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IN-VITRO NEMATICIDAL EFFICACY OF *Lantana camara* LEAF EXTRACT COMBINED WITH ENDOPHYTIC FUNGUS (*Colletotrichum nigrum*) AGAINST ROOT-KNOT NEMATODES

Waswa, Stanlous Juma[1,2,*], Waceke, J. Wanjohi[1], and Maina, M.[1]

[1]Department of Agriculture science and Technology, Kenyatta University,
P.O Box, 43844-00100, Nairobi, Kenya.

[2]Department of Biological and environmental science, Kibabii University,
P.O Box, 1699-50200, Bungoma, Kenya.

*Corresponding Author

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ABSTRACT

Root-knot nematodes (RKNs) are a major threat to crop productivity due to their polyphagous nature. However, hazards due to chemicals have necessitated farmers to search for alternative safe approaches to manage RKNs. This study evaluated nematicidal effects of *Lantana camara* L. leaf extract alone and in combination with endophytic *Colletotrichum nigrum* isolated from roots of tree tomato as an ecofriendly solution to manage RKNs. Extract from powdered *Lantana* leaves (20 grams/100 ml w/v) were evaluated at 25, 50, 75 and 100 % concentrations as standalone and in combination with 1×10^6 spores/ml of *C. nigrum* to leverage on their synergistic effect against second stage juveniles (J2s) of RKNs. One milliliter of each concentration of *Lantana* extract and or 1 ml of 1×10^6 spores/ml of *C. nigrum* were pipetted into sterile eppendorf tubes containing 1 ml of 50 J2s. The control treatment contained 50 J2s in 1 ml of sterile distilled water. Data was analyzed using Anova SAS software version 9.2. *Lantana* leaf extract (100 %) alone caused highest significant ($P\leq 0.05$) mortalities of 89 and 91 % J2s at 72 hours in experiment I and II, respectively, while the same concentration in combination with *C. nigrum* had 81 and 83% mortalities of J2s at 72 hours in both tests, respectively. *Colletotrichum nigrum* alone had 88 and 89 % mortalities at 72 hours relative to control. *Lantana* leaf extract and *C. nigrum* may be used singly or in combination in the RKN management to increase agricultural productivity.

Keywords: Root-knot nematodes, endophytic *Colletotrichun nigrum*, second stage juveniles, *Lantana* leaf extract.

1. INTRODUCTION

Root-knot nematodes are polyphagous pests affecting many crops in tropical and sub-tropical agricultural production systems by reducing the quality and quantity of their yields (Sikora and Fernandez, 2005). RKNs are difficult to manage due to their polyphagous nature, short life cycle and high multiplication rate (Trudqill and Blok, 2001). Several strategies have been used to manage RKNs. Chemical nematicides have been used to significantly control the nematodes in most intensive cropping systems. However, their prolonged use reduces their efficacy, high economic costs and toxicity to man, non-target organisms and the environment necessitates the use of safe alternative measures of control.

Research on plant extracts with nematicidal properties (Rafaat *et al*., 2020; Mahloatjie *et al*., 2019 Feizi *et al*., 2011; Chitwood, 2002) and endophytic fungi has gained attraction over the recent past (Naz *et al*., 2021; Khan *et al*., 2020a; Li *et al*., 2015). Different plant extracts have been evaluated for their nematicidal potential against root-knot nematodes (Bordoloi *et al*., 2021; Ansari *et al*., 2016; Juorand *et al*., 2004; Shaukat *et al*., 2002). Plant extracts contain bioactive nematicidal chemical compounds (Bordoloi *et al*., 2021; Liu *et al*., 2020; Chitwood, 2002). These plant extracts include, organic acids, phenolics, alkaloids, terpinoids and terpenes, coumarins and secondary metabolites (Bordoloi *et al*., 2021; Shaukat *et al*., 2002). *Lantana camara* contains camaric acid, camarinic acid, Lantanolic acid, Linaroside, Oleanoic acid, Lantadene A and B, betulinic acid, Lancamarolide and 11α-hydroxy-3-oxours-12-en-28-oic acid that are known to be nematotoxic (Gebreyohannes *et al*., 2023; Begum *et al*., 2015).

Several endophytic fungi in the genus *Trichoderma* have been used against plant parasitic nematodes (Khan *et al*., 2020b). Endophytic fungi exhibit different modes of biocontrol action which include production of antibiotics and lytic enzymes, competition for space and nutrients, induced resistance, paralysis and mycoparasitism (Latz *et al*., 2018). In antibiosis, they produce metabolites which inhibit nematodes (Khan *et al*., 2020b). These metabolites include alkaloids, phenols, peptides, flavonoids, steroids, quinones, polyketides, terpenoids, and volatile organic compounds (Lugtenberg *et al*., 2016; Latz *et al*., 2018). Secondary metabolites produced by endophytic fungi may be toxic to nematodes. Endophytic fungi also compete with plant pathogens for space and nutrients by colonizing different tissues thereby deriving nutrients and occupy space that could be used by other pathogens (Poveda *et al*., 2020). Presence of endophytic fungi in plants triggers improved defense mechanism against pathogen attack. Endophytic fungi may indirectly interact with pathogens through host plant by inducing plant resistance, that is, systemic acquired resistance-SAR and induced systemic resistance-ISR (Stirrling, 2018; Xiang *et al*., 2018.

