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INTENSITY AND DISTRIBUTION OF *Meloidogyne* Spp. IN COWPEA GROWING AREAS OF MOZAMBIQUE

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in warm marginal environments of the country. Rootknot nematodes (*Meloidogyne* spp.) are among the major constraints limiting cowpea yield in the country. Understanding rootknot nematode prevalence, distribution and damage intensity in major cowpea growing areas is crucial in making control decisions. A study was conducted to identify rootknot nematode species, determine prevalence, distribution and damage intensity (incidence and severity) in Mozambique. The study covered 8 districts belonging to 3 provinces namely: Gaza, Inhambane, and Nampula provinces. Out of the 72 cowpea fields surveyed, 56.9% were infested with rootknot nematodes. Inhambane province registered the highest root knot nematode prevalence at 74%. *Meloidogyne incognita* and *M. javanica* were the most frequent root knot nematode species encountered. *M. enterolobii* was another species observed to be associated with cowpea rootknot in this study. The highest frequency of *M. incognita* and *M. javanica* was observed in cowpea fields from Inhambane and Gaza provinces, respectively. Provinces differed significantly in terms of the intensity of rootknot nematode damage in which Inhambane province recorded the highest rootknot incidence and galling severity at 39.8% and 1.9, respectively. Rootknot nematode damage intensity differed significantly across districts in which Homoine district registered the highest rootknot nematode incidence and galling severity at 55.8% and 2.1, respectively.

Keywords: Cowpea, *Meloidogyne* Spp., distribution, damage intensity

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1.0 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a crop of great importance widely cultivated in Mozambique. The crop is mostly cultivated in provinces of Nampula, Inhambane, Zambézia, Gaza and Maputo, almost exclusively by smallholder women farmers (INE, 2008; Chiulele, 2010). It is primarily cultivated for household consumption in form of grain, fresh leaves and fresh pods, besides becoming an important source of family income through sell of its various products in open markets (Chiulele *et al.*, 2011). In addition, the crop improves cropping systems and soil fertility through weed suppression and nitrogen fixation, contributing to increased yields of nitrogen demanding crops grown with or after it (Tarawali *et al.,* 2002).

Despite its widespread cultivation and national importance, average on-farm cowpea yields have for long remained below 300 kg ha^{-1} (Muitia *et al.*, 2006) owing to numerous biotic production constraints such as nematodes. Rootknot nematodes (*Meloidogyne* Spp.) have been reported as one of the major biotic constraints limiting cowpea production in Mozambique (*Oever et al.*, 1998), posing a major food and nutritional security threat. Amidst this threat, current information on the extensive distribution, severity and identity of *Meloidogyne* species in smallholder farmers' fields in Mozambique is very limited. Such information would form an important component of nematode and or disease management and control programs, more so at such a time when cowpea is becoming increasingly important to the country's food, nutrition and income security. The study was aimed at identifying species, determining prevalence, distribution and damage intensity (incidence and severity) of rootknot nematodes in cowpea fields. The study covered cowpea fields in eight districts in three provinces of Mozambique.

2.0 MATERIALS AND METHODS

2.1 Description of the study area

The study was conducted in three of the main cowpea producing provinces namely: Nampula, Gaza and Inhambane. Nampula province is located 15.250°S and 39.500°E in northeastern Mozambique with an area of 79,010 km². The Province is bordered by Niassa Province to the northwest and west, Zambezia Province to the southwest, and the Indian Ocean to the east (Geohive, 2014; Macauhub.com, 2016). Two districts (Moma and Meconta) were selected from this province for this study, based on their importance in cowpea production. Moma is one of the coastal districts of the province and experiences average annual temperatures higher than 25oC and an annual rainfall range between 800 and 1200mm with an alfisol soil type. Meconta is one of the inland districts of the province with an annual rainfall of between 1000 to 1400mm (Maria & Yost, 2006).

