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CHANGES IN PROTEIN CONTENT AND OXIDASES ACTIVITIES WITH FRUIT DEVELOPMENT AND RIPENING IN THE LEAVES AND FRUITS OF OLIVE (*OLEA EUROPAEA* L. *CV. ZARD*) DURING "ON" AND "OFF" YEARS

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Abstract

Results of the studies on olive trees from four regions of Iran (Roudbar, Gilvan, Gorgan, and Zanjan) indicate that soluble protein content as well as the activity of peroxidase and polyphenoloxidase in the leaves and fruits increases and continues to do so until complete ripening of fruits. After ripening, protein content and enzyme activities don't change or slightly decrease until the harvest of fruits. The trend seems to be similar in the "on" and "off" years, but in the "off" years the protein content and enzyme activity in the leaves and fruits are greater reflecting the various metabolic status of the plant during floral induction and fruit ripening.

Introduction

Olive is an important plant with high nutritional and economical value. The protein content of olive is low but it plays an important role in inducing the defense responses wherein some enzymes such as peroxidase (EC1.11.17) and polyphenoloxidase (EC1.14.18.1) play an important role (Asada, 1992; Dell'Aquila & Spada, 1993). Floral induction and the fruit set in plants are important developmental processes which accompany the appearance of changes in the leaves (Ballard & Jenkins 1991). Early studies by Ross (1970) showed that protein inhibitors like cycloheximide suppress the flowering in *Xanthium strumarium*. Oota & Umemura (1970) demonstrated that a change in RNA base composition was associated with floral induction in *Pharbitis nill*. Studies on *Pharbitis nill* and *Hyocyamus niger* involving *in vitro* translation of mRNA and analysis of the protein products by SDS-PAGE has revealed both quantitative and qualitative changes in mRNA composition associated with floral induction and fruit formation (Van-loon, 1971; Warm 1984; Lay-yee *et al.*, 1987; Araki & Komeda, 1990).

Changes in protein contents during the ontogenic development and changes in gene expression in association with floral induction have been reported in *Amaranthus*, *Sinapis alba*, *Chrysanthenum segetum* (Pryke & Bernier 1978; Kohli *et al.*, 1980; Rembur & Nougarede, 1989; Ballard & Jenkins, 1991).

Studies on enzyme indicated that one of the major roles of peroxidase and polyphenolxidase is the control of cell growth. This control is affected by peroxidases through the change of amount of IAA (Barcelo *et al.*, 1990) and by polyphenol oxidases through the change in the synthesis of phenolic compounds which are important in many biosynthetic pathways (Sanchez-Ferrer *et al.*, 1993). In addition, these enzymes change both quantitatively and qualitatively by different stress factors (Abeles *et al.*, 1988; Apostol *et al.*, 1989). The activity of these enzymes is a suitable determinant in the study of plants infected by pathogens (Asada, 1992; Miyake & Asada 1992). The present report describes the amount of protein and the activities of peroxidase and polyphenoloxidase in the leaves and fruits of olive trees during flower and fruit formation.

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Material and Methods

Leaves and fruits of olive varieties from four regions of Iran (Roudbar, Gilvan, Gorgan and Zanjan) were collected every two weeks in various periods of flower formation and fruit growth and development and as well as 75 to 165 days after fruit set. The samples were stored at -20°C or in lyophylized form at -4°C.

Extraction for protein and enzyme assays was done on ice using Tris-Glycine buffer pH 6.8 and 5% PVPP (Lavee & Avidan, 1994). The supernatant was isolated by centrifugation of 41500 g for 45 minutes at -4°C (Kohli & Sawhney, 1979) and subsequently stored at -20°C. Appropriate samples were used for determination of protein content and enzyme activities. Protein content was determined using Lowry's method (Lowry *et al.*, 1951; Cooper 1977). PO activity was measured spectrophotometrically at 530 nm using the methods developed by Ewans (1968); Abeles *et al.*, (1991) and Brownleader *et al.*, (1993). PPO activity was determined spectrophotometrically at 400 nm using the methods of Van-loon (1971); Chabanet *et al.*, (1993) and Asaka & Aogama (1994).

ANOVA and Duncan's multiple range test (DMRT) were used to compare the protein content and PO and PPO activities at various times during flower formation and fruit growth and development in "on" and "off" year in leaves and fruits. The results were subjected to statistical analysis at the 5% and 1% levels.

