J. Crop Prot. 2017, 6 (3): 327-340

Research Article

Effect of organic and biological fertilizers on pomegranate trees: yield, cracking, sunburning and infestation to pomegranate fruit moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

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Abstract: To investigate the effect of different fertilizers on yield and health related traits of pomegranate fruit, a field experiment was carried out during 2014 and 2015 seasons on six years old pomegranate trees cv. 'Bajestani' growing in sandy loam soil under drip irrigation system in Khalilabad city, Khorasan, Iran. Organic fertilizers including: cow manure (25 kg/tree), vermicompost (5 kg/tree), and granulate humic (2 kg/tree) were applied without or with biofertilizers (azetobarvar, phosphobarvar and potabarvar at 1 liter/tree) by placement method. Results showed that plants treated with combination of organic and biofertilizers had higher yield and lower fruit drop than plants treated with organic fertilizers individually. The lowest fruit cracking (7.5%, 2.25%) as well as fruit infestation with *Ectomyelois ceratoniae* (Zeller) (27.5%, 25.34%) were observed in the plants treated with both biofertilizers and humic in 2014 and 2015, respectively. The most infestation to *E. ceratoniae* was observed in control treatment (38.07%, 31.44%) in both years, respectively. Fruit sunburn was not affected by nutrition of any type in both years. Results of leaf analysis revealed that plants treated by a combination of biofertilizer and humic had higher levels of macro and micronutrients compared with untreated plants. Altogether, our results suggest that biofertilizer in combination with organic fertilizers, especially granular humic, could be used in pomegranate orchards management to improve pomegranate yield, as well as to prevent crop losses resulting from cracking, nutrient deficiency and *E. ceratoniae* infestation.

Keywords: pomegranate, organic fertilizers, fruit cracking, sun burning, *Ectomyelois ceratoniae*

Introduction

Pomegranate *Punica granatum* L., a member of Lythraceae, is one of the oldest edible fruits that has been cultivated in the sub-tropical, arid and semi-arid regions of the world (Damania, 2005). Iran is the leading country in pomegranate cultivation and production in the world. The cultivation area in this country is about 82,000 ha with an annual production of around 941,000 tons (Jalili, 2016). Fruit sunburn, cracking and pomegranate fruit moth infestation are among the main constraints for

Handling Editor: Jafar Mohaghegh Neyshabouri

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Received: 29 November 2016, Accepted: 6 August 2017
Published online: 20 August 2017

[DOR: 20.1001.1.22519041.2017.6.3.8.8]

pomegranate production, which greatly reduce quality and marketability of this fruit (Gharesheikhbayat, 2006; Bakeer, 2016). Fruit cracking or splitting is one of the main disorders that affects pomegranate quality and quantity. Fruit cracking not only reduces marketability and consumer acceptance, but also provides ports of entry for insects and fungi and renders fruits more susceptible to the environmental stresses, hence causing a serious commercial loss to farmers (El-Rhman, 2010; Abubakar *et al*., 2013). Water deficiency, soil texture and structure, mineral deficiency as well as air humidity are among the factors that have a profound effect on pomegranate fruit cracking (Jalili, 2016). Sun burning is another limiting factor causes serious economic losses to the pomegranate producers all around the world (Melgarejo *et al*., 2004). Some of the pomegranate fruits are sensitive to the intense sun light, which is one of the obvious characteristics of the most pomegranate production regions. Sun burnt husks damages appear in the form of large dark spots on the fruit skin, which decreases marketability as well as juiciness of the arils resident at that location. Value of sunburnt fruits is reduced and these fruits are not marketable because of the poor fruit quality. Pomegranate fruit moth, *Ectomyelois ceratoniae* (Zeller) (Lep: Pyralidae), is the most important pest of Iran's pomegranate orchards (Azqandi *et al*., 2015). This pest greatly reduces the fruit quality and quantity causing considerable damage to the pomegranate producers annually (Shakeri, 2005). It is estimated that pomegranate fruit moth causes significant economic losses affecting 30-60% of the pomegranate production in Iranian orchards (Azqandi *et al*., 2015). This pest is also a major constraint for other pomegranate producer countries including Turkey, Tunisia, Iraq and Algeria (Demirel, 2016).

It is now well established that healthy, vigorous plants are better able to withstand biotic and abiotic stresses (Altieri and Nicholls, 2003). Due to the higher cost and hazardous effect of chemical fertilizers, application of biofertilizers has gained momentum in the recent years to enhance plant growth and yield (Aseri *et al*., 2008). Various biofertilizers have been successfully applied in different fruit crops. *Arbuscular mycorrhizae*, composts, vermin compost, humus, animal manure Tytanit® are among biofertilizers that have showed positive influence on vegetative growth and yield of fruits trees (Mosa *et al*., 2014). Organic fertilizers play significant role in maintaining the sustainability of soil and improving soil physico-chemical properties and micro-fauna activity (HI wale, 2015). It is reported that application of humus, vermicompost and cow manure improved the vegetative growth as well as fruit production in different fruit tree species (Mosa *et al*., 2014). Pomegranate is known as an organic crop in Iran and consumers prefer fruits that have not been subjected to the chemical substances. Most of the approaches that are used in sustainable agriculture have been found to provide a balanced plant nutrition, and at the same time to increase the availability of certain elements and improve the tolerance of plants to pests and disease (Osborn *et al*., 2003). Nutrients are important for growth and development of plants and also microorganisms, and they are important factors in disease control (Agrios, 2005; Dora's, 2009). Also, many studies have shown that plant resistance to pests and diseases is linked to optimal physical, chemical, and perhaps most importantly, biological properties of soil (Altieri and Nicholls, 2003; Zander *et al*., 2007). Several studies revealed various types of signaling between soil and plants mediated by soil organic matter that could be enhanced by management (Phelan *et al*., 1995 and 1996; Stone *et al*., 2004).

