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Determination of Nitrification Retarding Activities of Four Plant Essential Oils

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ABSTRACT

An Investigation was conducted to determine the nitrification retarding activities of the essential oils of Piper umbelatum, Piper nigrum, Pinus sylvestris and Monodora myristica. The essential oils were obtained from the plant materials by steam distillation and analyzed using GC-MS. The microbial analysis, chemical analysis and soil analysis were done by standard methods. The main composition of the essential oil of P. umbellatum were caryophyllene (10.44%) and aromadendrene (13.74%), linalool (21.73%) was found to be the main constituent of P.nigrum, P. sylvestris had terpinen-4-ol (21.82%), α terpineol (14.07%) and β terpineol (27.17%) as its key constituents, the essential oil of M. myristica had Z-13-octadecenoic acid (25.18%) and E-Sabinol (17.87%) as the chief constituents. The values of nitrosomonas, actinomycetes and nitrobacter population when urea fertilizer was coated with the essential oils after the 7^{th} to 21^{st} day of incubation ranged from ($1.6 x10^5$- $8.6x10^5$ cfu/g). The values of NO_3^- ranged from 6.22 – 23.06 mg/Kg while the quantity of NO_2^- ranged from 3.64 - 13.36 mg/Kg. The amount of NH_4^+ ranged from 15.16-78.44 while the amount of urea left in the soil ranged from 25.60 - 88.05 mg/Kg. The results revealed that all essential oils had nitrification retarding activity. The population of bacteria during the different days of incubation was higher in the controlled experiment than in the essential oil coated urea fertilizer, while P. umbellatum coated urea fertilizer had the least nitrification activity, the essential oil of P. sylvestris had the highest nitrification regulatory activity.

1. INTRODUCTION

In order to cope with the increasing world population, there is a need to strengthen the global food supply. In order to resolve the envisaged pending food scarcity in years to come, there is a need to increase food production by 100% (Roberts, 2009). To balance the gap between the export of nutrients from the field, the harvested crops, and the nutrients supplied by the soil, fertilizers are usually applied. In Africa, nitrogenous fertilizer is extensively used in farming. However, not all of the applied fertilizer is used up by the crops. A very essential crop nutrient required for growth and viability is nitrogen. Nitrogen (N) base fertilizers have tremendously improved the global growth of crop yield. For years, the increase in rate of fertilizer application was important for enhancing an improved yield of crops, but it was observed that the effectiveness of nitrogenous fertilizer was reduced when it is applied at a high dose (Hua *et al*., 2020).

A very high percentage of nitrogenous fertilizer applied to the soil for the crops is not utilized. Treusch *et al*. (2005) reported that about 25% of the fertilizer applied to the soil remains in the form of nitrate while the rest 25% of the fertilizer is leached away or denitrified. Plants are deprived of nitrate from nitrogenous fertilizer as well as the soil during periods of excess rainfall, this is as a result of leaching which may move nitrate out of the effective rooting zone. The microbiological change of nitrite and nitrate to vaporous forms of Nitrogen is known as denitrification. This is another way in which nitrogen is lost from the soils, it normally occurs in waterlogged soils (Cáceres *et al*., 2017).

Compounds that retard the conversion of ammonium, ammonia, and urea fertilizers used on the soil are known as nitrification inhibitors. Several methods have been employed for the improvement of nutrient use efficiency in the soil, one of these methods is the addition of nitrification inhibitors with fertilizers (Fu *et al*., 2020). These so called nitrification inhibitors can be of help in regulating nitrogen loss in soil that would otherwise be useful for plant growth (Turnera *et al*., 2010). Nitrification inhibitors are extensively used in the U.S. in cities like Illinois (Habibullah *et al*., 2018). Nitrification inhibitors improve the rate at which nitrogenous fertilizer applied to the soil stays longer in the soil. Some of the nitrogenous fertilizers lost their nutrients to the environment, and thereby contributing to the environmental problems observed, which led to climatic change. Deodhar *et al.*, (2020) revealed that the degree of effectiveness of the inhibitors is based on some external conditions.

