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Research article

Seed treatments with essential oils protect radish seedlings against drought

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Abstract: Establishment of seedlings of economic crops is often reduced if there is not a steady supply of water. Essential oils (EO) from plants are increasingly used instead of synthetic chemicals to protect plant and animal products against biotic and abiotic stresses. We investigated priming radish seeds by soaking or by matriconditioning with synthetic or natural compounds as a means of inducing resistance to drought stress, thus maintaining crop yield. Priming radish seeds for two hours in solutions of essential oils (EO) thymol and carvacrol derived from Origanum syriacum, with "oregano natural product" (ONP; a solution of the residue remaining after EO extraction), or with the gibberellin synthesis inhibitor trinexapac ethyl (TE), was much more effective in inducing drought resistance than was matriconditioning with the same compounds in sawdust for two days. The latter treatment induced considerable fungal and bacterial infection in treated seeds if the substrate-matrix was not heat-treated beforehand. The increase in specific leaf area in plants from treated seeds was mostly consistent with an increase in leaf water content. Seed treatments with EO, ONP, and especially TE led to a three-fold increase in radish seedling survival compared with water-treated controls, when 21 day-old seedlings were irrigated after 6 days of drought. Under drought conditions, seedlings from treated seeds had a 2–3-fold increase in relative water content increased 2–3-fold, while membrane permeability decreased 20–50-fold as a result of the treatments. However, the physical benefits of the treatments often did not correlate with treatment-induced increases in physiological parameters such as pigments (chlorophyll, carotenoid, anthocyanin), pigment ratios (chlorophyll a/b, carotenoid/chlorophyll), or antioxidant activity. Seed treatments with biostimulants can be as effective as treatments with synthetic compounds in inducing drought resistance in seedlings.

Keywords: Trinexapac-ethyl; carvacrol; thymol; Oregano syriacum; carotenoids; anthocyanin; DPPH; chlorophyll; relative water content; ion leakage; seed priming

1. Introduction

Establishment of seedlings of economic crops is often reduced if there is not a steady supply of water. Rainfall and temperatures over the next 10–100 years are predicted to shift to drier and warmer climate conditions, which will further exacerbate the establishment of the majority of the economic crops that are currently produced [1]. Breeding for abiotic stress resistance in crops is effective but time-consuming. Seed treatments to induce abiotic stress will permit continued use of current genotypes, or at least provide a stop-gap while plant breeders develop new stress-resistant varieties.

Seed treatments to induce abiotic stress resistance have been reviewed extensively [2,3]. Soaking, coating directly or with slow-release formulations of various chemicals, use of water-absorbing and water-resisting coatings—all have been attempted as means to induce stress resistance. Solid matrix priming, also known as matriconditioning, is a relatively recent development in seed treatments. It has been used to protect against cold stress during germination, to reduce the variability in emergence, and to supply plant growth regulating bacteria to the seed [3-5]. The matrix itself can range from granulated minerals (shale or clay) to available plant by-products such as rice husks or sawdust [4,5].

Essential oils (EO) from plants are increasingly used instead of synthetic chemicals to protect plant and animal products against biotic and abiotic stresses. Thymol and carvacrol have germicidal effects against fungi and bacteria, and also act as antioxidants, thus protecting sensitive membranes from damage during abiotic or biotic stresses [6,7]. However, treating seeds with excessively-high concentrations of EO can reduce germination and damage surviving seedlings [8]. Similar results are found with growth regulators, where the optimal concentration must be determined to avoid abnormal germination and development [9].

We hypothesized that seed treatments with compounds that strengthen membranes and enhance antioxidative activity by plants could protect radish seedlings against drought. Matriconditioning seeds with plant extracts that contain essential oils to induce drought resistance in seedlings has not been reported previously. Our objective was to compare standard imbibition and matriconditioning as means for treating radish seeds with EO from two chemotypes (carvacrol and thymol) of za'atar (*Origanum syriacum*), as well as extracts from the residue remaining after essential oils have been steam-distilled from harvested oregano. The latter oregano natural product (ONP) retains bioactive compounds [10]. We also treated the seeds with Moddus (trinexapac-ethyl, TE), an inhibitor of gibberellin synthesis that can induce drought resistance [11].

