Research Article

Toxicity Effect of *Eucalyptus globulus* on *Pratylenchus* spp. of *Zea mays*

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Abstract | Maize (*Zea mays* L.) is an important food crop widely consumed in various forms in many parts of the world. Its annual yield is largely hampered by nematode infestation, especially the *Pratylenchus* spp. Experiments were conducted in a screenhouse and under field conditions to evaluate the toxicity of fractions and essential oil of *Eucalyptus globulus* (Myrtaceae) against *Pratylenchus* spp. infecting *Zea mays*. The phytochemical basis of the plant was partly established using Infrared (IR) spectroscopy, Gas Chromatography – Mass Spectrometry (GC-MS), proton and carbon-13 Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C NMR) Spectroscopy analysis. Effect of application of plant phytochemicals on the planted maize was also examined in terms of growth rate and survival of *Pratylenchus* spp. The essential oil (ECSG/EO) exhibited significant ($p<0.05$) toxicity, reduced nematode population and increased grain yield (6.79 kg) compared to the standard, carbofuran (CBFN) (7.18 kg) The *Eucalyptus globulus* ethanol extract fractions (ECSG/EtOH) showed moderate effect, while the chopped leaf materials used as soil amendment (ECSG/AMDM) exhibited the least potency among all the treatments. Spectroscopic analysis by GCMS revealed the presence of terpenoids and phenolics as major constituents of *E. globulus*. The essential oil showed the presence of 1,8-cineole (23.3%), citronella (18.1%), geranial (17.6%), isopulegol (10.4%), myrcene (13.0%), cuminaldehyde (9.1%) and 2-pinene (8.5%) as major constituent. Results obtained in this research revealed that essential oil from *Eucalyptus* (a bio-nematicide) holds promise as an affordable and environmentally-friendly alternative for nematode control and increased crop yield particularly in maize production.

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Introduction

Maize, grown in tropical and sub-tropical regions of the world has the highest production level among other cereals as a result of its food value (CIMMYT, 1992; Keller, 2017/2018; Wang *et al*., 2018). Many varieties and hybrids have been developed and cultivated (Khan *et al*., 2018). It is in high demand due to its relevance for human consumption, animal feed and bio fuel production (Xiong *et al*., 2010). It is the most important cereal in Nigeria, mostly grown by small scale farmers due to its rapid growth rate and yield. It is an important source of carbohydrate, protein, phosphorus and crude fibre. Global production of maize is over 600 metric tonnes (McDonald and Nicol, 2005). Productivity was reported to rise from 1.2 tonnes/ha to 1.7 tonnes/ha over a period of twenty years (Alene *et al*., 2009). This minimal increase in productivity is associated with considerable crop loss to pest and diseases on the field during crop development. Plant parasitic nematodes are a major constrain to maize production especially in Nigeria, where they are known to induce close to 50% yield loss. Root lesion nematode *Pratylenchus* spp. has been implicated to occur frequently in maize fields and thus reducing productivity (Bridge *et al*., 2005; McDonald and Nicol, 2005; Nicol *et al*., 2011). Several species of lesion nematodes are pathogenic to corn. They are the widest spread corn parasite (Roth *et al*., 1993). Talwana *et al*. (2008) reported that *Pratylenchus zea* is the most common nematode affecting maize, while Alvey *et al*. (2003) in their findings suggested that they are the major parasite causing significantly low yield in maize production. These was corroborated by Khan *et al*. (2019), in their findings, they affirmed that *Pratylenchus zea* are the most common specie of nematodes encountered in maize fields. The yield of maize is lower than the general demand of the populace due to the incidence of *Pratylenchus* spp. on the field. The demand to increase maize production has however encouraged the use of synthetic nematicides in maize production. While the application of synthetic nematicide is often considered inappropriate on a low value crop such as maize due to the economic value, it is however unavoidable on many instances of massive nematode infestation. Moreover, the concern about health and environmental hazards posed by the use of synthetic pesticides warrants the search for more affordable and safer alternatives (Fabiyi *et al*., 2020). *Eucalyptus* spp. (Myrtaceae) are renowned for the abundance of essential oils which are applied as component of brands of mouthwash and cough suppressant (Mallard *et al*., 2018; Salem *et al*., 2018). It is used in the treatment of respiratory diseases, arthritis and as flavourings in tea. The antimicrobial activity of *Eucalyptus globulus* are well documented in literature (Caceres, 1991; Alkofahi, 1997; Ait-Ouazzou, 2011). However, there is limited information on its applications as a nematicide. Consequently, this research was undertaken to determine the efficacy of *Eucalyptus globulus* leaves as a potential control alternative against the pathogenicity of *Pratylenchus* spp infecting maize and also evaluate its effect on the growth and yield of maize.

