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ASSESSMENT OF FOUR LEMON GRASS PHYTOCHEMICALS AS ALTERNATIVE NEMATICIDE FOR ROOT-GALL NEMATODE CONTROL ON TOMATO (*Lycopersicon esculentum* L.)

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ABSTRACT

The pesticidal value of a plant is a function of the phytochemicals it contains. This study evaluated the effectiveness of lemon grass (*Cymbopogon citratus*) phytochemicals on M. incognita infection on tomatoes in in vitro and greenhouse experiments. Results showed that the commercial synthetic nematicide (Furadan), alkaloids, tannins, flavonoids, and saponins all indicated the highest nematode mortality at the highest concentration after 72-hour exposure *in vitro*. However, at the lowest concentration of 10 mg/ml, furadan and alkaloids gave the same results statistically, achieving the highest nematode mortality of 100%. Phytochemicals under screenhouse conditions reduced the number of galls and the root-gall index on tomatoes while enhancing plant growth parameters. Furadan and alkaloids were, however, not significantly ($P<0.05$) different in causing the least reduction recorded in the number of galls and root-gall index on tomato plants inoculated with 1000 infective juveniles (J2s). The impact of Furadan showed a significant decrease in the number of galls from 32.00 to 0.00 and for alkaloids, 34.00 to 0.80 at 5 ml/pot, respectively. The root-gall index was also reduced from the severely galled index of 4.00 in control plants to a "no infection status" of 0.00 on applying Furadan and alkaloids at 5 ml, respectively. Plant heights, number of leaves, shoot weights, and root weight were highest when furadan and alkaloids were applied compared to other phytochemicals and the untreated control. This study, therefore, suggests that *C. citratus*-based phytochemicals possess strong nematicidal effects and can be used effectively in an integrated disease management program against root-knot nematodes.

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to the family Solanaceae. The crop is universally cultivated, cherished, and regarded as one of the world's most consumed vegetable crops. It is a crop widely grown in fields and greenhouses after potatoes. Poojitha (2023) reported that its spread and distribution transcend geographical boundaries, thriving in tropical and temperate regions and being cultivated globally for local consumption and as an important export crop. Tomato is moderately balanced in vitamins, minerals, fiber, protein, essential amino acids, monounsaturated fatty acids, carotenoids, and phytosterols (Chaudhary *et al.,* 2018).

Todate, its nutritive value stands out as an essential ingredient and recipe for raw, cooked, or processed foods (Wang *et al*., 2022)

Despite the several hectares devoted to this invaluable crop, the yield potential has not been achieved in this part of the tropics. Although the yield potential is affected by several factors, including fungi, bacteria, viruses, and nematodes, its susceptibility to attacks by the root-knot nematodes (Meloidogyne spp.) has remained very destructive worldwide (Tsay *et al.,* 2004). The root-knot nematodes (RKNs) are obligate, semi-endoparasites reported to cause annual yield losses of US$125 billion (Moens *et al*., 2009). Its approximate distribution in Nigeria's agricultural soils has been reported to be 75 % (Adegbite and Adesiyan, 2005).

Chemical plant protection with synthetic nematicides and methyl bromide as active ingredients has for several years become the leading management strategy among farmers and scientists to combat the RKNs (Siji *et aI.*, 2010; Cetintas and Yarba, 2010; Desaeger *et al.,* 2020). Studies have shown that the high cost of synthetic nematicides and their hazardous environmental effects have led to a ban on non-target organisms by most African farmers (Adekunle and Fawole, 2003). Several measures have been explored in the management of root-knot nematodes such as crop rotation, planting of resistant varieties, and use of botanicals, amongst others (Siji *et al*., 2010). However, the use of botanicals in the management of *Meloidogyne incognita* is being promoted due to its reported effectiveness and environment-friendliness (Adegbite and Agbaje, 2007; Claudius-Cole *et al*., 2018)

Although some higher plants have been examined as sources of novel compounds with activity against parasitic nematodes (Adesiyan *et al*., 2000), only 6 % have been studied for biological activity, and 15 % among the estimated 4 million plant species investigated phytochemically (Uma, 2015). Over the years, examining plants for novel compounds with nematicidal action has not yielded adequate information or scientifically validated for developing an eco-friendly nematicide. While the use of plant extract as an alternative nematicide is gaining prominence and becoming widespread in use, little is however known about the assessment of lemon grass phytochemicals as an alternative nematicide for the control of root-gall nematode (*M. incognita*) disease in tomatoes.

This study is therefore concerned with assessing lemon grass phytochemicals as an alternative nematicide for the control of *M. incognita* on Tomato in *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Study Location

This study took place during the 2023 agricultural season. It was carried out in the screenhouse and laboratory of the Department of Crop Science and Technology, Federal University of Technology, Owerri, Nigeria, located on Latitude 5° 27' 50.23" North and Longitude 07° 02' 49.33" East, at 55 m above mean sea level.

Plant Materials

Fresh older leaves of Lemon grass were collected from the Teaching and Research Farm of Federal University of Technology, Owerri, and identified at the hebarium of Crop Science and Technology Owerri. The fresh plant leaves were washed thoroughly and air dried at room temperature of 28-30^{o}C under shade. It was

ground into powder using a Thomas Wiley laboratory grinding machine and thereafter sieved through with a laboratory sieve of 212 mm aperture.