Results and Discussion

The protein content in leaves of olive trees during the various periods of floral formation, fruit growth and development in "on" year is shown in Fig. 1. An increase in protein content occurs in June, the period of fruit formation and continues until September, the period of fruit ripening. It remains constant until November when the fruits ripen and decreases during the subsequent months. Protein contents in various periods showed significant differences. These changes are seen in the leaves of Roudbar, Gilvan and Gorgan olive trees in "on" year.

The protein content during the various periods of floral formation and fruit growth and development in leaves of olive trees in "off" year are shown in Fig. 2. The trend of change in protein content is similar in "on" and "off" years. The protein content in various periods and various regions showed significant differences. It is interesting to note that the protein content in "on" year is lower than in "off" year which is in agreement with the result of Lavee & Avidon (1994).

The results on the protein content during various periods of fruit growth and development in "on" year showed an increase in the protein content until September which remained constant until November, when the fruit completely ripened and decreased thereafter (Fig. 3). The trend of change in protein content during various periods of floral formation, fruit growth and development in fruits of olive trees is similar in "on" and "off" years, but is generally lower in "on" year than in "off" year (Fig. 4).

The activity of peroxidase in the leaves during various periods of flower formation and fruit growth and development in the olive trees in "on" year diminishes in the primary stages of fruit formation, but increases at the time of complete maturation and again decreases at the end. The statistical studies indicate that the enzyme activities have significant differences during these periods (Fig. 5).

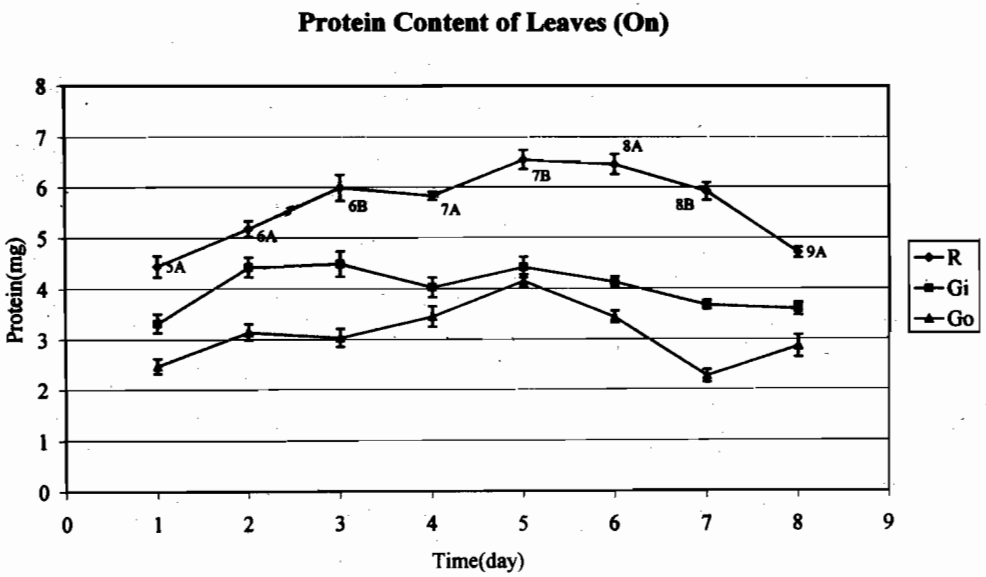

Fig.1. Protein content in the leaves of Roudbar, Gilvan and Gorgan in "on" year.
4A=8th June-23rd July, 5A=7th -22nd August, 6A=7th -22nd September 6B=23rd September-7th October, 7A=8th -22nd October, 7B=23rd October-6th November, 8A=7th -21st November, 8B=22nd November-6th December, 9A=7th December-21st December

