NSUK Journal of Science & Technology (NJST), Vol. 7: No. 2, 2020. pp. 46-52. Print: ISSN: 2756- 696X
On-line: ISSN2756-6951
Website: www.nsukjst.org.ng

OCCURRENCE OF ROOT-KNOT NEMATODE DISEASE ON SELECTED VEGETABLES AND EFFECTS OF RHIZOSPHERE INFESTED SOILS ON SOME OF THEM IN LAFIA, NASARAWA STATE

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ABSTRACT

A survey was carried out from September to December 2018 to assess the presence of root-knot nematode disease on roselle, okra, pepper and tomato in Lafia, Nigeria. Appearance of wilting symptom of crops was used to select plants that were sampled and examined for presence of root gall. Galling was used to confirm occurrence of root-knot disease. To determine threat of root-knot nematode infested fields to the crops, rhizosphere soil from two further selected species, roselle and okra were collected and used for pot experiment from February to August, 2019. Data were obtained on some growth and fruiting parameters of roselle and okra during the pot experiment, analyzed for variance and statistically significant treatment means were separated at 5% probability. The result showed wilting incidences of 2.63, 1.40, 1.83 and 2.30 % for roselle, okra, pepper and tomato respectively and gall indices of 3.82, 5.91, 3.01 and 3.71correspondingly. Okra had highest gall index but least wilt incidence. For the pot experiment, roselle plant height decreased from 120.33 cm in control, 0 kg nematode infested rhizosphere soil (0 kgis) to 98.77 cm in 3kg is.. Okra height was highest in control, at 81.67 cm and least in 3 kgis, 75.27 cm. Roselle number and weight of fruits decreased from 0 kgis to 3 kgis. For okra fruit parameters only fruit weight decreased significantly ($P \leq 0.05$) from 8.34 to 5.00 cm.Low wilt incidence despite high gall index with only fruit weight significantly affected showsokra is more tolerantof higher infection than others. Roselle was more susceptible, with highest wilt incidence and significant reductions in both fruit weight and numbers.

***Keywords**: Root-Knot Nematode, Roselle, Okra, Pepper, Tomato, Rhizosphere*

INTRODUCTION

Plant-parasitic nematodes are serious but unsung pathogens affecting over 3000 plant species including most cultivated crops and cause global food production losses of at least 12.3% , estimated at $157 billion annually (Sikora and Greco, 2005; Hassan *et al*., 2013; Cheng *et al*., 2015, Kumar, 2020).Root knot nematodes *Meloidogyne* spp. are the most predominant nematodes on many crops and are considered by some as probably the greatest threat to agriculture in sub-Saharan Africa. They are described as a malignant soil borne pest to vegetables (Coyne *et al*., 2018; Sikor*et al*., 2018).

Meloidogyne species are widely distributed in agricultural lands and on several ~~c~~rops as yam, maize, pineapples, cucumber, and vegetables (Sikora and Fernandez, 2005; Agbenin and Ogulana 2006; Daramola and Afolami, 2014; Duru *et al*., 2015; Suleiman *et al*., 2015; Aminu-Taiwo *et al*., 2015). Although, the female Meloidogyne spp spend most of their adult life in roots of hosts, the juveniles are found in the rhizosphere soil surrounding the roots of these crops and have been reported on rhizospheres of crops grown in Nigeria including (Ploeg, 2013; Duru*et al*., 2015; Suleiman *et al*., 2015).

Meloidogyne incognita is the most ubiquitous root-knot nematode. In a study in Pakistan it constituted up to 90% of nematode spp. affecting vegetables. In another study on roselle in northern Nigeria, it was 62.29%. The root-knot nematodes distribution on cowpea in 31 states of Nigeria, show *M. incognita* with 51.8 %, *M. javanica*, 44.1% and *M. arenaria* 4.1%. They caused galls on 64.59 % of five vegetable types in Niger State, Nigeria (Olowe, 2004; Anwar and Mckenry 2012; Kumar *et al*., 2014 Ogunsola *et al*., 2018). Nematodes (Juveniles) remain in the soil after harvest and decline gradually at off season but begin to build up again when conditions are favourable (Ploeg, 2013). It is known that the extent of the nematode damage to crops is related to the degree of infestation of the soil (Seinhorst, 1986)

