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RESPONSE OF *ZEA MAYS* UNDER *PRATYLENCHUS* SPP. INFECTION TO EXTRACTS OF *EUCALYPTUS GLOBULES*

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ABSTRACT

Experiments were conducted in the screenhouse and under field conditions, to evaluate the toxicity of *Eucalyptus globulus* constituents in form of fractions, essential oil and amendment, on the growth of maize and population of *Pratylenchus* spp. The essential oil (ECSG/EO) proved to be significantly ($p<0.05$) more effective and compared with the standard synthetic nematicide carbofuran (CBFN) in reducing nematode population and increasing grain yield. ECSG/EtOH fractions were also effective, while raw plant material used as soil amendment (ECSG/AMDM) was the least effective among all the treatments. The I.R, GC/MS, H^1 NMR and ^{13}C NMR results revealed that the leaves contain monoterpenoids, sesquiterpenes, triterpenoids, hydrocarbons and phenolics.

Keywords: *Eucalyptus globulus, Pratylenchus* spp., amendments, essential oil.

INTRODUCTION

Zea mays is grown in tropical and sub tropical regions of the world, it has the highest production level among other cereal (CIMMYT, 1992; Dixon *et al*., 2009). It is in high demand due to its use as animal feed; poultry feed and as a source of bio fuel (Dixon *et al*., 2009). Globally the production of maize is over 600 metric tonnes (McDonald and Nicol, 2005). It is the most important cereal in Nigeria, mostly grown by small scale farmers and in home gardens during the raining season; it is an important source of carbohydrate, protein, phosphorus and crude fibre. Productivity has risen from 1.2 tonnes/ha to 1.7tonnes/ha (Alene *et al*., 2009) over a period of twenty years. 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 in Nigeria, they are known to cause up to 50% yield loss in maize. Root lesion nematode *Pratylenchus* spp has been implicated to occur frequently on 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 most wide spread corn parasite (Roth *et al*., 1993). Talwana *et al*., (2008) also established *Pratylenchus zea* as the most common nematode on maize, while Alvey *et al*., (2003) in their findings suggested that they are the major parasite causing significant low yield in maize. The yield of maize is lower than the general demand of the populace due to the incidence of *Pratylenchus* spp on the field. Increasing production has however encouraged the use of synthetic nematicide in the bid to bring down nematode population.

The application of synthetic nematicide is often considered inappropriate on a low value crop such as maize because of the cost implication. Moreover the concern about health and environmental hazards posed by the use of synthetic pesticides warrants the search for cheaper alternatives. Consequently this research was undertaken to determine the efficacy of *Eucalyptus globulus* leaves as an alternative control method on the pathogenicity of *Pratylenchus* spp infecting maize and its effect on growth and yield of maize. *Eucalyptus* spp (family Myrtaceae) contains generally essential oils some of which are used as an ingredient in many brands of mouthwash and cough suppressant (Boland *et al*., 1991; MSDS, 2012). It is used in the treatment of respiratory diseases, arthritis and as flavourings in tea. The anti microbial and bactericidal effects of *Eucalyptus globulus* have been reported (Caceres, 1991; Alkofahi, 1997; Ait-Ouazzou, 2011), but there is no information on its nematotoxic or nematostatic activity.

MATERIALS AND METHODS

Preparation of Plant Materials

Eucalyptus globulus leaves were collected in large quantity from the University of Ilorin campus. The air dried leaves were pulverized in a steel man diesel engine milling machine with a 7 horse power capacity; model R175A. The powdered leaves were then divided into five equal parts for the different methods of extraction. Ten kilogram each was extracted separately in n-hexane, ethyl acetate and ethanol. Each extraction lasted five days. The extracts were concentrated using rotary evaporator and allowed to dry to constant weight. Another set of ten kilogram of plant material was set aside for hydro distillation, while the last set of plant material was reserved for direct soil amendment. The extracts were coded ECSG/hex (n-hexane extract), ECSG/EtOAc (ethyl acetate extract) and ECSG/EtOH (ethanol extract). The essential oil obtained from the hydro distillation was coded ECSG/EO (*Eucalyptus globulus* essential oil) and ECSG/AMDM for the plant material used for soil amendment. Clevenger apparatus was used for the hydro distillation; each set lasted 3hours until the plant materials for distillation was exhausted. The resulting oils were dried over anhydrous magnesium sulphate. The oil was a light yellow liquid oil with boiling point of about 177^0C and density 0.92g/mL, it is insoluble in water but miscible with anhydrous alcohol and glacial acetic acid. All crude extracts were fractionated on aluminium oxide (Al_2O_3) and the fractions obtained were subjected to I.R, GC/MS, H^1 NMR and ^{13}C NMR analysis.

