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LABORATORY SCREENING FOR INFECTIVITY OF SELECTED INDIGENOUS ENTOMOPATHOGENIC NEMATODE ISOLATES ON TUTA ABSOLUTA IN KENYA

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ABSTRACT

The American leaf miner (Tutaabsoluta)important invasive pest of tomato in Kenya. The pest infests the crop at any growing stage causing yield loss of up to 100%. Due to limited pest management options, farmers are currently using synthetic pesticides despite their negative effects to the environment. Infectivity of five indigenous entomopathogenic nematode (EPN) isolates (TK1, S86, 97, 75 and R52) on 2nd and 3rd larval stages of T. Absolute was evaluated under laboratory conditions. The larvae were subjected to different EPNs concentrations (100, 150, 200, and 250/ml) and the control. Larval mortality (infectivity) was recorded at 24 hour interval up to 120 hours. All the tested EPN isolates infected and reproduced within T. absolute. IsolateTK1 caused the highest mortality (100%;92±5%) while isolate 97 recorded the least mortality (40±2%; 48±8%), on 2nd and 3rd larval instars respectively at concentration 100 in the48 hour exposure time. Larval mortality increased overtime with isolate 97 recording >80% increase by the 120 hour. A significant difference($P<0.05$)in mortality between EPN isolates and different time intervals was recorded. There was no significant difference ($P>0.05$) between mortality of 2nd and 3rdT. absoluta instars. The findings of this study revealed that the five EPN isolates were infective to the T.absoluta larvae. The five EPN isolates are recommended for further development for use in the integrated management of T. Absoluta

Keywords: Tomato, Tutaabsoluta, Entomopathogenic nematodes, Infectivity, Infective juveniles

1. INTRODUCTION

Tomato (*Solanum lycopersicum*L.) belongs to the family Solanaceaeand is one of the most known and grown vegetables globally. In 2013, world tomato production was 163.4 million tonnes (Asgedom*et al*., 2011). The production of tomato is faced with many constraints includingdiseases and insect pests(Waiganjo*et al*., 2013;Wakil, *et al*., 2018). In the recent past,a devastating pest, the tomato leafminer (*T. absoluta*)has gained importance mainly inthe cultivated Solanaceae plants, tomato being the major host. The pest is native to South America from where it has spread to Europe, Asia, Middle East, East and South Africa (Desneux*et al.*, 2011; Retta and Berhe, 2015; Kichaoui*et al*, 2016; Mutamiswa*et al*., 2017; Visser*et al*., 2017;). The pest can cause economic losses of up to 100% in out and indoor tomato production (Retta and Berhe, 2015; Mutamiswa*et al*., 2017). In Kenya, *T. absoluta* was first reported in 2013

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inIsiolo, Embu, Meru, Garissa, Wajir and Marsabit Counties from where it has spread to all the tomato growing regions in the country. The pest is believed to have entered the country from Ethiopia where it had been reported earlier (Daily Nation, 2014).The current management of *T. absoluta* in most parts of the world is mainly by chemicalwith farmers using 8-25 sprays on tomato in a season (Gozel and Kasap, 2015; Retta and Berhe, 2015; Roditakis*et al*., 2015; PCPB, 2018). Use of synthetic pesticides poses health and environmental safety concerns. Also effectiveness of chemical control is limited due to *T. absoluta* nature of damage and its ability to develop insecticide resistant strains (Roditakis*et al*., 2015; USAID, 2016). Thus, there is need for integratedapproachesin the management of *T.absoluta*.

