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The study effects of some biological agents on Chickpea (*Cicer arietinum* L.) under semi-dry conditions in Kermanshah

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ABSTRACT

Among the environmental stresses, drought stress is one of the important factors that influence on the yield, yield components and physiological characteristic of the crops under rain-fed conditions. As regards, in the Iran the most cropping area of the chickpea is rain-fed, so, using of the biological agents with once performance supplemental irrigation during growth can be effective in improving the nutritional status of the chickpea. So that, a field experiment was conducted as split-factorial based on Randomized Complete Block Design with three replications, at the Experimental Farm at Campus of Agriculture and Natural Resources of Razi University, in 2010-2011. The main plots consisted of the supplemental irrigation at time of the flowering and non- irrigation and sub-plots were including use and non-use of the Mycorrhiza, Rhizobium and humic acid as factorial. Traits including relative water content (RWC), biological yield, grain yield, number of pods per plant, number of grains per plant, number of grains per pod, 100-grains weight and harvest index. The results showed that the main effect of the supplemental irrigation on yield, yield components (except 100-grains weight), RWC, N and P content were significant. Also, the effect of the humic acid was significant on RWC, biological yield, grain yield, harvest index and P content but the Rhizobium wasn't effective on the traits. The Mycorrhiza only was significant for grain yield and P content. Also the interaction effects between supplemental irrigation and humic acid were significant for biological yield and grain yield and P content. Overall, application of the supplemental irrigation with use of biological and symbiosis agents was effective in improving physiological traits.

Keywords: Chickpea; Humic acid; Mycorrhiza; Rhizobium; Supplemental irrigation.

INTRODUCTION

Chickpea is one of the most important pulses in the world. This product provides the cheap protein diet, especially for poor people. The total of amino acids in the wheat- chickpea diet provides the perfect combination of amino acids [4]. Also, due to capable of nitrogen fixation can be improved soil fertility [1]. Drought is one of the abiotic stresses that can reduce the production of chickpea [17]. As regards, the 90 percent of cultivation of chickpea is as rain-fed and it is cultivated in arid and semi- arid land [16], so that drought is a limited factor that influenced the yield of this product. In this regard, the use of efficiency supplemental irrigation in order to improve the chickpea yield which cultivated as rain-fed can be useful and the time of it should be when, with less water available at during critical stages of crop growth achieve optimum yield [19]. The grain yield of chickpea is affected by the number of pods per plant, the number of grains per plant and 100-grain weight and supplemental irrigation has been able effect on the pod formation and grain filling stage has increased the grain yield of chickpea [15]. Food shortage is also limiting growth of crop [23]. Phosphorus is one of the essential nutrient elements for crop that is required for growing grain legumes [12] and is involved in many physiological processes such as the use of sugar and starch, photosynthesis and saving, transfer of energy. Legumes due to the large energy consumption in fixing nitrogen need more phosphorus [24] and the reactions of it complex in acidic and alkaline soils [23]. Nitrogen is in structure of chlorophyll, protoplasm, protein and nucleic acid and it is associated with higher photosynthetic activity, power growth producing leaves and branches and also affect on grain protein quality [1]. On the other hand, plenty rats of nitrogen and phosphorus increased the grain yield even under deficit moisture conditions [10]. In the supply of crop nutrients use of biological fertilizers are preferred than chemical fertilizers [20]. Biological fertilizers are important aspects of economically and ecologically. These are natural fertilizers that including bacteria, algae, fungi alone or in combination with each other and increase access to nutrient for the crops. Materials humic are one of the biological fertilizers that can be effective in the access of availability of phosphorus, growth crop and finally in increasing of crop yield [13]. On the other hand, legumes such as chickpea can get tripartite symbiosis with fungi (*Glomus intraradices*) and bacteria (Rhizobium). This symbiosis is able to supply phosphorus and nitrogen for the crops [25]. This can increase the absorption of phosphorus. More phosphorus absorbed by effect on the nitrogen fixation, increased amount of nitrogen [18]. This experiment was conducted to investigate the effects of bio-fertilizer (humic acid) and symbiosis agents (Mycorrhiza and Rhizobium) on the yield and yield components of chickpea under supplemental irrigation.

