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Influence of Marine Brown Alga Extract (Dalgin) on Damping-off Tolerance of Tomato

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Abstract
In this research, effects of a seaweed extract, *Ascophyllum nodosum*, was investigated on *Phytophthora capsici*. 0.5% seaweed extract 80% significantly reduced severity of infections. Plants treated with seaweed extract showed enhanced activities of various defense-related enzymes including: β-1,3-glucanase, peroxidase, and polyphenol oxidase. Upregulation of various genes, including lipoxygenase (LOX), chitinase (Chi), galactinol synthase (GolS), and phenylalanine ammonia lyase (PAL), were observed in treated plants. The greatest level of expression level was observed for PAL in application. Based on our finding, we could conclude that seaweed extracts are able to induce resistant in tomato and are suitable candidate to control of plant fungal disease.

Keywords: *Ascophyllum nodosum*, Tomato, *Phytophthora capsici*, Biochemical assay

1. Introduction
Induced systemic resistance (ISR) is a phenomenon whereby disease resistance is induced by treatment with biotic and abiotic compounds [1-2]. Induction of defense enzymes causes plant resistant to pathogen attack [3-6]. Up until now, literature survey indicated that no research has been conducted on how these inducers may influence the induction of defense enzymes.

Phytophthora capsici causes damping-off of tomato in several parts of the world [7-8]. *Phytophthora* damping-off is controlled by synthetic fungicides. The regulations on the utilization of synthetic fungicides, justifies the work for new active molecules. There are earlier reports of ISR of plants to fungal diseases through macro-algal extract application [9-10]. In this research, we attempted to assay the effect of a commercial extract from a marine alga (dalgin) to tomato damping-off disease caused by *P. capsici*, by analysis of expression level of some defensive related gene and the change rate of oxidative enzymes.

2. Experimental
2.1. *Plant material and treatments*
Seeds of tomato (*Lycopersicon esculentum*) (Shannon) were sown into pots. Twenty-one days after sowing, tomato plants were treated (30 ml plant^{-1}) with 0.5 or 1 % dalgin. For fungicide, metalaxyl G5% (Iranshymih) was drenched at 2 g l^{-1} concentration. For treatments involving dalgin + fungicide, the plants were drenched with metalaxyl on the sixth day after inoculation. The experiments were conducted based on completely randomized block design with 10 treatments. Inoculation of tomato seedlings done with zoospore suspension of *P. capsici*. Disease severity was rated 34 days after planting [11].

2.2 *Enzyme and biochemical assays*

Peroxidase [PO] activity was assayed according to Reuveni [4]. Polyphenol oxidase [PPO] and β-1,3-glucanase activities were determined as previously described [12]. Total phenol was determined as previously described [13].

2.3. *Real-time PCR reactions*

Real-time PCR reactions were performed on the cDNA obtained from the tissues, by using Rotor-Gene 3000 (Corbett Robotics, Australia). The primers were designed by using primer3 software for the reference gene [14] and other genes of interest (Table 1).

Table 1: Primers used in the Real-time PCR studies

Name	Forward primer (5'–3')	Reverse primer (5'–3')	Amplicon length (bp)	PCR efficiency (%)	Tm (ºC)	Accession number
LOX	ATTCGGCACCGATTTCCCTC	CTCCAGCAGGGAAATACCCA	121	100	61	U37840
Chi	CAATGGACGCCATCCCCTAA	TTCGACTTTCCGCTGCAGTA	124	100	61	FJ849060
PAL	TTGAACCACCCTATTGATTTGTGC	TCTCCCTCACCTACCACACA	73	100	61	M90692
GolS	ACGAGTTCACCACATTGCAC	TCCCCAAACTTCATTTGCCA	132	100	61	AF447452
Ubiquitin	TCGTAAGGAGTGCCCTAATGCTGA	CAATCGCCTCCAGCCTTGTTGTAA	119	100	61	DQ115882

3. **Results and discussion**

In all treatment, disease severity was significantly reduced, when were compared to control (Fig. 1). The highest and lowest rate of disease severity was record for 01 % dalgin foliar spray and 0.5 % dalgin spray in alternation with metalaxyl, respectively (Fig.1). These results show that 0.5 % dalgin is more effective than 1 % dalgin for disease controlling (Fig.1). Fourteen days after inoculation, tomato control plants showed typical disease symptoms (Fig. 1). The highest levels of biomass were observed in tomato plants drenched with 0.5 % dalgin (Fig. 2).

