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Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: A multivariate approach

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ABSTRACT

Twenty-five essential oils extracted from different sources (*Aniba rosaeodora* wood, *Cananga odorata* flowers, *Cannabis sativa* inflorescences, *Cymbopogon citratus* leaves, *Citrus limon* leaves, *Citrus nobilis* leaves, *Cupressus sempervirens* leaves, *Curcuma longa* rhizomes, *Hypericum perforatum* leaves, *Illicium verum* fruits, *Melaleuca alternifolia* leaves, *Mentha* × *piperita* leaves, *Mentha spicata* leaves, *Monarda fistulosa* leaves, *Ocimum micranthum* leaves, *Ocimum basilicum* leaves, *Ocotea quixos* leaves, *Pelargonium capitatum* leaves (two samples), *Pinus nigra* leaves, *Pogostemon cablin* leaves, *Syzygium aromaticum* leaves, *Thymus vulgaris* leaves, *Vetiveria zizanoides* roots, *Zingiber officinale* rhizomes) were screened for their phytotoxic behavior against *Solanum lycopersicum* seeds and seedlings in order to assess their capacity to inhibit germination, damage germinated seeds and alter the normal development of plantlets. The oils were analyzed by means of GC and GC–MS and a relationship between some functional groups and the ability to affect the germination or the development of roots and hypocotiles was evidenced by means of multivariate data analysis. *A. rosaeodora*, *C. citratus*, *I. verum*, *M.* × *piperita*, *M. fistulosa*, *O. micranthum*, *S. aromaticum* and in particular both *Pelargonium* samples were the most effective in both pre- and post-emergence stages. Multivariate data analysis allowed the construction of a model according to which the higher content in monoterpene alcohols, aldehydes or phenylpropanoids can be used as a predictor of distinct phytotoxic activities.

1. Introduction

Environmental concerns, the demand for organic crops and the emergence of weed resistance to synthetic herbicides have spurred a lively interest for biodegradable, allelopathic substances capable to manage different kinds of weeds (Dayan and Duke, 2010; Petroski and Stanley, 2009; Duke et al., 2000). Under sustainable agricultural practices in particular, some weeds may be detrimental not only in terms of total crop performance but also for safety concerns, as in the case of some members of the Solanaceae family and their toxic tropane alkaloids (EFSA, 2008). Phytotoxicity is defined as a plant-plant interaction inducing detrimental effects on various physiological processes (e.g. seed germination, plantlet growth and development). Plants are actively engaged in a constant competition for space, light and soil resources and such perennial evolutionary pressure has forged a vast array of phytotoxic/allelopathic secondary metabolites. For human purposes, these substances may serve as a potential reservoir of natural herbicides and as a scaffold for the chemical manipulations needed to develop substances more potent and less harmful to other organisms (Duke et al., 2002; Chou, 1999; Vyvyan, 2002). The main secondary metabolites involved are released from living organs by root exudation, volatilization from secretory glands, leaching from epigean parts and by decomposition of plant residues like fallen leaves or fruits (Bais et al., 2006). A plethora of phytochemicals intervenes mainly within the rhizosphere and includes very different secondary metabolites, like ketones (e.g. sorgoleone, leptospermone), simple phenolics and polyphenols (*p*-coumaric acid, kaempferol, tannins), nitrogenated compounds (benzoxazinones), triterpenoids (quassin), dibenzofurans (usnic acid) and terpenes (cineoles) (Barnes and Putnam, 1987; Barton et al., 2010; Weston and Mathesius, 2013; Einhellig and Souza, 1992; Blum et al., 1999;

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Romagni et al., 2000). For some of these molecules a phytotoxic activity has been reported yet in the nanomolar range, but problems in terms of availability of adequate amounts of purified substances and commercial affordability may emerge. A further valuable class is represented by terpenes, which can provide a competitive advantage to essential oil bearing plants. Their use as phytotoxic inhibitors of germination and plantlet growth has been highlighted, with consistent activity in the ml/l range on various weed and model plants (Vokou, 1992; Vokou et al., 2003; Batish et al., 2008; Dayan et al., 2011; Angelini et al., 2003; Dudai et al., 1999). The relevance of essential oils within this field is stated by the fact that various natural alternatives to synthetic herbicides currently approved for organic agriculture rely on essential oils or their constituents (e.g.: Matratec, GreenMatchEX, Nature's Avenger) and are used as burn-down products applied in the post-emergence stage (Young, 2004; Lanini, 2012). However, due to the high cost and high use rates, the exploration of other plant secondary metabolites as potential bioherbicides is needed (Boyd and Brennan, 2006).

Essential oils are an extremely heterogeneous pool of secondary metabolites, protean both in terms of biosynthetic pathways, chemical structures or functional groups. Furthermore, distinct chemotypes are known yet within a single botanical species. The extreme plasticity of their chemical composition is the convergent evolutionary outcome of a number of stimuli, concurring in a synergistic way to the modulation of a large number of variables. Given their multiple roles, essential oils represent a paradigm of chemodiversity and can be considered as one of the most striking examples of the versatility of biological systems (Bakkali et al., 2008). As a consequence, both the intrinsic variability of the composition and the relative potency of different essential oils should be provided at the same time in order to estimate which species and which chemotype is best suited as a potential phytotoxic agent.

Many efforts have been made to identify the phytotoxic effect of single constituents of essential oils (Isman, 2000; Martino et al., 2010). However, the biological activity on seed germination or on seedling growth of essential oils is often the result of cumulative effects of the whole phytocomplex, by means of multifactorial processes including enzyme inhibition, chlorophyll degradation, electrolyte leakage and membrane damage (Poonpaiboonpipat et al., 2013). Thus, multiple classes or common functional groups may be involved at the same time and promote specific biological consequences (Karaman, 2008; Rolim de Almeida et al., 2010). The literature outlines different approaches within this trend and a wide range of different models has been applied to single essential oils. The overall consequence, in most cases, is that a comprehensive approach and a concurrent correlation with the phytochemical profile are somehow lacking. The results are scattered in a myriad of papers, making difficult to draw sound conclusions and reducing the possibility to infer which chemotype or which cultivar of an essential oil bearing plant is better suited to provide the best phytotoxic potential. Furthermore, such circumstances hinders the possibility to deduce which blends of different essential oils may provide a more effective phytotoxic activity.

According to the above described scenario, in the present study we report an *in vitro* screening on the phytotoxic effect at pre and post-emergence growth of twenty-five essential oils on plantlets of a standard target species (*Solanum lycopersicum)* representing the Solanaceae family, whose weed members constitute a potential danger in organic agriculture (Macias et al., 2000; EFSA, 2008.). Our objective was to evaluate a complete phytotoxicity profile of essential oils vastly differing in their phytochemical composition and develop, by means of multivariate data analysis, a predictive model correlating phytochemical composition and phytotoxic potential.

