# Foliar-Applied Mineral Oil Enhanced Hormones and Phenols Content and Hastened Breaking Bud Dormancy In "Astrachan" Apple Trees

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**Abstract**

The effect of mineral oil alone or in combination with Dintro-ortho-cresol (Universal) with different concentrations at early reaching break dormancy in buds of 'Astrachan' apple (*Malus sylvestris,* Mill) trees was investigated. Also, their effects on metabolic changes in the content of buds from promoter and inhibitor substances during their release from dormancy were detected. The efficiency of early bud break was noticed in varying degrees with mineral oil or Universal at different concentrations. It was observed that all applied concentrations lead to early bud break, short flowering duration, high percentages of bud break and fruit-set. In addition, high contents of total indoles, indole-3-acetic acid, gibberellic acid, total and conjugated phenols, as well as, low content of abscisic acid and free phenols were achieved. According to the results, it is recommended using 6% of mineral oil as operator safer bud break promoters for reach to the early break dormancy in buds of 'Astrachan' apple trees under Egyptian winter conditions and the maximum yield by regulating the hormonal and phenolic content in buds.

**Keywords:** Apple; Dormancy; Mineral oil; Universal; Hormones; Phenols; Yield.

## 1. Introduction

Dormancy is "the inability to initiate growth from meristems or other organs and cells with the capacity to resume growth under favorable conditions" [1]. The main physiological stages of dormancy have been recently termed paradormancy, endodormancy, and ecodormancy. Paradormancy the growth is regulated by plant factors originating outside the bud. While the endodormancy stage growth is repressed by physiological factors inside the bud. During the ecodormancy stage, bud break is avoided because of environmental factors are inadequate to support growth [2]. Poor bud break in locations have warm winters with insufficient winter chilling [3].

Mineral oil or mineral oil plus DNOC (Universal) is the most useful rest breaking treatment for apple and has been used effectively to supplement cold temperature, to achieve satisfactory bud break and to enhance cropping [4]. Several researchers justified the idea that dormancy breaking agents like Mineral oil and/or DNOC [5, 6, 7] may be spread within the plant.

Initiation and release of dormancy have been related in other plant species to changes in endogenous hormone concentrations [8]. In this respect, the endogenous hormonal change in the bud dormancy inducing and releasing processes was studied by many researchers [8, 9, 10]. They found that the occurrence, termination, regulation, and control of dormancy were regulated by hormones. A few number of studies have been done on the change of endogenous hormones from dormancy releasing to bud opining, but none on the relation between bud break and dynamic change of endogenous hormones, as well as the equilibrium of late-opining apple varieties. The relationship between plant phenols and bud dormancy as well as bud break was studied by several workers [11, 12].

Because of the winter in Egypt is short and does not meet the chilling requirements of buds, the delay in opening the buds of ‘Astrachan’ apple trees until late winter exposes them to damage under the influence of high temperature and/or delays them in entering in dormancy in the following year leads to some physiological defects that may result in weakness and death. This threatens the "Astrachan" apple productivity in Egypt, so this work focuses mainly to explain the behavior of the hormonal and phenols contents in buds and their reflections in the duration to full buds break, and the percentages of bud break and fruit-set as a result of spraying "Astrachan" apple trees with mineral oil or Universal at different concentrations elucidating their impacts in hastening the dormancy break.

