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Full Length Research Paper

Effects of whey on the colonization and sporulation of arbuscular mycorhizal fungus, *Glomus intraradices*, in lentil (*Lens orientalis*)

Semra Demir[1]* and Elvan Ozrenk[2]

[1]Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection, 65080, Van/Turkey.
[2]Yuzuncu Yil University, Faculty of Agriculture, Department of Food Engineering, 65080, Van/Turkey.

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The aim of this study is to research the effect of 2 different doses of whey [50 ml kg^{-1}(W_{50}) and 100 ml kg^{-1}(W_{100})], an important organic waste, on colonization and sporulation of arbuscular mycorhizal fungus (AMF) *Glomus intraradices*'(*G.i.*) inoculated to lentil plant and the effects of changing P ratio in the soil and plant as a result of whey application, changing salt, pH and $CaCO_3$ values on AMF colonization and sporulation. It is found that macro and micro nutrient elements have increased significantly compared to control plants when G.*i.* and whey are used alone and together. In the studies on whey's effect on the colonization and sporulation of fungal symbiont, it is found that low dose of whey (W_{50}) increases *G. i.*'s colonization and sporulation. Therefore regression analysis found that the effects of P contents in the plant and soil on AMF, especially on colonization are statistically significant and positive in some applications (G.i + W_{50}, G.i + W_{100}), but the effects of soil pH, saltiness and $CaCO_3$ content on both the colonization and sporulation are statistically insignificant.

Key words: Arbuscular mycorrhizal fungus (AMF), whey, colonization, spore density.

INTRODUCTION

The building and maintenance of good soil structure are the objectives of suitable agriculture. With emphasis on sustainable agriculture attention has been increasingly turning to agronomic practices, like the addition of organic residues for the improvement of soil physical conditions (Sonnleitner et al., 2003a). Expectations of soil physical improvement include increased soil moisture, improved soil structure and a concomitant increase in soil resistance to erosion (Lynch and Bragg, 1985; Tisdall, 1991; Sonnleitner et al., 2003b).

Recently, a variety of organic substrates as manure has been used to improve soil structure. The substrates that have been added to soil range from simple sugars (e.g. cellulose, chitin, starch) to complex organic materials (e.g. straw, peat, grass, compost, manure, sewage, sludge, urban refuse) (Sonnleitner et al., 2003b). The use of whey or whey spraying as manure for fields is another strategy to enhance nutrient outflow to plants. Whey may be defined broadly as the serum or watery part of milk remaining after the separation of the curd that results from the coagulation of milk by acid or proteolytic enzymes (rennet) (Zadow, 1994). The constituents of whey which are important for manuring and microbiologically growth are N, P, K, S, Ca, Na, Mg, lactose and proteins (Morris, 1985). It used for manuring purposes not only to encourage plant growth but may also increase microorganism population in the soil (Reddy et al., 1987; Özrenk et al., 2003).

AM fungi, one of the most widespread and functional symbiont in natural and agricultural ecosystems, are influenced by the physical, chemical and biological properties of soil, and the colonization and sporulation of these fungi reveal some of its status (Smith and Read, 1997).

The objectives of the present incubation study were (i) to research the effects of whey on the colonization and sporulation of AMF *Glomus intraradices* in lentil plants, (ii) to detect in parallel to whey application, the effects of changeable P in the plant and soil and changeable salt,

*Corresponding author. E-mail: semrademir@yyu.edu.tr. Fax: +904322251624.

pH, and $CaCO_3$ ratios within soil structure on colonization and sporulation of AMF, and (iii) to elucidate relations between organic waste material (whey) and symbiont fungus (AMF) which were added into soil.