This study evaluates the effectiveness of entomopathogenic nematodes (EPNs) for biological control of *T.absoluta*. The EPNs are free living roundworms in the family Steinernematidae and Heterorhabditidae that parasitize and kill many insect species (Hoctor, 2013; Valadas*et al*., 2013, Nikdeland Niknam, 2015, Garcia-del-Pino*et al*., 2018). These nematodes were first reported in Kenya in 1997 after a survey in Coastal and Central region (Waturu, 1998).Use of EPNs in pest management has been mainly on soil dwelling insects but over time research shows their potential use against foliar feeding pests (Arthurs *et al*., 2004; Lacey *et al*., 2015; Nikdel, 2015). Effectiveness of EPNs in pest management depends on lifecycle, environmental conditions during application, range of hosts, foraging ability and matching of species among others (Nikdeland Grewal *et al*., 2005a; Lacey and Georgis, 2012; Garcia-del-Pino*et al*., 2013;Hoctor, 2013;Niknam, 2015). These nematodes infect hosts by entering the body through orifices or directly through the cuticle in case of *Heterorhabditis* spp. The EPNs then release symbiotic bacteria which reproduces inside the host's body, killing them within 24 to 72 hours (Grewal *et al*., 2001Salvadori*et al*., 2012; Shapiro *et al*., 2012;Hoctor, 2013;Kalia*et al*., 2014;Berbercheck, 2015). Mortality/death is only in pests susceptible to EPNs infectivity. Thus, correct host–nematode combination is vital in enhancing infectivity of nematodes in the pests. Nematode infectivity, is determined by the percentage number of infected pest larvae which is indicated by their mortality (Dobes*et al*., 2012, Berbercheck, 2015). The rate of infectivity is affected by EPNs dosage and time. Low dosage gives reduced host mortality while a high one may lead to infection failure. For *G. mellonella*, a pest commonly used to evaluate EPNs infectivity,a dose of 25 -200 IJs/ larvae is adequate (Woodring and Kaya, 1988; Boffel*et al*., 2000). Indigenous strains of EPNs active against *T. absoluta* represent a reliable alternative to excess and inappropriate use of chemical insecticides for the management of this pest. Successful incorporation of EPNs in IPM is dictated by successful evaluation of novel species or strains for efficacy/infectivity against a target pest (Shapiro-Ilan., 2012). The objective of this study was therefore to determine the infectivity of five indigenous EPN isolates on *T. absoluta*, based on time,dosage and their reproduction potential (progeny recovery).

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2 MATERIALS AND METHOD

2.1 Entomopathogenic nematode (EPN) and *T. absoluta* cultures

The EPNs isolates for this study (S86, 75,97,R52 and TK1) were obtained from a stock maintained at Kenya Agricultural and Livestock Research Organisation(KALRO),Kandara. The EPNs had been earlier isolated from soils in a survey in Central highland counties and Kwale County of coastal region, Kenya (Waturu, 1997). They were reared and multiplied on greater wax-moth (*Galleria. Mellonella*) in the laboratory at 24 ±2^{o}C according to Woodring and Kaya, (1988). Suspensions of isolates Dauver juveniles were drawn from a stock suspension stored at 20°C and conditioned to room temperature for at least 1 hour. Viability of the EPN isolates was assessed compound microscope. A volume of the suspension was diluted with appropriate amount of distilled water. The diluted suspension of each of the EPN isolates was adjusted to 200 nematodes per ml which was added into a 9cm Petri dish padded with a white cotton cloth. Ten pre–pupating larvae of *G. mellonella* were placed in each Petri dish. After five days of incfection (baiting), infective juveniles (IJs) were recovered from *G. mellonella* using modifiedWhite trap method according to White,(1927). The recovered IJswere stored as suspension in 50ml of sterile distilled water in 250ml plastic at 20 ±2^{o}C for later use.

The *T.absoluta* life stages were collected from tomato infested plants from Embu and Kirinyaga Counties. The pest was maintained in tomato crop established in a screen house at KALRO Kandara, from where the larvae were obtained.

2.2 Plate assay forinfectivity of EPN isolates against *T. absoluta*

Insect mortality bioassay was carried out using five different EPN isolate: S86, 75, 97, R52 and TK1. Five larvae of *T.absoluta* were placed singly in a 9cm petri dish lined with white cotton cloth for each replicate per concentration of each isolate. The EPNs were subjected to second and third instars of*T. absoluta*,larvae in a completely randomized design (CRD) with five treatments; zero (control), 100, 150, 200 and 250 per ml of distilled water. In the control treatment only 1ml of distilled water was used to wet the filter papers before placing the *T. absoluta* larvae. Each treatment was replicated five times with five petri dishes for each replicate (N=25). Dead *T. absoluta* larvae were randomly selected from each treatment and dissected under the microscope to confirm mortality due to EPNs. Data on mortality against time and concentration were recorded in each EPN isolate at 24 hour interval for 5days. Data on mortality against exposure time and concentration was analysed using GenStat Computer Package, 15th edition. The means were separated using Fisher's protected least significant difference test at 5% significance level.

3 RESULTS

3.1 Nematode infected fresh *T. absoluta* cadavers

The study EPN isolates infected and caused death of *T.absoluta* as shown in plate 1A and B.