## 2. Materials and Methods

### *2.1. Trees selection and treatments*

The 15-year-old trees of "Astrachan" apple (*Malus sylvestris,* Mill) budded on "Baladi" apple were selected randomly and uniformly for preliminary study in 2015 and for farther studied in 2016 and 2017 seasons, in the orchard of the Horticulture Station in Aboksha Abshawi, Fayoum Governorate, Egypt. The selected trees were labeled in November 2015, foliar-spray applications (3 l tree$^{-1}$; six trees per treatment) were conducted as follows: Treatment 1 (control trees) did not receive any of the two compounds, only tap water; Treatment 2 was a foliar spray to run-off with 1% (v/v) mineral oil plus DNOC commercially known as "Universal" (regular winter oil, having a UR of 75% and containing 1.5% m/m of Dintro-ortho-cresol (DNOC) ammonium salt); Treatment 3 was a foliar spray with 6% (v/v) mineral oil (MO; Guangzhou Hanglian Chemical Industry Co. Ltd., Guangzhou, P. R. China);Treatment 4 was a foliar spraying with 2% (v/v) mineral oil plus DNOC commercially known as "Universal" was a foliar spraying with 2% (v/v) mineral oil plus DNOC commercially known as "Universal". All spray treatments were applied at 23 January with a volume of 4-liter tree$^{-1}$. Triton B [0.1% (v/v)] was added as a wetting agent to each spray solution. Sampled beginning from 14 February up to 28 March 2009 for the first study season. The experiment was repeated for the second one; 2017. From the preliminary study in 2016 – 2017, Universal, MO or Universal, were applied at 1% (v/v), 6% (v/v), or 2% (v/v), respectively, as these were found to be the most significant concentrations for later bud growth in 'Astrachan' apple trees (data not shown). These treatment levels were used later for the main study.

2.2. Morphological Characteristics and Yield Measurements

Buds count was made for each tree of all treatments. The dates of floral and vegetative buds started to open were recorded. Number of vegetative and floral buds was counted for all buds when it was opened and the percentages were estimated. The dormant buds were also counted and expressed as a percentage of the total number of buds. The dates at which flowering reached 25%, 50%, 75% and 100% of the total flowers were estimated in each treatment. Flowers whose calyx began to extend were tagged in order to measure the percentage of fruit-set. At harvest stage, apple fruits were harvested, counted and weighed for each examined tree.

2.3. Extraction and Determination of Endogenous Hormone in Apple Buds

In different experiments (2 g) floral buds were collected 7-day intervals beginning from 14 February up to 28 March for determining the metabolic changes in the hormonal content in buds. Buds were randomly sampled

and immediately transported to the laboratory. Floral bud samples were taken from each tree of each treatment, frozen in liquid nitrogen and stored at –20°C until extraction and assay as adopted in [13] and described in [14].

The buds were ground in cold 80% (v/v) aqueous methanol. The buds were transferred to a flask with fresh methanol and the volume was adjusted to 20 ml of methanol for each g fresh weight of sample. The buds were extracted for 24 hr at 0°C and then were vacuum filtered through filter paper Whatman No. 42. The residue was returned to the flask with fresh volume of methanol and stirred for 30 min with magnetic stirrer and then filtered again. The procedure was repeated once more and the combined extracts were evaporated to the aqueous phase in a rotatory flask evaporated. The aqueous phase (10 to 30 ml) was adjusted to pH 8.6 with 1% (w/v) NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate fraction was evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with 1% HCl (v/v) and re-partitioned three times with equal volumes of ethyl acetate. The remaining aqueous phase was discarded and the combined acidic ethyl acetate phase was reduced to 5 ml (fraction I) to be used for gas chromatography (GC) determination of gibberellic acid ($GA_3$), indol -3- acetic acid (IAA) and abscesic acid (ABA). To estimate the amount of $GA_3$ (fraction I), IAA and ABA, the plant hormone fractions and standard ones were methylated according to [15] to be ready for GC analysis. Flame ionization detector was used for identification and determination of acidic hormones using Helwett Packered Gas Chromatography (5890). The chromatography was fitted and equipped with HP-130 m x 0.32 mm x 0.25 μm capillary column coated with methyl silicone. The column oven temperature was programmed at 10°C/min from 200°C (5 min) to 260°C and kept finally to 10 min. Injector and detector temperature were 260 and 300°C, respectively. Gases flow rates were 30, 30, 300 cm/sec for $N_2$, $H_2$ and air, respectively and flow rate inside column was adjusted at 2 ml/min. Standards of $GA_3$, IAA and ABA were used. The retention times (RT) of peaks of authentic samples were used in identification and characterization of peaks of samples under investigation [16]. Peak identification was performed by comparing the relative retention time of each peak with those of $GA_3$, IAA and ABA standards. Peak area was measured by triangulation and the relative properties of the individual compounds were therefore obtained at various retention times of samples.