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Gaza province is located -23.0222° S, 32.7181°E, in the south of Mozambique with an area of 75,334 km2 with Xai-Xai district as its capital. The province is bordered by Inhambane province to the east, Manica Province to the north, Maputo Province to the south, South Africa to the west, and Zimbabwe to the northwest. The province is characterized by arid conditions with an annual rainfall of 400-600mm with soils of sandy texture (INE, 2006). From this province, Bilene, Chibuto and Mandlakazi districts were considered for the study, based on their importance in cowpea production.

Inhambane province is located -23.0° S, 34.3° E, on the Indian Ocean coast in the southern part of the country with an area of 68,615 km2 with Inhambane district as its capital. The province is bordered by Manica and Sofala provinces to the north, Gaza province to the west and the Indian Ocean coast to the south and east. The province is characterized by a warm rainy season between November and March and sandy textured soils (INE, 2006; visit mozambique.net, 2016). In this province, the study took place in 3 districts including: Inharrime, Jangamo and Homoine districts, based on their importance in cowpea production.

2.2 Sampling of study areas for rootknot nematode infestation

The study was conducted in a total of eight districts in three provinces. In each district, 3 localities were selected based on concentration of cowpea farmers. In each locality, 3 farmer fields were selected, thus 9 farmer fields were sampled from each district. For each of the provinces (Gaza and Inhambane), 27 cowpea fields were sampled, while 18 were sampled for Nampula province. For each field, a maximum of 0.5 acres occupied by cowpea plants were considered for sampling. A zigzag sampling pattern was used in each of the fields selecting between 5-10 plants to constitute a bulk sample per field. Along the zigzag transect, 5 -10 plants were selected uprooted using a shovel and combined together to form a bulk sample. All the root samples from each field were placed in polythene envelopes with the sample code, location name, sampling date, district name, and location coordinates and placed in a cooler box. The root samples were then taken to the laboratory for rootknot nematode evaluation.

2.3 Evaluation of galling severity, incidence and prevalence of rootknot nematodes

Overall, 511 cowpea plant samples were obtained from the three provinces (Table 1). Form the fields, cowpea root samples were taken to the phytopathology laboratory at the Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, Maputo, Mozambique. In the laboratory, root samples were cleaned to remove soil and other debris under a gentle stream of tap water before blotting them dry with blotting paper. Samples were then dipped in a 1 liter solution containing 15mg of phloxine B for 5 minutes to stain egg masses (Coyne *et al.*, 2007) in order to improve visibility of rootknot nematode damage. Galling severity was achieved by

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scoring individual cowpea plants (roots) for rootknot gall index. A scale ranging from 1 -5, modified from Bridge and Page (1980) was used; where 1: no galling damage, 2: root shows only small knots but clearly visible and main root is free of knots 3: larger knots predominant but main root is clean, 4: knotting observed on main roots and 5: majority of main roots knotted.

Table 1: Number of cowpea plant samples collected from surveyed areas

Province	District	Number of cowpea plant samples
Gaza	Bilene	66
	Chibuto	59
	Mandlakazi	59
Inhambane	Inharrime	63
	Jangamo	67
	Homoine	72
Nampula	Meconta	66
	Moma	59

Incidence rootknot nematode damage was evaluated as present (P) or absent (A) of damage based on the presence of rootknot galls or egg masses (stained pink by phloxine B) or both. Incidence was thereafter calculated as a ratio of rootknot infected plants to the total number of plants sampled in each respective farmer field and expressed as a percentage (Equation 1). Prevalence of root knot nematodes was calculated as a percentage of cowpea fields infested with rootknot nematodes (Equation 2). All data generated was recorded in Microsoft excel.

$$\text{Incidence of root knot nematode infestation} = \left(\frac{\text{Number of root knot nematode infected plants}}{\text{Total number of sampled plants per field}}\right) * 100 \quad (1)$$

$$\text{Prevalence of rootknot nematodes} = \left(\frac{\text{Number of cowpea fields infested with rootknot nematodes}}{\text{Total number of cowpea fields sampled}}\right) * 100 \quad (2)$$