Healthy appearance is one of the main factors affecting marketability of fruits. Sunburn, fruit cracking, and contamination with pomegranate fruit moth are three disorders reducing pomegranate quality and quantity in Iran. From the view point of sustainable agriculture, management of horticultural practices is one of the main ways to produce

[DOR: 20.1001.1.22519041.2017.6.3.8.8]

healthy crops. Therefore, the objective of the present study was to investigate the effect of organic fertilizers application (cow manure, vermicompost, and granule humic) individually or combined with Biofertilizer, on the yield, leaf minerals content, fruit drop, cracking, sunburn and infestation with pomegranate fruit moth.

Materials and Methods

Plant materials and treatments

This investigation was carried out during two successive growing seasons (2014 and 2015) on six years old pomegranate trees cv. 'Bajestani', one of the most important pomegranate cultivars in the North East of Iran, grown in sandy loam soil under drip irrigation system in a commercial orchard located at Khalilabad in Khorasan-Razavi Province Iran. Khalilabad is located at 35°15'21"N latitude 58°17'11"E longitude and 975 m above the sea level with an annual average temperature of 17.5 °C and an annual average precipitation of 190 mm which is mostly precipitated in the winter. Soil physical and chemical properties of the experimental orchard are presented in Table 1. The experimental trees were planted at 4 × 5 m spacing and received the same horticultural practices. The 48 trees were arranged in randomized complete block design (RCBD) and eight treatments were analyzed with six replications for each treatment (1 replication = 1 tree).

Three types of organic fertilizers were applied in this study viz. cow manure (CM) (25 kg/tree in the first year), vermicompost (V) (5 kg/tree in both years), and granule humic (GH) (2 kg/tree in both years). Cow manure is more stable in the soil and due to the economic issues, local gardeners use this fertilizer every couple of years. On the other hand, all of the biofertilizers used in this study were purchased from commercial companies and were applied according to the manufacturer recommendations; that suggest these products should be used at a defined dose every year.

Biofertilizer were formulated by Zist-Phanavar-Sabz Company under commercial names of Azotobarvar, Potabarvar, and Phosphatobarvar which consisted of bacteria extracted from Iranian soils with the ability to fix nitrogen (N), potassium (K) and phosphorus (P).

Treatments included the following: cow manure (CM), vermicompost (V), granule humic (GH), biofertilizer (B), cow manure + biofertilizer (CM + B), vermicompost + biofertilizer (V+B), granule humic + biofertilizer (GH+B), and Untreated plants (U). Analysis of organic fertilizers was performed prior the experiment (Table 2) and characteristics of biofertilizers obtained from manufacturing company and time of application was are listed in Table 3.

Table 1 Some of physical and chemical properties of the orchard soil used in the study (compound soil samples from depths of 0-30 and 31- 60 cm).

Particle size distribution (%)			Texture class	Ec (dS/m)	pH	Available nutrients							
Sand	Silt	Clay				Organic Carbon (%)	N (%)	P (mg/kg)	K (mg/kg)	Fe^{2+} (mg/kg)	Mn^{2+} (mg/kg)	CU^{2+} (mg/kg)	Zn^{2+} (mg/kg)
70	21	9	Sandy loam	1.54	7.72	0.3	0.03	19.1	350.95	2.9	8.5	0.83	4.06

Table 2 Analysis of organic fertilizers used in this investigation.

Fertilizers	N (%)	P (%)	K (mg/kg)	pH	EC (dS/m)
Cow manure	1.26	0.55	25.36	8.2	7.07
Vermicompost	1.2	0.52	0.82%	8.54	3.42
Granule humic	5	2	2%	3.38	4.28

Table 3 Characteristic of Biofertilizers used in this study and time of their application.

Biofertilizers	Trade name	Bacteria	Time of application		
			1	2	3
N biologic	Azetobarvar	*Azetobacter winland*: (04)	Emergence of first leaves	First fruiting	After 20 days
P biologic	Phosphate barvar	*Pantoa glomerance* (05) *Pseudomonas putida* (p13)	Budding spores	-	-
K biologic	Potabarvar	*Pseudomonas vancouverensis* *Pseudomonas coreensis*	After falling flowers	First fruiting	After 20 days

Fertilizers were applied in sub-soil placement method in February of each year. The placement regions were in the depth of 30 cm under tree canopy in a distance of 50 cm from trunk around the tree. To do this, a soil drill was used to bore holes in soil around four sides of trunk and according to the manufactured company, 250ml of biofertilizers were added to the organic fertilizers in each hole (one liter per tree). Trees were irrigated in a four days interval by four drippers per tree.