2.4. Extraction and Determination of Total Indoles in Apple Buds

Indoles was extracted from apple buds by grinding 2 g fresh buds 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 $Na_2SO_4$ (Aldrich).

Total indoles in buds were determined as µg /g fresh weight according to [17] 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 [18]. 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.

2.5. Estimation of Free, Conjugated and Total Phenolics Content in Apple Buds Using Folin-Ciocalteu Reagent

Total and free phenols in buds were determined as mg/g fresh weight using folin-ciocalteu reagent and Sodium carbonate solution according to [19] with some modification.

Weigh a random sample of (2g) buds without scales as a representative of the fresh material. Dry the buds at 64-65 ºC for 16 hours. Grind each sample to a very fine powder.

2.5.1. Extraction of Free Phenolics

Weigh 100 mg of each powder of samples in an eppendorf tube and add 6.5 ml of methanol (50%). Close the tubes and ensure no evaporation will take place during extraction. Vortex thoroughly the samples and place them in a thermomixer at 65 ºC with 900 rpm for 30 minutes. Take the tubes out of the thermomixer and let them to cooling at room temperature. Then, centrifuge the tubes at 14,000 rpm for 5 minutes and ensure the supernatant does not have sample particles floating in it; if it does, centrifuge again. Make the colorimetric reaction.

2.5.2. Extraction of Total Phenolics

For each sample, weigh 100 mg of powder in an eppendorf and add 6.5 ml of hydrochloric acid in methanol (10 ml of HCl 1.2 M with 90 ml methanol). Close the tubes and ensure no evaporation will take place during extraction. Vortex thoroughly the samples and place them in a thermomixer at 42 ºC and 1100 rpm for 30 minutes. Take the tubes out of the thermomixer 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. Take 2.5 mL of supernatant, put it in new eppendorf. Reduce to dryness and resuspend the precipitate resulting in 6.5 ml of methanol. Vortex thoroughly and make the colorimetric reaction.

2.5.3. Colorimetric Reaction

Take 1 mL of supernatant and carefully transfer into test tube. Then, 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 $Na_2CO_3$ (4.25 g of $Na_2CO_3$ (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 sec. 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.

These estimates represented total phenols (conjugated and free phenols) and thus conjugated compounds were obtained by subtraction free phenols from total phenols.

### *2.6. Statistical analysis*

The values of the determined characters were subjected to statistical analysis according to the standard procedure described in **[20]**. The ‘F’ test was applied to assess the significance of the treatment at 5% level of probability.

## 3. Results

Because of the matched trends of the results, the hormones and their relations are represented in combined analysis system for the two examined seasons.

### *3.1. Date of Floral Bud Break*

The foliar application with mineral oil plus DNOC (Universal) at (1and 2%) and mineral oil at 6% for "Astrachan" apple trees was hastened the floral bud break as comparison with the control in which trees were sprayed with tap water (Table 1). The period to the first floral bud break was shortened by 7–7 days and 10–10 days with Universal, at 1% and mineral oil at 6% and 12–12 days with Universal, at 2% in the two experimental seasons as comparison with the control trees, respectively. In addition, the period to full flowering were shortened by 8–7 days and 11–10 with Universal, at 1% and mineral oil at 6% and 13-13 days with Universal, at 2%, in the two experimental seasons, respectively as compared to the control. Universal, at 2% was found to be most effective in shortening the period of full flowering, since shortened flowering period to 20–21 days, while mineral oil at 6% was shortened it to 20–22 days and Universal at 1%

was shortened it to 21-23 days compared to 22-23 days in the control in the two experimental seasons, respectively.

Table 1. Effect of (universal at 1and 2% and mineral oil at 6%) treatments on the date of flower bud opening and flowering period in "Astrachan" apple trees.

