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The Inhibitory Efficacy of Plants Extracts in the Management of Root Knot Nematode (*M. Incognita*) Infected Crops

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Abstract

This in vitro study is to establish phytochemicals and the nematicidal potential of organic extract from Datura stramonium and Gongronema latifolium on egg hatch inhibition of M. incognita for an effective root knot nematodes control. The tested botanical extract were found to possess alkaloids, flavonoids, saponins, steroids, phenols, terpenoids and glycosides in different composition as shown from the phytochemical analysis of the extracts. The egg hatchability test shows that the extracts were very effective in inhibiting egg hatching in all concentrations as compared to the control of egg hatch percentage inhibition was at the level of dilution and time of exposure. Datura stramonium was more effective in inhibiting egg hatching of about 98% at 100% concentration compared to 76% inhibitory effect of Gongoronema latifolium which may be due to the presence of terpenoids and higher concentration of alkaloids, flavonoids and phenols from the D. stramolium extracts. The vast bioactive components present in the tested botanicals make them suitable for bio pesticides and nematicide synthesis for eco-friendly and sustainable agro development.

1. Introduction

Nematode is small unsegmented, multicellular and pseudocoelomic worms living in water, soil, plants and animals (Asif *et.al*, 2017; Khan *et. al.* 2019). In plants, Nematodes is a parasite; for the posed terrible threat to diversity of plant species in the agricultural sector due to their prolific reproducing abilities (over 2,000) species of plants (Ali *et al*, 2013). Over 4100 species of plant parasitic nematodes has been identified as parasites within 197 genera accounting for 7% of the phylum nematode (Decreamer & Hunt, 2006). Plant parasitic nematodes adopt different functional, molecular and genetic mechanisms in order, to subdue the natural, sophisticated protection/ defence mechanism developed in plant against several plant pathogens thereby invading host plant and causing serious economic losses to farmers globally. An estimated 150 billion US dollars annual loss is attributed to plant parasitic nematode in the agriculture sector globally (Oka *et.al*., 2000; Abad *et al*, 2008). The sedentary endo-parasite like the root knot nematodes (*Meloidogyne species*) is economically and scientifically important due to their wide hosting range, adaptability and high rate of reproduction (Luc, Sikora & Bridge, 2005; Farinde *et al*, 2007). The four (4) most destructive species in this genus are *M. javanica, M. arenenia, M. hapla* and *M. incognita.* The *M. incognita*, a single female lays about 500 to 5000 eggs in her life time (Ali *et.al.*, 2013).

M. incognita infection forms root galls below ground symptom, causing several damages to roots by restricting the uptake of nutrient and water resulting to above ground symptoms like leaf necrosis/chlorosis, stunted growth, wilting of plant parts thereby predisposing the plant to various

opportunistic pathogens such as bacteria and fungi in forming disease complex (Imafidor & Nzeakwo, 2020; Ali *et al*, 2019). Nematode management is important in order to limit crop losses and to meet up the high requirement for food and raw materials used in our industries; plant protection against nematode is difficult because nematodes cannot be completely eradicated from the field but can be controlled to threshold level. The monetary importance of *M. incognita* on plant crops is increasing as most of the synthetic nematicides, which were very effective in the control of *M. incognita* are facing prohibition restriction due to environmental problems (carcinogenic potential, toxic residues, resulting to hormonal imbalance, spermatoxicity and longer half-life shelf) and health reasons (Wachira, Kimenju & Okoth, 2009; Dubey *et.al.* 2011).

This has led to scarcity and high cost of the available chemical nematicides. The current situation now spurred several farmer and nematologist to search for an alternative nematological management strategy that will be very effective, cheap, accessible and eco-friendly. Among several ecologically based strategies for the management of nematodes is the use of nematicides from plants origin; an alternative approaches involving the use of antagonistic plants for their antinematicidal potential is gaining interest in the management of plant parasitic nematodes (Gommers, 1981; Sukul, 1992; Chitwood, 2002). Botanicals are environmentally friendly and safe for farmers uses. Several botanicals are known to possess nematicidal components, which may be utilized as organic amendments or bio-pesticides. Plant extracts are very effective, cheap, easily applied and eco-friendly for the management of plant parasitic nematode (Chitwood, 2002; Chitwood, 2003, Adekunle and Fawole, 2003, Fawole, 2009). Consequently, the extracts either enabled the plants to resist the nematode invasion or activated directly the defence mechanisms of the plants and enhanced growth (Hussain, Mukhtar & Kayani, 2011; Mukhtar, Kayani & Hussain, 2013).