Fruits were harvested on the First of November in both seasons and number of fruits per tree, fruit weight, and tree yield were analyzed. At harvest time, fruits per tree for each treatment were weighted and then average yield/tree as kg was estimated. Average fruit weight was expressed as a fraction of the fruit yield to the number of fruits/tree.

Also, the percent of sunburn, cracked, and fruit drop as well as fruit contaminated by pomegranate fruit moth was investigated and recorded.

Percentage of fruit drop was calculated as follow (Khattab *et al*., 2012):

$$Fruit\ drop(\%) = \frac{(TNFS - TNFH)}{TNFS} \times 100$$

Where TNFS is total number of fruit set and TNFH is number of fruit at harvest.

At harvest time, percentage of pomegranate fruit moth infestation, cracked fruits, sunburned fruits as well as cracked and sunburned fruits per tree for each treatment were calculated by following equation:

(Total number of damaged fruits per tree/Total number of fruits per tree) × 100.

Pomegranate fruit moth infestation was expressed as a fraction of infected fruits (both dropped and those retained on tree) to the total number of fruit/tree (Sobhani *et al*., 2015).

Leaf nutrients (N, P, K, Mg, Fe, Zn)

In order to analyze the nutrient status of plants, fifty leaves were collected from the sixth node from the base of shoots at the second week of May in both years. After washing with tap water and distilled water, leaf samples were dried in oven at 70 °C and subsequently were ground using mortar and pestle. One gram of powder was burned in muffle oven at 550± 25 °C. The resulting ash was then dissolved in 10 ml of 2 N HCl and adjusted to a volume of 100 ml with distilled water for determination of macro- and micronutrients (Chapman and Pratt, 1961). Total nitrogen content was determined using Kjeldhal method. Potassium was measured by flame photometer and phosphorous content analyzed by the molybdovanadate yellow color method by spectrophotometer. Zinc (Zn), Magnesium (Mg), and Iron (Fe) were determined using an atomic absorption spectrophotometer.

Statistical analysis

Analysis of variance (ANOVA) was performed by SAS software version 9.1 and means were compared using Duncan Multiple Range Test at $p < 0.05$ to determine the significance of differences between treatments.

Results and Discussion

Tree yield

For both years, tree yield showed significant differences among different treatments, (2014: $F_{7,35} = 4.64$; $P = 0.0009$; CV = 15.94). Biofertilizer individually had no significant positive effects on fruit characteristics but in

[DOR: 20.1001.1.22519041.2017.6.3.8.8]

combination with organic fertilizers improved some of the characteristics of pomegranate. Plants treated with both cow manure and biofertilizer had the highest tree yield (20.4 kg) in 2014, while plant treated with combination of granule humic and biofertilizer had the highest yield (53.12kg) in 2015 (Table 4). Control plants showed the lowest yield in both years. It is noteworthy that in 2014 some of the flowers from the first flowering peak were damaged because of a late spring frost. Therefore, some of the differences in the yield of the two successive years might be due to the frost, but we cannot ignore the effects of fertilization in the first year as well as maturation of trees.

The averages of tree yield in the combined treatments of vermicompost and granule humic with biofertilizer were higher than their individual treatments in the two studied years. Although in 2014, combination treatment of cow manure with biofertilizer had higher yield than cow manure alone, there were no significant yield differences between cow manure individually and its combination with biofertilizer (2015: $F_{7.35}$ = 22.42; P = 0.0001; CV = 14.68).

Our results indicated that the average yield (53.12 kg) of plants treated with granule humic and biofertilizer were 2.43 fold of control plants (21.79 kg) in the second year.

In agreement with our finding, Aseri *et al.* (2008) reported that biological treatment of *Azotobacter chroococcum* and *Glomus mosseae* significantly increased the fruit yield and other growth related traits of five years old pomegranate trees in the field conditions. Application of biofertilizer in combination with compost resulted in a better yield in olive trees compared to compost alone (Maksoud *et al.*, 2012). Also it is reported that Humic acid alone or in combination with biofertilizer and MSW (composted municipal solid waste) enhanced the yield of grapevine especially in the second season of experiment (Eman *et al.*, 2008). As compared with the individual treatments and control plants, combined treatments of NPK + manure + biofertilizer (*Azotobacter chroococcum* strain EB2) and manure + biofertilizer (*A. chroococcum* strain EB2) gave higher yield and improved some of the physical characteristics in mango fruits, including fruit weight, fruit firmness and peel thickness, (Omar and Bela, 2007). Results of our work and similar studies indicate that combined treatments of organic and biofertilizers had higher fruit yield than their individual treatments. For proper growth and reproduction, microorganisms in the biologic fertilizers need an appropriate condition which may have not been available in the sandy loam soils. Addition of organic matters to such soils will improve the physical properties of rhizosphere in favor of growth and development of microorganisms (Mader *et al.*, 2002; Baldock and Nelson, 2000). The following may be considered as some of the reasons for the increase in the second year than the first year: 1- No spring frost in second year 2- Increasing age of trees 3-Effect of fertilizers.