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A B

Plate 1: Nematode infected fresh *T. absoluta* cadaver (A) andInfective juveniles emerging from acadaver (Mg x40)

3.2 Effect of EPN isolate concentrations and time on mortality of *T. absoluta* 2nd instar larvae

The concentration 100 at 24, 48 and72 hours,isolate TK1 had the highest means of mortality of60±8.9, 92±4.9 and 100±0.0% respectively while isolate 97 had the least means (20±12.6, 48±8.0, 60±13, 76±4.0 and 96±4.0same period). Mortality of 100% was observed in isolateTK1 from 72nd hour, and isolatesS86 and R52 from 96th hour(Fig 1A). There was no significant difference (P>0.001) between isolates in all the exposure times for concentration 100.

The TK1 recorded highest mean (60±8.9 to 100%) at concentration 150 in all exposure time although a 100% mortality was reached at 72 hour. Isolate 97 caused least mortality (12±4.9, 36±4.0 and 68±12%)by 72ndhour. All the isolates recorded >50%mortality by the 72 hour (Fig 1.B). There was significant difference (P<0.001) between TK1 with all the other EPNs at 24 and 48 hour.

In concentration 200, the highest mortality mean of 60±11.0% was in S86 in the 24thhour but no significant difference (P>0.001) was recorded from the other isolates. Isolate 97 had the lowest % mortality of 12±8, 44±8 and 68%at 24th, 48th and 72ndhour. There was significant difference (P<0.001) between isolate S86 and 97 and later with the all isolates in the 24thhour. All isolates had a>50%mortality in the 72 hour and 100% by the 96thhour (Fig1.C).

From 24 to 96 hour of exposure, in concentration of 250, EPN 97 caused low mortality (4±4, 40±8.9 and 76±9.8) among the isolates. The isolate was significantly different (P<0.001) from the rest of isolates at 24 and 48 hours only. In the 72ndhour there was significant difference (P<0.001) between EPN 97 and R52, S86 and TK1. More than >50%mortality was registered by the EPNs in the 72nd hour with R52 recording 100%. All isolates attained 100% larval mortality by the 120thhour (Fig1.D).

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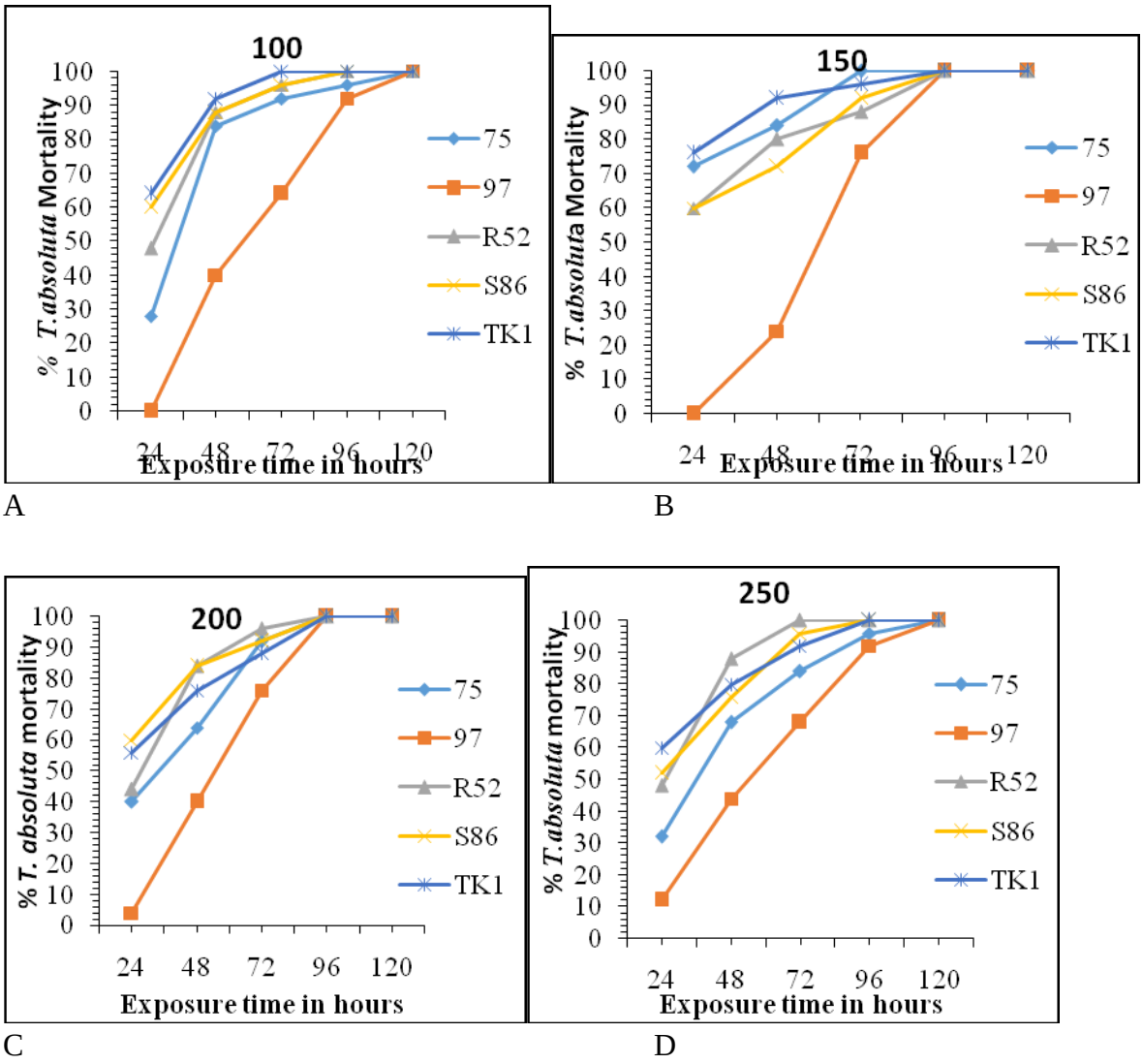