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

Mycoparasitism results to one fungus parasitizing another deriving nutrient from it and eventually killing it (Lecomete *et al*., 2016).

Use of nematicidal plant extracts and endonhytic fungi enhances safe and sustainable approach for nematode management. There has been no single method that has successfully managed RKNs and therefore there is need to combine different approaches to come up with sustainable and effective control. Botanicals in combined application with antagonistic fungi have been used in the management of RKNs. *Trichoderma harzianum* in combined application with botanicals of Rape seed, *Lantana*, African marigold and Neem reduced nematode disease parameters in tomatoes (Feyisia *et al*., 2016). Kiriga *et al*. (2018) also reported efficacy of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple in Kenya. Akram *et al*. (2022) demonstrated the efficacy of integrating botanical with biocontrol fungi and velum against root-knot nematode, *Meloidgyne graminicola* on wheat. Integrated application of metam sodium with neem cake and *purpureocillium lilacinum* significantly reduced RKN disease parameters and increased plant growth parameters in cucumber (Thakur *et al*., 2020). Combining *Lantana camara* leaf extract with *C. nigrum* could be utilized in integrated pest management strategies. The objective of this study was to determine the *in vitro* efficacy of *Lantana camara* L. leaf extract alone and in combination with endophytic fungus, *Colletotrichum nigrum* against RKN J2s. Standalone treatments of endophytic fungus, *Lantana camara* leaf extract and their combination could offer a safe ecofriendly alternative method of control compared to harmful chemicals.

2. MATERIALS AND METHODS

2.1 Isolation and preparation of endophytic *Colletotrichum nigrum* from tree tomato roots

Healthy fresh tree tomato roots (*Solanum betaceum* Cav.) were collected from tree tomato farms in Nyandarua County (0.1804ºS and 36.5230ºE) in Kenya. The roots were put into zip-lock bags and transported to Kenyatta University (1.1805º S and 369348º E) Agriculture Laboratory for processing. The roots were sterilized according to Dababat *et al*. (2008) protocol by cutting them into 5 cm length, thoroughly washing with tap water and sterilized with 70 % ethanol for 3 minutes to remove surface epiphytes. The roots were then sterilized in 1.5 % Sodium hypochlorite (NaOCl) for three minutes. Sterilized roots were rinsed three times with sterile distilled water; blot dried using sterile blotting papers and cut into 0.5 cm length using sterile scalpel blades (Dababat *et al*., 2008). Thin roots were sterilized for one minute each in 70 % ethanol and in 1.5 % NaOCl respectively. The 0.5 cm root pieces were evenly placed on potato dextrose agar (PDA) media (39 g of PDA, in 1L of sterile distilled water) that was sterilized for 15 minutes at 121 ºC in an autoclave. The media was amended with 150 mg/l each of streptomycin-sulphate to inhibit bacteria contamination. The PDA plates were sealed with parafilm and incubated at 25 ºC until endophytic

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

fungi emerged. The water from the last rinse of the roots was plated on PDA and incubated at 25 ^{o}C for seven days to evaluate the efficacy of sterilization. To obtain pure cultures, fungal cultures of the isolate were sub-cultured on a fresh PDA media using discs from the leading mycelia margins taken by flame sterilized 5 mm cork borer and incubated for two weeks. Pure cultures obtained were maintained at 4 ^{o}C in the refrigerator before further use.

For J2 mortality tests, spore concentrations were made by flooding the surface of seven-day old pure fungal cultures with 10 ml of sterile distilled water amended with 1 % tween 20. Then, a sterile microscope slide was used to dislodge the fungal spores by gentle scrapping of the mycelia on the surface of PDA media. The contents were then filtered through three layers of muslin cloth and the spore densities determined using a hemocytometer under the microscope (Niranja *et al*., 2009). Sterile distilled water was added to adjust the spore concentrations to 1×10^{6} spores per ml for use. Endophytic *C. nigrum* is eco-friendly and do not present threats like those caused by chemical nematicides. This fungus provides sustainable nematode management strategy as it readily occurs in tissues of its hosts. The fungus confers protection to plants onsite as they reside asymptomatically within plant tissues.

