THE INFLUENCE OF PLANT EXTRACTS ON ROOT BIOSTIMULATION IN DIFFERENT STRAWBERRY (*Fragaria × ananassa* Duchense) CULTIVARS

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ABSTRACT

The use of botanical extracts is considered an important tool to stimulate plant growth, reduce the use of synthetic pesticides, or both. The impact of hydro-alcoholic extracts of *Calendula officinalis*, *Salvia officinalis*, *Tagetes* sp., and *Taraxacum officinale* on growth and root development of plants of five strawberry cultivars ('Albion', 'Florence', 'Magnum', 'Rumba', and 'San Andreas') grown in semi-field controlled conditions was tested in the present study. The vigor and growth of the five strawberry genotypes were significantly affected by the extracts, with cv. Florence consistently producing more biomass than any other variety compared to the untreated control. The extracts also impacted the root system differently depending on the specific genotype. However, the *C. officinalis* flower extract consistently improved the root architecture, increasing the value of five out of six parameters compared to the control. The genotype-related response points to the strong influence of the "variety factor" on the possible effect of plant extracts considered for biostimulation, plant protection purposes, or both, prompting the need for additional work to unravel the bottlenecks in using botanicals.

Key words: *Fragaria × ananassa*, biostimulant, organic farming, root architecture

INTRODUCTION

The recent EU restriction on synthetic pesticides and the implementation of the principles of the European Green Deal, Farm to Fork, and Biodiversity Strategies developed by the EU Commission [European Commission 2020] have made the management of plant pests difficult, stimulating the search for alternatives to chemicals. The use of plant extracts and other natural compounds is considered an important tool to reduce the use of synthetic pesticides in the implementation of IPM practices [Seiber et al. 2014], as well as in strategies for plant protection in organic farming as this category of products is included among the compounds allowed by the European Union legislation for plant protection of organic crops [European Commission 2021]. In particular, for strawberry (*Fragaria × × ananassa* Duchense), one of the most cultivated berry crops around the world with about 9.2 Mt production, and in Europe with about 1.8 Mt [Food and Agriculture Organization of the United Nations 2021, Yuan and Sun 2022], the management of many severe pests in an open field and greenhouse cultivation has become difficult due to recent restrictions in agrochemical use and the lack of effective alternatives.

The most promising perspective for plant-derived biopesticides is based on using medicinal and aromatic plants or their derivatives. These species have

existed in human history since ancient times and have been widely cultivated due to their valuable, multi-functional secondary metabolites useful in pharmacy, perfumery, cosmetics, colorants, and crop protection products [Petrovska 2012]. An interesting application of plant-derived biopesticides could derive from exploiting species that can also be employed for ground-cover management practices in orchards and plantations [de Pedro et al. 2020, Mia et al. 2021]. The biomass produced by cover crops of medicinal and aromatic species could be used either as a source for commercial purposes or plant extract preparation by farmers to be directly utilized in the fruit growing system. Plant-derived biopesticides could have a multi-purpose function or mechanisms of action [Acheuk et al. 2022, Khursheed et al. 2022], improving plant growth or vigor [Godlewska et al. 2021] and, in this case, allowing to treat them as biostimulants.

In this study, we assessed the growth stimulation effect of five self-made plant extracts originating from medicinal or aromatic species (*Calendula officinalis*, *Salvia officinalis*, *Tagetes* sp., and *Taraxacum officinale*) known for bio-pesticidal activity on different strawberry varieties, with particular emphasis on root system development.

MATERIALS AND METHODS

Plant extract preparation. Plant extracts were obtained by mixing with a 1 : 5 ratio (w : v) the relevant plant material with an alcoholic solution (33% ethanol in water). After two weeks of extraction at room temperature in the dark, the extracts were separated from the plant material [Tartanus et al. 2022] and used in the experiment. The following plant species and parts were used for the extraction: *Calendula officinalis* (Scotch marigold) – flowers (anthodium), *Calendula officinalis* (Scotch marigold) – whole plant, *Salvia officinalis* (sage) – whole plant, *Tagetes* sp. (marigold) – whole plant, *Taraxacum officinale* (dandelion) – roots.

Analysis of the plant extracts chemical composition. A chemical analysis of the plant extracts was performed to determine the pH, total carbon, and nutrient elements content. A pH meter measured the pH (Accument 50, Fisher Scientific Poland). Total N and C content were determined by the conductometric method using a TruSpec CNS analyzer [Wright and Bailey 2001]. To analyze the nutrient elements, plant extracts were microwave digested in HNO_3 (1 : 10 v/v ratio), using closed Teflon vessels, and the macro- (P, K, Ca, Mg, S-SO4, Na) and microelements (Fe, Mn, Cu, Zn) were determined by an inductively-coupled plasma spectrometer (ICP Model OPTIMA 2000DV, Perkin Elmer, USA) as described by Kowalczyk et al. [2020].