Figure 1: Infectivity of EPN Isolates at different concentrations (A 100, B 150, C 200 and D 250) on 2nd larvae of *T. absoluta*

3.3 Effect of EPN isolate concentrations and time on mortalityof *T. absoluta* 3rdinstar larvae

At concentration 100, at the 24th hour,TK1 had the highest larval mortality of 64±12%, followed by S86, R52, 75 and 97 with 60±0.0,48±4.9% 28±10.2 and 0±0% respectively.There was a significant difference (P<0.001) between isolate 75, 97 There was no significant difference (P>0.001) between R52, S86 and TK1 at the 24th hour (Table 1). In the 48thand 72nd hours of exposure isolate, TK1 had a means of 92±5 and 100±0.0% respectively with a significant difference (P<0.001) between TK1 and 97 in both hours. Mortality of >90 percent in all the isolates was recorded in 96 and 120 hour (Table 1).

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At a concentration 150, on the 24 hour, TK1 caused the highest larval mean mortality 76±0.0%, followed by 75, 97, R52 and S86 causingmortality of 72±14.97, 0±0,60 and 60±10.95% respectively.(what about the others?). There was significant difference (P<0.001) between EPN 97 and all the other isolates. In the 48th hours TK1 led with a mean of 92±0.0% followed by isolate 75, R52, S86 and 97 with mean mortality of 84±7.5, 80±8.97, 72±4.9 and 24±7.5% respectively. The EPN 97 was significantly different (P<0.001) from all the other isolates. In the 72nd hour, highest mean (100±0%) was observed in EPN 75 while isolates, TK1, S86, R57 and 97 the order of means was 96%, 92±8, 88±8 and 76±14.7%. There was significant difference (P<0.001) between isolate 97and isolates TK1 and 75(Table 2). In the 96 and 120 hours mortality was 100 percent in all the isolates (Table 2).

In concentration 200, isolates R52 at hour 24, 48 and 72 gave highest mean larval mortality (64±12,96±4). The isolate was significantly different (P<0.001) from isolate 97 in the 24 and 72 hour and TK1in the 48hour (Table3).All the isolates recordedmeans ≥80% by the 72nd hourbut 100%mortality in 96th hour(Table 3).

On concentration 250, EPNsTK1, S86, R52, 75 and 97 had mortality of 64±4.0, 56±8.0, 52±10.19, 40±8.9 and 0±0 % in the 24th hour(Table 4). Significant difference (P<0.001) was between TK1 and 97, and 75. In the 48 hour, Isolate 97 and 75 had the least means of 40±12.6 and 76±7.5% respectively. There was significant difference (P<0.001) between isolate 97 and other isolates, and isolate TK1 with 97, R52 and S86 in the 48 hour. All the isolates recorded a mean of ≥60% by the 72nd hour but 100% was observed in the 120 hour (Table 4).