2.2 Rearing of the second stage juveniles of root-knot nematode

The J2s of RKNs were reared on a susceptible tomato variety (Cal-J) for three months in the agriculture greenhouse at Kenyatta University. Galled roots were used to obtain the J2s. An egg mass from a single female was picked using a needle to establish pure cultures for the experiments. The *Meloidogyne* egg mass was put in the sterile 2 sand: 1 soil mixture in pots (12 cm diameter) at the root zone of four-week-old transplanted susceptible tomato plants of cultivar Cal- J. and maintained in the greenhouse. After three months, plants were uprooted, galled roots were washed, chopped into 1cm, macerated in 1.5 % NaOCl solution in a blender and the suspension passed through 500 µm, 106 mµ and 20 µm sieves into a beaker (Hooper *et al*., 2005). The resultant suspension containing RKN eggs was incubated on plates lined with a serviette in darkness for 14 days and freshly hatched J2s were collected from fourth day every two days (Coyne *et al*., 2007). The numbers of freshly hatched J2s per ml were determined under the microscope at ×40 using grid-49 nematode counting dish (Hussey and Barker, 1973). The nematode suspension was adjusted with sterile distilled water to 50 J2s/ml for use in the laboratory. The nematode suspensions were stored at 4^{o}C before use.

2.3 Preparation of *Lantana camara* leaf extract

Mature *Lantana camara* L. (Verbenaceae) leaves were collected from their natural habitat at Kenyatta University agriculture farm along the hedges, dried in the shade to maintain the alkaloids and processed into powder with an electric grinder and kept in air-tight containers. A 20g of the

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

powder was soaked in 100 ml sterile distilled water for 24 hours in a 500 ml conical flask to extract active ingredients. The filtrate was passed through two folds of muslin clothes. The suspensions were then filtered through Whatman No. 1 filter paper and the filtrate was centrifuged at 2400 revolutions per minute (rpm) for 10 minutes. The extracted clear supernatant was treated as a "standard solution'' (100 percent concentration). Using sterile distilled water, suspensions at concentrations of 25, 50, 75, and 100 percent (Taye *et al*., 2012) were made from the standard solution.

2.4 Effect of different concentrations of *Lantana* leaf extracts on mortality of RKN J2s *in vitro*

One milliliter of 0, 25, 50, 75, 100 percent concentrations of *Lanata camara* L. extract were pipetted into sterile eppendorf tubes and 1 ml of juvenile suspension containing 50 freshly hatched live J2s pipetted into each. The treatments were as follows:

i) 25 % *Lantana* leaf extract + 50 J2s
ii) 50 % *Lantana* leaf extract + 50 J2s
iii) 75 % *Lantana* leaf extract + 50 J2s
iv) 100 % *Lantana* leaf extract + 50 J2s
v) Sterile distilled water + 50 J2s

2.5 Effect of combining different concentrations of *Lantana* leaf extracts with *Colletotrichum nigrum* on mortality of RKN J2s *in vitro*

One milliliter containing 50 J2s was pipetted into sterile eppendorf tubes containing 1 ml of varying concentration of *Lantana* leaf extract and 1 ml of 1×10^6 of the endophytic *C. nigrum*. The control treatment had 1 ml of sterile distilled water in eppendorf tubes containing 1 ml of 50 freshly hatched live J2s. The treatments of combining *L. camara* leaf extract with *C. nigrum* were as follows:

i) 25 % *Lantana* leaves extract + *C. nigrum* (1×10^6 spores/ml) + 50 J2s
ii) 50 % *Lantana* leaves extract + *C. nigrum* (1×10^6 spores/ml) + 50 J2s
iii) 75 % *Lantana* leaves extract + *C. nigrum* (1×10^6 spores/ml) + 50 J2s
iv) 100 % *Lantana* leaves extract + *C. nigrum* (1×10^6 spores/ml) + 50 J2s
v) *C. nigrum* (1×10^6spores/ml) + 50 J2s
vi) Sterile distilled water + 50 J2s

The experiments were laid out in completely randomized design (CRD) with four replicates in the laboratory. The dead and active J2s in each treatment were counted after 24, 48, and 72 hours. Nematode J2s were considered dead if they were straight and rigid without exhibiting any response

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

after probing the tail using a mounting needle (Abbasi et al., 2008). The experiments were repeated once. At the end of the experiment, the RKN dead J2s were transferred into sterile distilled water and left for 72 hours to check for any recovery. The dead J2s did not recover from mortality.

Data of dead J2s was converted into percentage mortality before statistical analysis using the formula by Abbot. (1925) as follows:

$$\textbf{Percent J2 mortality} = \frac{\textbf{Dead J2s}}{\textbf{Total J2s}} \times \textbf{100}$$

3. STATISTICAL ANALYSIS

The data was organized in MS excel sheets and subjected ANOVA using SAS version 9.2 computer software. Tukey's Honestly Significant Differences test (HSD, where $P \leq 0.05$) was used to separate the significant means. Regression and correlation analysis were done to evaluate the relationship between mortalities of RKN J2s against concentrations and time of exposure.