MATERIALS AND METHODS

Study Site: This experiment was conducted at the Research Farm of Campus of Agriculture and Natural Resources of Razi University, Kermanshah, Iran with the longitude 34° 21´ N and 47° 9´ E and the elevation 1319 m above the sea. The soil texture was silt-clay with a pH of 7.9. The rates of nitrogen, phosphorus and potassium in the soil before the experiment were 0.11%, 8.6 and 410 ppm, respectively. Monthly temperature (°C) and total rainfall (mm) in year of the experiment are shown in Figure 1.

The experiment design: A field experiment was conducted as split-factorial based on Randomized Complete Block Design with three replications, in 2010-2011. The main plots consisted of the supplemental irrigation at time of the flowering and non-use of supplemental irrigation and sub-plots were including use and non-use of the Mycorrhiza, Rhizobium and humic acid as factorial. The space between planting rows was 25 cm. The density of plants was 50 plants per square meter. The seeding was performed manually in November 24, 2010.

Application of treatment: The Rhizobium used as seed treatment at sowing time, Mycorrhiza fungi (*Glomus intraradices*), was applied on seeds at sowing time and also humic acid was applied as foliar application at early vegetative growth stage. The seeds chickpea were Azad cultivar. This cultivar is a new chickpea cultivar for dry-land moderate and semi warm climate of the Iran.

Traits: At the harvesting time a square meter separated from the soil surface of each plot and seed yield, biological yield were obtained. The numbers of pods per plant, seeds per pod counted of ten plants of each plot were obtained. The harvesting index was calculated by dividing the seed yield on the biological yield as a percentage. Leaf relative water content (RWC) was determined according to the methods of Cornic (1994) [5], based on the following equation:

$$RWC = (FW - DW) / (SW - DW) \times 100$$

Where FW; Leaf fresh weight and DW; Dry weight of leaves after drying at 85°C for 3 days, SW; turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20°C). The percentage of stem nitrogen was determined by Kjeldahl method [3] at flowering stage. The percentage of stem phosphorus also determined at flowering stage [2]. The data analysis, mean comparisons were conducted with the software's SAS 9.1 and MSTAT-C.

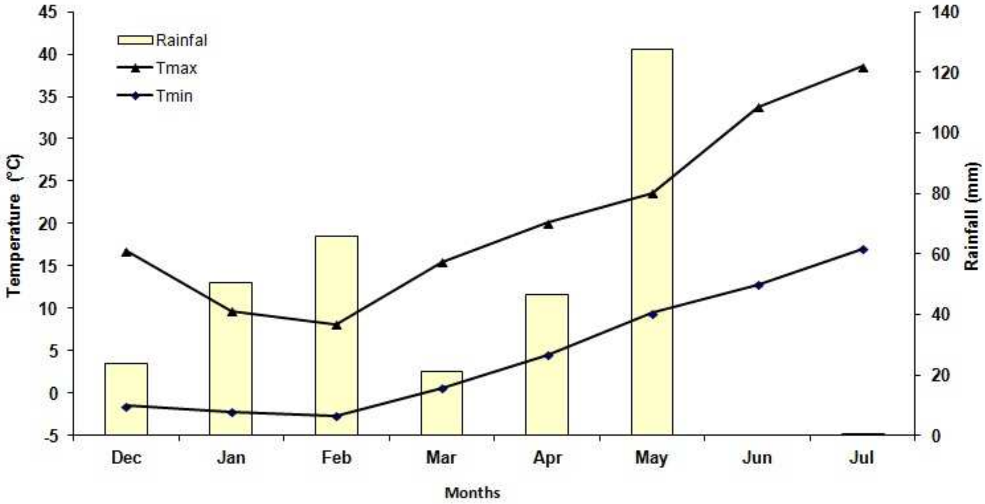

Figure 1. Monthly total rainfall (mm) and temperatures (°C) (Max and Min), in year of experiment

RESULTS AND DISCUSSION

Number of pods per plant: The effect of supplemental irrigation was significant on the number of pods per plant (table 1). The number of pods is one of the most important yield components that can have much influence on the yield. Jalilian et al., founds, the supplemental irrigation increased the pods per plant of chickpea [11]. Due to increased temperatures during pod growth, respiration rate increases and the amount of photosynthetic material available for transfer to the developing seeds is increased [26].