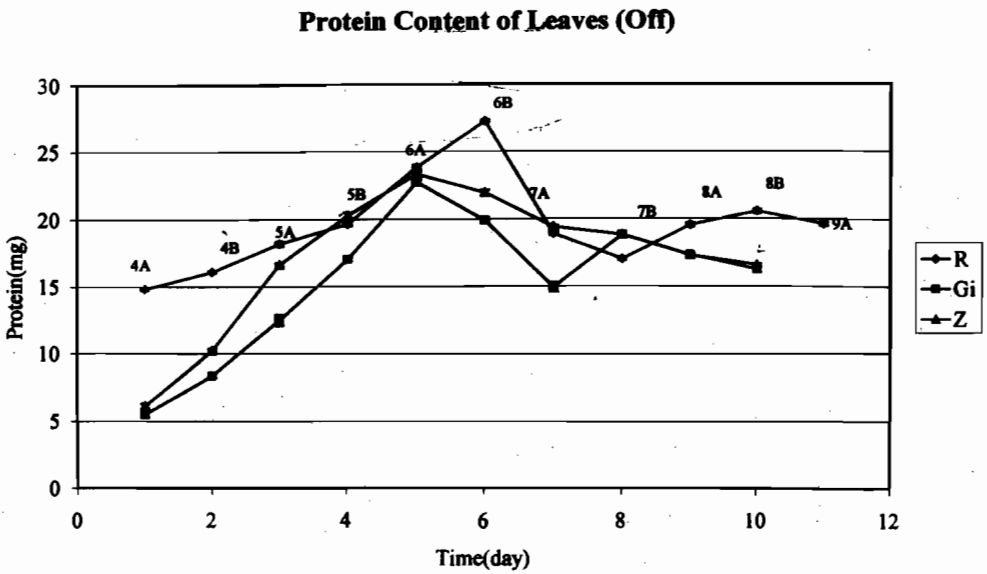

Fig.2. Protein content in the leaves of Roudbar, Gilvan and Zanjan in"off" year.

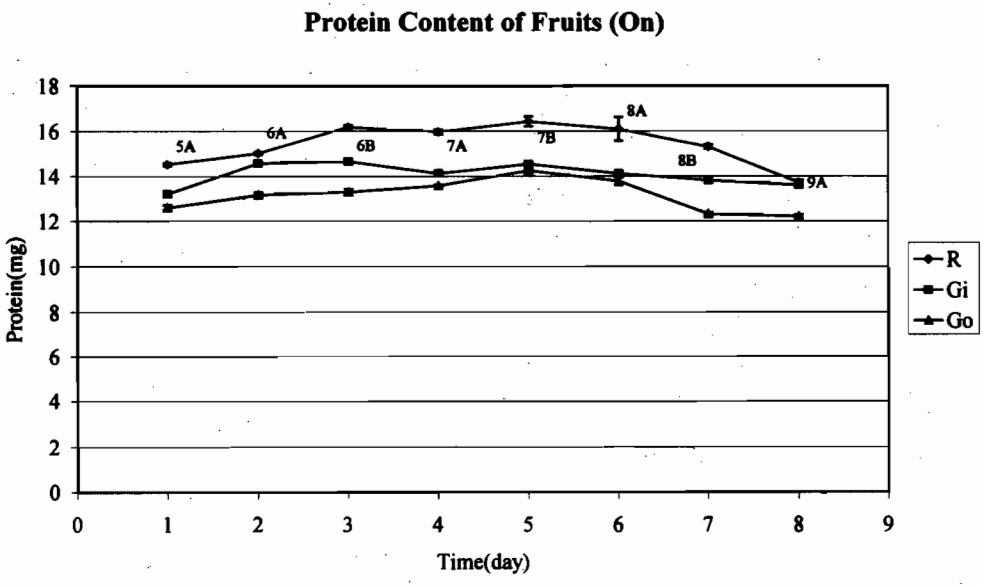

Fig.3. Protein content in fruits of Roudbar, Gilvan and Gorgan in "on" year.

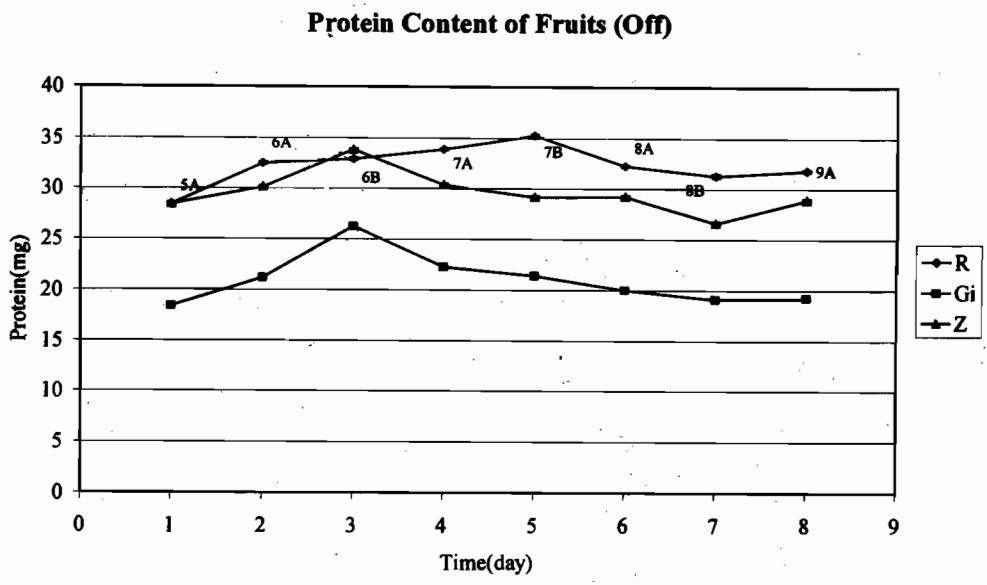

Fig.4. Protein content in fruits of Roudbar, Gilvan and Zanjan in "off" year.