Screenhouse experiment

Sandy loam soil was collected from the back of the Faculty of Agriculture, University of Ilorin (Lat 8^0, 29^1 N of the Equator; Long: 4^0, 40^1 E of the Greenwich Meridian). It was pasteurized with an electric soil sterilizer at 60^0C for 2hours. The soil was allowed to cool down and stored away in bags for stabilization within a period of four weeks, after which they were weighed at 20kg each into twenty five litre buckets. The experimental design was a randomized complete block design made up of six treatments at four levels and each was replicated three times (6x4x3). A total of seventy two buckets were used in the experiment. The first and second trials which were planted between April and August 2013 and 2014 had the same design and arrangement in the screen house. At harvest the total number of nematodes in treated and untreated pots was determined.

Field Experiment

Two trials were conducted at the University of Ilorin Teaching and Research Farm, Bolorunduro, kwara state Nigeria, in two raining seasons, between April to August 2013 and at the same period in 2014. The experimental design was also a 6X4X3 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 10m long ridges spaced at 0.5m apart with 30cm plant spacing within the rows. Four maize seeds were sown per hole and were thinned down to two plant stand after two weeks of germination. Plants used for data taken were tagged accordingly on each plot. At harvest soil cores, 15cm deep were collected for nematode population at the base of each maize plant. A diagonal transect method of sampling was used. The soils were thoroughly mixed to give a composite sample for each plot, and total number of nematodes at harvest was determined.

Preparation of Inoculums and Inoculation

Pratylenchus spp used for inoculation was collected from infected maize roots, obtained from IITA maize field, they were extracted using the modified Baermann sieve method (Coyne *et al.,* 2007) and multiplied on maize plants grown in plastic pots. After multiplication they were re-extracted. Number of nematodes per unit volume was estimated by taking aliquots of one ml three times and counting under a stereomicroscope using Doncaster's (1962) counting dishes. The maize plants were inoculated after two weeks of planting with approximately 300 nematodes using the method of Iheukwumere *et al*. (1995).

Treatment application

Carbofuran 3G bought from Ilorin metropolis was applied at 0.3, 0.5, and 1.0kgai/ha-^{1} in the screenhouse, while on the field 1.0, 1.5 and 2.0kga.i/ha-^{1} was used. The dosage of application of plant material used as soil amendments was the same as carbofuran in the screenhouse and on the field. Fractions obtained from column chromatography of crude extracts and the essential oils were applied at 30, 20 and 10mg on the field and the screenhouse had 15, 10 and 5mg, inoculated untreated maize fields served as control.

Statistical Analysis

Data was collected on plant height, days to 50% tasselling, maize yield kg/ha^{-1}, nematode population in 200g soil and nematode population in 10 gram root sample. All parameters were subjected to analysis of variance (ANOVA). Treatment means where necessary were separated using the new Duncan's multiple range test at 5% level of probability (Gomez and Gomez, 1984).

Instruments

The fractions were analysed on GC-6890N-system. Injector (250^{0}C), attached to a mass- selective detector 5973. Identification of compounds was by comparison with NIST library data. I.R spectra were recorded on 8400s Fourier Transform Infrared (FTIR). Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) were determined using JEOL 400 MHz. The chemical shifts were recorded in ppm relative to TMS, while the coupling constants are in Hz

Spectroscopic Results

The GCMS analysis of *E. globulus* showed the presence of fifteen major constituents of which 1,8-cineole (23.3%), citronella (18.1%), geranial (17.6%), isopulegol (10.4%), myrcene (13.0%), cuminaldehyde (9.1%) and pinene (8.5%) are the major constituents and the others are detected in traceable amount. The I.R (KBr cm-^{1}) indicated the presence of hydroxyl functional groups at 3353, 3200 and 1061 cm-^{1}. The ^{1}H- NMR showed methyl and methylene signals at δ 0.71, 0.83, 1.04, 1.22ppm and diagnostic ^{13}C-NMR signals appeared around 116, 120, 174, 191ppm representing unsaturated double bonds and carbonyl functional groups.