MATERIALS AND METHODS

Cultivation of pants

Lentil plants (*Lens orientalis* cv. Sazak 91) were grown in plastic pots (18 x 18) on sterilized mixture of soil and sand (1/1, v/v). 10 experimental replicates were prepared for each treatment (each lentil plant was in a separate pot) according to completely randomized experimental design. The characteristics of the mixture having pH 7.08 value were as follows (kg $^{-1}$) 0.059% salt, 13.29% $CaCO_3$, 0.031% N (total), (kg mg^{-}1), 5.94 P, 162.70 K, 18.23 Ca, 1.05 Cu, and 0.34 Zn. Seeds of pepper were surface sterilized with 0.05% sodium hypochloride for 45 min before sowing them into 5 cm depth of growth media. The plants were grown in a greenhouse under natural photoperiods (23.5/18°C day/night, 4000 - 6000 lux light intensity) for 10 week during which only distilled water was applied. In addition, twice a week, each pot was supplied with 100 ml of nutrient solution containing (mg l^{-1}) 720 mg $MgSO_4$ 7.H_2O, 12.2 mg KH_2PO_4, 295 mg $Ca(NO_3)_2$.$4H_2O$, 240 mg KNO_3, 0.75 mg $MnCl_2$.$4H_20$, 0.75 mg KI, 0.75 mg $ZnSO_2$.H_2O, 1.5 mg H_3BO_3, 0.001 mg $CuSO_4$.$5H_20$, 4.3 mg FeNaEDTA and 0.00017 mg Na_2MoO_4.$2H_2O$.

The symbiotic fungal partner, *G. intraradices* (Isolate No: OM/95), was produced in a soil : sand mixture (1/1, v/v) using maize as the host. Inoculum of *G. intraradices*, consisted of spores, external mycelium and AMF colonized roots, was laid into around the seed (10 g). The same amount of sterilized inoculum was laid into the control pots.

Whey

Sweet whey which was coagulated with yeast was used in this study. Analyses of whey were made according to Kosikowski (1982), Case et al. (1985), and Scienkiewicz and Riedel (1990). The characteristics of the whey were as follows (ml^{-1}); dry matter 3.69%, water 96.3%, fat 0.1%, fatless dry matter 3.52%, protein 0.88%, ash 0.46%, lactose 3.60%, N 0.13%, P 36.3 µg, Fe 0.82 µg, Zn 4.3 µg, Mn 0.7 µg, Mg 41.75 µg, K 948.5 µg, Ca 233.81 µg, acidity 0.33%, pH 5.10. Whey was applied as 2 doses in the experiment [(50 ml kg^{-1} soil (W_{50}), 100 ml kg^{-1} soil (W_{100})] 2 weeks after the seed planting (Konar and Arıoglu, 1987). Moreover, sterile water was applied to control pots instead of whey.

The six groups involved in the study were:

Control pots (Untreated) (T1)
Application of W_{50} (T2)
Application of W_{100} (T3)
Inoculation with *G. i.*(T4)
Inoculation with *G. i.* + W_{50} (T5)
Inoculation with *G. i.* + W_{100} (T6)

Harvesting and analyses

At the end of the experiment, plants were harvested 10 wk after seed sowing. Plant shoots were separated, dried (at 70°C for 48 h) and weighed. The vanadate-molybdate-yellow procedure was used for P analysis in Jenway 6405 UV/Vis spectrophotometer (Lott et al., 1956). Total nitrogen contents (Kjeldahl-N) of the plant and soil materials were determined according to standard methods described as in Kacar 1972 and Bremner 1965 respectively. Soil pH-value, organic matter, $CaCO_3$ and totally salt were determined by Grewelling and Peech (1960), Jackson (1962), Çağlar (1949) and Richard (1954), respectively. Moreover, available P, K, Ca, Cu, and Zn of soil mixture were determined by Kacar (1972).

Spores of *G. intraradices* were isolated by wet sieving (Gerdemann and Nicholson, 1963) and centrifugation (Jenkins, 1964). Root length (%) colonized by AM fungus was determined using the gridline-intersect method (Giovanetti and Mosse, 1981) after clearing all 0.5 cm root pieces from the borders of the upper and lower thirds of the pot in 10% (w/v) KOH for 2 h (Douds and Schenck, 1990) and staining with tryphan blue (Phillips and Hayman, 1970).