| | Date of flower bud opening | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Beginning | | 25% | | 50% flowering | | 75% flowering | | End | | Flowering period (day) | |
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| Control | 5 | 4 | 11 | 12 | 15 | 16 | 20 | 21 | 26 | 26 | 22 | 23 |
| Universal 1% | April 29 | Apri 28 | April 8 | Apri 8 | Apri 14 | April 15 | Apri 16 | April 17 | Apri 18 | Apri 19 | 21 | 23 |
| Mineral oil 6% | Marc 26 | Mar 25 | April 6 | Apri 7 | Apri 12 | April 11 | Apri 14 | April 15 | Apri 15 | Apri 16 | 20 | 22 |
| Universal 2% | Marc 24 | Mar 23 | April 6 | Apri 6 | Apri 11 | April 9 | Apri 12 | April 13 | Apri 13 | Apri 13 | 20 | 21 |
| | Marc | Mar | April | Apri | Apri | April | Apri | April | Apri | Apri | | |

3.2. Floral Bud Break and Fruit Set

Table 2 shows that, all tested compounds significantly increased the percentages of floral bud break and fruit-set when compared to the control. However, Universal, at 2% was the most effective than all others. It surpassed the tap water by 18–18% and 36–34% for bud break and fruit-set in the two experimental seasons, respectively. While mineral oil at 6% was surpassed the tap water by 17–16% and 33–31% for the same two parameters. Universal at 1% was less effective among the three compounds. It exceeded the control by 14–15% and 14–14% for the same two parameters in the two experimental seasons, respectively.

Table 2. Effect of (universal at 1and 2% and mineral oil at 6%) treatments on the percentage of bud break and fruit set in "Astrachan" apple trees.

| Treatments | Bud break (%) | | Fruit-set (%) | |
|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 |
| Control | 68.21c | 70.00b | 13.33c | 14.56c |
| Universal 1% | 78.11b | 80.66a | 15.25b | 16.66b |
| Mineral oil 6% | 80.33ab | 81.25a | 17.77b | 19.11a |
| Universal 2% | 80.88a | 83.15a | 18.20a | 19.63a |

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

3.3. Fruit yield

Table 3 shows that, all tested compounds increased number of apple fruits tree$^{-1}$ and total fruit yield tree$^{-1}$ when compared to the control. Universal, at 2% was most effective in this concern. It exceeded the control by 15–16% and 29–31% for number of fruits tree$^{-1}$ and fruit yield tree$^{-1}$ in the two experimental seasons, respectively, while mineral oil at 6% was surpassed the tap water by 14–15% and 27–28% for the same two parameters. Universal at 1% was found to be less effective when compared to Universal at 2 and mineral oil at 6%, although it exceeded the control by only 10–9% and 10–8% for the same two parameters in the two experimental seasons, respectively.

Table 3. Effect of (universal at 1and 2% and mineral oil at 6%) treatments on No. of fruit tree$^{-1}$ and total yield tree$^{-1}$ of "Astrachan" apple trees.

| Treatments | No. of Fruit tree$^{-1}$ | | Total yield tree$^{-1}$ (kg) | |
|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 |
| Control | 119.50c | 126.09c | 10.77c | 11.28c |
| Universal 1% | 132.35bc | 138.11b | 11.95b | 12.29b |
| Mineral oil 6% | 137.20b | 145.08a | 13.70b | 14.52b |
| Universal 2% | 138.56a | 146.84a | 13.91a | 14.86a |

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

3.4. Hormonal Content in Buds

Foliar-applied Universal at 1and 2% and mineral oil at 6% increased the contents of total indols, indole-3-acetic acid (IAA) and gibberellic acid ($GA_3$) and decreased the contents of abscisic acid (ABA) in the floral buds of "Astrachan" apple trees when compared to the control in which trees were sprayed with tap water as shown in Table 4. The results of all hormones obtained with Universal at 2% treatment were surpassed the results found with mineral oil at 6% and Universal at 1% in most of the sampled dates. The maximum contents of IAA, $GA_3$ and total indoles were obtained from buds applied with Universal at 2% and collected in 10 March (buds were released from dormancy), while their minimum contents were obtained from buds applied with mineral oil at 6% and Universal at 1% and sampled in 14 & 21 February (buds were still dormant) as shown in Table 4 and 5. For ABA, the opposite result was found. In addition, the maximum ratios of IAA/ABA and $GA_3$/ABA were noted in the 28 March sample (buds were released from dormancy), while the minimum ones were found in the 21 & 28 February samples (buds were still dormant) as shown in **Table 4**.