Number of grains per pod: The simple effect of supplemental irrigation also, was significant for this trait (table 1). But other treatment effects were not significant on the number of grains per pod (Table 1). The highest number of the grains per pod (1.17) was related to the irrigation (Table 2). The grain number is one of the yield components that have the more variability to the grain weight and it can affect the yield [6]. In the chickpea the number of seeds per pod in addition, it is under genetic control, are controlled by environmental factors such as deficit water [27]. When there is a limitation of photosynthetic material, the seeds that are formed initially aborted and reduced the number seeds per pod. So, drought stress at the reparative stage, as way will reduced average of the number of seeds per pod [28].

Table 1. Analysis of Variance for yield and yield components and harvest index (MS)

S. O. V	df	Number of pods per plant	Number of grains per pod	Number of grains per plant	100-grains weight	Grain yield	Biological Yield	Harvest Index
Ir	1	234.08**	0.35*	810.16**	48.56^{ns}	6919804.68**	16042968.75**	450.18*
Error (Ea)	2	3.52	0.32	125.27	144.54	26875.00	66832.75	11.43
Hu	1	5.33^{ns}	0.07^{ns}	74.00^{ns}	18.30^{ns}	1890117.18**	2180268.75**	500.52*
Rh	1	7.36^{ns}	0.07^{ns}	74.50^{ns}	6.13^{ns}	196992.18^{ns}	56718.75^{ns}	88.02^{ns}
M	1	4.08^{ns}	0.07^{ns}	69.12^{ns}	1.82^{ns}	73242.18*	18018.75^{ns}	42.18^{ns}
Ir×Hu	1	0.33^{ns}	0.07^{ns}	47.20^{ns}	0.00^{ns}	354492.18*	486018.75**	172.52^{ns}
Ir×Rh	1	0.08^{ns}	0.07^{ns}	38.52^{ns}	0.00^{ns}	117.18^{ns}	18.75^{ns}	6.02^{ns}
Ir×M	1	0.08^{ns}	0.07^{ns}	43.32^{ns}	0.05^{ns}	117.18^{ns}	18.75^{ns}	1.68^{ns}
Hu×Rh	1	0.33^{ns}	0.00^{ns}	0.10^{ns}	0.20^{ns}	1054.68^{ns}	468.75^{ns}	0.02^{ns}
Hu×M	1	0.33^{ns}	0.00^{ns}	0.00^{ns}	0.45^{ns}	117.18^{ns}	468.75^{ns}	0.52^{ns}
Rh×M	1	0.08^{ns}	0.00^{ns}	0.00^{ns}	0.45^{ns}	9492.18^{ns}	918.75^{ns}	9.18^{ns}
Ir×Hu×Rh	1	0.33^{ns}	0.00^{ns}	0.10^{ns}	0.05^{ns}	1054.68^{ns}	468.75^{ns}	0.52^{ns}
Ir×Hu×M	1	1.33^{ns}	0.00^{ns}	3.20^{ns}	0.20^{ns}	117.18^{ns}	168.75^{ns}	0.52^{ns}
Ir×Rh×M	1	0.08^{ns}	0.00^{ns}	0.00^{ns}	0.20^{ns}	117.18^{ns}	468.75^{ns}	0.52^{ns}
Hu×Rh×M	1	0.33^{ns}	0.09^{ns}	36.40^{ns}	0.20^{ns}	5742.18^{ns}	168.75^{ns}	4.68^{ns}
Ir×Hu×Rh×M	1	0.33^{ns}	0.09^{ns}	36.40^{ns}	0.45^{ns}	117.18^{ns}	4218.75^{ns}	2.52^{ns}
Error (Eb)	28	2.40	0.04	25.42	12.90	52366.07	60612.93	73.74
CV (%)	–	7.63	20.39	22.70	13.22	14.01	7.38	17.75

*ns : Non- significant, * and **: Significant at 5 and 1% probability levels, respectively; Ir: Supplemental Irrigation; Hu: Humic acid; Rh: Rhizobium; M: Mycorrhiza*

Table 2. Mean comparisons for yield and yield components in chickpea under supplemental irrigation and Bio-fertilizers