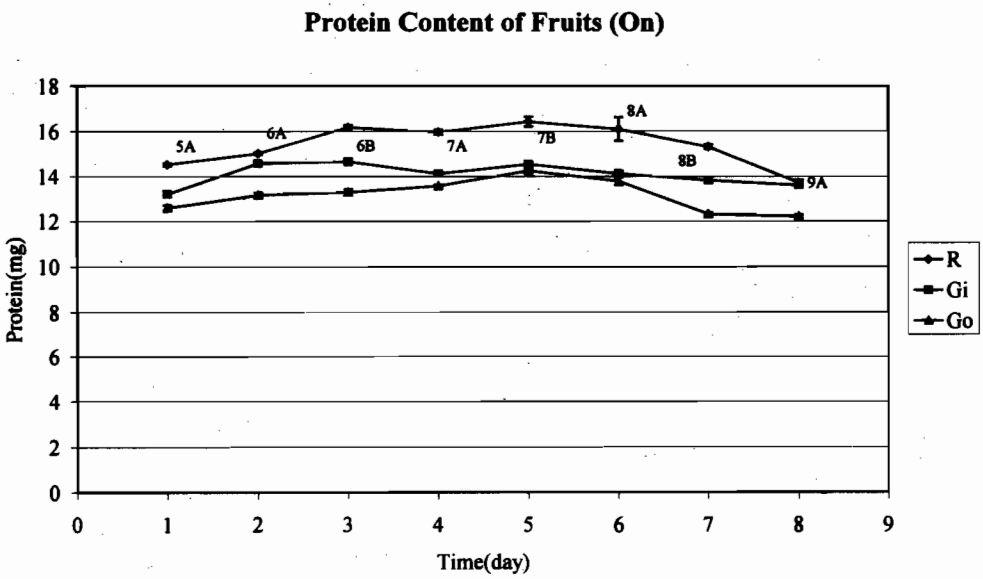

Fig.3. Protein content in fruits of Roudbar, Gilvan and Gorgan in "on" year.

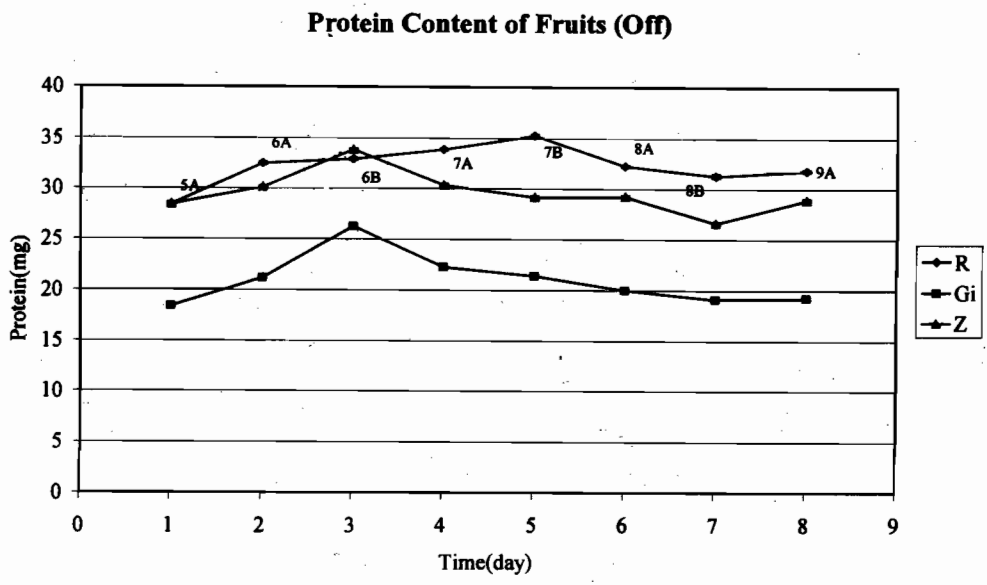

Fig.4. Protein content in fruits of Roudbar, Gilvan and Zanjan in "off" year.

