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Garlic extract as a novel strategy to hasten dormancy release in buds of 'Anna' apple trees

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ABSTRACT

A 2-season orchard trial was carried out to verify the effects of garlic extract (GE), as a novel dormancy-breaking substance, at different concentrations; 0, 50, 100, 150 and 200 ml l^{-1} on bud break dormancy and the metabolic alterations in buds of 'Anna' apple trees. Water content, total carbohydrates, reducing and total sugars, anthocyanins, total free amino acids, free proline, total indoles and free phenols were analyzed after the application of GE. The obtained results showed that, GE treatments hastened date of floral bud break and increased percentage of bud break, fruit set, total number of fruits and fruit yield per tree. Except for free phenols, GE also increased the abovementioned chemical components in buds. The best results were obtained from the treatment of 150 ml l^{-1} GE. Therefore, it is concluded that the use of GE at 150 ml l^{-1} could be recommended for improving the bud break, growth and yield of apple trees cv. "Anna".

1. Introduction

The term dormancy, as applied to plants, implies that growth is arrested and the plant enters in a state which the bud meristem and/or the vascular cambium are at rest. Dormancy is an adaptive mechanism that enables woody plants to survive to the freezing temperatures of winter. This complex process is characterized by the cessation of meristem activity, which is accompanied by winter bud set, extensive metabolic remodeling, hardiness to low temperatures and; in deciduous trees, by leaf senescence and abscission.

The induction of dormancy occurs in response to seasonal environmental signals. In most woody plants, shortening of the photoperiod induces growth cessation, bud set, and in some degree, cold acclimation. The subsequent drop in temperature then leads to a greater tolerance to cold and leaf fall (Allona et al., 2010).

Uses of bio-extracts containing beneficial micro- and macro-elements in spite of active substances such as volatile compounds, instead of synthetic chemicals, are known to improve plant growth through the supply of plant nutrients and may help to sustain environmental health and soil productivity. In addition, using breaking synthetic chemicals (Seif El-Yazal and Rady, 2012) demonstrated to be high cost and cause an environmental contamination. Therefore, it has been focused attention on foliar application of bio-extracts (Rady and Seif El-Yazal, 2013) such as onion and garlic extracts.

On the other hand, Vargas-Arispuro et al. (2008) reported that different products derived from garlic (*Allium sativum* L.) were obtained and evaluated as bud break agent in table grapes (*Vitis vinifera* L.). The isolated compounds were chemically identified and included allicin, diallyl disulfide, diallyl trisulfide, 3-vynil-[4H]-1,2-ditiin and 2-vynil-[3H]-1,3-ditiin, S-methyl cysteine sulfoxide, dimethyl disulfide, dimethyl trisulfide and dimethyl thiosulfonate. The volatile compounds from S-methyl cysteine sulfoxide promoted 100% of bud break from all cultivars. The compounds from garlic that stimulated bud break in grapevines in this work include sulfur in their molecules; therefore it is assumed that sulfur could play a key role in breaking bud dormancy of grape cultivars evaluated in this study.

Many deciduous, perennial fruit crops require winter chilling for adequate bud break and flowering. Recent research has shown that changes in sugar and amino acid profiles are associated with the release of buds from dormancy (Judd et al., 2010). The beneficial effect of garlic extract on bud break, growth, yield and some chemical constituents of different fruit species was studied by several authors (Botelho et al., 2007; Botelho and Muller, 2007a,b; Vargas-Arispuro et al., 2008; Botelho et al., 2009, 2010; Biasi et al., 2010; Abd El-Rzek et al., 2011).

Accordingly, the work herein was planned to study the effect of exogenous application of garlic extract at different rates; 0, 50, 100, 150 and 200 ml l^{-1} on date and percentage of floral bud break and metabolic

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changes in some chemical components in "Anna" apple buds during different stages of dormancy and dormancy release.

2. Materials and methods

2.1. Tree selection, garlic extract preparation and their applications

Ten-year-old trees of "Anna" apple variety (*Malus sylvestris*, Mill.) grafted on Malling-Merton 106 (MM 106) rootstock and grown on loamy sand soil were randomly selected for this research study in the 2006/2007 and 2007/2008 seasons. In an attempt to reach break dormancy at the appropriate time, trees grown in the Horticultural Station of the Faculty of Agriculture, Fayoum University, Fayoum, Egypt were selected ($n = 6$ for each treatment) and labeled in November and received foliar treatments during December 2006 and 2007, and were then sampled from 8 January to 3 March 2007 and 2008. The experiment was repeated exactly in the two studied seasons with 30 trees in each. Trees of each experiment were arranged in a complete, randomized design. Each tree was designed as one replicate, and each treatment included six trees.

Egyptian white garlic was harvested in April and cloves were cold-stored at −3 °C until mid-December. Samples of cloves were ground using mortar and pestle and the active ingredients were extracted by ethyl alcohol (95%). The garlic ethanol mixture was filtered and the alcohol was removed by evaporation under vacuum (30 °C) using rotary evaporator, Buchi model 011. The produced paste was termed as garlic extract which was kept cool in refrigerator (4 °C) until use. Garlic extract was diluted in water to give the final concentrations required (50, 100, 150 and 200 ml garlic extract l^{-1} tap water) directly before use. A surfactant super film at 0.1% was added to the spraying solution. The trees were sprayed using a back gum sprayer to the spur surface until well wetted. All the agricultural and horticultural practices were carried out as usual.