Since the organic fertilizers slowly release their elements in soil, they gradually affect yield (Steiner *et al.*, 2007) therefore are more effective in second year. As we did not observe any changes in climate conditions within two years, differences between treatments in one year may be related to the effects of fertilizers. Therefore, combined treatments of organic with biofertilizers showed to be more effective than their individual treatments.

Average of fruit weight

Plants treated with combination of biofertilizer and Granule Humic had the largest and heaviest fruits (209.49 g), while control plants had the least fruit weight (157.98g). Mean values for the remaining treatments had no significant differences (2014: $F_{7.35}$ = 1.42; P = 0.2295; CV = 19.85). In the second year, the mean values for fruit weight were significantly higher in the combined treatments, while, individual treatments were not significantly different from control plants. The highest fruit weight was observed in the plants treated with combination of biofertilizer plus Vermicompost (389.36g) and granule humic (373.88g) (Table 4). ($F_{7.35}$ = 4.87; P = 0.0007; CV = 13.66).

Table 4 Effects of different fertilizer treatments on the yield related traits in 2014 and 2015.

Treatments	Number of fruits / tree		Fruit weight (g)		Fruit drop (%)		Yield (kg)	
	2014	2015	2014	2015	2014	2015	2014	2015
Vermicompost (V)	57.33 b	114.67 bc	165.58 ab	213.54 b	31.195 ab	20.23 a	12.12 bcd	27.00 cd
Cow Manur (CM)	102.33 a	213.33 a	173.61 ab	193.41 b	23.90 abcd	17.67 ab	17.48 ab	40.27 b
Granule Humic (GH)	70.67 ab	239.17 a	193.93 ab	245.46 b	25.85abcd	14.54 ab	12.31 bcd	38.20 b
Biologic (B)	76.17 ab	126.67 bc	172.72 ab	230.92 b	26.34 abc	19.70 a	11.31 cd	29.20 c
B + V	73.83 ab	128.00 bc	174.28 ab	389.36 a	17.03 cd	14.52 ab	12.70 bcd	30.30 c
B + CM	105.50 a	172.67 ab	195.51 ab	302.32 ab	22.61 bcd	11.23 ab	20.40 a	40.70 b
B + GH	79.00 ab	130.17 bc	209.49 a	373.88 a	13.25 d	8.81 b	15.43 abc	53.12 a
Untreated	57.00 b	81.17 c	157.98 b	191.61 b	35.80 a	20.08 a	8.65 d	21.79 d

The values followed by the same letter do not show statistically significant differences (Duncan's multiple range test, $P < 0.05$).

Fruit drop

In 2014, the lowest (13.25%) and the highest (35.8%) fruit drop were recorded in the combined treatment of granule humic plus biofertilizer and control plants, respectively ($F_{7.35} = 3.29$; $P = 0.0085$; CV = 19.74). In the second year, the lowest fruit drop (8.81%) was observed in the plants treated with combined Granule Humic and biofertilizer, while, there were no significant differences between plants treated with individual biofertilizer and vermicompost with control plants ($F_{7.35} = 1.96$; $P = 0.0895$; CV = 17.40). In fact adding biofertilizer to the organic fertilizers could improve the plant performance and will result in the retaining more fruit per tree (Table 4).

Number of fruit per tree

Most of the treated plants had higher number of fruits per tree compared with the control plants in the first season. The only exception was vermicompost treated plants which showed no significant differences with control plants ($F_{7.35} = 2.76$; $P = 0.0217$; CV = 14.33). The highest number of fruits per tree (105.5) was obtained in the plants treated with cow manure in combination with biofertilizer. In the first season, the number of fruits per tree was not significantly affected by treatment of cow manure alone or in combination with biofertilizer, that was the same for Granule Humic individually or in combination with biofertilizer. In the second season, all of the treated plants had significantly higher number of fruits per tree compared with the control plants ($F_{7.35} = 4.83$; $P = 0.0007$; CV = 19.40). Singular treatment of granule humic had the highest number of fruits per tree (239.17) while control plants had the lowest o (81.17). In a similar experiment Fawzi *et al.* (2010) reported that combined treatments of compost (or CM), biofertilizer and Mg significantly increased pear fruit yield (number and weight of fruits) compared with the individual treatments of compost and CM.

Among fertilizer treatments, vermicompost treated plants showed the lowest number of fruits per tree (114.67) in both years. This treatment also resulted in the lowest tree yield and fruit weight as well as the highest fruit drop among different treatments. It is noteworthy that this fertilizer had the lowest nutrient and the highest pH among different organic fertilizers used in this study (Table 2). Therefore, for improving yield related traits, vermicompost was less effective than other organic fertilizers and this low efficiency could be attributed to its composition, especially the scanty nutrients and high pH, which is not appropriate for calcareous soils.