Table 4. Effect of (universal at 1and 2% and mineral oil at 6%) treatments on hormonal content in buds of "Astrachan" apple trees.

| Treatments | Indole acetic acid (IAA) (µg g$^{-1}$ DW) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 14 Feb | 21 Feb | 28 Feb | 7 Mar | 14 Mar | 21 Mar | 28 Mar |
| Control | 1.41c | 1.35 c | 4.13 b | 4.56b | 4.88 a | 4.12b | 4.02 b |
| Universal 1% | 1.63 c | 1.44 c | 4.25 b | 4.69a | 5.09 a | 4.36 a | 4.25 b |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mineral oil 6% | 1.81 c | 1.56 c | 4.49 b | 4.74 a | 5.12 a | 4.64 a | 4.37 b |
| Universal 2% | 1.85 c | 1.67 c | 4.51 b | 4.78 a | 5.14 a | 4.66 a | 4.40 b |
| Gibberellic acid ($GA_3$) (µg $g^{-1}$ DW) | | | | | | | |
| Control | 1.33 d | 1.08 d | 1.55c | 1.86 c | 3.44a | 3.31 a | 2.60b |
| Universal 1% | 1.38 d | 1.13 d | 1.77 c | 1.91 c | 3.74 a | 3.51 a | 2.69 b |
| Mineral oil 6% | 1.41 d | 1.23 d | 1.84 c | 1.98 c | 3.94 a | 3.71 a | 2.74 b |
| Universal 2% | 1.43 d | 1.25 d | 1.87 c | 2.04 c | 3.96 a | 3.73 a | 2.77 b |
| Abscisic acid (ABA) (µg $g^{-1}$ DW) | | | | | | | |
| Control | 3.61 b | 4.33 a | 4.96 a | 2.91 c | 1.98 d | 1.90 d | 1.44d |
| Universal 1% | 3.33 b | 3.57 b | 4.86 a | 2.65 c | 1.78 d | 1.74 d | 1.31 d |
| Mineral oil 6% | 2.99 b | 3.48 b | 4.51 a | 2.46 c | 1.60 d | 1.48 d | 1.05 d |
| Universal 2% | 2.93 b | 3.40 b | 4.39 a | 2.40 c | 1.52 d | 1.46 d | 1.01 d |
| IAA/ABA ratio | | | | | | | |
| Control | 0.39 d | 0.31 d | 0.83 d | 1.56 c | 2.46 c | 2.16 c | 2.79 c |
| Universal 1% | 0.48 d | 0.40 d | 0.87 d | 1.76 c | 2.85 c | 2.50 c | 3.24 b |
| Mineral oil 6% | 0.60 d | 0.44 d | 0.99 d | 1.92 c | 3.20 b | 3.13 b | 4.16 a |
| Universal 2% | 0.63 d | 0.49 d | 1.02 d | 1.99 c | 3.38 b | 3.19 b | 4.35 a |
| $GA_3$/ABA ratio | | | | | | | |
| Control | 0.36 d | 0.24 d | 0.31 d | 0.63 c | 1.73 b | 1.74 b | 1.80 b |
| Universal 1% | 0.41 d | 0.31 d | 0.36 d | 0.72 c | 2.01 b | 2.01 b | 2.05 b |
| Mineral oil 6% | 0.47 d | 0.35 d | 0.40 d | 0.80 c | 2.46 a | 2.50 a | 2.60 a |
| Universal 2% | 0.48 d | 0.36 d | 0.42 d | 0.85 c | 2.60 a | 2.55 a | 2.74 a |

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

### 3.5. Phenolic Content in Buds

Total soluble phenols and conjugated phenols increases with increasing of foliar-applied Universal from 1 to 2%, while the free phenols decrease in the floral buds of "Astrachan" apple trees when compared to the control as shown in Table 5. The data of all phenols obtained with Universal 2% treatment were surpassed the data found with mineral oil at 6% and Universal 1% in most of the sampled dates. The maximum contents of total soluble phenols and conjugated phenols were obtained from buds applied with Universal at 2% and collected in 21 March (buds were released from dormancy), while their minimum contents were obtained from buds applied with mineral oil at 6% and Universal at 1%, and sampled in 21 February (buds were still dormant). For free phenols, the opposite result was found. In addition, the maximum ratios of conjugated phenols /free phenols were noted in the 21 March sample (buds were released from dormancy), while the minimum ones were found in the 14 & 21 February samples (buds were still dormant) as shown in **Table 5**.