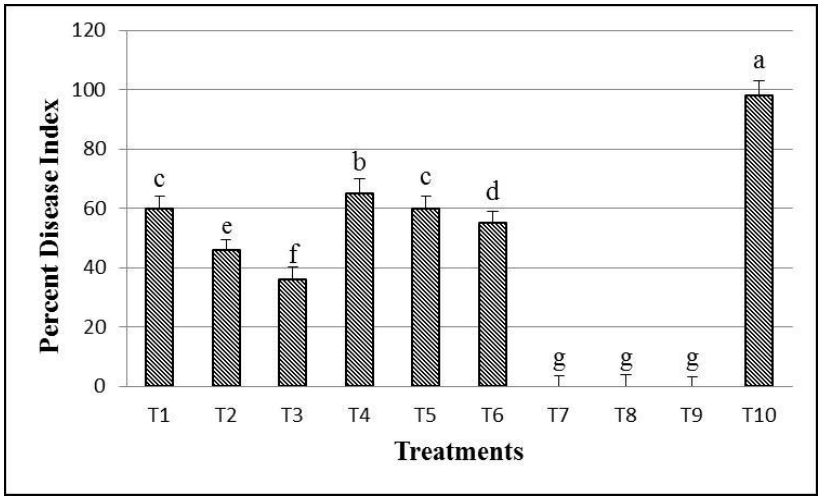

Figure 1: Effect of dalgin on the severity of *Phytophthora capsici* in tomato. Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. *Bars* indicate the standard deviations (±SD). Data are means of three replicates. Treatments: T1, 0.5% foliar spray; T2, 0.5% drench; T3, 0.5% spray + drench; T4, 1% foliar spray; T5, 1% drench; T6, 1% spray + drench with Dalgin ; T7, 0.5% Dalgin spray alternating with fungicide (metalaxyl , 2 g L^{-1}) drench; T8, 1% Dalgin spray alternating with fungicide (metalaxyl, 2 g L^{-1}) drench; T9, fungicide (metalaxyl , 2 g L^{-1}) drench control; and T10, water control. Two independent trials were conducted. Dalgin was applied 6 h before inoculation and 5 and 10 days after inoculation. Disease severity was recorded on 10 days after inoculation. For fungicide treatment (T9), metalaxyl was drenched at 6 days after inoculation.

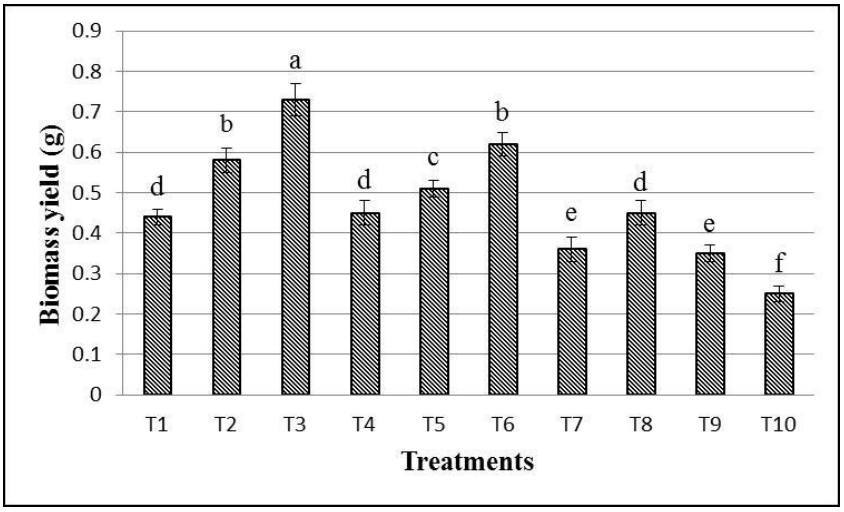

Figure 2: Effect of Dalgin and *Fusarium* inoculation in tomato on plant biomass. Mean root dry biomass as grams. Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. *Bars* indicate the standard deviations (±SD). Data are means of three replicates. Treatment and observation details as in Fig. 1

The activities of the defense enzymes PO, PPO and β-1,3-glucanase are shown in Table 2-4. The activities of the defense enzymes in treated plants with dalgin were significantly increased at 48, and 72 h after treatment. Enzyme activities were significantly decreased at 96h after treatment. So, these results suggest can confirmed key rol of these oxidative enzymes in plant resistance system. The biochemiacal analysis showed that spray + drench treatment has more effective on total phenolic production than spray/drench treatment and control. The greatest rate of total phenolic cotenant for spray + drench was recorded at 72h after treatment. Total phenolic contenant was significantly decreased 96h after treatment (Table 5).