Root-knot nematode destruction is reflected in the crop losses they cause as a group or as individual species. Globally, nematodes, of which *Meloidogyne*spp are the most common and destructive, cause an estimated 14% crop losses, amounting to almost $100 billion dollars annually (Mitkowski and Abawi, 2011). In India, the annual crop loss due to plant-parasitic nematodes is estimated at 21.3%,equivalent to $1.58b of which vegetable crops suffer comparatively more losses; while in the USA, they are estimated to cause $4.973b on solanaceous vegetables alone (Koenning *et al.*, 1999; Kumar *et al*., 2020). *Meloidogyne incognita* as a specie has a yield loss potential of between 25 – 100 % on tomato (Seid *et al*., 2015). *Meloidogyne* spp. were found to cause up to 56% in four vegetables including okra and tomato and up to 45% avoidable losses have been recorded for okra in particular, while the annual losses for the crop is estimated at $38.41m equivalent in India (Sharma and Baheti, 1992; Baheti and Bhati 2017; Kumar *et al*., 2020)

In Nigeria, the root knot nematode has long been associated with substantial yield reductions in vegetables and other crops across various regions (Babatola 1983; Swarup 1983, Hassan *et al*., 2010; Kumar *et al*., 2012; Bawa *et al*., 2014; Tanimola and Akarekor, 2014). However, this information is not available for vegetables in Nasarawa state and Lafia in particular. The threat of root-knot nematodes needs to be assessed for every farming community and especially for vegetable production. This research aims to assess the presence of root knot nematode disease on roselle, okra, pepper and tomato, in Lafia and the impact of these nematodes' presence in the rhizosphere of okra and roselle, two vegetables purposively chosen because of their relatively high use as traditional soup vegetable for okra and as emerging drink significance for roselle in the area.

MATERIALS AND METHODS

Four randomly selected villages, Gunji, Kwandere, Ombi II and Shabu in Lafia metropolis, Lafia LGA were surveyed for Root knot nematode infections on roselle, okra, pepper and tomato between September and December 2018. In each village 10 farmers' fields were selected. In each farm, plants wilting in the midst of otherwise healthy growing plants were suspected to be infected by nematode, after the method of Agbenin and Ogunlana (2006). They were uprooted and examined for root galls (which indicate root-knot nematodes as the reason for wilting) and fitted to Gall Index (GI) scale adopted by Coyne *et al*.(2007). The wilting incidence per farm was also calculated using the formula below:

$$Incidence = \frac{Number\ of\ wilted\ plants}{Total\ number\ of\ plants\ per\ farm\ area\ surveyed} x\ 100$$

Soils from the rhizospheres of infected roselle and okra plants from farmers' fields were collected at a depth of 0-15cm using a soil auger for pot experiment. Top soil was also collected and sterilized in a half drum (55 gallons capacity) metal container, which was covered and heated until steaming was achieved and steaming was maintained for at least 30 minutes thereafter. Sterilized soil was weighed into four plastic pots at 10kg, 9kg 8kg and 7kg, to which rhizosphere soils ("is") from infected crop were added as treatments at 0kg, 1kg, 2kg and 3kg (0 kgis, 1 kgis, 2 kgis and 3 kgis) for each of the two crops, Roselle and Okra. These were replicated three times each, arranged in a completely randomized design

Three seeds of the test crop were sown per pot at the nursery of the College of Agriculture Lafia and later thinned to two for each set. The set up was watered at least twice a week from sowing date of 19th February 2019 until the arrival of rains in mid-April. Normal management practices were carried out. NPK 15:15:15 fertilizer was applied 4weeks after sowing and insecticide was used to spray against insects at recommended rates.

Data was collected on a plant from each pot on plant height from week 2 to week 24. At harvest (mid – August), number of fruits, fruit length and fruit weight were taken. The data was analyzed using SPSS for descriptive statistics and Analysis of variance ANOVA.

