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Volatile Constituents and Potatoes Tuber Sprout Suppressant Activity of *Pimenta racemosa* (Mill) J.W. Moore

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Abstract: Essential oil was obtained by hydrodistillation from air dried aerial part of *Pimenta racemosa* (mill) J.W Moore. The oil was analysed for its constituents by means of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (Gc -Ms). Sesquiterpene compounds (63.1%) occurred in higher proportion. The major constituents of the oil were Germacrene D (10.6%), β-elemene (8.8%), germacrene A (7.3%), selin-11-en-4-α-ol (6.3%), δ-cadinene (5.9%), β-caryophyllene (5.8%), germacrene B (5.3%) and α-copaene (5.2%). The essential oil of *P. racemosa* applied as dust formulation, was effective as a potato tuber sprout suppressant.

Key words: Myrtaceae · *Essential oil* · *Pimenta racemosa* · Sesquiterpene · Germacrene D (10.6%) · β-elemene (8.8%) · Germacrene A (7.3%) · Selin-11-en-4-α-ol (6.3%) · TritonX-100 · Potato tuber sprout suppressant

INTRODUCTION

Pimenta racemosa (Mill) J.W. Moore (family Myrtaceae) is a small narrow and medium size upright tree or shrub, about 15-25 in tall. It has a dark green and shiny evergreen leaves of about 1-3 in long which produce a wonderful spicy aroma when crushed. *P. racemosa* belongs to the botanical spice-group of plants with different names such as bayrum tree (West Indians), bay (English) and Bayrum (baum) as well as Kronpiment (German) [1]. *P. racemosa* is grown or cultivated especially in Indonesia, West indies, Venezuela, Mexico, Puerto, Rico,Guayana, Jamaica and Africa [1].

Previous studies have shown that *P. racemosa* leaves and fruit oils are used in perfumes, after shave lotion enhancing growth and strength or acting against hair loss and commercial food flavouring [1, 2]. It is also used in the treatment of rheumatism or for tooth ache as well as for its anti-inflammatory and analgesic properties [3]. There are literature reports on the biological activities [4, 5] and chemical constituents of essential oil of *P. racemosa* [5-10], *P. racemosa* var. *racemosa* [11-15], *P. racemosa* var. *hispaniolensis* [12], *P. racemosa* var. *ozua* [12], *P. racemosa* var. *grisea* [12, 15] and *P. racemosa* var. *terebintha* [15]. The bay oil and its different varieties origin normally contains diverse monoterpenoid compounds such as eugenol, myrcene, terpinen-4-ol, geranial, neral, 1, 8-cineole, α-terpinene, methy chavicol, chavicol and isoeugenol, while Sesquiterpene are less common.

Early sprouting is one of the major problems facing stored potato tubers intended for processing as a result of relatively high temperature (25°C) required for the storage [16]. At this temperature, tropical sprouting of tubers is favourable and it causes major losses in stored tubers. Several reports have shown that the contents of amino acids in potato tubers crops which are important for the protein balance of diet depends on the performance of the cultivar as well as on growing and storage conditions [17]. The storage quality and dormancy period of stored tubers has been previously enhanced using chemical products called inhibitors [17]. It is essential to prevent tuber crops by other means, since chemical plant protection products are banned from use in this farming system [18]. As a result, inhibitors of natural origin could be one of the solutions to this problem.

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The aim of this research was to determine the chemical constituents and sprout suppressant activity of *P. racemosa* oil on potato tubers during the storage period.

Experimental

Plant Material: Aerial parts of the *P. racemosa* of unknown variety and potato tubers were purchased from Iyana Iba Market, Badagry Expressway Lagos in April 2009. The plant sample was identified by Mr. Oluwa of Department of Boatny, Lagos State University, Ojo, Lagos.

Extraction of the Essential Oil: Aliquots (350 g) of the air-dried and pulverized plant sample were subjected to hydrodistillation for 3hr using a Clevenger-type apparatus in accordance with the British Pharmacopoeia specification (19) to obtain a pale yellow essential oil.

Analysis of the Oil Sample

Gas Chromatography (GC) Analysis: GC analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30m x 0.25mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/ min to 220°C. The injector and detector temperatures were maintained at 250°C; carrier gas nitrogen (2mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 μL. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis: GC-EIMS analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30m x 0.25 mm; film thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature 220°C and 240°C, respectively; oven temperature was programmed from 60°-240°C at 3°C/min; carrier gas was helium at a flow rate of 1mL/min.; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was 30-300 m/z at a scan rate of 1 scan/sec.