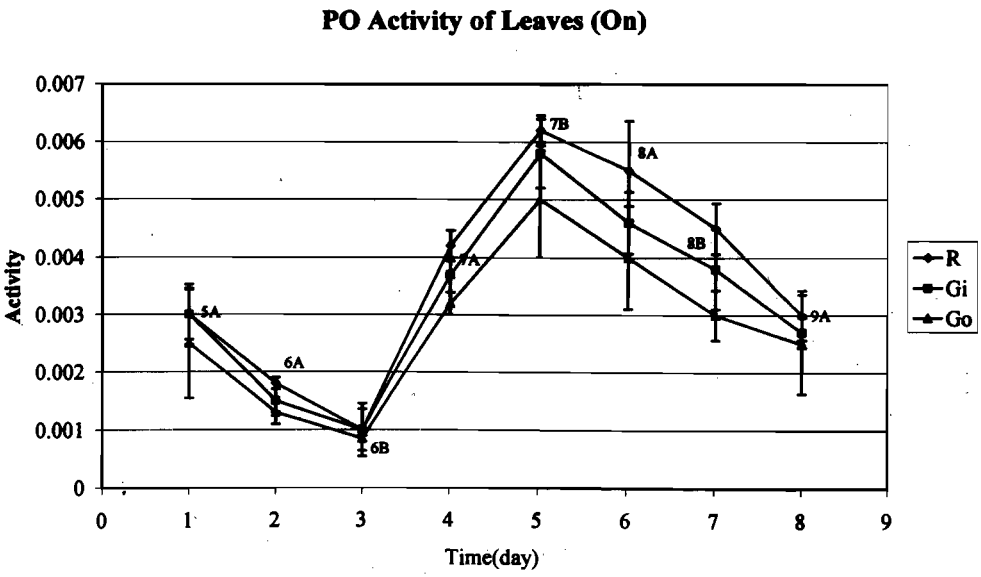

Fig.5. Activity of peroxidase from the leaves of Roudbar, Gilvan and Gorgan in "on" year.

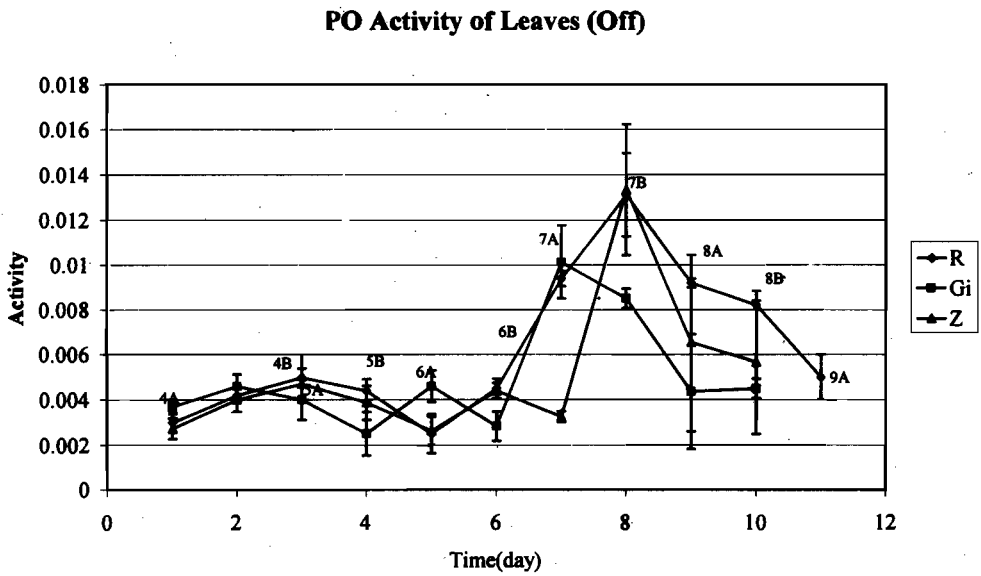

Fig.6. Activity of peroxidase from the leaves of Roudbar, Gilvan and Zanjan in "off" year.

Table 1. Mean value of protein content in leaves and fruits of different regions, in "on" and "off" year.

	Leaves		Fruits	
Region	**"on"**	**"off"**	**"on"**	**"off"**
Roudbar	5.629[a]	18.98[a]	15.65[a]	31.99[a]
Gilvan	3.989[b]	15.49[e]	14.08[b]	21.48[c]
Gorgan	3.100[e]	17.85[b]	13.15[c]	3.66[b]

Table 2. Mean value of protein content in the leaves and fruits of different months, in "on" and "off" year.