2. Materials and methods

2.1. Essential oils

Pure essential oils were obtained via steam distillation from a number of commercial sources. Specimen samples have been kept for future reference at the University of Ferrara, Dipartimento di Scienze della Vita e Biotecnologie. *Aniba rosaeodora* (AR), *Cymbopogon citratus* (CC), *Citrus limon* (CLI), *Citrus nobilis* (CN), *Cupressus sempervirens* (CS), *Curcuma longa* (CL), *Ocimum micranthum* (OM), *Ocotea quixos* (OQ), *Zingiber officinale* (ZO) essential oils were purchased from Fundacion Chankuap, Macas, Ecuador and came from locally cultivated plants. *Cannabis sativa* (CSA), *Hypericum perforatum* (HP), *Illicium verum* (IV), *Mentha* × *piperita* (MP), *Mentha spicata* (MS), *Monarda fistulosa* (MF), *Pinus nigra* (PN), *Thymus vulgaris* (TV) were obtained from Pam'Innov, Le Chaffaut Saint Jurson, Provence, France; *Pelargonium capitatum* (PCS, PCB) samples were purchased from Biotrade, Mirandola, Italy; *Pogostemon cablin* (PC), *Syzygium aromaticum* (SA), *Vetiveria zizanoides* (VZ) were acquired from Globar Agro Resources, Medan, Indonesia and came from locally grown plants; *Cananga odorata* (CO), *Ocimum basilicum* (OB), *Melaleuca alternifolia* (MA) essential oils were supplied by CTM, Verona, Italy. The essential oil samples were stored in glass vials with teflon-sealed caps at $-18 \pm 0.5\,^{\circ}C$ in the absence of light. Given their well-known phytotoxic behavior and use in commercial products, *C. citratus* and *S. aromaticum* were included as references (Poonpaiboonpipat et al., 2013; Boyd and Brennan, 2006). A different array of essential oils was selected, ranging from those with a typical monoterpene hydrocarbon pattern (*C. limon*, *P. nigra*) or rich in aldehydes (*C. citratus*), phenylpropanoids (*I. verum*, *S. aromaticum*), phenolics (*T. vulgaris*, *O. basilicum*), alcohols (*A. rosaeodora*, *M. fistulosa*, *M. alternifolia*, *P. capitatum*), ketones (*M. spicata*, *M.* × *piperita*), aliphatic and oxygenated sesquiterpenes (*C. odorata*, *C. longa*, *P. cablin*, *V. zizanoides*, *Z. officinalis*) to those characterized by a more balanced presence of different classes of compounds and chemical moieties (e.g. *O. quixos*, *O. micranthum*). Essential oils were in fact chosen in order to obtain a pool representing the widest possible chemodiversity.

2.2. Gas chromatography

Essential oil samples were analyzed and the relative peak areas for individual constituents averaged. The relative percentages were determined using a ThermoQuest GC-Trace gas-chromatograph equipped with a FID detector and a Varian FactorFour VF-5 ms poly-5% phenyl-95%-dimethyl-siloxane bonded phase column (i.d. 0.25 mm; length, 30 m; film thickness, 0.15 μm). Operating conditions were as follows: injector temperature 300 °C, FID temperature 300 °C, Carrier (Helium) flow rate 1.0 ml/min and split ratio 1:50. Oven temperature was initially 55 °C and then raised to 100 °C at a rate of 1 °C/min; then raised to 250 °C at a rate of 5 °C/min and finally held at that temperature for 15 min. One microliter of each sample dissolved in 1 ml of CH_2Cl_2 was injected. The percentage composition of the oils was computed by the normalization method from the GC peak areas, without using correction factors.

2.3. Gas chromatography/mass spectrometry analysis

Essential oil constituents were then analyzed by a Varian GC-3800 gas chromatograph equipped with a Varian MS-4000 mass spectrometer using electron impact and hooked to NIST library. The constituents of the volatile oils were identified by comparing their GC relative retention times, Kovats Index (KI) and by matching the MS fragmentation patterns, as well as retention indices, with the above mentioned mass spectra libraries and with those in the literature (Adams, 2001). In order to determine the KI of the components,

a mixture of alkenes (C8–C24) was added to each sample before injecting in the GC–MS equipment. The operating conditions were the same reported for GC analysis and the same column was used. The MS conditions were as follows: ionization voltage, 70 eV; emission current, 10 μAmp; scan rate, 1 scan/s; mass range, 29–400 Da; trap temperature, 150 °C, transfer line temperature, 300 °C.

2.4. Bioassay of seed germination (pre-emergence)

The phytotoxic evaluations were performed on *S. lycopersicum*, chosen as a standard reference species for the Solanaceae family (Macias et al., 2000). This family hosts copious annual weed species considered noxious for many commercial crops and their relevance is magnified under organic cultivation regimes, due to their poisoning potential even at very low concentrations (Caligiani et al., 2011). Tomato was also chosen in place of common weeds in order to resort to a more general model, offering a genetically uniform pool with the reliable germination rates guaranteed by commercial crop seeds. Fifteen tomato seeds where allowed in Petri dishes on two filter paper (sterilized at 121 °C, 25 min, 1.033 kg/cm^{-2}) moisted with 4 ml of distilled water or a Tween 20 solution at 100 mg/l. Dishes were covered, sealed by Parafilm®, and placed in a growth chamber (25 ± 1 °C with a 16 h photoperiod under fluorescent tubes at a light intensity of 27 μmol m^{-2} s^{-1}). Two series of controls were performed: one with distilled water alone and another with a solution of Tween 20. The first control was performed to obtain the maximum level of germination, and second to exclude an inhibitory effect on tomato seed germination exerted by Tween 20. New germinated seeds were recorded daily; emerging (1 mm) visible radicle was used as a criterion for germination and hypocotyl and root lengths were measured; these measurements allowed to establish the daily growth rate of seedlings grown in experimental conditions. Mean germination time (MGT) was calculated as previously reported (Ellis and Roberts, 1980). This growth curve was used as a reference for subsequent phytotoxicity test, to exclude that any growth inhibition was due to a delay in germination rather than a toxic effect due to allelochemicals. Essential oils were suspended in distilled water at 1000 mg/l and to improve their dissolution, each suspension was added with 100 mg/l of Tween 20; solutions were made fresh and strongly agitated prior to any experiment. The duration of experiment was 15 days. Each experiment was replicated four times.