4. RESULTS

4.1 Effect of different concentrations of *Lantana* leaf extracts on mortality of RKN J2s *in vitro*

The results showed that different concentrations of *Lantana* leaf extract applied alone were able to kill J2s of RKN after 24, 48 and 72 hours of exposure (Table 1). There was a significant difference ($P \leq 0.05$) in the juvenile mortality treated with different concentrations of *Lantana* leaf extract after 24, 48 and 72 hours in experiment I and II respectively (Table 1). In both experiments the highest mortality of J2s was observed at the 100 % followed by 75 %, 50 %, and 25 % concentrations in decreasing order. *Lantana* leaf extract at 100 % concentrations caused the highest mortality of J2s in experiment I and II after 24, 48, and 72 hours respectively.

Table 1: Effect of different concentrations of *Lantana camara* leaf extract on mortality of RKN J2s *in-vitro*

% Mortality of Root-knot nematode J2s						
	Experiment I			Experiment II		
Concentrations of *Lantana* leaf extract	**24 Hours**	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**
25%	27.00±1.29d	47.00±1.00d	57.0±01.29d	27.00±0.58d	48.00±0.82d	56.50±0.96d
50%	46.00±2.00c	57.00±1.29c	66.50±0.96c	45.50±0.96c	56.50±0.96c	68.00±1.83c
75%	58.00±0.96b	67.50±1.00b	78.50±0.96b	56.00±1.15b	70.00±0.82b	80.00±0.82b
100%	68.00±0.82a	79.00±1.29a	89.00±1.29a	69.00±0.58a	81.00±2.58a	91.500±1.71a
Control(distilled water)	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Data are means ± SE of four replicates. Means followed by different letter(s) in the same column are significantly different according to Tukey's Honestly significant difference (HSD) test at P≤0.05).

On the effect of time, it was observed that the length of time of exposure had a correlation on the rate of mortality of J2s and hence the duration of 72 hours produced the highest mortality of J2s among the concentrations as compared to the control in both experiments (Figure 1 and Table 1).

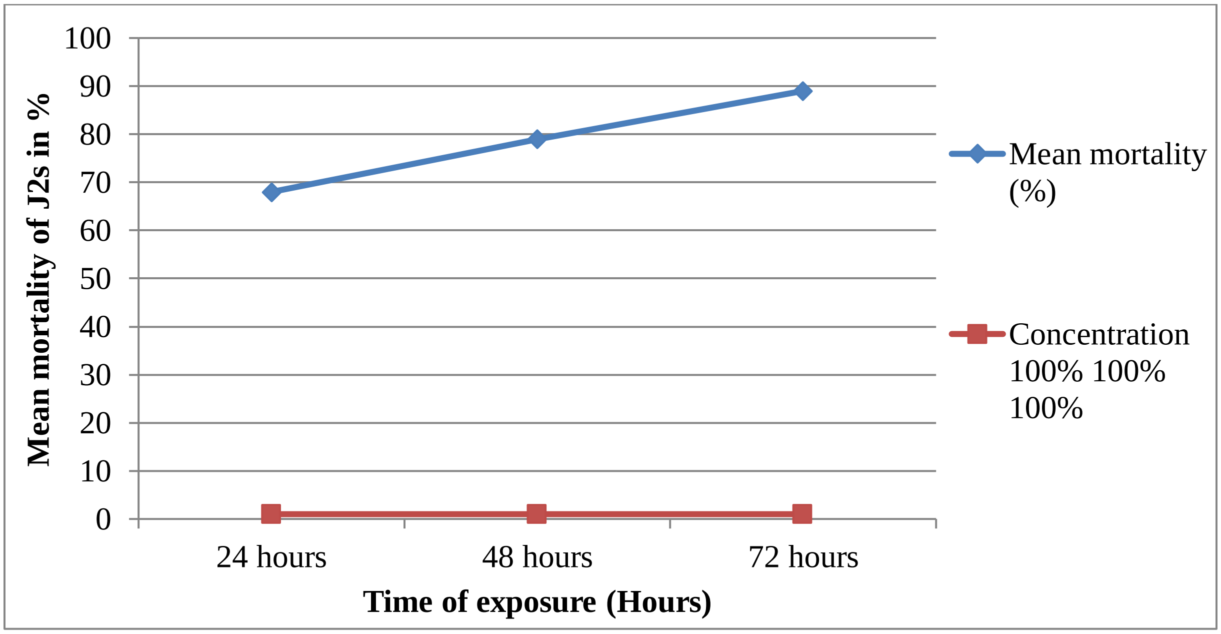

Figure 1: A line graph showing the relatioship between time of exposure to *Lantana* leaf extract and mortality of J2s *in vitro*

In both experiments, there was a general trend of increasing mortality of J2s with increasing *Lantana* leaf extract concentrations and vice versa (Figure 2 and Table 1). The 100% concentrations had the highest mortality of J2s compared to other concentrations (Figure 2).