Phytochemical Extraction and Isolation

The extraction, phytochemical test, and Isolation followed standard procedure according to A.O.A.C, (2007) and Musa (2015) where the ground powder of lemon grass (30g) was defatted with N-Hexane (250 ml) using Soxhlet extraction. The extraction was conducted for about six hours at a temperature between 65 ˚C and 70ºC. The defatted marc was further extracted with Methanol (250 ml) at 60 ˚C, and the extract's solvent was evaporated in a water bath. Methanolic extract (6g) was chromatographed over Silica gel Column (200g: 230-400 mesh) and eluted with the solvent mixture of (70:30:1, V/V). This procedure was repeated, for Tannins, Saponins, Flavonoids and Alkaloids, where the N-Hexane extract was used. The various fractions were then purified using the recrystallization method.

Test for Tannins (Sofowora, 1984)

Two milliliters of the extract were treated with two drops of 5 % FeC13. The formation of a green precipitate on dilution indicates the presence of Tannins.

Test for Saponins (Trease and Evans, 1995)

The presence of Saponins in the leaf extracts of Lemon grass was tested by boiling 2g of the extract with 20 ml of distilled water in a bath. It was then filtered using filter paper. Ten milliliters of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. Three drops of olive oil were then added to the froth and shaken vigorously again. The formation of an emulsion indicated the presence of Saponins.

Test for Flavonoids (Herbone, 1980)

The plant extract (2 ml) was acidified with 1 % HCl and dissolved in 20 % NaOH. It was observed to be canary yellow in color, which indicates the presence of Flavonoids.

Test for Alkaloids (Trease and Evans, 1995)

Two grams of leaf extracts were warmed with 20 ml of 1% Tetraoxosulphate (VI) acid in a conical flask in a water bath for 2 minutes. It was intermittently shaken and centrifuged to obtain the supernatant. A drop of Meyer’s reagent was added to 0.1 mL of the supernatant in a test tube and observed for a cream precipitate.

Nematode culture

Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 was reared under screenhouse conditions on Tomato (*Lycopersicon esculentum* cv. Roma vf) Eggs were extracted from infected tomato roots with 5 % commercial bleach solution (Hussey and Barker, 1973). Eggs released from the roots were collected on a 450-mesh sieve and transferred into distilled water. Second stage juveniles were obtained by placing egg masses into hatching vessels that were put in petri dishes and adding 4 ml of water. After 1 day, the second stage juveniles were collected from water. Nematode species were identified by perineal pattern morphology (Jepson, 1987). Ten to fifteen adult females were dissected and identified using standard procedure and nematode identification key (Naz *et al.,* 2013a).

Preparation of Phytochemical Dilutions

Phytochemicals (160 mg each) of Lemon grass were diluted in dimethylsulphur oxide (99% DMSO, Merck), and stock solutions (1:1 vv) were

prepared following standard methods (Naz *et al.*, 2013a). The stock solutions were diluted in distilled water, and three final concentrations, 10, 20, and 30 mg/mL, were formed from each stock solution using the formula V1 C1 = V2 C2 (Naz *et al*., 2013b).

Nematicidal Assay (*In vitro*)

Twenty nematode juveniles of *M. incognita* were exposed to each phytochemical concentration in separate and simultaneous *in-vitro* experiments. Treatments were arranged in a Completely Randomized Design (CRD) with three replications respectively. Furadan 3G (Carbofuran) dissolved in 1% DMSO was used as a positive control, whereas inoculum treated with simple distilled water dissolved in DMSO (H_2 0: DMSO) was regarded as a negative control. Efficacy of alkaloids, saponins, tannins, flavonoids and Carbofuran (Standard) were tested against the 20 juveniles (J2s) after 12, 24, 36, 48, 60 and 72 h of incubation at 0 (control) 10, 20, and 30 mg/ ml in the laboratory. All petri dishes were maintained on laboratory bench at ambient temperature (±30 °C). Dead as well as live juveniles (J2) were identified after each incubation duration or period of being exposed to each treatment using an electronic stereomicroscope. Juveniles without any movement or appearing straight were regarded as dead (Pan *et al.,* 2016). Data on J2s mortality was calculated. Percentage mortality = X/N x 100/1. Where X represents number of dead juveniles. N= Origininal number of juveniles (J_2)

Experimental Design and Layout in the Screenhouse (*In vivo*)

Treatments applied comprise Phytochemicals (Factor A); Tannins, Saponins, Alkaloids, Flavonoids, and Furadan (Standard). Rates (Factor B); 0.00 ml (control), 4.00 ml, and 8.00ml at 30 mg/ml concentration. Thus, a 5X3 factorial experiment arranged in Completely Randomized Design with 5 replications was laid out in the screen house thereby giving a total of 75 pots containing 5 kg of steam sterilized soil. The treatments were randomly assigned to the experimental pots of 12.5 cm diameter. Three weeks old tomato (Roma vf) seedlings, raised in nursery trays containing steam sterilized soil, were transplanted into each potted soil. One week after transplanting, all test plants were inoculated with 1000 (J_2) infective juvenile of the nematode and treated with various treatments under study using a syringe calibrated in ml. Inoculated plants without treatment or zero levels of treatment application served as the control. A mean temperature of ±31 ^{0}C was recorded in the soil. Data was collected on plant heights, number of leaves, shoot weights, root weights, number of galls and root-gall index.