Materials and Methods

Plant materials

Eucalyptus globulus leaves were collected within the premises of the University of Ilorin, Nigeria and air dried for two weeks in the laboratory at room temperature. The dried leaves were finely grinded in a steel man diesel engine milling machine with a 7 horse power capacity; model R175A (Fabiyi *et al*., 2018). Ten (10) Kilogram each of the powdered leaf material was weighed into three different glass jars and extracted separately with n-hexane, ethyl acetate and ethanol for five days at room temperature. The extracts were concentrated using rotary evaporator and allowed to dry to constant weight. Another 10 kg of plant material was subjected to hydro-distillation for 3 hrs using Clevenger apparatus, the essential oil obtained was dried over anhydrous magnesium sulphate and kept at room temperature in the laboratory until further analysis. The remaining dry plant material was reserved for soil admix. The extracts gleaned from the different solvents extraction were coded ECSG/hex (n-hexane extract), ECSG/EtOAc (ethyl acetate extract) and ECSG/EtOH (ethanol extract) while the essential oil obtained from the hydro distillation was coded ECSG/EO (*Eucalyptus globulus* essential oil) and ECSG/AMDM for the plant material used as soil admix. The crude extracts were fractionated separately on aluminium oxide (Al_2O_3) using solvents of increasing polarity and the fractions obtained were analysed using Infrared (IR) spectroscopy, Gas Chromatography–Mass Spectrometry (GC-MS), proton and carbon-13 Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C NMR) Spectroscopy. The IR was used to identify the characteristic and diagnostic functional groups and deduce which functional groups play vital role in the bioactivity being studied; NMR was employed to elucidate the proton environment and characterize the hybridisation of each carbon.

Instruments

The essential oil obtained from the plants were analysed on Agilent GC-6890N-system (with injection port temperature 250°C) attached to a mass-selective detector 5973 mass spectrometer, with 105 m × 0.530 mm id capillary columns. Identification of compounds was achieved by the comparison of the mass spectra with those of NIST 08 library. Infrared spectra of column chromatography fractions were recorded on 8400s Fourier Transform Infrared (FT-IR) while Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) were determined on a JEOL 400 MHz taking chemical shifts in ppm relative to tetramethylsilane (TMS).

Screenhouse experiment

Sandy loam soil collected from Faculty of Agriculture, University of Ilorin, Ilorin (80, 291 N; 40, 401 E) was pasteurized using an electric soil sterilizer at 60°C for 2 hrs. The soil was allowed to cool down and stored in bags for stabilization for four weeks, before they were weighed at 20 kg each into twenty-five litre buckets. The experimental design followed was a factorial design with six treatments at four levels and each replicated three times. A total of seventy-two buckets were used in the experiment. The first and second trials which were planted between April and August of 2013 and similar period of 2014 had the same design and arrangement in the screen house. At harvest the total number of nematodes in treated and untreated pots was evaluated.

Field experiment

Two trials were conducted at the University of Ilorin Teaching and Research Farm, Bolorunduro, Kwara State Nigeria, between April to August 2013 and 2014. The experimental design was a Randomized Complete Block Design (RCBD) which consists of six treatments at four levels and each replicated three times. This gave a total of seventy-two plots. Each experimental plot consisted of five 10 m long ridges spaced at 0.5 m apart with 30 cm plant spacing within the rows. Four seeds were sown per hole and were thinned down to two plant stand after two weeks of germination. Plants used for data recording were tagged accordingly in each plot. At harvest, soil cores, 15 cm deep were collected for nematode population count at the base of each maize plant using a diagonal transect sampling method. The soils were thoroughly mixed to give a composite sample for each plot, and total number of nematodes at harvest was counted.