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

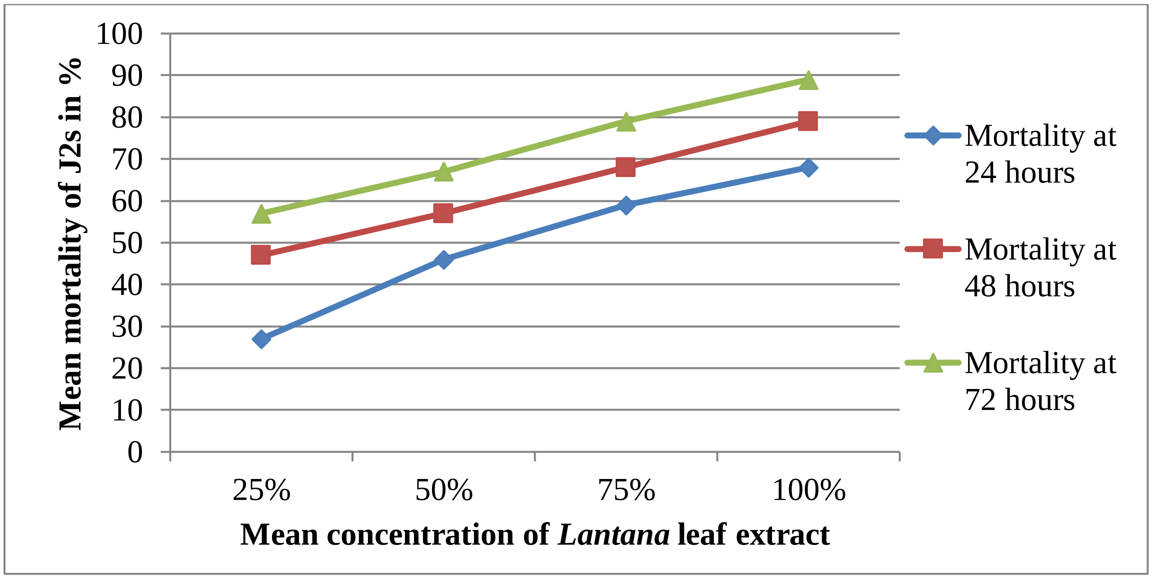

Figure 2: Line graphs showing the relationship between various concentrations of *Lantana* leaf extract and mortality of J2s *in vitro*

Regression analysis revealed a positive linear relationship between *Lantana* leaf extract concentration and the duration of exposure. On further analysis, the time of exposure with regard to mortality of J2s was found to have a significant positive correlation (r=0.91, P≤0.05) with concentration in *in vitro* test (Figure 3) and the same trend was observed in *in vitro* test II.

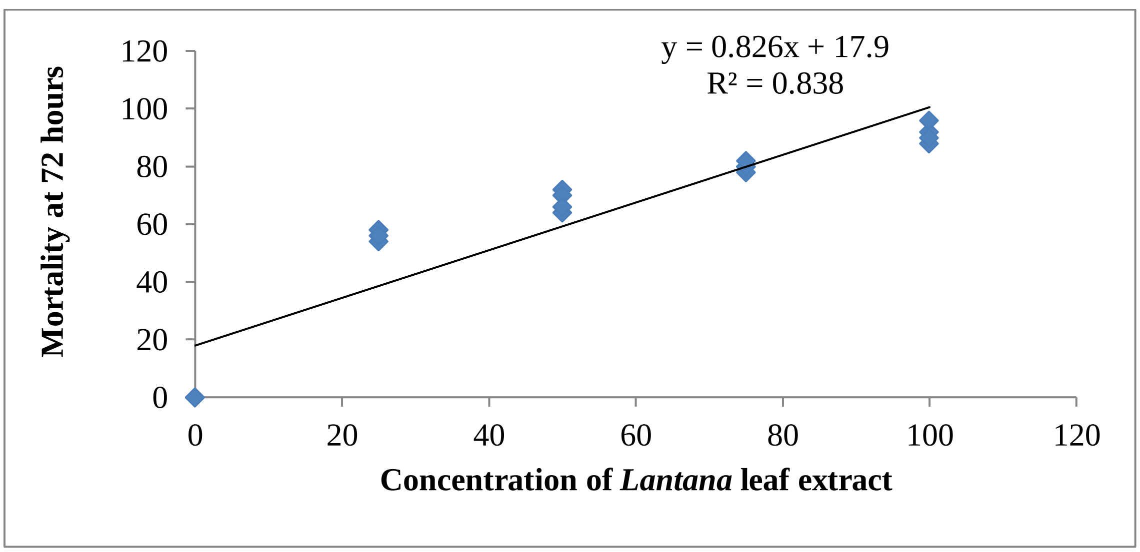

Figure 3: Relationship between the time of exposure and concentration of *Lantana* leaf extract on mortality of J2s *in vitro*