Statistically analyses

Data were analyzed by linear regression and analysis of variance ($P < 0.05$). Characteristics for which significant treatment effects were found were further separated using Duncan's multiple range test ($P < 0.05$) (SAS, 2005).

RESULTS

Soil characteristics

In the soil analyses conducted before and after applying *G. intraradices* and whey into the growth media, it was detected that chemical composition of the soil displayed some differences in considerable ratio, concentration of the elements other than Ca (P, N, K, Cu, Zn) increased compared to untreated soil group; Ca is more in the control soil. While P, N, and K concentrations of soil samples considerably increased in T6 application in which *G. intraradices* and W_{100} were applied together (34, 100 and 58.3%, respectively), Cu and Zn amounts reached the highest increase, respectively, in T4 and T5, T2 applications (Table 1). While the $CaCO_3$ ratio of the soil, salt concentration and pH value displayed some increases in all applications compared to the control group, these increases were especially detected more in T6 application and this was found statistically significant ($P < 0.05$).

Plant analyses

There were some differences in both macro (P, N, K and Ca) and micro elements (Zn and Cu) according to the applications in plants analysed within the scope of the study. In T4 application, *G. intraradices* inoculation was applied, a statistically significant increase (200%) was recorded in terms of P concentration ($P < 0.05$) (Table 2). Both *G. intraradices* and whey increased the nutrient status of the plants (Table 2).

Sporulation and colonization of AM in the root systems

The highest colonization and sporulation values belong-

Table 1. Means of total P, N, K, Ca, Zn and Cu concentrations, CaCO3 Salinity pH of group soils [control (T1), application of W_{50} (T2), application of W_{100} (T3), inoculation with *G. i.* (T4), inoculation with *G. i.* + W_{50} (T5), inoculation with *G. i.* + W_{100} (T6)].

Group	P (mg kg^{-1})	N (%)	K (mg kg^{-1})	Ca (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	$CaCO_3$ (%)	pH	Salinity (%)
T1	5.91 ± 0.25 e	0.03 ± 0.00 c	162.70 ± 0.87 f	18.23 ± 0.14 a	1.05 ± 0.01 d	0.34 ± 0.01 e	13.29 ± 0.12 de	7.08 ± 0.03 d	0.059 ± 0.01 e
T2	6.97 ± 0.11 d	0.04 ± 0.00 b	211.50 ± 0.82 d	14.42 ± 0.12 d	1.26 ± 0.01 c	1.05 ± 0.01 a	14.51 ± 0.11 c	7.66 ± 0.03 c	0.069 ± 0.01 c
T3	7.96 ± 0.04 c	0.04 ± 0.00 b	230.50 ± 0.96 c	15.44 ± 0.12 c	1.46 ± 0.01 b	0.99 ± 0.01 b	15.49 ± 0.09 b	7.76 ± 0.03 b	0.071 ± 0.01 c
T4	8.05 ± 0.06 bc	0.04 ± 0.00 b	187.15 ± 0.99 e	13.56 ± 0.13 e	2.14 ± 0.06 a	0.74 ± 0.01 d	13.43 ± 0.08 d	7.10 ± 0.03 d	0.060 ± 0.01 d
T5	8.90 ± 0.03 b	0.06 ± 0.00 a	244.94 ± 0.44 b	15.55 ± 0.07 bc	1.45 ± 0.01 b	1.02 ± 0.01 a	15.54 ± 0.07 b	7.79 ± 0.02 b	0.089 ± 0.02 b
T6	9.31 ± 0.18 a	0.06 ± 0.00 a	258.27 ± 0.44 a	16.53 ± 0.12 b	1.32 ± 0.01 bc	0.90 ± 0.01 c	16.46 ± 0.08 a	7.98 ± 0.03 a	0.098 ± 0.03 a

Each number represents the mean of 15 observations and numbers with same letter within a column are not significantly different (P < 0.05, Duncan's multiple range test).

