

Contact Toxicity and Ovideterrent Activity of Three Essential Oil-Based Nano-Emulsions against the Olive Fruit Fly *Bactrocera oleae*

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Abstract: The control strategies for the olive crop key pest, *Bactrocera oleae*, involve synthetic chemical insecticides and few eco-sustainable alternatives, such as ovideterrents and lures. In the last few decades, the interest concerning the formulation of botanical based biopesticides increased, but little research investigated the suitability of these approaches for *B. oleae* control. This research aimed to investigate the residual contact toxicity and the oviposition deterrence of three essential oil (EO)-based nano-emulsions (*Pimpinella anisum*, *Foeniculum vulgare*, *Mentha x piperita*) against *B. oleae* adult flies. All the nano-emulsions possessed optimal physical characteristics, with droplets dimensions ranging from 115 to 152 nm and low PDI values (<0.2), even after 1 year of storage. Although no notable residual contact toxicity was noted, all the tested formulations reduced the number of oviposition puncture in no-choice tests (percent repellence: mint < fennel < anise). In choice trials, olives treated with fennel and anise EO-formulations at the highest concentration (7.5%, 75 g of EO/L) were less attractive respect to control fruits and a significant reduction of olive punctures was recorded. Nano-biopesticides are promising eco-friendly tools to integrate *B. oleae* pest management programs and to reduce the use of harmful conventional active ingredients.

Keywords: nano-insecticide; botanical; repellence; Tephritidae; IPM

Table S1. GC-MS analysis of the anise (*Pimpinella anisum*) essential oil.

LRI= Linear Retention Index. NI= Not Identified. tr=traces.

Compound	LRI Calculated	LRI Literature	Peak Area (%)
Anisole	921	917	tr
α -Thujene	925	931	0.01
α -Pinene	931	939	0.69
Camphene	947	953	0.02
Linaloyl oxide	969	971	0.01
Sabinene	972	976	0.05
β -Pinene	976	980	0.17
β -Myrcene	990	991	0.09
α -Phellandrene	1005	1005	0.35
δ -3-Carene	1010	1011	0.18
α -Terpinene	1016	1018	0.05
o-Cymene	1024	1022	0.14
Limonene	1027	1031	1.55
Eucalyptol (1,8-Cineole)	1029	1033	0.29
(Z)- β -Ocimene	1036	1040	0.01
(E)- β -Ocimene	1047	1050	0.01
γ -Terpinene	1056	1062	0.20
cis-Sabinene hydrate	1067	1068	tr
cis-Linalool oxide	1071	1070	0.02
Terpinolene	1088	1088	0.08
Linalool	1099	1098	2.51
α -Fenchol	1114	1117	0.01
trans-p-Mentha-2,8-dien-1-ol	1122	1125	tr
2,4-Heptadienal, 2,4-dimethyl-	1133	1129	tr
Pinocarveol	1138	1137	0.01
Camphor	1143	1143	0.04
Isopulegol	1146	1145	tr
Menthone	1153	1154	0.02
Borneol	1164	1165	0.03
Isomenthone	1167	1164	0.01
Dihydro- γ -Terpineol	1171	1178	0.04
Terpinen-4-ol	1175	1177	0.19
α -Terpineol	1189	1192	0.15
Estragole	1198	1196	5.49
Bornyl formate	1228	1228	0.01
Nerol	1236	1228	0.01
Neral	1248	1240	tr
Linalyl acetate	1252	1257	1.28
(Z)-Anethole	1257	1258	0.10
p-Anisaldehyde	1266	1263	0.06
NI 1	1270		0.02
Geranial	1276	1270	0.03

