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Changes in Metabolic Processes during Break Dormancy in Apple Buds under Foliar-Applied Garlic Extract

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Abstract

The effect of garlic extract at different concentrations for reaching early break dormancy in buds of "Anna" apple (*Malus sylvestris*, Mill) trees and their effects on metabolic changes in the content of shoots from (water content %, total carbohydrates, total sugars, reducing sugars, anthocyanine, total free amino acids, free proline, total indoles and free phenoles) during their release from dormancy were investigated. The trees were grown in loamy sand soil, and sprayed with five treatments; 0, 5, 10, 15 and 20% garlic extract. Generally, it was found that all studied flowering parameters; date of flower buds break, percentage of bud's break, fruit set, total number of fruits/tree, yield/tree (kg) and seasonal changes in some chemical constituents of shoots; water content %, total carbohydrates, total sugars, reducing sugars, anthocyanine, total free amino acids, free proline and total indoles were increased with the application of different treatments. The best results were obtained from the treatments of garlic extract at 15 % flowed by 20 %. On the contrary, the same treatments decreased free phenols in shoots as compared to the control. It could be recommended to use garlic extract at the rate of 15 % for improving bud break, growth, yield and chemical constituents of apple shoots.

Keywords: Apple (*Malus sylvestris*, Mill); Dormancy; Garlic extract; Bud break; Flowering; Yield; Chemical constituents.

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1. Introduction

The term dormancy, as applied to plants, implies that growth is arrested and the plant enters a state in which the meristems of buds and/or the vascular cambium are at rest, dormancy is an adaptive mechanism that enables woody plants to survive 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, an acquired high tolerance to cold 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 some degree of 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, the high cost of dormancy breaking agent (chemicals) and their environmental pollution have been focused attention on foliar application of bio-extracts such as garlic extract.

On the other hand**,** Vargas-Arispuro et al. (2008) reported that different products derived from garlic (*Allium sativum* L.) were obtained and evaluated as stimulate budbreak of "Table grape" (*Vitis vinifera* L.). The isolated compounds were chemically identified and include allicin, diallyl disulfide, diallyl trisulfide, 3-vynil-[4H]1.2- ditiin and 2-vynil-[3H]-1.3-ditiin, S-methyl cysteine sulphoxide, dimethyl disulfide, dimethyl trisulfide and dimethyl thiosulfonate. Cuttings with six buds were used to evaluate the compounds. All evaluated compounds promoted budbreak in the cuttings of grapes. The volatile compounds from S-methyl cysteine sulfoxide promoted 100% of budbreak of cultivars. The compounds from garlic that stimulated budbreak in grapevines in this work include sulphur in their molecule; therefore, we propose that sulphur 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 budbreak 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 were studied by several workers **(**Botelho et al., 2007; Botelho and Muller, 2007a & b; Vargas-Arispuro et al., 2008; Botelho et al., 2009; Ahmed et al., 2009; Botelho et al., 2010; Eshghil et al., 2010; Corrales-Maldonado et al., 2010; Biasia et al., 2010; Abd El-Rzek et al., 2011).

Accordingly, the present work was planned to study the effect of exogenous application of garlic extract at different rates on flowering and yield, as well as some chemical constituents of apple trees during different stages of growth at both 2007 and 2008 seasons.

2. Materials and methods

2.1. Growth conditions and treatments

This study was carried out during the two successive seasons of 2014 and 2015 in the orchard of the Horticulture Farme of Faculty of Agriculture, Fayoum University in an attempt to break dormancy of "Anna" apple variety (*Malus sylvestris,* Mill) grafted on Malling-Merton 106 (MM 106) rootstock. The trees were 10 years old when experiment started and grown in loamy sand soil. Trees were selected in November, 2013 a uniform as possible for spray treatments.

Garlic extract was applied in five concentrations 0.0% (water), 5%, 10%, 15% and 20% in water using garlic extract. Each tree received (4) liter of the assigned solution. Garlic extracts were sprayed at two equal doses, the first was sprayed before the end of dormancy (nearly 24th of December), and the second was applied one week later with a volume of 4 L/tree for each one in both seasons.

Each treatment was replicated on four individual (tree) and receiving only one of the following treatments:

1- Control

2- Garlic extract at 5%

3- Garlic extract at 10%

4- Garlic extract at 15%

5- Garlic extract at 20%

2.2. Preparation of garlic extracts

Garlic samples 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 evaporating under vacuum (30C$^{\circ}$) using rotary evaporator, Buchi model 011. The extract was kept cool in refrigerator (4C$^{\circ}$) until use. Garlic extract was diluted by water to give the final concentration required (5, 10, 15 and 20%) 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.

The following parameters were determined to evaluate the effect different spray treatments on flowering, yield, and chemical constituents of apple shoots.

2.3. Morphological characteristics

In both the two seasons, bud counts were made for each tree. The dates on which flower and vegetative bud started to open were recorded. Number of vegetative and flower buds was counted when all buds were opened and the percentages were estimated. The dormant buds were also counted and were expressed as percentage from the total number of buds. The dates at which flowering reached 25, 50, 75 and 100 percent of the total flowers were estimated in each treatment. Flowers whose calyx began to extend were tagged in order to determine the percent of fruit set. The yield of fruits in kg/tree as well as, number of mature fruits/ tree were recorded when fruits reached the commercial colour to be picked.

2.4. Chemical analysis

Samples of one-year-old shoots, ~1 m long, were collected from each replicates 9 days intervals after spraying till march 3 in the two studied years for determination the seasonal changes in bud components. Samples of one-year-old shoots were taken at random and immediately transported to the laboratory for the following determination.

2.4.1 Total carbohydrate determination in apple shoots

Procedure: Total carbohydrate was extracted from apple shoots by placed 10 mg dry sample with 10 mL of H_2SO_4 (0.1N) in test tube and extracted on a boiling water bath for 30 minutes. The sample was filtered to remove the insoluble material. After filtering the solution was completed to 100 ml by distilled water.

Carbohydrate contents were determined by phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Briefly, to 1 ml of sugar solution, 50 µl 80% phenol and then 3 ml 98% sulfuric acid was added. The mixture vortexed were kept at room temperature for 30 min and the absorbance read at 490 nm.

2.4.2 Total and reducing sugars determination

Total and reducing sugars were determined as mg/g fresh weight using phosphomolybdic acid reagent (A.O.A.C., 1995). Briefly, sample (500 mg) of frozen shoots was crushed in a porcelain mortar and extracted with 50 ml of 80% (v/v) boiling ethanol for 5 minutes. The sample was filtered to remove the insoluble material. The extract was centrifuged at 10,000rmp for 10 minutes. Then the volume of the supernatant was adjusted to 100 ml. Protein was precipitated by adding 1.0 ml of ethanol extract with 3 ml of basic lead acetate (137g/L) and the excess lead acetate was precipitated with a solution of 1M sodium phosphate monobasic (141.7g /L). The mixture was centrifuged and the volume of the supernatant was completed to 10

ml. For determining reducing sugars 1ml of the filtrate was mixed with 1ml of copper sulphate solution (13.2g sodium sulphate and 6.0g copper sulphate were dissolved in one liter) and 1 ml of alkaline tartarate solution (12g sodium potassium tartarate, 20g anhydrous sodium carbonate, 20g sodium bicarbonate and 18g potassium oxalate were dissolved in one liter), then the mixture was heated in boiling water bath for 10 minutes. After cooling, 2ml of phosphomolybdic acid reagent (23g molybdic acid and 5g sodium tungestatc were dissolved in 200ml of 10% sodium hydroxide and boiled for 20-30 min. After cooling 125ml of phosphoric acid were added and the volume was completed to 500ml with water) was added and the developed blue colour was measured at 540 nm. For determining total sugars, 1ml of the filtrate was mixed with 1ml 1N HCL then the mixture was neutralized with sodium bicarbonate solution (1N), then the volume was completed to 5 ml. The total sugars were determined using a known volume (1ml), as described in the method of reducing sugars determination.