Determination special distribution of rootknot nematodes was accomplished by processing of survey coordinates using software, Quantum GIS, version 2.16 (QGIS Development Team, 2009. QGIS Geographic Information System. Open Source Geospatial Foundation). A map showing rootknot nematode distribution was produced

2.4 Identification of rootknot nematode species

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This was accomplished by extracting triplicates (per root sample) of engorged female rootknot nematodes from root samples using a pin, surgical blade, and a pair of forceps under a stereo microscope. By selecting one plant from each cowpea field, galled plants from the 41 rootknot nematode infested cowpea fields were used for the extraction of female nematodes used in the identification process. However, not all the root sample where heavily infested to provide sufficient numbers of rootknot nematode females. Due to this, females were extracted from only 36 individual plant root samples. These therefore provided a total of 36 female rootknot nematode samples. Female rootknot nematode samples were preserved in absolute alcohol, in labeled, tight sealing Eppendorf tubes. These samples were then sent to the Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium for identification, using a method described by Janssen *et al.* (2016). Out of the 36 samples, only 22 provided viable DNA material for PCR (Polymerase Chain Reaction) analysis.

2.5 Determination of rootknot nematode species frequency index

A total of 72 cowpea fields were surveyed for rootknot nematode infestation. Of the 72 cowpea fields, 41 (56.9%) fields were found to be infested with rootknot nematodes. The frequency index of identified rootknot nematode species was calculated at province and district level, but also, an overall index was calculated taking into account a total of the 72 cowpea fields sampled. Rootknot nematode species frequency index was calculated (Equation 3.) and expressed as a percentage.

$$\text{Meloidogyne species frequency index} = \left(\frac{\text{Number of cowpea fields infested with a particular Meloidogyne species}}{\text{Total number of cowpea fields sampled}}\right) * 100 \quad (3)$$

2.6 Data analysis

Data collected was entered in Microsoft excel and analyzed using STATA statistical package (version 12). All data did not conform to parametric ANOVA models and hence were analyzed following Kruskal Walis approach. Post hoc multiple comparisons and separation of means was done using Dunn's test with no adjustments. Data was presented as tables of means, graphs and maps were applicable.

3.0 RESULTS

3.1 Distribution of rootknot nematodes in the study area

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Rootknot nematodes were found to be widely distributed among all surveyed areas (Figure 2). Out of the 72 cowpea fields surveyed, 41 (56.9%) were infested with rootknot nematodes. Twenty (20), 16 and 5 cowpea fields were infested with rootknot nematodes in Inhambane, Gaza and Nampula provinces, respectively, resulting into 88.8%, 59.2% and 27.7% prevalence, respectively (Table 2). The highest (8) number of rootknot nematode infested cowpea fields was observed from Homoine district while the least (2) was from Moma district.