Table 5. Effect of (universal at 1and 2% and mineral oil at 6%) treatments on total indole, total, free and conjugated phenols content in buds of "Astrachan" apple trees.

| Treatments | Total Indole (μg $g^{-1}$ DW) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 14 Feb | 21 Feb | 28 Feb | 7 Mar | 14 Mar | 21 Mar | 28 Mar |
| Control | 46. 12 c | 40. 17 c | 46. 33 c | 53. 64b | 62. 12a | 57.42 b | 55.23 b |
| Universal | 47. 55 c | 44. 13 c | 50. 20 b | 53.64 b | 62.81a | 61. 77 a | 60.10 a |
| Mineral oil | 49. 94 b | 47. 16 c | 53. 28 b | 55.11b | 65.91a | 64. 25 a | 62.57 a |
| Universal | 50. 41 b | 48. 22 c | 54.22 b | 57.76b | 66.98a | 63. 81 a | 62.91 a |
| | Total phenols (mg $g^{-1}$ FW) | | | | | | |
| Control | 3.25 a | 2. 84 b | 2. 20 c | 2.95 a | 3.20 a | 3. 66 a | 2.74 b |
| Universal | 3.31 a | 2. 85 b | 2. 21 c | 3. 06 a | 3.49 a | 3.74 a | 2.81 b |
| Mineral oil | 3.42 a | 2. 90 b | 2.33 c | 3.16 a | 3. 52 a | 3.80 a | 2.83 b |
| Universal | 3. 44 a | 2. 91 b | 2.37 c | 3.21 a | 3.53 a | 3.82 a | 2.85b |
| | Free phenols (mg $g^{-1}$ FW) | | | | | | |
| Control | 1.13 a | 1.06 a | 0.90 b | 0.82 b | 0.87 b | 0.83 b | 0.81 b |
| Universal | 1.07 a | 0.91b | 0.83 b | 0.80 b | 0.80 b | 0.78 c | 0.74 c |
| Mineral oil | 1.05 a | 0.85 b | 0.81 b | 0.80 b | 0.78 c | 0.74 c | 0.71 c |
| Universal | 1.04 a | 0.81 b | 0.80 b | 0.79 b | 0.76c | 0.73 c | 0.70 c |
| | Conjugated phenols (mg $g^{-1}$ FW) | | | | | | |
| Control | 2.12 c | 1.78 c | 1.30 d | 2.13 c | 2.33 b | 2.83 a | 1.93c |
| Universal | 2.24b | 1.89 c | 1.38 d | 2.26 b | 2.69b | 2.96 a | 2.07c |
| Mineral oil | 2.37b | 2.05 c | 1.52d | 2.36 b | 2.74b | 3.06 a | 2.12 c |
| Universal | 2.40b | 2.10 c | 1.57 b | 2.42b | 2.77 b | 3.09 a | 2.15c |
| | Conjugated /Free phenols ratio | | | | | | |
| Control | 1.87d | 1.67d | 1.44d | 2.59b | 2.67 b | 2.77 b | 2.38 b |
| Universal | 2.09c | 2.07c | 1.66d | 2.82b | 3.36 a | 3.79 a | 2.79 b |
| Mineral oil | 2.25c | 2.41c | 1.87d | 2.95b | 3.51 a | 4.13 a | 2.98 b |
| Universal | 2.30c | 2.59b | 1.96c | 3.06b | 3.64 a | 4.23 a | 3.07 b |