2.4.3 Total anthocyanins determination in apple shoots

Total anthocyanin content was analysed by a method described by (Mancinelli, 1984) with some modifications (Horbowicz et al., 2008, 2009). The apple shoots were extracted 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 A530 - 0.25A657 was used to compensate for the absorption of chlorophyll degradation products (Mancinelli, 1984). Anthocyanin content was expressed as mg of cyaniding 3-glucoside in 100 g of dry matter, using 29,600 as molecular extinction coefficient.

2.4.4 Total free amino acids determination by ninhydrin reaction in apple shoots

Total free amino acids were determined by reaction with ninhydrin using glycine as standard (Jayarman, 1981; Chen et al., 2009) with some modification. Briefly, sample (500 mg) of frozen shoots 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. This was followed by addition of 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 of SnCl2_2H2O. The mixtures in the volumetric flasks were 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 of these blue-purple products were measured against a reagent blank at 550 nm.

2.4.5 Free proline determination in apple shoots

Free proline was determined as described by Ennajeh et al. (2006). Briefly, sample (200 mg) of frozen shoots 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 reaction 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 acid. The tubes were closed and heated for 30 min in a water bath set to 100 °C. The sample was cooled on ice, then 3 ml of toluene was added and the mixture was shaken vigorously. The colored toluene phase (upper phase) was saved and dehydrated with anhydrous Na_2SO_4. The extracts were kept in the dark for a minimum of 2 h before their absorbance was measured at 528 nm. Proline concentration of the fresh shoot was calculated based on a standard calibration curve with concentrations ranging from 0 to 0.025 mg ml^{-1}.

2.4.6 Extraction and determination of total indoles in apple shoots

Total indoles was extracted from apple shoots by grinding 2 g fresh shoots with 50 mL toluene and 5 mL 5% TCA for 1 min. The purée was centrifuged for 30 min at 3500 rpm (2534 × g) to separate the toluene from the apple pulp. The toluene layer was decanted and filtered through a 0.45 µm syringe filter into a beaker containing anhydrous Na2SO4 (Aldrich).

Total indols in shoots were determined as µg /g fresh weight according to Snellings et al. (2003) with some modification. The derivatizing reagent used in the colorimetric method consisted of 1.25 g (4-dimethyl-amino- benzaldehyde (DMAB)) in 100 mL MeOH and 25.6 mL concentrated HCl (Snell and Snell, 1967). In the procedure, 4mL apple extract was diluted to 10 mL with toluene, after which 2 mL was vortexed for 15 min with 2 mL derivatizing reagent. The resulting mixture was centrifuged for 6 min at 3500 rpm to separate the MeOH and toluene layers. The absorbance of the MeOH (bottom) layer was measured with spectrophotometer at 567 nm.

2.4.7 Free phenolics determination using folin-ciocalteu reagent in apple shoots

Free phenols in shoots were determined as mg/g fresh weight using folin-ciocalteu reagent and Sodium carbonate solution according to Galicia et al. (2009) with some modification.

Procedure: Weight a random sample of 20-30 shoots as a representative of your material. Dry the shoots at 64-65 ºC for 16 hours. Grind each sample to a very fine powder.

Extraction of free phenolics: For each sample, weigh 100 mg of powder in an Eppendorf tube. Add 6.5 ml of methanol (50%). Close the tubes, ensuring no evaporation will take place during extraction. Vortex thoroughly the samples and place them in a thermo mixer at 65 ºC and 900 rpm for 30 minutes. Take the tubes out of the thermo mixer and let them cool at room temperature. Centrifuge the tubes at 14,000 rpm for 5 minutes. Ensure that the supernatant does not have sample particles floating in it; if it does, centrifuge again. Make the colorimetric reaction.

Colorimetric reaction: Take 1 mL of supernatant and carefully transfer it to test tube. Add 0.8mL of 25 % Folin-Ciocalteu reagent (dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85% phosphoric acid and 10 ml concentrated hydrochloric acid. Reflux for 10 hr. Add 15 g lithium sulfate, 5 ml water and 1 drop bromine. Reflux for 15 min. Cool to room temperature and bring to 100 ml with water. Then take 2.5 ml of F-C 2N with 7.5 ml of deionized water and vortex thoroughly). The F-C reagent should be added before the alkali to avoid the air-oxidation of phenolics. Add 2.2 mL of 400 mM Na2CO3 (4.25 g of Na2CO3 (99.9%) in 100 ml of deionized water). Cover the tubes with adhesive aluminum tape to avoid dropping of samples. Vortex the tubes at 800 rpm for 10 seconds. Incubate tubes at 42 ºC for 9 minutes for color development. Take the tubes out of the oven and let them cool at room temperature, protect them from direct light. Read absorbance at 765 nm in a spectrophotometer.

2.5. Statistical analysis

The values of the determined characters were subjected to statistical analysis according to the standard procedure described by Gomez and Gomez (1984). The 'F' test was applied to assess the significance of the treatment at 5% level of probability.

3. Results

3.1. Date of flower bud break

Data presented in Table (1) clearly indicated that spraying apple trees with all concentrations of the tested substance hastened the beginning of flower bud break as compared to the control. This earliness reached about 19, 25, 30 and 27 days over the control in the first season and 17, 23, 27 and 22 days over the control in the second season for garlic extract at 5, 10, 15 and 20% respectively.

Treatment	Date of flower bud opening											
	Beginning		25% flowering		50% flowering		75% flowering		End		Flowering duration (day)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	17 Mar	16 Mar	20 Mar	22 Mar	3 Apr	5 Apr	7 Apr	8 Apr	9 Apr	11 Apr	23	26
Garlic extract 5%	26 Feb	28 Feb	28 Feb	3 Mar	8 Mar	11 Mar	15 Mar	17 Mar	20 Mar	22 Mar	22	23
Garlic extract 10%	26 Feb	22 Feb	22 Feb	25 Feb	4 Mar	6 Mar	12 Mar	14 Mar	14 Mar	16 Mar	22	23

Garlic extract 15%	15 Feb	18 Feb	19 Feb	22 Feb	22 Feb	27 Feb	5 Mar	6 Mar	9 Mar	11 Mar	22	22
Garlic extract 20%	18 Feb	23 Feb	20 Feb	23 Feb	1 Mar	3 Mar	10 Mar	12 Mar	12 Mar	14 Mar	22	20

Table 1. Effect of garlic extract treatments on time of flower bud opening in "Anna" apple trees

As regards to the effect of the tested substances on 50% bud break, the present results clearly show that all treatments hastened 50 % bud break as compared to the control. This earliness reached about 27, 30, 37 and 34 days over the control in the first season and 25, 30, 37 and 33 days over the control in the second season for garlic extract at 5, 10, 15 and 20% respectively.

