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EFFECTS OF HUMIC ACID, WHEY AND ARBUSCULAR MYCORRHIZAL FUNGI (AMF) APPLICATIONS ON SEEDLING GROWTH AND FUSARIUM WILT IN ZUCCHINI (*Cucurbita pepo* L.)

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ABSTRACT

The effects of Arbuscular Mycorrhizal Fungi (AMF, *Gigaspora margarita*), whey (50 ml kg^{-1}), and Humic acid (HA, 500 mg kg^{-1}) applications on seedling growth and Fusarium wilt (FON, *Fusarium oxysporum* sp. *niveum*) in hybrid zucchini (*Cucurbita pepo* L.) cultivar Focus F_1 were investigated. The study was consisted of total 16 treatments including different combinations of FON, AMF, humic acid and whey. There were significant effects of the treatments on seedling growth and some nutrient contents. The treatment which had the highest suppression on Fusarium wilt was the single AMF treatment followed by triple application of AMF, HA, and whey.

Key words: AMF, fusarium wilt, humic acid, whey, zucchini.

INTRODUCTION

In seedling production in vegetables, obtaining suitable growth medium is essential. The addition of organic residues could improve physical conditions of growth medium (Sonnleitner *et al.* 2003a). Recently, a variety of organic substrates as manure has been used to improve growth medium. The substrates that have been added to soil range from simple sugars to complex organic materials (Sonnleitner *et al.* 2003b).

Humic acid (HA) ameliorates the nutrient uptake of plants, the plant growth, and plant resistance, which has been revealed in several studies (Turkmen *et al.* 2005; Tufenkci *et al.* 2006; Abdel-Monaim *et al.* 2011).

The use of whey being important for nutrition and microbiological growth is another strategy to enhance nutrient outflow to plants. It has been used as fertilizer not only to encourage plant growth but may also increase microorganism population in the soil (Özrenk *et al.* 2003).

Arbuscular mycorrhizal fungi (AMF) are the most widespread root fungal symbionts and are associated with most plant species. Mycorrhizal symbiosis plays a significant role in the nutrition and development of host plants. Arbuscular mycorrhizal fungi (AMF) have been apparent to improve soil properties (Miller and Jastrow 2000), and nutrient uptake of plants (Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) additionally support plants to cope with both biotic and abiotic stresses: they may fight with some soil-borne pathogens (Garmendia *et al.* 2004; Hu *et al.* 2010), minimize some nutrient deficiencies (especially phosphorous and micro nutrients), and ameliorate drought, salinity, and pollution tolerance (Turkmen *et al.* 2005; Sensoy *et al.* 2007; Turkmen *et al.* 2008; Sensoy *et al.* 2011).

Cucurbita pepo L. is an important vegetable in Turkey and in the world (FAOSTAT 2010). *Fusarium oxysporum* f.sp. *niveum* causes fusarium wilt in *Cucurbita pepo* L. (Martyn and McLaughlin 1993). Effectiveness of AMF is much better in a sterile growth medium (Wang *et al.* 2008). Inoculation with AMF, in some vegetables, may improve growth performance (Temperini *et al.* 2009). Recent studies have suggested that AMF-inoculated zucchini could benefit from association with AMF (Colla *et al.* 2008; Cardarelli *et al.* 2010; Sensoy *et al.* 2011) by enhancing its nutrition in abiotic stress conditions. Sensoy *et al.* (2011) revealed the response of four zucchini (*Cucurbita pepo* L.) hybrids to different arbuscular mycorrhizal fungi, and these researchers stated that *Gigaspora margarita* inoculated cv. Focus F_1 had the highest relative mycorrhizal dependency.

The aim of the present study was to reveal the effects of AMF (*Gigaspora margarita*), whey and HA applications on seedling growth and Fusarium wilt (*Fusarium oxysporum* sp. *niveum*) in hybrid zucchini (*Cucurbita pepo* L.) cultivar Focus F_1.