Infection Assessment

Root systems were separately assessed according to the severity of root-gall index (Infection) and by using the root-gall rating scale (0-4) for comparison (Agu and Ogbuji,1996). Rating scale: 0 = No galls i.e. infection free. 1 = Rare (1-3 galls), 2 = Mild infection (4-10 galls). 3=Moderate infection (11-30 galls) and 4=Severe infection (over 30 galls).

Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA) using SPSS version 22.0 and GENSTAT Edition 4. Where significant, means were separated using Fisher's Least Significant Difference (LSD) at the 5 % level of probability, respectively.

RESULTS AND DISCUSSION

In vitro Experiment

Nematicidal effect of four lemon grass (*Cymbopogon citratus*) phytochemicals and their concentrations on the mortality of *M. incognita* is shown in Tables (1 to 5). Results revealed a significant ($p<0.05$) effect of phytochemicals of Lemon grass against nematode (J2s) mortality of *M. incognita* at 10, 20 and 30 mg/ml concentration. Interactive effects of all phytochemicals and exposure duration at 12, 24, 36, 48, 60, and 72 h of incubation were also significant. Alkaloids, Tannins, Saponins, Flavonoids and Furadan were all lethal to the nematodes and differed significantly from one another in their effectiveness. The effectiveness of phytochemicals in both aqueous and methanolic extracts of *Azadirachta indica* has been reported by Danahap *et al.,* (2024), who observed that nematode mortality was significantly higher than those of the untreated control at concentrations above 50 mg/ml.

An increase in concentration resulted in increased juvenile mortality. Phytochemicals of lemon grass killed the highest J2s of *M. incognita* at the highest concentration of 30 mg/ml and 72 h of incubation. Donahap *et al*., 2024 also reported 100% nematode mortality at the highest concentration of 300mg/ml. *In vitro* study showed that the nematicidal activities increased with an increase in concentration as well as time of exposure. This agreed with the report of Okechalu *et al*., (2020), who reported similar findings in the leaf extracts of *Ricinus communis* and *Azadirachta indica*. This, however, differed significantly with mortality (12.33/20) recorded in the untreated control group at 72 h of incubation. Among different phytochemicals, the highest (20/20 i.e. 100 %) J2s mortality was attained with Furadan and Alkaloids at the lowest concentration of 10 mg/ml and 60 h of incubation Tables (1 and 2). This was followed by Tannins, flavonoids, and saponins respectively Tables (3 and 5). The efficacy of Alkaloids even at much lower concentrations and exposure is a testament to their effectiveness against root-knot nematodes as reported by other workers (Wang *et al.,* 2012: Ogwudire *et al*., 2022). Kuljanabhagavad and Wink (2009) further stated that the mechanism of action might be related to their cytonematoxic effect.

Nematode mortality (J2s) significantly increased with longer incubation/exposure periods to treatments at the three levels of concentration in all phytochemical treatments. As the contact time increased from 12 to 72 hours, nematode mortality increased irrespective of concentration level. However, at a longer exposure period of 72 hours, all three levels of concentration attained (20/20 i.e. 100 %) the highest nematode mortality. This suggests that longer exposure periods enhance the nematocidal activity of these extracts. This trend could be attributed to the time needed for the bioactive compounds present in the extracts to exert their effects on the nematodes. This though differed significantly with mortality (12/20) recorded in the untreated control group containing distilled water. Interestingly, it required shorter periods of exposure (36 and 60 hours) with carbofuran and alkaloid treatment to attain complete nematode mortality of 100 %.

Table (6) shows the relative potency of nematocidal activity of phytochemical extracts in comparison with the synthetic nematicide Furadan (Standard). The relative

potency (RP) which is a measure of how much of the *Cymbopogon* extract is needed to produce a mortality effect compared to the Furadan was determined. The data in Table (2) therefore underscores the positive correlation between contact time and nematocidal efficacy, showing that longer exposure durations of 12-72h enhanced the Cymbopogon extract's time-kill kinetics ability on *M. incognita* juveniles. The percentages represent the level of nematode mortality or effectiveness in controlling nematodes at different concentrations of phytochemicals and Furadan, as well as in the control group.