2.5. Bioassay of in vitro tomato seedling (post-emergence)

Seed germination experiments were carried out on ¼ strength MS medium, solidified with 0.8% (w/v) agar (PhytoAgar, Duchefa). The pH of the medium was adjusted to 5.8 with 1.0 M NaOH before autoclaving at 120 °C for 25 min at 1.033 kg/cm^{-2}. Cultures were maintained in a growth chamber at 25 ± 1 °C with a 16 h photoperiod under fluorescent tubes at a light intensity of 27 μmol m^{-2} s^{-1}. Fifteen seeds were placed in each 500 ml glass jars (7 cm high, 9 cm diameter) containing 90 ml of medium and incubated in a growth chamber for 4 days. After this time 10 ml of the essential oil emulsions, prepared as described above, were poured on the surface of the medium. The plants were put back in the climatic chamber for additional six days (ten days after sowing). After this period plantlets were removed from the agarized medium and the parameters listed in Tables 3 and 4 were recorded. Each experiment was replicated four times.

2.6. Multivariate data analysis

Datasets were appropriately transformed for the statistical treatment using the multivariate data analysis software program SIMCA-P (version 11, Umetrics AB, Umeå, Sweden). Multivariate analysis was carried out using Principal Component Analysis (PCA) and Partial least Squares regression (PLS) to elaborate mutual relationship amongst the quantities of the compounds and classes of components in the essential oils to obtain an overview of how the samples were correlated to each other with regard to the different phytotoxic assays.

3. Results and discussion

3.1. Phytochemical composition

The composition of the 25 samples is reported in Table 1, while Table 2 summarizes the principal classes of compounds and the sum of most relevant functional groups. Most essential oils showed only minor changes in composition with respect to data reported in literature for their most representative chemotypes, and for most chemodiverse species the samples were assigned as follows: *P. capitatum* samples were attributed to rose-scented cultivars, *O. basilicum* was attributed as linalool chemotype; *T. vulgaris* as a *p*-cymene, low thymol chemotype; *M. fistulosa* was attributed to a geraniol chemotype and *C. odorata* oil was classified as a third grade sample.

3.2. Pre-emergence effects – germination tests

All the tested essential oils induced a marked extension of germination time in *S. lycopersicum* seeds; mean germination time (MGT) was higher or comparable to those of the two reference oils (*C. citratus* and *S. aromaticum*), for which a 8.6 and a 11.2 MGT was respectively recorded. *P. capitatum* sample B provided the strongest activity, with a complete inhibition of germination after 15 days, while *M. fistulosa* and *P. capitatum* sample A scored a MGT of 11.5 and 10 days respectively. Moderate performances were recorded also for *M.* × *piperita*, *O. basilicum*, *O. micranthum*, *P. cablin* and *A. rosaeodora*, with MGT above 8 days, that is two times more than both negative controls and comparable to those of one of the positive controls (*C. citratus)*. The lowest MGT values were noticed for *C. limon*, *C. sempervirens*, *H. perforatum*, *P. nigra* and *T. vulgaris*, which however still induced a 30–40% extention of the germination time compared to untreated tomato seeds. A similar order of efficacy was reported for germination. Germinated seeds were always inhibited in their growth if compared to untreated seeds, and the most relevant pre-emergence consequences were noticed in *A. rosaeodora*, *M. fistulosa*, *P. capitatum* and *S. aromaticum*, with an almost complete inhibition of both root and hypocotyl length. On this regard, good performances were noticed again for *P. cablin*, and *M.* × *piperita*, whose administration induced inhibitions in pre-emergence growth of *S. lycopersicum* seeds above 75%. On the contrary, *C. limon*, *C. sempervirens*, *H. perforatum*, *P. nigra* and *T. vulgaris* only slightly reduced the length of root and hypocotyle. On the whole, *P. capitatum* and *M. fistulosa* samples emerged as the most relevant candidates for pre-emergence phytotoxic potential, with performances comparable or better than those of positive controls. The trend observed confirms literature data in which oils rich in volatile phenolics like thymol, carvacrol, eugenol, alcohols or ketones evidenced a strong antigerminative behavior on different weed seeds, while aliphatic monoterpenes and compounds like 1,8-cineole and limonene were almost inactive (Kordali et al., 2008; Angelini et al., 2003; Azirak and Karaman, 2008; Azirak et al., 2010; Rolim de Almeida et al., 2010).

3.3. Post-emergence phytotoxic activity–seedling growth

Four oils, namely *C. odorata*, *I. verum* and both *P. capitatum* samples caused a complete or almost complete mortality in plantlets. *A. rosaeodora*, *C. longa*, *C. citratus*, *M.* × *piperita*, *M. fistulosa*, *S.*

Table 1
Phytochemical composition of 25 essential oils screened for phytotoxicity.

Compound	KI	AR	CC	CL	CLI	CN	CO	CS	CS	HP	IV	MA	MF	MP	MS	OB	OM	OQ	PC	PCB	PCS	PN	SA	TV	VZ	ZO
Octane, 2-methyl	855									35.65																
Nonane	900									3.29																
Tricyclene	927																					1.03				
α-Thujene	930				1.09	1.77						1.02						0.96						0.58		
α-Pinene	939			1.1	2.69	3.56		26.37	2.51	25.64		2.61		0.52	0.54		0.83	4.31			0.5	22.31		2.32		3.31
Camphene	954							1.35														4.2		1.79		9.98
2,4(10)-Thujadiene	960																					0.6				
Benzadehyde	963																	0.93								
Sabinene	975				36.11			3.01						0.72				4.64								
β-Pinene	979				3.35	3.08			1.13			0.69		0.95	1.05		2.49					13.91		0.61		0.52
3-Octanone	984																							0.57		
Nonane, 2-methyl	987									7.16																
Myrcene	991		15.48	0.69	2.56	0.73		1.38	2.63						1.88		0.83	0.79		0.7		2.64		1.57		0.92
3-Octanol	991														0.92											
α-Phellandrene	1003			20.42														2.05								
3-Carene	1007							7.46										0.71								
α-Terpinene	1017			1.26	2.8							8.96						1.27						1.61		
p-Methylanisole	1020						1.29																			
p-Cymene	1025			3.61		4.13		2.82				3.5						3.01				1.95	1.23	15.33		
Limonene	1029				24.08	2.77		6.4	0.53			1.01		0.9								9.44		1.95		
β-Phellandrene	1030										0.77	0.79														7.67
1,8-Cineole	1031			10.3								3.64		5.1	11.25	5.08	15.57	8.21		1.08				1.91		
2-Octanol, 2-methyl	1033																2.96									
cis-Ocimene	1037				0.76	0.75										0.61										
trans-Ocimene	1050				3.18	10.91											0.61									
γ-Terpinene	1060			1.01	3.91	14.3						19.64						2.5				0.52	0.68	5.63		
Artemisia ketone	1062									1.37																
Decane, 2-methyl	1064									4.83																
cis-Sabinene hydrate	1070				0.78									0.77	1.78									0.65		
p-Cymenene	1086					0.85																0.83				
Linalool oxide A	1086	1.05																								
Terpinolene	1089			6.19	0.82	1.56			0.78			3.3						0.91						1.04		
Linalool oxide B	1095	1.01																								
Linalool	1097	75.97	1.28		4.73	41.58	1.03	0.93	3.03		2.34		0.71		0.83	49.88	7.55	0.93		2.59	5.96					
Undecane	1100									1.81																
cis-Thujone	1102									0.89														7.26		
trans-Thujone	1114																							0.74		
cis-Rose oxide	1119																				0.51					
Phenylethyl alcohol	1130																			6.75						
allo-Ocimene	1132																4.7									
trans-Pinocarveol	1139																					1.25				
Camphor	1146							1.19								0.63								3.08		
Citronellal	1149				3.55																					
Menthone	1153								4.55					21.42	1.6											
iso-Menthol	1163													2.99												
iso-Menthone	1163								0.72					2.93							10.71					
neo-Menthol	1166								1.16					4.11												
Borneol	1169																							2.69		1.02
Umbellulone	1171							4.02																		
Menthol	1172								12.54				1.72	38.61	5.54							0.9				
Terpinen-4-ol	1177				3.93			1.66				36.8		0.81				1.26				1.6		1.32		
neo-iso-Menthol	1183													0.54								1.28				
p-Cymen-8-ol	1183							0.57																		
α-Terpineol	1189	5.58						0.75		0.61	0.28	3.4				0.73	0.78	1.18						1.33		