Experiment design. The experiment was conducted at the National Institute of Horticultural Research in Skierniewice (central Poland, 51°57'36" N, 20°8'59"E). The soil for the pot experiment was collected from the experimental farm belonging to the Institute. It was characterized as silt soil with 1.43% soil organic matter and a pH 6.1. No specific pests were present in it. Plants of five strawberry cultivars ('Albion', 'Florence', 'Magnum', 'Rumba', and 'San Andreas') were planted in 2.5 l pots (one seedling per pot) on 22 April 2020 and grown under an open plastic tunnel. After acclimation and initial growth, three plants per variety were treated with specified plant extract. Each strawberry plant received 50 mL of a plant extract diluted ten times and applied to the soil for five consecutive days (from 6th to 10th July 2020). Control plants were watered with an ethanol solution diluted as in the extracts (2.75% ethanol in water). The plants were grown further for the next three weeks and then collected for analysis. The plants were neither fertilized nor protected from diseases and pests during the experiment. Soil water capacity was maintained at a steady level, watering manually the pots 2–3 times per week with approx. 50 mL of water. The average monthly temperature during the experiment was 16.9°C.

Growth and root architecture analysis. The plants were collected three weeks after the treatment, and the fresh weight of the whole plant and root system was measured to determine plant growth. The root system was scanned with an EPSON EXPRESSION 10000 XL root scanner, and the root morphological parameters (root length, root surface area, root diameter, root volume, and number of root tips) were determined using the WinRhizo software (Regent Instruments Inc., Canada, 2009).

Statistical analysis. The data obtained were analyzed statistically using the R software version 4.0.2 [R Core Team 2020]. To visualize the differences in

the chemical composition of the plant extracts, a hierarchical clustering of the mineral elements was performed by applying the Ward method and Euclidean distance (using *dist* and *hclust* functions from the *stats* package). For the plant growth parameters (including the results obtained from WinRhizo software), the Shapiro-Wilk test was used to verify if the data followed a normal distribution, and Levene's test was used to verify the homogeneity of variances. The non-parametric Kruskal–Wallis analysis with Fisher's least significant difference post hoc test with significance set at $p \leq 0.05$ was performed (using the *kruskal* function from the *agricolae* package). Principal component analysis was performed using the *prcomp* function from the *stats* package. Two-dimensional PCA was visualized using the *autoplot* function from the *ggfortify* package, and three-dimensional PCA was visualized using the *pca3d* package. Other results were visualized using the *ggplot2* package.

RESULTS

The mineral composition of the plant extracts is shown in Table 1. Two groups of extracts could be identified based on the pH value: one acidic, with value around 5.5, including the extracts from *C. officinalis* flowers and *S. officinalis*, and one with pH sub-neutral, which included the extracts from the other two species and the whole plant of *C. officinalis*. The lowest C_{tot} content was found in the extract of the *C. officinalis* flowers (5.46%), while it ranged around 9% for the other four extracts.

The extract from *C. officinalis* flowers resulted in having the highest amount for the majority of mineral elements, in particular P, K, Mg, and the microelements, with concentrations up to five times than those of the other extracts, including that from the whole plant of the same species (Tab. 1). Calcium content was similar among the *C. officinalis* extracts and that of *Tagetes* sp. (around 280 mg/L), about twice that of sage plant and dandelion roots.

The Ward method of Euclidean distance based on all parameters of the extracts' mineral composition discriminated them into three groups: the most diverse formed by the flower extract of *C. officinalis*, a second formed by the extracts from *Tagetes* sp., *T. officinale* and *C. officinalis* whole plant, and an intermediate group including *S. officinalis* extract (Tab. 1).

Table 1. Chemical characteristics of the plant extracts used as biostimulants for strawberry plants and their clustering according to Ward's method for Euclidean distances
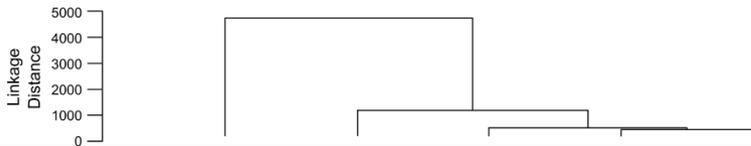
Parameter [elements mg/L if not specified otherwise]	*Calendula officinalis* flowers	*Salvia officinalis*	*Tagetes* sp.	*Taraxacum officinale*	*Calendula officinalis* plant
pH	5.55	5.41	6.49	6.40	6.68
C_{tot} [%]	5.46	7.99	9.69	10.00	9.37
$N-NO_3$	13	33	8	17	60
P	119	65	12	55	22
K	1523	1087	552	500	635
Ca	289	173	280	130	279
Mg	330	130	95	53	81
Na	851	19	36	28	110
$S-SO_4$	430	345	365	84	113
Fe	5.59	1.03	1.19	1.06	0.98
Mn	1.91	0.36	0.31	0.29	0.25
Cu	1.19	0.20	0.18	0.27	0.16
Zn	2.98	0.84	1.88	0.53	0.44

Effect of plant extracts on plant growth. The vigor and growth of the five genotypes were significantly different, with cv. 'Florence' consistently produces more above and below-ground biomass than any other variety, compared to the untreated control, while the cv. 'Albion' showed the lowest growth (Fig. 1a and Fig. 1b). However, this growth pattern was not fully reflected considering the root-shoot ratio (Fig. 1c). The varieties 'Magnum' and 'Florence' resulted in a significantly higher ratio compared to the others, which were similar among them, pointing to a more developed root system.