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

## 4. Discussion

It is clear nowadays that a wide variety of factors can break dormancy, in particularly environmental and hormonal influences have to be analyzed to understand the complex mechanism which start when the bud resume growth. During the process of the release of buds from dormancy, many changes in some chemical components in floral buds, particularly the contents of endogenous hormones (Total natural indoles, IAA, $GA_3$ and ABA) (**Table 4** and **5**) and endogenous phenols (Total, free and conjugated phenols) (**Table 5**) play a vital role in regulating dormancy and bud break. Several studies focused on the relationship between the endogenous hormones and dormancy either in buds **[21]**, in tubers **[22]** or in seeds **[23]**. During the whole testing period, IAA and $GA_3$ concentrations in buds of "Astrachan" apple at their release from dormancy was higher than those of before bud break. In contrast, ABA concentration in floral buds was higher before bud break than that of at dormancy releasing (**Table 4**). This suggested that higher IAA and $GA_3$ contents and lower

ABA content were needed for release of "Astrachan" apple buds from dormancy. In this concern, Dong et al. [21] showed that, growth-promoting hormones such as $GA_3$ and IAA found to be gradually increased, while growth-inhibiting hormones such as ABA decreased during bud break. GAs and ABA had the same precursor (Mevalonic acid) for their synthesis and the increase in the lighting time promoted the synthesis of $GA_3$ in buds. Moreover, the results showed that when the dormancy released, IAA/ABA ratio had the same changing tendency to IAA content (**Table 4**). Moreover, $GA_3$/ABA ratio in buds had the same changing tendency to $GA_3$ content during release from dormancy (**Table 4**). So, it could be presumed that IAA/ABA and $GA_3$/ABA ratio determined the metabolism direction. In addition, the ratios of IAA/ABA and $GA_3$/ABA in apple buds were decreased in dormant buds, while increased in opening ones. In this concern, it has been suggested that the balance of several hormones played a more important role than the level of certain single hormone in the procedure of dormancy releasing and opining of buds [21]. Moreover, the control of apple bud dormancy induction, maintenance and release therefore is mediated, at least in part, by changes in hormone signaling as it is also known for tree bud dormancy [24]. High levels of endogenous IAA and $GA_3$ and low level of endogenous ABA contents (**Table 4**) which noticed in "Astrachan" apple trees applied with mineral oil and Universal at different concentrations agreed with the explanation in [25] which assumption that mineral oil and DNOC application increased all substances stimulate flower formation, such as $GA_3$, auxins…. etc., and decreased the flowering inhibitor such as ABA…etc. In this concern, it has been concluded that the induction of flowering could be correlated with a natural rise in gibberellin which promotes flower formation in plants by either facilitating the formation of flowering hormone in the leaves or expressing it in the growing buds [26]. The physiological effect of oil-DNOC on bud break is via respiration; therefore, its effect is strongly influenced by the prevailing temperatures and during the week following application. It has been shown that a high day-time temperature is essential for a good effect [27]; the cause for the effect is a temporary anaerobic condition in the buds. This leads to ethanol production which in turn causes the effect [28]. Ethanol production explains the sensitivity of buds to treatments with oil-DNOC during excessive temperature and when the soil is water logged. Under normal situations the phytotoxic risk is low, enabling application even at bud swell. It was shown that at this stage the swelling terminal buds are inhibited (by DNOC) and this may increase the potential of the laterals to break [29]. Moreover, Abbott [30] suggested that the dormancy breaking effect of spraying with DNOC can be attributed to the caustic action of this compound in killing the scales and so preventing competitive resorption of nutrients. In this concern, it has been found that the DNOC sprays certainly led to very rapid shriveling of bud-scales [31]. On the other hand, chilling is not an absolute requirement for bud break, because high temperatures [32], bud scale removal [33], which implies wounding stress, and anaerobic conditions [34] can