Identification of the Constituents: The identification of the constituents of the essential oil sample was made possible by the use of homemade library mass spectra built up from pure substances and components of known oils and MS literature data (20-22). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Sprout Suppression Activity: Potato tubers were just beginning to break dormancy at the start of the trial. Small quantities of six diseases free uniform sized tubers (average of 65g per tuber) were held in loosely covered cardboard boxes (110 × 60 × 90 mm). 60g of alumina which serves as carrier was weighed into wide-necked jars covered with lids. 0.5 mL of acetone solution containing 0.3 mL of *P. racemosa* essential oil was added to the jar. The contents of the jar were mixed and then exposed to air for a few minutes for the solvent to evaporate. TritonX-100 (0.75 g) was added and the content of the jar was mixed for about one hour. Formulation without TritonX-100 was also prepared using Whatman filter paper. Control experiments were set up as described above without additive and the essential oil.

Treatment and Assessment of the Formulation for Sprout Suppression: The formulations with TritonX-100 were dusted evenly over the potato tubers, while the filter paper formulation was placed on the bottom of the cardboard boxes. The boxes were kept in ventilated room at 24 ±1°C and 65 ±5% (r.h). Three replicate boxes per application were prepared. Assessment of tuber after 18 days for longest sprout length, number of sprouting per tuber and number of rot were examined. The mass of sprout per unit mass of tuber was assessed for determination of release rate properties.

Statistical Analysis: Data analysis was performed with SPSS 12.0 software package, Microsoft Excel version 2003. The results were expressed as mean ± standard deviation. Statistically significant difference was determined using the student *T* test and analysis of variance (ANOVA) with 95% confidence level.

RESULTS AND DISCUSSION

Essential oil from the aerial parts of *P. racemosa* was obtained by hydrodistillation in a yield of 0.56% (v/w). Table 1 displays the result of the GC-MS analysis. Sixty seven volatile constituents accounting for 91.1% of the total oil contents were identified from the oil sample. Germacrene D (10.6%), β-elemene (8.8%), germacrene A (7.3%), selin-11-en-4-α-ol (6.3%), δ-cadinene (5.9%), β-caryophyllene (5.8%), germacrene B (5.3%) and α-copaene (5.2%) were the major compounds of the essential oil. There are significant quantities of α-selinene (3.9%), β-selinene (3.8%) and τ-cadinol (2.8%).

Table 1: Relative percentage of the main constituents of essential oils of *P. racemosa* as separated and identified with GC-MS

S/N	Constituents	Percentage composition (%)	R.I[a]
1	ρ-cymene	tr	1029
2	limonene	0.3	1034
3	phenyl acetaldehyde	tr	1048
4	Linalool	tr	1103
5	nonanal	0.2	1106
6	borneol	tr	1170
7	naphthalene	tr	1185
8	decanal	tr	1207
9	thymol	0.4	1295
10	carvacrol	tr	1303
11	δ-elemene	0.4	1343
12	thymol acetate	0.4	1352
13	α-cubenene	0.7	1354
14	α-ylangene	0.2	1372
15	α-copaene	5.2	1378
16	α-isocomene	0.5	1386
17	β-cubebene	1.3	1392
18	β-elemene	8.8	1394
19	*n*-tetradecane	0.1	1400
20	β-caryophyllene	5.8	1421
21	(*E*)-α-ionone	0.6	1431
22	γ-elemene	0.2	1432
23	*trans*-α-bergamotene	0.3	1439
24	aromadendrene	0.2	1447
25	γ-muurolene	1.6	1478
26	germacrene D	10.6	1483
27	β-selinene	3.8	1486
28	α-selinene	3.9	1498
29	α-muurolene	1.0	1502
30	germacrene A	7.3	1505
31	*trans*-γ-cadinene	0.7	1515
32	(*Z*)-γ-bisabolene	0.1	1516
33	7-epi-α-selinene	0.1	1522
34	δ-cadinene	5.9	1525
35	selina-3,7(11)-diene	0.2	1542
36	δ-calacorene	0.4	1543
37	*cis*-muurolol-5-en-4-β-ol (syn= *cis*-cupressol)	0.1	1552
38	germacrene B	5.3	1557
39	*trans*-nerolidol	0.4	1567
40	spathulenol	1.1	1578
41	caryophyllene oxide	2.2	1583
42	viridiflorol	0.5	1590
43	*n*-hexadecane	0.4	1600
44	β-atlantol	0.3	1608
45	humulene epoxide II	0.4	1609
46	1, 10-di-epi-cubenol	0.4	1616
47	1-epi-cubenol	1.7	1631
48	caryophylla-4(14),8(15)-dien-5-α-ol	0.3	1637
50	_τ-cadinol	2.8	1643
51	α-muurolol	0.6	1645
52	selin-11-en-4-α-ol	6.3	1657
53	cadalene	0.2	1674
54	elemol acetate	0.5	1681
55	(*Z*)-trans-α-bergamotol	0.7	1691
56	*n*-heptadecane	0.2	1700
57	pentadecanal	1.3	1719
58	curcumenol	0.5	1734
59	(*E*)-sesquilavandulyl acetate	0.4	1741
60	14-hydroxy-α-muurolene	0.4	1780
61	*n*-octadecane	0.2	1800
62	hexahydrofarnesylacetone	1.0	1848
63	α-chenopodiol	0.2	1857
64	methyl hexadecanoate	0.2	1927
65	*n*-heicosane	tr	2000
66	abietatriene	0.6	2054
67	(*E*)-phytol acetate	2.4	2218
	Total identified	91.1%	