The extracts affected the growth of the five varieties (Tab. 2). The two most diverse cultivars in terms of vigorousness, 'Florence' and 'Albion', were those whose growth was more influenced by the extracts. *C. officinalis* flowers increased roots and shoots biomass of 'Florence' extract (about 45%); shoots biomass increase in 'Albion' was maximized by *T. officinale* root extract (about 48%); *Tagetes* spp. increased shoot biomass in both varieties (about 40%) more than control. 'Magnum' and 'San Andreas' biomass were not significantly affected by the extracts, even though an increasing trend of both shoot and root weight was
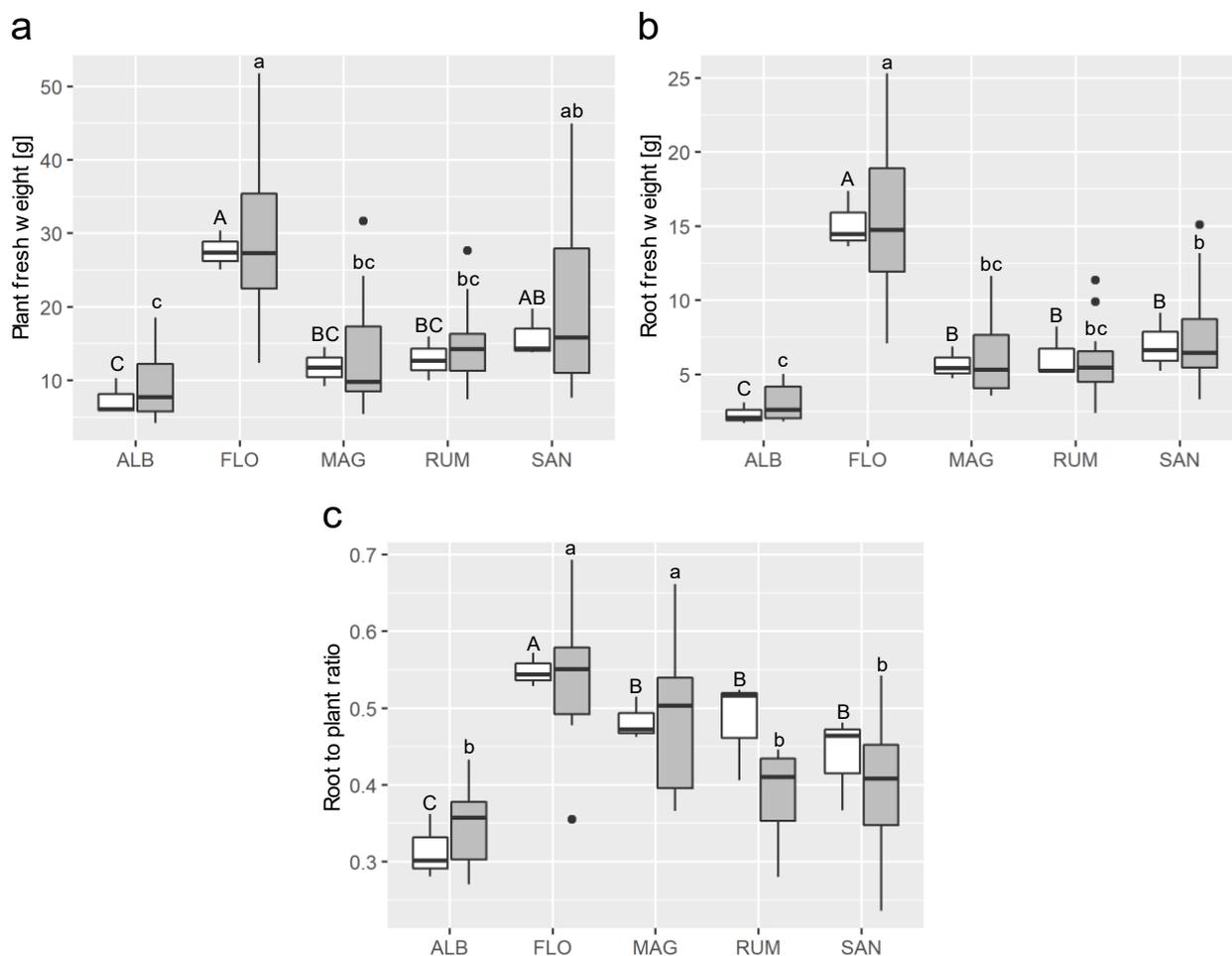

Fig. 1. Effect of the genotype on plant growth response after applying plant extracts. Grey boxes represent treatment with extracts and white the control. Cultivars: ALB – 'Albion', FLO – 'Florence', MAG – 'Magnum', RUM – 'Rumba' and SAN – 'San Andreas'. White or grey boxes with different letters (ABC or abc, respectively) are significant differences at $p \leq 0.05$ between cultivars treated (small letters) or control (capital letters) are shown