Leaf minerals

Results of leaf analysis indicated that some of the treatments significantly increased the uptake of some minerals in the fertilized plants (Table 5). In the first year, only plants treated with Cow Manure and Granule Humic either individually, or in combination with biofertilizer (CM + B and GH + B) had optimum levels of N in their leaves (2-2.5%) (Table 6). The highest amount of N (2.63%) was recorded in the plants treated with combined Granule Humic and biofertilizer, while control plants had the lowest N content (1.75%) ($F_{7.35}$ = 19.08; P < 0.0001; CV = 8.55). In the second year, only plants treated by granule humic alone and in combination with biofertilizer had the optimum level of N and the remaining plants had N deficiency (below 2%) ($F_{7.35}$ = 21.54; P < 0.0001; CV = 12.37). An adequate level of N is required for vegetative growth, flowering, and fruit yield also N causes flower drop in pomegranate trees (Zekri, 2012).

Table 5 Effects of different fertilizers on leaf nutrients of pomegranate cv. 'Bajestani' in two successive years.

Treatments	N (%)		P (%)		K (%)		Mg (%)		Fe (mg/kg)		Zn (mg/kg)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Vermicompost (V)	1.76 e	1.49 cd	0.13 d	0.198 cb	1.33 cd	0.98 ab	0.42 c	0.52 bc	109.00 cd	223.33 bc	33.66 b	9.66 c
Cow Manure (CM)	2.14 bc	1.62 c	0.19 bc	0.22 ab	1.41 cd	1.11 a	0.47 ab	0.65 ab	111.33 bcd	271.67 b	33.33 b	10.00 c
Granule Humic (GH)	2.03 dc	2.35 a	0.15 cd	0.168 dc	1.48 c	0.96 bc	0.45 abc	0.61 abc	138.66 a	339.33 a	21.66 c	45.66 a
Biologic(B)	1.75 e	1.52 cd	0.14 d	0.162 dc	1.46 c	0.96 bc	0.44 bc	0.51 bc	122.33 abc	229.67 bc	23.33 c	12.34 c
B + V	1.90 de	1.28 cd	0.15 cd	0.13 d	1.26 d	1.00 ab	0.43 c	0.49 bc	125.66 abc	242.67 bc	42.00 ab	8.00 c
B + CM	2.26 b	1.71 c	0.25 a	0.25 a	1.65 b	1.01 ab	0.48 ab	0.64 ab	129.00 ab	260.67 b	43.00 a	18.33 b
B + GH	2.63 a	2.06 b	0.21 b	0.14 d	1.63 b	1.10 a	0.483 a	0.73 a	140.00 a	222.00 bc	46.66 a	11.00 c
Untreated	1.75 e	1.19 e	0.14 d	0.17 bcd	1.86 a	0.85 c	0.36 d	0.44 c	95.66 d	184.33 c	20.66 c	11.00 c

The values followed by the same letter do not show statistically significant differences using (Duncan's multiple range test, P < 0.05).

Table 6 Optimum level of studied nutrients in pomegranate tree.

Nutrients	Optimum range
N (%)	2-2.5
P (%)	0.1-0.2
K (%)	0.6-1.5
Mg (%)	0.3-0.4
Fe (mg/kg)	60-120
Zn (mg/kg)	40-70

Analysis of leaf samples showed that all of the studied plants were in the optimum ranges of P in two consecutive seasons. In 2014, the highest level of P was recorded in the leaves of plants treated with combination of cow manure and biofertilizer (0.25%), while the lowest value was in Vermicompost treated plants (0.13%) ($F_{7.35}$ = 9.87; P = 0.0001; CV = 18.18). In 2015, the highest and the lowest P contents were recorded among combined treatments of Cow Manure plus biofertilizer (0.25%) and vermicompost plus biofertilizer (0.13%) ($F_{7.35}$ = 7.90; P = 0.0001; CV = 18.94). Phosphorus deficiency adversely affected the general growth of trees and roots, also caused decreased flowering and delayed maturation of fruits. Phosphorus absorption is limited in acidic and alkaline soils (Jones, 2012).

Leaf analysis showed that K content decreased in all studied plants in 2015; however, all of the treated plants had significantly higher K in their leaves compared with control plants. Plants treated by Granule Humic in combination with biofertilizer and Cow Manure showed significantly higher K in their leaves. K is one of the main nutrients for pomegranate fruit production (Zekri, 2012). Using different nutrients in pomegranate cv. 'Kandhari', Dhillon *et al.* (2011) reported that trees receiving no K produced the least fruit size and weight. Granule humic in combination with biofertilizer resulted in the heaviest fruits and gave the highest fruit yield that could be attributed to the higher uptake of nutrients such as K in this treatment. It is reported that optimum level of K in pomegranate leaves is in the range of 0.6-1.5% and our results showed that all of the plants had optimum levels of K in their leaves for the two studied years (2014: $F_{7.35} = 14.5$; $P < 0.0001$; CV = 14.5, and 2015: $F_{7.35} = 4.11$; $P = 0.0022$; CV = 9.77). It does not seem that fruit formation is directly influenced by potassium but if tree growth is weak, fruit set generally decreases. Trees with potassium deficiency more sensitive to injuries caused by cold winter and spring frost (Babalar and Pirmoradian, 2009).