(E)-Anethole	1294	1283	82.51
δ-Elemene	1334	1339	tr
α-Terpinyyl acetate	1346	1350	0.04
Neryl acetate	1363	1365	0.03
Isoledene	1369	1373	tr
α-Copaene	1372	1376	0.09
Geranyl acetate	1383	1383	0.02
β-Elemene	1388	1392	0.02
NI 2	1402		0.01
α-Gurjunene	1405	1409	0.02
cis-α-Bergamotene	1410	1415	0.08
Caryophyllene	1415	1418	0.47
β-Gurjunene	1423	1432	tr
trans-α-Bergamotene	1431	1439	0.52
Aromadendrene	1434	1439	0.04
NI 3	1438		0.01
α-Humulene	1449	1454	0.04
(E)-β-Farnesene	1453	1458	0.07
Alloaromadendrene	1456	1461	0.01
NI 4	1469		0.01
γ-Murolene	1472	1477	0.01
Germacrene D	1477	1480	tr
(Z-E)-α-Farnesene	1480	1482	tr
β-Selinene	1482	1485	tr
NI 5	1484		0.01
cis-β-Guaiene	1487	1490	tr
Viridiflorene	1492	1493	0.12
α-Murolene	1496	1499	0.01
(Z)-α-Bisabolene	1504	1504	0.13
cis-γ-Cadinene	1510	1514	0.03
δ-Cadinene	1519	1524	0.07
Cadina-1,4-diene	1528	1532	tr
α-Cadinene	1534	1538	0.01
Elemol	1546	1549	0.01
Ledol	1556	1565	tr
(E)-Nerolidol	1563	1564	0.08
Spathuneol	1574	1576	0.02
Globulol	1580	1583	0.05
Viridiflorol	1588	1590	0.03
Guaiol	1590	1595	0.02
Rosifoliol	1598	1599	0.02
Carotol	1611	1612	tr
epi-γ-Eudesmol	1620	1622	tr
epi-Cubenol	1624	1627	tr
γ-Eudesmol	1629	1630	0.02
epi-α-Muurolol (T-MuuroloI)	1639	1641	0.04

α -Muurolol	1643	1645	0.01
β -Eudesmol	1648	1649	0.01
α -Cadinol	1652	1653	0.08
Foeniculin	1679	1684	1.29
TOTAL IDENTIFIED			99.93

Table S2. GC-MS analysis of the fennel (*Foeniculum vulgare*) essential oil.

LRI= Linear Retention Index. NI= Not Identified. tr= traces.

Compound	LRI Calculated	LRI Literature	Peak Area (%)
Tricyclene	923	926	0.01
α -Thujene	926	931	0.05
α -Pinene	933	939	2.79
Camphene	948	953	0.39
Thuja-2,4(10)-diene	954	957	tr
Sabinene	973	976	0.13
β -Pinene	977	980	1.06
β -Myrcene	990	991	1.21
α -Phellandrene	1006	1005	3.33
δ -3-Carene	1011	1011	0.36
α -Terpinene	1017	1018	0.46
o-Cymene	1025	1022	2.43
Limonene	1031	1031	23.98
(Z)- β -Ocimene	1036	1040	0.68
(E)- β -Ocimene	1047	1050	0.06
γ -Terpinene	1058	1062	0.19
cis-Sabinene hydrate	1067	1068	tr
cis-Linalool oxide	1072	1070	0.01
Fenchone	1090	1090	10.14
Linalool	1099	1098	0.82
α -Pinene oxide	1108	1105	tr
α -Fenchol	1113	1117	0.04
β -Fenchol	1118	1122	0.01
trans-p-Mentha-2,8-dien-1-ol	1121	1125	0.08
Allo-Ocimene	1130	1132	0.01
cis-limonene oxide	1133	1134	0.03
cis-p-Mentha-2,8-dien-1-ol	1135	1138	0.05
trans-limonene oxide	1138	1139	0.03
trans-Pinocarveol	1139	1139	0.03
Camphor	1144	1143	0.19
Menthone	1155	1154	tr
Borneol	1165	1165	0.01
3-Thujanol	1169	1166	0.01
Dihydro- γ -Terpineol	1172	1178	0.04
Terpinen-4-ol	1176	1177	0.16
p-Cymen-8-ol	1186	1183	0.04
α -Terpineol	1190	1192	0.17
Estragole	1198	1196	3.15
trans-Dihydrocarvone	1202	1201	0.27
trans-Piperitol	1208	1205	0.01
α -Fenchyl acetate	1218	1220	0.01