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

4.2 Effect of combining *Lantana* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in vitro*

The various concentrations of *Lantana* leaf extract in combination with *C. nigrum* had significant mortality of RKN J2s after 24, 48, and 72 hours in experiment I and II respectively (Table 2). The 100 % *Lantana* leaf extract in combination with *C. nigrum* had the highest significant ($P \leq 0.05$) mortalities of J2s in both experiments after 24, 48, and 72 hours, respectively (Table 2). The 25 % concentrations of *Lantana* leaf extract in combination with *C. nigrum* had the least mortality of J2s in both experiments.

In comparison the 100 % concentration of *Lantana* leaf extract applied alone had the highest ($P \leq 0.05$) mortalities of J2s in experiment I and II after 72 hours (Tables 1 and 2) as compared to when combined with *C. nigrum* in both experiments after 72 hours of exposure.

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

Table 2: Efficacy of combining *Lantana camara* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in-vitro*

% Mortality of Root-knot nematode J2s						
	Experiment I			**Experiment II**		
Concentrations	**24 Hours**	**48 Hours**	**72 Hours**	**24 Hours**	**48 Hours**	**72 Hours**
25% LE + *C. nigrum*	18.50±0.96e	35.0±01.29d	46.00±2.16e	19.50±0.50e	33.50±1.71e	42.00±1.41e
50% LE + *C. nigrum*	32.00±1.41d	41.50±0.96c	53.00±1.29d	33.00±1.29d	45.00±1.26d	62.50±1.71d
75% LE + *C. nigrum*	45.00±1.29c	63.00±2.08b	69.00±1.29c	44.50±1.71c	57.00±1.29c	71.00±1.29c
100% LE + *C. nigrum*	52.00±4.14b	67.00±0.58a	81.00±1.29b	51.00±1.29b	66.00±1.41b	83.00±1.29b
1×10^6 *C. nigrum*	53.00±1.29a	67.50±3.50a	88.00±2.08a	53.50±2.50a	67.00±0.82a	89.00±2.38a
Control	0.00±0.00f	0.00±0.00e	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00f
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Data are means ± SE of four replicates. Means followed by different letter(s) in the same column are significantly different according to Tukey's Honestly significant difference (HSD) test at P≤0.05. **LE** = *Lantana* leaf extract

The results indicate that duration of exposure to concentrations of *Lantana* leaf extract combined with *C. nigrum* had an effect on mortality of J2s with the 72-hour period producing the highest mortality.

In both experiments, there was a general trend of increasing mortality of J2s with increasing *Lantana* leaf extract concentrations combined with *C. nigrum* and vice versa (Figure 4).

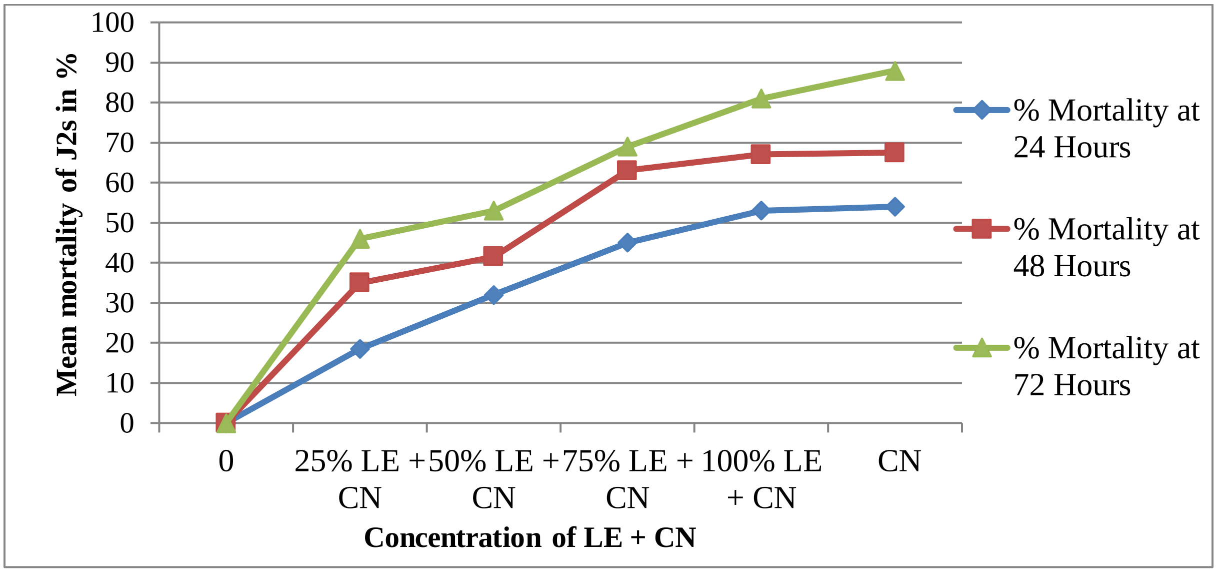

Figure 4: Line graphs showing the effect of various concentrations of *Lantana* leaf extract combined with *C. nigrum* on mortality of J2s *in vitro*