Table 2. Means of total P, N, K, Ca, Zn and Cu concentrations of lentil plants [control (T1), application of W_{50} (T2), application of W_{100} (T3), inoculation with *G. i.* (T4), inoculation with *G. i.* + W_{50} (T5), inoculation with *G. i.* + W_{100} (T6)].

Group	P (%)	N (%)	K (mg kg^{-1})	Ca (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
T1	0.11 ± 0.02 e	1.95 ± 0.02 d	0.09 ± 0.01 d	0.32 ± 0.01 c	6.68 ± 0.01 f	3.47 ± 0.05 f
T2	0.15± 0.03 d	3.04 ± 0.03 a	1.28 ± 0.02 bc	0.38 ± 0.01 bc	12.98 ± 0.02 b	5.35 ± 0.01 e
T3	0.19 ± 0.01 c	2.51 ± 0.03 bc	1.32 ± 0.01 b	0.42 ± 0.01 b	12.70 ± 0.02 c	5.54 ± 0.01 c
T4	0.33 ± 0.02 a	2.40 ± 0.05 c	1.00 ± 0.01 c	0.33 ± 0.01 c	12.03 ± 0.01 e	5.43 ± 0.01 d
T5	0.23 ± 0.03 b	2.55 ± 0.04 bc	1.31 ± 0.01 b	0.59 ± 0.01 a	13.14 ± 0.02 a	7.08 ± 0.02 b
T6	0.22 ± 0.03 b	2.75 ± 0.24 b	1.45 ± 0.01 a	0.41 ± 0.01 b	12.26 ± 0.01 d	7.17 ± 0.01 a

Each number represents the mean of 15 observations and numbers with same letter within a column are not significantly different (P < 0.05, Duncan's multiple range test).

ing to AMF *Glomus intraradices* were detected in T5 application in which W_{50} dose of symbiont and whey was applied (Table 3). Here, we observe that low doses of whey have positive effects both on sporulation and colonization of *G. intraradices*.

The effect of P content in the soil especially in T4 application was found important and positive on the sporulation of *G. intraradices* (P<0.05), but it was detected as negative in T5 and T6 applications (Table 5). The pH of the soil affected the sporulation negatively in all applications, and this effect was found also statistically significant in T6 application (Table 5). Even though the effect of total P in lentil plants on sporulation was found statistically insignificant, it was detected as positive in all applications. The $CaCO_3$ content created a negative effect on the sporulation similar to colonization (Table 5). In the relationship of salt ratio in the soil with sporulation, a negative effect was seen in T4 application in which whey was not applied but this effect turned into positive in other two applications in which higher and lower doses of whey was applied.

Regression analyses were conducted in order to introduce the relationship of this positive effect of whey on breeding structures of symbiont micro-organisms with total P changing in the soil and plant, salinity ratio, pH, and $CaCO_3$ content of the soil (Tables 4 and 5). According to the results, the effect of P contents belonging to the plant in all three processes (T4, T5, T6) on the colonization of *G. intraradices* was found significant (P<0.05). This effect was determined as negative in T4 application, and positive in T5 and T6 applications (Table 4). In addition to this, the effect of P content in the soil on AMF colonization was also

Table 3. Mean of % AMF colonized root of lentil plants and spore density in soil inoculated with *G.i* receiving whey.

Group	Colonization (%)	Spore density (cm^{-3} soil)
T4	61.41 ± 0.30 b	83.40 ± 3.53 c
T5	70.71 ± 0.39 a	132.90 ± 3.67 a
T6	57.69 ± 0.88 c	101.00 ± 2.58 b

Each number represents the mean of 15 observations and numbers with same letter within a column are not significantly different ($P < 0.05$, Duncan's multiple range test).