Table 2: Peroxidase (PO) activities in tomato plants treated with Dalgin (0.5%).

Time after treatment	PO			
	control	Spray	Drench	Spray + Drench
0	60.97± 4.37n	61.33± 7.43n	61.30± 6.83n	62.03± 6.74mn
24	60.83± 7.94n	66.80± 7.94k	74.87± 5.65j	81.87± 7.66i
48	62.93± 5.85m	79.64± 8.12i	89.17± 5.59h	96.73± 9.65g
72	62.04± 9.32mn	124.64± 8.38c	139.2± 8.87b	167.0± 6.87a
96	64.80± 6.72l	105.33± 6.41f	113.03± 9.04e	118.22± 6.99d

Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. Values are means ±SD. Data are means of three replicates.

Table 3: Polyphenol oxidase (PPO) activities in tomato plants treated with Dalgin (0.5%).

Time after treatment	PPO			
	control	Spray	Drench	Spray+Drench
0	2.190± 0.50l	2.171± 0.39l	2.191± 0.25l	2.167± 0.23l
24	3.431± 0.27k	3.331± 0.44k	4.189± 0.56j	7.533± 1.41g
48	3.426± 0.41k	6.431± 0.36h	8.59± 0.69f	12.40± 1.15d
72	3.320± 0.32k	10.367± 0.31e	17.00± 1.03c	30.00± 2.54a
96	3.410± 0.40k	4.567± 0.40i	8.38± 0.59f	22.35± 1.37b

Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. Values are means ±SD. Data are means of three replicates.

Table 4: β-1,3-glucanase activities in tomato plants treated with Dalgin (0.5%).

Time after treatment	β-1,3-glucanase			
	control	Spray	Drench	Spray+Drench
0	51.30± 3.71pq	51.67± 4.95op	51.30± 5.49pq	51.13± 3.81q
24	52.03± 7.32no	57.06± 6.82l	62.08± 4.19k	75.02± 7.12j
48	52.27± 6.87n	92.09± 5.99f	104.00± 9.17c	118.0± 5.88a
72	52.20± 5.91n	83.08± 6.36i	95.00± 5.87e	109.10± 4.89b
96	53.80± 3.87m	86.33± 7.01g	85.33± 4.20h	99.39± 7.34d

Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. Values are means ±SD. Data are means of three replicates.

Table 5: Total phenol specific activity in tomato plants treated with Dalgin (0.5%).

Time after treatment	Total phenol			
	control	Spray	Drench	Spray+Drench
0	3.060± 0.39kl	3.067± 0.61kl	3.030± 0.44l	3.117± 0.57jk
24	3.140± 0.52j	3.246± 0.36i	3.319± 0.59h	4.571± 0.82c
48	3.141± 0.34j	3.542± 0.85g	3.660± 0.67f	5.033± 0.52b
72	3.116± 0.71jk	3.835± 0.48e	4.569± 0.61c	5.717± 0.94a
96	3.080± 0.42jkl	3.056± 0.52kl	3.335± 0.46h	4.022± 0.46d

Values followed by the *same letter* do not differ significantly ($P \leq 0.05$) according to the least significant difference test. Values are means ±SD. Data are means of three replicates.

Real-time PCR confirmed a multifold increase of lipoxygenase (LOX), chitinase (Chi), galactinol synthase (GolS), and phenylalanine ammonia lyase (PAL) gene expression in dalgin-treated plants. Gene expression data analysis showed that with the passage time from 0 to 48 hours after inoculation, the expression levels of Chi, and GolS transcripts (Fig. 3a, d) were increased. The Data analysis in the control plant did not show any significant increase in gene expression level. The increase in levels of PAL, and LOX, transcripts were observed on the very next day after treatment with dalgin. The transcripts of PAL, and LOX (Fig. 2 b, c) were augmented in all treatments at 24–72 h after treatment.

Disease severity was remarkably reduced in all treatment, when compared to the control plants. *Ascophyllum* extract is able to increase resistance to *P. capsici* in pepper plants [15]. In this study, oxidative enzymes and various defense-related genes showed high secretion in treated plant with dalgin. The results of this study demonstrated that increase of LOX, Chi, GolS and PAL transcript levels are related to induction of resistance. The results of previous studies have shown that expression level of LOX, Chi, GolS and PAL in were elevated in the present of inducers [16-19]. Dalgin-treated plants also showed higher contents of total phenols and various enzymes PO, PPO, and β-1,3-glucanase. The results of this study demonstrated that increase of defense-related enzyme and phenolic activity is related to induction of resistance. These results are congruent with the results of previous studies [20-21].