Table 1 (*Continued*)

Compound	KI	AR	CC	CL	CLI	CN	CO	CS	CS	HP	IV	MA	MF	MP	MS	OB	OM	OQ	PC	PCB	PCS	PN	SA	TV	VZ	ZO
Myrtenol	1195																					0.61				
Estragol	1196										6.58					2.03										
Isomenthone	1196													1.16												
γ-Terpineol	1199																			0.83						
trans-Dihydrocarvone	1201														2.75											
Verbenone	1205									0.78																
Citronellol	1226				0.66															35.37	20.9					
Nerol	1230	1.32											0.8								0.58					0.63
Dihydro citronellol	1231																			0.73						
1,7-Heptanediol	1233																			0.75						
Tetrahydrogeraniol	1233																			1.4						
Thymol methyl ether	1235					1																		0.62		
Neral	1238		32.28										0.58								0.61					
Carvone	1243														47.43							3.14				
Carvacrol methyl ether	1245																							1.47		
p-Anisaldehyde	1250										1.77															
Piperitone	1253													0.58												
Geraniol	1253		3.35										89.54							23.55	18.4			8.28		1.11
Linalyl acetate	1257								9.51						0.96							0.59				
trans-Cinnamaldehyde	1266																	2.51								
Dodecane, 2-methyl	1266									1.77																
Geranial	1267		41.28																	0.75	0.84			0.56		
Citronellyl formate	1281																			4.82	5.33					
Isobornyl acetate	1283																			1.34						
Anethol	1285										78.8															
Bornyl acetate	1289							0.87								1.18						1.9		0.81		
Thymol	1290					9.02																	2.57	6.84		
Menthyl acetate	1295								3.64					5.97	0.79											
2-Undecanone	1295																					0.88				
Geranyl formate	1298																			1.53	4.19					
Carvacrol	1299																							7.96		
Tridecane	1300									0.58																
iso-Menthyl acetate	1305													0.89												
Dihydrocarveol acetate	1307														2.26							0.52				
δ-Elemene	1338																4.75	0.73								
Dimethylanthranilate	1341																									
α-Terpinyl acetate	1349							2.2																1.11		
a-Cubebene	1351																									0.84
cis-dihydronorzizaene	1354																								1.36	
Eugenol	1355						0.91									12.32	19.5		2.01				69.78			
trans-Dihydronorzizaene	1359																								0.85	
Carvyl acetate	1368														1.55											
α-Ylangene	1375						1.92																0.54			
α-Copaene	1377	1.9																2.15	0.67		0.64					
Cinnami acid methyl ester	1379																	1.7								
Geranyl acetate	1381						1.34		0.64															3.94		
β-Patchoulene	1382																		3.16							
β-Bourbonene	1388														2.39						1.34	1.37				
β-Elemene	1391						0.61									3.31	0.76	0.76	0.62	2.27		0.51				1.19
Sesquithujene	1392																									0.51
Jasmone	1393														0.7											
Elemene	1405																8.55									
Cycloseychellene	1406																		0.77							
Isocaryophyllene	1409								0.63																	
α-Gurjunene	1410											0.54														
α-Cedrene	1412							1.26																		

β-Funebrene	1415						0.63																			
2-Norzizaene	1417																							2.13		
Caryophyllene	1419			0.62	0.89	32.38	0.5	14.25	0.57	0.53	0.76	0.52	1.61	2.54	0.89	10.3	10.06	3.96	0.59	1.49	2.84	18.94	3.19	1.39		
β-Ylangene	1421																								0.68	
Thujopsene	1431						0.67																			
β-Copaene	1432					0.64			0.51					0.52							0.52						
trans α Bergamotene	1435							2.09							4.09											
α-Guaiene	1437															1.1			2							
γ-Elemene	1437															1.74									0.91	
β-Humulene	1439														0.97		4.89						1.48			
Aromadendrene	1441										1.8					0.64									0.53	
trans-Cinnamyl acetate	1443																10.02									
β, cis-Farnesene	1443																								0.67	
Prezizaene	1444																							2.73		
Seychellene	1449																	8.06								
Muurola -3,5-diene	1454					0.66																				
α-Humulene	1455					8.8		4.87								2.25		0.79			0.78	2.57				
α-Patchoulene	1456																	5.6								
cis-β-Farnesene	1457							1.81						1.38			1.19									
trans-β-Farnesene	1457							0.58													0.71					
allo-Aromadendrene	1460							1.12			0.8							2.11		0.58					0.53	
Muurola-4,5-diene	1465					0.67																				
9-epi-Caryophyllene	1467																	1.64								
trans-Cadina-1,6 4-diene	1473					2.68					0.55															
γ-Gurjunene	1475																	0.61								
γ-Muurolene	1480					9.31	3.22																		1.81	
ar-Curcumene	1481																								8.93	
γ-Curcumene	1483			0.7																						
α-Amorphene	1484																							3.5		
Germacrene D	1485													0.89	1.86	1.29				4.6	1.25					
α-Vetispirene	1489																							3.08		
α-Curcumene	1489			2.9																						
β-Selinene	1490															1.01	5.01									
β-cis Guaiene	1493																	14.43								
β-Vetispirene	1494																							5.99		
Zingiberene	1494			6.9													0.55								23.94	
Methyl Isoeugenol	1495																2.27		0.71							
γ-Amorphene	1496																							0.87		
2-Tridecanone	1497																				0.59					
Viridiflorene	1497	1.25									1.53									2.29						
Pseudowiddrene	1499	0.94																								
Bicyclogermacrene	1500					0.62					1.11					3.01	1.13			1.89						
δ-Guaiene	1501															1.87		0.73								
α-Muurolene	1502					1.56																				
γ-Patchoulene	1503																	3.63								
β-trans Guaiene	1504					0.94																				
Bergamotene derivative	1504					5.83																				
β-Bisabolene	1506			1.23												0.65							1.36		11.4	
cis, cis α-Farnesene	1506					4.98																				
Bulnesene	1510														1.51			15.84								
Benzene, (3-methyl-4-pentenyl)-	1510																		1.08							
δ-Amorphene	1512																1.14							4.29		
γ-Cadinene	1514					1.45									2.48										3.84	
Fenantrene	1515				1.23																					
cis-γ-Bisabolene	1515																								0.61	
endo, 1-Bourbonanol	1516																				1.11					