Wide-ranging varieties of plant species, representing 57 plants families possess nematicidal compounds (Sukul, 1992; Ntallie *et.al.,* 2011). Consequently, promising number of plants/plant parts have been screened for nematicidal activities globally (Nour El-Deen & Darwish, 2011; Nour El-Deen, Omaima & Abdel-Kafie, 2013). Unfortunately, very few of such plants had been reported to suppress plant parasitic nematodes in Nigeria (Adekunle and Fawole, 2003; Fawole, 2005; Adegbite and Adesiyan, 2005; Imafidor and Nzeakwo 2020). This enthuse the study of investigating the inhibitory effect of the extracts from *Gongronema latifolium* and *Datura stramonium* on egg hatching of *M. incognita* for the control of root knot nematode.

2. Materials and Methods

2.1 Procurement of experimental plant

Fresh leaves of *Datura stramonium and Gongronema latifolium* were collected anonymously in farm from Kolo Town ; Ogbia Local Government Area, Bayelsa State; species were identified botanically using keys like size, shape, venation and other leaves structures. They were further authenticated and classified using taxonomic guide with a deposited voucher specimen at the department of Crop and Soil Science, University of Port Harcourt, Rivers State.

2.2 Extraction/Collection of plant extracts

The leaves were washed properly and shade dried under laboratory condition for 15 days. Each of the dried leaves were turned into fine powder by an electrical blenders, 20g of each pounded plant material was introduced into a flash containing 200 ml of 90% ethanol (Zeck, 1971; Kepenekçi, Erdoğuş & Erdoğan, 2016), and was allowed to soak for 48 hours. The content was filtered using a whatman NO1 filter paper before agitating it, in an orbital shaker for 4 hours. The content was then centrifuged at 1500 rpm for 25 minutes to collect the supernatant which was diluted in 92% concentration of 25 ml of dimethyl solfoxide (DMS) making up the standard stock solution stored at a temperature of 4^{o}c in a refrigeration (Baideo *et. al.,* 2017).

2.3 Preparation of root knot nematode inoculum

The egg/juveniles of *M. incognita* inoculum were extracted by sodium hypochlorite method (Hung & Rohde, 1973; Hussey & Barker, 1973). Heavily galled roots of okra plants collected from an anonymous farm were carefully washed to remove soil particles. The infected roots were stained with a mixture of 1.0% Hel and 80% lactophenol for 2 minutes (Southy, 1970; Barthels *et al*, 1997). The

stained root, were gently washed to remove all the stains and they were kept in clean laclophenol for 24 hours, the female adult was identified to species level using the perineal pattern characteristics as *M. incognita.* Pure culture of *M. incognita* for the study was raised by infecting seedling of okra planted in pots with egg/juveniles of identifies *M. incognita species.* The volume of the suspension was standardized by adding water to 100 ml. 1 ml of the suspension was pipette into a county tray after bubbling into the suspensions (Asif *et.al*, 2017; Imafidor and Nzeakwo, 2020). Three counts were done to have a mean representing the number of egg/juveniles present in 1 ml suspension.