3.2. Percentage of bud break and fruit set

Data presented in Table (2) clearly show that all treatments gave a high percentage of flower bud break compared with the control. The maximum increases were recorded with garlic extract at 15%, which recorded 7.77 and 7.21 % in both seasons over the control, respectively.

Treatments	Bud break (%)		Fruit-set (%)	
	2014	2015	2014	2015
Control	81.42b	81.65b	14.66b	15.94b
Garlic extract 5%	82.95b	83.10b	15.18b	16.81b
Garlic extract 10%	84.80a	84.50b	16.23ab	17.05ab
Garlic extract 15%	87.75a	87.54a	19.16a	19.21a
Garlic extract 20%	86.38a	86.45a	18.89a	18.93a

Mean pairs followed by different letters are significantly different ($P \leq 0.05$) by Duncan's test; n=6

Table 2. Effect of garlic extract treatments on the percentage of bud break and fruit setting, in "Anna" apple trees

3.3. Yields and its components

Data in Table (2) indicated that all the tested substances increased apple yield and its components (fruit-setting and fruit number) as compared to the control trees. Such trend was true during the two studied seasons. The maximum increases were recorded with garlic extract at 15%, which recorded 30.69 and 20.51 for fruit-setting, 12.88 and 6.06 % for fruit number and 26.94 and 8.73% for apple yield/tree in the first

season, respectively over the control trees. Moreover, in the second season such increases were 3.27 and 4.37 for fruit-setting, 11.66 and 11.51% for fruit number and 26.94 and 20.72 % for yield/tree, over the control trees, respectively.

Treatments	Yield per tree (Kg)		Total number of fruits / tree	
	2014	2015	2014	2015
Control	146.33c	148.66b	15.66b	15.78b
Garlic extract 5%	151.14b	151.15b	16.22b	16.52b
Garlic extract 10%	154.25b	159.20ab	17.55a	17.41a
Garlic extract 15%	165.18a	169.35a	19.88a	19.05a
Garlic extract 20%	160.11a	166.25a	18.44a	18.61a

Mean pairs followed by different letters are significantly different ($P \leq 0.05$) by Duncan's test; n=6

Table 3. Effect of garlic extract treatments on No. of fruit tree^{-1} and total yield tree^{-1} of "Anna" apple trees

3.4. *Chemical constituents of buds*

3.4.1 Water content

Water content which were found in the examined shoots at 9 days intervals from 8th January till 3th March for apple variety as shown in Fig (1) clearly show that water content rapidly increased at early spring during bud breaking, from the first sample reaching its maximum content at (22th of February) then decreased at last sample (after bud break).

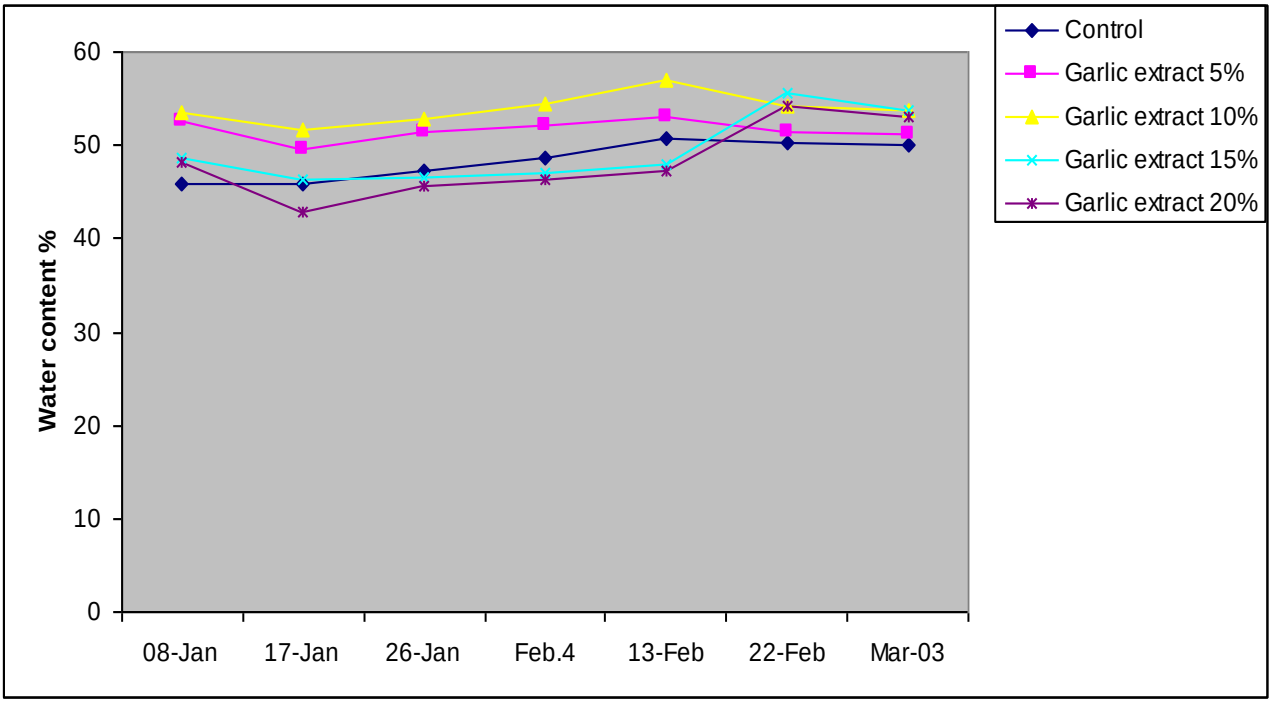

Figure 1. Effect of garlic extract on shoot shoot water content of "Anna" apple trees.

3.4.2 Total carbohydrates

The data in Fig (2) indicated that the amount of total carbohydrates in "Anna" apple variety gradually increased from the first sample till 17th January, thereafter it decreased till 26th January, then it increased gradually reaching its maximum values at 22th February followed with a decrease towards the last sample.