Table 1 (*Continued*)

Compound	KI	AR	CC	CL	CLI	CN	CO	CS	CS	HP	IV	MA	MF	MP	MS	OB	OM	OQ	PC	PCB	PCS	PN	SA	TV	VZ	ZO
Nootkatene	1516																								1.75	
β-Curcumene	1516			0.51																						
δ-Cadinene	1523	0.64					6.17					1.86									0.76	2.36			1.85	
β-Sesquiphellandrene	1523			5.45																						10.86
Zonarene	1530						0.66																			
γ-Vetivenene	1531																								4.26	
cis-Nerolidol	1533															0.69										
α-Cadinene	1538																					0.68				
Calamenene	1540							3.49				0.64														
α-Calacorene	1544																								0.65	
β-Vetivenene	1555																								10.92	
Vetivenene derivative	1557																								2.71	
Cubenol derivative	1566																								0.63	
2-Pentadecanone	1699																					0.6				
Vetiselinenol	1728																								2.32	
Khusimol	1741																								12.55	

AR, *Aniba rosaeodora*; CC, *Cymbopogon citratus*; CL, *Curcuma longa*; CLI, *Citrus limon*; CN, *Citrus nobilis*; CO, *Cananga odorata*; CS, *Cupressus sempervirens*; CSA, *Cannabis sativa*; HP, *Hypericum perforatum*; IV, *Illicium verum*; MA, *Melaleuca alternifolia*; MF, *Monarda fistulosa*; MP, *Mentha* × *piperita*; MS, *M. spicata*; OB, *Ocimum basilicum*; OM, *O. micranthum*; OQ, *Ocotea quixos*; PC, *Pogostemon cablin*; PCB, *Pelargonium capitatum* A; PCS, *P. capitatum* B; PN, *Pinus nigra*; SA, *Szyzygium aromaticum*; TV, *Thymus vulgaris*; VZ, *Vetiveria zizanoides*; ZO, *Zingiber officinalis*. Compounds, identified on the basis of comparison with MS database spectra, retention indices and pure reference chemicals, are listed in order of elution from a Varian FactorFour VF-5 ms column; KI: Kovats Index. Substances with relative abundance below 0.5% were omitted.

aromaticum and *V. zizanoides* had herbicide performances comprised between 50 and 73%. Most aliphatic monoterpene-rich and a sesquiterpene-rich sample (*Z. officinalis)* were completely ineffective in causing the dieback of plantlets and induced only a moderate inhibition of hypocotyle length. The administration of essential oils on top of agar medium was responsible of limited consequences on hypocotyle growth and in some cases, also in plantlets that subsequently died, the root length was not remarkably affected. As previously reported, the mechanism of action of most essential oil constituents is related to a combination of electrolyte leakage, inhibition of photosynthesis and enzymatic inactivation, but given the great chemical diversity encountered, different and simultaneous mechanisms could be involved, including also stimulatory effects (Macias et al., 2000). Some of the tested essential oils may thus determine, in *S. lycopersicum*, a switch from epigean to hypogean growth as a defensive response to stress, but no clear trend emerged on this regard. A great deal of research has been performed dealing with the herbicide effect of essential oils. Only a few publications, however, offer a direct comparison between different oils. Data described in Table 2 are in agreement with the literature, as most polar constituents of essential oils, in particular those with a free hydroxyl group, are usually described as the most effective post-emergence phytotoxic agents (Dudai et al., 1999; Vokou et al., 2003; Tworkoski, 2002).

3.4. Phytotoxic activity

The overall results outlined some trends and, interestingly, some oils gave different results in pre and post-emergence trials. For example, *I. verum* and *C. odorata* had a moderate efficacy in pre-emergence, while inducing instead a complete dieback when leached on germinated plantlets. On the contrary, both *Ocimum* species were fairly capable to inhibit seed germination and pre-emergence growth, but no plantlet died after their application. It must be noticed that positive controls *S. aromaticum* and *C. citratus* were not the best performers in both pre and post-emergence evaluation, while *P. capitatum* samples gave a complete inhibition of both pre and post-emergence growth of plantlets, thus highlighting the best all-around phytotoxic potential. If compared with the literature, these evidences suggest that a different performance can be expected also by essential oils obtained from the same species and confirm that a concurrent phytochemical characterization is needed to assess the phytotoxic potential of essential oils. In particular, this is evident for *T. vulgaris* and its poor results in our screening, despite being a species for which the literature outlines a good phytotoxic capability due to his profile rich in phenols (Tarayre et al., 1995). This behavior could be related to the low phenolic/oxygenated content of our sample, likely due to a poor selection of the collection time of the starting plant material, whose content in volatile phenolic compounds increases during flowering time but may be drastically lower during other phenological stages (Angelini et al., 2003; Dudai et al., 1999).

