) Mi+	Fresh shoot weight (g) %change	Dry shoot weight (g) Mi-	Dry shoot weight (g) Mi+	Dry shoot weight (g) %change	Fresh root weight (g) Mi-	Fresh root weight (g) Mi +	Fresh root weight (g) %change
1. 59	109.50^{bcde}	89.00^{def}	(-)18.7	46.45^{c}	34.21^{cd}	(-)26.5	13.33^{a}	7.80^{de}	(-)41.5	9.32^{abc}	5.41^{e}	(-)42.0
2. 11	130.75^{b}	102.25^{cde}	(-)21.8	40.00^{bc}	24.61^{e}	(-)38.5	11.19^{a}	8.47^{cde}	(-)24.3	8.95^{abcd}	6.20^{cde}	(-)30.7
3. 110	109.75^{bcde}	115.50^{bc}	(+) 5.2	31.53^{de}	23.70^{e}	(-)24.8	8.54^{cde}	9.86^{bcd}	(-)15.5	8.93^{abcd}	5.12^{e}	(-)42.7
4. 34	114.50^{bc}	111.50^{bcd}	(-) 2.6	43.69^{b}	30.38^{de}	(-)30.5	10.74^{bc}	7.68^{de}	(-)28.5	6.08^{cde}	5.80^{de}	(-) 4.6
5. 154	100.75^{cde}	105.00^{cde}	(+) 4.2	28.58^{de}	30.06^{de}	(+) 5.2	7.62^{de}	7.62^{de}	0	9.10^{abc}	7.14b^{cde}	(-)21.5
6. 13	103.25^{cde}	87.50^{er}	(-)15.3	26.67^{de}	25.54^{de}	(-) 4.2	7.24^{de}	6.05^{e}	(-)16.4	4.80^{e}	4.88^{e}	(+) 1.6
7.Betag	163.25^{a}	165.38^{a}	(+) 1.3	46.45$_{b}$	55.99^{a}	(-)20.5	7.68^{de}	13.77^{a}	(+)44.2	11.22^{a}	9.18^{abc}	(-)18.2
8. CLG	74.50^{e}	69.25^{e}	(-) 7.0	33.71^{cd}	24.45^{e}	(-)27.5	7.55^{de}	5.82^{e}	(-)22.9	9.94^{ab}	4.54^{fg}	(-)54.3

Var/ ABLge	Dry root weight (g) Mi-	Dry root weight (g) Mi+	Dry root weight (g) %change	# of pods Mi-	# of pods Mi+	# of pods %change	Weight of pods (g) Mi-	Weight of pods (g) Mi +	Weight of pods (g) %change
1. 59	0.25^{cde}	0.09^{g}	(-)64.0	2.86^{ab}	2.61^{ab}	(-)8.7	11.50^{bc}	7.75^{cde}	(-)32.6
2. 11	0.23^{cde}	0.23^{cde}	0	2.59^{ab}	2.61^{ab}	(+)0.77	4.25^{e}	9.75^{bcd}	(+)129.4
3. 110	0.37^{abcd}	0.20^{de}	(-)46.0	2.82^{ab}	2.95^{a}	(+)4.7	8.50^{cde}	14.50^{ab}	(+)70.6
4. 34	0.37^{abcd}	0.13^{de}	(-)64.7	2.88^{a}	2.71^{ab}	(-) 5.9	10.00^{bcd}	11.50^{bc}	(-)15.0
5. 154	0.42^{abc}	0.16^{de}	(-)61.9	1.94^{b}	2.81^{ab}	(+)44.8	5.50^{de}	14.25^{ab}	(+)159.1
6. 13	0.40^{abcd}	0.19^{de}	(-)52.5	2.64^{ab}	2.99^{a}	(+)13.3	4.75^{de}	13.00^{bc}	(+)173.7
7.Betag	0.54^{ab}	0.63^{a}	(+)16.7	2.87^{ab}	3.00^{a}	(+)4.5	11.50^{bc}	18.75^{a}	(+)63.0
8. CLG	0.36^{bcde}	0.15^{de}	(-)58.3	2.54^{ab}	2.43^{ab}	(-)4.3	12.00^{bc}	5.50^{de}	(-)54.2

Data are means of 4 replicates and transformed to the √x+.5 prior to statistical analysis. Means of the same letter do not differ significantly at P>0.05 according to Bonferroni and DMRT.

Fig. 2. Roots of garden pea cultivars at 60 days after *M.incognita Mi* inoculation. Left: dense root sytstem of Betag (susceptible variety) Right: sparse and galled roots of CLG (highly succeptible variety)

Nematode Parameters

The different garden pea cultivars/advanced breeding lines exhibited various reactions to nematode inoculation (Table 2). Apparently, garden pea cultivar CLG favored the growth and development of *M. incognita* having the highest total number of nematodes in the roots. This was followed by CGP 59, CGP 13, Betag, CGP 154, and CGP 11. The lowest nematode population was noted in CGP 110. Using the gall and egg mass indices(Coyne and Claudius-Cole, 2007),CGP 59, CGP 11, CGP 13, and CLG were categorized as highly susceptible and CGP 110, CGP 34, CGP 154 and Betagas susceptible to M. incognita infection. On the other hand, based on the criteria of Robinson (1980) all garden pea cultivars/advanced breeding lines are efficient hosts of *M. incognita.* (Figure 3).