The mean relative potency of Furadan and the tested phytochemical extracts at varying concentrations of 10, 20 and 30 mg/ml in order of potency is as follows. Furadan (79.40%, 87.20%, 93.10%), Alkaloids (81.90%, 79.60%, 83.50%), Tannins (81.10%, 79%, 80.60%), Flavonoids (74.50%, 77.30%, 83.50%), Saponins (63.10%, 68.60%, 71.60%). The control group showed a low level of nematode mortality, with a mean of 30%. This represents the natural mortality in the absence of any treatment. This result indicates the comparative effectiveness of Alkaloids and Tannins with the standard synthetic chemical nematicide, Furadan. These results indicate that the phytochemicals have the potential to be effective alternatives to Furadan in the development of eco-friendly nematocidal agents and/or specific nematode species

In vivo Experiment

Results of an *in vivo* study showed that plants treated with various rates of *C. citratus* phytochemicals and carbofuran significantly ($P<0.05$) differed in their ability to control *M. incognita* infection on assessed roots while increasing growth parameters on tomato plants compared to the severely galled untreated control plants Table (7). The significant result obtained with phytochemicals, and carbofuran when compared with the severely galled untreated control, implies that the treatments exerted nematicidal effect that might have suppressed the development of the nematode. This is in line with the work described by other studies, which stated that phytochemicals in tomato plants with nematicidal characteristics increased plant growth (Pakeerathan *et al.,* 2009). More so, phytochemicals of higher plants have been shown to contain nematicidal principles that are lethal to nematodes (Chitwood, 2002).

Furadan and alkaloids achieved 100 % root-gall nematode disease control on tomatoes respectively. The Impact of furadan application showed a significant reduction in the number of galls from 32 to 0.00 while alkaloids from 34 to 0.80 at 5 ml respectively. Again, the root-gall index was also reduced from the severely galled index of (4.00) in control plants to - no infection status of 0.00 on the application of furadan and alkaloids at 5 ml. The 100 % root-gall nematode control attained by furadan and alkaloids on the infected tomato resulted in a no-infection status. This might have been due to their adverse impact on *M. incognita* leading to the reduction in their population to a level that cannot hurt growth and and yield. Thus, the treated plants were able to perform their basic physiological processes aiding growth and yield (Adegbite and Agbaje, 2007).

Although the performances of furadan and alkaloids were statistically the same, they, however, differed significantly ($P<0.05$) from tannins, flavonoids and

saponins at 5 ml. It is, therefore, likely that the effectiveness of furadan and alkaloids over others might have been due to their nematocidal constituents and/or mechanism of action. Furadan has been confirmed by many workers to be an effective nematicidal agent containing the active ingredient carbofuran (Radwan *et al*., 2007; Tanimola and Godwin-Egein, 2009). On the other hand, disruption of protein synthesis, stability of biomembranes and metabolically important enzymes have been attributed to alkaloids' mechanism of action on micro-organisms (Oyedeji *et al*., 2011). The untreated control plants, however, recorded the highest number of galls and root-gall index. The conspicuous giant cells (galls) produced on roots and excessive lateral root growth has been reported as a major symptom of root-knot nematode infection (Castillo *et al.,* 2001).

An increased rate of phytochemical application was observed to have resulted in a decrease in the number of galls and root-gall index. As the rate increased from 2.5 ml to 5 ml, there was a corresponding increase in nematode mortality and consequent reduction in *M. incognita* infection. This agrees with similar findings by Salim *et al*., (2016) who reported that increased concentrations of aqueous extract from 5 to 10 significantly increased mortality of *M. incognita.* However, the highest reduction in the number of galls and root gall index was also recorded with furadan and alkaloids at 2.5 ml. This was followed by other phytochemicals whose nematicidal control significantly differed from plants in the untreated control group. The nematocidal effect of *C. citratus* phytochemicals revealed their pesticidal abilities against root-gall nematode (Chitwood, 2002, Adeniyi *et al*., 2010; Ukpabi *et al*., 2012).

Table (7) also showed that plants treated with various rates of *C. citratus* phytochemicals had reduced root gall incidence and produced significantly ($p<0.05$) higher plant heights than the severely galled untreated control tomato plants. Alkaloid treatment application at 5 ml (31.26 cm) and 2.5 ml (23.45 cm) produced highest plant heights significantly higher than those obtained with furadan at 5 ml (28.39 cm) and 2.5 ml (21.11cm) application respectively. This was followed by tannins, flavonoids and saponins which also controlled root gall nematode disease and gave higher plant heights compared to plants in the untreated control group. The observed increases in plant heights in the treated than untreated could be attributed to the adverse effect of the phytochemical extracts on the activities of the nematodes thereby creating conditions for optimal plant growth (Ihejirika *et al*., 2006). Highest mean plant heights were also attained with alkaloids (21.83 cm), this was closely followed by furadan (20.09 cm) and tannins (18.25 cm). Least mean plant heights were, however, obtained with flavonoids (14.39) and saponins (14.34) respectively.

Several leaves of tomato plants were significantly affected by *C. citratus* phytochemicals and rate at different galling incidences Table (2). Results also showed that plants treated with various rates of phytochemical extracts had reduced root-gall index and produced significantly ($p<0.05$) higher number of leaves than the severely galled untreated control plants. The *M. incognita*- infected tomato treated with carbofuran and *C. citratus* botanical extracts improved the growth of the tomatoes evident in an increased number of leaves at a reduced galling index (Adebowale and Papadopoulos, 2014).