Treatments	Number of pods per plant	Number of grains per pod	Number of grains per plant	100-grain weights (g)	Grain yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Irrigation							
–	18.10	1.00	18.10	26.16	1253.13	2756.25	45.29
+	22.51	1.17	26.31	28.17	2012.50	3912.50	51.41
lsd $_{(5\%)}$	2.33	0.70	13.90	14.93	203.62	321.10	4.20
Humic acid							
–	19.97	1.04	20.96	26.55	1434.38	3121.25	45.12
+	20.64	1.12	23.45	27.78	1831.25	3547.50	51.58
lsd $_{(5\%)}$	0.91	0.13	2.98	2.12	135.32	145.58	5.07
Rhizobium							
–	19.91	1.04	20.96	26.81	1568.75	3300.00	47.00
+	20.70^{a}	1.12	23.45	27.52	1696.88	3368.75	49.70
lsd $_{(5\%)}$	0.91	0.13	2.98	2.12	135.32	145.58	5.07
Mycorrhiza							
–	20.01	1.04	21.00	26.97	1593.75	3315.00	47.41
+	20.60	1.12	23.40	27.36	1671.88	3353.75	49.29
lsd $_{(5\%)}$	0.91	0.13	2.98	2.12	135.32	145.58	5.07

+ , application and –,no application

Table 3. Analysis of variance for N, P content and Relative Water Content (RWC) in chickpea under supplemental irrigation and Bio-fertilizers (MS)

S. O. V	df	N	P	RWC
Replication	2	0.06^{ns}	0.0003^{ns}	75.7^{ns}
Ir	1	1.17**	0.03**	2148.4**
Error (Ea)	2	0.03	0.0003	94.1
Hu	1	0.005^{ns}	0.009**	3058.7**
Rh	1	0.03^{ns}	0.0006^{ns}	134.1^{ns}
M	1	0.01^{ns}	0.001*	23.5^{ns}
Ir×Hu	1	0.001^{ns}	0.002**	0.2^{ns}
Ir×Rh	1	0.0002^{ns}	0.0001^{ns}	4.2^{ns}
Ir×M	1	0.01^{ns}	0.0003^{ns}	39.5^{ns}
Hu×Rh	1	0.0002^{ns}	0.00007^{ns}	32.9^{ns}
Hu×M	1	0.0018^{ns}	0.00007^{ns}	2.3^{ns}
Rh×M	1	0.0002^{ns}	0.00007^{ns}	11.8^{ns}
Ir×Hu×Rh	1	0.02^{ns}	0.0003^{ns}	13.8^{ns}
Ir×Hu×M	1	0.001^{ns}	0.0001^{ns}	20.8^{ns}
Ir×Rh×M	1	0.005^{ns}	0.0003^{ns}	76.1^{ns}
Hu×Rh×M	1	0.0002^{ns}	0.00007^{ns}	32.9^{ns}
Ir×Hu×Rh×M	1	0.005^{ns}	0.0001^{ns}	8.7^{ns}
Error (Eb)	28	0.04	0.007	55.9
CV (%)	–	15.96	7.77	9.4

*ns: Non- significant, * and **: Significant at 5 and 1% probability levels, respectively; Ir: Supplemental Irrigation; Hu: Humic acid; Rh: Rhizobium; M: Mycorrhiza*

Table 4. Mean comparisons for N content, P content and Relative Water Content in chickpea under supplemental irrigation and Bio-fertilizer

Treatments	N (%)	P (%)	RWC (%)
Irrigation			
–	1.20	0.18	72.4
+	1.51	0.23	85.79
lsd $_{(5\%)}$	0.22	0.02	12.05
Humic acid			
–	1.35	0.20	71.11
+	1.37	0.22	87.08
lsd $_{(5\%)}$	0.12	0.009	4.41
Rhizobium			
–	1.33	0.210	77.42
+	1.38	0.217	80.77
lsd $_{(5\%)}$	0.12	0.009	4.41
Mycorrhiza			
–	1.34	0.20	78.40
+	1.37	0.22	79.80
lsd $_{(5\%)}$	0.12	0.009	4.41

+, application and –, no application

Table 5. Interaction effects of supplemental irrigation × humic acid on grain yield, biological yield and P content