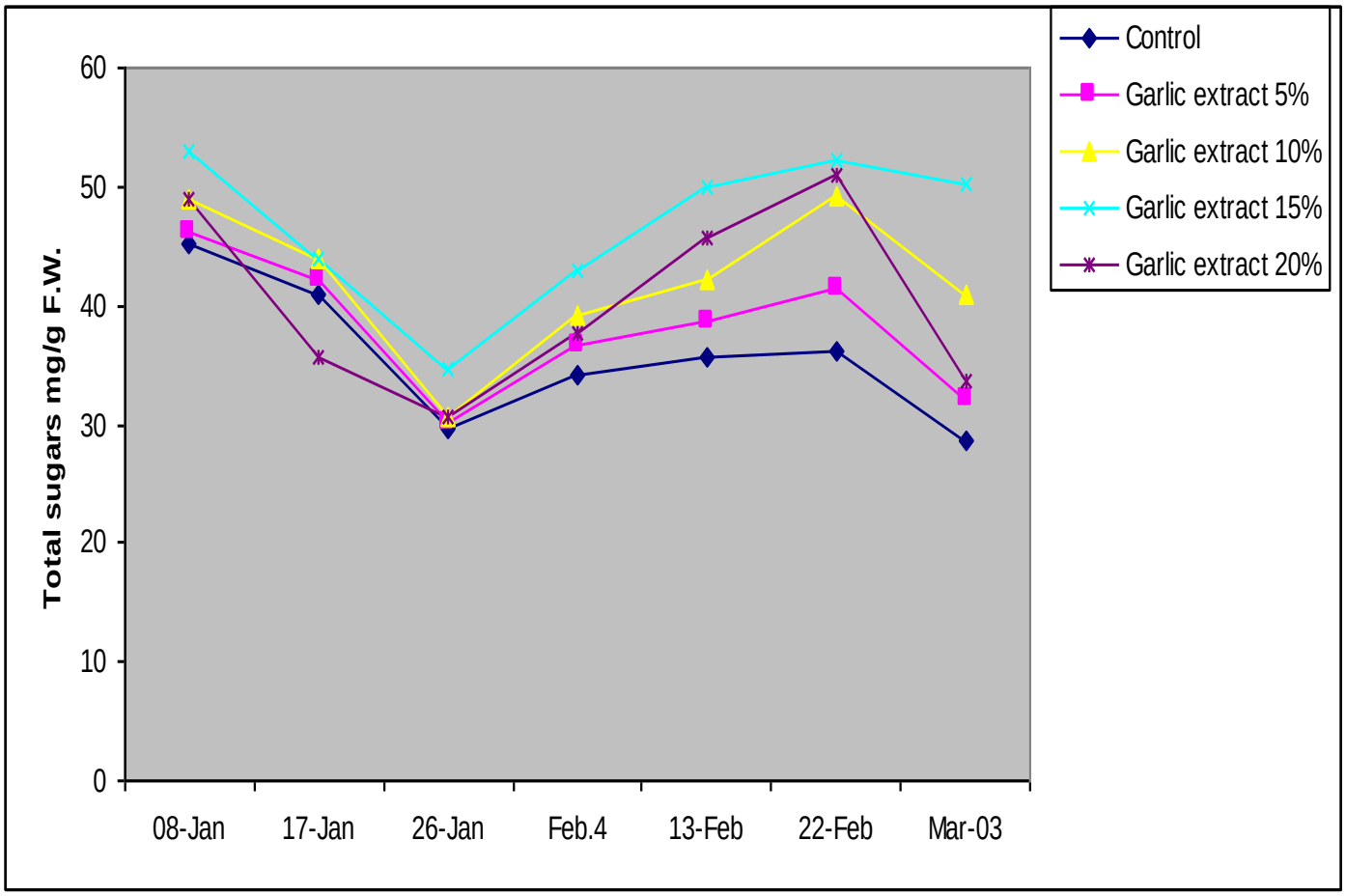

Figure 2. Effect of garlic extract on shoot total carbohydrates of "Anna" apple trees.

3.4.3 Total and reducing sugars

The data in Fig (3 and 4) clearly show that total sugars content in "Anna" apple variety gradually decreased from the first sample till 26th January, thereafter it increased gradually reaching its maximum values at 22th February followed with a decrease towards the last sample. On the other hand, reducing sugars content in" Anna" apple variety gradually decreased from the first sample till 4st February and thereafter it increased till the last sample.

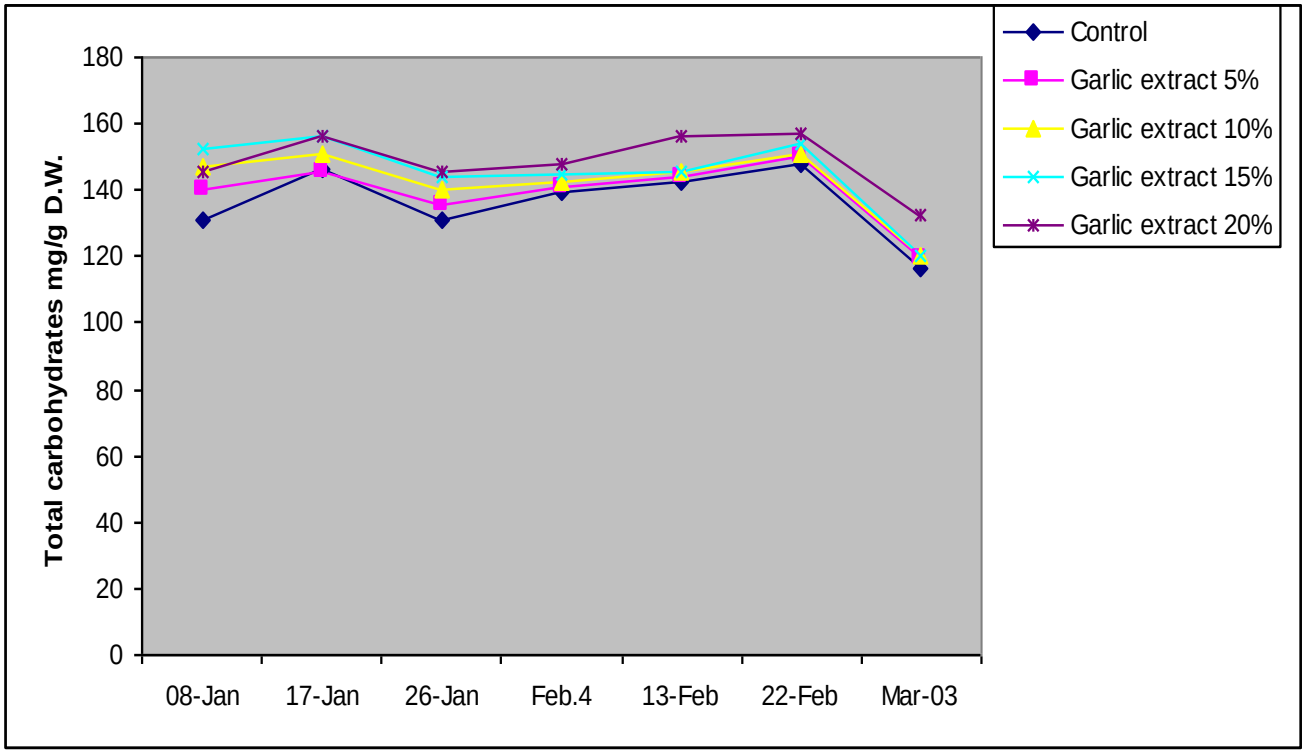

Figure 3. Effect of garlic extract on shoot total sugars of "Anna" apple trees.