Different kinds of synthetic chemicals that have the ability to inhibit the hydrolysis of urea and nitrification processes in soils have been reported. Some of the chemicals used as nitrification inhibitors include nitrapyrin and dicyandiamide. Due to the high cost involved in the use of these chemicals, the scarcity, and their adverse effect on beneficial soil microorganisms, they have only been used experimentally (Upadhyay *et al*., 2011). In this study, the four essential oils were selected based on the microbial activities they possess. These products have been restricted to the experimental stage according to Cui *et al*. (2022). This use of botanical plants has bridged the gap between the cost implications and the easy accessibility of nitrification inhibitors. This study aimed at determining the nitrification regulatory ability of four different essential oils coated on urea fertilizer.

2. METHODOLOGY

Plant Materials

African Nutmeg (*Monodora myristica*) seeds, *Piper umbellatum* seeds were bought from Orisumbare Market in Osogbo, Osun State. Pine needle (P*inus sylvestris*) was obtained from its tree at Awo Hall Obafemi Awolowo University, Ife, Osun State. Black pepper (*Piper nigrum*) was purchased from Agbalata Market Badagary, Lagos State. The seeds were authenticated at the Department of Plant Biology, Osun State University, Osogbo, Nigeria. The seeds were air-dried and ground to a powder. Urea fertilizer was bought from an agricultural store at, Station Road, Osogbo, Osun State, Nigeria.

Extraction of Essential Oils

The ground powders of the seeds were extracted using the steam distillation method. The ground seed was poured into a 1 L round bottom flask with the aid of a funnel, distilled water was then added, while the solution was mixed with a glass stirring rod. The heating mantle was connected to a power source and turned on, the flow of water through the condenser of the Clevenger commenced and was fitted into the round bottom flask, which was then placed on the heating mantle. The mixture was heated gradually while extraction was allowed for 8 hours, after which the extract was collected.

GC-MS Analysis

Gas chromatography and mass spectrometry gas chromatographic analysis was done on an Agilent 6890N instrument equipped with a flame ionization detector and HP-5MS (30m × 0.25mm × 0.25μm) capillary

column. The essential oil components were identified on an Agilent Technologies 5973N mass spectrometer. The GC was set at an initial oven temperature of 60 °C for 1 min and it was ramped at 10 °C min−1 to 180 °C for 1 min, followed by another ramping at 20 °C min−1 to 280 °C for 15 min. The injector temperature was left at 270 °C. The sample (1 μL) was injected with a split ratio of 1:10. Helium was the carrier gas with a flow rate of 1.0 mL min−1, the Spectra were scanned from 20 to 550 m/z. The constituents were identified by retention indices with those of the literature. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 08 and Wiley 275 libraries or with mass spectra from literature. Component relative percentages were calculated based on GC peak areas without using correction factors.

Coating of Urea and Microbial Analysis

The urea fertilizer was weighed (5 g) and mixed manually with 0.263 g of the different essential oils to form 5% (w/w) formulation. The mixture was then mixed with 100 g of the air dried soil and placed in a plastic container and maintained at 50% water holding capacity and the container was loosely capped to allow aeration. The formulation was placed in an incubator at 25^{o}C and collected in triplicate for the microbial and chemical analysis after 7, 14 and 21 days of incubation. A control experiment was conducted with same formulation but without essential oil coating. Samples of soil were picked from the incubated samples, the soils were analyzed for population of nitrosomonas, nitrobacter and actinomycetes using serial dilution method and adopting the method of Aneja, 2003.