Table 2. Effect of plant extracts on the growth of different strawberry cultivars. Letters show statistically significant differences for $p \leq 0.05$. Means ±SD

| Treatment	Shoots fresh weight (g)	Roots fresh weight (g)	Roots/Shoots ratio
'Albion'			
Control	7.4 ±2.5 ab	2.3 ±0.7 a	0.31 ±0.04 b
Tagetes sp.	10.3 ±7.1 ab	3.1 ±1.7 a	0.32 ±0.07 ab
T. officinale	11.0 ±2.8 a	3.7 ±1.0 a	0.33 ±0.03 ab
C. officinalis – F	7.3 ±4.1 ab	2.8 ±1.5 a	0.39 ±.0.0 ab
C. officinalis – W	10.8 ±3.6 ab	3.2 ±1.1 a	0.30 ±0.01 b
S. officinalis	5.8 ±1.0 b	2.2 ±0.4 a	0.38 ±0.00 a
'Florence'			
Control	27.6 ±2.6 ab	15.2 ±2.0 ab	0.55 ±0.02 abc
Tagetes sp.	39.9 ±10.7 a	17.5 ±5.4 ab	0.44 ±0.08 c
T. officinale	21.6 ±4.3 b	13.4 ±4.2 b	0.61 ±0.07 a
C. officinalis – F	40.1 ±10.6 a	22.0 ±3.3 a	0.56 ±0.06 ab
C. officinalis – W	27.0 ±5.7 b	14.4 ±4.2 ab	0.53 ±0.04 bc
S. officinalis	20.5 ±7.5 b	11.5 ±4.0 b	0.56 ±0.02 ab
'Magnum'			
Control	11.8 ±2.7 a	5.7 ±1.1 a	0.48 ±0.03 ab
Tagetes sp.	8.9 ±0.8 a	4.3 ±0.9 a	0.48 ±0.06 ab
T. officinale	11.6 ±3.6 a	6.0 ±1.7 a	0.52 ±0.02 a
C. officinalis – F	19.0 ±4.8 a	7.2 ±1.9 a	0.38 ±0.02 b
C. officinalis – W	15.7 ±13.9 a	7.1 ±4.0 a	0.53 ±0.14 a
