

Research Paper

The effect of the aquatic and alcoholic extracts of bipod nettle weed on the growth and some biochemical features of tomato plant under the greenhouse condition

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Introduction

In recent years, researchers are looking to decrease the use of chemical compounds by applying safe organic substances (organic farming) that are safe for human health and are compatible with the environment. Besides the noticeable beneficial effects on the yield production in agriculture, organic substances are degradable after their final effect on the treated plant (Godlewska *et al.*, 2021). With a 60 genera and more than 700 species, bipod nettle weed (*Urtica dioica* L.) is among the plants native to Iran. Bipod nettle weed (*Urtica dioica* L.) is a self propagated herbaceous plant which grows through in dim and damp regions (Bisht *et al.*, 2012). Results of the previous study indicated the prominent effect of the crude extracts prepared from different parts of this plant on the growth indices of several crop plants (Maricic *et al.*, 2021). Although there are a few studies on the effect of the plant extracts on the growth factors of the tomato plant but no research has yet been done on the effect of the Iranian bipod

nettle weed (*Urtica dioica* L.) extract as a medicinal plant on improving the growth potential of the tomato plant. In this research, we tried to explore the effects of aquatic and ethanolic extracts of the nettle weed (*Urtica dioica* L.) on some tomato growth indices as well as its biological potential. Different effects of nettle weed extract on cytokinin production were also evaluated by assessing the transcript level of the SIHK4-Cytokinin inducer gene.

Materials and Methods

Bipod nettle weed (*U. dioica*) was collected during the growing season from its natural habitat in Mazandaran province in the north of Iran and aquatic and ethanolic extracts were prepared from the aerial parts (stem and leaf) of it according to the published protocol (Leser and Treutter, 2005). The Super chef cultivar of the tomato plant was used for this research. Planting, cultivation, and treatment of the tomato seedlings were all performed under the standard conditions of the greenhouse. Tomato seedlings were distinctly treated with the 100, 300, and 500 ppm (of the pod total volume) concentrations of the aquatic and alcoholic extracts of the nettle weed at 4 to 6 leaf stages. Nettle extracts were injected directly into the pods in the rhizosphere medium of the soil. The effect of different concentrations of bipod nettle weed aquatic and alcoholic extracts on the RGR (Relative growth rate) was evaluated at 3-, 6-, 9- and 12-day post treatment according to the available protocol (Gardner *et al.*, 1985). Using the spectrophotometer (Shimadzu, UV Mini 1240, Japan), the amount of a and b chlorophylls was measured and the results were stated as milligram over gram of the wet weight of the treated samples according to the published protocol (Lichtenthaler and Buschmann, 2001). The total phenol content of tomato seedlings treated with each of the aquatic and alcoholic nettle extract was measured (Duarte *et al.*, 2008). Peroxidase (POX) and Polyphenoloxidase (PPO) enzymes specific activity was distinctly assessed by Quaiacol and Catechol substrates, respectively (Rakhshandehroo *et al.*, 2012; Chandrashekaraiah *et al.*, 2009). Specific activity for each of the enzymes was assessed based on the absorbance changes in the refraction in minute per microgram of the total protein. The transcript level of the SIHK4-Cytokinin inducer gene was also assessed by semi-quantitative RT-PCR (RT-sqPCR) for the tomato seedlings treated with the aquatic and ethanolic nettle extracts (Antiabong *et al.*, 2016). In this study, the values of standard deviation (\pm SD) for the treated samples were counted on the basis of the intensity of PCR products of the SIHK4-Cytokinin inducer gene over the intensity of 18S rRNA (as for internal control) in agarose gel which was measured by Image J software. Results stated as the value of one for each of the tested samples with three replications. Each test in this study was

done with three replications with at least five samples for each treatment. The treatment mean values were compared with the complete random design (DRC) at the 1% level.

Results and discussion

On the basis of the obtained results in this study, aqueous and ethanolic extracts of nettle weed significantly increased the relative growth rate in treated tomato seedlings. Aquatic and ethanolic extracts at 500 ppm and just aquatic extract at 300 ppm concentration induced the highest growth level and approximately two-fold compared to the non-treated negative control tomato 14 days after treatment. Treatment of tomato seedlings with the 300 ppm and 500 ppm concentrations of aqueous and ethanolic extracts of nettle significantly increased the chlorophyll a and b contents in comparison to the negative control tomato plant. In this regard, the best effect was obtained by applying the 500 ppm of aqueous and ethanolic extract which was about 3.5-fold in treated samples compared to the control. Treating the tomato seedlings with 300 ppm and 500 ppm concentrations of aquatic and ethanolic extracts resulted in a significant increase in total phenol content of the treated samples which was 2.5-fold more than the negative control at 6-, 9- and 14-day post treatment, respectively. Results also indicated a significant increase in the POX specific activity by treating the tomato seedlings with 300 ppm and 500 ppm concentrations of aquatic and ethanolic extracts compared to the negative control at 6, 9, and 14 days post treatment. Although PPO specific activity was increased by applying both extracts at the determined concentrations, the highest increase was observed by applying the 300 ppm and 500 ppm concentrations of aquatic nettle extract with 3.5- and 4-fold increase compared to the negative control, respectively 14 days post treatment. In this study, the transcript level of the SIHK4-Cytokinin inducer gene was also assessed as a criterion of growth regulator production in tomato seedlings treated with the nettle extracts. Treating the tomato seedlings with 300 ppm and 500 ppm concentrations of aquatic and ethanolic extracts also resulted in a significant increase in the expression of this gene in comparison to the control plant 14 days post treatment. Results of the previous research showed that the increased level of cytokinin as a growth regulator may increase the chlorophyll and phenol contents in the tissue of the plants and may inhibit the oxidative stress that is responsible for the death of the tissues (Honig et al., 2018). In line with this, the results of the present study also showed that as much as the cytokinin level increased the amount of the chlorophylls a, b, and total phenol increased in the tomato tissues treated with aquatic and alcoholic extracts in which the highest level of incensement was observed by applying the 500 ppm of nettle aquatic extract. Hence, we can suggest that aqueous and alcoholic extracts of nettle weed might have increased the

tomato metabolism and growth vigor by improving its biological potency by means of inducing the genetic expression system that led to the increased level of cytokinin and induction of phenolic compounds biosynthesis. Previous research revealed that aquatic and alcoholic extracts of the bipod nettle weed leaf contain phenolic compounds such as Quercetin and Fumaric acid (Ogles and Yalcin, 2012). The positive effects of several phenolic compounds on different crop plants have been elucidated before (Ertani *et al.*, 2016; Makoi and Ndakideimi, 2007; Sulusoglu, 2014). The effect on the induction of growth in treated tomato seedlings may be related to the presence of phenolic compounds in the aqueous and ethanolic extracts of the bipod nettle weed extracts.

Conclusion

Here, we explored the effect of different concentrations of aquatic and ethanolic Iranian bipod nettle weed extracts on the biological and growth features of the tomato seedlings to offer a suitable growth regulator that can be used by the farmers who produce greenhouse crops. Totally, the 500 ppm concentration of aquatic and ethanolic extracts of nettle weed enhanced the growth of treated tomato seedlings by increasing the tomato metabolism. The observed positive effect of nettle weed alcoholic and aquatic extracts on enhancing the tomato growth features can be linked to their effects on the antioxidant activities, phenolic compound and cytokinin accumulation in treated plants.

Keywords: *Gene expression, Nettle, Peroxidase enzyme, Polyphenol oxidase enzyme, Total phenol*

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