3.5. Multivariate data analysis

As described by PLS plot in Figs. 1 and 2, the multivariate cross-linking of chemical and biological data suggests the emergence of a slightly different behavior between pre- and post-emergence application of essential oil constituents. Concerning their potential use as germination inhibitors (Fig. 1), linear monoterpene alcohols (MLA) provide the strongest correlation with all the parameters evaluated in our experiments, with phenylpropanoids (PP) and the total oxygenated monoterpene (MO) content being also good predictors of antigerminative properties. On the contrary, ME showed a limited involvement with the biological property of the essential oils if compared to MLA, PP and MO, given the minor correlation with

Table 2
Principal classes of compounds and functional groups of 25 essential oils screened for phytotoxicity.

	Part	Monoterpenes						Phenylpropanoids		Phenols	Other	Sesqiiterpenes		
		Total	Non oxygenated	Oxygenated	Easters	Linear alcohol	Ketones	Aldehydes				Total	Non oxygenated	Oxygenated
Aniba roseaeodora (AR)	W	87.32	0.9	86.42	0.37	85.71	0	% 0	0.09	0	0	6.57	5.66	0.91
Cananga odorata (CO)	F	4.35	1.34	3.01	1.34	1.67	0	0	6.84	1.01	0	86.5	79.66	6.84
Cannabis sativa (CSA)	AP	46.6	8.57	38.03	14.67	17.41	5.51	0	0.24	0.39	2.1	38.31	12.63	25.68
Citrus limon (CLI)	L	97.18	81.91	15.27	0.52	9.88	0	3.77	0	0.16	1.23	1.58	1.58	0
Citrus nobilis (CN)	L	87.65	45.64	42.01	0	41.93	0	0	0	10.02	0.48	1.73	1.4	0.33
Cupressus sempervirens (CS)	L	61.13	49.69	11.44	3.07	0	5.21	0	0	0	0	24.9	12.67	12.23
Curcuma longa (CL)	R	44.93	34.63	10.3	0	0	0	0	0	0	0	47.99	29.15	18.84
Cymbopogon citratus (CC)	L	93.67	15.48	78.19	0	4.63	0	75.56	0	0	0.43	0	0	0
Hypericum perforatum (HP)	AP	30.95	26.84	4.11	0.37	0.48	2.53	0.29	0	0	0	5.25	4.17	1.06
Illicium verum (IV)	F	5.12	1.77	3.35	0	2.94	0.41	0	87.15	72.53	3.43	1.16	1	0.16
Melaleuca alternifolia (MA)	L	87.33	42.55	44.78	0.14	40.58	0.41	0	0	0	0	12.32	11.41	0.91
Mentha spicata (MS)	AP	81.03	4.69	76.34	5.56	6.37	50.73	0	0	0	0.92	9.38	0.87	8.51
Mentha × *piperita* (MP)	AP	90.88	4.27	86.61	7.24	47.53	33.33	0	0	0	0.12	2.48	2.48	0
Monardafistulosa (MF)	AP	94.91	1.1	93.81	0.46	92.77	0	0.58	0	0	0.27	0.88	0	0.88
Ocimum basilicum (OB)	AP	59.35	1.74	57.61	1.18	50.72	0.63	0	14.84	12.32	0	22.69	5.99	16.7
Ocimum micranthum (OM)	AP	33.85	9.68	24.17	15.57	8.6	0	0	19.5	19.5	3.14	40.16	39.23	0.93
Ocotea quixos (OQ)	L	33.36	21.63	11.73	0	3.52	0	0.93	18.13	0.21	0.4	37.68	28.44	9.24
Pelargonium capitatum Bourbon (PCB)	L	81.54	1.17	80.37	11.51	72.37	0.82	1.11	7.56	0	1.08	1.86	1.81	0.05
Pelargonium capitatum S.Africa (PCS)	L	74.54	0.5	74.04	11.52	48.11	11.13	1.45	0	0	0	25.65	16.12	9.53
Pinus nigra (P	L	87.29	70.73	16.56	5.06	8.16	1.48	0.6	0	0	0	2.85	1.81	1.04
Pogostemon cablin	AP	0.42	0.42	0	0	0	0	0	2.14	2.37	0	92.68	63.64	29.34
Szyzygium aromaticum	L	2.6	2.1	0.5	0	0.5	0	0	70.12	2.7	0.71	21.51	20.41	1.1
Thymus vulgaris	AP	66.21	33.24	32.97	5.96	12.56	11.2	0.56	0	16.89	0	8.02	0.54	7.48
Vetiveria zizanoides	R	0	0	0	0	0	0	0	0	0	1.08	0	56.5	42.42
Zingiber officinalis	R	27.01	23.45	3.59	0.29	3.13	0	0.17	0	0	0.91	68.52	0.23	68.29

Sums were obtained taking into account the substances listed in Table 1 and also those having a relative abundance below the 0.5% threshold.

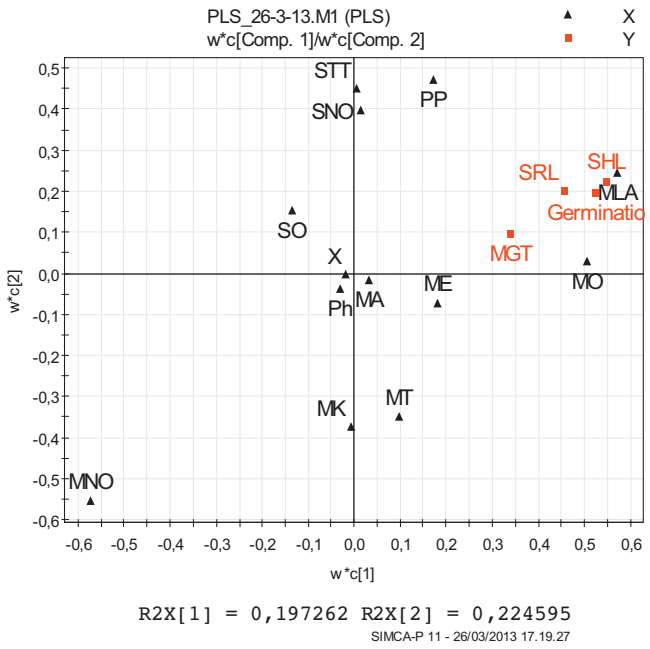

Fig. 1. PLS correlation between bioactivities and principal classes of compounds in the 25 essential oils tested in pre-emergence. *Legend*: Chemical classes localized close to and on the same side of the biological data with reference to the y-axis (right side) are mainly involved in the biological activity as a consequence of their higher positive correlation with the germination inhibition. MK, monoterpene ketones; MLA, monoterpene alcohols; SO, oxygenated sesquiterpenes; STT, total sesquiterpenes; SNO, non-oxygenated sesquiterpenes; MA, monoterpene aldehydes; MNO, non-oxygenated monoterpenes; MT, Total monoterpenes; Ph, phenols; PP, phenyl propanoids; MO, oxygenated monoterpenes; ME, methyl esters; X, other; MGT, mean germination time; SRL, root length; SHL, hypocotyle length; GER, germination rate.