2. Materials and Methods

Seed treatment. Radish seeds (*Raphanus raphanistrum* subsp. *sativus*, cv. Varda) were obtained from the Official Israel Seed Testing Laboratory. Seeds were imbibed in solutions as described below for 2 hours at room temperature (20–23 ºC) or were matriconditioned with solutions for two days at 15 ºC in sawdust obtained from a local carpenter shop at a ratio of 3:1:3 seed: matrix: solution [5]. Seeds were then air-dried to their original weight.

Treatment solutions. Seeds were treated with 0.5% (v/v) aqueous extracts from carvacrol and thymol chemotypes of *Origanum syriacum*, 0.5 or 5% (v/v) solutions of oregano natural product (ONP, Bakto Flavours, Inc.), or 0.15% (375 ppm active ingredient) or 0.3% (750 ppm a.i.) Moddus (Syngenta brand of trinexapac-ethyl). The Oregano syriacum chemotypes had respectively 70% carvacrol, 0% thymol or 40% thymol and 20% carvacrol in their essential oil fractions, while the ONP had no carvacrol or thymol, but did have bioactive phenols and antioxidative activity [10].

Treated seeds (four replicates of 20 seeds each) were germinated for seven days at 20 ºC on Anchor germination paper, after which germination was categorized and calculated as percent normal or abnormal. Fungus-infected seeds were counted for calculation of percent total infection. Separate replicate was sown on MTMB and MFS media to check for specific (*Xanthomonis campestris*) or non-specific bacterial infection, respectively.

Seeds were also sown in 150 ml pots with a 20:80 peat: sand mix, and grown in a 20 ºC chamber under fluorescent grow-lights with a 16 h: 8 h light: dark regime. Each treatment was replicated in four pots. Five seeds were sown per pot. Percent emergence was calculated after seven days. After emergence, seedlings were thinned to three plants/ pot. At 15 days after emergence, the thickness of the first true leaf was measured with a digital caliper. Discs 1 cm diameter were cut from the first true (avoiding the main veins), weighed, and placed in the dark at room temperature for 24–48 hours in methanol to determine chlorophyll and carotenoids/xanthophylls or in methanol-1% HCl to determine anthocyanins [12,13]. The methanol extract was also used to determine antioxidant activity in the leaf [14]. A 40 µL aliquot of methanol extract was added to 960 µL of DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution, incubated for 20 minutes, and read at 517 nm. The results were expressed on a mm^2 basis as the difference in absorbance between blank (methanol only) and sample. Specific weight (mg/mm^2) was calculated from the weight of the 1-cm diameter (0.79 mm^2) discs.

After three weeks of growth, total drought was imposed for 6 days. At the end of that time, the second true leaf was taken for measurement of relative water content. Leaves were weighed, floated for two hours on deionized water in a petri dish, blotted and reweighed, and then dried overnight at 85 ºC before final weighing. Relative water content was calculated as [(fresh weight − dry weight) ÷ (turgid weight− dry weight)]. Discs 1 cm diameter were cut from the third true leaves (avoiding the main veins), weighed, and placed in 2 ml of deionized water in sealed vials for 6 hours at room temperature. The vials were then shaken to mix the contents, and the electrical conductivity (ion efflux) of the solution was measured with a Cyberscan CON 110 meter (Eutech Instruments). Ion efflux was expressed as µS/mg/hr. The vials with the tissue discs were then frozen at −20 ºC for 12–18 hours to rupture the plant cells, allowed to come to room temperature, and percent of total ion leakage was measured as initial efflux (before freezing)/ final efflux (after freezing and thawing).