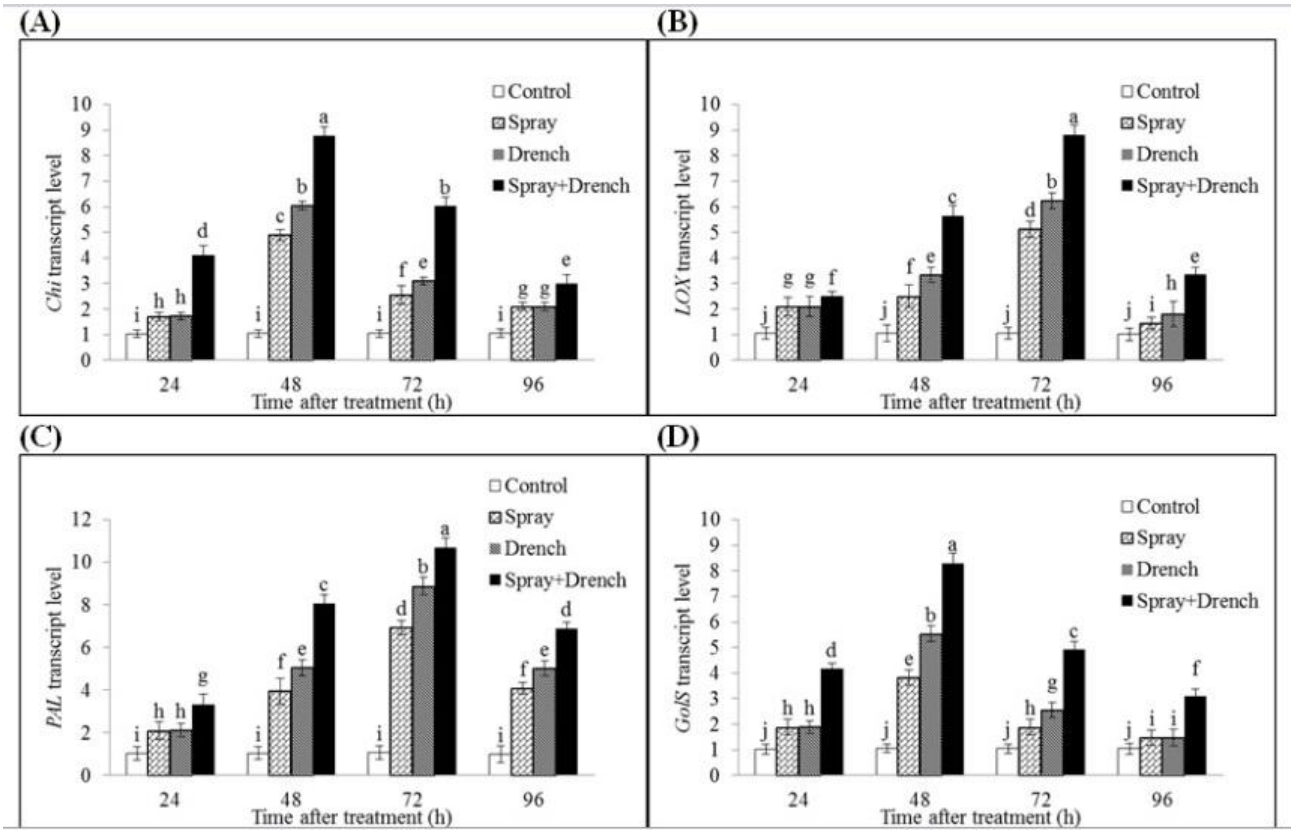

Figure 3: Real-time PCR analyses of the expression of transcripts of defense genes in Dalgin-treated tomato leaves. The defense genes were as follows:
Chi, chitinase (A); *LOX*, lipoxygenase 2 (B); *PAL*, phenylalanine ammonia lyase (C); *GolS*, galactinol synthase (D). Each value is the mean of three biological replicates and three technical replicates. *Bars* indicate the standard deviations (±SD). Data are means of three replicates. Means with *different letters* are statistically significantly different according to the analysis of variance test ($P < 0.05$).

Conclusions

According to the obtained results in this study, we conclude that use of *Ascophyllum* extract causes ISR against the *P. capsici* and could be useful as an alternatives to chemical fungicides in integrated pest management.

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