Preparation of inoculums and inoculation

Pratylenchus spp used for inoculation was collected from infected maize roots, obtained from IITA maize field and extracted using the modified Baermann sieve method (Coyne *et al*., 2007) and applied on maize plants grown in plastic pots. After multiplication they were re-extracted and the number of nematodes per unit volume was estimated by taking aliquots of 1 mL three times counting under a stereomicroscope using Doncaster's counting dishes. The maize plants were inoculated after two weeks of planting with approximately 300 nematodes using the method of Iheukwumere *et al*. (1995) and Fabiyi *et al*. (2019). Briefly, a slight trough was made round the base of each plant and nematodes were dispensed in to it in the screenhouse and field.

Treatment application

Fractions and the essential oil were applied at 10, 20 and 30 mg/kg soil on the field and at 5, 10 and 15 mg/kg soil for the screenhouse, while inoculated untreated maize plots served as control. Carbofuran 3G obtained from Ilorin metropolis, Nigeria was applied at 0.3, 0.5, and 1.0 Kg ai/ha^{-1} in the screenhouse, while in the field 1.0, 1.5 and 2.0 Kg a.i/ha^{-1} was adopted.

Data collection and statistical analysis

Data which include plant height, days to 50% tasselling, maize yield kg/ha^{-1}, nematode population in 200 gm soil and nematode population in 10 gm root sample were collected and all parameters were subjected to analysis of variance (ANOVA) using GenStat 5.32. Treatment means, where significant differences were found, were separated using the new Duncan's multiple range test at 5% level of probability (Gomez and Gomez, 1984).

Results and Discussion

Phytochemical characterisations

The light yellow essential oil had density of 0.92 g/mL, insoluble in water but miscible with anhydrous alcohol. The GC-MS analysis of *E. globulus* showed the presence of fifteen major constituents of which 1,8-cineole (or eucalyptol) (23.3%), citronellal (or rhodinal) (18.1%), geranial (or trans-citral) (17.6%), myrcene (or beta-geraniolene) (13.0%), isopulegol (or p-menth-8(9)-en-3-ol) (10.4%), cuminaldehyde (or cumal) (9.1%) and 2-pinene (or sylvapine A) (8.5%) (Figure 1) are the major constituents while the others were detected in trace amount. The IR (KBr cm^{-1}) of the fractions indicated the presence of hydroxyl functional groups at 3353-3200 cm^{-1} corresponding to aliphatic and phenolic alcohol. The ^{1}H-NMR spectral of the fractions showed prominent methyl and methylene signals at δ 0.71, 0.83, 1.04, 1.22 ppm and diagnostic ^{13}C-NMR signals appeared around 116 and 120 for unsaturated double bonds and at 174 and 191 ppm corresponding to carbonyls.

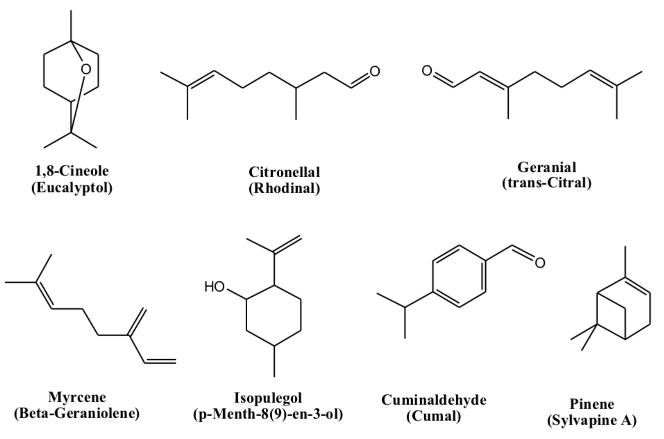

Figure 1: *Structures of major compounds obtained from the essential oil of Eucalyptus globulus with their alternate name in parenthesis*