After the drought period, plants were watered for a further 6 days, whereupon the number of revived (turgid) plants were counted to calculate % survival after drought. The above-ground portion of each plant was weighed, dried for 18 hours at 85 ºC, and reweighed to determine the percent dry weight.

All data were analyzed as a factorial design by ANOVA [15] and means separated by LSD. Main variables were seed treatment (matriconditioning vs soaking), while secondary variables were the seed treatment compounds. With the exception of percent total infection, there were no main effect differences between matriconditioning and soaking treatments. Significant differences between seed treatment compounds are indicated by different letters within columns, while significant interactions at the seed treatment X compound level are indicated by asterisks.

3. Results and Discussion

Germination and emergence. Normal germination greater than 85% (the lowest commercially acceptable percentage) was observed in all treated seeds except those exposed to carvacrol and trinexapac-ethyl (TE, Moddus) (Table 1). The lower concentration of TE reduced germination more than the higher, a paradoxical situation that has been observed with other hormonally-regulated phenomena [16,17]. However, the higher concentration of TE caused the greatest amount of abnormal germination, which included deformed roots or stunted shoots. Emergence of seedlings in pots generally paralleled the results obtained with germination on paper (Table 1), but tended to be greater. This is probably because germination was measured after six days, while emergence was determined after 14 days. The greatest incidence of fungal infection was observed in control and ONP treatments, with both thymol and carvacrol extracts causing a significant reduction in infection, as has been reported [18]. The reduction in fungal infection resulting from TE treatment was surprising, but may be due to fungitoxic effects of the low pH of TE solutions (as indicated in the Material Safety Data Sheet for TE). Infection by *Xanthomonas capestris* was similar to controls (3000 cfu) in all solution treatments except thymol and carvacrol, which completely suppressed bacterial growth (data not shown). Matriconditioning did not cause significant changes in results compared with priming in solution, with the exception of fungal and bacterial infections, which were considerably increased in all treatments based on plant extracts. Both rice husk and sawdust matrix material turned out to harbor fungal contaminants (mostly *Rhizopus* and *Pennicillium*) and non-specific bacteria, which were significantly (rice husk) or totally (sawdust) suppressed by two days heating at 100 ºC (Figure 1). Rice husk was also a physically difficult material to work with in treating small seeds such as radish. We therefore used heat-treated sawdust for matriconditioning seeds that were sown in pots for the growth and drought experiment.

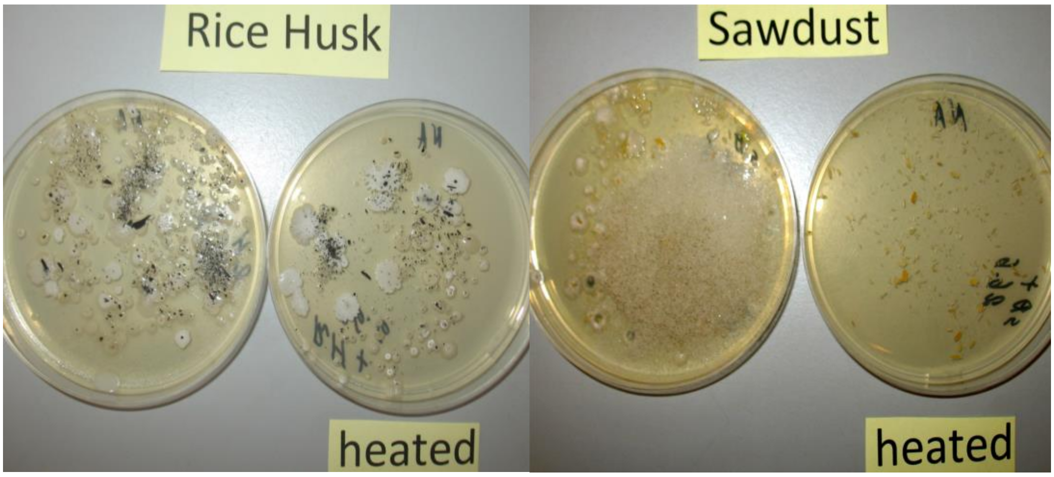

Figure 1. Reduction or elimination of fungi and bacteria in matriconditioning substrates rice husk and sawdust, after two days heating at 100 ºC followed by incubation on agar at 20 ºC for five days.