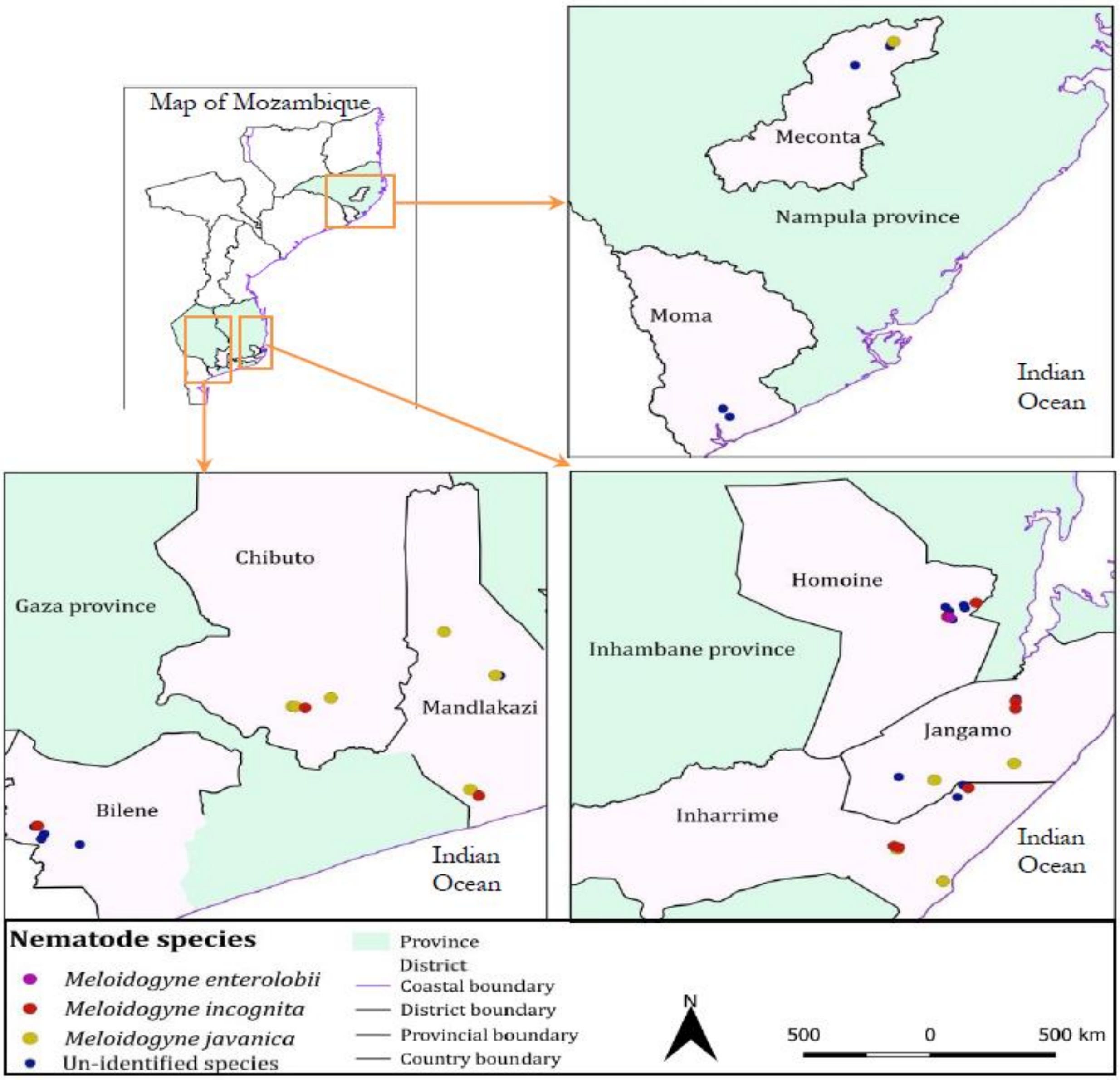

Figure 2: Distribution of *Meloidogyne* species

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Table 2: Prevalence of rootknot nematodes

Province	District	Number of Cowpea fields infested with rootknot nematodes	Prevalence (%)	
			District	Province
Gaza	Mandlakazi	6	66.6	59.2
	Bilene	5	55.5	
	Chibuto	5	55.5	
Inhambane	Inharrime	6	66.6	74.0
	Jangamo	6	66.6	
	Homoine	8	88.8	
Nampula	Meconta	3	33.3	27.7
	Moma	2	22.2	

3.2 Rootknot nematode incidence and galling severity

Significant differences in mean rootknot nematode incidence and galling severity were observed among provinces. Inhambane province recorded the highest mean incidence (39.88%) and galling severity (1.9) of rootknot nematodes which differed significantly from Gaza and Nampula provinces (Graphs A and B).