2.4 In Vitro Hatching Test

Distilled water was added into the stock solution of 40 mm petri dishes with three replications at different concentrations (10%, 25%, 50% and 100%). 3 ml of *M. incognita* egg suspension containing 150 eggs were transferred to each of the petri dishes containing different concentrations of the extract while the petri dishes with distilled water served as control. The set up was kept in the laboratory under root temperature and the juveniles hatching was counted at 24, 48 and 72 hours of exposures. The percentage egg hatch inhibition over distilled was calculated.

$$\% = \frac{Total\ No\ of\ egg-hatched\ egg\ x\ 100}{\text{Total No of eggs}}$$

2.5 Data Analysis

The percentage egg hatched inhibition was calculated per 24, 48 and 72hours of exposure at different concentrations (10%, 25%, 50% and 100%). The Genstare statistical package (Edition 7) was used to analyse the collected data; the mean difference comparison was done at 5%. The square root transformation of $\sqrt{(x+0.5)}$, (where x is the mean) was used for counting and transforming data. Thereafter, the generated data from the quantitative phytochemical screening was analysed using Two Ways Anova to determine variation among phytochemicals from the botanicals

2.6 Qualitative phytochemical screening

2.6.1 Test for alkaloids: (Mayer's test). The presence of green colour precipitate when little drops of Mayer's reagent is added into a test tube containing 1ml of plant extract and 2 ml of concentrate (H_2SO_4) shows the presence of alkaloids (Vijayalakshmi, Mishra & Parasad, 1979)

2.6.2 Test for flavonoid: (sodium hydroxide test), the change of colour observed from yellow to colourless when diluted hydrogen chloride acid is added into the test-tube containing 5ml of filtered extract and 2 ml of 10% Sodium hydroxide shows the presence of flavonoid (Khan *et. al.*, 2019).

2.6.3 Test for saponins: Yadav & Agarwala, (2011) description was duly followed as stable foam formation indicates the presence of saponins when crude aqueous extract is vigorously shaken for about 3 minutes ().

2.6.4 Test for tannins: 5 drops of 1% lead acetate is added into a test-tube containing 1ml of plant extract and the formation of white precipitate represents tannins presents (Latif *et al*, 2019).

2.6.5 Test for steroids: Concentrated H_2SO_4 is added through the side of a test-tube containing equal volume of plant extract and chloroform. The formation of red colour at the lower chloroform layer indicates the presence of steroids when. (Yadav & Agarwala, 2011).

2.6.6 Test for glycoside: (Liebermann's test). The change in colour observed from violet, blue and green indicates the presence of glycosides when H_2SO_4 is added into a cooled test-tube containing crude extracts and a mixture of 2 ml of chloroform and 2 ml of acetic acid (Yadav & Agarwala, 2011).

2.6.7 Test for phenol: (Ferric chloride test). The formation of a deep blue or black colour when 3 ml of 5% aqueous ferric chloride was added into a test-tube containing 2 ml of crude plant extract indicates the presence of phenolic compounds in the extract. (Yadav & Agarwala, 2011).

2.6.8 Test for terpenoid: (Salkowki's test). The formation of pink colour indicates the presence of terpenoid when 1 ml of chloroform was added into a test-tube containing 2 ml of plant extracts (Dixit & Ali, 2010; Kepenecki, 2011).

3. Results And Discussion

The result of the effect of the extracts of *Datura stramonium* and *Gongronema latifolium* in egg hatch inhibition of *M. incognita* shows officious in inhibiting eggs hatching at all concentration which is concomitant with the report of Asif *et.al*, (2017). The analysis (quantitative and qualitative) in plants parts (leaf and stem) extracts from *Datura stramonium* and *Gongronema latifolium* shows several varied phytochemicals.

Table 1: Qualitative analysis of Phytochemicals present from *Datura stramonium* and *Gongronema latifolium.* Extracts

Botanical	*Plant part*	*Phytochemicals present in plant extracts*							
		AL	*FL*	*SA*	*TA*	*ST*	*PH*	*TER*	*GL*
Gongronema latifolium	*Stem*	+++	+++	++	++	+	+++	-	+
Datura stramonium	*Leaf*	+++	+++	+	+	+	+++	+++	+++

Source: Researcher, 2022. Where: AL = Alkaloids, FL = Flavonoids, SA = Saponins, TA = Tannins, ST = Steroids, PH = Phenols, TER = Terpenoid and GL = Glycosides. . +++ = abundantly present, ++ = moderately present, + = traceable and - = not detected.