Table 4. Relationships between AMF colonization ratio and contents of total plant and soil P, soil pH, soil salinity, and soil $CaCO_3$ in mycorrhizal lentil plants.

Groups	y =	a +	b.x +	$c.x^2$ +	$d.x^3$	R^2	R
T4 x Plant P ^{P3}		5353	-261.1	4.2	-0.0230	0.575	0.758 *
T5 x Plant P ^{P2}		62.7	-1.752	0.0123	-	0.652	0.807 *
T6 x Plant P ^{P3}		-417.52	22.23	-0.394	0.0023	0.659	0.811 *
T4 x Soil P ^{P2}		291.9	-9.24	0.0752	-	0.724	0.850 *
T5 x Soil P ^{P2}		-11.8	0.584	-0.0041	-	0.063	0.250
T6 x Soil P ^{P2}		9.231	0.022	-0.0004	-	0.147	0.383
T4 x pH ^{P3}		-36.75	177.17	-.288	0.0157	0.107	0.327
T5 x pH ^{P3}		-32.81	136.19	-1.91	0.004	0.506	0.711
T6 x pH ^{P2}		21.396	-0.474	-0.0042	-	0.355	0.595
T4 x salinity ^{P3}		24.20	-118.64	1.933	-0.01	0.160	0.400
T5 x salinity ^{P2}		126.84	-3.55	0.0251	-	0.0334	0.182
T6 x salinity ^{P2}		3.1075	-0.084	0.0008	-	0.299	0.546
T4 x $CaCO_3$ ^{P2}		-96.42	3.51	-0.028	-	0.057	0.238
T5 x $CaCO_3$ ^{p2}		-138.64	4.38	-0.032	-	0.068	0.260
T6 x $CaCO_3$ ^{P2}		-60.96	2.66	-0.023	-	0.175	0.418

^{P2}: Polynomial fit (degree 2), ^{P3}: Polynomial fit (degree 3).
*: Correlation coefficient is significant ($P < 0.05$).

found significant and positive in T5 and T6 applications (Table 4). While the effect of other factors like salinity ratio, pH, and $CaCO_3$ content of the soil on the colonization of *G. intraradices* was found insignificant, the effect of pH was detected as positive in T4 and T5 applications and negative in T6 application. The effect of soil salinity on the colonization of *G. intraradices* was determined as negative in T4 application but positive in T5 and T6 applications. The effect of $CaCO_3$ ratio in the soil was found as negative in all three applications.

The effect of P content in the soil on sporulation of *G. intraradices* was found significant and positive especially in T4 application ($P < 0.05$), but determined as negative in T5 and T6 applications (Table 5). Soil pH affected the sporulation in negative direction in all applications and this effect was also found statistically significant in T6 application (Table 5). While the effect of total P in lentil plants on sporulation was found statistically insignificant, it was detected as positive in all applications. The $CaCO_3$ content had a negative effect on sporulation similar to colonization (Table 5). In the relationship of salinity ratio of the soil with sporulation, while a negative effect was seen in T4 application in which whey was not applied, this turned into positive in other 2 applications in which higher and lower doses of whey were applied.

DISCUSSION

It is known that whey applied on the soil with the aim of fertilization improves soil structure, and increases water holding capacity of the soil and porosity in addition to its effect of increasing productivity (Watson, 1978; Sienkiewicz and Riedel, 1990). Due to its characteristics, whey is effective not only in terms of plant nutrition but as nutrient of microorganisms existing in soil micro flora. Morris (1985) argued that protein nitrogen existing in whey is converted into inorganic nitrogen which can be used by the plant. It is stated that whey is also significant in terms of microbial nutrition with rich nutrition materials existing in whey and some carbon compounds especially lactose are used as an energy source for microorganisms

Table 5. Relationships between AMF spore density and contents of total plant and soil P, soil pH, soil salinity, and soil $CaCO_3$ in mycorrhizal lentil plants.