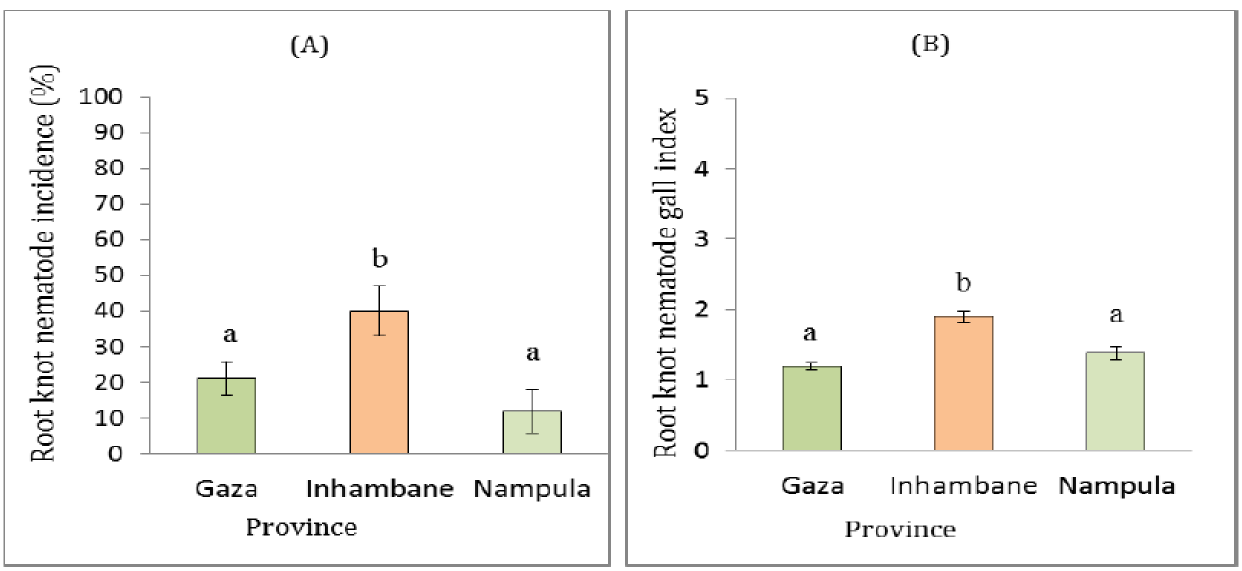

Figure 1: Incidence (A) and galling severity (B) of rootknot nematodes among provinces. Bars with the same letters are not significantly different (p>0.05)

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Among districts, significant differences were observed in terms of rootknot nematode incidence and galling severity. Homoine district registered the highest mean incidence (55.88%) and galling severity (2.11) which differed significantly from the rest of the sampled districts (Table 2).

Table 2: Mean variation of rootknot nematode incidence and galling damage in cowpea across districts

Province	District	Rootknot nematode Incidence	Rootknot nematode Gall Index (1-5)
Gaza	Mandlakazi	21.77^{ab}	1 (1.29)^{abc}
	Bilene	24.33^{ab}	1 (1.12)^{ab}
	Chibuto	17.33^{ab}	1 (1.20)^{abc}
Inhambane	Inharrime	24.66^{ab}	2 (1.60)^{bcd}
	Jangamo	39.11^{ab}	2 (1.96)^{de}
	Homoine	55.88^{b}	2 (2.11)^{e}
Nampula	Meconta	18.88^{ab}	2 (1.70)^{cde}
	Moma	04.77^{a}	1 (1.03)^{a}
	P.value (a = 0.05)	*0.0347*	*0.0001*
	Number of observations (n)	*72*	*511*
	Degrees of Freedom (df)	*7*	*7*
	Chi-squared value(χ^2)	*15.105*	*81.384*

Means with the same letters in a column are not significantly different (P>0.05)

3.3 Rootknot nematode species identified on cowpea plants

Three (3) species of rootknot nematode, namely: *M. incognita* , *M. javanica*, and *M. enterolobii* were identified to be associated with rootknot of cowpea roots. Of the 27 cowpea fields sampled from each province, *M. javanica* was observed from 4 and 6 cowpea fields of Inhambane and Gaza provinces, respectively. Of the 18 cowpea fields sampled from Nampula province, *M. javanica* was observed only from one field. *M. incognita* was from 7 and 3 cowpea fields of Inhambane and Gaza provinces, respectively. *M. enterolobii* was observed only in 1 cowpea field from Inhambane province. Of the 72 cowpea fields sampled, *M. javanica*, *M. incognita* and *M. enterolobii* were observed in 15.3%, 13.8% and 1.4%, respectively, of the cowpea fields surveyed (Table 4).