Table 2. Number of developmental stages, galls and egg masses of *M. incognita* in the soil and roots 60 days after inoculation with 1500 J2s/plant

	Nematode parameters										
Var/ ABL (CGP)	**Mi in soil[1]**	**J2s in roots[1]**	**J3-J4[1]**	**Adult f.[1]**	**Total # of *Mi (soil & roots)***	**# of galls[1]**	**RKI[2]**	**# of egg masses[1]**	**EMI[3]**	***Rf*[4]**	**Pathologic reaction (PR)[5]**
1. 59	0.60^{b}	0.00^{b}	39.50^{bc}	82.50^{b}	4178	9.59^{c}	2.18^{b}	7.95^{c}	2.12^{c}	2.78	highly susceptible
2. 11	0.60^{b}	0.00^{b}	40.00^{bc}	47.00^{c}	2146	11.89^{b}	2.35^{a}	8.37^{bc}	2.12^{c}	1.43	highly susceptible
3. 110	0.60^{b}	0.00^{b}	43.50^{bc}	40.00^{c}	1983	7.98^{d}	2.12^{b}	4.00^{d}	1.87^{d}	1.32	susceptible
4. 34	14.40^{a}	0.00^{b}	36.75^{bc}	32.75^{cd}	2220	10.13^{c}	2.23^{ab}	9.69^{b}	2.23^{b}	1.41	susceptible
5. 154	6.80^{ab}	0.00^{b}	43.50^{bc}	48.00^{c}	2801	9.51^{c}	2.18^{b}	7.26^{c}	2.12^{c}	1.83	susceptible
6. 13	0.80^{b}	1.40^{b}	49.00^{b}	81.00^{b}	3371	13.00^{ab}	2.35^{a}	8.53^{bc}	2.13^{c}	2.24	highly susceptible
7.Betag	0.20^{b}	0.00^{b}	32.50^{c}	20.00^{cd}	2940	7.23^{e}	2.12^{b}	4.00^{d}	1.80^{d}	1.96	susceptible
8. CLG	0.00^{b}	5.40^{a}	71.50^{a}	123.00^{a}	4921	13.88^{a}	2.35^{a}	13.16^{a}	2.35^{a}	3.28	highly susceptible

[1]Data are means of 4 replicates and transformed to the √x+.5 prior to statistical analysis. Means of the same letter do not differ significantly at P>0.05 according to Bonferroni and Duncan's Multiple Range Test (DMRT). 2 Root-knot index (RKI). 3 Egg mass index (EMI).4 Reproductive factor (Rf) = Pf/Pi where: Pf- final nematode population; Pi- initial nematode population 5 Pathologic reaction (PR) was based on root gall scoring by Coyne et al., (2007).

Fig. 3. Stand of garden pea cultivars/ advanced breeding lines 60 days after inoculation showing that all cultivars/advanced breeding lines are efficient hosts of *M. incognita.*

DISCUSSION

Use of resistant cultivars is expected to be a vital component of nematode management program in the future. Host response could be appropriately measured by the ability of the plants to suppress development or reproduction of the nematodes (Roberts, 2002 as cited in Dong et al., 2007). In the study, the results were highly variable along number of galls and egg masses on the different cultivars/advanced breeding lines evaluated. The highest mean number of galls, egg masses, J2, J3-J4, and adult females were obtained from CLG variety 60 days after inoculation which differed significantly from the rest of the cultivars/advanced breeding lines. Majority of the J2s that penetrated the root system had developed into J3-J4 and adult females. The rapid development and reproduction of the nematodes in the roots indicated that none of the eight garden pea cultivars/ advanced breeding lines tested was resistant to *M. incognita.*

CGP 11, CGP 110, CGP 154, CGP 13 and Betagshowed an increased pod weight even when infected with nematode. These results are similar to the findings of Pedrocheet al., (2012) that at lower concentration, nematodes have a stimulatory effect on the growth and development of the plants. On the other hand, a significant reduction in growth and yield was observed in CLG cultivar when inoculated with *M. incognita*. It gave a maximum reduction in plant height and a maximum decrease of fresh and dryroot weights, which wasdue to highly efficient nematode reproduction. Moreover, CLG had the lowest plant height. The pod weight obtained in CLG may not be the lowest but it was comparable to CGP 11. The results obtained on the pathologic reaction of garden pea cultivars/advanced breeding lines to *M. incognita* corroborate the findings of Rehmanet al.,(2006); Zazzerini and Tosi (1997); and Montasseret al.,(1985).

Based on root-knot gall and egg mass indices, advanced breeding lines CGP 59, CGP 11, CGP 13, and CLG variety were highly susceptible while advanced breeding lines CGP 110, CGP 34, CGP 13, and Betag variety were susceptible to *M. incognita* infection. However, among the eight garden pea varieties/advanced breeding lines tested, Betag variety was the most tolerant to root-knot nematode infection.

