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Effect of Humic Acid Applications on the Root-Rot Diseases Caused by *Fusarium* spp. on Tomato Plants

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Abstract: A study was conducted to elucidate the relations between the effects of humic acid applications and incidents of root rot diseases of tomato in greenhouse soils contaminated with soil-borne pathogens. For this purpose, commercially available powder preparations containing 80% humic acid and fulvic acid were used in the experiment. After the application of three different doses (80, 160, 240 mg active ingredient, a.i., plant^{-1}), with five replications, healthy and diseased plants were counted and root weights were recorded to determine the disease development followed by fruit maturity. The disease percentage of the control group was found as 30.4 while those of the treated groups were 29.6, 30.8 and 34.4 with respect to doses of 80, 160 and 240 mg a.i. plant^{-1}, respectively. Average root fresh weight in the control group was found 110 g plant^{-1} while those of the treated groups were found as 135, 156, 215 g plant^{-1}, respectively. The results clearly showed that the application of humic acid caused an increment in root fresh weight while making plants to susceptible to the root rot pathogens.

Key words: Humic acid, *Fusarium* spp., root rot, tomato

INTRODUCTION

Soil organic contents are one of the most important parts that they directly affect the soil fertility and textures with their complex and heterogen structures although they occupy the minor percentage of soil weight. They also play very crucial roles for the microbial activities in the soil and soil aggregation (Boyle *et al*., 1989; Tejada *et al*., 2006).

In recent years, many advances have been achieved to improve the quality and quantity in agriculture. Advances and developments in agriculture not only depend on mechanization and new hybrid seeds but also improvement the soil properties which also help to increase the crop productivity. Unsuitable soil conditions for plant development generally arise from the lack of organic contents in the soil. To solve this problem, humic substances have started to be given to the soil in Turkey and as well as in the other parts of the world to improve the crop yield. For example, humic and fulvic acid preparations have commonly been used in many greenhouses in Turkey. Majority of them have been produced domestically although some of them have been imported.

From the point of view of producers, these chemical preparations have been perceived and accepted as a kind of hormone promoting the growth rather than improving the chemical and physical conditions of the soil. Thus, growers have been intensively using these chemicals from the beginning of seedling stage until the end of vegetative growth. Especially in winter seasons, these substances have been commonly and extensively used to promote the root development in cold-affected soils.

In many studies, humic and fulvic acid preparations were reported to increase the uptake of mineral elements (Maggioni *et al*., 1987; De Kreij and Basar, 1995; Mackowiak *et al*., 2001), promoted the root length (Vaughan and Malcolm, 1979; Canellas *et al*., 2002) and increased the fresh and dry weight of crop plants (Kauser *et al*., 1985; Chen *et al*., 2004a, b). Due to positive effect of humic substances on the visible growth of plants, these chemicals have been widely used by the growers instead of other substances such as pesticides etc. This, however, led to growers use more of these substances.

Until now, the beneficial effects of humic substances have been mentioned but excessive use of these chemicals might lead to the environmental pollution and other undesired consequences in the near future.

In this study, the relations between root rot pathogens of tomato caused by *Fusarium* spp. and humic acid applications were elucidated after the establishment of diseases in contaminated soils.

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MATERIALS AND METHODS

This study was conducted in a grower's greenhouse soil where it was heavily contaminated with *Fusarium roseum* and *F. oxysporum* f. sp. *radicis* root rot diseases for subsequent two years in Fethiye county between 2005-2006. Before the start of experiment, it was noticed that the greenhouse soil had great potential to cause infections on tomato plants via preliminary experiments. Tomato seedlings from a local variety (MAY Tomato seed M 1103 F1) were transferred to contaminated greenhouse soil. Growth was continued for a further two weeks before seedlings were used for the experiments. Humic and fulvic acid preparations were then applied as 100 mL with doses of 80, 160 and 240 mg a.i plant^{-1}. The second dose was divided into two and the third dose was divided into three doses and applied with 20 day intervals. The main reason for this application was due to the behaviour of the growers who applied the chemicals more than once in one growing season. In general, when applications were made, the solutions were carefully applied to the lower stem area in the soil. The plants were then irrigated with the drip irrigation system.