MATERIALS AND METHODS

Arbuscular Mycorrhizal Fungi (AMF, *Gigaspora margarita*), whey (50 ml kg^{-1}), and Humic acid (HA, 500 mg kg^{-1}) applications on seedling growth and Fusarium wilt (FON, *Fusarium oxysporum* sp. *niveum*) in hybrid zucchini (*Cucurbita pepo* L.) cultivar Focus F_1 were

examined. The study was consisted of total 16 treatments including different combinations of FON, AMF, HA and whey. The data was analyzed with one-way ANOVA (Table1-3). Moreover, the experiment could also be analyzed as four-factor ANOVA (Table 4).

Growth medium comprised of an autoclaved mixture of perlite and peat moss (1:1 *v/v*) in seedling trays (Each individual plant cell measures approximately 4.5 cm W x 5.5 cm L x 5.5 cm D) covered by vermiculite. The experiment used a completely randomized design with three random replications of fifteen cells each, for a total of 720 cells. One seed was sown per cell, each of which contained 80 cm^3 of sterilized growth medium. AMF inocula consisted of spores, extraradical mycelium and mycorrhizal roots. In the AMF-inoculated samples, five g (25 spores g^{-1}) of inoculums were placed in the growth medium before the seeds were sown (Demir and Onogur, 1999).

Sweet whey which was coagulated with yeast was used in this study. The characteristics of the whey were as follows (ml-1); dry matter 7.06%, water 92.9%, fat 0.4%, protein 1.04%, ash 0.52%, lactose 4.33%, N 0.16%, acidity 0.13%, pH 6.62. Whey was applied as 1 dose in the experiment (50 ml kg^{-1} growth medium) 2 weeks after the seed sowing (Konar and Arıoğlu 1987).

Seedling trays were placed in a growth room at a temperature of 22±2 ^oC with 12 h fluorescent illumination (8,000 lux light intensity), and irrigated with distilled water. Each seedling was fertilized twice with a 5 ml of nutrient solution (for 1 L: 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 $MnCl_2.4H_2O$, 0.75 mg KI, 0.75 mg $ZnSO_2.H_2O$, 1.5 mg H_3BO_3, 0.001 mg $CuSO_4.5H_2O$, 4.3 mg FeNaEDTA, and 0.00017 mg $Na_2MoO_4.2H_2O$) modified by Vosatka and Gryndler (1999).

Zucchini seedlings were artificially inoculated by sand culture (Turhan and Grosman 1987) of *Fusarium oxysporum* sp. *niveum* (The isolate of FON was obtained from Dr. Sener Kurt, Mustafa Kemal University Plant Protection Department). In the sand culture method, corn meal (20 g), agarose (20 g), and washed coarse river sand (120 g) were combined in 350 ml glass bottles, and were watered and then sterilized by autoclaving. Seven to ten day-old FON isolates grown on potato dextrose agar (PDA) cultures were mixed to the prepared sterilized medium at the rate of one fourth petri dish to each bottle. Final mixture was incubated at 25±2 ^oC for 4 weeks. These four-week old cultures were mixed with growth medium at the rate of 5% (v/v). The six-week-old seedlings were transferred into the pots containing 300 cm^3 of growth medium, and half of the treatments were inoculated with FON. The seedlings were harvested 3 weeks after FON inoculation.

Stem diameters, shoot heights, root lengths, leaf numbers, shoot dry weights, and root dry weights of seedlings were determined after harvesting. Samples were then oven-dried at 68ºC for 48 h, ground, and nitrogen (N) contents of shoots measured using Kjeldahl method; potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), cupper (Cu), zinc (Zn), and manganese (Mn) contents of shoots measured using atomic absorption spectrophotometer; phosphorous (P) contents of shoots measured using the vanadate-molybdate-yellow procedure with spectrophotometer (Kacar and Inal 2008).

Zucchini roots were dyed to detect AMF presence using a modification of Phillips and Hayman's (1970) method, and the percentage and intensity of mycorrhizal colonization was estimated using the gridline intersect method (Giovanetti and Mosse 1980).