Determination of Different Forms of Nitrogen, Routine Soil Analysis and Statistical Analysis

Samples of soil were collected from all the incubated samples and analyzed for the different forms of nitrogen by the method of Jackson (1973). The various soil parameters which include measurement of soil pH, determination of organic carbon, particle size analysis, determination of nitrogen, determination of estimated cation exchange capacity, determination of exchangeable base and determination of total elements were determined by the method of FAO (2004) and Jackson (1973). One-way analysis of variance (ANOVA) with SPSS statistics was used to analyze the data obtained in three replicates. The differences in the values of the experiments were considered to be significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the GC-MS analysis of the major components of four essential oils. The major components detected in the essential oil of *M. myristica* are Z-13-octadecenoic acid, linalool, sabinol, and n-hexadecanoic acid (25.18%, 9.11%, 17.87%, and 7.66% respectively). Igwe *et al*. (2005) reported the presence of 61 components from the hydrodistillation of the essential oil *M. myristica* with the main component being 3.20% of α-phellandrene expoxide. The analysis of the essential oil of *P. nigrum* showed that the major components are, linalool (21.73%), γ-Bisabolene (8.75%), β-Caryophyllene (7.35%). Fan *et al*. (2011) reported limonene (35.06%) as the main constituent of the fruits of *P. nigrium*. On the other hand, Jirovetz *et al*. (2002) observed that germacrene (11.01%) was the main component obtained from the essential oil of dried fruit of *P. nigrium.*

The GC-MS analysis of *P. sylvestris* identified the major components as terpinen-4-ol, β terpineol, Borneol, α terpineol, longifolene. Awojide *et al*. (2023) reported that isoborneol is the major constituent of *P. sylvestris* essential oil, while other components, are α-terpineol, terpine-1-ol and fenchol. The essential oil of *P. umbelattum* (Table 1) indicated the presence of thirty-seven components of which, of the five major constituents, linalool (8.55%) is an oxygenated monoterpene while others are hydrocarbon sesquiterpenes with aromadendrene (13.74%) being the component with the highest value, as reported by Awojide *et al.* (2015). The differences in the composition may be as a result of the chemotypes for the same or different species, they could also be as a result of environmental and physiological differences (Fayemiwo *et al*., 2014).

Soil testing is an important step in nutrient management and can be used as a diagnostic tool to identify trends over time (Brady and weil, 2002). The soil physiochemical properties in the absence of urea fertilizer, soil mixed with urea fertilizer, and soil mixed with coated fertilizer is shown in Table 2. It is important to know if the application of fertilizer or the essential oil coating on fertilizer will affect the physicochemical parameters of the soil sample used in the experiment. From the result, the value of Cation exchange capacity (CEC) for the soil used ranged between 8.12-8.34 cmol/mg. When the CEC value is high, this indicates the ability of the soil to hold cations, a CEC of more than 10 meq/100 g is said to be good enough for the soil (Khaledian *et al.*, 2017), and it shows that the soil is either fine sandy loam or loam and silt loams (Havlin *et al*., 2011).

The CEC change with soil pH between 6 and 7.5 is considered to be the best to grow most crops. The soil pH has an effect on the micro and macro nutrients. The result revealed that the soil used for the various tests was suitable for use in the growing of crops, the soil is termed slightly alkaline by The United States Department of Agriculture (Tony *et al*., 2020). Nitrogen fixation supplies nitrogen in the form of nitrate (NO_3^-)) and ammonium (NH_4^+)) and it is best with soils of pH 6-8 (Brady and Weil, 2002). The result shows that the required micronutrients needed to supply plants are present in all categories of the soil analyzed. The soil was suitable for the growth of agricultural plants, as seen in the estimated values. The results of the parameters in the soil without urea fertilizer, soil mixed with urea fertilizer, and soil mixed with essential oil coated urea fertilizer were significantly not different.