Each dose was carried out with five repetitions in which two planting rows containing 22 plants in a parcel were accepted as one repetition. Control plants were not treated with any substances.

Treated plants were observed until the first sign of fruit maturity such as colour and taste changes. The plants showing disease symptoms of each repetition was recorded. Disease incidence was evaluated on the basis of diseased and healthy plants. Isolations were then made from the roots of diseased plants using Penta Chloro Nitro Benzene (PCNB) Agar Culture Medium (Nelson *et al*., 1983) to determine the causing organisms.

When the fruits became visibly mature, five randomly selected plants from each treatment were uprooted from the soil and their roots were washed and the soil particles were gently removed under tap water. The plants were then laid over the paper tissues to remove the wetness and their fresh weights were recorded.

The results were analysed using Analysis of Variance including Duncan's Multiple Range Test to determine the significant differences between treatments with SPSS Package Program 10.0 (SPSS is a registered trademark of SPSS inc.).

RESULTS AND DISCUSSION

A positive relationship was found between the increasing concentrations of humic substances and the appearance of the diseases caused by soil borne pathogens (Table 1). When humic acid was applied with doses of 80, 160 and 240 mg a.i. plant^{-1}, the percentage disease symptoms of plants in each respective dose were recorded as 29.6, 30.8 and 34.4%, respectively, while the percentage disease symptoms in control plants were found as 30.4% (Table 1). No statistical difference was found between the application of humic acid (80, 160 mg a.i. plant^{-1}) and the control plants. In fact, the disease progress showed similar patterns with the control group. A further increase in the dose (240 mg a.i plant^{-1}) resulted in a quite significant root fresh weight increment while making the plants quite susceptible to the root rot pathogens.

Table 1: Effect of humic acid on *Fusarium* root rot diseases*

Humic acid treatments (mg a.i. plant^{-1})	Disease incidence (%)	Root fresh weight (g)
0 (Control)	30.4^{a}	110^{a}
80	29.6^{a}	135^{b}
160	30.8^{ab}	156^{c}
240	34.4^{b}	215^{d}

Values followed by the same letter(s) in columns are not significantly different at $p<0.05$, *When the fruits became visibly mature, Five randomly selected plants from each treatment were uprooted for their disease incidents and fresh weight determinations.

It was clearly shown that humic acid might play an important role in increasing the disease incidence in heavily contaminated soils over 160 mg a.i. plant^{-1}. One of the main reasons for this result could be attributed to the positive effect of humic acid which encourages the tap and lateral root development as well as root proliferation while resulting in cracks and wounds on them as a result of vigorous development. One of the main evidences which supports this idea could be the appearances of infection sites in the main root area which is supposed to be strong and hard (Fig. 1). For example, average root fresh weight in the control group was found 110 g plant^{-1} while those of the treated groups were 135, 156 and 215 g plant^{-1}, respectively. It was evident that the increase in fresh weight of roots was resulted from the vigorous development of lateral roots on the main root system.

When the isolations were made after the completion of experiment, *Fusarium* spp. were recovered from all diseased plants, from which 65% were *Fusarium oxysporum* f. sp. *radicis* and 42% were *Fusarium roseum*.

Soil humic substances have two important roles for the development of plants, either directly or indirectly (Nardi *et al*., 1996). The direct effect on plants has generally on the development and promotion of root systems and uptake of mineral elements. However, the indirect effect is generally on the improvement physical and chemical textures of soil (Zhang, 1994). The indirect beneficial effects of these substances had generally taken a high profile and became quite common among the

growers; however, their effects on plant metabolism have not known widely (Malcolm and Vaughan, 1979). The stimulating effect on plants was reported from earlier researchers (Lee and Bartlett, 1976). In many works, the effect of humic acid on germination, rooting and total plant biomass of various plants has been reported. But, the mechanism still remains unclear. So far hormone-like substances have been elucidated to understand the mechanism of humic substances in plant metabolism. But, no growth regulators or growth regulators-like substances have been reported by the workers (Chen *et al*., 2004a, b). Another hypothesis proposed for the mechanism was the facilitated uptake of microlements. It was reported that the uptake of iron and zinc elements increased by the addition of humic substances to the soil. In a study carried out on tomato plants, humic acid not only promoted the plant growth but also increased the uptake of iron (Jakson and William, 1997). In the studies of Adam *et al*. (1998) and Turkmen *et al*. (2004) the concentration of iron was found 113-123% more than that of the control group. It was thought that reduction of Fe^{3+} to Fe^{2+} played important roles. As results of these findings, the positive effects of humic substances on plant growth have been cited in various studies (Linehan, 1976; Hartwigson and Evans, 2000).