Table 1. Germination and fungal infection percentage of solution-primed or matriconditioned radish seeds after seven days on germination paper. Emergence was determined from seeds sown in a sand: peat 80:20 potting mix in 10 cm pots. ONP = Oregano natural product; TE = trinexapac-ethyl. Four replications of 50 seeds each for germination on paper; four replications of five seeds each per pot; mean separations within columns by LSD, $p \leq 0.5$. Asterisks indicate significant ($p \leq 0.5$) differences between solution and matriconditioning treatments within specific treatment materials.

Treatment material	Normal germination (%)		Abnormal germination (%)		Total germination (%)		Total Infection (%)		Emergence (%)	
	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix
Water	95.0a	88.3a	1.7a	5.0a	96.7a	93.3a	48.3a	43.3b	100a	100a
ONP 0.1%	90.0a	96.7a	5.0a	1.7a	95.0a	98.3a	36.7ab	83.3a*	95a	95a
ONP 5.0%	96.7a	95.0a	3.3a	5.0a	100.0a	100.0a	46.7a	73.3a*	100a	100a
Thymol	88.3a	90.0a	5.0a	8.3ab	93.3a	98.3a	18.3c	78.3a*	100a	100a
Carvacrol	60.0b	96.7a*	16.7b	3.3a	76.7b	100.0a	26.7bc	68.3a*	70b	100a*
TE 0.15%	8.3d	6.7b	5.0a	8.3ab	13.3d	15.0b	16.7c	36.7b	40c	60b
TE 0.3%	20.0c	0.0b	20.0b	15.0b	40.0c	15.0b*	20.0c	25.0b	45bc	35c

Leaf structure. Leaf thickness and specific area were both enhanced in plants that grew from seeds treated with TE (Table 2), while ONP 0.1 and carvacrol only enhanced specific area. TE treatments lead to increased thickness of the epidermal layer of leaves by inducing wax and cutin formation [19,20]. There was no correlation between leaf thickness and weight per cm^2. Exogenous applications of carvacrol and phenol-containing compounds such as ONP can change the fatty-acid structure of plant tissues [21], in addition to inducing suberization of xylem tissues [22]. Strengthening membranes and water-retention capability of tissues might partially account for enhanced specific weight in the absence of increased leaf thickness, although this would be an epigenetic effect on the plant resulting from the original seed treatment.

Drought survival. Soaking seeds in all solutions except ONP 0.1% resulted in an average seedling survival rate of more than 70% (Table 3), compared with 19% for untreated seeds. In contrast, matriconditioning enhanced seedling survival only with thymol and carvacrol extracts. However, fresh weight of surviving seedlings was enhanced in comparison with control only in plants grown from TE- and ONP 0.1% solution-primed seeds. Matriconditioning was effective in enhancing seedling fresh weight only in the TE 0.3% treatment. In parallel with enhanced seedling fresh weight, % seedling dry weight was 25–50% less in plants from solution-primed seeds in comparison with control, while matriconditioning decreased % dry weight only in the TE 0.3% treatment. Solution priming was therefore more effective in preserving the relative capacity of the seedling to retain moisture. This is further highlighted by the more than 100% increase compared with controls in relative water content in plants from seeds solution-primed with thymol, carvacrol and TE. Matriconditioning was similarly effective only with TE treatments. Membrane permeability, which correlates with plant water loss or a decrease in plant fresh weight, was measured by ion efflux and total percent ion leakage. The two measurements provided parallel results, in which almost all treatments resulted in substantial decreases in membrane permeability compared to controls. However, there was no correlation between measurements of membrane permeability and relative water content, plant fresh weight or percent survival.