Screenhouse and field

The maize plants treated with *E. globulus* E. oil and fractions showed different degrees of increased vegetative growth compared to the untreated control plants (Figure 2). Plants treated with carbofuran and essential oil (CBFN and ECSG/EO) were significantly ($p<0.05$) taller, followed by plants administered with fractions from ethyl acetate extract (ECSG/EtOAc). Maize plants in soil amended with *E. globulus* materials exhibited lower plants heights. Similarly, on the field, ECSG/EO and CBFN exhibited taller plants throughout the period of observation (Figure 3). Early tasselling (days to 50% tasselling) was observed in ECSG/EO and CBFN treated plants (50.73+05 and 51.22+12), while ECSG/AMDM treated plants took longer to tassel (72.11+14). Field experiment revealed tasselling was earlier in essential oil and carbofuran treated plants (Figures 4 and 5). There was a significant relationship between early tasselling and yield in the screenhouse. Higher grain yield was recorded in plants treated with extracts as compared to the yield of plants in the amended soil and untreated control plants (Figures 6 and 7). On the field, ECSG/EO treated plants had higher grain yield than all other treatments, yield was directly proportional to the rate of treatment application. Significant differences ($p<0.05$) (0.000) were observed in nematode population in 250 g soil sample and in the 15 g root sample of treated and untreated plants. Number of recovered nematodes reduced significantly in EO treated plants in the screenhouse and on the field. There was no significant difference between number of nematodes recovered from the soil of ECSG/EtOH and EtOAc fraction treated plants. Nematodes were completely absent in the 15-gram root sample of plants treated with EO and CBFN in the screenhouse (Figure 8), while a few were recovered from the roots of other treatments, however ECSG/AMDM treated soils had the highest number of recovered nematodes among all treatments 26+03 (Figures 8). The untreated control plants had higher number of nematodes in their soil (810.26 and 123.11) and roots respectively; there was however no nematodes in the soil and roots of plant treated with the highest concentration of extract (Figure 7b). Plants treated with the highest dose had significantly higher height, earlier tasselling, higher yield and fewer nematode population in the screenhouse and on the field (Figures 2b, 3b, 4b, 5b, 6b, 7b, 8b and 9b). Number of small black lesions varied significantly among treatments. A few rot was associated with severe cases which were seen in untreated plants in the screenhouse and field. In general, plants treated with essential oil (EO) had significantly robust vegetation than other treatment materials.

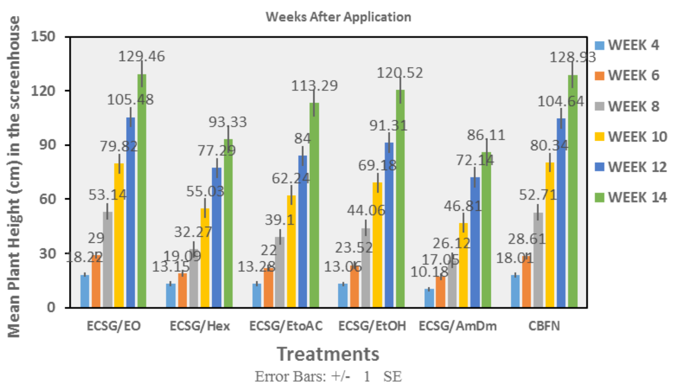

Figure 2: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on height of maize in the screenhouse.*

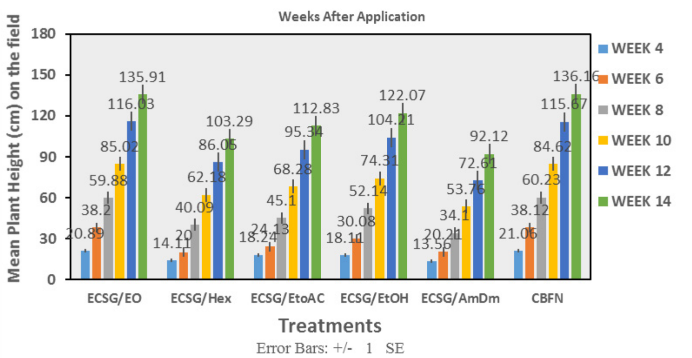

Figure 3: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on height of maize on the field.*

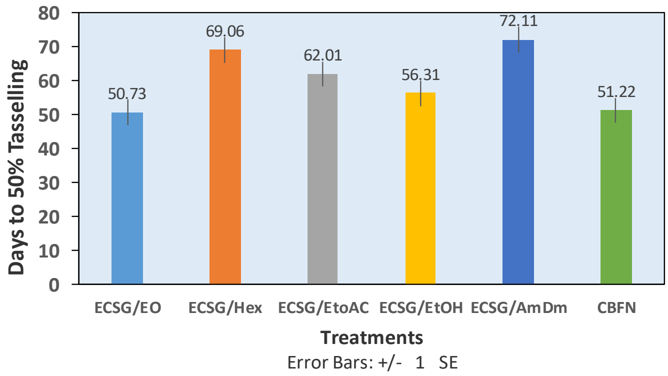

Figure 4: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on days to 50% tasseling in the screenhouse*