Fusarium wilt disease severity was determined with a 0-3 scale (Demir *et al.* 2006) and disease incidence was calculated according to the following equation;

Disease incidence (%) = $((0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3)) \times 100 / n \times 3$

where n_0 was the number of plants with no symptoms, n_1 was the number of plants having one third necrosis at the stem-root-neck, n_2 was the number of plants having two third necrosis at the stem-root-neck, n_3 was the number of dead plants, n was total plant number, and 3 was the highest value at the scale.

Data were analyzed using the SAS statistical program, with variance analysis conducted for all data. Differences between treatments were determined using Duncan's Multiple Range Test (SAS Software 1997).

RESULTS AND DISCUSSION

The treatments had significant effects on seedling shoot height (Table 1). The highest seedling shoot height was observed in the treatment combined AMF + HA. The treatments had also significant effects on seedling phosphorous content (Table 2). The highest seedling phosphorous content was observed in the treatment combined AMF + whey followed by single HA application, while single whey applications had the lowest seedling phosphorous content.

The treatments had significant effects on iron and copper contents, mycorrhizal colonization and disease incidence (Table 3). The highest iron content was observed in the treatment combined HA + whey, while the lowest iron content was observed in the treatment combined AMF + HA. The highest copper content was observed in the single HA treatment, while the lowest copper contents was generally observed in the treatments infected with FON. The lowest mycorrhizal colonization was observed in the single AMF treatment, while the treatments containing HA and whey had higher mycorrhizal colonization rates than that of the single AMF treatment, and FON infected treatments had even higher mycorrhizal colonization rates than those of the disease free ones. The lowest disease incidence was observed in the single AMF treatment followed by triple

application of AMF + HA + whey. On the other hand, there was a lit bit increase in disease incidence in the treatment combined AMF and whey.

In four-factor ANOVA (Table 4), it was seen that FON was negatively affected most of the traits (Leaf number, shoot an root dry weights, P,K, Zn, Cu, Mg, and Ca contents, mycorrhizal colonization, and disease incidence); AMF application was significant on N, and Cu contents, and mycorrhizal colonization; HA application was significant on Cu content; Whey application was significant on Fe content; FON*AMF interaction was significant on root dry weight and mycorrhizal colonization; FON*HA interaction was significant on P content; FON*Whey interaction was significant on root dry weight; AMF*Whey interaction was significant on P content; HA*Whey interaction was significant on Cu content; FON*AMF*HA interaction was significant on N and P contents; FON*AMF*Whey interaction was significant on Fe and P contents and disease incidence; FON*HA*Whey interaction was significant on Fe and Zn contents; AMF*HA*Whey interaction was significant on Mn, Fe, Zn, and Mg contents and disease incidence; FON*AMF*HA*Whey interaction was significant on disease incidence.

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 (Ocak and Demir 2012). Due to its characteristics, whey is effective not only in terms of plant nutrition but as nutrient of microorganisms existing in soil micro flora. Demir and Ozrenk (2009) studied the effects of whey on the colonization and sporulation of AMF, *Glomus intraradices*, in lentil (*Lens orientalis*), and recorded that both sporulation and colonization ratios of AM fungus increased especially with the application of low doses of whey. These researchers also stated that nutrition status within plant increase due to use of whey also affected the development of AM fungus which is an obligate microorganism. In the present study, although the single whey applications had the lowest seedling phosphorous content, the significant effects of whey treatments combined with AMF and HA were observed on seedling height, some nutrient contents, mycorrhizal colonization, and disease incidence.