Table 1: Effect of EPN isolate concentrations (100) and time on mortalityof *T. absoluta*3rd instar

	Exposure time in hours				
EPN Isolates	24	48	72	96	120
75	28±10.20d	84±7.5 a	92±4.9 a	96±4.0 a	100±0.0 a
97	0±0.0 e	40±14.1cd	64±14.7b	92±4.9 a	100±0.0 a
R52	48±4.9bc	88±8.0 a	96±4.0 a	100±0.0 a	100±0.0 a
S86	60±0.0b	88±8.0 a	96±4.0 a	100±0.0 a	100±0.0 a
TK1	64±11.7b	92±4.9 a	100±0.0 a	100±0.0 a	100±0.0 a
P value	< 0.001	< 0.001	< 0.001	> 0.001	>0.001

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 2 Effect of EPN isolate concentrations (150) and time on mortalityof *T. absoluta* 3rd instar(Mean ± SEM)

Exposure time in hours

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EPN_isolates	24	48	72	96	120
75	72±14.97 de	84±7.48 abcd	100±0 a	100±0.0 a	100±0.0 a
97	0±0 g	24±7.48 f	76±14.70 cde	100±0.0 a	100±0.0 a
R52	60±0.0 e	80±8.94 bcd	88±8.0a bcd	100±0.0 a	100±0.0 a
S86	60±10.95 e	72±4.90 de	92±8.0 abc	100±0.0 a	100±0.0 a
TK1	76±0.0 cde	92±0.0 abc	96±0.0 ab	100±0.0 a	100±0.0 a
P value	< 0.001	< 0.001	< 0.001	> 0.001	>0.001

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 3: Effect of EPN isolate concentrations (200) and time on mortalityof *T. absoluta* 3rd instar (Mean ± SEM)

	Exposure time in hours				
EPN_isolates	24	48	72	96	120
75	52±4.90 ef	88±8 abc	96±4 ab	100±0.0 a	100±0.0 a
97	0±0.0 g	40±8.94 f	80±6.33 bcd	100±0.0 a	100±0.0 a
R52	64±11.66de	96±4.0ab	100±0.0 a	100±0.0 a	100±0.0 a
S86	56±16.0 ef	92±4.90 abc	96±4.0 ab	100±0.0 a	100±0.0 a
TK1	56±7.48 ef	76±7.48 cd	88±8.0 abc	100±0.0 a	100±0.0 a
P value	< 0.001	< 0.001	< 0.001	> 0.001	>0.001

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 4 Effect of EPN isolate concentrations (250) and time on mortality of *T. absoluta*3rd instar (Mean ± SEM)

	Exposure Time in hours				
EPN isolates	24	48	72	96	120
75	40±8.944 f	76±7.483bcd	96±4.0a	96±4.0 a	100±0.0 a
97	0±0.0 g	40±12.649f	68±12cde	100±0.0a	100±0.0 a
R52	52±10.198ef	92±4.889ab	100±0.0a	100±0.0a	100±0.0 a
S86	56±9.798ef	88±8.0ab	96±4.0a	100±0.0a	100±0.0 a
TK1	64±4.0de	68±4.899cde	84±9.798abc	100±0.0a	100±0.0 a
P value	< 0.001	< 0.001	< 0.001	> 0.001	>0.001

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Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

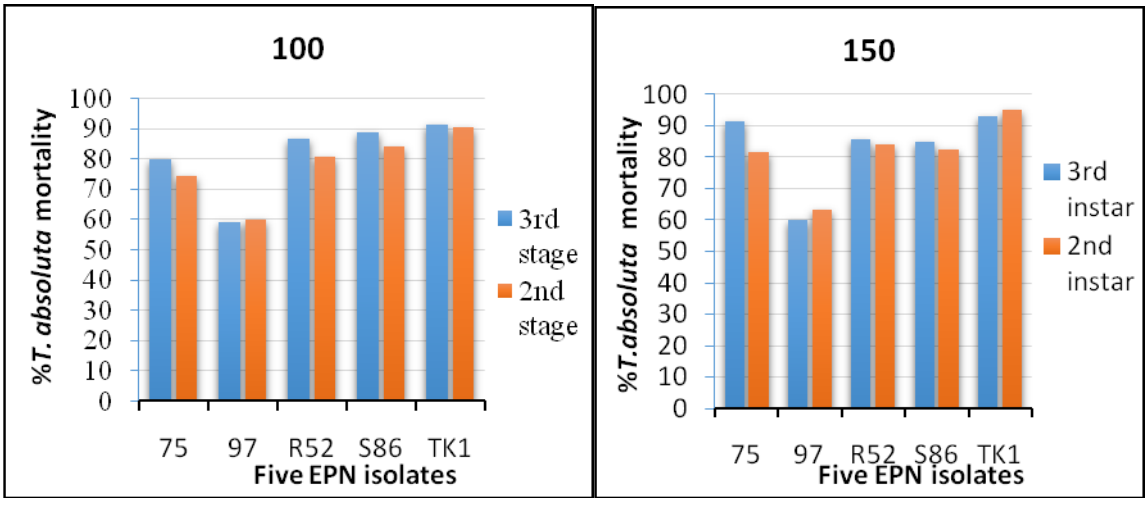
3.4 Comparison between the effects of nematode isolates concentrations on 2nd and 3rd instar larvae of *T. absoluta*