Irrigation	Humic acid	Grain yield (kg ha^{-1})	Biological yield (kg ha^{-1})	P (%)
Rain fed				
	–	968.8[d]	2443[d]	0.18[c]
	+	1538[c]	3070[c]	0.19[c]
Supplemental irrigation				
	–	1900[b]	3800[b]	0.21[b]
	+	2125[a]	4025[a]	0.26[a]

Means at least one common letter in each column, based on Least Significant Difference (LSD) test at 5% level are not significant; +, application and –, no application

Number of grains per plant: The number of grains per plant only was affected by supplemental irrigation (Table 1). In an experiment the application of humic acid increased the number of grains per plant of common bean [14].

Grain yield: The simple effects of supplemental irrigation, humic acid and mycorrhiza on the grain yield were significant (Table 1). Also, the interaction effects of irrigation and humic acid also, was significant (Table 1). The highest grain yield was related to use of supplemental irrigation and application of humic acid (2125 kg ha-1) (Table 5).

The application of humic acid can be increased the yield of grain (Table 2). Also, in different experiment, the yield and yield components of bean, soybean and vetch increased by application of humic acid [7, 9].

Biological yield: The biological yield of chickpea was significantly affected by supplemental irrigation and humic acid (Table 1). Also, the interaction effects of these treatments were significant. The highest and lowest of biological yield, were related to use of supplemental irrigation and application of humic acid (4025 kg ha^{-1}) and rain-fed conditions and no application of humic acid (2443 kg ha^{-1}) (Table 5). The effect of supplemental irrigation on increasing of biological yield of peas, also reported by Pezeshkpour et al. [21].

100-grains weights: None of the effects of treatments on 100-grains weight were not significant (Table 1).

Harvest index: The simple effects of irrigation and humic acid only were significant on the harvest index (Table 1). The highest rate of harvesting index was obtained for irrigation and for humic acid related to application of it with amounts (51.4% and 51.6%), respectively (Table 2). Raey et al., in their research studies on the harvest index of chickpea reached similar conclusions about the usefulness of the supplemental irrigation and the density [22].

N and P content: The effect of irrigation was significant on the N and P content and also effects of humic acid, mycorrhiza and interaction effect of irrigation and humic acid were significant for P content. The effect of Rhizobium was not significant for this trait (Table 3). The highest rate of P content related to application of humic acid, mycorrhiza and irrigation condition (Table 4) and for N content rate obtained irrigation (Table 4). The application of mycorrhiza increased the content of phosphorous of the chickpea [25]. Also, the rate of phosphorous and availability of it increased in corn by application of humic acid [8].

RWC: RWC also was affected by irrigation and humic acid (Table 3). The highest rate of it related to use of irrigation and humic acid (Table 4). Turkan et al. [30], stated that water deficit obviously decreased RWC in bean. Zaree et al. [31], also found drought stress reduces relative water content.

CONCLUSION

According to these results, the use of supplemental irrigation at flowering stage had positive effects on the nutrients, yield and yield components of chickpea. Also, the plant in this condition had higher RWC than rain-fed conditions. Shoot nutrients analysis data showed that, supplemental irrigation had significant effect on the rats of steam nitrogen and phosphorus of chickpea. Also, the supplemental irrigation increased numbers of pods per plant, grains per pod, grains per plant, grain yield, biological yield and harvesting index. These results also, reported by Rasaei et al. [29]. Application of mycorrhiza and humic acid, also, improved the grain yield and rate of phosphorous of chickpea. The use of irrigation and application of humic acid can be effective on the rat of steam phosphorous, grain yield and biological yield. In general, the use of supplemental irrigation at the beginning of flowering stage and application of bio-fertilizer can be useful in nutritional needs of chickpea and it's as way to increase yield of chickpea in the rain-fed conditions.