Many crops benefit from AMF in a variety of biotic and abiotic stress conditions (Meghvanski *et al.* 2008; Miyauchi *et al.* 2008; Wang *et al.* 2008; Long *et al.* 2010). The limited number of studies on the effects of AMF in zucchini has suggested that AMF-inoculated zucchini could benefit from association with AMF in abiotic stress conditions (Colla *et al.* 2008; Cardarelli *et al.* 2010). Colla *et al.* (2008) studied the effects of AMF on salt stressed zucchini plants grown at low and high phosphorous concentration. These researchers stated that the beneficial effects of AM on zucchini plants could be due to an improvement in water and nutritional status. Cadradelli *et al.* (2010) studied the effects of AMF on alkaline stressed zucchini plants grown under mineral and organic fertilization, and demonstrated that AMF inoculated treatments under alkaline conditions had higher total, marketable yield and total biomass compared to non-inoculated AMF treatments, which might be due to a better nutritional status. Sensoy *et al.* (2011) revealed the response of four zucchini (*Cucurbita pepo* L.) hybrids to different arbuscular mycorrhizal fungi, and these researchers stated that *Gigaspora margarita* inoculated cv. Focus F_1 had the highest relative mycorrhizal dependency. The significant effects of AMF inoculation on the most of nutrients (especially on P, K, Ca, Fe, and Zn), cotyledon width and stem diameter were detected in that study (Sensoy *et al.* 2011). Cardarelli *et al.* (2010) also demonstrated that AMF inoculated zucchini plants had higher nutrients, cotyledon, stem diameter, and root fresh weight compared to non-inoculated AMF plants. Wang *et al.* (2008) also found that N and P contents in cucumber roots and Mg, Cu, Zn contents in cucumber shoots were increased by inoculating AMF. There are many other benefits of AMF in agriculture. AMF may positively affect crop development, even in phosphorous-rich soils, and the development of cultivars with improved symbiotic qualities would insure the production of good crop yields while improving sustainability. (Hamel and Strullu 2006). In the present study, the significant effects of AMF treatments and its combination with Whey and HA on seedling growth and some nutrient contents were observed. Moreover, AMF treatments had also suppression on Fusarium wilt.

Humic acid (HA) ameliorates the nutrient uptake of plants, the plant growth, and plant resistance to biotic and abiotic stress conditions (Wang 1995; Turkmen *et al.* 2005; Tufenkci *et al.* 2006; Abdel-Monaim *et al.* 2011). Turkmen *et al.* (2005) studied the effects of AMF and HA on the seedling development and nutrient content of pepper grown under saline soil conditions. These researchers stated that the most of the seedling growth parameters and nutrient contents were positively affected by AMF and HA applications, and HA increased the effects of AMF in saline conditions. Tufenkci *et al.* (2006) studied the effects of humic acid doses and application times on the plant growth, nutrient and heavy metal contents of lettuce grown on sewage sludge-applied soils. These researchers stated that HA had positive effects on plant growth parameters and nutrient contents. Abdel-Monaim *et al.* (2011) studied the effects of HA on *Fusarium* wilt in soybean, and stated that HA could induce systemic resistance against the disease. In the present study, the significant positive effects of HA treatments either single or combined with AMF and HA were observed on seedling growth, some nutrient contents, mycorrhizal colonization, and disease incidence.

In conclusion, Arbuscular Mycorrhizal Fungi (AMF), Humic acid (HA) and whey could offer enhancement in seedling production and resistance to Fusarium wilt in zucchini (*Cucurbita pepo* L.). Effective use of these symbiotic soil fungi and organic substrates could be an essential element for sustainable agriculture. AMF, HA, and whey may enhance seedling traits and plant resistance in vegetable species. Therefore, the appropriate doses of HA and whey need to be tried and found in the initially identified appropriate cultivar-AMF combinations in order to derive the utmost benefit from symbiosis in future vegetable studies.

Table 1. Effects of different combinations of *Fusarium oxysporum* sp. *niveum* inoculated (FON), Arbuscular mycorrhizal fungi (AMF), Humic acid (HA), and whey on seedling stem diameter, leaf number, shoot height, root length, and shoot and root fresh weights of *C. pepo* plants.