RESULTS AND DISCUSSION

Wilt incidence and gall index

Characteristic *Meloidogyne* spp. infestation symptoms like wilting and root galls were observed among roselle, okra, tomato and pepper plants in the surveyed area (Fig. 1).The mean incidences of wilting for all farms surveyed were 2.63, 1.40, 1.83 and 2.30 for roselle, okra, pepper and tomato respectively. The mean root gall indices were correspondingly 3.82, 5.91, 3.01 and 3.71. The occurrence of the root knot disease in all areas studied is similar to earlier reports in other northern states of Nigeria including, Benue, Jigawa, Kaduna, Kano, Katsina, Niger and Sokoto, were RKN disease have been reported in surveys and other studies on roselle, okra, tomato, pepper and other vegetables (Atungwu *et al*., 2013; Kumar *et al*., 2014; Bem *et al*., 2014; Suleiman *et al*., 2015; Ogunsola, *et al.*, 2018).

Okra had the least wilting incidence (1.40 %) while roselle had the highest (2.63 %).Pepper had the least root gall index (3.01) and okra had the highest (5.91). That okra had the highest gall index, meant it had much attack resulting in many galls, yet had the least wilting incidence, implies that the okra varieties had some level of tolerance. This agrees with the findings of Mukhtar *et al*. (2013) that okra cultivars have varying tolerance to root knot nematodes and the conclusions of Atungwu*et al*. (2011), with sunflower cultivars, which had high gall index, but had no yield difference with control set up.The study shows that wilting does not necessarily become directly proportional to gall index and can indicate the tolerance of the crop (crop doing well despite infection). Traditionally, the root gall index has been the principal instrument of root knot damage assessment and with reproductive factor, used to rate resistance or tolerance (Bridge and Page 1980; Coyne *et al*., 2007; Traunfeld, 2017). Many scientists increasingly see yield and other crop performance indicators as important determinants of tolerance and have adopted or called for their use as determinants of resistance and tolerance (Afolami *et al*., 2004; M

The Gall index (3.82) and wilt incidence (2.63%) of roselle were higher than that of both pepper (3.01;1.83) and tomato, (3.71 and 2.30 %) respectively, suggesting that though all are more susceptible than okra. Pepper and tomato have shown more resilience in their response than roselle. Roselle response to root knot nematodes varies in literature, depending on cultivars. Wilson and Menzel (1964) have since discussed this variability in kenaf and roseelle. Vawdrey and Stirling 1992) reported resistance, while Heffers *et al*. (1991) and Adegbite *et al*. (2008), reported susceptibility. However, pepper and tomato are known to have tolerant and resistant cultivars, and in the case of tomato, the Mi gene is known to be responsible for resistance (Roberts *et al*., 1986; Kaloshian*et al*., 1998; Nombella *et al*., 2003, Aminu-Taiwo, 2015).

Plant height of roselle and okra

The plant height of roselle was affected by all treatments (Table1). From week 2 to week 24, 1 kgis, 2 kgis and 3 kgis treatments had lower plant heights than the control, 0 kgis. The 3kg infested soil (3 kgis) recorded the least plant height through all weeks of the study, ranging from 7.47 cm in week 2 to 98.77 cm in week 24; compared to the control, 0 kgis, which recorded between 8.80 cm and 120.33 cm in week 24. The difference in height at the end of the study between the highest treatment 3 kgis and the control is 21.56 cm (or 17.92 % reduction) The plant heights of 3 kgis however did not significantly ($P \leq 0.05$) differ from those of 2 kgis treatments from weeks 2 to 12, but differed from weeks 14 to 24. Except for week 4, plant heights were statically higher for 1 kgis than for 2kgis and 3 kgis between weeks 2 and 12. This means for every 1 kg increase in infested rhizosphere soil, representing increased nematode population, there was increase in damage, which agrees with previous works (Trudgill and Phillips, 1992; Atungwu *et al*., 2011). They were however statistically similar with 2kgis and different from 3kgis treatments from weeks 14 to 24. This implies some level of threshold damage activity, giving the population of 2kgis within the host root that is not too far from what 1 kgis can achieve and only a higher population like the 3 kgis could enable a significantly higher limitation on height. The control (0 kgis) had better height for all weeks than all other treatments, which was significantly ($P \leq 0.05$) better in weeks 2, 6, 8 & 12, but did not differ significantly from 1kgis in week 4 and from weeks 14 to 24. This suggests that for week 14 -24, the "few" nematodes in 1 kg soil may have had their greatest impact and possibly met growth needs for their population and reached or passed the "plateau" phase of population growth. In week 4 however, it may be a balance between the capacity of the nematodes to inflict harm on the plant and the ability of the plant to defend itself against (resist) the invasion which was being activated that caused a lull or slow down on the impact on plant height. Nematode host relationships like these have long been explained in literature (Barker, 1976; Seinhorst, 1986; Ehwaeti *et al*., 2000; Xing and Westphal, 2012).