S. officinalis	13.1 ±9.9 a	6.1 ±3.1 a	0.53 ±0.13 a
'Rumba'			
Control	12.9 ±3.0 ab	6.2 ±1.8 a	0.48 ±0.07 a
Tagetes sp.	13.3 ±5.1 ab	4.7 ±2.4 a	0.35 ±0.08 a
T. officinale	12.2 ±1.8 b	5.3 ±1.0 a	0.43 ±0.02 a
C. officinalis – F	12.4 ±1.8 ab	4.9 ±0.7 a	0.39 ±0.03 a
C. officinalis – W	20.4 ±6.3 a	7.9 ±3.1 a	0.44 ±0.06 a
S. officinalis	15.6 ±5.9 ab	6.5 ±3.1 a	0.40 ±0.06 a
'San Andreas'			
Control	15.9 ±3.3 a	7.0 ±2.0 a	0.44 ±0.06 a
Tagetes sp.	17.0 ±6.2 a	6.8 ±1.2 a	0.42 ±0.08 a
T. officinale	19.4 ±18.0 a	6.4 ±2.9 a	0.44 ±0.17 a
C. officinalis – F	25.3 ±13.0 a	9.7 ±4.7 a	0.39 ±0.03 a
C. officinalis – W	28.4 ±18.8 a	9.4 ±5.3 a	0.36 ±0.09 a
S. officinalis	16.1 ±6.6 a	5.2 ±0.7 a	0.35 ±0.11 a
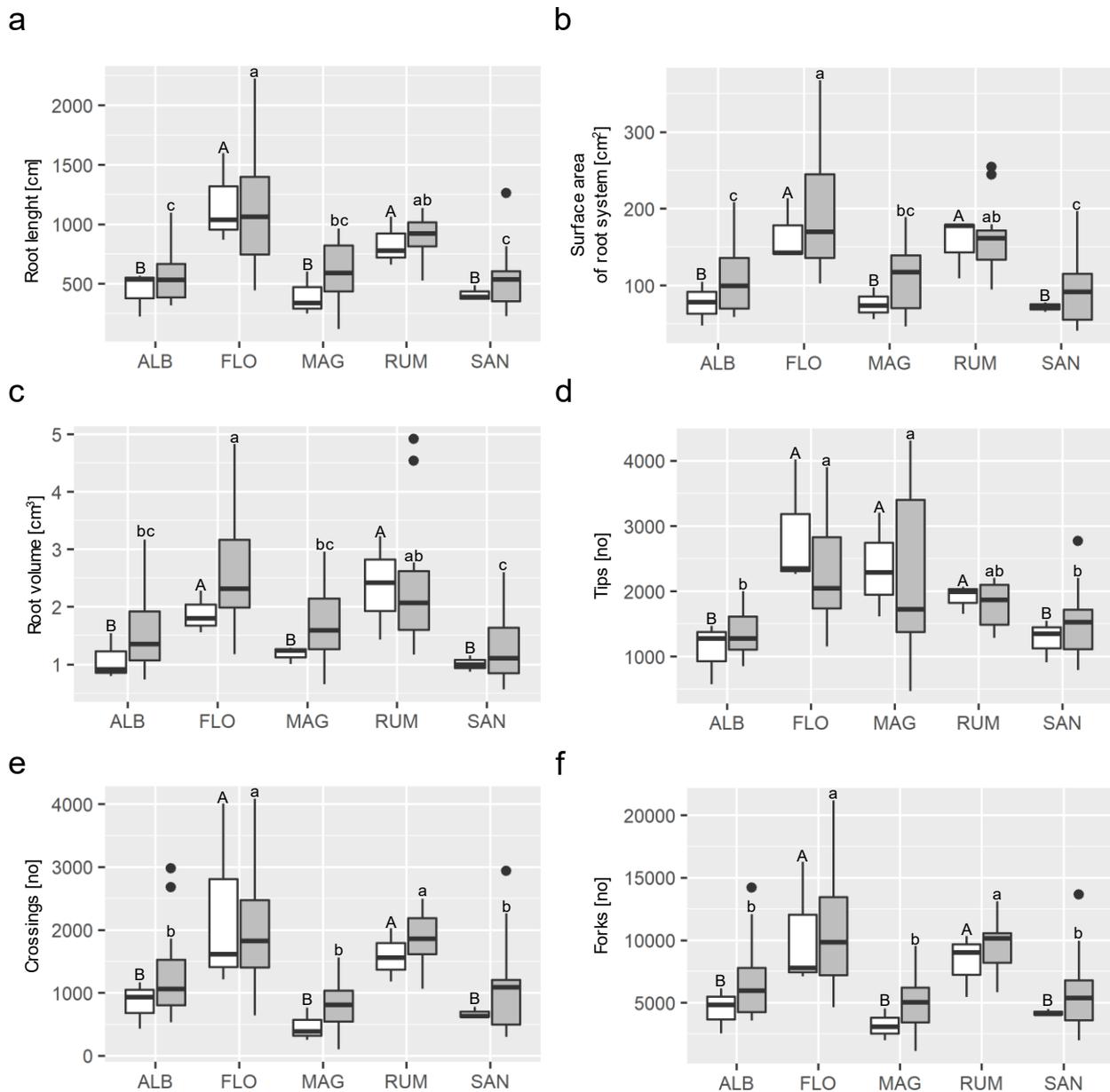
Fig. 2. Effect of the genotype on root system architecture after applying plant extracts. Grey boxes represent treatment with extracts and white the control. Cultivars: ALB – 'Albion', FLO – 'Florence', MAG – 'Magnum', RUM – 'Rumba' and SAN – 'San Andreas'. White or grey boxes with different letters (ABC or abc, respectively) are significant differences at $p \leq 0.05$ between cultivars treated (small letters) or control (capital letters) are shown