Table 1: Major constituents of four essential oils

Components	% In Sample			
	Piper Umbellatum	*M myristica*	*Piper Nigrium*	*Pinus Sylvestris*
Linalool	8.55	9.11	21.73	-
Caryophyllene	10.44	-	7.35	-
Aromadendrene	13.74	-	-	-
γ Bisabolene	8.82	-	8.75	-
Germacrene	6.56	-	-	-
Sabinol-cis	-	17.87	-	-
tri-1,3-octadecenoic acid	-	25.18	-	-
Palmitic acid	-	7.66	-	-
β Farnescene	-	-	6.12	-
Terpinen-4-ol	-	-	-	21.82
β Terpineol	-	-	-	14.07
Borneol	-	-	-	6.72

Table 3 shows the result of the bacterial population in the soil after the urea fertilizer was coated with essential oils mixed with the soil and then left to incubate for 7 days. The result revealed that for the different bacterial counts, the control soil had the highest population of microbial counts. In the treated urea soil samples, the *P. umbellatum* urea fertilizer coated soil had the highest microbial population while the least microbial population, was observed in the *P. sylvestris* treated soil. The results generally revealed that the essential oil treated Urea samples reduced the population of bacteria present in the soil. The result showing the values of the different forms of nitrogen present after 7 days of incubation of the essential oil treated Urea mixed with soil is shown in Table 4. The result revealed that the value of Urea present in the control soil sample had the lowest value of 45.44 mg/Kg while those of the essential oil treated soil samples had higher values of Urea. The values of other different forms of Nitrogen (NO_3^-, NO_2^- and NH_4^+) in the control experiment, were higher than those recorded for essential oil treated samples. The essential oil of *P.*

umbellatum had the highest values of the different forms of nitrogen, while the essential oil of *P. sylvestris* had the lowest values.

Table 2: Physical and chemical properties of the soil

Parameters	Soil without Fertilizer	Soil mixed with Fertilizer	Soil mixed with coated Fertilizer
pH	7.85±0.05	7.81±0.12	7.50±0.30
ECEC (cmol/Kg)	8.34±0.05	8.12±0.01	8.30±0.20
Organic C (%)	1.38±0.02	1.35±0.11	1.36±0.02
N (%)	0.16±0.01	0.14±0.10	0.12±0.01
Exchangeable bases			
Ca (cmol/Kg)	7.21±0.01	7.25±0.02	6.96±0.03
Mg (cmol/Kg)	0.63±0.02	0.64±0.02	0.64±0.01
Na (cmol/Kg)	0.22±0.01	0.24±0.02	0.23±0.02
K (cmol/Kg)	0.31±0.01	0.32±0.01	0.33±0.01
Particle size			
Sand (%)	86.80±1.20	84.40±1.22	86.40±1.24
Silt (%)	9.00±0.11	9.40±0.02	8.90±0.12
Clay (%)	5.20±0.01	5.20±0.01	5.00±0.20
Micro-nutrients			
P (mg/kg)	10.84±0.11	12.73±0.78	11.74±012
Fe (mg/kg)	166.00±9.12	170.30±3.23	178.21±9.20
Cu (mg/kg)	3.50±0.02	3.30±0.12	3.20±0.01
Zn (mg/kg)	19.8±0.01	19.16±0.02	19.27±0.23
Mn (mg/kg)	779.23± 11.23	780.75±10.20	783.35±10.50

Table 3: Microbial analysis of incubated soil on the 7th day

Essential Oil	Nitrosomonas count (cfu/g)	Actinomycetes count (cfu/g)	Nitrobacter count (cfu/g)
Pinussylvestris	$2.4x10^5 \pm 700^a$	$1.6x10^5 \pm 000^a$	$3.1x10^5 \pm 500^a$
Monodoramyristica	$2.9x10^5 \pm 1000^b$	$2.1x10^5 \pm 900^c$	$4.2x10^5 \pm 700^c$
Piper umbellatum	$4.3x10^5 \pm 900^d$	$2.7x10^5 \pm 2000^e$	$5.5x10^5 \pm 700^e$
Piper nigrum	$3.1x10^5 \pm 300^c$	$2.5x10^5 \pm 400^d$	$5.2x10^5 \pm 900^d$
Control soil	$6.3x10^5 \pm 400^f$	$4.0x10^5 \pm 300^g$	$6.4x10^5 \pm 1000^g$

The result shows the mean ± SD of three replicates. Data within a column followed by the same letter are not significantly different at $P < 0.05$.