Table 4: Rootknot nematode species frequency indices in surveyed provinces (%)

	Meloidogyne spp. frequency index (%)		
Province	*M. javanica*	*M. incognita*	*M. enterolobii*
Gaza	22.2	11.1	0
Inhambane	14.8	25.9	3.7
Nampula	5.5	0	0
Overall	15.3	13.8	1.4

Within districts, the highest occurrence of *M. javanica* was observed from 33% of cowpea fields surveyed from Chibuto and Mandlakazi districts, respectively. Cowpea fields from Jangamo district registered the highest occurrence of *M. incognita* at 33%. Only 11% of cowpea fields from Homoine district were infested with *M.enterolobii* (Table 5).

Table 2: Rootknot nematode species frequency indices in surveyed districts (%)

		Meloidogyne spp. frequency index (%)		
Province	District	*M. javanica*	*M. incognita*	*M. enterolobii*
Gaza	Bilene	0.00	11.00	0.00
	Chibuto	33.00	11.00	0.00
	Mandlakazi	33.00	11.00	0.00
Inhambane	Inharrime	22.00	22.00	0.00
	Jangamo	22.00	33.00	0.00
	Homoine	0.00	22.00	11.00
Nampula	Meconta	11.00	0.00	0.00
	Moma	0.00	0.00	0.00

3.4 Other field observations made during the survey

During the field survey, it was observed that the majority of the fields were infested with weeds ranging from a few to many weeds including both herbaceous and grass types of weeds. Weeds such as *Amarantus* spp. were observed infested with rootknot nematodes. Only one farmer applied fertilizer to cowpea crops while all other farmers applied neither fertilizer nor pesticides. Farmers, especially in Inhambane and Gaza provinces practiced mixed cropping, with groundnuts, cassava, and maize as common crops across all sampled areas, while pigeon pea, water melons and Bambara ground nut were generally less frequent. Sandy soils appeared to be dominant across all sampled areas. It was also observed that cowpea fields were generally cultivated on sandy soils.

Generally, the rootknot damage appeared to be more severe on erect cowpea varieties than prostrate ones. Apart from farmers in Nampula province, majority of farmers in Gaza and Inhambane did not practice recommended practices for disease control, such as crop rotation. Mixed cropping of other rootknot nematode host crops (of different growth cycle lengths) was commonly observed in Gaza and Inhambane provinces. It was observed that the majority of farmers in Nampula province included fallows and rotation within their agricultural practices. Apart from rootknot galling damage, signs of fungal and viral diseases were evident on cowpea plants. Viral symptoms such as chlorosis, yellowing and mottling of plant leaves appeared were widespread in Gaza province as opposed to the other provinces.

4. DISCUSSION

Results of this study have revealed that *M. incognita* and *M. javanica* are dominant rootknot nematode species affecting cowpea plants in Mozambique. In close agreement with the current study, these two species have been reported in a number of studies as some the most encountered rootknot nematode species affecting different crops in several tropical countries all over the globe (Adam *et al.,* 2007; Ma *et al.,* 2006; Ploetz, 2009; Onkendi *et al.,* 2014). Besides *M. incognita* and *M. javanica*, the current study also identified another species *M. enterolobii* on one of the cowpea plant samples from Homoine district. The current study therefore further partially agrees with findings by Onkendi *et al.* (2014), Onkendi & Moleleki (2013b) that reported presence of *M. enterolobii* on potatoes and guava from Mozambique, Burkina Faso, the Democratic Republic of Congo, Malawi, Senegal, South Africa and Togo. Findings from the current study probably constitute the first report of the presence of *M. enterolobii* specifically in cowpea in Mozambique. The occurrence of these species on cowpea is an indication of a likelihood of wide spread cultivation of rootknot nematode susceptible varieties of cowpea in the country and possibly, the presence of aggressive populations of *Meloidogyne* spp. that may have evaded any potential resistance.