Table 3. Effect of plant extracts on root morphology and architecture. Letters show statistically significant differences for $p \leq 0.05$. Means ±SD

Treatment	Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Tips (n.)	Crossings (n.)	Forks (n.)
			'Albion'			
Control	442.1 ±190.0 a	76.9 ±28.7 a	1.1 ±0.4 a	1108 ±472 ab	842 ±379 a	4505 ±1818 a
Tagetes sp.	783.9 ±293.8 a	132.6 ±43.9 a	1.8 ±0.6 a	1786 ±207 a	1860 ±907 a	9146 ±3945 a
T. officinale	698.5 ±309.7 a	118.0 ±67.4 a	1.6 ±1.2 a	1496 ±281 a	1802 ±1067 a	8481 ±4720 a
C. officinalis – F	484.4 ±151.4 a	98.9 ±23.3 a	1.6 ±0.3 a	1179 ±138 ab	917 ±394 a	5570 ±1800 a
C. officinalis – W	501.1 ±126.5 a	105.5 ±36.8 a	1.8 ±0.8 a	1474 ±458 ab	852 ±208 a	5536 ±1152 a
S. officinalis	431.5 ±96.9 a	75.1 ±35.6 a	1.1 ±0.9 a	1002 ±96 b	900 ±254 a	4791 ±1827 a
			'Florence'			
Control	1169.2 ±381.0 bc	165.6 ±41.8 b	1.9 ±0.4 b	2877 ±987 ab	2279 ±1513 ab	10391 ±5094 ab
Tagetes sp.	1243.9 ±156.5 ab	193.8 ±38.7 ab	2.4 ±0.8 ab	2663 ±325 ab	2237 ±380 ab	11790 ±1824 ab
T. officinale	880.2 ±145.1 bc	150.4 ±24.5 b	2.1 ±0.3 ab	1844 ±190 c	1592 ±233 b	8811 ±1144 b
C. officinalis – F	1857.3 ±320.0 a	310.5 ±56.9 a	4.2 ±1.0 a	3150 ±769 a	3291 ±814 a	17278 ±3435 a
C. officinalis – W	985.2 ±411.6 bc	174.1 ±71.9 b	2.5 ±1.0 ab	2052 ±750 bc	1483 ±649 b	8817 ±3992 b
S. officinalis	769.2 ±362.6c	142.4 ±49.5 b	2.3 ±0.9 ab	1649 ±437 c	1528 ±930 b	7798 ±3675 b
			'Magnum'			
Control	396.9184.3 a	75.7 ±20.7 a	1.2 ±0.2 a	2368 ±797 ab	468 ±262 a	3212 ±1271 a
Tagetes sp.	552.1 ±82.7 a	110.3 ±21.2 a	1.8 ±0.4 a	1329 ±344 b	753 ±83 a	4484 ±851 a
T. officinale	775.7 ±315.0 a	131.3 ±63.4 a	1.8 ±1.0 a	2014 ±607 ab	998 ±544 a	5821 ±2578 a
C. officinalis – F	796.3 ±70.0 a	122.8 ±22.3 a	1.5 ±0.4 a	3973 ±377 a	1099 ±235 a	6334 ±1464 a
C. officinalis – W	424.2 ±467.7 a	96.6 ±80.3 a	1.9 ±0.9 a	1867 ±1809 b	609 ±825 a	4047 ±4767 a
S. officinalis	473.0 ±113.4 a	91.8 ±37.0 a	1.5 ±0.9 a	2478 ±860 ab	644 ±191 a	4069 ±1073 a
			'Rumba'			
Control	835.1 ±206.4 a	155.6 ±40.3 a	2.4 ±0.9 a	1906 ±219 a	1588 ±421 b	8284 ±2526 ab
Tagetes sp.	790.4 ±73.8 a	135.6 ±33.1 a	1.9 ±0.7 a	1724 ±205 ab	1736 ±154 ab	8573 ±742 b
T. officinale	881.7 ±307.6 a	145.1 ±43.9 a	1.9 ±0.5 a	1958 ±408 a	1721 ±598 ab	8875 ±2638 ab
C. officinalis – F	905.3 ±103.0 a	162.9 ±15.9 a	2.4 ±0.6 a	1391 ±90 b	2006 ±451 ab	10423 ±872 ab
C. officinalis – W	1043.5 ±90.8 a	183.4 ±63.8 a	2.7 ±1.7 a	2067 ±168 a	2314 ±175 a	11376 ±1510 a
S. officinalis	885.0 ±71.4 a	169.3 ±65.6 a	2.7 ±1.9 a	1866 ±186 a	1635 ±149 b	9280 ±2343 ab
			'San Andreas'			
Control	412.4 ±65.0 a	72.0 ±6.4 a	1.0 ±0.1 a	1269 ±325 ab	672 ±93 b	4201 ±255 b
Tagetes sp.	480.9 ±171.0 a	78.5 ±23.2 a	1.0 ±0.2 a	1394 ±406 ab	880 ±458 ab	4831 ±2192 ab
T. officinale	351.9 ±210.1 a	71.2 ±42.1 a	1.2 ±0.7 a	1112 ±408 b	535 ±411 b	3657 ±2543 b
C. officinalis – F	801.5 ±401.3 a	128.3 ±59.4 a	1.6 ±0.7 a	2046 ±626 a	1785 ±1002 a	9016 ±4039 a
C. officinalis – W	560.5 ±204.7 a	106.6 ±43.2 a	1.7 ±1.0 a	1539 ±583 ab	1260 ±784 ab	6311 ±2653 ab
S. officinalis	532.5 ±264.2 a	77.7 ±32.6 a	0.9 ±0.3 a	1500 ±427 ab	1198 ±954 ab	5902 ±3725 ab

observed in the plants treated with *C. officinalis* flowers and *C. officinalis* whole plant extract (Tab. 2).

A much diverse impact of both genotypes and extracts resulted when analyzing the root system morphology and architecture (Fig. 2). The varieties 'Rumba' and 'Florence' were characterized by a root system with significantly higher total root length, surface area, and, consequently, root volume compared to the other three varieties (Fig. 2a–c). Both 'Florence' and 'Rumba' varieties also showed more crossings and forks than the others (Fig. 2e–f). The small root system observed for cv. 'Albion' and cv. 'San Andreas' matched with the lowest number of tips. However, this was not the case for cv. 'Magnum', which has several tips comparable to that of 'Florence' and 'Rumba' (Fig. 2d).

'Florence' appeared to be the variety most susceptible to the extracts' effect when considering the root morphology and architecture (Tab. 3). It was the only variety showing an effect of extracts on root total length, surface area, and root volume. The extract of *C. officinalis* flowers increased the value of all these parameters compared to the control, *Tagetes* spp. positively affected root length and volume; S. *officinalis* whole plant extract reduced significantly total root

length. The extracts affected the number of tips in all varieties. Scotch marigold flower extract increased the value with 'Florence', 'Magnum', and 'San Andreas', but decreased it in 'Rumba' and not modified in 'Albion', compared to the control. An increased number of tips was induced in 'Albion' by *Tagetes* spp. and *T. officinale*. All other extracts induced a similar number of tips to control for the remaining varieties. These results were reflected in the number of crossings and forks: 'Florence', 'Rumba', and 'San Andreas' appeared to be affected, with *C. officinalis* flowers and *C. officinalis* whole plant extracts increasing in general both parameters compared to control (Tab. 3).