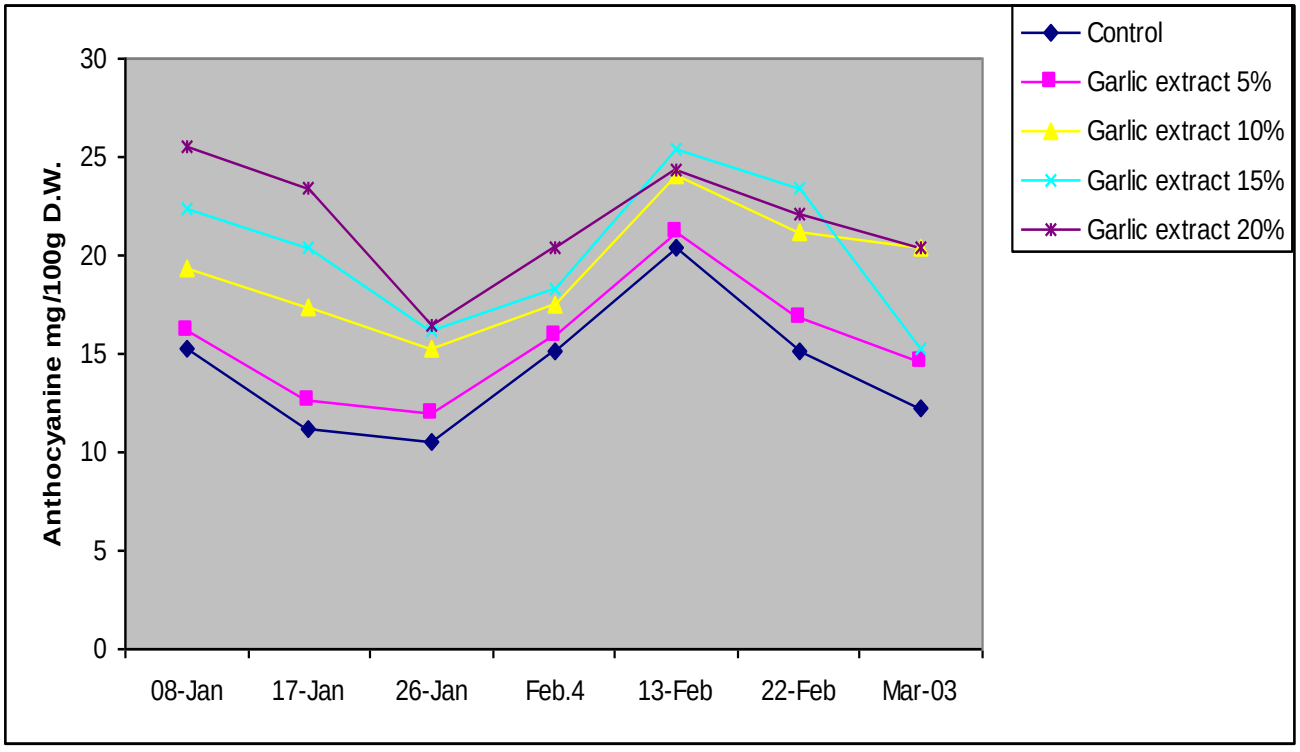

Figure 4. Effect of garlic extract on shoot reducing sugars of "Anna" apple trees.

3.4.4 Anthocyanin

Data presented in Fig (5) generally show that anthocyanine in shoots of "Anna" apple variety gradually decreased from the first sample till 26th January thereafter it increased gradually reaching its maximum values at 13th February followed with a decrease towards the last sample.

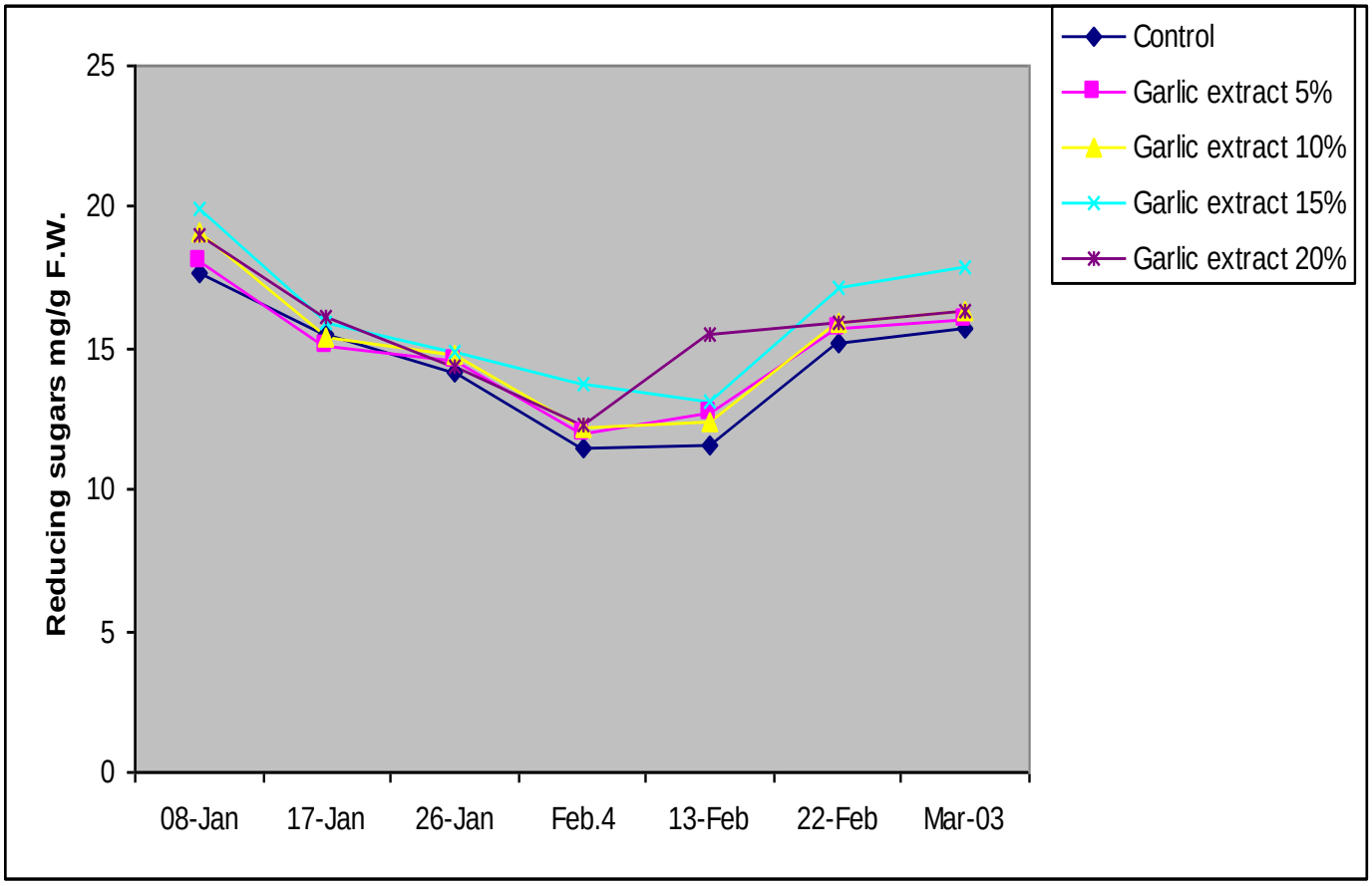

Figure 5. Effect of garlic extract on shoot anthocyanine of "Anna" apple trees.