Plant height of Okra was consistently high in the untreated (control), throughout the study, ranging from 7.10 – 81.67 cm from week 2 to week 24 (Table 2). This was significantly ($P \leq 0.05$) higher than other treatments except for 1kgis at the last week (24) to which it was statistically similar. Week 24 is maturity period for the plant. At a plateau to decline population growth phase, as earlier stated, this can be expected, especially that 1 kgis is the nearest to 0 kgis (starting- population of nematodes).Curiously 1kgis in week 2, 1kgis and 2 kgis in weeks 4 and 8, expected to have lower populations of root knot nematodes (RKNs), had significantly ($P \leq 0.05$) lower plant height than 3 kgis with expectedly higher RKN population. Trudgill and Phllips (1992) explain that nematode population dynamics are density dependent and are influenced by host growth, the reproductive potential of the species and by various environmental factors. The environmental factors, more likely than the others could contribute to this situation. Seinhorst (1986) had reviewed these occurrences, showing that, it is not always that a direct and straightforward relationship between crop performance and nematode population are obtained. From weeks 12 to 14, the height of the three test treatments, 1 kgis, 2 kgis and 3 kgis did not significantly ($P \leq 0.05$) differ from one another. However, from week 16 to the end of the experiment in-week 24, 3 kgis had significantly ($P \leq 0.05$) lower heights than all other treatments. The reduction in height due to the 3 kgis compared to the untreated (0 kgis) was 6.4 cm (or 7.84 %). Odeyemi et al. (2016), found reductions in okra plant height due to *M. incognita* infection ranging from 13.69% to 75.64%. The disparity in ratio may be due to the starting inoculation populations. However, the findings in this study showed that at a threshold population, possibly achieved at week 16 for 3 kgis, the impact was distinct for okra plant height. It again agrees with the damage/population density relationship, which has even been used for modelling crop damage in watermelon (Trudgill and Phillips 1992; Enwaeti, *et al*., 2000)

Number and weight of roselle fruits

Both number and weight of roselle fruits decreased with increasing inoculum of root knot nematode infested soil (i.e. nematode populations) from 0 kgis (control), to 3 kgis (Table 3). The control had the highest number of fruits (30.67) which was significantly ($P \leq 0.05$) higher than all others, while 3 kgis had least number of fruits (10.33), a reduction of 66.32%. However, number of fruits of 1kgis did not differ from that of 2kis; likewise, that of 2kgis did not differ from that of 3kgis, but that of 1 kgis differed significantly ($P \leq 0.05$) from that of 3 kgis. The control, 0 kgis also had the heaviest weight of fruits (278.97g). The 1, 2 and 3 kgis treatments had 195.90, 148.13 and 100.17g fruit weights respectively. The percentage reduction in fruit weight ranged from 29.78% between the control and 1 kgis treatment to 64.09% between the control and 3 kgis. All treatments, 0, 1, 2, and 3 kgis significantly ($P \leq 0.05$) differed from each other descending in magnitude in that order. This result is similar to Vawdrey and Stirling (1992) and Adegbite *et al*. (2008) who obtained a yield reduction of up to 48.7% of roselle due to root knot nematodes.

Fruit weight, length and diameter for okra.

The fruit parameters measured for okra (Table 4,), showed apparent decrease from the control 0 kgis through the highest inoculum, 3 kgis, (fruit length 6.07 cm to 5.13 cm; fruit diameter, 1.40 cm to 1.27 cm) but the difference were not statistically significant except fruit weight (8.40 cm to 5.00 cm or 40.48 %) which was significant ($P \leq 0.05$). Odeyemi *et al*. (2016) similarly found fruit weight to significantly reduce by 12.96% to 53.29%. The absence of significant levels of difference in other fruit parameters can be attributed to the known tolerance of okra cultivars to root knot nematodes making them to perform despite being afflicted by the nematodes (Afolami and Adigbo 1999; Mukhtar *et al*., 2013).