Table 2. Leaf thickness and leaf specific area of first true leaf of radish plants grown from solution-primed or matriconditioned seeds, after 14 days of growth. ONP = Oregano natural product; TE = trinexapac-ethyl. N = 4; mean separations within columns by LSD, $p \leq 0.5$. Asterisks indicate significant ($p \leq 0.5$) differences between solution and matriconditioning treatments within specific treatment materials.

Treatment material	Leaf thickness (μm)		Specific area (mg/cm^2)	
	Solution	Matrix	Solution	Matrix
Water	175 a	175a	11.3a	14.6a
ONP 0.1%	175	183a	19.9b	17.5a
ONP 5.0%	191ab	193ab	15.5ab	18.0a
Thymol	176 a	175a	17.1ab	13.4a
Carvacrol	180 ab	181a	56.0d	15.2a
TE 0.15%	197 bc	210 c	30.4c	24.2b
TE 0.3%	211 c	205bc	29.8c	39.2c*

Pigments. Chlorophyll concentration was enhanced by the higher TE concentration in solution priming and by all ONP and thymol treatments (Table 4). Carotenoid concentrations were enhanced by TE 0.3% in solution, by thymol in matriconditioning, and by ONP in both solution and matrix priming (Table 4). Both chlorophyll a/b and carotenoid/ chlorophyll ratios are considered indicators of abiotic stress resistance [23,24]. The chlorophyll a/b ratio did not vary between treatments, nor did it correlate with any direct or indirect measurement of drought resistance: neither % plant survival nor relative water content nor membrane permeability (data not shown). Similar lacks of correlation have been reported for other crops such as pearl millet [25] and oil palm [26], among others. The carotenoid/chlorophyll ratio of many treatments was greater than that of controls (Table 4), but it, too, did not correlate with measurements of drought resistance (data not shown), as others have found [26]. Anthocyanin concentration of plants from treated seeds was different from control only in the case of ONP 0.1% from solution. Although anthocyanins are considered to be sources of antioxidant activity [27], there was no correlation with DPPH reduction (Table 4). Antioxidant activity did not vary in tissues from matriconditioned seeds, and rose above control levels only in thymol and TE 0.15% solution-primed seeds (Table 4).

4. Conclusions

Not only was the material used in matriconditioning a source of pathogen infection (which can be mitigated by a time-consuming but necessary heat treatment of the substrate; Figure 1), the method itself did not enhance drought resistance, nor did it enhance physiological parameters to the same extent as solution priming. All the seed treatment compounds enhanced seedling survival after drought, compared to control (Table 2), although TE reduced overall germination (Table 1). Even in the absence of the highly bioactive EOs thymol and carvacrol, extracts from oregano residue still had biostimulatory activity to induce drought resistance. The physiological basis for increased drought resistance in seedlings from primed seeds is still unclear. Certain seed treatments, especially carvacrol and trinexapac-ethyl, caused enhanced pigmentation and increased antioxidative activity, but these protective mechanisms were not consistently correlated with the seedling's ability to recover from drought, as exemplified by leaf relative water content and membrane permeability. Although both carvacrol and TE treatments reduced final emergence, sowing a certain percentage more of treated seeds in drought-prone regions could compensate for this reduction, since the extra amount of treated seed would be less expensive than resowing the entire field in the event of drought.

Table 3. Percent survival, fresh and dry weight, relative water content (RWC), and membrane permeability (ion efflux rate and percent leakage) of radish plants grown from solution-primed or matriconditioned seeds after 6 days of drought (RWC, membrane permeability), followed by 7 days of irrigation and recovery (survival and plant weight). ONP= Oregano natural product; TE = trinexapac-ethyl. N = four replications of three plants per pot; mean separations within columns by LSD, $p \le 0.5$. Asterisks indicate significant ($p \le 0.5$) differences between solution and matriconditioning treatments within specific treatment materials.