	Leaves		Fruits	
Time	**"on"**	**"off"**	**"on"**	**"off"**
23rd July-6th August		8.81[e]		
7th August-22nd August	3.409[c]	14.40[f]	13.45[f]	25.08[g]
23rd August-6th September		18.26[d]		26.10[f]
7th September-22nd September	4.427[d]	21.45[b]	14.26[b]	27.94[d]
23rd September-7th October	4.580[c]	23.97[a]	14.72[b]	31.00[a]
8th October-22nd October	4.433[c]	19.43[c]	14.53[c]	28.82[b]
23rd October-6th November	5.037[a]	16.94[e]	15.08[a]	28.12[c]
7th November-21st November	4.674[b]	18.56[d]	14.78[b]	27.16[c]
22nd November-6th December	3.896[e]	18.15[d]	13.82[e]	
7th December-21st December	3.720[f]		13.18[g]	

The letters of a, b, c, d, e, f, and g indicate the significance levels among various treatment means.

Table 3. Mean values of peroxidase and polyphenoloxidase activities in leaves and fruits of different regions in "on" and "off" year.

	PO activity in leaves		PPO activity in leaves		PPO activity in fruits	
Regions	**"on"**	**"off"**	**"on"**	**"off"**	**"on"**	**"off"**
Roudbar	0.00359[a]	0.00623[a]	0.001262[a]	0.002072[a]	0.0007975[a]	0.0007188[a]
Gilvan	0.00326[a]	0.00442[b]	0.001055[ab]	0.001800	0.0006533[ab]	0.0006555[a]
Gorgan	0.00280[a]		0.0009050[b]		0.0006592[b]	
Zanjan		0.00502[b]		0.001833[a]		0.0006382

The activity of peroxidase in the leaves during various periods of the floral formation and fruit growth and development in the olive trees are similar as is "on" and "off" years (Fig. 6). It showed significant differences during the various periods and various regions. Peroxidase activities determined in fruits in the different stages of fruit growth and development in "on" and "off" years did not show such variation.

The activity of polyphenoloxidase in the leaves during various periods of the fruit growth and development in "on" year is similar to peroxidase activity with some fluctuations in the beginning and after it increased during the fruit ripening time it decreases slightly (Fig. 7). The activity of this enzyme showed significant differences in the various periods with highest activity observed during the first half of November.

The activity of polyphenoloxidase during various periods of floral formation and fruit growth and development in the leaves of Roudbar, Gilvan and Zanjan olive trees in "off" year showed that the activities of this enzyme are similar in "on" and "off" years, while they showed significant differences in the various periods with no significant differences in behavior in various regions (Fig. 8).

Table4. Mean value of peroxidase and polyphenoloxidase activities in leaves and fruits of different months in "on" and "off" year.

	PO activity in leaves		PPO activity in leaves		PPO activity in fruits	
Regios	**"on"**	**"off"**	**"on"**	**"off"**	**"on"**	**"off"**
8th June-23rd July		0.002733^{f}	0.0006^{gh}	0.0006gh		0.00003^{e}
23rd July-6th August		0.003933^{c}	0.0007833^{fg}	0.0007833^{fg}		0.00025^{e}
7th August-22nd August	0.002833^{d}	0.004733^{de}	0.0006233^{e}	0.00098^{f}	0.0001144^{e}	0.0001522^{f}
23rd August-6thSeptember		0.004067^{e}		0.00040^{h}		0.001033^{a}
7th September-22nd September	0.001533^{e}	0.002433^{f}	0.0004233^{f}	0.00040^{h}	0.0005500^{d}	0.0008433^{b}
23rd September-7th October	0.0009556^{f}	0.004433^{de}	0.0004233^{f}	0.0016^{e}	0.0006667^{c}	0.0008893^{b}
8th October-22nd October	0.003700^{c}	0.005200^{cd}	0.0009^{d}	0.0033^{b}	0.001013^{a}	0.001097^{a}
23rd October-6th November	0.005667^{a}	0.010500^{a}	0.002333^{a}	0.004133^{a}	0.0009367^{a}	0.00022997^{b}
7th November-21st November	0.004533^{b}	0.008678^{b}	0.001467^{b}	0.003422^{b}	0.0008300^{h}	0.0006922^{c}
22nd November-6th December	0.003767^{c}	0.006033^{c}	0.0013^{c}	0.00252	0.00006556^{c}	0.00071^{c}
7th December-21st December	0.002739^{d}	0.004733^{de}	0.001017^{d}	0.0025^{d}	0.0004600^{d}	0.0049^{d}

The letters a, b, c, d, e, f, g, and h indicate the levels of significance among various treatment means.