Table 2: Quantitative analysis of the Phytochemicals present from Datura stramolium and Gongronema latifolium. Extracts

Botanicals	Quantitative analysis							
	AL	FL	SA	TA	ST	PH	TER	GL
Datura stramonium	269.05	266.72	0.79	0.03	2.06	379.93	73.02	146.66
Gongronema latifolium	31.2	44.3	18.1	16.5	7.2	32.9	0.00	0.16

Source: Researcher, 2022. Where: AL = Alkaloids, FL = Flavonoids, SA = Saponins, TA = Tannins, ST = Steroids, PH = Phenols, TER = Terpenoid and GL = Glycosides.

Table 3: Effect of leaf and stem extracts from *D. stramonium* and *G. latifolium* on egg hatch inhibition percentage at different concentration (10%, 25%, 50% and 100%) and Duration (24, 48 and 72 hours)

Botanical	Exposure Time	10%	% inhibition	25%	% Inhibition	50%	% inhibition	100%	% inhibition
D. stramolium	24	82	45.33	76	49.33	58	61.33	43	71.33
	48	13	92	11	92	10	93.33	9	94
	72	10	93.33	10	93.33	7	95.33	3	98
G. latifolium	24	115	23.33	103	31.33	83	44.66	64	57.33
	48	101	32.66	91	39.33	66	56	50	66.66
	72	86	42.66	64	57.33	54	64	42	72

Source: Researcher, 2022. Values are presented in mean of three replicates; therefore, the in vitro results of the nematicidal efficacy of *D. stamonium* and *G. latifolium* shows inhibitory efficacy against egg hatching of *M. incognita* in all the concentrations and this also showed a significant difference in egg hatch inhibition as compared with the control.

Table 4: Analysis of Variance Table for Two-Way ANOVA

Source	SS	d.f	M.S	*F*-ratio	5% F-limit
B/W Phytochemicals	77896.09	7 (8-1)	11128.01	1.023	3.45 (7,7)
B/W Botanicals	61574.84	1 (2-1)	61574.84	5.661	5.59 (1,7)
Residual	76135.504	7 (7x1)	10876.50		
Total	215606.43		83579.35		

Source: Researcher, 2022.