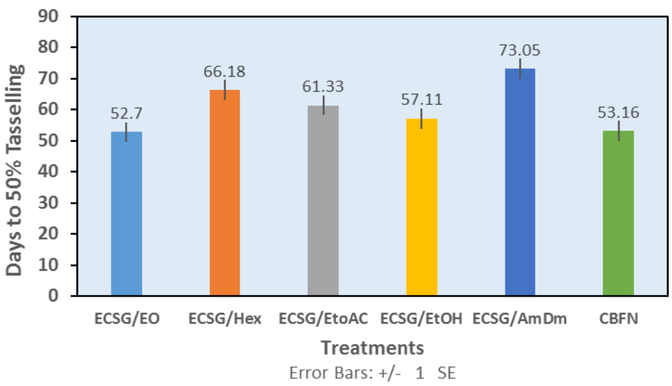

Figure 5: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on days to 50% tasseling on the field.*

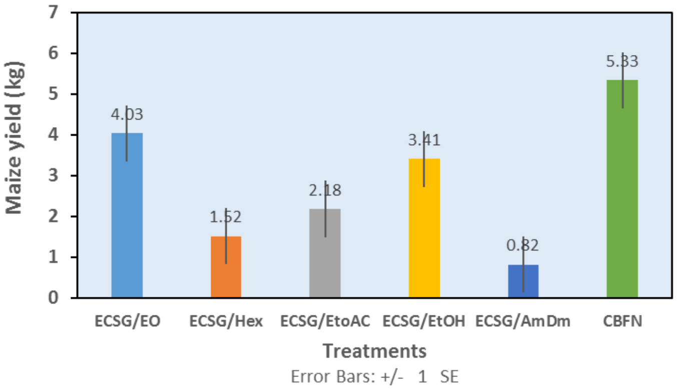

Figure 6: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on yield of maize in the screenhouse.*

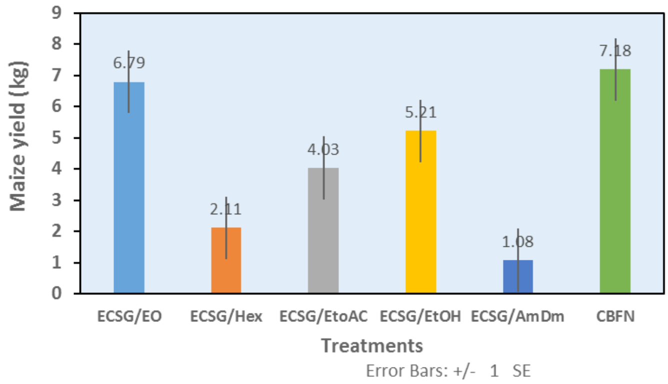

Figure 7: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on yield of maize on the field.*

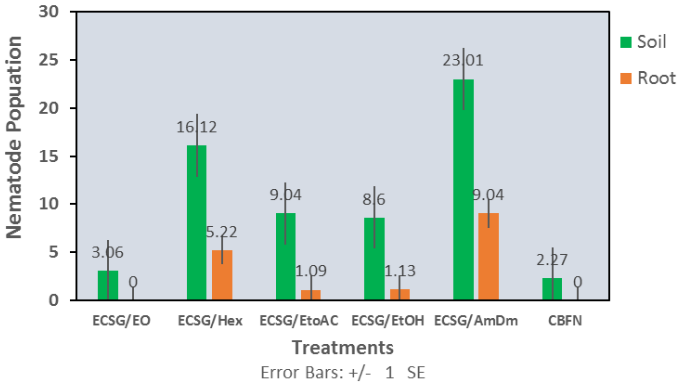

Figure 8: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on nematode population of maize in the screenhouse.*

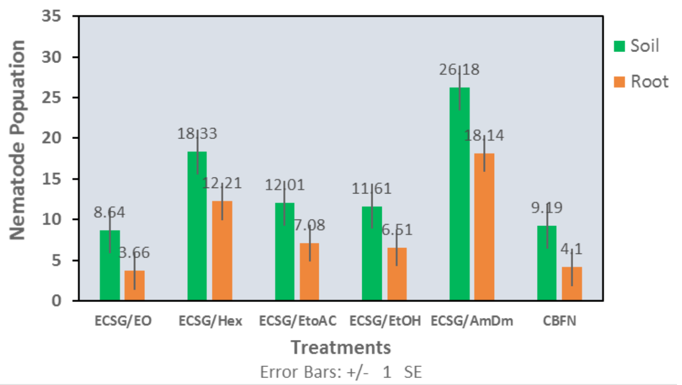

Figure 9: *Effect of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on nematode population of maize on the field.*