Significant differences were observed regarding Fe content of different treatments for the two successive years. The highest value was recorded in the combined treatment of Granule Humic plus biofertilizer (140 mg/kg) and singular treatment of Granule Humic (339.33 mg/kg) in 2014 and 2015 respectively, while the lowest content was observed in the control plants for both years (95.66 and 184.33 mg/kg, respectively). The higher content of Fe in the leaves of Granule Humic treated plants might be attributed to the positive effect of this fertilizer in reduction of pH in the root media, which finally results in the higher uptake of Fe from rhizosphere (2014: $F_{7.35} = 6.27$; $P = 0.0001$; CV = 12.32, and 2015: $F_{7.35} = 5.01$; $P = 0.0005$; CV = 20.36).

The iron is essential for chlorophyll synthesis and its deficiency causes leaf chlorosis (Jalili, 2016). In the first year, the combined treatment of granule humic and biofertilizer resulted in the highest amount of Zn (46.66 mg/kg) and untreated plants had the lowest value (20.66 mg/kg), while in the second year, Zn content was the highest (45.66 mg/kg) in granule humic treated plants and the lowest (8.00 mg/kg) in the combined treatments of vermicompost with biofertilizer (2014: $F_{7.35} = 12.68$; $P < 0.0001$; CV = 21.44, and 2015: $F_{7.35} = 84.86$; $P < 0.0001$; CV = 21.05). Zn regulates fruiting and growth of tree and deficiency of Zn causes premature fruit drop there may be 80% fruit drop (Babalar and Pirmoradian, 2009). Combined treatment of cow manure with biofertilizer and control plants had the highest (0.48 mg/kg) and the lowest (0.36 mg/kg) Mg content, respectively in the first season, while combined treatment of granule humic with biofertilizer and control plants showed the highest (0.73 mg/kg) and the lowest (0.44 mg/kg) Mg contents in the second season (2014: $F_{7.35} = 9.91$; $P < 0.0001$; CV = 6.76, and 2015: $F_{7.35} = 3.06$; $P = 0.0127$; CV = 23.83).

Results of leaf analysis form two consecutive years revealed that plants treated by granule humic individually or in combination with biofertilizer had higher content of macro and micro nutrients; hence this treatment might have facilitated the uptake of elements from root media. It is reported that individual application of cow manure and Compost as well as their combinations with biofertilizer significantly increased N, P, K, and Mg contents of pear leaves (Fawzi *et al*., 2010). Compared to the chemical fertilizers, organic fertilization at high level plus humic acid enhanced navel orange leaves nutritional status through increasing their nitrogen, phosphorus and potassium contents (Barakat *et al*., 2012). Also, Fayed (2005) found that applying different organic manures on apple trees cv. 'Anna' increased leaf macro elements (N, P, and K) contents as compared with unfertilized trees. In fact, healthy soils high enough in organic matter and with a biologically diverse microorganisms support plant health and nutrition better than soils low in organic matter and microbial diversity.

Our results revealed that granule humic application effectively promoted nutrient uptake from root media and resulted in the higher content of N, K, Mg, Fe, and Zn ions in the leaves of treated plants. Iran is situated in the arid regions and most of the lands used for pomegranate cultivation have calcareous soils and are alkaline (pH ≥ 7); so trace element deficiency is a common phenomenon in such soils which have a negative effect on crop yield (Fayed, 2005; Malakuti and Tabatabaie 2005). Analysis of organic matters revealed that granule humic had pH 3.38; therefore, continuous use of this acidic fertilizer, can modulate the root media especially in favor of trace elements absorption, eventually

[DOR: 20.1001.1.22519041.2017.6.3.8.8]

resulting in the higher uptake of these type of nutrients. In fact all of the studied nutrients were higher in the granule humic treated plants in 2015 compared with 2014. Due to the accumulative effect of successive biological fertilization of the root media, the remarkable effects of biological products are observed some years after their application (Mosa *et al*., 2014).

Fruit cracking

Plants treated with granule humic plus biofertilizer showed the lowest percent of cracked fruits (7.5 and 2.25% in 2014 and 2015, respectively), while control plants had the highest percent of fruit cracking (22.67 and 16.47% in 2014 and 2015, respectively) (Table 7). For all studied plants, fruit cracking was lower in the second year of experiment. It is noteworthy that fruit cracking was much higher in the plants treated by cow manure than other treatments, especially in the second year (12.41%). Irregular irrigation is among the main causes of fruit cracking in pomegranate (Abubakar *et al*., 2013). Biofertilizers contains beneficial microorganisms; by adding organic matters, the soil structure will improve and provide a better condition for microorganisms to grow and increase in population (Ehteshami and Chaiechi, 2011). Addition of organic matters such as Humic will improve the water holding capacity of the root media and has additional benefit in the arid and semi-arid regions which are usually faced with limited water resources (Abdel-Nasser and Harhash, 2001; Raziei *et al*., 2005). Nutrient deficiency is another important factor in pomegranate fruit cracking (Khadivi-Khub, 2015). Therefore effects of organic fertilizers in reduction of fruit cracking may be somewhat attributed to the positive effects of these fertilizers for providing more nutrients. Results of leaf mineral analysis also support this idea. On the other hand, combined treatments that increased plant nutrients, also decreased fruit cracking. (2014: $F_{7.35} = 1.64$; $P = 0.1569$; CV = 16.51, and 2015: $F_{7.35} = 1.78$; $P = 0.1220$; CV = 13.94).