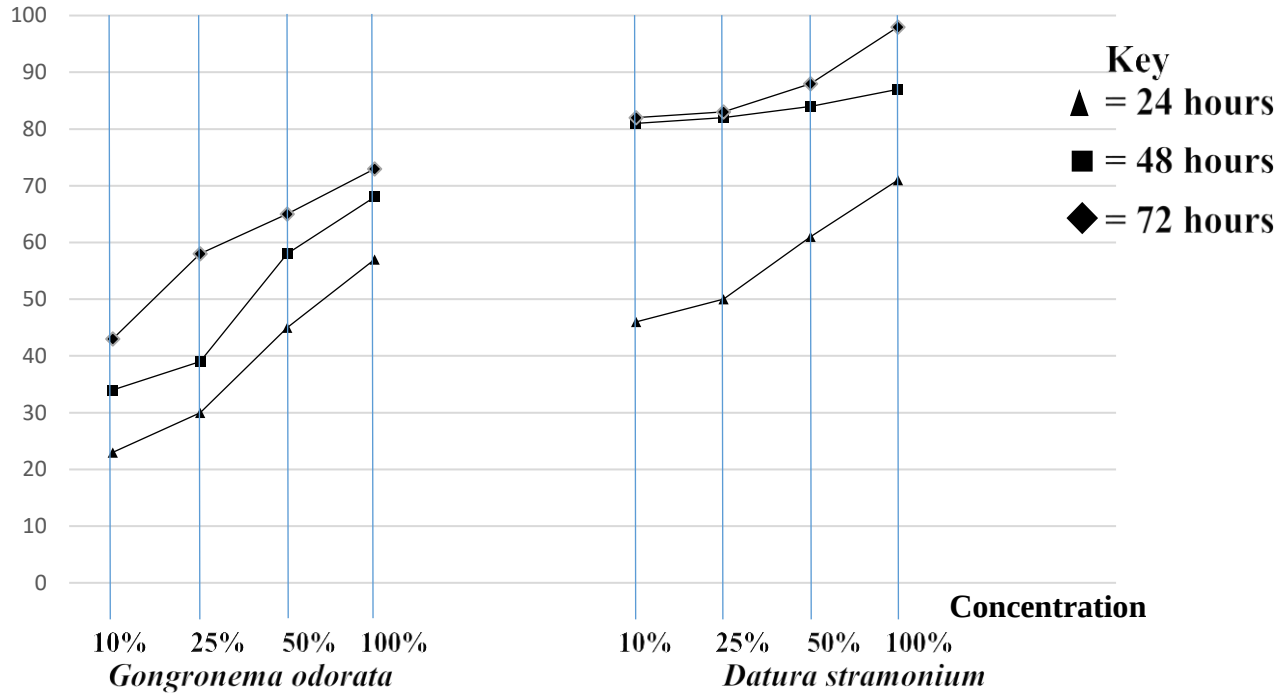

Figure 1: The effect of different concentration (10%, 25%, 50% and 100%) of *D. stramolium* and *G. latifolium* on egg hatch inhibition of 72 hours of exposure.

Results shows the effect concentration on the egg hatching inhibition of *M. incognita* at 10%, 25%, 50% and 100% and the effect is concentration dependence (i.e) the higher the concentration the more fail to hatch.

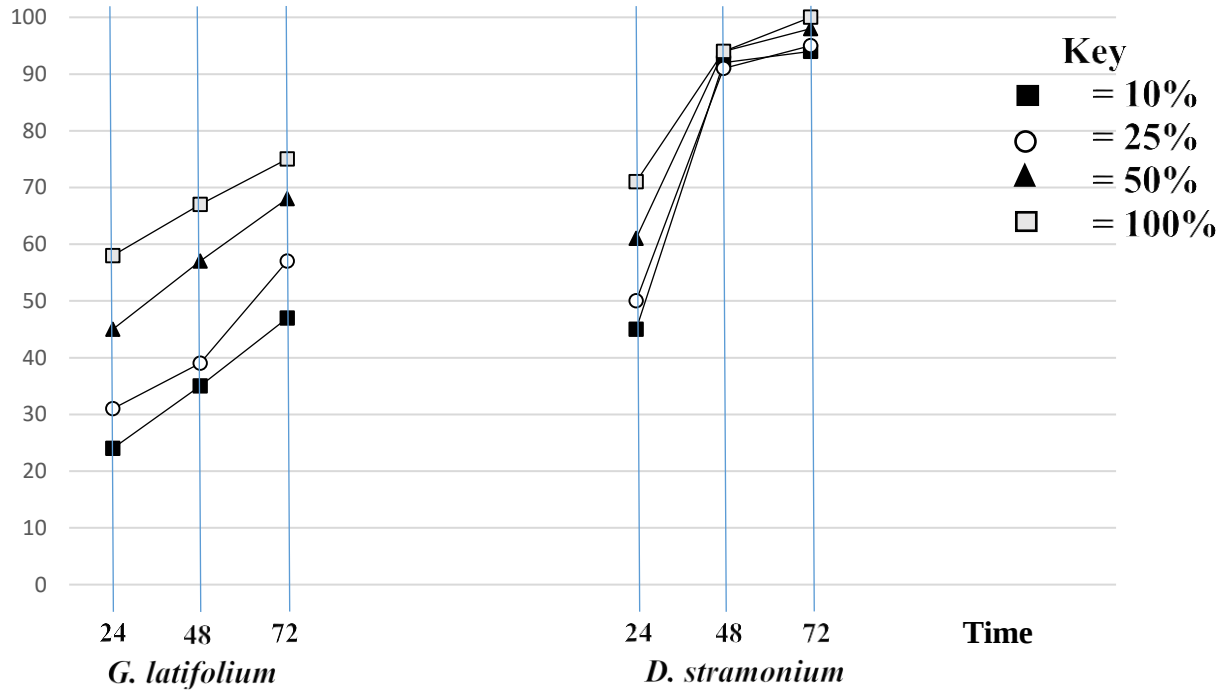

Figure 2: The effect of exposure time of treatment (24, 48 and 72 hours) on the percentage egg hatching inhibition of *M. incognita* in vitro.