However, the application of alkaloids produced the least galling responses and the highest number of leaves at both 5 ml (43.73) and 2.5 ml (30.07) and they differed significantly from those attained with furadan treatment at 5 ml (36.53) and 2.5 ml (23.33) respectively. This was followed by tannins, flavonoids and saponins. Alkaloids recorded the highest mean number of leaves (28.93), followed by furadan (24.29), tannins (20.28) and flavonoids (16.96). The least mean number of leaves was, however, obtained with saponins treatment (15.61). The least galling responses and highest number of leaves attained with alkaloids might have been due to its adverse effect on the *M. incognita,* thereby creating favorable conditions for nutrient transmission necessary for growth. Alkaloids have been reported to interfere with processes such as DNA replication and RNA transcription which are vital to micro-organisms (Ibrahim *et al*., 2014).

The relationship between *C. citratus* phytochemicals and rates on shoot weights in *M. incognita* infected tomato is shown in Figure (1). Plants in pots treated with both 5 ml (45.04 g) and 2.5 ml (35.38 g) of furadan and 5 ml (44.88 g) and 2.5 ml (33.32 g) of alkaloids attained maximum shoot weights at least galling incidence which significantly differed from other phytochemicals (tannins, flavonoids and saponins) and the untreated plants in the control group. Plants in the untreated control group recorded the least mean shoot weights of 20.69 g compared to mean shoot weights recorded at 2.5 ml (26.15 g) and 5 ml (37.12 g) of treatment application respectively. Application of phytochemicals revealed that shoot weights of tomato were markedly increased with increased rates/dose of application. These results are also in line with the work of Pakeerathan *et al.*, (2009), who reported that phytochemicals in tomato with nematicidal characteristics increased plant growth.

The relationship between *C. citratus* phytochemicals and rates on root weights in *M. incognita* infected tomato is shown in Figure (2). Interestingly, the highest root weights were obtained in the severely galled (4.00) untreated control plants with the highest nematode parasitism (number of galls and root-gall index). This contrasted with the lowest root weights recorded on treated plants. Mukhtar *et al.,* (2013), reported increases in fresh root weights of okra inoculated with nematode as observed in the untreated control. He further stated that increases in fresh weights are directly proportional to the level of inoculation.

However, both furadan at 2.5 ml (6.74 g) and 5 ml (7.33 g) and Alkaloids at 2.5 ml (6.73 g) and 5 ml (7.30 g) produced root weights higher than those produced by tannins, flavonoids, and saponins treatment. Plants in the untreated control group recorded the highest mean root weights of 8.21 g compared to mean root weights recorded at 2.5 ml (6.31 g) and 5 ml (6.83 g) respectively. Apart from the furadan, the performance of alkaloids as biopesticides proved to be most effective by recording less root weight of galls in the roots since the presence of galls in the roots not only increases root weights but prevents adequate water and nutrient uptake resulting in stunted growth and reduced yield. This report is similar to the findings of Ononuju *et al*., (2015) which stated that the number of galls, eggs in the root, and nematode larvae in soil were drastically reduced as a result of termidust- a chemical pesticide application.

Table (1): Effect of Furadan on Mortality of Root-Knot Nematodes (J2) at Different Hours of Exposure

Duration of Exposure (Hours)							
Furadan 3G (mg/ml)	12h	24h	36h	48h	60h	72h	Mean
0 (Control)	0.44	2.33	4.67	6.33	10.00	12.33	6.00
10	5.33	13.33	17.00	18.67	20.00	20.00	16.44
20	5.67	15.67	20.00	20.00	20.00	20.00	17.45
30	7.67	18.33	20.00	20.00	20.00	20.00	18.33
Mean	7.67	12.41	15.42	16.25	17.50	18.08	
$LSD_{0.05}$ (Furadan Conc.)			0.79				
$LSD_{0.05}$ (Exposure Dur.)			0.96				
$LSD_{0.05}$ (Furadan Conc.× Exp. Dur.)			1.93				

Table (2): Effect of Alkaloids on Mortality of Root-Knot Nematodes (J2) at Different Hours of Exposure.

Duration of Exposure (Hours)							
Furadan 3G (mg/ml)	12h	24h	36h	48h	60h	72h	Mean
0 (Control)	0.44	2.33	4.62	6.33	10.00	12.33	6.02
10	5.33	8.00	11.67	14.67	20.00	20.00	13.28
20	5.67	9.33	15.33	16.67	20.00	20.00	14.50
30	7.67	11.67	16.33	18.00	20.00	20.00	15.61
Mean	4.78	7.83	12.00	13.92	17.50	18.08	
$LSD_{0.05}$ (Furadan Conc.)			0.43				
$LSD_{0.05}$ (Exposure Dur.)			0.53				
$LSD_{0.05}$ (Furadan Conc.× Exp. Dur.)			1.05				

Table (3): Effect of Tannins on Mortality of Root-Knot Nematodes (J2) at Different Hours of Exposure.