trans-Carveol	1220	1217	0.02
2-Hydroxy-1,8-cineole	1224	1229	0.02
β -Fenchyl acetate	1232	1232	0.05
Nerol	1235	1228	0.01
cis-Carveol	1239	1233	0.01
Carvone	1245	1242	0.02
(Z)-Anethole	1253	1258	0.21
p-Anisaldehyde	1260	1263	0.24
NI 1	1271		0.02
(E)-Anethole	1290	1283	43.81
2,3-Pinenediol	1315	1319	0.27
NI 2	1340		0.02
δ -Elemene	1346	1339	tr
Euguenol	1356	1356	0.99
α -Copaene	1372	1376	0.08
Geranyl acetate	1381	1383	0.01
β -Cubebene	1386	1390	0.01
β -Elemene	1388	1391	0.03
NI 3	1395		0.03
cis- α -Bergamotene	1410	1415	0.03
Caryophyllene	1415	1418	0.25
β -Copaene	1424	1429	0.01
trans- α -Bergamotene	1431	1439	0.23
Aromadendrene	1434	1439	0.02
α -Humulene	1449	1454	0.03
(E)- β -Famesene	1454	1458	0.04
Alloaromadendrene	1455	1461	0.01
γ -Muurolene	1472	1477	tr
Germacrene D	1477	1480	0.03
γ -Curcumene	1480	1480	0.01
NI 4	1482		0.01
Valencene	1489	1496	tr
Viridiflorene	1491	1493	0.03
α -Muurolene	1496	1499	0.01
(Z)- α -Bisabolene	1504	1504	0.04
cis- γ -Cadinene	1510	1514	0.01
δ -Cadinene	1519	1524	0.05
Eugenol acetate	1530	1524	0.03
Elemol	1546	1549	0.01
(E)-Nerolidol	1561	1564	0.04
Spathuneol	1574	1576	0.03
β -Caryophyllene oxide	1579	1581	0.06
Viridiflorol	1588	1590	0.02
Guaiol	1590	1595	0.01
Rosifoliol	1598	1599	0.01
epi- γ -Eudesmol	1619	1622	tr

epi-Cubenol	1624	1627	tr
γ -Eudesmol	1629	1630	0.01
epi- α -Muurolol (T-MuurololI)	1639	1641	0.02
α -Muurolol	1644	1645	tr
β -Eudesmol	1648	1649	tr
α -Cadinol	1652	1653	0.04
Foeniculin (1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene)	1680	1684	0.54
NI 5	1789		0.01
TOTAL IDENTIFIED			98.4

Table S3. GC-MS analysis of the mint (*Mentha × piperita*) essential oil.

LRI= Linear Retention Index. NI= Not Identified. tr= traces.