Results shows that the rate of egg hatch inhibition of *M. incognita* in vitro response per time of exposure. as the exposure time is directly proportionate to percentage egg hatch inhibition.

4. Discussion

The present research work evaluate potential beneficial efficacy on two botanical extracts (*Datura stramonium* and *Gongronema latifolium*) in the management of root-knot nematode (*M. icognita*) through nematicidal effect on egg hatchability. An abroad spectrum material such as botanicals is used in the management of pest due to the unique action, cheap, readily available, easily degradable and environmentally friendly (Ali *et. al.,* 2007). Locally available botanicals and their parts have been widely used in the past to manage crop plants from damage caused by plant pest and pathogen (Ali *et. al.,* 2007). The extracts of the tested botanicals affected significantly on the egg hatch inhibition of *M. incognita.* Current results revealed that botanical extracts especially higher concentrations displayed the greatest activity on egg hatch inhibition at 72 hours and above exposure period in an in vitro condition. All the tested botanical extracts exhibited a juvenile hatching capacity value lower than the control as a nematicidal index appropriate in the control of root knot nematodes; which where once reported by Khurma and Singh (1997); Kepenekçi, Erdoğuş and Erdoğan (2016). The result also showed that percentage rate in egg hatch inhibition is directly proportional to the concentration of the extracts and the times of exposure. This corroborates with the finding of E-Elling *et.al.* (2009) and Ganai *et.al.* (2013); that inhibitory effect on the egg hatching of *M. incognita* concentration is dependent which implies that the efficacy of plant extract is in contingent with exposure duration and concentration of the nematode. Asif *et. al.,* (2017) also reported that higher exposure time of treatment promote juvenile mortality and decrease juvenile hatching from egg. Similarly, Belay, Sakhuja and Tefera, (2013) also put it clear that the efficacy of plant extracts on egg hatch inhibition was concentration and exposure time dependent.

The inhibitory effect of the extracts on egg hatching is likened to the toxic substances present in the extract. This agrees with the reports of Adegbite and Adesiyan (2005) that the inhibitory efficacy on egg hatching is due to chemicals present in the extracts of the botanical possessing ovicidal and larvicidal properties. It could be that the plant extract induces some mechanisms to effect the inhibition of egg hatching. This also corresponds with the finding of Susan, Khurma and Singh (1997) and Chedekal (2013). The results on the phytochemical analysis of the tested botanical extracts showed that the extract contained some phytochemicals viz alkaloids, flavonoids, saponins, phenols, steroids, terpenoid of different composition in the extracts. This could be the reason why the extracts are highly officious to egg hatch inhibition of *M. incognita.* This agrees with the earlier finding of (Asif *et.al.,* 2011; Ebuete, Ebuete & Berezi, 2022), who reported that presence of several phytochemicals of nematicidal importance on several plant species from different families varies significantly between the same species, varieties, plant parts and geographical location. Adegbite (2003), also supported the finding with his initial reports that the phytochemicals from botanicals extract inhibit egg hatching at the embryonic stage; killing the egg or dissolving egg masses to allow easy penetration of the extracts through the shell membrane. Zhang *et al,* (2012), also supported this finding from their reports that, the overall efficacy of plant extract in deterring egg hatching and promoting juvenile mortality is linked to the biological intricacy among interacting phytochemicals in the plant extracts.