On further analysis, the time of exposure with regard to mortality of J2s was found to have a significant positive correlation (r=0.94, P≤0.05) with concentration in *in vitro* test I (Figure 5) and the same trend was observed in *in-vitro* test II.

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

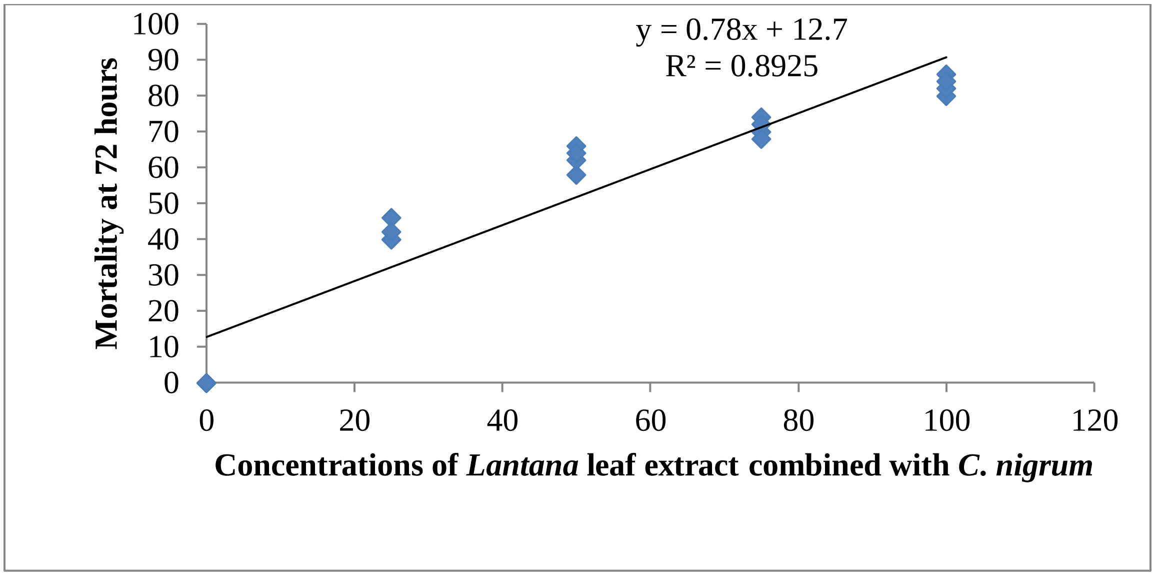

Figure 5: Relationship between the time of exposure to various concentrations of *Lantana* leaf extract combined with *C. nigrum* in *in vitro* test

5. DISCUSSION

5.1 Effect of different concentrations of *Lantana* leaf extracts on mortality of RKN J2s *in vitro*

In-vitro test revealed that 25, 50, 75 and 100 % concentrations of *Lantana* leaf extract had significant mortality of J2s at 24, 48 and 72 hours of exposure (Table 1). The 100 % *Lantana* leaf extract concentration was the most effective against the J2s (Table 1; Figure 2). This could be due to 100 % having the highest concentration of nematicidal compounds. Increase in the time of exposure directly increased the rate of mortality of J2s (Table 1; Figures 3). This could have been due to the fact that the longer exposure of J2s to nematicidal compounds in *Lantana* leaf extract allowed time for maximum action. These findings are in agreement with Feysia *et al*. (2016) who reported 96 % mortality of J2s at 72 hours of exposure to *Lantana* leaf extracts. Khan *et al*. (2019) on evaluation of some botanicals (*Coccinia grandis*, *Commelina benghalensis*, *Leucas cephalotes*, *Phyllanthus amarus* and *Trianthema portulacastrum*) against RKN, *Meloidogyne incognita* on carrot found out that 5000 ppm concentration of the botanical extracts had the highest mortality of J2s compared to 1000 ppm concentration. A research by Bordoloi *et al*. (2021) reported 91.6 % mortality of J2s *in-vitro* in 100 % concentration after 96 hours when working on mechanism of *L. camara* leaf extract in the management of *M. incognita* on tomato. Plant extracts contain nematicidal chemical compounds (Bordoloi *et al*., 2021; Chitwood, 2002). It has been shown that plant extracts contain bioactive compounds with nematicidal effects such as organic acids,