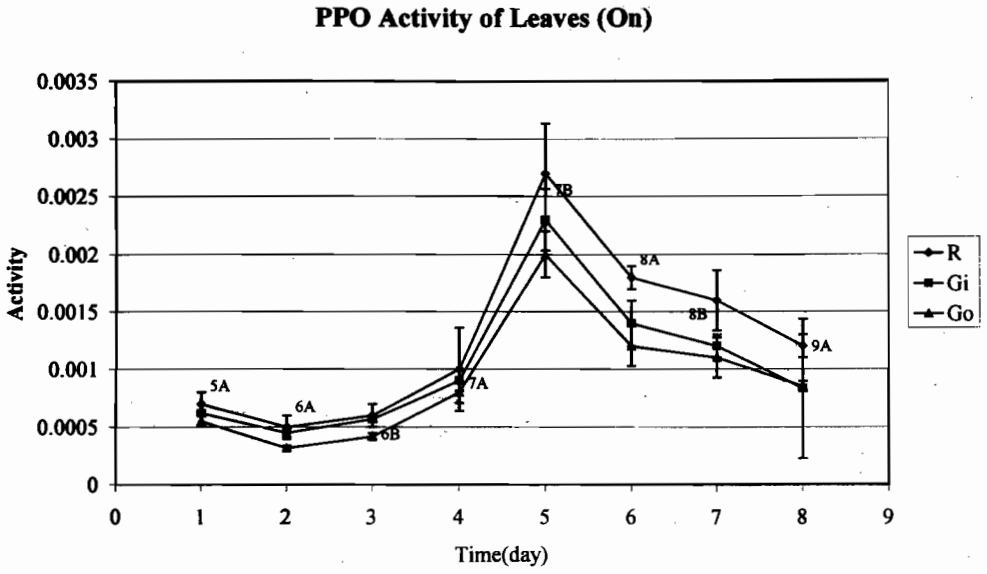

Fig.7. Activity of polyphenoloxidase from the leaves of Roudbar, Gilvan and Gorgan in "on" year.

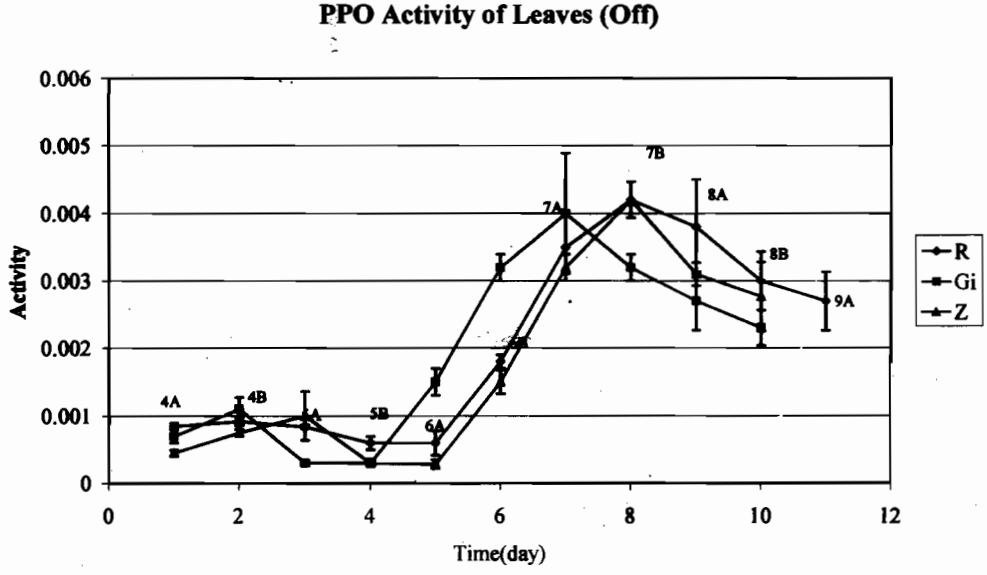

Fig.8. Activity of polyphenoloxidase from the leaves of Roudbar Gilvan and Zanjan in "off" year.