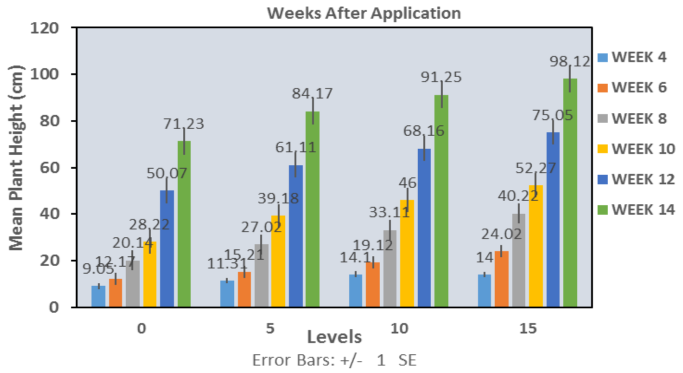

Figure 1b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on height of maize in the screenhouse.*

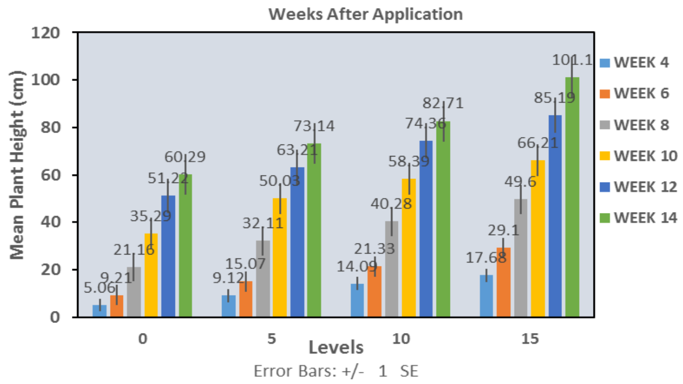

Figure 2b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on height of maize on the field.*

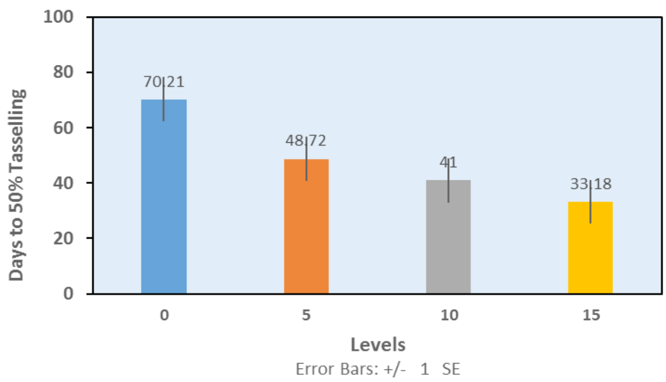

Figure 3b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on days to 50% tasseling in the screenhouse.*

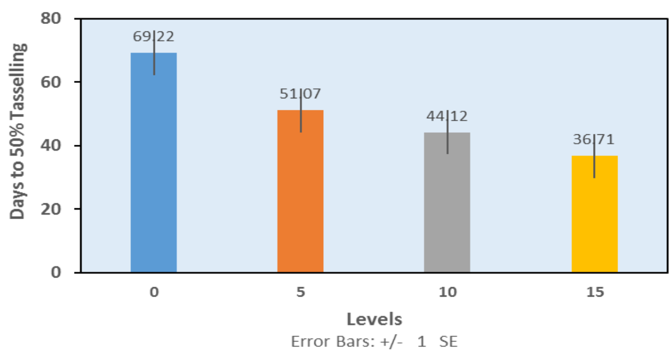

Figure 4b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on days to 50% tasseling on the field.*