Table 4: Chemical analysis of incubated soil on the 7th day

Essential Oil	NO_3-N mg/kg	NO_2-N mg/kg	NH_4-N mg/kg	Urea-N mg/kg
Pinussylvestris	6.22 ± 0.21^a	3.96 ± 0.01^a	15.16 ± 0.01^a	88.05 ± 0.11^d
Monodoramyristica	8.11 ± 0.11^b	6.18 ± 0.11^c	17.08 ± 0.03^b	82.67 ± 0.11^d
Piper umbellatum	8.57 ± 0.01^b	6.71 ± 0.13^c	$18.78\pm0.21b^c$	82.19 ± 0.23^d
Piper nigrum	6.65 ± 0.02^a	3.88 ± 0.01^a	15.89 ± 0.11^a	86.71 ± 0.21^d
Control soil	18.32 ± 0.13^f	11.65 ± 0.14^f	54.56 ± 0.14^f	45.44 ± 0.03^a

Table 5 shows the result of the bacterial population in the soil after the urea fertilizer was coated with essential oils mixed with the soil and then left to incubate for 14 days. The result revealed that for the different bacterial counts, the control soil had the highest population of microbial counts. In the treated urea soil samples, the *P. umbellatum* urea fertilizer coated soil had the highest microbial population, while the

least microbial population was observed in the *P. sylvestris* treated soil. The results generally revealed that the essential oil treated urea samples reduced the population of bacterial present in the soil, which indicated an increase in urease activity.

The result showing the values of the different forms of nitrogen present after 14 days of incubation of the essential oil treated Urea mixed with soil is shown in Table 6. The result revealed that the value of Urea present in the control soil sample had the lowest value of 38.14 mg/Kg while those of the essential oil treated soil samples had higher values of urea. The values of other forms of Nitrogen (NO_3^-, NO_2^- and NH_4^+) in the control experiment were higher than those recorded for essential oil-treated samples. The essential oil of *P. umbellatum* had the highest values of the different forms of nitrogen except urea, which has the lowest value, while the essential oil of *P. sylvestris* had the lowest values of the different forms of nitrogen except urea, which has the highest value. This result indicated that the urea-coated essential oil of *P. umbellatum* had the least activity as an inhibitor, while *P. sylvestris* had the highest retarding activity.

Table 7 shows the result of the bacterial population in the soil after the urea fertilizer was coated with essential oils mixed with the soil and then left to incubate for 21 days. The result revealed that for the different bacterial counts, the control soil had the highest population of microbial counts. In the treated urea soil samples, the *P. umbellatum* urea fertilizer-coated soil had the highest microbial population, while the least microbial population was observed in the *P. sylvestris* treated soil. The result generally revealed that the essential oil-treated Urea samples reduced the population of bacteria present in the soil, but at a different rate.

Table 5: Microbial analysis of incubated soil on the 14th day

Essential Oil	Nitrosomonas count (cfu/g)	Actinomycetes count (cfu/g)	Nitrobacter count (cfu/g)
Pinussylvestris	$2.8x10^5 \pm 1000^a$	$1.8x10^5 \pm 700^a$	$3.7x10^5 \pm 800^a$
Monodoramyristica	$3.3x10^5 \pm 500^b$	$2.4x10^5 \pm 800^b$	$4.5x10^5 \pm 200^b$
Piper umbellatum	$4.7x10^5 \pm 900^d$	$3.2x10^5 \pm 700^d$	$6.2x10^5 \pm 400^d$
Piper nigrum	$3.9x10^5 \pm 700^c$	$2.8x10^5 \pm 400^c$	$5.6x10^5 \pm 600^c$
Control soil	$7.7x10^5 \pm 600^f$	$4.1x10^5 \pm 300^e$	$8.2x10^5 \pm 700^f$