CONCLUSION

The findings of this study overall confirms that root knot nematodes attack four vegetables, roselle, okra, pepper and tomato, in Lafia agreeing with many authors including Atungwu et la., 2013 Kumar *et al*., 2014, Coyne *et al*., 2018 and Sikora *et al*., 2018, that vegetables are subject to attack and their production constrained by root knot nematodes, as they also occur widely in sub-Saharan Africa and in Nigeria (Mingochi and Jensen, 1986; Sikora and Greco 2005, Suleiman *et al*., 2015; Coyne e*t al.*, 2018; Ogunsola *et al*., 2018). The results of the survey showed roselle had the highest wilting incidence (2.63%) and okra had the lowest (1.4%); but also showed that okra had the highest gall index of 5.91 against that of roselle, 3.82. It therefore showed okra had higher tolerance to nematode infection, while roselle is more susceptible. Pepper and tomato are in-between the two.

The affected crops, roselle and okra in the pot studies generally had reductions in growth and fruit parameters. Plant height of roselle and okra declined from 120.33 cm and 81.67 cm in the control to 98.77 cm and 75.27 cm. in the highest treatment 3 kgis, respectively. Roselle had 17.92 % reduction in growth while okra had 7.84 % growth loss. The numbers of fruits for roselle were even more affected, reduced by 66.32%., while its fruit weight reduced by 29.78%. Of three fruit parameters taken for okra, only fruit weight, had a significant reduction of 40.48 %, the other two, fruit length and diameter, did not significantly differ at the two extremes of treatments. This study shows all the vegetables and more crucially the two further studied, respond differently to root-knot nematode attack or disease.

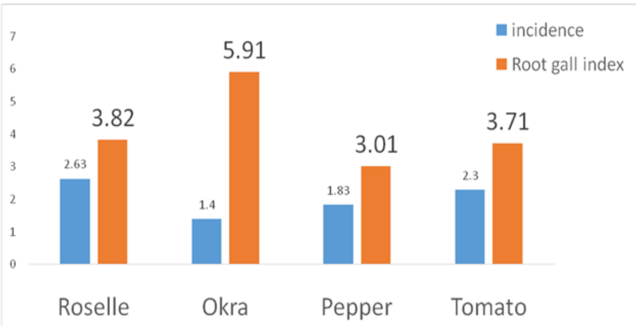

Figure 1: Mean wilting incidence (%) and mean Root gall index of Roselle, Okra, Pepper and Tomato in Lafia Nasarawa State.

Table 1: Plant height of roselle treated with various levels of nematode infested rhzospere soil

Treatment	2WAS	4WAS	6WAS	8WAS	10WAS	12WAS
3 kgis	7.47±0.404^{c}	11.60±0.557^{b}	16.90±0.777^{c}	23.27±1.105^{c}	29.53±1.135^{c}	39.30±0.351^{c}
2 kgis	7.60±0.328^{c}	12.00±0.529^{b}	17.10±1.159^{c}	23.67±1.450^{c}	30.07±0.968^{c}	39.60±1.115^{c}
1 kgiss	7.89±0.296^{b}	12.30±0.173^{ab}	18.00±0.404^{b}	24.87±0.612^{b}	32.50±0.289^{b}	41.83±0.664^{b}
0 kgis	8.80±0.153^{a}	13.80±0.265^{a}	20.00±0.577^{a}	26.67±0.882^{a}	34.43±1.445^{a}	42.57±4.081^{a}
LSD	0.692	0.719	3.349	1.454	2.179	2.545

Means followed by different letters within the same column are significantly different at 5% level of probability.
WAS = Week after sowing   kgis = Kilogram nematode infested rhizosphere soil

Table 1: Plant height of roselle treated with various levels of nematode infested rhizospere soil continued