replace the chilling requirements. Ethylene production increases following various disturbances or stresses in plants. These disturbances can be induced by abiotic or biological agents [35]. Environmental stresses that induce ethylene production include physical wounding and cutting, chilling, drought, and water flooding [36]. Moreover, stress resulting from many rest-breaking treatments like tree defoliation, shoot decapitation or application of certain chemicals is known to increase ethylene levels in plants [37]. Also, Hillman et al. [38] concluded that ethylene appears to play an important role during normal bud break following shoot decapitation. Moreover, 1-Aminocyclopropane-1-carboxylic acid (ACC), a precursor for ethylene synthesis, increased during the transition from dormancy to the active state in *Prunus avium* L. and *Prunus serrulata* Lindl [39]. In this respect, El-Shereif et al. [40] reported that bud scale removal increased bud break and 1-aminocyclopropane-1-carboxylic acid (ACC) content in buds. Also, the results indicate that bud break of grapevine seems to be associated with the promotion of ethylene biosynthesis caused by wounding stress. Moreover, the mode of action of mineral oil spray in increasing bud break are to the reduction in the amount of water lost from the buds and mostly prevent them from drying out, so that, they can continue their normal metabolic activities and mobilization of nutrients to the developed buds [41]. In general, dormancy-breaking agents and time of application positively affected advancing bud break as well as flowering as a result of increased yield. Even though mineral oil gives a similar effect on bloom timing, I would suspect that it works in a different way. I would guess that dormant oil increases the boundary layer temperature of the tree, making it "feels" warmer than actual ambient air temperature. So, if chill hours have been accumulated, and the nights are short enough, the tree would bloom since it is now warm enough [26].

The most of phenol compounds have been isolated from bud scales and have growth inhibitor role in buds. Literature reported by researchers and our works (**Table 5**) indicate that phenol compounds increased during rest in flower buds, then decreased after rest and completely eliminated at blooming. The chilling period influences during disappear them. Therefore, the previous works were undertaken to determine if such a relationship exists between bud break and the phenol contents or not [42]. Moreover, Codignola et al. [43] reported that, phenol composition has been increased from November to February and then, have been decreased in March and have been eliminated on blooming stage in peach buds. Several studies have shown correlation between bud break and seasonal variation of phenols in trees [44]. The evaluation of dormant buds has shown that, phenol compounds rarely occur in a free state within the cell; rather they are commonly conjugated with other molecules. This finding could be taken some evidence, that, phenols could be play important role to protection buds during winter, dormant season and bud break. The decrease in free phenols and the increase in conjugated phenols after mineral oil or Universal at different concentrations treatments (**Table 5**) may be due to that the reduction in free phenols contrasted with the increase in total indoles (**Table**

**5)** i.e. endogenous promoters increased and consequently indigenous inhibitors decreased in the buds which led to increasing in plant growth parameters. In this respect, Kefeli and Kutacek **[45]** suggested that plant phenol may be decided into three groups; promotive, inhibitor and inactive. They added that promotion of plant growth by phenols may proceed through the modulation of either IAA biosynthesis or its destruction. Moreover, Wang et al. **[46]** 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 stimulated the oxidation process of phenols by increasing the peroxidase activity.

The favorable effect of mineral oil or Universal at different concentrations on the date of flower bud opening (**Table 1**) may be due to their stimulation effect on natural $GA_3$. In this connection, Subha-Drabandhu **[26]** concluded that the induction of flowering could be correlated with a natural rise in GAs in plants to facilitate the formation of flowering hormone in the leaves or expressing it in the growing buds. In our study, mineral oil or Universal at different concentrations positively affected the date of flower bud opening. This may be due to the increase in $GA_3$ and IAA and the decrease in ABA contents (**Table 4**). The increase in percentage of bud break and fruit set (**Table 2**) may be attributed to their stimulation effect of buds opening on formation of sink in shoot tip. In this concern, it has been 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 **[47]**. The improving effect of mineral oil or Universal at different concentrations on yield and its components (**Table 2 and 3**) was mainly attributed to its positive action on enhancing growth parameters and photosynthetic pigments.

## 5. Conclusion

The foliar application with mineral oil or Universal at different concentrations for "Astrachan" apple trees hastened the bud break, shortened the period of flowering and improved bud growth and fruit set. Universal at 2% and Mineral oil at 6% was found the most effective and significantly improved the contents of endogenous IAA, $GA_3$ and conjugated phenols as well as reduced ABA and free phenols content. This is led to the increasing in the percentages of the bud break and fruit set, as well as the reduction in the period of flowering, and finally the increasing in the yield. Therefore, we recommend using mineral oil at 6% as operator safer bud break promoters for the increase in "Astrachan" apple trees productivity. It may provide a well strategy for the increase in the percentages of bud break and fruit set, and the reduction in the flowering period to protect the floral buds against the high temperature in late winter in Egypt.

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