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Response of maize (*Zea mays* L. *saccharata* Sturt) to different concentration treatments of deltamethrin

Ragbet Ezgi Duran *, Semra Kilic, Yasemin Coskun

Faculty of Arts and Sciences, Biology Department, Suleyman Demirel University, Isparta 32 260, Turkey

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ABSTRACT

The aim of this study was to investigate the effect of the deltamethrin pesticide on the biological properties of maize (*Zea mays* L. *saccharata* Sturt). Maize seeds were exposed to environmentally relevant dosages (0.01, 0.05, 0.1 and 0.5 ppm) of deltamethrin. On the 7th day of germination, morphological, anatomical and physiological responses were determined. All seedling growth characters were decreased with increasing deltamethrin levels. The most negative effect on the radicle length of maize was observed by the highest deltamethrin concentration with a 61% decrease ($P < 0.05$). Both stomatal density and stomatal dimension reduction were caused by increasing concentrations of deltamethrin. Moreover, the pigments like chlorophyll a, chlorophyll b, total chlorophyll and caretonoids decreased with the increase in deltamethrin concentration. Conversely, anthocyanin and proline content increased in parallel with deltamethrin concentration. As a result, all morphological traits and pigments except for proline and anthocyanin were significantly reduced with an increase in pesticide concentration, compared to control ($P < 0.05$).

1. Introduction

Pesticides are extensively used to increase agricultural production by preventing losses due to pests [1]. Deltamethrin [(S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] is one of the important pyrethroid pesticides that are widely used for crop protection and to increase harvest productivity and has become very popular with pest control operators. Synthetic pyrethroid insecticides are potent insecticides that kill insects through dermal contact, and are used against a wide range of pests. Synthetic pyrethroids are widely used as the broad-spectrum pest control agents in agricultural production because of their selective insecticidal activity, rapid biotransformation and excretion by the mammalian catabolic system and non-persistence in the environment [2]. Moreover, the pyrethroid insecticides have greater photostability and a relatively low toxicity when compared to organochlorine and organophosphorus insecticides [3,4]. However, there is a risk of pesticide residues being present in the consumed food, due to overuse and accumulation in the food chain.

Pyrethroids are similar to pyrethrins which are present in the flowers of *Chrysanthemum cinerariaefolium*. Since the 1970s, pyrethroid pesticides have been ranked among important insecticides used to control insect pests. However, chemicals that are used to improve yield or to protect plants from the harmful effects of irrigation and drainage systems can contaminate and may adversely affect the biotic and abiotic components of water [5]. Pyrethroid pesticides are highly toxic and considered as a potential risk to both human health [6] and aquatic organisms [7–9], particularly fish. Even small amounts of contamination in the aquatic environment by deltamethrin result in adverse effects on freshwater aquatic life. In addition, this substance could affect the fertility of organisms exposed to it [10]. The antioxidative response of a popular pulse crop species (*Glycine max* (L.) Merr.) during pre-flowering, flowering and post-flowering has a strong correlation with increasing deltamethrin concentrations except for catalase [11].

As pyrethroids are widely used as insecticides, they are present in the environment in considerable amounts [12]. However, the effects of insecticides, particularly deltamethrin, on seed germination, and anatomical (stomata) and physiological properties (chlorophyll a and b, carotenoid, proline) in maize are yet to be investigated. Therefore, in the present study we tried to evaluate the toxicity of deltamethrin in maize seedlings.

2. Materials and methods

2.1. Plant material and plantation

Uniform-sized seeds (n = 25) of a commercial variety of maize (*Zea mays* L. *saccharata* Sturt) were used as the model plant. In the present study, Decis EC2.5 insecticide, whose active substance is

* Corresponding author. Suleyman Demirel University, Biology Department, Faculty of Arts and Sciences, Isparta 32 260 C, Turkey. Fax: +902462371106.
E-mail address: ezgiduran@sdu.edu.tr (R.E. Duran).

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deltamethrin, was used. The Decis was obtained from Bayer. Other chemicals were purchased from Sigma (USA) and Fluka AG (Buchs, Switzerland). Stock solution of deltamethrin was prepared as 2.5 g/ml in 100 ml as per the instructions of the manufacturer. Treatment concentrations were prepared from this solution as 0.01, 0.05, 0.1, 0.5 ppm and 0.0 ppm for the control group.

Seeds of healthy maize plants were surface sterilized with 0.5% sodium hypochloride for 10 min, followed by extensive washing with sterile distilled water. They were placed into a clean 50 ml glass beaker and pre-treated for 72 hours with four different deltamethrin concentrations.

At the end of the treatment, seeds were sown in 12 cm petri dishes lined with two layers of filter paper (Whatman 1) that were moistened with 10 ml of distilled water, and then placed in an incubator at 25 °C for 7 days. Three replicates for each group including the control were performed for different concentrations of deltamethrin. Seeds were considered to be germinated with the emergence of the radicle. At the end of the 7th day, the radicle and coleoptile lengths of seedlings were measured with a millimetric ruler, and germination percentages were calculated according to Khan and Ungar [13].