Fruit Sunburn

Results of on the percent of sunburnt fruits revealed that no significant differences among different treatments and control plants. (2014: $F_{7.35} = 2.56$; $P = 0.0307$; CV = 18.94, and 2015: $F_{7.35} = 0.87$; $P = 0.5391$; CV = 19.47). These observations reveal that fertilization with organic materials have no significant positive effects on sunburn of fruit in pomegranate, and other factors should be taken into account to reduce this disorder. In fact, to combat this adverse character, we need the methods to reduce the direct sunlight; for example shading and mulching with kaolin are among the horticultural practices that have been considered in the recent years (Melgarejo *et al.*, 2004; Parashar and Ansari, 2012; Ghorbani *et al*., 2015).

Table 7 Effects of different fertilizer treatments on fruit cracking, sun burn and infested fruit by crab moth in 2014 and 2015.

Treatments	Cracking (%)		Sunburn (%)		Cracking & sunburn (%)		Infestation (%)	
	2014	2015	2014	2015	2014	2015	2014	2015
Vermicompost (V)	11.72 ab	5.66 ab	20.76 a	18.74 a	10.67 b	22.59 a	30.30 ab	37.31 a
Cow Manure (CM)	19.22 ab	12.41 ab	17.13 a	21.22 a	9.20 b	18.61 ab	28.92 abc	35.61 a
Granule Humic (GH)	16.98 ab	2.80 b	15.90 a	14.42 a	15.90 ab	23.17 a	28.33 bc	33.97 a
Biologic (B)	18.37 ab	5.76 ab	19.15 a	19.31 a	14.55 ab	23.84 a	27.59 bc	35.48 a
B + V	12.16 ab	5.66 ab	19.88 a	21.93 a	12.33 ab	13.77 ab	28.20 bc	33.60 a
B + CM	19.66 ab	7.45 ab	20.58 a	16.69 a	11.16 b	12.17 ab	27.86 bc	32.53 ab
B + GH	7.50 b	2.25 b	16.96 a	15.57 a	13.70 ab	9.71 b	27.50 c	25.34 b
Untreated	22.67 a	16.47 a	21.81 a	21.08 a	18.48 a	23.99 a	31.44 a	38.07 a

The values followed by the same letter do not show statistically significant differences using (Duncan's multiple range test, $P < 0.05$).

Pomegranate fruit moth infestation

Pomegranate fruit moth infestation was affected by fertilizer treatments and untreated plants had the highest infected fruits (31.44%), while, combined treatment of granule humic plus Biofertilizer had the lowest infestation (27.5%) (2014: $F_{7,35} = 2.46$; $P = 0.0369$; CV = 18.17). Totally, combined treatment of organic and biofertilizers showed lower infected fruits than individual organic treatments. Fruit infestation was increased in the second year for most of the treatments, mainly due to the outbreak of pest in the region, the exception was granule humic plus biofertilizer treated plants which showed lower fruit infestation than in the first year. Although, there were no significant difference between most of the treated and control plants in the second year, ($F_{7,35} = 2.73$; $P = 0.0226$; CV = 7.23). Combination of granule humic with biofertilizer significantly reduced the number of infected fruits. In the second year, the lowest infection was recorded in the combined treatment of granule humic with biofertilizer (25.34%) and the highest degree of infestation was observed in the control plants (38.07%).

Soil organic matter has been directly and positively correlated to the soil fertility and agricultural productivity potential. It is reported that soils with high organic matters and active soil biology, generally exhibit good soil fertility and crops grown in such soils generally exhibit lower abundance of several insect herbivores (Altieri and Nicholls, 2003).

A healthy plant can be more tolerant to the pest attacks compared to an unhealthy plant (Mattson, 1980). Balanced nutrition is one of important plant health indices. A hypothesis about relationship between pests and plant nutrition indicate that if balanced nutrient is provided for plants according to the plant nutritional needs, pest pressure will be decreased in plant (Syrovy and Prassad, 2010). There are some evidences supporting this hypothesis in different plants such as C*hilo suppressalis* (Walker) with rice and *Leptinotarsa decemlineata* (Say) with potato (Phelan *et al*., 1996; Alyokhin *et al*., 2005). There are similar reports for fruit trees. For instance, it is reported that balance between macro and micro nutrients in Citrus decreased pests' injury (Schumann *et al*., 2010). Presence of organic matter in the root environment will aid retaining of nutrient balances in plants. Humic materials increase soil fertility by enhancing its cation exchange capacity (CEC) and maintenance the proper pH of nutrient solution and root media, thereby have important role in nutrient balance (Sharif *et al*., 2002).

According to the given optimum level of nutrients in pomegranate leaves (Table 6) and compared to Table 5 it is obvious that control plants (no nutrient treatment) are apt to show deficiency symptoms, especially in the second year. This is directly related to the lack of proper nutrition in the two consecutive years that increase the losses caused by PFM.