Duration of Exposure (Hours)							
Furadan 3G (mg/ml)	12h	24h	36h	48h	60h	72h	Mean
0 (Control)	0.33	2.33	4.67	6.33	10.00	12.33	6.00
10	4.67	7.66	13.67	14.67	19.33	20.00	13.33
20	5.67	9.33	13.30	16.67	20.00	20.00	14.22
30	7.33	7.66	15.33	17.33	20.00	20.00	14.61
Mean	4.50	6.75	11.81	13.75	17.33	18.08	
$LSD_{0.05}$ (Furadan Conc.)			0.43				
$LSD_{0.05}$ (Exposure Dur.)			0.53				
$LSD_{0.05}$ (Furadan Conc.× Exp. Dur.)			1.05				

Table (4): Effect of Flavonoids on Mortality of Root-Knot Nematodes (J2) at Different Hours of Exposure.

Duration of Exposure (Hours)							
Furadan 3G (mg/ml)	12h	24h	36h	48h	60h	72h	Mean
0 (Control)	0.33	2.33	4.67	6.33	10.00	12.33	6.00
10	4.33	6.67	9.67	14.67	18.67	20.00	12.34
20	6.67	8.67	13.33	16.33	19.33	20.00	14.50
30	7.67	11.67	16.67	17.67	20.00	20.00	15.61
Mean	4.75	7.35	11.09	13.75	17.00	18.08	
$LSD_{0.05}$ (Flavonoid Conc.			0.48				
$LSD_{0.05}$ (Exposure Dur.)			0.58				
$LSD_{0.05}$ (Flavonoid Conc.×Exp. Dur.)			1.16				

Table (5): Effect of Saponins on Mortality of Root-Knot Nematodes (J2) at Different Hours of Exposure.

Duration of Exposure (Hours)							
Furadan 3G (mg/ml)	12h	24h	36h	48h	60h	72h	Mean
0 (Control)	0.33	2.33	4.67	6.33	10.00	12.33	6.00
10	2.67	5.33	9.33	12.00	16.33	20.00	10.94
20	4.33	7.33	11.67	13.33	18.33	20.00	12.50
30	6.33	9.00	12.33	13.67	18.67	20.00	13.33
Mean	3.42	6.00	9.50	11.33	15.83	18.08	
$LSD_{0.05}$ (Saponins Conc.)			0.54				
$LSD_{0.05}$ (Exposure Dur.)			0.67				
$LSD_{0.05}$ (Saponins Conc.×Exp. Dur.)			1.33				

Table (6): Relative Potency of Nematocidal Activity of Phytochemical Extract in comparison with Furadan 3G (Standard).

Extracts	**Contact Time (hrs)**						
Alkaloid	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
10 mg/ml	84.20%	60.02%	68.65%	78.58%	100%	100%	81.9%
20 mg/ml	57.79%	59.54%	76.65%	83.35%	100%	100%	79.6%
30 mg/ml	65.72%	63.67%	81.65%	90.00%	100%	100%	83.5%
Tannin	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
10 mg/ml	73.78%	57.46%	80.41%	78.58%	96.65%	100%	81.1%
20 mg/ml	63.00%	59.43%	68.35%	83.35%	100%	100%	79.0%
30 mg/ml	62.81%	57.46%	76.65%	86.65%	100%	100%	80.6%
Saponin	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
10 mg/ml	42.18%	39.99%	54.88%	60.00%	81.65%	100%	63.1%
20 mg/ml	48.11%	46.78%	58.35%	66.50%	91.65%	100%	68.6%
30 mg/ml	57.07%	49.10%	61.65%	68.35%	93.35%	100%	71.6%
Flavonoid	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
10 mg/ml	68.40%	50.04%	56.88%	78.20%	93.35%	100%	74.5%
20 mg/ml	66.67%	55.33%	66.65%	81.65%	93.35%	100%	77.3%
30 mg/ml	65.72%	63.67%	83.35%	88.35%	100%	100%	83.5%
Furadan	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
10 mg/ml	31.50%	66.50%	85.00%	93.35%	100.00%	100%	79.4%
20 mg/ml	45.00%	78.35%	100.00%	100.00%	100.00%	100%	87.2%
30 mg/ml	58.35%	100.00%	100.00%	100.00%	100%	100%	93.1%
Control	**12 hrs**	**24 hrs**	**36 hrs**	**48 hrs**	**60 hrs**	**72 hrs**	**Mean**
C	1.65%	11.65%	23.35%	31.65%	50.00%	62%	30.0%

Relative Potency= MT/MB X 100/1. Where MT= Mortality by Test extract, MB= Mortality by Standard (Furadan 3G).

Table (7): Effect of C. citratus Phytochemicals and Rates on plant heights, number of leaves and M. incognita infection on Tomato