3.4.5 Total free amino acid and free proline

Data presented in Fig. (6 &7) generally show that total free amino acids and free proline content in "Anna" apple variety gradually decreased from the first sample till 26th January, thereafter it increased gradually reaching its maximum values at 13th February followed with a decrease towards the last sample.

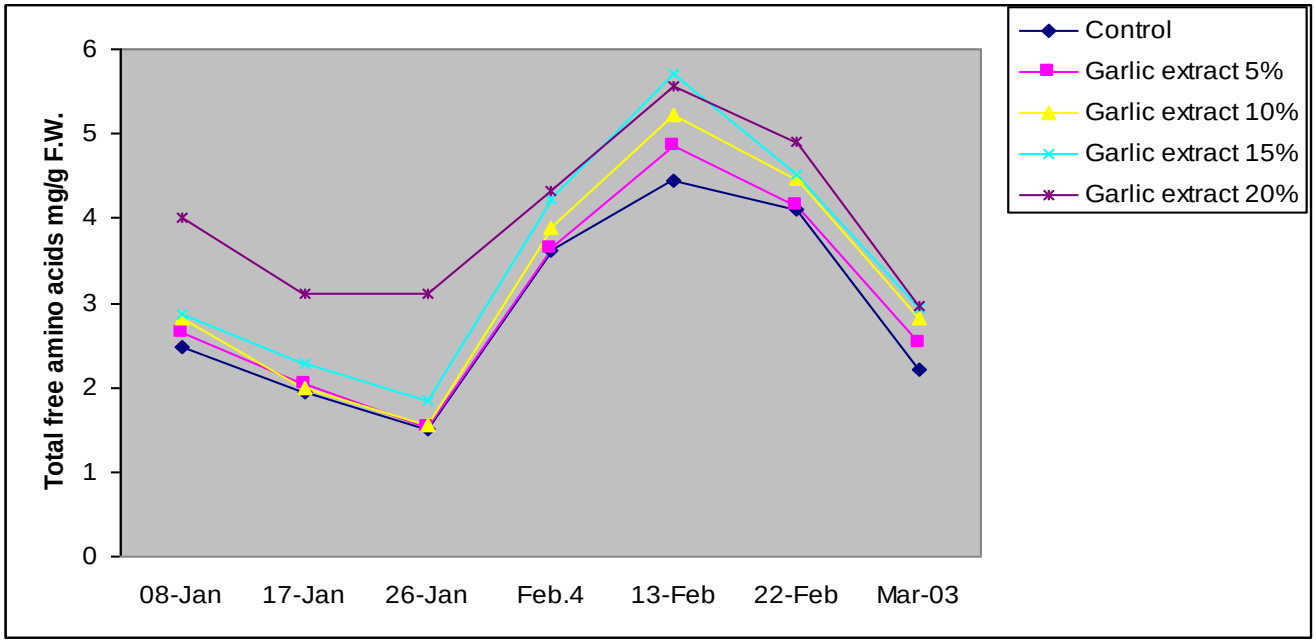

Figure 6. Effect of garlic extract on shoot total free amino acids of "Anna" apple trees.

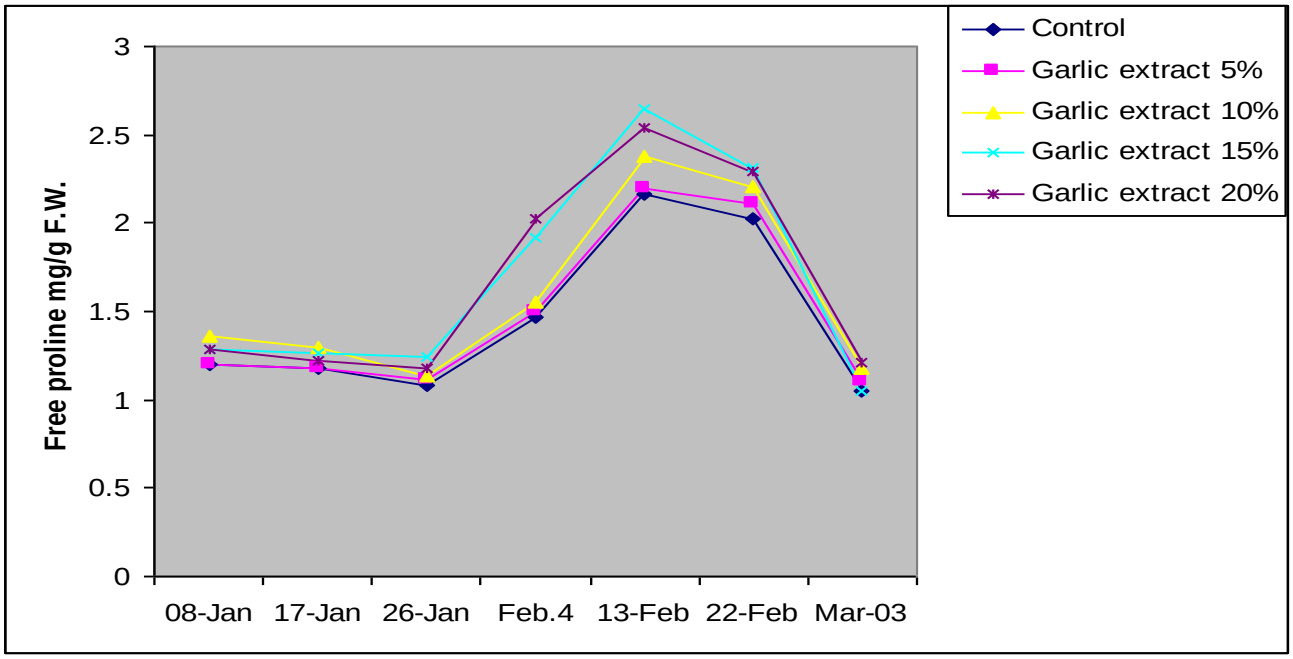

Figure 7. Effect of garlic extract on shoot total free proline of "Anna" apple trees.

3.4.6 Total indoles

Data presented in Fig (8) generally show that total indoles in "Anna" apple variety gradually decreased from the first sample till 26th January, thereafter it increased gradually reaching its maximum values at 13th February followed with a decrease towards the last sample.