ISSN: 2455-6939

Volume: 10, Issue: 06 "November-December 2024"

phenolics, alkaloids, terpinoids and terpenes, coumarins and secondary metabolites that possess nematicidal properties (Bordoloi *et al*., 2021; Shaukat *et al*., 2002). *Lantana camara* has been shown to release camaric acid, camarinic acid, Lantanolic acid, Linaroside, Oleanoic acid, Lantadene A and B, betulinic acid, Lancamarolide and 11α-hydroxy-3-oxours-12-en-28-oic acid that have nematicidal properties (Gebreyohannes *et al*., 2023; Begum *et al*., 2015). However, Bordoloi *et al*. (2021) reported that lower concentrations (25-50 %) of *Lantana camara* extracts stimulated plant growth on tomato while higher concentrations of 75-100 % inhibited tomato plant growth. The author further noted that peroxidase, polyphenoloxidase and total phenol which are nematicidal were highest in 100 % concentrations and lowest in 25 % and 50 % concentrations. This could explain the highest mortality of J2s in 100 % concentrations (Table1; Figure 2). From these results, it is evident that *Lantana* leaf extract have nematicidal effects and this could be due to the presence of nematotoxic phytochemicals that are soluble in water. Use of plant extracts is an economical and safe method of controlling RKNs. Shaukat *et al*. (2002) showed that plant extracts that contain alkaloids and flavonoids have orvicidal and larvicidal properties on RKN eggs. The reason for *Lantana* leaf extract having nematicidal activity could also be attributed to lipophilic properties of oxygenated compounds which dissolve cytoplasmic membranes of nematodes thus interfering with enzyme protein structure. Plant extracts suppress acetylcholine esterase enzyme activity. These mechanisms could be the cause of nematode J2 mortality in this research.

5.2 Effect of combining *Lantana* leaf extract with *Colletotrichum nigrum* on mortality of RKN J2s *in vitro*

The combination of different concentrations of *Lantana* leaf extract with *C. nigrum* significantly ($P \leq 0.05$) killed J2s although insignificantly lower than when applied as standalone treatments (Table 2; Figure 4). The length of time of exposure was directly proportional to the mortality of J2s (Table 2; Figure 5). The 72 hour exposure allowed enough time for the active compounds in *Lantana* leaf extract and *C.nigrum* to act on the nematodes and hence higher mortality. These results are in agreement with Feysia *et al*. (2016) who reported that the combination of *Lantana* extract with *Trichoderma harzianum* had 66 % mortality of J2s compared to *Lantana* alone which had 96 %. The 100 % concentration of *Lantana* leaf extract combined with *C. nigrum* had the highest mortality of J2s due to the fact that both components contained high concentration of active nematicidal compounds. The reduced mortality of J2s in the combination of *Lantana* leaf extract and *C. nigrum* could be due to either of them or both of them having some level of inhibition to each other. Feysia *et al*. (2016) also found out that botanicals in combination with *Trichoderma harzianum* had lower mortality of J2s as compared to when applied alone after 24, 48 and 72 hours. This is in agreement with the findings of this study where *Lantana* leaf extract applied singly reduced J2 populations more than when combined with endophytic *C. nigrum*. Since the anticipated additive effect against J2s was not achieved, there is need to investigate which compounds both in *Lantana* leaf extract and *C. nigrum* could have interfered with effective synergism. However, the reduction in mortality of J2s in the combined treatments was not significant and therefore co-application of *Lantana* leaf extract with *C. nigrum* has potential against RKNs. *Lantana* leaf extract is known to contain alkaloids and phenolic compounds some of which may have antagonized the fungal metabolites. For conclusive results, the treatments used in these experiments needs to be tested under greenhouse and field conditions.

6. CONCLUSION

The use of *Lantana camara* leaves extract and *C. nigrum* as standalone and in combination was able to paralyze J2s of RKN. *Lantana* leaf extract could be having allelochemicals that are nematicidal while endophytic *C. nigrum* could be possessing secondary metabolites that are toxic to nematodes. *Lantana camara* leaf extract and endophytic *C. nigrum* should be adopted by farmers as an ecofriendly method of managing RKNs. The combined treatment of *Lantana camara* leaf extract and endophytic *C. nigrum* significantly paralyzed root-knot nematodes and is therefore recommended as a safe non-chemical alternative in the management of RKNs. The finding of this study revealed a slight and insignificant antagonism between *Lantana camara* leaf extract and endophytic *C. nigrum*. The level of either of the two components inhibiting each other should be established to ascertain which compounds inhibit each other or others.

Endophytic *Colletotrichum nigrum* should be considered for commercialization and be made available to control nematodes. This endophytic fungus should also be incorporated into breeding programs especially in nursery establishments. Farmers can start using *Lantana* leaf extract against RKNs. *Lantana camara* is readily available and farmers can easily make the leaf extract with minimal instructions for use.

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