Our results indicate that lack of proper nutrient for two consecutive years not only reduced the yield but also increased loss due to PFM in the control plants and it could be concluded that control plants are more attractive hosts for PFM. More investigation is needed to find out about mode of host selection that is used by PFM. Long-term management of organic matter can lead to better plant resistance against pests (Altieri and Nicholls, 2003). Our results from the second year revealed that combined treatment of granule humic plus biofertilizer reduced pomegranate fruit moth infestation by 13% compared to control plants. Therefore, this combined treatment has potential for use in better management of pomegranate orchards to reduce the pomegranate fruit moth injuries.

Conclusion

Effects of different organic fertilizers individually and or in combination with biofertilizer were analyzed on pomegranate trees cv. 'Bajestani' and some of the yield related traits, leaf minerals, cracking and sunburnt fruits, as well as pomegranate fruit moth-infected fruits were investigated. Our results indicated that biofertilizer alone had no

[DOR: 20.1001.1.22519041.2017.6.3.8.8]

significant positive effects on fruit characteristics but its combination with other organic fertilizers improved growth and yield of pomegranate and reduced some pomegranate disorders. Among organic fertilizers used in this study (cow manure, vermicompost, and granule humic), combined treatment of granule humic plus biofertilizer resulted in better uptake of nutrients and fruit yield. This treatment also reduced fruit cracking and infestation of pomegranate by fruit moth in pomegranate cv. 'Bajestani'. Among the organic fertilizers used, granule humic was the most effective one that improved the uptake of measured nutrients from calcareous soils especially in the second year. Our observations revealed that positive effects of biofertilizers were higher in the second year compared to the first year. In fact, in the alkaline sandy loam soils, the positive effects of biofertilizers appear gradually by time. Organic fertilizer management had no significant effects on fruit sunburn and it seems that other horticultural practices such as proper pruning and shading would be more effective than fertilization for reducing sunburnt fruits. Altogether, our results suggest that biofertilizer in combination with organic materials could be used in horticultural practices to prevent crop losses resulting from cracking, nutrient deficiency as well as pomegranate fruit moth infestation, and may have a significant practical application.

Acknowledgements

This research was supported by university of Tehran and authors acknowledge Mr. Parhizi orchard man of pomegranate and all employees of the orchard for cooperation during this study.

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تأثیر کودهای آلی و زیستی بر درختان انار: عملکرد، ترک‌خوردگی، آفتاب‌سوختگی و آلودگی به کرم گلوگاه انار *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

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چکیده: جهت بررسی اثر کودهای متفاوت روی عملکرد و سلامت صفات مرتبط با میوه انار، یک پژوهش مزرعه‌ایی در طی سال‌های ۱۳۹۳ و ۱۳۹۴ روی انارهای ۶ ساله رقم بجستان پرورش یافته در خاکی با بافت لوم شنی و سیستم آبیاری قطره‌ایی در شهرستان خلیل‌آباد استان خراسان رضوی انجام شد. کودهای ارگانیک شامل کود حیوانی (۲۵ Kg/tree)، ورمی کمپوست (۵ Kg/tree)، هیومیک گرانوله (۲ Kg/tree) به‌تنهایی و در ترکیب با کودهای زیستی (ازتوبارور، فسفوبارور، پتابارور) به اندازه یک لیتر برای هر درخت به روش چالکود استفاده گردید. نتایج نشان داد که گیاهان تیمار شده با ترکیب کودهای ارگانیک و زیستی عملکرد بهتر و ریزش کم‌تر نسبت به گیاهان تیمار شده به‌وسیله کودهای آلی به‌صورت انفرادی داشتند. تیمار ترکیب کود زیستی و هیومیک کم‌ترین ترک‌خوردگی میوه (۷/۵، ۲/۲۵ درصد به‌ترتیب در سال ۱۳۹۳ و ۱۳۹۴) را داشت. هم‌چنین کم‌ترین آلودگی به کرم گلوگاه انار *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) در همان تیمار مشاهده شد (۲۷/۵، ۲۵/۳۴ درصد به‌ترتیب در ۱۳۹۳ و ۱۳۹۴). بیش‌ترین میزان آلودگی به کرم گلوگاه انار در تیمار عدم‌تغذیه (۳۱/۴۴، ۳۸/۰۷ درصد) در هر دو سال مشاهده گردید. آفتاب سوختگی میوه تحت تأثیر نوع تغذیه و عدم تغذیه قرار نگرفت. نتایج آنالیز برگ آشکار کرد که گیاهان تیمار شده به‌وسیله ترکیب کود زیستی و هیومیک سطح بالاتری از عناصر پرمصرف و کم‌مصرف در مقایسه با گیاهان تیمار نشده دارند. در مجموع نتایج ما پیشنهاد می‌کند که کود زیستی در ترکیب با کودهای آلی مخصوصاً هیومیک گرانوله می‌تواند در بهبود عملکرد و جلوگیری از زیان حاصل از ترک‌خوردگی، کمبود عناصر غذایی و آلودگی به کرم گلوگاه انار در مدیریت باغات انار مورد استفاده قرار گیرد.

واژگان کلیدی: انار،کود آلی، ترک‌خوردگی میوه، آفتاب سوختگی، کرم گلوگاه انار

[DOR: 20.1001.1.22519041.2017.6.3.8.8]