At concentration 100, all the EPN isolates caused mortality >50 % **on 2nd and 3rd instar larvae** The TK1 caused highest % mortality 91.2±3.67 and 90.4±3.67% on2nd and 3rd instar larvae respectively. Lowest % mortality (59.2±8.37 and 60±6.43) was observed in EPN 97 (Fig 2 A). There was no significant difference (P>0.001) between % mean mortality in 2nd and 3rd instar larvae of *T. absoluta* by each EPN isolate.

All the nematodes caused >60 % mortality in the two instars at concentration 150. Nematode TK1 had the greatest % mean mortality of 95.2±2.09 and 92.8±3.24%, while isolate 97 had the least mean of 60±8.87 and 63.2±7.54% in both larval stages (Fig 2B). There was no significant difference (P>0.001) between % mean larval mortality among the EPNs.

Similarly, all the nematodes registered >60% mortality in the two instars at concentration of 200. Nematode R52 recorded highest % mortality of 84.8±4.8 and 92±3.65%. It was followed by nematode S86 with mean larval mortality of 87.±3.98 and 89±4.63 (Fig2C). Isolate 97 was the least infective with mortality of 64±8.0 and 64±8.16%. There was no significant difference (P>0.001) between 2nd and 3rd instar mean mortality among the nematode isolates.

Percent mortality of >63% was recorded at concentration 250 by all EPN isolates. Isolate R52 had the highest %mortality of 87.2±4.45 and 89±4.33, followed by TK1 (86.4±3.6 and 83.2±3.74). Isolate 97 recorded least %mortality of 63.2±7.37 and 63.2±7.37 (Fig 2D). There was no significant difference (P>0.001) between 2nd and 3rd instars % mortality in all EPN isolates.



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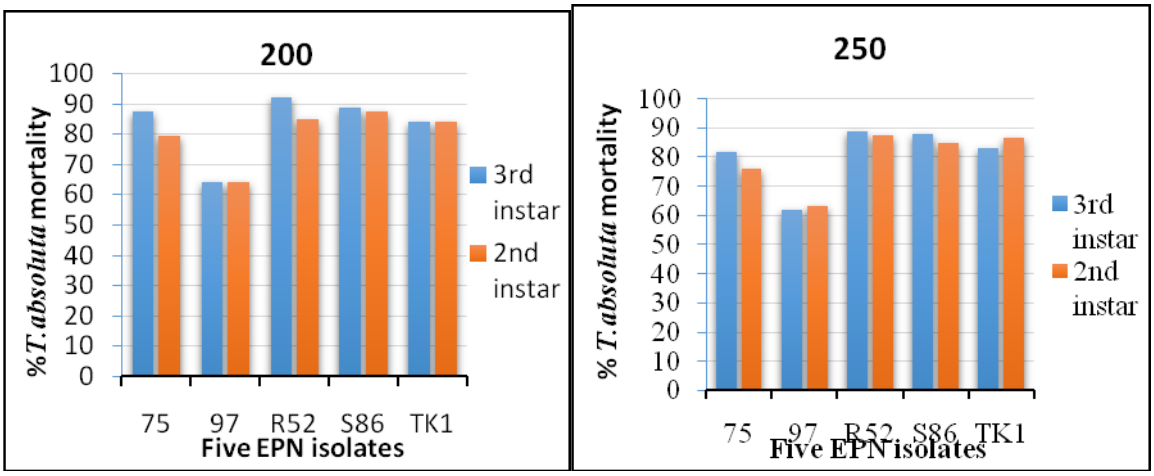
A B



C D

Figure2: Comparison between the infectivity of EPN isolates on 2nd and 3rd larvae of *T.absoluta* at different concentrations (A 100, B 150, C 200 and D 250)

3.5 Comparison between the effect of EPN and time on mortality of 2nd and 3rd instar larvae of *T.absoluta*

At 24hour all the EPN isolates caused mortality on *T. absoluta* apart from isolate 97 on the 3rd larval instar. Isolate 97 caused least % mortality 0 ±0 and 9.6±2.99 while TK1 led with mean of 52±3.35% and 51.2±4.08% on **2nd and 3rd instar** larvae respectively. There was no significant difference (P>0.05) between mortality of the two larval stages by each isolate at 24hour (Table 5).

