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RESEARCH ARTICLE

IDENTIFICATION OF *MELOIDOGYNE* SPECIES ATTACKING CHICKPEAS IN NAKURU COUNTY, KENYA

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ABSTRACT

Chickpea (*Cicer arietinum L.)* is a rich source of nutrients such as carbohydrates, proteins, fats, mineral ions and vitamins. Chickpeas crop yield has been affected by root knot nematodes infestation which account for approximately 13% of crop loss. Four main *Meloidogyne* species, namely *M. hapla, M. javanica, M. arenaria* and *M. incognita* attack chickpeas. In Kenya, chickpeas are cultivated after the main crops. This study was conducted in a glass house to characterize and identify the root knot nematodes attacking chickpeas in Nakuru County. Characterization and identification were done using perineal patterns procedure on female root knot nematodes. Thirty samples were taken and all resulted onto uniform perineal patterns of *M. javanica* distinguished from other species by a distinct lateral ridge separating dorsal and ventral arch. It was therefore concluded that *M. javanica* is the main root knot species attacking chickpeas in Nakuru County.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third important legume after beans (*Phaseolus vulgaris*) and field pea (*Pisum sativum* L.) (FAOSTAT, 2008). It is an important source of proteins (Kumar *et al*., 2005) and carbohydrates which constitutes 80% of the total dry seed mass (Grusak, 2002). Reports indicate that chickpea is cholesterol free and a source of dietary fiber, vitamin and minerals (AAFC, 2006). It is consumed in different forms in many countries where it is grown (Muehlbauer and Tullu, 1997). In the Indian subcontinent for example, chickpea cotyledons are used as flour for making paste for snacks (Chavan *et al.,* 1986). In Asia and Africa it's consumed as stew, salads or in roasted, boiled or fermented forms (Hulse, 1991). Chickpea serves health benefits than the nutritional ones, it comprises of components which improve health such as dyspepsia and relieve for diabetes (Jukanti *et al*., 2012). Total fat content in raw chickpea seeds varies from 2·70 to 6·48% (Alajaji and El-Adawy, 2006). Shad *et al*. (2009) reported lower values (about 2·05 g/100 g) for crude fat content in Desi-type chickpea varieties. Wood and Grusak (2007) reported a fat content of 3·40–8·83 and 2·90–7·42% in Kabuli and Desi-type chickpea seeds respectively. Fatty acids are also present with linoleic acid (LA) being higher in Kabuli than in Desi types, linoleic acid being dominant and a source of fatty acid with the highest fraction (51.2% LA) than other edible lentils such as peas and beans (Wang and Daun, 2004). Chickpeas supplement provision of vitamins when consumed with other foods especially cereals (Singh and Diwakar, 1993). They are good sources of riboflavin (B2), pantothenic acid (B5) folic acid, niacin and pyridoxine (B6) (Lebiedzinska and Szefer, 2006).Other components such as minerals including zinc, iron, magnesium and calcium are also present in chickpea diet (FAO, 2002; USDA, 2010). Desi type has higher amounts of calcium than Kabuli type though there no significant differences between the two types for the other minerals (Ibanez *et al.,* 1998). In additional to the nutritional benefits to the body, chickpeas serve health benefits. Foods rich in dietary fibre (DF) are associated with low basal metabolic index (BMI) (Howarth *et al.,* 2001). Chickpeas are said to have DF and low glycaemic index (GI) therefore it is important in reduction of weight hence obesity reduction. Sulphur containing amino acids (SCFA) including butyrate, produced after chickpeas consumption, helps to suppress cell proliferation (Cummings *et al.,* 1981). Butyrate also inhibits DNA compaction and gene expression by histone deacetylase suppression (Mathers, 2002). Consumption of fibre foods leads to reduced levels of plasma cholesterol. Foods rich in saponins reduce cholesterol (16 to 24%) (Thompson, 1993). The mechanism involves inhibition of fatty acids synthesis in the liver by fiber components such as butyrate and SCFA hence reduced cholesterol (Crujeiras *et al.,* 2007).