CONCLUSION AND RECOMMENDATIONS

Advanced breeding lines of Chinese Green Pea (CGP) 59, CGP 11, CGP 13, and Chinese Light-green (CLG) were highly susceptible while CGP 220, CGP 34, CGP 134, and Betag were susceptible to *M. incognita* infection. Among the cultivars/advanced breeding lines tested, none showed resistance to *M. incognita.* Continuous screening and characterization of other locally available varieties that could be used as commercial sources of resistance to *M.incognita* is imperative for it to become a component of a sustainable nematode management system.

ACKNOWLEDGMENTS

With gratitude, the authors sincerely acknowledge:

PGMA Graduate Scholarships in Science and Technology through the Department of Science and Technology (DOST) Accelerated Science and Technology Human Resource Development Program (ASTHRP) for the financial assistance;

The Saint Louis University, Baguio City

The Semi-temperate Vegetable Research and Development Center (STVRDC), Benguet State Universityfor allowing the senior author to use the research facilities.

The Highland Crops Research Station, Institute of Plant Breeding BSU, La Trinidad, Benguet for providing the seeds used in the study.

LITERATURE CITED

Agdebite, A.A., G.O.Agbaje, M.O.Akande, N.A. Amusa, J.A. Adetumbi, and O.O.Adeyeye.2005.Expression of Resistance to Meloidogyne incognita to Kenaf Cultivars (Hibiscus cannibinus) under Field Conditions. World Journal of Agricultural Sciences 1 (1): 14-17

Cardwell, D.M.&R. E. I n g h a m. 1 9 9 7. Reproduction of Meloidogyne chitwoodi onPopcorn Cultivars. Supplement to the Journal of Nematology 29 (4): 657-661

Coyne, D.L.,J.M. Nicol, andJ. M. C l a udius-Cole. 2007. Practical Plant Nematology: A F i e l d and Laboratory Guide. S P - I P M Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin

Davide, R.G. 1988. Nematode P r o b l e m s Affecting Agriculture in the Philippines. Journal of Nematology 20 (1): 214-218

Dong, W., C.C. Holbrook, P.Timper, T.B.Brenneman, and B.G.Mullinix. 2007. C o m p a rison of Methods for Assessing Resistance to Meloidogynearenaria in Peanut. Journal of Nematology 39 (2):169-175

Hussey, R.S. and K.R. Barker.1973. A Comparison of Methods of Collecting Inocula of Meloidogyne spp. including a New Technique. Plant Disease Reporter 57, 1025-8

Khan, M.R. 2008.Methods of Screening of Jute (Corchorus sp.)Germplasms forResistant Sources against Nematodes. http://www.articlebase. com/science-Articles

Khan, S.A.2009. Screening of Tomato Cultivars against Root-Knot Nematode and theirBiological Management. Ph.D. Thesis, University of Agriculture, Pakistan Repository, Faisalabad

Montasser, S.A., A.A. Al-Sayed, and A.M. Kheir.1985. Study on Races of Root- K n o t Nematode Meloidogyneincognita in India with particular Reference to Karnataka. Abstracts of papers presented at the 3rd Nematology Symposium Solan p. 58 November 24-26.

Niblack, T.L., R.S. Hussey, and H.R.Boerma. 1986. Effects of Environments on Meloidogyne incognita Inoculums levels and Glycine maxGenotype on Root-knot Nematode-Soybean Interaction in Field Microplots. Journal of Nematology 18: 338-346.

Pedroche, N.B., L.M. Villanueva, and D. De Waele.2009.Management of Root-Knot Nematode Meloidogyne incognita i n C a r r o t Communications in Agricultural and Applied Biological Sciences 74 (2): 605-615

Rehman, A., R.Bibi, and M.H.Ullah. 2006. S c r e e n i n g of Sunflower Cultivars against Root- knot nematode Meloidogyneincognita Journal of Agriculture and SocialSciences 2 (3): 182-184

Robinson, R.A. 1980. New Concepts in Breeding for Disease Resistance. Annu. Rev. Phytopathology 18: 189-210

Theis, J.A., S.B. Merrill, and E.L. Corley 2002. Red Food Coloring Stain: New, Safer Procedures for Staining Nematodes in Roots and Egg Masses on Root Surfaces. Journal of Nematology 34 (2): 179-181

Upadhay, R. D. and K. Dwivedi. 1987. Analysis of Crop Losses in and gram due to Meloidogyne incognita. International Nematology Network Newsletter 4:6-7.

Verma,K.K. and D.C. Gupta. 1993. Germplasm Evaluation of some Rabi Pulses against Root-knot Nematode , Meloidogynejavanica. Indian Journal of Nematology 23: 209-210.

Zazzerini, A. and L.Tosi. 1997. First Report of Root- knot Nematodes (Meloidogyne spp.) on Sunflowers in Mozambique. Plant Disease 81 (11): 1333