In this study, the composition of humic and fulvic acid had increased the root rot disease by promoting and increasing the lateral root developments by causing cracks on the tap root rather than increasing the virulence of the pathogen. The cracks on the root system probably resulted in a gap for the entry of pathogens (Fig. 1). In addition to that, facultative parasites might have also entered the root system due to increased surface area. The humic substances might have also limited the aeration in the root area by increasing lateral root numbers, thus, humidity in the area might have played an important role for the pathogens to develop and sporulate freely. The strong evidence supporting this proposal is that there was no disease development on lateral roots, however, tap root was severely affected by the pathogens.

The development of disease might have also been encouraged by the type of soil in which the humidity and microbial activities were remarkably higher than that of ordinary field soils.

The start and place of disease are quite important issues. If the root rot starts from the root tips and goes upward where the lateral rooting is proliferated, then the severity of disease would be lessen and eventually diminished. These kinds of symptoms would come out when the seedlings were deeply transplanted or just transplanted to the contaminated soils. However, if the infection starts in tap root or heavily lateral rooted area (Fig. 1, 2), then the symptoms would become more severe and the colonization would be much quicker.

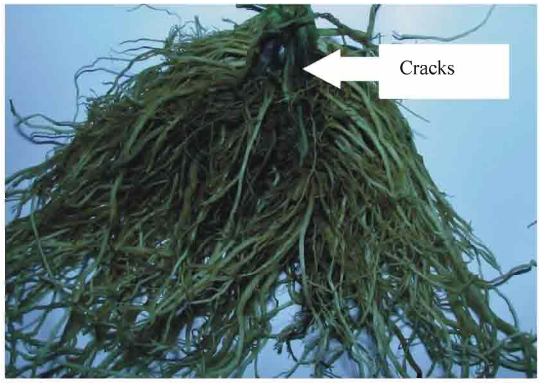

Fig. 1: Shows root rot symptoms and cracks on the tap root caused by humic acid application (240 mg a.i. $plant^{-1}$)

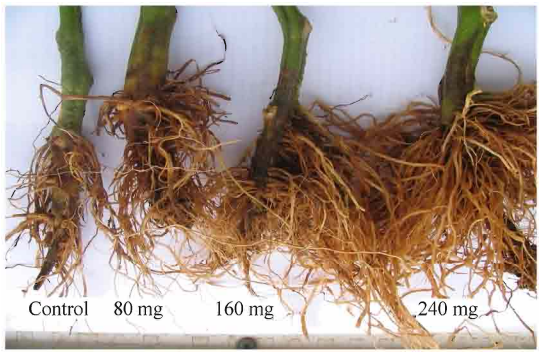

Fig. 2: Root rot infections of tomato plant in humic acid applied soils (From left; Control, 80, 160 and 240 mg a.i. $plant^{-1}$)

From the above findings, it should not be interpreted that the humic substances are harmful to plants. Instead, these substances promote the root and shoot development by increasing the surface root area which allows and facilitates the uptake of water and mineral elements as long as it is used in right concentrations and amounts in right time.

In this study, tomato plants which lack of ability to establishing lateral root systems were stimulated with the humic acid preparations, however, they were badly affected by the greenhouse temperature and the root rot pathogens. As a result of that, they dried out quickly (Fig. 2). Naturally, humic substances were used to increase the microbial activities in the soil.

Experimental findings clearly showed that continuous applications of root promoting substances such as humic

acid etc., although they increased the microbial activities, should be avoided in heavily contaminated soils with the soil-borne pathogens.

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