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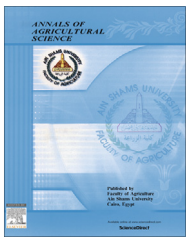

Phytotoxicity of *Euphorbia helioscopia* L. on *Triticum aestivum* L. and *Pisum sativum* L.

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Abstract Invasive field weeds, such as *Euphorbia* sp., represent main threat for crop productivity. The present work was conducted to evaluate the phytotoxicity of *Euphorbia helioscopia* on wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.). The influence of aqueous extract from shoot of *E. helioscopia*, at different rates (1.0%, 2.5%, 5.0%, and 10.0%; w/v), on germination and early seedling growth of wheat and pea as well as some of the synchronized physiological aspects was investigated. *E. helioscopia* aqueous extract severely affected the germination in a concentration dependent manner. Plumule and radicle length, as well as their fresh and dry masses were markedly reduced. Moreover, amylase activity and total soluble sugars were significantly reduced in response to treatment with aqueous *Euphorbia* extract, in both test plants, whereas, proteolytic activity showed marked improvement. The linear regression analysis revealed the presence of positive linear correlation between germination rate and amylase activity. The stress markers such as proline, phenolics and flavonoids were markedly accumulated upon treatment with *E. helioscopia* extract. The increment in the level of total phenolics was concomitant with an improvement in phenylalanine ammonia lyase and peroxidase activities. High performance liquid chromatography analysis of *E. helioscopia* extract revealed the presence of two cinnamic acid derivatives (caffeic and *p*-coumaric acids), two benzoic acid derivatives (vanillic and syringic acids) and a flavanonol (Dihydroquercetin). To our knowledge, this is the first study to investigate the physiological implications underlying the phytotoxicity of *E. helioscopia*.

Introduction

Plants are essential elements of the earth ecosystem. In nature, plants grow next to each other forming the different communities of the plant kingdom. Nature, in turn, affects the plant growth and development by different types of stresses that could limit the agricultural productivity (AL-Wakeel et al., 2013; Saleh and Madany, 2015). Duration, severity and rate of the imposed stress are the factors underlying the plant response to stress (Omezzine et al., 2014). Within their communities, plants, including weeds, compete together for space, moisture, nutrients and light. They can also interact, chemically, by releasing secondary metabolites

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(allelochemicals) into the growth environment, a phenomenon called allelopathy (Einhellig, 1996; Tanveer and Rehman, 2010; Harun et al., 2014). Allelochemicals can be beneficial or detrimental to the receptor plants (Omezzine et al., 2014), imposing their effect on cellular, molecular and physiological levels (Barkosky et al., 2000; Batish et al., 2006). The novel weapon hypothesis suggests that allelopathy is one of the effective tools that allow plants to invade and establish in new ecosystems and eventually determines the building and composition of the invaded plant community (Callaway and Ridenour, 2004).

Wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) are of the most ancient crops known to man. They are among the most important food crops, representing a significant percentage of the world trade. This economic importance made scientists devote considerable attention for these crops in their research (Al-Wakeel et al., 2007; Nigro et al., 2014). Despite their economic values, many threats are facing these important crops (Saleh and Madany, 2015). One of these threats is the invasive field weed, such as *Euphorbia* sp., (de Albuquerque, 2010; Pudeko et al., 2014). Several species of *Euphorbia*, the largest genus in the family "Euphorbiaceae" have attracted much attention for their antimicrobial, antiviral, antitumor, cytotoxic, pesticidal and phytotoxic activities (for review see Mohamed et al., 2012). *Euphorbia helioscopia* (Sun Spurge) is native to most of Europe, northern Africa, and east of Asia (Blamey and Grey-Wilson, 1989). It is a common weed in Egypt that emerges from February to April and invades many important winter crops and vegetables, such as wheat, chickpea, faba bean and pea (Mahmoud, 1996; Boulos, 2000).