SCREENHOUSE AND FIELD RESULTS

The maize plants treated with fractions showed different degrees of increased vegetative growth compared to the untreated control plants. Table 1 depicts comparative effectiveness of all treatments on the plant height of maize in the screenhouse. 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 plant materials were however not as tall as other plants. Similarly on the field, ECSG/EO and CBFN exhibited taller plants (Table 4). Early taseling was observed in ECSG/EO and CBFN treated plants (50.73 and 51.22), while taseling ability was delayed in ECSG/AMDM treated plants (72.11), this was the observation on the field as well where taseling was earlier in essential oil and carbofuran treated plants (Tables 2 and 5). There was a significant relationship between early taseling and yield. Higher grain yield was recorded in plants treated with fractions as opposed to the yield of plants in the amended soil and untreated control plants. In the screenhouse and on the field, EO treated plants had higher grain yield than all other treatments (Tables 2 and 5) the magnitude of yield was directly proportional to the rate of treatment application. Significant differences were observed in the effect of the various treatments on nematode population in 250 g soil sample and in the 15g 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 treated plants (Tables 3 and 6). Nematodes were completely absent in the 15gram root sample of plants treated with EO and CBFN in the screenhouse, 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. 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 fractions. 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.

Screen house

Table 1: Effect of *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on plant height of maize in the screenhouse.

Treatments	4th WAP	6th WAP	8th WAP	10th WAP	12th WAP	14th WAP
ECSG/EO	18.22^{a}	29.00^{a}	53.14^{a}	79.82^{a}	105.48^{a}	129.46^{a}
ECSG/Hex	13.15^{b}	19.09^{cd}	32.27^{d}	55.03^{d}	77.29^{d}	93.33^{d}
ECSG/EtoAc	13.28^{b}	22.00^{b}	39.10^{c}	62.24^{c}	84.00^{c}	113.29^{c}
ECSG/EtOH	13.06^{b}	23.52^{b}	44.06^{b}	69.18^{b}	91.31^{b}	120.52^{b}
ECSG/AmDm	10.18^{c}	17.05^{d}	26.12^{e}	46.81^{e}	72.14^{e}	86.11^{e}
CBFN	18.01^{a}	28.61^{a}	52.71^{a}	80.34^{a}	104.64^{a}	128.93^{a}
S.E.M	0.05	0.06	0.14	0.19	0.26	0.31
Level/Mg						
0	9.05^{c}	12.17^{d}	20.14^{d}	28.22^{d}	50.07^{d}	71.23^{d}
5	11.31^{b}	15.21^{c}	27.02^{c}	39.18^{c}	61.11^{c}	84.17^{c}
10	14.10^{a}	19.12^{b}	33.11^{b}	46.00^{b}	68.16^{b}	91.25^{b}
15	14.00^{a}	24.02^{a}	40.22^{a}	52.27^{a}	75.05^{a}	98.12^{a}
SE.M	0.03	0.09	0.11	0.16	0.23	0.29

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

Table 2: Effect of *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on days to 50% tasseling and yield of maize in the screenhouse.

Treatments	Days to 50% Tasseling	Maize Yield in (kg)
ECSG/EO	50.73^{a}	6.79^{a}
ECSG/Hex	69.06^{d}	2.11^{d}
ECSG/EtoAc	62.01^{c}	4.03^{c}
ECSG/EtOH	56.31^{b}	5.21^{b}
ECSG/AmDm	72.11^{e}	1.08^{e}
CBFN	51.22^{a}	7.18^{a}
S.E.M	1.16	0.07
Level/Mg		
0	70.21^{d}	0.31^{d}
5	48.72^{c}	1.27^{c}
10	41.00^{b}	3.06^{b}
15	33.18^{a}	5.14^{a}
SEM	0.27	0.03

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

Table 3: Effect of *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on nematode population of maize in the screenhouse.