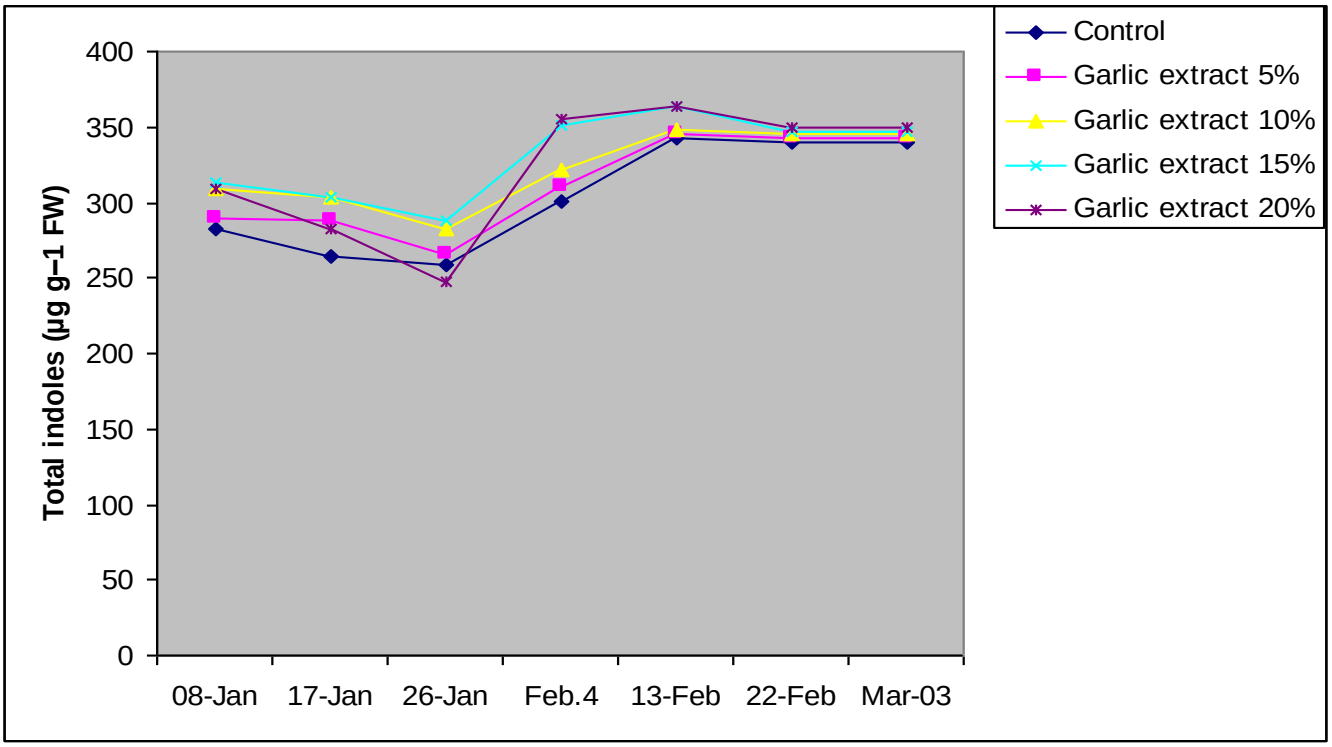

Figure 8. Effect of garlic extract on shoot total indoles of "Anna" apple trees.

3.4.7 Free phenols

The data in Fig (9) indicated that the amount of free phenols in "Anna" apple variety gradually decreased from the first sample8th January till the last sample3th March. Concerning the effect of the spray treatments on water content %, total carbohydrates, total sugars, reducing sugars, anthocyanine, total free amino acids, free proline and total indoles, it is clear from the present data that nearly all treatments gave higher values from these constituents when compared with the control trees. The best results were obtained by spraying the trees by garlic extract at the rate of 15 % flowed by 20 %. On the other hand, all treatments gave lowest values of free phenols if compared to the control trees. The best results were obtained by spraying the trees by garlic extract at the rate of 15 %.

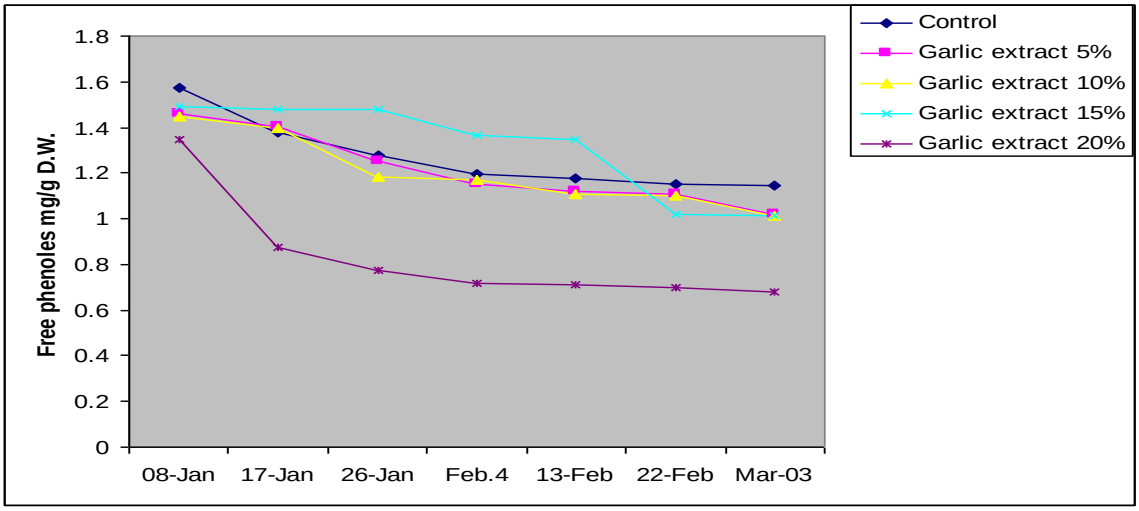

Figure 9. Effect of garlic extract on shoot free phenoles of "Anna" apple trees.

4. Discussion

There are strong evidences that one of the principal mechanisms involved in the dormancy bud breaking of temperate climate fruit plants is related to the induction of an oxidative stress (Pinto et al. 2007). In agreement with Pinto et al. (2007), H_2O_2 would function as a chemical sign activating the expression of genes directly or, indirectly, triggering metabolic alterations that can be detected by other molecules, as for instance, a kinase, that would activate or repress the expression of genes responsible for the dormancy breaking. Besides, a momentary increase in H_2O_2 levels precedes the overcome of the endodormency of buds. According to Kubota et al. (1999) the active substances in garlic, responsible for breaking bud dormancy in grapevines are sulfur- containing compounds with an allyl group (CH_2CHCH_2), particularly diallyl mono-, di-, tri-, and tetra-sulfides, but only trace amounts of dimethyl mono- and di-sulfides were present. Exposure to volatiles of diallyl di- and tri-sulfides was the most effective treatment in promoting bud break, irrespective of the concentration and the duration of exposure. However, the effects of dimethyl sulfide and diallyl sulfide on budbreak varied among the concentrations and the duration of exposure. Spraying apple trees with garlic extract at (5, 10, 15 and 20%) resulted in heavy flowering as well as high productivity with good fruit quality for apple trees. Treatments increased the measured flowering characters; this was due to the fact that these treatments resulted in more availability of some nutrients, i.e., sulfur, to trees. Enhancement of flowering parameters with garlic extract (as sulfur natural compound) would be expected since sulfur is a constituent of the amino acids cysteine, cystine and methionine and hence proteins. Both of these amino acids are precursors of other sulfur-containing compounds such as coenzymes and secondary plant products. Sulfur is a structural constituent of these compounds (e.g., R^1-C-S-C-R^2) or acts as