Treatment material	Survival (%)		Fresh weight (mg)		Dry weight (%)		Relative water content (%)		Ion efflux(μS/mg/hr)		Ion leakage (%)	
	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix
Water	19 a	33 a	133 a	209 a	34a	22a	26a	40a	5.11a	1.88a*	67a	52a
ONP 0.1%	25 a	11 a	330 b	336 a	17b	16ab	43ab	46a	0.19b	0.18b	31ab	21b
ONP 5.0%	70 b	20 a*	274 ab	376 a	18b	11ab	37ab	31a	0.26b	0.24b	34ab	14b
Thymol	80 b	76 b	302 ab	218 a	11b	21ab	48b	32a	0.27b	0.17b	15b	19b
Carva-crol	69 b	70 b	242 a	272 a	6b	18ab	76c	36a*	0.11b	0.36b	11b	16b
TE 0.15%	67 b	53 a	397 bc	347 a	8b	13ab	69bc	57ab	0.09b	0.42b	8b	23b
TE 0.3%	75 b	45 a*	557 c	817 b*	9b	5b	50bc	67b	0.14b	0.13b	19b	19b

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Conflict of interest

All authors declare no conflicts of interest in this paper.

Table 4. Pigment (chlorophyll, carotenoid, anthocyanin) concentrations, carotenoid/chlorophyll ratio and antioxidant activity in extracts from first true leaves of radish plants grown from solution-primed or matriconditioned seeds, 15 days after emergence. ONP = Oregano natural product; TE = trinexapac-ethyl. N = four replications of three plants per pot; mean separations by LSD, $p \leq 0.5$. Asterisks indicate significant ($p \leq 0.5$) differences between solution and matriconditioning treatments within specific treatment materials.

Treatment material	Total chlorophyll (μmol/mm²)		Carotenoid (μmol/mm²)		Carotenoid/Chlorophyll ratio		Anthocyanin (nmol/mm²)		Antioxidant activity (units/mm²)	
	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix	Solution	Matrix
Water	26.0a	27.0a	1.91a	1.78a	0.07a	0.07a	7.33	7.24	30.6a	40.4*
ONP 0.1%	32.7b	30.9b	2.57a	3.30b	0.08a	0.11b*	8.38	7.92	41.1ab	47.1
ONP 5.0%	31.4b	34.2b	3.53b	4.09b	0.11b	0.12b	8.01	8.88	39.2a	47.7
Thymol	29.7b	31.8b	2.19a	3.75b	0.07a	0.12b*	7.82	8.26	53.1b	51.6
Carvacrol	27.9ab	28.6ab	2.81a	2.04a	0.10b	0.07a*	7.76	7.66	31.8a	39.2
TE 0.15%	27.0ab	26.7a	2.52a	2.08a	0.09ab	0.08a	7.33	7.32	48.8b	42
TE 0.3%	29.7b	28.9ab	3.74b	2.58a	0.12a	0.09ab*	8.13 ns	7.53ns	35.7a	43.3ns

References

1. Knight H, Knight MR (2001) Abiotic stress signaling pathways: specificity and cross-talk. *Trends Plant Sci* 6: 262-267.
2. Paparella S, Araújo SS, Rossi G, et al. (2015) Seed priming: state of the art and new perspectives. *Plant Cell Rep* 34: 1281-1293.
3. Taylor AG, Allen PS, Bennett MA, et al. (1998) Seed enhancements. *Seed Sci Res* 8: 245-256.
4. Taylor AG, Klein DE, Whitlow TH (1988) SMP: Solid matrix priming of seeds. *Sci Hortic* 37: 1-11.
5. Ilyas S (2006) Seed treatments using matriconditioning to improve vegetable seed quality. *J Agronomi Indonesia (Indonesian J Agron)* 34: 124-132.
6. De Azeredo GA, Stamford TL, Nunes PC, et al. (2011) Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Res Int* 44: 1541-1548.
7. Ye X, Ling T, Xue Y, et al. (2016) Thymol mitigates cadmium stress by regulating glutathione levels and reactive oxygen species homeostasis in tobacco seedlings. *Molecules* 21: 1339-1353.
8. Martino LD, Mancini E, Almeida LFRD, et al. (2010) The antigerminative activity of twenty-seven monoterpenes. *Molecules* 15: 6630-6637.
9. Arteca RN (2013) *Plant growth substances: principles and applications*. Springer Science & Business Media.
10. Havkin-Frenkel D, Bakto Natural Preservatives Llc (2017) Recovery of residual plant components after distillation of essential oils. U.S. Patent 9,572,849 [P].