Isolate 97 had the lowest mean, 28.8±4.63% and 33.6±4.12% withR52 having the highest mortality of 71.2±0.80% only on 3rd instar and TK1 with a mean of 68.8±3.88% on 2nd instar. Apart from isolate 97 all the other isolates caused mortality>50%. There was no significant difference (P>0.05) in mortality of the two instars by each EPN at 48 hour (Table 6).

Isolates R52, TK1, S86 and 75 had mean mortality >65% in both larval instars. The EPN 97 caused least mortality (57.6±3.9 and 54.4±5.88%) though it was >50% at 72 hour. There was no significant difference (P>0.05) in mortality of the two instars by each EPN at 72 hour (Table 7).

All the EPNs caused >70% mean mortality on both instars at 96hour. The R52, TK1, and S86 had the highest mortality of >80% with 97 recording the least mortality (78.4±0.98 and 73.6±1.6%). There was significant difference (P<0.05) in mortality by EPN 97 on 3rd and 2nd larval only (Table 8).

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Percent mortality of 80% was realized by isolatesS86, TK1 and R52 on both larval instars apart from 75 and 97 that had a mean of 79% on the 2nd instar. There was no significant difference (P>0.05) in mortality by all the EPNs on 3rd and 2nd larval instar in the 120 hour (Table 9).

Table 5:Effect of EPN and time on mortality of 2nd and 3rd instar larvae of *T. absoluta* at 24 hour (Mean ± SEM)

Isolate	3rd stage	2nd stage	P-Value
Control	0±0a	0±0a	>0.05
75	38.4± 2.04b	29.6±3.92b	
97	0 ±0b	9.6±2.99b	
R52	44.8±1.50b	36.8±3.44b	
S86	46.4±5.31b	42.4±4.83b	
TK1	52±3.35b	51.2±4.08b	

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 6:Effect of EPN and time on mortality of 2nd and 3rd instar larvae of *T. absoluta* at 48 hour (Mean ± SEM)

Isolate	3rd stage	2nd stage	P-Value
Control	0±0	0±0	>0.05
75	66.4±30a	56±5.66a	
97	28.8±4.63a	33.6±4.12a	
R52	71.2±0.80a	64.8±1.96a	
S86	68±1.79a	61.6±2.99a	
TK1	65.6±1.60a	68.8±3.88a	

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 7:Effect of EPNs and time on mortality of 2nd and 3rd instar larvae of *T. absoluta* at 72 hour (Mean ± SEM)

Isolate	3rd stage	2nd stage	P-Value
Control	0±0a	0±0a	>0.05

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75	76.8±1.497a	68.8±3.666a
97	57.6±3.919a	54.4±5.879a
R52	76.8±1.497a	75.2±1.497a
S86	76±1.265a	74.4±2.04a
TK1	73.6±2.04a	76±2.191a

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 8:Effect of EPNs and time on mortality of 2nd and 3rd instar larvae of *T. absoluta* at 96 hour (Mean ± SEM).

Isolate	3rd stage	2nd stage	P-Value
Control	0±0	0±0	< 0.05
75	78.4 ±0.98a	77.6±1.6a	
97	78.4±0.98a	73.6±1.6b	
R52	80±0a	80±0a	
S86	80±0a	80±0a	
TK1	80±0a	80±0a	

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

Table 9:Effect of EPN and time on mortality of 2nd and 3rd instar larvae of *T. absoluta* at 120hour(Mean±SEM)

Isolate	3rd stage	2nd stage	P-Value
Control	0±0	0±0	>0.05
75	80±0a	79.2±0.8a	
97	80±0a	79.2±0.8a	
R52	80±0a	80±0a	
S86	80±0a	80±0a	
TK1	80±0a		
	80±0a		

Means with different letters are considered significant by ANOVA and post ANOVA Fisher's protected least significant difference test