Table 6: Chemical analysis of incubated soil on the 14th day

Essential oil	NO_3-N mg/kg	NO_2-N mg/kg	NH_4-N mg/kg	Urea-N mg/kg
Pinussylvestris	6.47 ± 0.21^a	3.88 ± 0.01^a	15.28 ± 0.11^a	82.59 ± 0.01^d
Monodoramyristica	8.23 ± 0.01^c	5.76 ± 0.01^{bc}	17.43 ± 0.01^b	80.33 ± 0.01^d
Piper umbellatum	8.69 ± 0.11^c	6.54 ± 0.03^c	19.86 ± 0.03^c	79.82 ± 0.03^d
Piper nigrum	6.78 ± 0.02^a	4.03 ± 0.02^a	16.29 ± 0.04^b	81.51 ± 0.02^d
Control soil	21.79 ± 0.11^f	13.28 ± 0.03^e	63.59 ± 0.22^f	38.18 ± 0.11^a

Table 7: Microbial analysis of incubated soil on the 21st day

Essential oil	Nitrosomonas count (cfu/g)	Actinomycetes count (cfu/g)	Nitrobacter count (cfu/g)
Pinussylvestris	$3.5x10^5 \pm 800^a$	$2.4x10^5 \pm 200^a$	$3.9x10^5 \pm 500^a$
Monodoramyristica	$4.1x10^5 \pm 800^b$	$2.8x10^5 \pm 500^b$	$5.2x10^5 \pm 900^c$
Piper umbellatum	$5.4x10^5 \pm 400^d$	$3.8x10^5 \pm 600^d$	$7.0x10^5 \pm 300^e$
Piper nigrum	$4.6x10^5 \pm 500^c$	$3.2x10^5 \pm 400^{bc}$	$6.1x10^5 \pm 300^d$
Control soil	$8.6x10^5 \pm 500^f$	$5.1x10^5 \pm 1000^e$	$8.6x10^5 \pm 400^f$

The result showing the values of the different forms of nitrogen present after 21 days of incubation of the essential oil treated urea mixed with soil is shown in Table 8. The result reveals that the value of urea present in the control soil sample had the lowest value of 25.60 mg/kg, while those of the essential oil treated soil samples had higher values of urea. The values of other different forms of Nitrogen (NO_3^-, NO_2^- and NH_4^+) in the control experiment were higher than those recorded for essential oil treated samples. The essential oil of *P. umbellatum* had the highest values of the different forms of nitrogen, while the essential oil of *P. sylvestris* had the lowest values. The population of bacteria that are responsible for the oxidation process of the different forms of nitrogen was recorded. The result showed that the population of bacteria in the control experiment for the 7th, 14th, and 21st days of incubation was higher than that observed in the essential oil treated urea fertilizer, while the population of bacteria kept increasing with days of incubation in the control experiment (Tables 4, 6 and 8) and the values of the population of bacteria reduced in the essential oil treated samples. This indicated that the essential oil used to incubate the soil controlled the growth of bacteria, thereby reducing the urease activity of the bacteria that are responsible for the oxidation of ammonium to nitrite (nitrobacter) and nitrite to nitrate (Nibrosomonas). The essential oil with the highest activity against bacterial growth is *P. svlvestris,* while *P. umbellatum* had the least activity against bacterial growth during the days of incubation (7th, 14th, and 21st).