Groups	y =	a +	b.x +	c.x^2 +	d.x^3	R^2	R
T4 x Plant P [P2]		7.3934	-0.1673	0.001	-	0.353	0.594
T5 x Plant P [P2]		3.6159	-0.0501	0.0002	-	0.0096	0.097
T6 x Plant P [P2]		2.6320	-0.0471	0.0002	-	0.0074	0.086
T4 x Soil P [P2]		36.59	-0.68	0.0041	-	0.578	0.760 *
T5 x Soil P [P2]		-4.75	0.20	-0.0007	-	0.225	0.474
T6 x Soil P [P2]		4598	-136.11	1.3451	-	0.410	0.640
T4 x pH [P2]		2.233	0.1155	-0.0007	-	0.0764	0.276
T5 x pH [P2]		-1.464	0.136	-0.005	-	0.245	0.494
T6 x pH [P3]		1512	-44.82	0.4452	-0.0015	0.670	0.818*
T4 x salinity [P3]		167.027	-6.0115	0.0721	-0.0003	0.322	0.567
T5 x salinity [P2]		74.7336	-1.0832	0.0040	-	0.125	0.353
T6 x salinity [P3]		-867.04	25.866	-0.2569	0.0009	0.210	0.458
T4 x $CaCO_3$ [P2]		17.3823	-0.0744	0.0003	-	0.0875	0.295
T5 x $CaCO_3$ [P2]		-178.96	2.9257	-0.0110	-	0.345	0.587
T6 x $CaCO_3$ [P2]		-79.11	1.8378	-0.0088	-	0.333	0.577

[P2]: Polynomial fit (degree 2), [P3]: Polynomial fit (degree 3).
*: Correlation coefficient is significant ($P < 0.05$).

(Morrissey, 1985; Iwabuchi and Yamauchi, 1987). Moreover, it was observed in some studies that whey addition to soil promotes bacteria and fungus population (Sonnleitner et al., 2003a, 2003b).

In this study, it was recorded that both sporulation and colonization ratios of AM fungus increase especially with the application of low doses of whey (Table 3). Nutrition status within plant increase due to use of whey also affected the development of AM fungus which is an obligate microorganism. Thus, it was seen in the regression analyses that total P within the plant had a significant effect on colonization of fungus in all 3 applications (Table 4). In parallel to whey application, while the effects of various P, salt, pH, and $CaCO_3$ ratios on colonization and sporulation were different, pH had a negative effect on sporulation especially in T6 application (Table 5). AM fungus generally prefer the environments with acidic structure, therefore negative effect of pH on sporulation in T6 application where a higher dose of whey was used could be dependent on different reasons. Clark et al. (1999) argued that some AMF isolates are also effective in very acidic soils and this effect could be different according to isolates. Therefore, the negative effect on sporulation could be dependent on its isolate characteristics.

In conclusion, it has become an obligation to create evaluation areas in agricultural fields for this product which is an important dairy waste with high nutritive. This is certainly accurate when its positive effects on micro-organism population within soil structure are taken into account. However, the most important point required to be taken into account during these applications is to use whey in necessary and sufficient amounts. Since its excessive use can create negative effects on micro-organism population in soil structure, attention should be paid in order to avoid exceeding required amount.

REFERENCES

Bremner JM (1965). Methods of Soil Analysis, Part: 2, American Soc. of Agro Inc., Publisher Medison, Wisconsin, USA.

Case RA, Bradley RL, Williams RR (1985). Chemical and physical methods. In: Richardson GH (ed) Standarts methods for the examination of dairy products, 15 th edn. American Public Health Association, Washington D.C., pp. 327-402.

Clark RB, Zeto SK, Zobel RW (1999). Arbuscular mycorrhizal fungal isolate effectiveness on growth and root colonization of *Panicum virgatum* in acidic soil. Soil Biol. Biochem. 31: 1757-1763.

Douds D, Schenck NL (1990). Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. New Phytol. 116: 621-627.