a functional group (e.g., R- SH) directly involved in metabolic reactions. About 2% of the organic reduced sulfur in the plant is present in the water-soluble thiol (- SH) fraction, and under normal conditions he tripeptide glutathione accounts for more than 90% of this fraction (De Kok and stulne, 1993). Cysteine, the first stable product of the assimilatory sulfate reduction, acts as a precursor for the synthesis of all other organic compounds containing reduced sulfur, as well as for other biosynthetic pathways, such as the formation of ethylene (Miyazaki and Yang, 1987)**.**Sulfate reduction in the leaves leads to export in the phloem of reduced sulfur compounds, mainly as glutathione to sites of demand for protein synthesis (e.g., in the shoot apex, fruits, but also roots) and probably also for regulation of sulfate uptake by roots (Rennenberg, 1989). On the other hand, sulfolipids are particularly abundant in the thylakoid membranes of chloroplasts, about 5% of the chloroplast lipids (Schmidt, 1986). Sulfolipids may also be involved in the regulation of ion transport across bio membranes. Sulfolipids levels in roots have been shown to be positively correlated with plant salt tolerance, the higher the level the greater the tolerance (Erdei et al., 1980; Stuiver et al., 1981). Also, the favorable effect of the used substances on date of flower bud opening may be due to their stimulation effect of natural gibberellin. In this connection Luna et al. (1993) and Subha-Drabandhu (1995), concluded that the induction of flowering could be correlated with a natural rise in gibberellin which promote flower formation in plants by either facilitating the formation of flowering hormone in the leaves or expressing it in the growing buds. Gibberellins also may be a primarily responsible for bolting which may be essential for the formation of the floral stimulus in leaves. Also, Skene (1969) reported that when a bud opens and attains the shape of shoot, its tip acts as a strong sink for metabolites and thus being interception center for photosynthates and nutrient results in earlier start of the bloom. Also, garlic extract used in this study, have an improving effect on chemical constitutes. This may be attributed to the essential role of these substances in the synthesis of some amino acid and consequently, formation of growth regulators especially auxin, and ethylene. The improving effect of garlic extract on yield and its components was mainly attributed to its positive action on enhancing growth parameters and photosynthetic pigments. The improving effect of garlic extract on buds' water content may be due to the early activity**.** In this concern George et al. (1990) suggested that water and nutrients may also be mobilized to the growing points at the expense of the developing fruits. Moreover, Borkowaska (1980) found that the transition of buds from the dormant stage to the bursting process is related to an increase in the water content in the tissue, mobilization of nutrients, and activation of hydrolytic enzymes and intensification of respiration. The stimulating effect of garlic extract as foliar spray on total carbohydrates concentrations in buds of sprayed trees may be directly or indirectly due to certain enzymes which activate the anabolic processes leading to the accumulation of these substances. In this connection Bachelard and Wightman (1973) concluded that the period of increase in dry weight of both flower and vegetative buds appear to be due to the movement of metabolites could have come from twigs,

branches and roots of trees. The stimulating effect of garlic extract as foliar spray on total and reducing sugars may be due to that these substances stimulate the conversion of the non-soluble carbohydrates to the soluble ones by activating the hydrolytic enzymes**.** In this concern**,** Bazy (1984) showed that a considerable increase in reducing sugars were evident during active growth periods whereas these sugars were very low during the period of retarded growth. Moreover, Young (1989**)** and Whitworth and Young (1992) working on apple trees found that starch is being used as source of metabolites during chilling. They also added that sucrose and hexose (glucose + fructose) are important sugars during early growth. Also, O'kennedy et al. (1975) and **Gemma** (1995) pointed out that numerous changes in the level of carbohydrates occurred in buds as they based from the non-growing stage beginning of growth, they added that starch accumulated during the period of photosynthetic activity and is used for regeneration of growth in the spring. Moreover, El-Mansy and Walker (1969) and Abo-Hussein (1970) they concluded that reducing sugars induce several multibiological responses which ultimately lead to the promotion of flowering. Moreover the increase in total free amino acids in buds after garlic extract treatments may be due to the increase in cystein , cystine and methionine since sulfur is a constituent of these amino acids. In this concern, Hill-Cattngham (1968) found that there was a decrease in the nitrogen concentration of the woody tissues in the spring, particularly in the bark tissues of shoots. This was attributed to the movement of nitrogenous compounds from the bark and wood to the developing flower buds and growing points. Also, Kuroi (1974) indicated that the nitrogen (including amino acid) was low level in buds or roots during dormant stage and reached maximum just prior to bud break, the nitrogen stored mostly in the roots and translocated to buds before bud break and early growth. Moreover, Tromp (1970); Bachelard and Wightman (1973) who observed that a probable correlation could exist between the state of dormancy and the dominant total amino acids in the buds. Moreover, they added that significant decrease in the dormant status of buds occurred and this was accompanied by an increase level of catabolic metabolism of protein. A further change to anabolic metabolism nearly two weeks before bud burst resulted in a net synthesis of cellular constituents in preparation for bud burst. Moreover, Marschner (1995) reported that, reduced sulfur is a structural constituent of several coenzymes and prosthetic groups such as ferredoxin, biotin (Vitamin H), and thiamine pyrophosphate (Vitamin B_1). In many enzymes and coenzymes such as urease, sulfotransferases and coenzymes-A**.** The decrease in free phenols after garlic extract treatments may be due to that the reduction in free phenols contrasted with the increase in total indoles i.e. indogenous promoters increased and consequently endogenous inhibitors decreased in the buds which led to increasing in plant growth parameters. In this respect Sagi and Garay (1961) showed that phenolic effect on plant growth was contributed to either antagonism with I.A.A. activity. Moreover, Wang et al. (1991) on apple found that dormant buds contained a high amount of phenolic substances which

decreased after bud break then increased until the start of bud expansion. Phenolic compounds are found to be patent modifiers of catalase, peroxidase and polyphenol oxidase activity, as both inhibitors and stimulators in apple buds. Moreover, these substances may be stimulating the oxidation process of phenols by increasing the peroxidase activity. The stimulating effect of garlic extract as foliar spray on total indole may be attributed to the increase in total free amino acids in buds specially tryptophan amino acid. In this concern, Tuner (1972) reported that natural breaking of dormancy was accompanied with increasing buildup of endogenous gibberellins and the breakdown of starches to sugars. Also**,** Baz (1984) reported that, for breaking dormancy naturally or chemically, a pronounced increase in the endogenous gibberellins occurred with emergence from the dormant state to sprouting. Moreover, Robert and Francis (1985**)** reported that the increase in endogenous GA, content which induce the synthesis of hydrolytic enzymes, increase the mobilization of these sugars to developed buds. According to Dokoozlian et al. (1998), the scales of the buds of vines, that protect them against dehydration and injury by the extreme climatic conditions, are a barrier against the products used for the dormancy

5. Conclusions

As a substitution of harmful synthetic growth regulators, it has been concluded that the results of this study give evidence to the role of garlic extract as one of the natural and safety substances in breaking bud dormancy. Thus**,** using garlic extract greatly increased growth and apple yield as well as improved chemical constituents. The constituents of the used substances participate in the different metabolic processes which increased syntheses of carbohydrates, sugars, total free amino acids, and plant hormones so that the use of garlic extract could increase apple productivity.

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