Global chickpea production by 2006-2009 was 9.6 million metric tons with an average yield of 849kg/ha (FAO, 2011). In Kenya, Kimurto *et al*. (2013) reported that chickpea is a relatively new crop grown by small scale farmers in Eastern and Rift Valley regions. Its spread however has been recorded in dry highlands and dry lowlands where rainfall ranges from 250-550mm per annum (Kibe and Onyari, 2007; Onyari *et al*.,

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2010). Kenya's chickpea production was reported to be 55,000 tons according to ICRISAT (2008) statistics. In Nakuru, cultivation has been done in Naivasha and Egerton-Njoro (Mulwa *et al*., 2010; Kimurto *et al*., 2013). Drought resistant chickpea is also found in the country which is an essential food supplement; the crop is a bonus crop as it is planted immediately after the main crop such as maize is harvested (Kimurto *et al*., 2004).

Chickpea varieties are susceptible to ectoparasitic and endoparasitic nematodes such as *Heterodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Meloidogyne* spp. (root knot), (Rehman *et al*., 2012). Root knot nematodes are sedentary endoparasites that induce root-knot symptoms and cause serious agricultural damage (Trudgill and Blok, 2001) with over 100 species (Karssen *et al*., 2013) which result to root knot disease. *M. javanica, M. arenaria, M. incognita* and *M. hapla* are four major species which accounts for to 95% of all crops loss (Agrios, 2005) and 13.7% of chickpeas yield loss (Rehman *et al*., 2012) and translating to annual loss of 157 billion dollars globally (Abad *et al*., 2008; Okendi *et al*., 2014).The species are identified on the basis of their perineal patterns, the morphology which is located at the posterior body region of adult females (De Ley and Blaxter, 2002). The posterior region comprises the vulva, anus, lateral lines, phasmids, tail and surrounding cuticular striae (De Ley and Blaxter, 2004), these parts differ in different *Meloidogyne* spp. and are useful in identification. In Kenya details of rootknot nematodes attacking chickpeas has not been documented, therefore the aim of this study was to identify the *Meloidogyne* spp. attacking chickpeas in Nakuru County, Kenya on the basis of the perineal patterns.

MATERIALS AND METHODS

The study was conducted in a glass house at Egerton University, Njoro, Nakuru County in Kenya. Heavily infected roots of chickpea plants were collected from Egerton University Biological plot, Fields 3, 7, Gilgil and Naivasha by random selection. Infected plants were uprooted and samples put in labeled polythene bags for nematode extraction. The samples were preserved at 5^0 C in the refrigerator.

The extraction was done using the method described by Hussey and Barker (1973). Galled roots of chickpeas were washed and galls cut open using a scalpel and a dissecting needle to tease out adult female nematodes in a petri dish containing water. *Meloidogyne* females' perineal patterns were cut using a method described by Taylor and Netsch (1974). Cuticles of the female nematodes ruptured by cutting the anterior part and gently pushing out body tissues. Thirty samples of cuticles were then placed in 45% lactic acid in a petri dish, lactic acid aided in facilitating removal of body tissues and allowed to stand for half an hour. After tissues removal, the cuticle were transferred to a drop of glycerin on a glass slide where they were carefully trimmed so as to be only slightly larger than the perineal pattern. The piece of cuticle with the perineal pattern was transferred to a drop of glycerin on a slide. Observations were made on a compound microscope for identification as described by Taylor and Netsch (1974) and photographs taken.

RESULTS

Root knot nematodes collected from the study area are depicted in Plate 1with Figure 1 and 2.