Growth Parameters									Root infection Assessment							
Phytochemicals	Plant height (cm)			Mean	Number of Leaves			Mean	Number of galls			Mean	Root-gall index (0-4)			Mean
	Rates (ml)				Rates (ml)				Rates (ml)				Rates (ml)			
	0 Control	2.5	5		0 Control	2.5	5		0 Control	2.5	5		0 Control	2.5	5	
Alkaloids	10.77	23.45	31.26	21.83	12.99	30.07	43.73	28.93	34.00	3.00	0.80	12.60	4.00	0.40	0.00	1.47
Flavonoids	10.74	12.52	19.92	14.39	13.13	16.53	21.20	16.96	39.00	13.20	8.40	20.20	4.00	2.40	2.00	2.80
Furadan	10.77	21.11	28.39	20.09	12.99	23.33	36.53	24.29	32.00	0.80	0.00	10.93	4.00	0.40	0.00	1.47
Saponins	10.73	14.87	17.41	14.34	12.95	14.40	19.47	15.61	41.80	26.80	19.80	29.47	4.00	3.00	2.40	3.13
Tannins	10.71	18.97	25.09	18.25	12.97	20.40	27.47	20.28	36.20	7.60	5.00	16.27	4.00	1.80	1.40	2.40
Mean	10.74	18.18	24.41		13.01	20.95	29.68		36.60	10.28	6.80		4.00	1.60	1.16	
LSD $_{0.05}$ (Phytochemical)		0.53				1.26				2.41				0.31		
LSD $_{0.05}$ (Rates)		0.41				0.98				1.87				0.24		
LSD $_{0.05}$ (Phytochemical X Rates)		1.91				2.20				4.18				0.54		

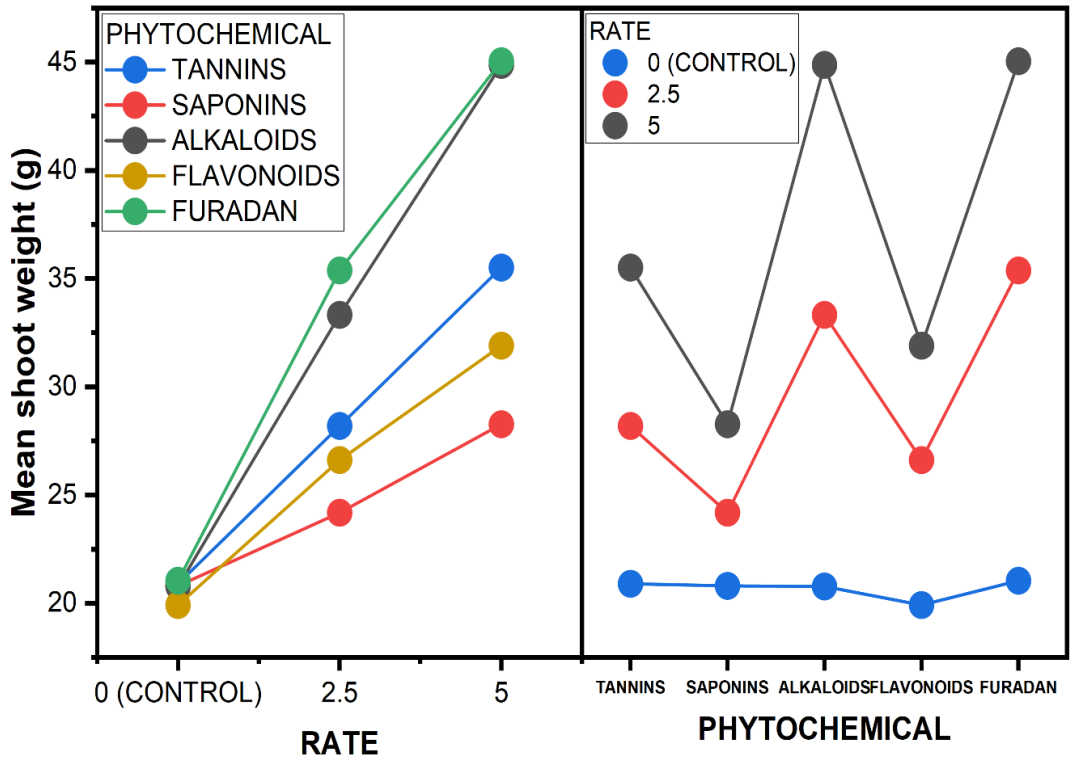

Figure (1): Relationship between *C. citratus* phytochemicals and rates on shoot weight in *M. incognita* infected Tomato