11. Rademacher W (2016) Chemical regulators of gibberellin status and their application in plant production. *Ann Plant Rev* 49: 359-404.
12. Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzimol* 148: 350-382.
13. Stafford HA (1966) Regulatory mechanisms in anthocyanin biosynthesis in first internodes of Sorghum vulgare: effect of presumed inhibitors of protein synthesis. *Plant Physiol* 41: 953-961.
14. Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 26: 211-219.
15. Rorden C (2007) Available from: http://www.cabiatl.com/mricro/ezanova/
16. Aharoni N (1989) Interrelationship between ethylene and growth regulators in the senescence of lettuce leaf discs. *J Plant Growth Regul* 8: 309-317.
17. Klein JD, Shalev YR, Cohen S, et al. (2016) Postharvest handling of "etrog" citron (*Citrus medica*, L.) fruit. *Isr J Plant Sci* 63: 64-75.
18. Horvathova E, Navarova J, Galova E, et al. (2014) Assessment of antioxidative, chelating, and DNA-protective effects of selected essential oil components (eugenol, carvacrol, thymol, borneol, eucalyptol) of plants and intact *Rosmarinus officinalis* oil. *J Agric Food Chem* 62: 6632-6639.
19. Heckman NL, Horst GL, Gaussoin RE (2001) Influence of trinexapac-ethyl on specific leaf weight and chlorophyll content of *Poa pratensis*. *Int Turfgrass Soc Res J* 9: 287-290.
20. Phavaphutanon L, Hebbe Y, Cohen S, et al. (2004) Trinexapac-ethyl and paclobutrazol protect against drought in hot pepper plants. *HortScience* 39: 897.
21. Martín JA, Solla A, García-Vallejo MC, et al. (2012) Chemical changes in *Ulmus minor* xylem tissue after salicylic acid or carvacrol treatments are associated with enhanced resistance to *Ophiostoma novo-ulmi*. *Phytochem* 83: 104-109.
22. Martín JA, Solla A, Domingues MR, et al. (2008) Exogenous phenol increase resistance of *Ulmus minor* to Dutch elm disease through formation of suberin-like compounds on xylem tissues. *Environ Exp Bot* 64:97-104.
23. Gitelson AA, Gamon JA, Solovchenko A (2017) Multiple drivers of seasonal change in PRI: Implications for photosynthesis 1. Leaf level. *Remote Sens Environ* 191: 110-116.
24. Todaka D, Zhao Y, Yoshida T (2017) Temporal and spatial changes in gene expression, metabolite accumulation and phytohormone content in rice seedlings grown under drought stress conditions. *Plant J* 90: 61-78.
25. Ashraf M, Ahmad A, McNeilly T (2001) Growth and photosynthetic characteristics in pearl millet under water stress and different potassium supply. *Photosynthetica* 39: 389-394.
26. Silva PA, Oliveira IV, Rodrigues KC (2016) Leaf gas exchange and multiple enzymatic and non-enzymatic antioxidant strategies related to drought tolerance in two oil palm hybrids. *Trees* 30: 203-214.
27. Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. *J Agric Food Chem* 51: 28-633.