The extracts from parts of *Gongronema latifolium* is used extensively as medicinal plant for several illnesses. Eze and Nwanguma (2013) reported on the bacteriocidal and fungicidal properties of the extracts on different parts of *Gongronema latifolium* on *S. aureus* and *C. albican* at a concentration of 400 mg/g. Illodibia *et.al.* (2015), reported on the microbial efficacy of leaf extract of *Gongronema latifolium* on collectorichum species isolate form spoilt tomatoes. Adebolu and Oladimeji (2005) also had it that the extracts of *Gongronema latifolium* possess both antifungal and antimicrobial (antioxidant) to several microbes that are pathogenic to animals and human. There is a well-documented report regarding the use of several species of *Datura* in the management species of plant parasitic nematodes; of such work Kamau *et. al.* (2020), reported that at 10% concentration, the aqueous leaf extracts of *D. metal* reduced egg hatching of *M. incognita.* Saeed, Awadh and Al-Thobhani (2015), also determine the nematicidal effect of *D. metal* on egg hatch inhibition and juvenile mortality at 25,000 and 150,000 mg/kg concentration within10 days of exposure. Bakr (2021) evaluates the nematicidal potential of the seed and leaf extracts of *D. metal* and discovered that extracts of *D. metal* inhibited egg hatching of *M. incognita* up to 24.4 and 35.7% at 7 days of exposure. The nematicidal efficacy of *D. innoxia* on egg hatching inhibition and juvenile mortality of *M. incognita* at

low concentrations (5 and 10%) was reported by Babaali *et.al.*(2017). Saeed, Awadh and Al-Thobhani (2015), evaluated the anti-parasitic effects of ethanol and crudes from the extracts of *D. Meloidogyne incognita* and reported an inhibitory capabilities on egg hatching and juvenile mortality at 80% *Helicotylenchus dihystera.* The root and leaf extracts of *D. alba* were reported by Kepenekçi, Erdoğuş and Erdoğan (2016) to inhibit egg hatching at 86.67 and 94.48% extraction standard after 2 and 6 days of exposure period. The nematicidal potential from the leaf extracts of *Datura stramonium* have been reported by Prasad, Ram and Imtiyaz (2002) to have inhibitory action against egg hatching of *M. incognita.* In another studies, Chrisostomos, *et.al.*(2018) evaluated the aqueous and ethanol extracts of *Datural stramonium* and revealed that hot water extracts induced 75 to 100% egg hatching inhibition as against the 80-100% ethanolic extracts. Bakr (2021) also reported on the nematicidal efficacy of the extracts of different *Datura* species and recorded a highest percentage *M. incognita* egg hatching. In a recent studies Ramadan (2021), have reported on the negative effects of *Datura* species on various stages in the life cycle of *M. incognita* in vivo and in vitro.

The study revealed also that extract of *Datura stramolium* is more effective on inhibiting egg hatching than extracts from *Gongronema latifolium* (98% and 72% respectively). This could be due to the high presents of alkaloid, flavonoids, phenoids and glycosides in the extract of *Datura stramolium* or it could be due to the absent of terpenoids in the extracts of *Gongronema latifolium.* At 0.05% limits, the variation among plant extracts (Phytochemicals) was insignificant (*F*-ratio 1.023< P3.45) (*interpolation method*); however, the variation differences concerning Botanicals were significant (*F*-ratio 5.661>P 5.59) (table. 4).

5. Conclusion

The study revealed that extracts of *Datura stramonium* and *Gongronema latifolium* are effective on inhibiting the egg hatching of *M. incognita* in vitro. On that note, extracts from the tested botanicals represents good replacement to chemical nematicides, these tested botanicals which are antagonistic to *M. incognita* egg hatching are excellent candidates because they can be used in crude form or in developing nematicides and can serve as raw materials in the development of synthetic nematicides. The use of the extracts of the tested botanicals may well provide one of the efficient, cost effective and eco-friendly nematode management strategies. The use of phytochemicals from extracts of botanicals to control *M. incognita* instead of synthetic chemicals may benefit farmers in creating additional economic benefit beyond just nematode control. Findings in the present studies is important in targeting inhibiting egg hatching of *M. incognita;* a significant stage in the life cycle of nematode by reducing hatching of larva been the infective stage in all genera of plant parasitic nematode.

6. Recommendations

1. Extracts from the tested botanicals should be synthetically developed and utilized as Nematocides
2. More research be conducted to determine other anti-parasitic capacities from the plants extracts.

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