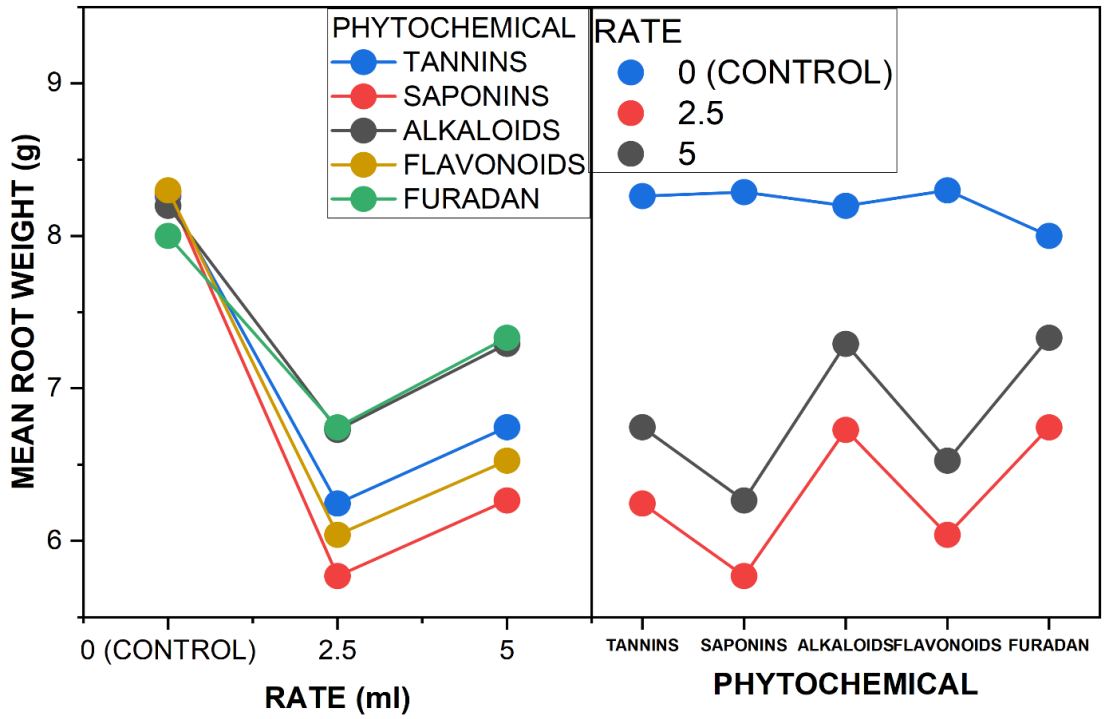

Figure (2): Relationship between *C. citratus* phytochemicals and rates on root weights in *M. incognita* infected Tomato

CONCLUSIONS

This present study has shown that Lemon grass (*C. citratu*s) contains bio-active compounds with nematicidal activity against the root-knot nematode *M. incognita*. Alkaloids, Tannins, Flavonoids and Saponins phytochemicals all controlled nematode parasitism and improved growth of tomatoes in both *in vitro* and *in vivo experiments*. However, it can be concluded that alkaloids comparatively attained a 100 % level of nematode control with the commercial synthetic nematicide furadan, while boosting the growth parameters of tomato. Given these promising results, synthetic nematicide such as furadan should be de-emphasized considering the hazards caused to man and the environment. Current study therefore suggest that phytochemicals could be useful in the development of new environmentally friendly and sustainable nematicidal agent. Further study is required, required on the type of action, mechanisms, and activity relationships in the suppression of nematodes with phytochemicals of *C citratus.* Field experiments can also be carried out to consolidate the gains of this work.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

تقييم أربع مركبات كيميائية نباتية من عشبة الليمون كبديل لمبيدات النيماتودا لمكافحة نيماتودا العفص الجذري في الطماطم (*Lycopersicon esculentum* L.)

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الخلاصة

قيمة المُبيدة للنبات تعتمد على المواد الكيميائية النباتية التي يحتويها. قيّمت هذه الدراسة فعالية المواد الكيميائية النباتية لعشبة الليمون (Cymbopogon citratus) في إصابة الطماطم بدودة الخيطية (M. incognita) في التجارب المعملية وفي البيوت المحمية. أظهرت النتائج أن مُبيد النيماتودا الصناعي التجاري الفوردان (Furadan)، والقلويدات، والعفص، والفلافونويدات، والسابونين، جميعها أظهرت أعلى معدل وفيات للنيماتودا عند أعلى تركيز بعد 72 ساعة من التعرضفي المختبر. ومع ذلك، عند أدنى تركيز (10 ملغ/مل)، أعطى الفوردان والقلويدات النتائج نفسها إحصائيًا، محققين أعلى معدل وفيات للنيماتودا بنسبة 100%. قللت المواد الكيميائية النباتية في ظروف البيوت المحمية من عدد العقد ومؤشر عقد الجذور على الطماطم، مع تحسين

معايير نمو النبات. ومع ذلك، لم يكن هناك اختلاف معنوي (P <0.05) بين الفوردان والقلويدات في التسبب في أقل انخفاض مسجل في عدد العقد ومؤشر العقد الجذرية على نباتات الطماطم الملقحة بـ 1000 من صغار النباتات المصابة (J2s). أظهر تأثير الفيرودان انخفاضًا كبيرًا في عدد الأورام من 32.00 إلى 0.00 وللقلويدات من 34.00 إلى 0.80 عند 5 مل/وعاء، على التوالي. تم تقليل مؤشر الجذور المتورمة أيضًا من مؤشر التورم الشديد البالغ 4.00 في النباتات "عدم وجود عدوى" البالغة 0.00 عند تطبيق الفيرودان والقلويدات بتركيز 5 مل، على التوالي. كانت ارتفاعات النباتات، وعدد الأوراق، وأوزان البراعم، ووزن الجذور هي الأعلى عند تطبيق الفيرادان والقلويدات مقارنةً بالمبيدات النباتية الأخرى والمجموعة الضابطة غير المعالجة. تشير هذه الدراسة إلى أن المواد الكيميائية النباتية المستخلصة من C. citratus تمتلك تأثيرات قوية مضادة للديدان ويمكن استخدامها بفعالية في برنامج إدارة متكامل للأمراض ضد ديدان الجذور العقدية.

الكلمات المفتاحية: *Cymbopogan cytratus*، Meloidogyne incognita، الطماطم، المواد الكيميائية النباتية، دودة العقد الجذرية.

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