Treatments	Stem diameter (mm)	Leaf number	Shoot height (cm)	Root length (cm)	Shoot fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})
1: Control	2.53 NS	8.38 NS	9.31 ^{d*}	14.79 NS	2.72 NS	0.56 NS
2: AMF	3.06	8.04	14.21 ^{ab}	17.72	4.42	0.73
3: HA	2.55	8.61	9.67 ^{cd}	15.67	2.59	0.42
4: Whey	2.99	8.16	11.16 ^{bcd}	17.78	3.27	0.56
5: AMF + HA	2.91	8.90	15.73 ^{a}	17.19	4.36	0.59
6: AMF + Whey	2.73	9.68	10.02 ^{cd}	16.07	3.65	0.56
7: HA + Whey	3.06	9.35	11.44 ^{bcd}	18.23	3.77	0.59
8: AMF + HA + Whey	3.36	8.85	11.39 ^{bcd}	17.72	4.08	0.53
9: Control + FON	2.72	7.35	11.17 ^{bcd}	14.73	2.40	0.49
10: AMF + FON	3.41	8.37	13.11 ^{abcd}	15.11	4.20	0.61
11: HA + FON	3.13	8.19	12.88 ^{abcd}	16.83	4.55	0.59
12: Whey + FON	3.12	7.63	14.56 ^{ab}	16.39	3.76	0.48
13: AMF + HA + FON	3.24	7.57	13.49 ^{abc}	16.71	4.03	0.96
14: AMF + Whey + FON	2.80	6.88	13.04 ^{abcd}	18.29	2.67	0.57
15: HA + Whey + FON	3.21	7.85	12.23 ^{abcd}	18.53	3.04	0.72
16: AMF + HA + Whey + FON	3.30	6.48	10.90 ^{bcd}	13.90	3.47	0.39

Values are the means of three replicate samples. NS: nonsignificant. *:*P<0.05*(significant)

Table 2. Effects of different combinations of *Fusarium oxysporum* sp. *niveum* inoculated (FON), Arbuscular mycorrhizal fungi (AMF), Humic acid (HA), and whey on seedling nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) contents of shoots in *C. pepo* plants.

Treatments	**Nitrogen (%)**	**Phosphorous (%)**	**Potassium (%)**	**Calcium (%)**	**Magnesium (%)**
1: Control	2.27 NS	1.80 ^{abcd*}	0.81 NS	5.97 NS	2.80 NS
2: AMF	2.61	1.75 ^{abcd}	0.89	7.94	3.66
3: HA	2.48	2.50 ^{ab}	0.94	6.64	3.15
4: Whey	1.80	0.60 ^{d}	0.77	7.23	3.46
5: AMF + HA	1.79	1.45 ^{abcd}	0.86	6.87	2.91
6: AMF + Whey	2.59	2.90 ^{a}	0.83	6.63	3.33
7: HA + Whey	2.01	1.70 ^{abcd}	0.84	5.98	3.02
8: AMF + HA + Whey	1.92	2.00 ^{abcd}	0.90	7.05	3.63
9: Control + FON	2.71	0.90 ^{cd}	0.89	6.98	3.63
10: AMF + FON	1.72	1.22 ^{bcd}	1.03	8.19	3.99
11: HA + FON	1.76	2.00 ^{abcd}	1.02	7.80	3.79
12: Whey + FON	2.52	0.80 ^{d}	1.06	7.59	4.09
13: AMF + HA + FON	2.11	2.45 ^{abc}	1.01	7.95	3.65
14: AMF + Whey + FON	2.21	0.75 ^{d}	0.97	7.77	3.72
15: HA + Whey + FON	1.61	1.10 ^{bcd}	1.01	7.03	3.59
16: AMF + HA + Whey + FON	1.96	1.35 ^{bcd}	1.01	6.60	3.60

Values are the means of three replicate samples. NS: nonsignificant. *:*P<0.05*(significant)

Table 3. Effects of different combinations of *Fusarium oxysporum* sp. *niveum* inoculated (FON), Arbuscular mycorrhizal fungi (AMF), Humic acid (HA), and whey on mycorrhizal colonization, disease incidence and seedling iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) contents of shoots in *C. pepo* plants.