The growth pattern genetics at the base of the variety response to the extracts were confirmed by two- and three-dimensional principal component analyses using the data of untreated plants (Fig. 3). The diversity between 'Florence' and 'Albion' and between both; the other three varieties emerged with this multivariate analysis, which pointed out also some differences between 'Rumba' and the other intermediate varieties.

On the other hand, when merging the results of all varieties, only *C. officinalis* flower extract consistently modified the morphology and architecture of the root
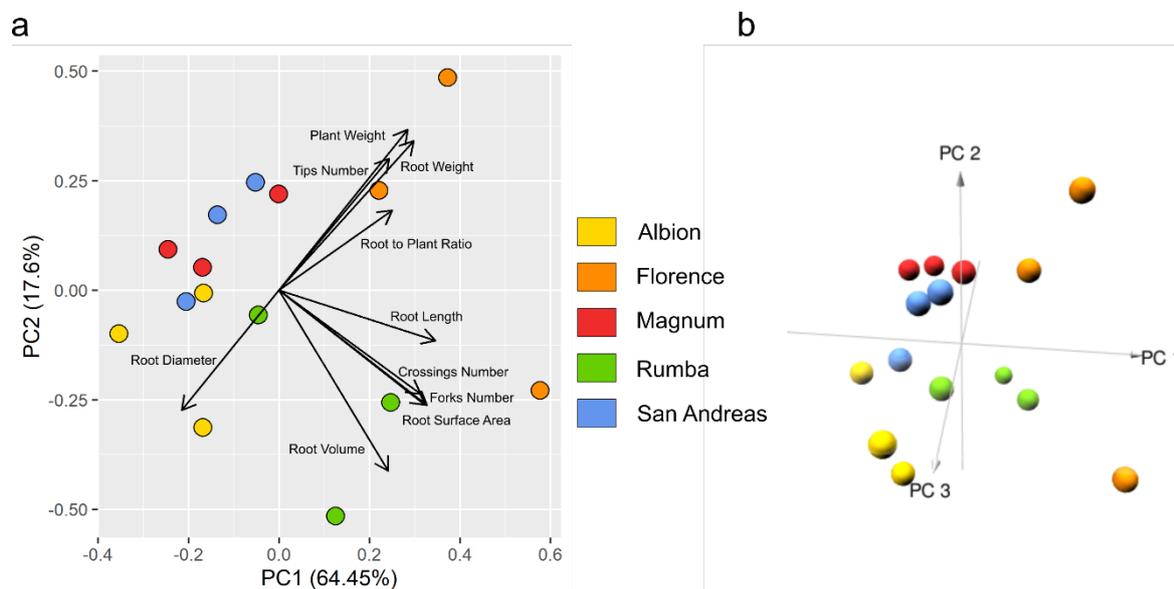

Fig. 2. Discrimination of the five strawberry varieties (untreated control) by principal component analyses of the plant growth and root morphological parameters: (A) two-dimensional PCA and (B) representative snapshot of a 3D model from three-dimensional PCA

Table 4. Effect of plant extracts on root morphology and architecture merging data from all varieties. Letters show statistically significant differences for $p \leq 0.05$. Means ±SD

Treatment	Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Tips (n.)	Crossings (n.)	Forks (n.)
Control	651.1 ±371.0 ab	109.1 ±50.7 a	1.5 ±0.1 b	1906 ±869 a	1170 ±931 b	6119 ±3661 b
Tagetes sp.	770.2 ±315.4 ab	130.2 ±51.9 a	1.8 ±0.1 ab	1779 ±562 a	1493 ±759 ab	7765 ±3564 ab
T. officinale	717.6 ±300.4 ab	123.2 ±48.2 a	1.7 ±0.1 ab	1685 ±484 a	1330 ±705 ab	7129 ±3143 ab
C. officinalis – F	968.9 ±523.3 a	164.7 ±86.0 a	2.3 ±0.1 a	2348 ±1173 a	1820 ±1021 a	9724 ±4848 a
C. officinalis – W	702.9 ±376.6 ab	133.2 ±66.7 a	2.1 ±0.2 ab	1800 ±866 a	1304 ±771 ab	7217 ±3809 ab
S. officinalis	618.2 ±261.3 b	111.3 ±54.0 a	1.7 ±0.1 ab	1699 ±645 a	1181 ±658 ab	6368 ±3048 b

system, increasing the value of five out of six parameters compared to control (Tab. 4).

The extract from *C. officinalis* flowers resulted in inducing in 'Magnum' a total biomass and root weight about twice that of the control, resulting in the lowest root/plant ratio, significantly different from the other extracts, and paralleled by the highest number of tips, significantly higher than the control.

'Florence' was the most sensitive variety to the application of plant extracts: statistically significant changes were observed in 10 out of 11 studied parameters (Tab. 2 and Tab. 3), while other varieties showed a limited response to them (3 or 4 significantly changed parameters).

DISCUSSION

The selection of the plants and their organs for preparing the extracts was based on their potential effects on protecting strawberries from pests [Turchen et al. 2020]. However, we evaluated the effects of the extracts on the plant, considering the possible interactions with the root system of different genotypes and the contribution of the mineral elements contained in the extracts.