Cowpea fields from southern Mozambique, more so from Inhambane province, were infested with multiple species of rootknot nematodes, namely, *M. javanica*, *M. incognita* and *M. enterolobii*. This may have happened as a consequence of the presence of several alternative hosts including crop plants and weeds which may harbor different response or resistance profiles to nematode attack. Rootknot nematode host crops such as maize, cassava, groundnuts in addition to cowpea were mixed cropped for more than one growing season. Poor cropping practices such as the ones observed herein may render use crop resistance inefficient. It has been observed that repeated cultivation of resistant cultivars for 3-8 years may to evoke outbreak of resistant rootknot nematode strains arising from selection pressure exerted against native perhaps initially less virulent rootknot nematode populations (Eddaoudi, 1997). In the fields, cowpea in addition to other crops were observed to be generally cultivated on sandy soils besides being infested with rootknot nematode susceptible weeds such as *Amaranthus* spp. Nematodes are known to be ubiquitous members of the soil fauna, light textured soils being their favorite habitat ((Viketoft, 2007; Olabiyi & Oladeji, 2014). The presence of multiple hosts with varying growth cycles in addition to conducive edaphic conditions (sandy soils) provides such a favorable environment that supports multiplication of rootknot nematode species with differential host preferences and hence the observed damage levels involving multiple species. This therefore poses an urgent need for field sanitation practices such as regular weeding, besides cautious mixed cropping practices that will see reduction of soil nematode populations to such levels that do not cause significant economic damage to crops.

In general, low rootknot damage intensity was observed in Gaza and Nampula provinces, this may in part be due to possible limitations in wide self-transmissibility of rootknot nematodes. By virtue of their nature, nematodes as soil borne plant pathogens have limitations as far as their self-dispersal is concerned. They move slowly and are present in small areas. Besides, their level of genetic flow is also limited. Their dispersal is usually aided by external agents such as windy rains and erosions amongst other agents (Agrios, 2005). It is thus clear that temporal and spatial occurrence of the right combination of a set of dispersal conditions may be difficult and may not synchronize with the right developmental stages of the nematode. This leaves transmission of these pathogens in the hands of unscrupulous agricultural practices that may not be common across agro-ecologies, resulting into the low infection levels where observed.

5. CONCLUSIONS

Rootknot nematodes were found widely spread over all sampled localities with an overall prevalence of 56.9%. Three rootknot nematode species, namely: *M. incognita*, *M. javanica*, and *M. enterolobii* were identified to be associated with cowpea rootknot in the surveyed cowpea fields. High rootknot nematode diversity was observed from Inhambane province. Over all, Inhambane province registered the highest rootknot nematode prevalence, incidence and gall indices, and hence rootknot nematode damage intensity.

6. RECOMMENDATIONS

Screening of cowpea and other host crops grown with or after it for existing resistance against rootknot nematode will help in identification of resistance and possible abatement of rootknot nematode damage. Conducting of rootknot nematode resistance research must be under taken by

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cowpea breeders before variety release, especially in areas severely infested with rootknot nematodes. This should be conducted under field and greenhouse conditions for several successive crop seasons in order to test the durability of observed resistance. Identifying cowpea genotypes with multiple resistances to rootknot nematode species should be underscored in the search for resistance. When conducting resistance trials, isolates of rootknot nematode populations should collected from the various cowpea growing areas of southern Mozambique, as these have been observed to be infested with multiple species of rootknot nematodes. In addition to that, infested areas should be prioritized for receiving currently available rootknot nematode resistant cowpea cultivars. As part of agronomic research, conducting awareness and training of farmers on proper rootknot nematode management by agronomists will help in reducing rootknot nematode damage.

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