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REFERENCES

[1] A Ali; Z Ali; J Iqbal; M Ahmad Nadeen; N Akhtar; HM Akram; A Sattar, *J Agric. Res*, **2010**, 48, 3, 335-343.
[2] AOAC. *Association of official analytical chemists*. 15th ed. **1990**, 1, 657-687.
[3] AOAC. *Official methods of analysis. 11th ed, association of analytical chemists, Washington D. C*, **1970**, 123-128.
[4] MS Baloch; M Zubair, *The Journal of Animal and Plant Sciences*, **2010**, 20, 3, 208-210.
[5] G Cornic, *Bios. Oxford*, **1994**, 297-313.
[6] T Dore; MJ Meynard; M Sebillotte, *Euro, J Agron*, **1998**, 8, 29-37.
[7] GL Dplessis; AF Mackenzie Can, *J. Soil Sci*, **1983**, 63, 749-751.
[8] I Erdal; MA Bozkurt; KM Cimrin; S Karacas; M Saglam, *Turkish J. Agric*, **2000**, 24, 663-668.
[9] S Haghighi; T Saki Nejad; Sh Lack, *Life science journal*, **2011**, 8, 3, 583-588.
[10] M Islam; S Mohsan; S Ali; R Khalid; F Ul-Hassan; A Mahmood, *International Journal of Agriculture and Biology*, **2011**, 13, 725-730.
[11] J Jalilian; SAM Modarres Sanavy; S Sabagh pour, *J Agric Sci Natur Resour*, **2005**, 12, 5, 1-9. (Text in Persian with English abstract).
[12] D Jiang; H Hengsdijk; TB Dai; W deboer; Q Jing; WX Cao, *Pedosphere*, **2006**, 16, 25-32.
[13] CA Jones; JS Jacobsen; A Mugoas, *Fertilizer*, **2004**, 32, 37-39.
[14] M Kaya; M Atak; KM Khawar; Y Cemalettin; S Ozcan, *International J. Agric. Biol*, **2005**, 7, 6, 875-878.
[15] A Khourgami; M Rafiee, *African Crop Science Conference Proceedings*, **2009**, 9, 141-143.
[16] J Kumar; S Abbo, *Adv. Agron*, **2001**, 72, 107-138.
[17] A Mafakheri; Af Siosemardeh; B Bahramnejad; PC Struik; E Sohrabi, *Australian Journal of Crop Science*, **2010**, 4, 8, 580-585.
[18] PE Mortimer; MA Perez-Fernandez; AJ Valentine, *Soil Biol, Biochem*, **2008**, 40, 1019-1027.
[19] T Oweis; Supplemental Irrigation, *International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria*, **1997**.
[20] N Pereira Stamford; P Ribeiro Dos Santos; A Muniz Mendes Freire De Moura; C Etiene De Rosalia Esilva Santos; D Santiago De Freitas, *Scientia Agricola*, **2003**, 60, 4, 767-773.
[21] P Pezeshkpour; SK Mousavi; SH Shahabu; M Kalhor; A Khourgami, *Iranian J. Agric*, **2008**, 39, 2, 389-397. (Text in Persian with English abstract).
[22] Y Raey; N Demaghsi; R Seied Sharifi, *Iranian J. Agric Sci*, **2008**, 9, 4, 371-381. (Text in Persian with English abstract).
[23] MS Sarir; MI Durrani; A Ishaq Mian, *Journal of Agricultural and Biological Science*, **2006**, 1, 1, 29-31.
[24] J Schulze; G Temple; SJH Beschow; CP Vance, *Ann. Bot*, **2006**, 98, 731-740.
[25] AR Tavasolee; N Aliasgharzad; GhR Salehijouzani; M Mardi; A Asgharzadeh, *African Journal of Biotechnology*, **2011**, 10, 39, 7585-7591.
[26] MC Saxena, *John wiley and sons. New York*. NY. **1993**, 3-14.
[27] Y Gan; PR Miher; BG Mc Conkey; RP Zentner; PH Liu; CL Mc Donald, *Can. J. Plant Sci*. **2003**, 83, 39-46.
[28] B Ney; C Duthion; O Ture, *Crop Sci*, **1994**, 34, 141-146.
[29] A Rasaei; ME Ghobadi; M Ghobadi, *AJAR*. **2012**, 2353-2358.
[30] I Turkan; B Melike; F Ozdemir; H Koca, *Plant Sci*, **2005**, 2353-2358.
[31] A Zaree; M Nasri; N Ne'mati, *Pelagia Research Library, Advances in Applied Science Research*, **2011**, 2, 4, 598-612.