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4 DISCUSSION

The study results revealed thatthe five indigenous EPNswere infective against *T. absoluta* larvae as they caused larval mortality and infective juveniles recovered. This conforms to observations made byNyasani*et al*., (2007) on 5 EPN isolates against Diamond Backmoth (DBM) larvae and Sudheer and Prabhu, (2008) on*H. indica*and *Steinernema* species in red hairy caterpillar. According to Grewal *et al*., (1993), EPNs may not be infective in some insects due to host resistance making it hard for nematodes to develop and reproduce within the host. It is suggested that the production of proteolytic enzymes by the symbiotic bacteria in the nematodes helps to overcome the problem of host resistance thus making it possible for nematodes to reproduce in insect cadavers.Thus in this study,*T. absoluta*was susceptible and did not have resistance against the study isolates, R52, S86, 75, 97 and TK1. The ability of EPN to infect and reproduce within the host pest ensures persistence and recycling ability in the natural environment. This is critical for effective pest control and commercial production efficiency of EPNs (Kalia*et al*., (2014).

Isolate TK1 was found to be more infective as it caused mortality of between 60 and 90% to the 2nd and 3rd larvae of *T. absoluta* at low concentration (100 and 150IJs) in the first 48 hours of exposure. Isolate 97caused lowest mortality of 0 - 48% at the same exposure time and concentration though infectivity increased with concentration and exposure time. Pesticides with fast knock down effect at low concentration reduces crop damage and is economical to use. The S86, 75 and R52 were found to be cause variable infectivity of *T. absoluta* and they could be belonging to the generalist category of EPNs. Effectiveness of EPN in pest management depends, range of hosts, foraging ability and matching of species among others (Nikdeland Niknam, 2015;Garcia-del-Pino*et al*., 2013; Hoctor, 2013; Lacey and Georgis, 2012; Grewal *et al*., 2005a)

Results indicated that concentration of 200 at 72nd hourresulted in the highest pest mortality in isolates TK1, S86, 75, 97 and R52. Thus a positive relationship between nematode concentration and larval mortality was observed. The positive relationship was attributed to more EPNs penetrating the *T.absoluta*larvae leading to increased mortality. Kalia*et al*., (2014),reported that EPNs reproduction in host pest contribute to recycling ability which is key to effective pest control, commercial production efficiency and for their crucial survival in the environment. Screening for infectivity of nematode species or strain against a specific insect pest is a crucial step toward successful biocontrol strategy.There was a slight decrease in mortality of *T. absoluta*dose 250, probably due to overcrowding of EPNs in the Petri dish and increased competition for entry into the host body, leading to of the nematodes death hence low host infection.

The percentage mortality of *T. absoluta* increased with exposure period in all the EPN isolates. Larval mortality was least at 24 hour and highest at 96 hours but differed among EPN isolates. For instance Isolate 96 caused least mortality (0-20%)in the 24th hour mortality but reached 100% by the 120th hour thus it effectiveness is highly exposure time dependent.Infectivity of EPN differ with exposure time (Mahar *et al*., 2004; Nyasani*et al*., 2007).

Mortality of *T. absoluta* larvae differed among EPN isolates. Isolate 97 in terms of EPN type caused lowest mortality in both larval stages compared to all the other isolates regardless

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concentration and exposure time. Different EPN species or strains behave differently toward host pest. This is in relation to mode of attack, entry point in to the pest, pathogenicity of the symbiotic bacteria harboured and the class of pest being infected. Nematode species contribute to the ability of IJs to penetrate the host where it is through body openings or abrasion through the cuticle in case of *Heterorhabditis*. Again toxins and enzymes related to the symbiotic bacterium of the EPNs are vital in suppression of host pest immunity hence death hence difference in infectivity of EPNs as reported by Cappaert and Koppenhofer, (2003); Lacey and Georgis, (2012); Karimi and Salari, (2015).

5 CONCLUSION

The study demonstrates that indigenous EPNs isolates have potential in pest management. This is so because the EPNs were infective to the larvae of tomato leafminer. Finding indigenous EPNs species adapted to local climatic conditions is believed to be a crucial step for effective biological pest management options. The EPNs can be utilized in the management of *T. absoluta* as biological pest control agents.Proper match of EPN species, dosage and exposure time are important factors for successful utilization of EPNs. Infectivity of the isolates on *T. absoluta* egg and pupal stages be evaluated andidentification of these native EPN isolates to species level is recommended. Also, screen house and field trials to be conducted to verify the laboratory results.

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