Treatment	14WAS	16WAS	18WAS	20WAS	22WAS	24WAS
3 kgis	49.67±1.281^{c}	60.30±2.511^{c}	70.87±3.149^{c}	79.33±4.145^{c}	90.87±3.779^{c}	98.77±8.694^{c}
2 kgis	51.90±1.358^{b}	63.00±1.365^{b}	72.83±1.386^{b}	81.33±2.444^{b}	91.43±2.033^{b}	105.40±8.072^{b}
1 kgis	55.70±3.323^{ab}	65.67±5.044^{ab}	75.77±4.504^{ab}	83.03±3.562^{ab}	95.43±3.175^{ab}	113.50±5.594^{ab}
0 kgis	59.00±1.528^{a}	68.17±1.497^{a}	77.13±2.267^{a}	86.90±1.646^{a}	97.77±0.769^{a}	120.33±7.535^{a}
LSD	5.737	4.801	4.170	5.499	5.689	15.067

Table 2: Plant height of okra treated with various levels of nematode infested rhizospere soil

Treatment (Rhizosphere soil)	2WAS	4WAS	6WAS	8WAS	10WAS	12WAS
3 kgis	6.47±0.371^{b}	10.33±0.088^{b}	14.73±0.536^{b}	21.10±1.601^{b}	27.17±1.954^{b}	33.37±1.821^{b}
2 kgis	6.33±0.240^{b}	9.77±0.260^{c}	14.77±0.623^{b}	19.37±0.829^{c}	26.40±1.301^{b}	32.60±1.222^{b}
1 kgis	5.83±0.267^{c}	9.83±0.726^{c}	14.50±0.874^{b}	20.20±0.964^{c}	26.40±1.127^{b}	33.90±1.266^{b}
0 kgis	7.10±0.493^{a}	11.50±0.265^{a}	16.13±0.521^{a}	22.00±1.850^{a}	29.57±1.273^{a}	35.60±2.166^{a}
LSD	0.379	1.036	0.654	0.773	1.014	1.253

Means followed by different letters within the same column are significantly different at 5% level of probability.   WAS = Week after Sowing

Table 2: Plant height of okra treated with various levels of nematode infested rhizospere soil Continued

Treatment (Rhizosphere soil)	14WAS	16WAS	18WAS	20WAS	22WAS	24WAS
3 kgis	38.13±2.085^{b}	43.43±2.285^{c}	52.97±3.017^{c}	62.07±2.359^{c}	66.83±4.310^{c}	75.27±4.390^{c}
2 kgis	38.07±0.968^{b}	44.77±1.534^{b}	54.40±2.261^{b}	63.07±3.123^{b}	68.50±3.342^{b}	77.20±4.842^{b}
1 kgis	38.97±1.244^{b}	45.00±1.539^{b}	54.87±1.605^{b}	63.33±1.856^{b}	69.10±1.193^{b}	79.97±3.183^{ab}
0 kgis	41.23±1.962^{a}	48.00±1.000^{a}	56.43±1.560^{a}	64.13±1.954^{a}	72.33±3.275^{a}	81.67±4.842^{a}
LSD	2.612	1.958	2.225	1.015	1.465	4.604

Table 3: Number and weight of fruits per pot for roselle with various levels of nematode infested rhzospere soil

Treatment	Numberof fruits	Mean weight (g)
3 kgis	10.33±2.728^{c}	100.17±29.272^{d}
2 kgis	15.66±2.333^{bc}	148.13±22.632^{c}
1 kgis	20.33±3.528^{b}	195.90±32.439^{b}
0 kgis	30.67±3.180^{a}	278.97±22.972^{a}
LSD	8.110	86.185

Means followed by different letters within the same column are significantly different at 5% level of probability.

Table 4: Fruit weight, length and diameter of okra with various levels of nematode infested rhizospere soil

Treatment	Mean weight per fruit (g)	Fruit length (cm)	Fruit diameter (cm)
3 kg inoculants	5.00±0.153^{c}	5.13±0.176^{a}	1.27±0.067^{a}
2 kgis	6.20±0.723^{b}	5.37±0.318^{a}	1.33±0.088^{a}
1 kgis	6.57±0.467^{b}	5.70±0.153^{a}	1.33±0.088^{a}
0 kgis	8.4 3±1.073^{a}	6.07±0.384^{a}	1.40±0.058^{a}
LSD	1.208	0.365	0.152

Means followed by different letters within the same column are significantly different at 5% level of probability

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