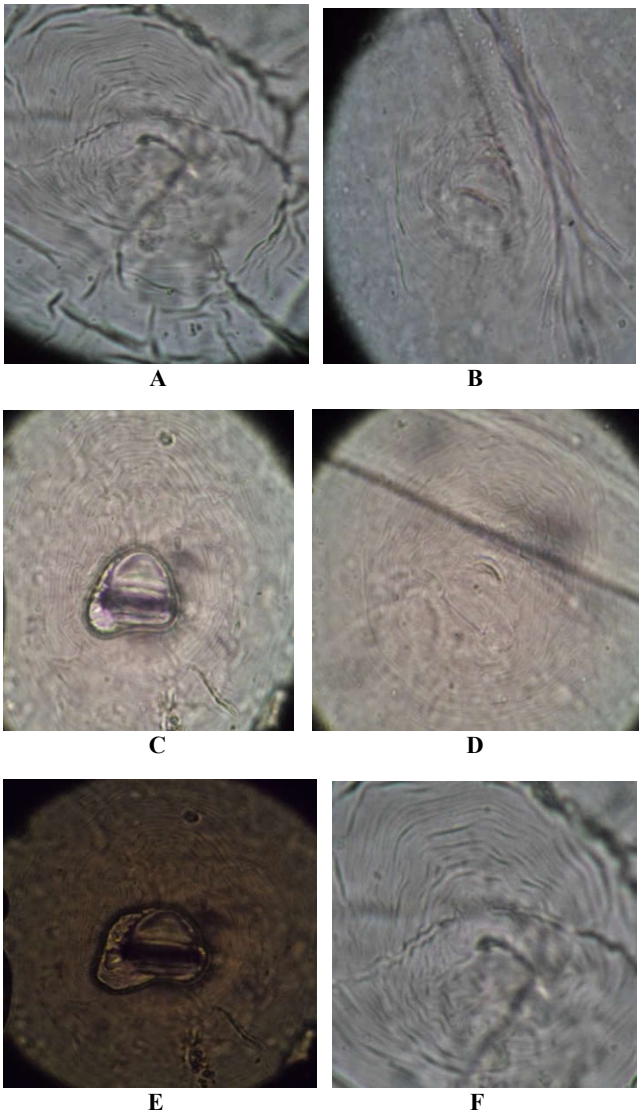
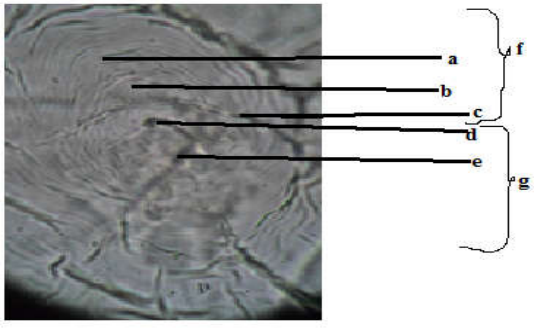


Figure 1. Perineal patterns showing *M. javanica.*



LEGEND
a- arch
b- tail tip
c- lateral line
d- anus
e- vulva
f- dorsal side
g- ventral side

Figure 2. Features in the perineal patterns

The perineal patterns (A, B, C, D, E and F) in figure 1 had distinct lateral ridges which divide the dorsal and ventral striae, ridges ran the entire width of the pattern. The striae were smooth to slightly wavy and some bent towards the vulval edges. The dorsal arch contained a whorl in the terminal area. The perineal patterns had uniform taxonomic features that are characteristic of *M. javanica.*

DISCUSSION

The perineal patterns were found to have distinct lateral ridges that run the entire width of the pattern and divide the dorsal and ventral striae unlike in *M. incognita*, *M. hapla* and *M. arenaria* which do not have distinct lateral lines. They also have low and rounded dorsal arch unlike *M. incognita* whose dorsal arch is high and squarish, the striae were coarse, smooth to slightly wavy and bend towards the vulva (as shown in Fig 1 and 2) which is unlike *M. hapla* which has fine dorsal and ventral striae that meet at an angle as was reported by Eisenback (1985). There was consistency in the perineal patterns of the root knot nematodes extracted from chickpea grown in Nakuru indicating the occurrence of a single species. The features were characteristic of *M. javanica*. In Kenya, CABI (2002) *M. javanica* was associated with the damage of broad beans, tomato and cabbage and also attack cowpeas and pigeon peas in Mbeere, Mwea, Gachoka and Siakago areas (Waseke *et al*., 2013).Ngundo and Taylor (1974) reported infestation of *M. javanica* and *M. incognita* in beans in Thika.

This is evident that *M. javanica* is present in Kenyan soils. Ansari *et al.* (2012) reported that local chickpea cultivars produced low yield in *M. javanica* infested fields, however Sharma and Sharma (1988) reported that *M. javanica* is the second species predominant in chickpea losses in India. Susceptibility of chickpea cultivars to *M. javanica* was reported to be high (Sharma *et al*., 1993). Reports from Maheshwari *et al*. (1997) indicate that inoculation of *M. javanica* juveniles prior to *Fusarium oxysporum* f. sp. *ciceri* caused greater wilt incidence in susceptible cultivars and induced vascular discoloration in roots of resistant cultivars of chickpea. Inoculation of *M. javanica* and *Rhizoctonia bataticola* in chickpea seedlings reduce plant growth with tap root devoid of lateral roots and appearing dark and rotting (Ali *et al*., 2003). It was therefore concluded that *M. javanica* is the main *Meloidogyne* spp. attacking chickpeas in the study area, therefore paving way for further studies of managing it.

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