Table 8: Chemical analysis of incubated soil on the 21st day

Essential oil	NO_3-N mg/kg	NO_2-N mg/kg	NH_4-N mg/kg	Urea-N mg/kg
Pinussylvestris	6.96±0.01[a]	3.64±0.11[a]	16.71±0.21[a]	82.12±0.0[d]
Monodoramyristica	8.47±0.11[b]	5.81±0.11[c]	17.96±0.03[a]	79.61±0.01[d]
Piper umbellatum	8.82±0.21[c]	6.59±0.13[d]	21.22±0.01[c]	76.76±0.11[d]
Piper nigrum	6.89±0.12[a]	4.65±0.05[b]	19.38±0.11[b]	80.94±0.02[d]
Control soil	23.06±0.01[f]	13.36±0.03[f]	78.44±0.04[f]	25.60±0.05[a]

The result shows the mean ± SD of three replicates. Data within a column followed by the same letter are not significantly different at $P < 0.05$.

From the values of different forms of nitrogen obtained, it showed that during all days of incubation, the value of urea left in the control experiment reduced drastically compared to the values obtained in the essential oil-coated fertilizer. The value reduction from 45.44 mg/Kg observed in the 7th day of incubation to 38.18 mg/Kg in the 21st day indicated that urea fertilizer was easily converted to other forms of Nitrogen due to the higher population of bacteria in the essential oil coated fertilizer. Higher values of urea were available in the soil, which could be a result of the lower bacterial population present in the soil sample. Ammonium nitrogen is converted to nitrite by Nitrobacter. The value of NH_4^+ present in the control experiment increased with days of incubation; this increase is in relation to the increase in the population of Nitrobacter. It should be noted that NH_4^+ can easily be lost by volatilization in the form of NH_3 or can rapidly be converted to Nitrite. The values of NH_4^+ present in all treated soil samples throughout the days of incubation were lower than the corresponding control experiment, indicating a lesser absence of volatilization of the NH_4^+. Nitrite cannot be easily used by plants, so it is normally converted to nitrate, which is required by plants. The control experiment during all the days of incubation had a higher value of NO^-_2 corresponding to the higher value of nitrobacter present in the control experiment. The same trend was observed in the values of NO_3^- recorded. A higher value of nitrate in the control experiment indicated that a higher population of nitrosomonas was present in the control sample. Nitrate, which cannot be easily held by the plant, can be lost through leaching.

The essential oil-coated fertilizer was able to control these conversion processes of the different forms of nitrogen, thereby allowing the fertilizer applied to be present for the plant's use for a longer duration. Records have it that Pyrethrum flowers have nitrification inhibitory activities, which enable nitrogen to be more readily available in the soil (Li *et al.,* 2021). The control experiment during all days of incubation

had a higher value of NO_2^- corresponding to the higher value of nitrobacter present in the control experiment. The same trend was observed in this bioassay. The main constituents of *P. sylvestris*, which is the most active essential oil in nitrification retarding activity, were terpinen-4ol, α-terpineol and β-terpineol, while the main components of *P unbellatum*, which is the least active, were caryophyllene, aromadendrene, and linalool. Opoku *et al.* (2014) revealed that the use of *M. spicata* oil was able to increase the efficiency of nitrogen. Similar results were observed when the essential oils of *P. umbelatum*, *P. nigrum*, *P. sylvestris*, and *M. myristica* were used to coat urea. Some products that are termed natural have been investigated to possess urease retarding properties (Patra *et al.*, 2009). In the assessment of Patra *et al.* (2009), the soil microbial activity of all natural materials used in the coating of fertilizer influenced the counts of nitrosomonus and nitrobacter responsible for nitrification, as well as actinomycetes and total bacteria. Nitrsomonus population decreased.

4. CONCLUSION

The results obtained showed that all the four essential oils reduced the population of bacteria responsible for the conversion of urea fertilizer into all forms of nitrogen. Making the urea fertilizer more available in the soil for a longer duration of time. Finally, an account of the total bacterial population indicates significant antibacterial properties of the synthetic and natural products. This means that all essential oils used are nitrification retardants, making the urea fertilizer applied retain its usefulness for a longer duration.

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