Compound	LRI Calculated	LRI Literature	Peak Area (%)
2,5-Diethyltetrahydrofuran	898	898	0.03
Anisole	919	917	0.01
α -Thujene	925	931	0.03
α -Pinene	932	939	0.99
Camphene	948	953	0.02
Dehydrosabinene	951	957	0.02
Sabinene	972	976	0.4
β -Pinene	977	980	1.25
2-Menthene	983	985	0.01
β -Myrcene	990	991	0.3
3-Octanol	995	993	0.37
p-Mentha-1(7),8-diene (Pseudolimonene)	1005	1004	0.02
δ -3-Carene	1011	1011	0.01
α -Terpinene	1017	1018	0.04
o-Cymene	1024	1022	0.12
Limonene	1028	1031	3.64
Eucalyptol (1,8-Cineole)	1031	1033	4.73
(Z)- β -Ocimene	1036	1040	0.04
(E)- β -Ocimene	1047	1050	0.03
γ -Terpinene	1057	1062	0.08
cis-Sabinene hydrate	1066	1068	0.19
1-Octanol	1071	1070	0.09
Terpinolene	1088	1088	0.06
3-Nonanol	1098	1099	0.01
Linalool	1100	1098	0.2
Isopentyl isovalerate	1107	1103	0.02
β -Thujone	1117	1114	0.01
trans-p-Mentha-2,8-dien-1-ol	1121	1125	0.06
cis-p-Mentha-2,8-dien-1-ol	1136	1138	0.03
cis-Sabinol	1141	1143	0.1
Isopulegol	1145	1145	1.52
Menthone	1156	1154	18.23
Isomenthone	1166	1164	13.56
Lavandulol	1168	1166	0.16
Menthol	1179	1173	36.72
Isomenthol	1184	1178	1.05
Neoisomenthol	1189	1183	0.24
α -Terpineol	1191	1192	0.48
Estragole	1197	1196	0.05
trans-Dihydrocarvone	1202	1201	0.04
trans-Piperitol	1206	1205	tr
NI 1	1212		0.02

NI 2	1214		0.07
trans-Carveol	1221	1217	0.02
Citronellol	1230	1228	0.01
cis-3-Hexenyl isovalerate	1234	1238	0.01
Pulegone	1238	1237	1.2
Carvone	1244	1242	0.06
Piperitone	1253	1252	1.02
Neomenthol acetate	1272	1275	0.19
(E)-Anethole	1284	1283	2.64
Menthyl acetate	1292	1294	5.79
Isomenthol acetate	1306	1305	0.06
cis-Pinocarvyl acetate	1310	1309	0.01
NI 3	1317		0.01
NI 4	1324		tr
δ -Elemene	1333	1339	0.07
α -Cubebene	1346	1351	tr
Euguenol	1348	1356	0.01
Piperitone oxide	1350	1363	0.01
α -Ylangene	1367	1372	0.01
α -Copaene	1371	1376	0.04
β -Bourbonene	1381	1384	0.34
β -Cubebene	1386	1390	0.01
β -Elemene	1388	1392	0.1
Longifolene	1395	1402	0.01
Isocaryophyllene	1402	1404	0.01
α -Gurjunene	1405	1409	0
cis- α -Bergamotene	1410	1415	0
Caryophyllene	1415	1418	1.55
β -Copaene	1424	1432	0.09
trans- α -Bergamotene	1431	1439	0.02
Aromadendrene	1434	1439	0.01
(E)-geranylacetone	1440	1453	0.05
α -Humulene	1445	1454	tr
α -Patchulene	1449	1456	0.21
(E)- β -Farnesene	1453	1458	0.04
Alloaromadendrene	1456	1461	0.02
α -Acoradiene	1459	1463	0.01
γ -Gurjunene	1462	1473	0.03
γ -Muurolene	1472	1477	0.04
Germacrene D	1477	1480	0.52
β -Selinene	1482	1485	tr
Valencene	1492	1491	0.26
α -Muurolene	1496	1499	0.03
NI 5	1501		0.01
cis- γ -Cadinene	1510	1514	0.03
Cubebol	1513	1515	0.01

δ -Cadinene	1519	1524	0.11
Cadina-1,4-diene	1529	1532	tr
α -Cadinene	1534	1538	0.01
α -Calacorene	1541	1542	tr
Sesquisabinene hydrate	1549	1547	0.01
Aromadendrene epoxide	1563	1590	tr
Spathuneol	1575	1576	0.04
β -Caryophyllene oxide	1579	1581	0.12
Viridiflorol	1589	1590	0.05
α -Humulene oxide	1606	1606	0
epi-Cubenol	1626	1627	0
epi- α -Cadinol (T-Cadinol)	1641	1640	0.02
α -Cadinol	1655	1653	0.01
TOTAL IDENTIFIED			98.7