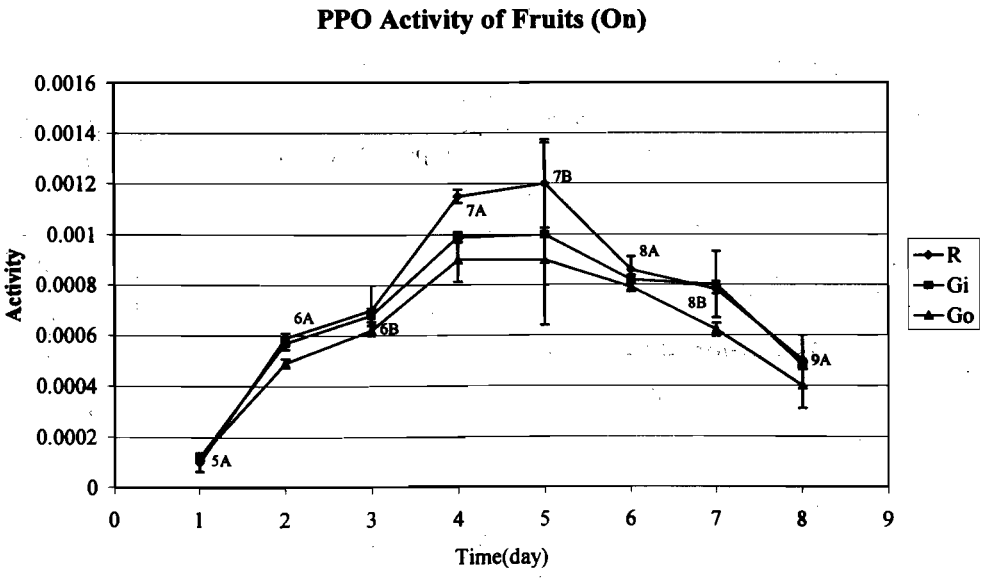

Fig.9. Activity of polyphenoloxidase from fruits of Roudbar, Gilvan and Gorgan in "on" year.

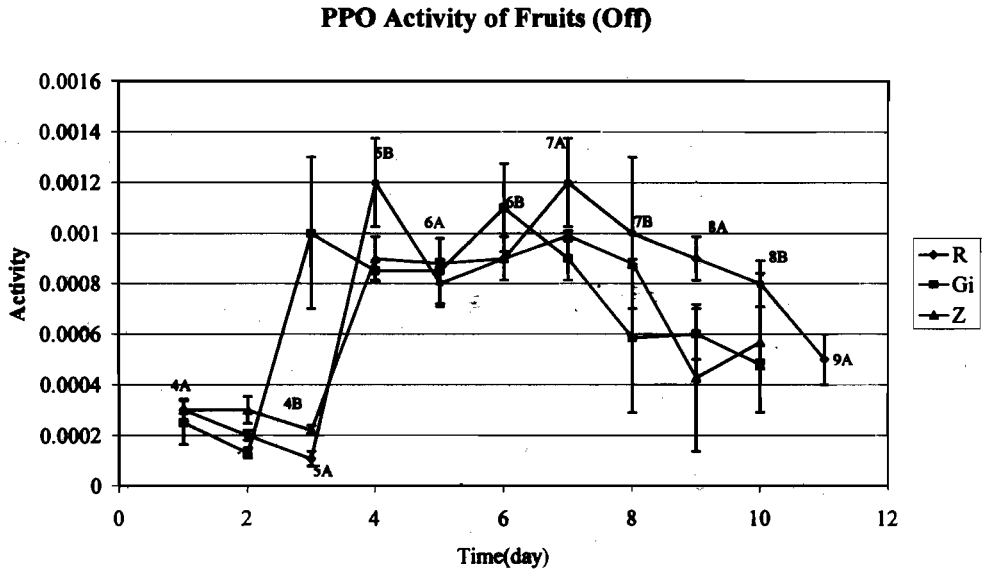

Fig.10. Activity of polyphenoloxidase from fruits of Roudbar, Gilvan and Zanjan in "off" year.

The activity of polyphenoloxidase in the fruit of olive trees during various periods of floral formation and fruit growth and development in "on" year showed an increase during fruit formation and ripening stages with decrease in later periods of sampling (Fig. 9). Enzyme activity showed significant differences in the various periods.

The activity of polyphenoloxidase during various periods of the floral formation and fruit growth and development in the fruits of Roudbar, Gilvan and Zanjan olive trees in "off" year showed that the activity of enzyme is similar in "on" and "off" years (Fig. 10). It showed significant differences in the various periods with no significant differences observed in trees from various regions.

Peroxidase and polyphenoloxidase activities in the leaves and fruits of olive trees in "on" and "off" years are similar to those of *Amarrantus caudatus* found by (Ballard & Jenkins, 1991) and of *Silene coeli-rosa* (Taylor *et al.*, 1990). Similarly the results of protein studies in olive trees are similar to those on *Hyoscyamus* during the flowering period (Warm, 1984) and *Pharbitis nill* cotylendon (Lay-Yea *et al.*, 1987).

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