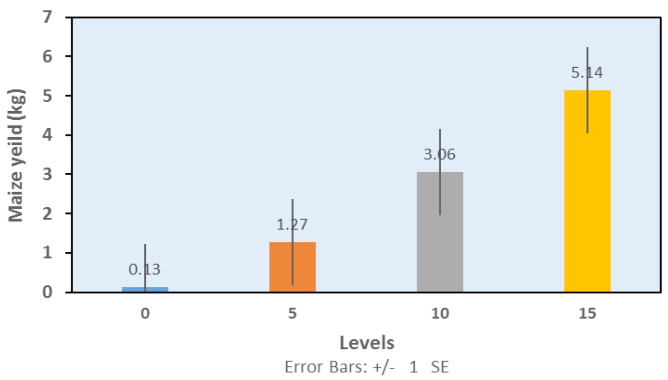

Figure 5b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on yield of maize in the screenhouse.*

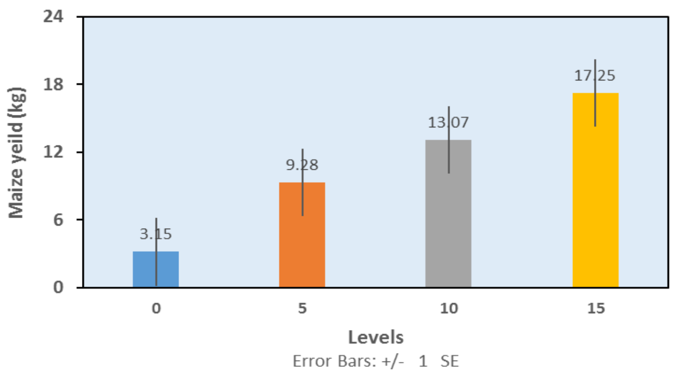

Figure 6b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on yield of maize on the field.*

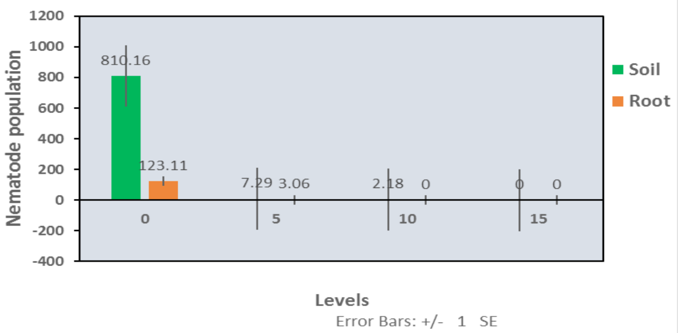

Figure 7b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on nematode population of maize in the screenhouse.*

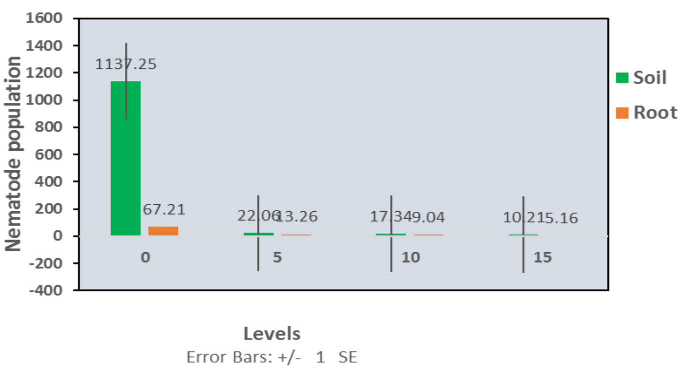

Figure 8b: *Effect of dosage of application of Eucalyptus globulus essential oil, fractions, leaf materials and carbofuran on nematode population of maize on the field.*