[a] Retention indices on CP-Sil-5 capillary column; tr trace amount (< 0.1%)

Previous studies (Table 3) on the essential oils of *P. racemosa* and its variety of different origins, have reported the abundant of monoterpenoid compounds mostly the cyclic oxygenated derivatives [5-15]. This class of compounds includes chavicol, 1, 8-cineole, neral, terpinen-4-ol, geranial, thymol, eugenol, isoeugenol, methyl eugenol and methyl isoeugenol. On the other hand, myrcene, γ-terpinene and limonene among the monoterpenes and sesquiterpene, β-caryophyllene were the hydrocarbon terpenoids that have been characterised from the diffrent oil samples. The present oil sample contained a large proportion of hydrocarbon sesquiterpene compounds (63.1%). However, the results indicated that region of origin and growing conditions could significantly affect the content of the oils. It could be seen that about twenty chemotypic forms of essential oils of *P. racemosa* and their variety have existed in literature (Table 2).

After 18 days of storage at 24°C, during which the potatoes tubers received treatment with *P. racemosa* essential oil, a mean sprout length of 0.95cm was found in the box containing oil with alumina and TritonX-100 (as additives) while 1.06 cm sprout length was found in the box containing oil soaked with filter paper, with the control box having a sprout length of 1.77cm. The number of sprouting spotted after 18 days of application were higher in control compared to the treated (Table 3). The number of rots tubers in control was higher with respected to treated boxes.

The trend today is to minimize the use of chemicals in stored fresh produce [23] and to find alternatives to currently used potatoes sprouting inhibitors [24]. Results of the present study show that the essential oil of *P. racemosa* applied as dust formulation is effective as a sprout suppressant (Table 3).

Formulation with TritonX-100 produced significant greater sprout suppression than the corresponding formulation without additive. This is because the formulation with TritonX-100 as additive was found to produce consistently lower release rates of the volatile oil, compare to the corresponding formulation without additives. The lowering of the release rate will enhance potatoes sprout suppression at high temperature [25]. The release rate *P. racemosa* oil from non-additive formulation was relatively high and almost constant; this made the effectiveness of the oil to lapse quickly before the period of 18 days. Reports shown that the dust formulation of Chlorpropham under tropical temperature is effective in reducing sprouting of potatoes during storage [26].