The high nutrient content of the *C. officinalis* flower extract could be one of the factors that contributed to the generally positive response of the plants to its application. The acidic reaction of the extract could have also contributed to the availability of some mineral elements (e.g., P) and eventually transiently modified the rhizosphere conditions, favoring the development of a population rich in beneficial microorganisms in the rhizospheric soil [Hinsinger et al. 2003]. However, the same acidic reaction of the *S. officinalis* whole plant extract was insufficient to promote plant growth and improve root morphological parameters. The high nitrogen content in *C. officinalis* whole plant extract as well as of other elements, a feature also shared by other extracts, could only partly be assumed as playing a role in the modification of roots growth since the impact of these extracts was, in general, limited to few plant characteristics, mainly on 'Florence.' Therefore, the effect measured on the plant growth could likely derive from some specific components of the extracts [Hartmann 2007].

Phytochemical studies have demonstrated the presence of several classes of chemical compounds, the main ones being polyphenols, terpenes, quinones, carotenoids, and volatile and essential oils, in all the plants used as a basis for the extracts [Afonso et al. 2019, Nelofer et al. 2017, Salehi et al. 2018]. These compounds can be extracted using different methods [Azmir et al. 2013], including hydro-alcoholic solutions such as those utilized in this study. Secondary metabolites affect how plants interact with soil microorganisms [Cheynier et al. 2013], for example, by promoting growth through ACC deaminase [Glick et al. 1998] or indole-3-acetic acid (IAA) production [Lambrecht et al. 2000], which foster better mineral nu-

trition because of a more developed plant root system. The frequently observed impact of the extracts on the root tips number and the fork number can be considered an indirect confirmation of such a hypothesis. It would thus support the emerging hypothesis for variation in root functional traits [Weemstra et al. 2016], which assumes that high specific root length, thin root diameters, and high root tips number should be traits indicative of a fast-growth plant and should relate to higher rates of root respiration (i.e., microbial interactions).

The genotype-related response to the extracts, which was particularly visible in the most and less vigorous varieties ('Florence' and 'Albion', respectively), points to the strong influence of the "variety factor" on the possible effect of the extracts. Moreover, the different varieties reacted diversified to the extracts, sometimes increasing the root growth in other shoots. Notably, some varieties modified the root morphology to respond to the extract application, which concerned the total length and the number of tips. The root system architecture represents the spatial arrangement of roots [Osmont et al. 2007] as determined by a genetic component and the interaction with environmental cues [Malamy 2005], which allows the plant to display a high level of root plasticity [Gruber et al. 2013]. Changes in nutrient availability as an effect of the extracts' application or the modifications induced at the rhizosphere level could thus both at the basis of the observed effects [López-Bucio et al. 2003], differently modulated by the five strawberry genotypes tested. Indeed, strawberry cultivars could present distinct growth characteristics for the whole plant and root system [Ariza et al. 2021, Klamkowski and Treder 2008], with root architecture, in particular length density, being highly correlated with fruit yield [El-Miniawy et al. 2014, Mattner et al. 2018]. Nevertheless, the observed response of the plants to the extracts could also be proportionally lower and represented only by trends challenging to distinguish from within cultivar variability, as also observed with the same cv. 'Albion' after treatment with a seaweed extract [Mattner et al. 2018].

The renewed interest in using plant-derived substances that are known to interfere with the host plant (repellents) and the feeding activity of a pest as well as functioning as growth and yield promoters should thus foster additional work to unravel the bottlenecks in using botanical extracts, considering the complex interactions between plant genetics, soil characteristics, and microbiome to make such research more valuable from the practical point of view.

CONCLUSIONS

In this study, five different plant extracts were tested to assess their impact on five different strawberry cultivars, particularly on root system development. The genotype affected the response of strawberry plants to different plant extracts known as potential improvers of plant health. Applying a hydro-alcoholic extract of *C. officinalis* obtained only from flowers or, to a lower extent, the whole plant showed a positive impact on plant growth, particularly on root characteristics associated with nutrient uptake. This effect was especially evident in the cv. 'Florence', a vigorous variety, rather than with less vigorous genotypes, is likely due to the mineral nutrient composition of the extract and the interaction of its components and pH with the rhizosphere microbiome.

Applying plant extracts derived from officinal or medicinal plants to support the integrated control of soil-borne pests could have a potentially positive effect on vigorous varieties, as they favor the development of the root system, contrasting thus the negative impact of the pests. However, further studies are needed to determine which component/s or properties (e.g. pH) of the extracts are the most effective for these purposes. Analyzing the physiological mechanisms based on the effect or the impact on the rhizospheric microbiome could improve our understanding and support the definition of application protocols in practice.

AUTHOR CONTRIBUTION

Conceptualisation: E.M.F., M.T., E.M.; developing methodology: E.M.F., M.T.; conducting research: E.M.F., M.T.; data analysis: E.M.F.; original draft preparation and revision of the article: E.M.F., M.T., E.M. The authors declare that E.M.F. and M.T. have contributed equally to the article.

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