Gerdemann LW, Nicholson TH (1963). Spores of mycorrhizal Endogene extraeted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46: 235-244.

Giovanetti M, Mosse B (1981). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Grewelling T, Peech M (1960). Chemical Soil Test. Cornel Univ. Agr. Exp. Sta. Bull. Handbook, 60. U.S. Dept. of Agriculture.

Iwabuchi S, Yamauchi F (1987). Electrophoretic analyses of whey proteins present in soybean globulin fractions. Journal of Agricultural and Food Chemistry 35(2): 205-209.

Jackson ML (1962). Soil Chemical Analysis. Prentice Hall. Inc. Engle Wood Cliff, New Jersey.

Kacar B (1972). Chemical Analyses of Plant and Soil II. Soil Analyses, Publications of Agriculture Fac. of Univ. of Ankara, No: 453 Ankara/Turkey.

Konar A, Arıoglu H (1987). A preliminary study on the use of cheese whey as fertilizer on soybean. J. Agric. Cukurova Univ. 2(2): 1-3.

Kosikowski FV (1982). Cheese and fermented milk foods, New York, p. 134.

Lott WL, Nery JP, Gallo JR, Medcaff JC (1956). Leaf analysis technique

in coffie researches. IBEC Research Ins. 29: 21-24.
Lynch JM, Bragg, E (1985). Microorganisms and soil aggregate stability. Adv. Soil Sci. 2: 287-297.
Morrissey PA (1985). Lactose, chemical and physico-chemical properties. In: Morrisey PA (ed) Developments in dairy chemistry-3. lactose and minor constituents, Appl. Sci. Publ. London, pp. 1-34.
Morris S (1985). Whey, feed or fertilizer. Proceed. of the Ruakura Farmer's Conference, New Zealand 37: 113-116.
Ozrenk E, Demir S, Tufenkci S (2003). The Effects of Whey Application and Inoculations of *Glomus intraradices* and *Rhizobium cicer* on the Some Growth Parameters of Chickpea. J. Agric. Sci. Yuzuncu Yil Univ., 13(2): 127-132.
Phillips JM, Hayman DS (1970). Improved procedure for cleaning roots and staining parasitic and vesicular - arbuscular mycorrhizal fungi for rapid assesment of infection. Trans. Br. Mycol. Soc. 55: 158-161.
Reddy GV, Deshmukh VR, Joshi RN, Kayama R (1987). Utilization of alfalfa (*Medicago sativa* L.) whey as a fertilizer in irrigation. Jpn. Soc. Grassland Sci. 33(1): 32-37.
Richard LA (1954). Diagnosis and Improvement of Saline and Alkaline Soils. Handbook 60. U.S. Dept. of Agriculture.
SAS (2005). SAS/STAT Software: hangen and enhanced SAS, Inst. Inc. Cri. NCI.
Sienkiewicz T, Riedel CL (1990). Whey and Whey Utilization. Verlag Th. Mann, Gelsenkichen-Buer, Germany.
Smith SE, Read DJ (1997). Mycorrhizal Symbiosis. Academic Press, New York.
Sonnleitner R, Lorbeer E, Schinner F (2003a). Monitoring of changes in physical and microbiological properties of a Chernozem amended with different organic substrates. Plant Soil, 253: 391-402.
Sonnleitner R, Lorbeer E, Schinner F (2003b). Effects of straw, vegetable oil, and whey on physical and microbiological properties of a chernozem. Appl. Soil Ecol. 22: 195-204.
Tisdall JM, Smith SE, Rengasamy P (1991). Aggregation of soil by fungal hyphae. Austr. J. Soil Res. 35: 55-60.
Watson KS (1978). Continuing impact of the environmental area on the dairy industry. Proocedings Whey Products Conference, Minneapolis-Minesota pp. 30-52.
Zadow JG (1994). Utilization of milk components: Whey. In: Robinson RK (ed) Advances in milk processing (Modern Dairy Technology), pp. 313-317.