Pratylenchus zea, *P. brachyurus*, and *P. penetrans* are known as the major pests of maize which parasitizes and damages the maize root. Findings by De Waele *et al.* (1998) confirmed that *P. zea* is the most common specie of *Pratylenchus* associated with the maize roots. Similarly, Poudyal (2007) established that a high percentage of *Pratylenchus* species are dominant in maize field. The report of McDonald *et al.* (2005) reiterated that *Pratylenchus* spp are the main nematode parasites of maize. Owing to the established damage and economic importance of *Pratylenchus* spp. on maize, the effectiveness of fractions from the different extracts of *Eucalyptus globulus* was demonstrated in this study to safeguard against environmental pollution of synthetic nematicides. All fractions and rate of application showed efficacy in decreasing nematode population around the soil and roots of maize plant. Generally, this observation can be attributed to the effect of the terpenes present in *Eucalyptus globulus.* This was supported by the results of the infra-red, GCMS and NMR analysis which established the presence of high levels of phenolics, pentacyclic triterpenes, hydrocarbons, monoterpenoids and phenylpropanoids. Citronella and isopulegol have been stated to be part of the constituents of *Eucalyptus* spp. essential oil (Bossou *et al.*, 2013), while Dagne *et al.* (2000), Pino *et al.* (2002) and Singh *et al.* (2009) established the presence of 1, 8-cineole thus corroborating the result of this research. Terpenoids, hydrocarbons, triterpenes and sesquiterpenes have been shown to possess nematicidal activity. Fabiyi *et al.* (2012) and (2014) in their study on chromatographic fractions and orange peel oils, reported a significant decrease in nematode populations at the highest rate of treatment application (75% conc. and 35 mg/mL, respectively. Terpenes are established to possess high nematicidal activity. Ntalli *et al.* (2010), found pure terpenes (L-carvone and pulegone) isolated from EOs from Lamiaceae species and tested individually to be highly nematicidal after 96 hours of exposure to *M. incognita* eggs and juveniles. Carvone, a terpene from *Mentba spicata* inhibited motility of reniform nematodes, the soil stages were more affected by the EO (Abd-Elgawad and Ome, 1995). Essential oils have been widely used as a means of control of plant parasitic nematodes against the toxic synthetic nematicide. Ozdemir and Gozel (2018), reported the nematicidal activities of essential oil (EO) from various plants and they found EO from *Eucaluptus citiriodora* to be toxic to juveniles and eggs of *M. incognita* among all the EOs tested. Essential oils from petroleum ether extract were found effective in the control of *M. incognita* eggs and juvenile's in-vitro, juvenile mortality was highest at 5 mL concentration (Joymati, 2009). Also, studies by Avato *et al.* (2017), established 75% mortality of *Pratylenchus vulnus* juveniles after 96 hours of exposure to 15 μg ml^{-1} of essential oil from *Rosmarinus officinallis*. Laquale *et al.* (2018), equally affirmed the effectiveness of essential oil from *Monarda didyma* and *Monarda fistulosa* on *Meloidogyne incognita* and *Pratylenchus vulnus*, both EOs were significantly active at 15.7 and 12.5μL mL respectively. In a similar study by Youssef *et al.* (2013), number of galls, egg masses, females and developmental stages of *M. incognita* were significantly reduced when leaf extracts of *Eucalyptus* spp was applied at 20 mL concentration in the screenhouse, there was also an increase in yield and vegetative growth. The nematicidal activity of extracts from *Chenopodium ambrosioides* was stated by Mello *et al.* (2006), there was a significant reduction in the population of *Pratylenchus brachyurus* with the highest dose of extract. The effect of nematode infection was seen clearly in the untreated control plants which had reduction in height, late tasselling and reduced yield. The observation of Kimenju *et al.* (1998) is in accordance with this, they encountered growth retardation in maize infected with *Pratylenchus* spp. in their study on impact of lesion nematode on maize. In the same vein, Youssef (2013), reported that the higher the population of *Pratylenchus zea* on maize field the lower the vegetative growth and yield. Norton (1983) also reiterated that reproduction of higher numbers of plant parasitic nematodes on maize automatically results in lower yield. Thus considerable control can be achieved by the application of essential oil.

Conclusions and Recommendations

Essential oil and fractions from *Eucalyptus globulus* have been found to possess nematicidal properties against *Pratylenchus* spp. infecting maize. The phytochemicals responsible for the activity particularly in the essential oil of the plant includes 1,8-cineole, citronella, geranial, isopulegol, myrcene, cuminaldehyde and pinene. The plant is a potential promising source of alternative to synthetic nematicides. The efficacy of this potential bio-nematicidal plant may be further explored for possible development for commercial purpose.

Novelty Statement

The probable use of *E. globulus* fractions in the management of *Pratylenchus* spp. on maize was demonstrated in this study.

Author's Contribution

Oluwatoyin Adenike Fabiyi: Conceptualization, research design, sample preparation, research execution, bench work, data interpretation, manuscript writing, manuscript proofreading
Olubumi Atolani: Research laboratory support, data analysis and interpretation, manuscript writing, manuscript proofreading.
Gabriel Ademola Olatunji: Research execution, data interpretation, manuscript proofreading.

Conflict of interest
The authors have declared no conflict of Interest.

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