Treatments	Iron (mg kg^{-1})	Copper (mg kg^{-1})	Zinc (mg kg^{-1})	Manganese (mg kg^{-1})	Mycorrhizal colonization (%)	Disease incidence
1: Control	12.80 $^{cde\ **}$	29.5 $^{abcd\ ***}$	43.0 NS	52.5 NS	-	-
2: AMF	17.40 ^{bcd}	37.0 ^{abc}	47.5	76.5	25.28 ^{c*}	-
3: HA	12.30 ^{cde}	45.0 ^{a}	46.8	63.0	-	-
4: Whey	16.80 ^{bcd}	41.7 ^{ab}	46.5	70.0	-	-
5: AMF + HA	10.95 ^{e}	20.3 ^{cde}	41.0	57.5	43.37 ^{bc}	-
6: AMF + Whey	12.70 ^{cde}	35.7 ^{abc}	40.5	57.5	43.93 ^{bc}	-
7: HA + Whey	23.30 ^{a}	25.0 ^{bcde}	47.0	69.0	-	-
8: AMF + HA + Whey	17.80 ^{bc}	15.0 ^{de}	69.0	73.5	43.86 ^{bc}	-
9: Control + FON	14.50 ^{bcde}	16.5 ^{de}	46.0	71.5	-	68.34 ^{ab**}
10: AMF + FON	15.00 ^{bcde}	9.0 ^{e}	53.5	72.0	60.06 ^{ab}	49.66 ^{c}
11: HA + FON	14.05 ^{bcde}	14.5 ^{de}	56.5	75.0	-	60.74 ^{ab}
12: Whey + FON	14.70 ^{bcde}	12.5 ^{de}	56.5	74.5	-	61.11 ^{ab}
13: AMF + HA + FON	13.25 ^{cde}	10.0 ^{e}	48.5	71.0	60.99 ^{ab}	60.74 ^{ab}
14: AMF + Whey + FON	18.80 ^{ab}	22.0 ^{cde}	55.5	62.5	76.43 ^{a}	70.37 ^{a}
15: HA + Whey + FON	12.10 ^{de}	9.0 ^{e}	47.5	65.0	-	66.44 ^{ab}
16: AMF + HA + Whey + FON	14.80 ^{bcde}	10.5 ^{e}	53.0	74.5	55.85 ^{abc}	59.26 ^{b}

Values are the means of three replicate samples. NS: nonsignificant. *:*P<0.05;* **:*P<0.01*; ***:*P<0.001*(significant)

Table 4. Significance levels of *Fusarium oxysporum* sp. *niveum* inoculated (FON), Arbuscular mycorrhizal fungi (AMF), Humic acid (HA), and whey on some mycorrhizal colonization, seedling growth and nutrient content parameters in *C. pepo* plants.

Factors and their interactions	LN	SDW	RDW	P	N	K	Mn	Fe	Zn	Mg	Ca	MYC	DI
FON	**	*	NS	*	NS	***	NS	NS	*	**	*	**	***
AMF	***	NS	*	NS	NS	NS	NS	NS	*	NS	NS	***	NS
HA	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
Whey	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
FON*AMF	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
FON*HA	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
FON*Whey	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AMF*HA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AMF*Whey	NS	NS	NS	*	NS	NS	NS	NS	*	NS	NS	NS	NS
HA*Whey	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FON*AMF*HA	NS	NS	NS	*	**	NS	NS	NS	NS	NS	NS	NS	NS
FON*AMF*Whey	NS	NS	NS	*	NS	NS	NS	*	NS	NS	NS	NS	*
FON*HA*Whey	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
AMF*HA*Whey	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	**
FON*AMF*HA*Whey	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

LN: Leaf Number; SDW: Shoot Dry Weight; RDW: Root Dry Weight; P: Phosphorous; N: Nitrogenous; K: Potassium; Mn: Manganese; Fe: Iron; Zn: Zinc; Mg: Magnesium; Ca: Calcium; MYC: Mycorrhizal Colonization; DI: Disease Incidence. NS: nonsignificant. *:*P<0.05;* **:*P<0.01*; ***:*P<0.001*(significant)

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