Table 2: The major constituents and chemotypes of essential oil of *P. racemosa*

Species	Major Constituents	Chemotype	Reference
P. racemosa	Myrcene (21.3%), 1, 8-cineole (9.7%), isoeugenol + eugenol (33.8%), chavicol (8.9%)	Isoeugenol / eugenol/myrcene	Buttery *et al.*, 1974
	1, 8-cineole (20.42%), α-terpineol (10.78%), methyl chavicol (10.78%), terpinen-4-ol (20.7%)	Terpinen-4-ol/1, 8-cineole	Bello *et al.*, 1998
	Neral (31.70%), geranial (41.30%)	Geranial/neral	Chapnon *et al.*, 1998
	Neral (28.50%), geranial (54.10%)	Geranial/neral	Chapnon *et al.*, 1998
	Eugenol (45.60 %), myrcene (24.97 %), chavicol (9.31 %)	Eugenol /myrcene/chavicol	Jirovetz *et al.*, 2007
	Eugenol (60.4%), chavicol (10.4%), myrcene (6.3%)	Eugenol/chavicol	Delespaul *et al.*, 2000
	1, 8-cineole (20.42%), α-terpineol (10.78%), methyl chavicol (10.78%), terpinen-4-ol (20.7%), chavicol (10.13%), eugenol (10.71%)	Terpinen-4-ol/1, 8-cineole	Bello *et al.*, 1995
P. racemosa	Eugenol (52.7%), myrcene (26.6%)	Eugenol/myrcene	Ayedoun *et al.*, 1996
var. *racemosa*	Nyrcene (16.17%), chavicol (15.51%), eugenol (44.41%)	Eugenol/myrcene/chavicol	Tucker *et al.*, 1991
	Eugenol (68.93%), methyl eugenol (11.88%)	Eugenol/methyl eugenol	Tucker *et al.*, 1991
	1, 8-cineole (20.4%), ñ-cymene (8.0%), terpinen-4-ol (20.7%), α-terpineol (10.1%)	Terpinen-4-ol/1, 8-cineole	Bello *et al.*, 2001
	Chavicol (17.1%), eugenol (56.1%), myrcene (6.4%), linalool (6.1%)	Eugenol/chavicol	Abaul *et al.*, 1995
	Limonene (4.6%), neral (31.7%), geranial (40.3%)	Geranial/neral	Abaul *et al.*, 1995
	Myrcene (12.8%), methyl chavicol (32.8%), methyl eugenol (48.1%)	Methyl eugenol/ methyl chavicol	Abaul *et al.*, 1995
P. racemosa	Terpinen-4-ol (5.9%), α-terpinyl acetate (12.7%),	α-terpinene/ α-terpinyl acetate/methyl eugenol	Garcia *et al.*, 2002
var. *terebinthina*	methyl eugenol (12.6%), α-terpinene (20.0%)		
P. racemosa	1, 8-cineole (37.96%), terpinen-4-ol (29.98%),	1, 8-cineole/terpinen-4-ol	Tucker *et al.*, 1991
var. *hispaniolensis*	α-terpinene (7.54%), ñ-cymene (6.81%)		
	Thymol (44.02%), ñ-cymene (8.89%), γ-terpinene (16.27%)	Thymol/γ-terpinene	Tucker *et al.*, 1991
	Methyl eugenol (63.88%), 1, 8-cineole (17.87%), methyl chavicol (5.13%)	Methyl eugenol/1, 8-cineole	Tucket *et al.*, 1991
	1, 8-cineole (27.50%), terpinen-4-ol (16.21%), methyl chavicol (22.61%), (*E*)-methyl isoeugenol (8.11%)	Methyl chavicol/1, 8-cineole/ terpinen-4-ol	Tucker *et al.*, 1991
P. racemosa	(*E*)-methyl isoeugenol (85.08-86.32%),	(*E*)-methyl isoeugenol	Tucker *et al.*, 1991
var. *grisea*	methyl eugenol (2.46-2.47%)		
	Methyl eugenol (82.80%)	Methyl eugenol	Tucker *et al.*, 1991
	Geraniol (85.52%)	Geraniol	Tucker *et al.*, 1991
	Methyl eugenol (4.52%), methyl isoeugenol (75.23%), β-caryophyllene (3.59%)	Methyl isoeugenol	Garcia *et al.*, 2002
P. racemosa	1, 8-cineole (47.24-55.93%), α-terpineol (6.65-15.12%),	(i) 1, 8-cineole/limonene	Tucker *et al.*, 1991
var. *ozua*	limonene (3.62-30.07%), terpinen-4-ol (4.00-15.67%)	(ii) 1, 8-cineole/terpinen-4-ol	
		(iii) 1, 8-cineole/ α-terpineol	
		(iv) limonene/ terpinen-4-ol	

Table 3:Anti sprouting Inhibitory activity of *P. racemosa* essential oil formulations on stored potato tubers at 24°C after 18days

Formulation	Sprout length (cm)	Number of sprout spotted	Number of rot	Mass of sprout per tuber mg/g
Control	1.17 ± 0.23	22.00 ± 8.55	6.67 ± 3.57	12.25
Oil supported with filter paper	1.06 ± 0.33	16.00 ± 4.62	5.00 ± 1.34	11.09
Oil supported with alumina and TritonX-100	0.95 ± 0.22	9.50 ± 3.55	3.33 ± 1.67	10.36

CONCLUSION

The results above show considerable promise for improved essential oil of *P. racemosa* on sprout suppression at high temperatures by the use of formulation. The limited release of essential oil of *P. racemosa* immediately after application may decrease the rotting of the potatoes. At $p \geq 0.05$, there was no significant difference among the treatments, associated with the little quantity of the oil used. This problem may be solved if the oil could be obtained in larger quantities for a better result. Further work is necessary to optimise

the combination of suppressant dose, carriers and additives loading for high temperature storage. Research work is ongoing aimed at identifying the main constituent(s) responsible for the sprout suppressant activity of the oil of *P. racemosa*.

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