The seedlings of each application were placed in 4-L pots containing perlite with Hoagland's nutrient solution. Plants were cultivated in a climate chamber under controlled conditions (photoperiod 12-h, temperature 25 ± 2 °C, relative humidity 60 ± 5%, light intensity 160 µmol/m^{-2}/s^{-1}) for 45 days. New nutrient solution was added on a regular basis during seedling growth. Epidermal tissue was stripped from the adaxial and abaxial surfaces of leaf lamina pieces to determine leaf stomatal density, expressed as the number of stomata per unit leaf area, mounted on a glass slide, immediately covered with a coverslip, and then lightly pressured with fine-point tweezers. For each independent measurement, numbers of stomata (s) and epidermal cells (e) for each film strip were counted from both the adaxial and abaxial surfaces in a 0.04 mm^2 unit area. The leaf stomatal index was calculated using the formula $[s/(e+s)] \times 100$, as defined by Meidner and Mansfield [14]. Stomata magnitudes (length and width) were defined in µm using an ocular micrometer under light microscope (40× object and 10× ocular).

2.2. Pigment determination

For Chlorophyll a (Chl a), Chlorophyll b (Chl b), carotenoids and anthocyanin measurements, the tissue samples (1.0 cm^2 in most cases) were ground in 2 ml cold acetone/Tris buffer solution (80:20 vol:vol, pH = 7.8), centrifuged to remove particulates, and the supernatant diluted to a final volume of 6 ml with additional acetone/Tris buffer. The absorbance of the extract solutions was measured with the spectrometer using an external cuvette holder and the pigment contents were determined with the formula below [15].

$$\text{Anthocyanin}\ (\mu\text{mol ml}^{-1}) = 0.08173\,A_{537} - 0.00697\,A_{647} - 0.002228\,A_{663}$$

$$\text{Chl } \underline{\mathbf{a}}\ (\mu\text{mol ml}^{-1}) = 0.01373\,A_{663} - 0.000897\,A_{537} - 0.003046\,A_{647}$$

$$\text{Chl } \underline{\mathbf{b}}\ (\mu\text{mol ml}^{-1}) = 0.02405\,A_{647} - 0.004305\,A_{537} - 0.005507\,A_{663}$$

$$\text{Carotenoids}\ (\mu\text{mol ml}^{-1}) = (A_{470} - (17.1 \times (\text{Chl } \underline{\mathbf{a}} + \text{Chl } \underline{\mathbf{b}}) - 9.479 \times \text{anthocyanin}))/119.26$$

2.3. Proline determination

The proline content was determined using the method of Bates et al. [16]. Proline was extracted from leaf samples of 100 mg FW with 2 ml of 40% methanol. 1 ml extract was mixed with 1 ml of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3:2, v/v) and 25 mg ninhydrin. After 1 h incubation at 100 °C, the tubes were cooled and 5 ml toluene was added. The absorbance of the upper phase was spectrophotometrically determined at 528 nm and total proline amount was calculated with the help of a standard curve.

2.4. Statistical analysis

The statistical analysis was carried out by one-way classification of ANOVA to describe the growth parameters reported in the present paper and to calculate statistical significance. Duncan's Multiple Range Test was applied for means (±SD) with at least two independent assays with three replicates using SPSS Software 13.0. Graphs for all experimental data were constructed to determine whether the mean values between different treatment concentrations and also the control group held a significant difference. The statistical significance level was taken as $P < 0.05$.

3. Results

3.1. Effects on seed germination and seedling growth parameters

Effects of different concentrations of deltamethrin on seed germination and seedling growth after a 72 hour application compared to the control group are shown in Fig. 1. Germination rate was decreased by 2%, 16%, 48% and 92% at 0.01, 0.05, 0.1 and 0.5 ppm concentrations of deltamethrin, respectively. The most destructive effect of deltamethrin on germination was at the highest concentration (0.5 ppm) with 92% decrease ($P < 0.05$). Radicle length decreased in parallel with increasing deltamethrin concentrations, compared to control. This decrease was 8%, 26%, 50% and 98% at 0.01, 0.05, 0.1 and 0.5 ppm, respectively. The most destructive effect on radicle length was a 61% decrease at the 0.1 ppm–0.5 ppm deltamethrin concentrations ($P < 0.05$). Increasing concentrations of deltamethrin affected coleoptile percentage and radicle count in the same direction with other parameters. Moreover, coleoptile percentage and radicle count were determined as 74% and 66% at 0.5 ppm deltamethrin, respectively ($P < 0.05$).

3.2. Effects on anatomical parameters

To determine the effects of deltamethrin on leaf parameters; stomata count, epidermis count and stomata sizes (width/length) of maize seeds pre-treated with different concentrations of deltamethrin for 96 hours and grown in perlite medium were

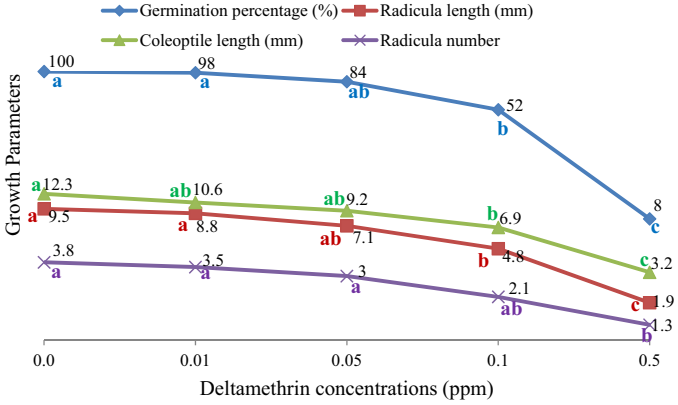

Fig. 1. Effects of different concentrations of deltamethrin on germination and seedling growth. Values that are followed by the same letter do not differ statistically at a significance level of 0.05.