Treatments	Nematode Population in 250 g soil	Nematode Population in 10 g root
ECSG/EO	3.06^{a}	0.00^{a}
ECSG/Hex	16.12^{c}	5.22^{c}
ECSG/EtoAc	9.04^{b}	1.09^{b}
ECSG/EtOH	8.60^{b}	1.13^{b}
ECSG/AmDm	23.01^{d}	9.04^{d}
CBFN	2.74^{a}	0.00^{a}
S.E.M	0.13	0.04
Level/Mg		
0	810.16^{d}	123.11^{c}
5	7.29^{c}	3.06^{b}
10	2.18^{b}	0.00^{a}
15	0.00^{a}	0.00^{a}
SEM	0.19	0.15

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

Field

Table 4: Effect of *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on plant height of maize on the field

Treatments	4th WAP	6th WAP	8th WAP	10th WAP	12th WAP	14th WAP
ECSG/EO	20.89^{a}	38.20^{a}	59.88^{a}	85.02^{a}	116.03^{a}	135.91^{a}
ECSG/Hex	14.11^{c}	20.00^{d}	40.09^{d}	62.18^{d}	86.05^{d}	103.29^{d}
ECSG/EtoAc	18.24^{b}	24.13^{c}	45.10^{c}	68.28^{c}	95.34^{c}	112.83^{c}
ECSG/EtOH	18.11^{b}	30.08^{b}	52.14^{b}	74.31^{b}	104.21^{b}	122.07^{b}
ECSG/AmDm	13.56^{c}	20.21^{d}	34.10^{e}	53.76^{e}	72.61^{e}	92.12^{e}
CBFN	21.06^{a}	38.12^{a}	60.23^{a}	84.62^{a}	115.67^{a}	136.16^{a}
SEM	0.13	0.17	0.12	0.18	0.21	0.27
Level/Mg						
0	5.06^{d}	9.21^{d}	21.16^{d}	35.29^{d}	51.22^{d}	60.29^{d}
10	9.12^{c}	15.07^{c}	32.11^{c}	50.03^{c}	63.21^{c}	73.14^{c}
20	14.09^{b}	21.33^{b}	40.28^{b}	58.39^{b}	74.36^{b}	82.71^{b}
30	17.68^{a}	29.10^{a}	49.60^{a}	66.21^{a}	85.19^{a}	101.10^{a}
SEM	0.00	0.03	0.09	0.12	0.08	0.11

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

Table 5: Effect *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on days to 50% tasseling and maize yield on the field.

Treatments	Days to 50% Tasseling	Maize Yield in (kg)
ECSG/EO	52.70^{a}	24.11^{a}
ECSG/Hex	66.18^{d}	10.03^{d}
ECSG/EtoAc	61.33^{c}	13.08^{c}
ECSG/EtOH	57.11^{b}	18.15^{b}
ECSG/AmDm	73.05^{e}	7.29^{e}
CBFN	53.16^{a}	23.81^{a}
S.E.M	0.31	0.19
Level/Mg		
0	69.22^{d}	3.15^{d}
10	51.07^{c}	9.28^{c}
20	44.12^{b}	13.07^{b}
30	36.71^{a}	17.25^{a}
SEM	0.28	0.07

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

Table 6: Effect *Eucalyptus globulus* essential oil, fractions, leaves and carbofuran on nematode population on maize in the field.

Treatments	Nematode Population in 250 g soil	Nematode Population in 10 g root
ECSG/EO	8.64^{a}	3.66^{a}
ECSG/Hex	18.33^{c}	12.21^{c}
ECSG/EtoAc	12.01^{b}	7.08^{b}
ECSG/EtOH	11.61^{b}	6.51^{b}
ECSG/AmDm	26.18^{d}	18.14^{d}
CBFN	9.19^{a}	4.10^{a}
S.E.M	0.12	0.09
Level/Mg		
0	1137.25^{d}	67.21^{d}
10	22.06^{c}	13.26^{c}
20	17.34^{b}	9.04^{b}
30	10.21^{a}	5.16^{a}
SEM	0.16	0.05

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$ using the new DMRT.
DMRT=Duncan's multiple range test. Each value is a mean of three replicates and an average of data taken over a two year period.

DISCUSSION

The effectiveness of fractions from the different extracts of *Eucalyptus globulus* was demonstrated. All fractions and rate of application exhibited efficiency in reducing 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 reduction in nematode populations at the highest rate of treatment application (75% conc. and 35 mg/mL, respectively. 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 5mL concentration (Joymati, 2009). In a similar study by Youssef and Lashein (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 effect of nematode infection was seen clearly in the untreated control plants which had reduction in height, late taseling 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. Norton, (1983) also reiterated that reproduction of plant parasitic nematode on maize automatically results in lower yield.

CONCLUSION

Fractions from *Eucalyptus globulus* have been found to possess nematicidal properties. It is a potential promising source of alternative compound to synthetic nematicides. The efficacy of the essential oil is most likely based on their terpenoid composition. Some of these components have been reported to be nematicidal.

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