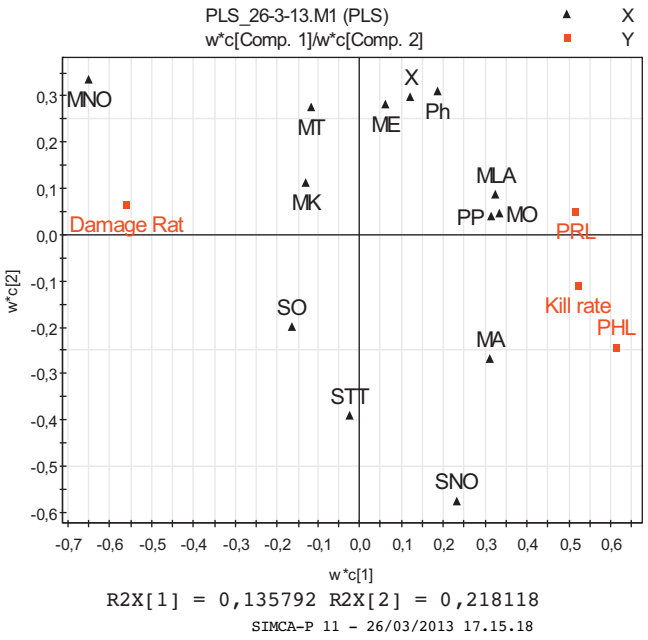

Fig. 2. PLS correlation between bioactivities and principal classes of compounds in the 25 essential oil tested in post-emergence. *Legend*: Chemical classes localized close to and on the same side of the biological data with reference to the y-axis (right side) are mainly involved in the biological activity as a consequence of their higher positive correlation with the inhibition of plantlets growth. MK, monoterpene ketones; MLA, monoterpene alcohols; SO, oxygenated sesquiterpenes; STT, total sesquiterpenes; SNO, non-oxygenated sesquiterpenes; MA, monoterpene aldehydes; MNO, non-oxygenated monoterpenes; MT, total monoterpenes; Ph, phenols; PP, phenyl propanoids; MO, oxygenated monoterpenes; ME, methyl esters; X, other; PRL, root length; PHL, hypocotyle length; DR, damage rate; MOR, rate of mortality.

Table 3
Pre-emergence effects of essential oils applied at 1 ml/l on *S. lycopersicum* seeds.

	MGT[a]	Germination	Root length	Hypocotyle length
	Days		Inhibition%	
Aniba roseaeodora	9.4	86.6	90.4	96.9
Cananga odorata	6.9	24.3	55.3	38.1
Cannabis sativa	6.8	3.6	47.9	34.9
Citrus limon	5.3	0.5	24.3	12.4
Citrus nobilis	8.3	6.6	74.16	54.28
Cupressus sempervirens	5.8	0.5	0.6	14.1
Curcuma longa	7.80	16.3	68.6	49.2
Cymbopogon citratus[c]	8.60	35.7	72.7	71.7
Hypericum perforatum	5.60	1.5	8.4	20.6
Illicium verum	7.8	17.8	69.2	67.3
Melaleuca alternifolia	8.4	44.8	71.6	62.6
Mentha spicata	7.20	10.3	64.2	48.4
Mentha × piperita	9.00	66.4	93.4	85.0
Monarda fistulosa	11.50	98.0	99.0	99.5
Ocimum basilicum	8.9	42.3	85.0	78.8
Ocimum micranthum	9	55.1	91.1	76.5
Ocotea quixos	7.5	12.3	84.9	44.5
Pelargonium capitatum Bourbon	10	98.3	98.6	98.2
Pelargonium capitatum S.Africa	N.G.[b]	100.0	100.0	100.0
Pinus nigra	5.5	2.0	56.9	30.0
Pogostemon cablin	9.40	79.4	74.3	74.2
Szyzygium aromaticum[c]	11.20	93.0	96.9	90.2
Thymus vulgaris	5.90	1.0	5.6	18.5
Vetiveria zizanoides	7.70	6.7	40.0	56.9
Zingiber officinalis	7.50	4.2	73.8	58.2
Control[d]	3.5			

[a] Mean germination time.
[b] N.G., no germination recorded after 15 days.
[c] Reference essential oils.
[d] Both controls provided the same results.

Table 4
Post-emergence effects of essential oils applied at 1 ml/l on *S. lycopersicum* plantlets.

	Root length	Hypocotyle length	Kill rate[a]	Damage rate[b]
	Inhibition %			
Aniba roseaeodora	33.6	48.6	61.5	A
Cananga odorata	−5.6	57.9	98.2	B
Cannabis sativa	11.2	38.4	0.0	B
Citrus limon	−21.2	3.9	0.0	C
Citrus nobilis	14.3	34.4	7.3	B
Cupressus sempervirens	−2.5	13.6	0.0	C
Curcuma longa	13.3	59.6	72.8	A
Cymbopogon citratus[c]	65.0	79.6	72.1	A
Hypericum perforatum	−21.3	28.7	0.0	C
Illicium verum	46.3	62.8	100.0	A
Melaleuca alternifolia	23.8	30.8	5.6	B
Mentha spicata	−26.2	36.3	10.0	C
Mentha × piperita	50.5	61.5	76.7	A
Monardafistulosa	32.7	56.8	64.5	A
Ocimum basilicum	9.1	46.2	0.0	B
Ocimum micranthum	27.3	46.9	0.0	A
Ocotea quixos	−11.2	46.6	30.5	C
Pelargonium capitatum Bourbon	43.4	61.1	100.0	A
Pelargonium capitatum S. Africa	35.4	61.4	100.0	A
Pinus nigra	24.9	19.0	0.0	B
Pogostemon cablin	32.9	65.5	38.3	A
Szyzygium aromaticum[c]	36.1	59.9	53.3	A
Thymus vulgaris	13.0	0.1	0.0	C
Vetiveria zizanoides	36.1	57.8	50.5	A
Zingiber officinalis	−12.5	30.8	0.0	C

[a] Rate of mortality after the essential oil application on top of agar soil.
[b] Visual score of damages induced by essential oil exposure A: 100–70%, B: 70–40, C: 40–0.
[c] Reference essential oils.

antigerminative capacity than other chemical classes. These indications seem to strengthen and corroborate previous suggestions in which essential oils rich in oxygenated compounds were seemingly more active than their hydrocarbon rich counterparts (Verdeguer et al., 2009). These data, however, were inferred from experiments in which only one outcome variable was evaluated. Furthermore, various authors have suggested that monoterpene-rich essential oils are more prone to induce phytotoxicity than sesquiterpene-rich essential oils and albeit some exceptions could be made, this trend seems to be confirmed by our results, in particular