Four rates; treatments of garlic extract (50, 100, 150 and 200 ml l^{-1}) and tap water, as a control, were applied. Garlic extracts were sprayed at two equal doses; the first was applied on 24 December and the second was applied 7 days later with an extract volume of 4 l $tree^{-1}$ in both seasons.

2.2. Morphological characteristics and yield measurements

In both main studied seasons, bud counts were made for each tree ($n = 6$) in all treatments. The dates on which floral and vegetative buds started to open were recorded. Numbers of buds were counted when all the buds had opened, and the percentages of buds opened were estimated. The dates on which flowering reached 25, 50, 75 and 100% of the total final number of flowers were estimated in each treatment. Flowers in which the calyx began to extend were tagged, in order to measure the percentage of fruit-set. At harvest, all the apple fruits were harvested from each tree, counted, and weighed.

2.3. Preparation of bud samples for chemical analyses

Bud samples were collected at 9-day intervals beginning from 8 January up to 3 March from each replicate of each treatment to determine the seasonal changes in bud contents of total carbohydrates, reducing and total sugars, anthocyanin, total free amino acids, free proline, total indoles and free phenols. Samples of floral buds were randomly taken and immediately transported to the laboratory for the aforementioned determinations.

2.4. Total carbohydrate determination

Total carbohydrate was extracted from apple buds by placing 10 mg dry sample with 10 ml of H_2SO_4 (0.1 N) in test tube on a boiling water bath for 30 min. The samples were filtered to remove the insoluble material and the solution was completed to 100 ml by distilled water. Carbohydrate contents (mg g^{-1} dry matter) were determined by phenol–sulfuric acid method (Rao and Pattabiraman, 1989). Fifty microliters of 80% phenol and 3 ml of 98% sulfuric acid were added to 1 ml of sugar solution. The mixture was kept at room temperature for 30 min and then the absorbance read at 490 nm.

2.5. Sugar determination

Reducing and total sugars were determined (mg g^{-1} fresh buds) using phosphomolybdic acid reagent (A.O.A.C., 1995). Sample (0.5 g) of frozen buds was crushed in a porcelain mortar and extracted with 50 ml of 80% (v/v) boiling ethanol for 5 min. The sample was filtered to remove the insoluble material. Protein was precipitated by adding 1 ml of ethanol extract with 3 ml of basic lead acetate (137 g l^{-1}) and the excess was precipitated with a solution of 1 M sodium phosphate monobasic. The mixture was centrifuged and the volume of the supernatant was completed to 10 ml.

For determining the reducing sugars, 1 ml of the filtrate was mixed with 1 ml of copper sulfate and 1 ml of alkaline tartarate solution, then the mixture was heated in boiling water bath for 10 min. After cooling, 2 ml of phosphomolybdic acid reagent and 125 ml of phosphoric acid were added. The mixture volume was completed to 500 ml with distilled water and the absorbance was measured at 540 nm.

For determining the total sugars, 1 ml of the filtrate was mixed with 1 ml of 1 N HCl then the solution was neutralized with sodium bicarbonate (1 N), and the volume was completed to 5 ml. The total sugars were determined as described in the method of reducing sugar determination (A.O.A.C., 1995).

2.6. Anthocyanin determination

Total anthocyanin content was analyzed as described by Mancinelli (1984) with some modifications (Horbowicz et al., 2008, 2009). Bud extracts were prepared with 1% HCl–MeOH for 24 h at room temperature, in darkness with occasional shaking. The extracts were carefully decanted and their absorbance was measured at 530 and 657 nm. The formula ($A_{530} - 0.25A_{657}$) was used to compensate for the absorption of chlorophyll degradation products (Mancinelli, 1984). Anthocyanin content was expressed as mg of cyanidin-3-glucoside in 100 g of dry matter, using 29,600 as molecular extinction coefficient.

2.7. Total free amino acid determination

Total free amino acids were determined by reaction with ninhydrin using glycine as a standard (Jayarman, 1981) with some modifications (Chen et al., 2009). Sample (0.5 g) of frozen buds was extracted with 50 ml of 80% (v/v) ethanol. The sample was filtered to remove the insoluble material. In a 25-ml volumetric flask, 1.0 ml of ethanol extract was added. Then, 0.5 ml of 1/15 mol/l phosphate buffer solution (pH 8.04) and 0.5 ml of 2% ninhydrin solution containing 0.8 mg ml^{-1} of $SnCl_2 \cdot 2H_2O$ were also added. The mixture in the volumetric flask was then placed on a boiling water bath for 15 min. The probes were quickly cooled with cold water, and adjusted to 25 ml with water. After they were left to stand still for 10 min, the absorbance values were measured against a reagent blank at 550 nm.

2.8. Free proline determination

Free proline was determined according to the method of Bates et al. (1973) slightly modified by Ennajeh et al. (2006). Sample (0.2 g) of frozen bud was extracted with 5 ml of 40% (v/v) methanol heated to 80 °C for 30 min in hermetically sealed tubes. The supernatant (1 ml) was mixed in a test tube with 2 ml glacial acetic acid, 1 ml ninhydrin solution (25 mg ml^{-1}) and 2 ml of a mixture consisting of 24% (v/v) distilled water, 60% (v/v) glacial acetic acid and 16% (v/v) orthophosphoric