The influence of different weeds on wheat and pea growth has been extensively investigated (Wisler and Norris, 2005; Tanveer and Rehman, 2010). However, studies about the potential of *E. helioscopia* upon growth and biochemical aspects of these crops are rare and a lot of additional information is required to stand on the impact of this weed upon its community, specifically wheat and pea. The present work was, therefore, undertaken mainly to study the influence of the water extract of the aerial parts of *E. helioscopia* on wheat and pea germination and seedling growth as well as some of the underlying physiological parameters.

Materials and methods

Plant material

Fresh shoots of *E. helioscopia* were collected from its natural habitats, in the field of the Faculty of Agriculture Experimental Station, Giza, Egypt, during vegetative stage. The aerial shoots of *E. helioscopia* were air-dried then ground to fine uniform texture and stored in glass jars until use. Seeds of wheat (*T. aestivum* L.*)* (Sakha 93) and pea (*P. sativum* L.) (Lincoln) were kindly obtained from the Department of Vegetables, Agriculture Research Center and used throughout the experiment.

Preparation of extract

Stock aqueous extract of *E. helioscopia* was prepared by soaking 20 g air-dried shoot in 200 mL distilled water (10%; w/v) at room temperature (27 ± 2 °C) for 24 h with occasional shaking. The mixture was filtered twice through Whatman No. 3 filter paper. The filtrate was centrifuged at 3000*g* for 20 min to remove the particulate material. Different concentrations (1.0%, 2.5%, 5.0%; w/v) were prepared from the stock solution in addition to the control (distilled water). The water extracts were individually bottled, labeled and stored at −20 °C until use.

Germination experiment

For germination experiment, 10 seeds of both wheat (*T. aestivum* L.) and pea (*P. sativum* L.) were surface sterilized using 0.1% (w/v) $HgCl_2$, rinsed several times with distilled water. The sterilized seeds were divided into five groups and separately soaked for 24 h in either water (control) or one of the different extract concentrations (1.0%, 2.5%, 5.0% and 10%). The primed seeds of each group were arranged in 3 Petri-dishes, 15 cm diameter, lined with two disks of Whatman No. 1 filter paper saturated with 10 ml of the same concentration. The Petri-dishes were incubated under controlled conditions, with a day/night temperature of 25 ± 2 °C. During the experimental period, the Petri-dishes were observed daily and extracts/distilled water were added when needed. Ten days after incubation, the germination percentage, plumule and radicle length, as well as fresh and dry weights were recorded. Dried samples were ground to fine powder and kept under dry conditions. Other fresh samples were quickly frozen and kept at −20 °C until used for enzyme assay.

Amylase and protease assays

Protease was extracted by homogenizing 10-day-old wheat and pea seedlings in 20 mM phosphate buffer, pH 7.6, with a pre-chilled pestle and mortar. For amylase extraction, 100 mM acetate buffer, pH 6.0, was used instead of the phosphate buffer. Proteolytic activity was assayed using bovine serum albumin (BSA) as substrate. The reaction mixture contained 0.5 ml of the crude extract and 2 ml of the substrate solution (20 mM phosphate buffer, pH 7.0, containing 10 mg/ml BSA). After 60 min of incubation at 40 °C, the reaction was stopped by adding 2.0 ml of 10% trichloroacetic acid and heating briefly in boiling water to precipitate undigested albumin. After centrifugation, the concentration of the resulted soluble peptides was measured by the modified Folin-Lowry method adopted by Hartree, (1972). Amylase activity was measured by mixing 0.5 ml of the crude extract with 0.5 ml of 0.5% soluble starch prepared in 0.1 M of acetate buffer, pH 6.0, containing 5 mM $CaCl_2$. The reaction was terminated by $HgCl_2$ after 30 min of incubation at 40 °C. The resulting reducing sugars were estimated by the Nelson's method (Clark and Switzer, 1977).

Extraction and estimation of total soluble sugars

Water-soluble reducing sugars were extracted according to the method outlined by Radwan et al. (2007). A known weight of oven dry powdered tissues was boiled in 10 ml of distilled water for 1 h on a water-bath. After centrifugation, the supernatant was made up to a known volume with distilled water. Reducing value of each extract was determined according to