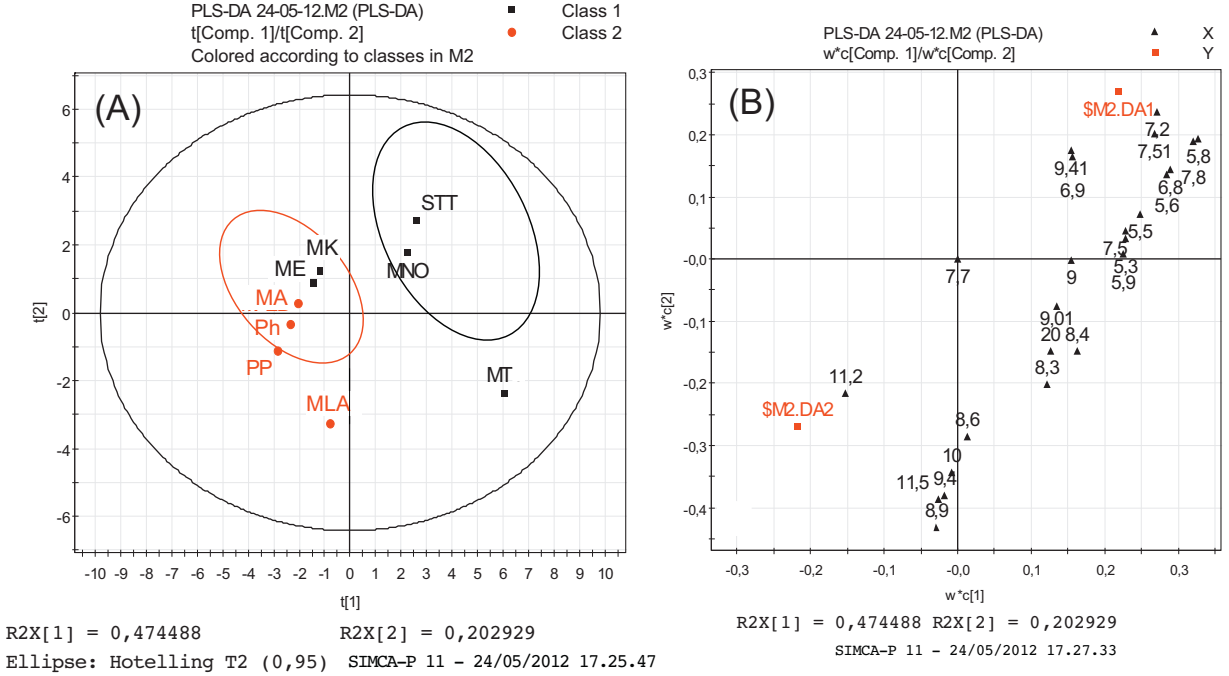

Fig. 3. PLS-Discriminant Analysis correlation showing the relationship between phytochemical profile and bioactivities. *Legend*: The loading plot (B) represents the dispositions of the values of the variable MGT, which contribute to separation between two different classes in the corresponding score plot (A): class circled in red (MA, Ph, PP, MLA) is more correlate with higher values of MGT, indicating an inhibition of the germination in *S. lycopersicum* plants. Class circled in black, poor correlation with a phytotoxic behavior; class circled in red, good correlation. MK, monoterpene ketones; MLA, monoterpene alcohols; SO, oxygenated sesquiterpenes; STT, total sesquiterpenes; SNO, non-oxygenated sesquiterpenes; MA, monoterpene aldehydes; MNO, non-oxygenated monoterpenes; MT, total monoterpenes; Ph, phenols; PP, phenyl propanoids; MO, oxygenated Monoterpenes; ME, methyl esters; X, other; PRL, root length; PHL, hypocotyle length; DR, damage rate; MOR, rate of mortality.

when multiple variables are simultaneously evaluated (Verdeguer et al., 2009). In fact, an higher sesquiterpene content (SO) provided slightly negative correlation with pre-emergence phytotoxicity, while MNO had always a negatice correlation with the tested bioactivities. Aliphatic monoterpenes (MNO) emerge also as the least effective constituents, and despite being often reported as good phytotoxic agents, their abundance contribute far less than that of other more polar compounds like phenylpropanoids, aldehydes and alcohols (Fig. 1). At post-emerge stage (Fig. 2), a slightly different trend emerged, as the dieback of plantlets and their growth parameters are still correlated positively with oxygenated monoterpene content and the contribution of the aldehydic moiety and of the phenylpropanoidic core appears to be much more evident. The contribution of monoterpene aldehydes (MA), that was negligible in pre-emergence, is instead relevant when essential oils are leached on germinated plantlets (Tables 3 and 4).

According to the PLS-DA plot in Fig. 3 (A, score plot), the phytochemical profile of the screened essential oils clusterizes in two classes. The first, described by parameters like non-oxygenated terpenes (MNO), total sesquiterpenes (STT) and total monoterpenes (MT) offers a poor correlation with a phytotoxic behavior. On the contrary, the second class described by total aldehydes (MA), phenolics (Ph), phenylpropanoids (PP) and monoterpene alcohols (MLA) offers a good correlation with bioactivities. Therefore, an higher content in these essential oil constituents represents the most desirable trait when screening for essential oils capable of both pre- and post-emergence phytotoxicity. In fact, as shown by the loading plot diagram (Fig. 3B) the second class of compounds (MA, Ph, PP, MLA) is positively correlated with the highest mean germination time (MGT) values, evidencing an greater involvement in the biological activity considered (germination inhibition).

4. Conclusions

Rose-scented *P. capitatum* and *A. rosaeodora* have been detected as potential natural herbicides endowed with very strong activities against *S. lycopersicum* both in pre and post-emergence stages, with potency greater or comparable to those of other renewed phytotoxic essential oils already used in commercial natural herbicides. According to the predictive model, an higher content in monoterpene alcohols, aldehydes or phenylpropanoids can be used as a predictor of phytotoxic activity in essential oils. In particular, while monoterpene alcohols are active on both seeds and plantets, aldehydes exert their activity mostly on germinated plantlets. Rather than randomly bioassaying essential oils, these structural and activity clues can be used to maximize chances of finding phytotoxic compounds. Furthermore, an artificial mixture of these classes of essential oil constituents may be envisaged as an ideal phytotoxic agent against weeds from the Solanaceae family, given its potential capability to act at different levels of pre and post-emergence plant growth.

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