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Effect of humic acid and mulches on characteristics of tall fescue (*Festuca arundinacea* Schreb.)

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Abstract

Application of biotic growth regulators (e.g. humic components) and appropriate mulches is recommended to improve turfgrass quality especially in arid andsemi-arid climate zones. However, limited number of studies have investigated their effect on lawn establishment. To investigate the effect of humic acid (HA) and selected mulches on characteristics of *Festuca arundinacea* in its planting stage, a factorial experiment based on a completely randomized block design with three replications was performed. The first factor was mulch types including vermicompost, leaf compost, cow manure, and sand (control) which were used to cover the seeds. The second factor was a HA solution (100 ml l^{-1}) sprayed monthly over the period of the experiment. Plant height, fresh and dry weight of lawn clippings, photosynthetic index, leaf texture, and overall turfgrass quality were measured. Spraying HA significantly improved the measured factors except the dry weight and photosynthetic index of the plants. Among the mulches, vermicompost provided better impressions on improving the characteristics of this turfgrass species including 48% increase in fresh weight, 18% increase in height, 48% increase in total quality, and 10% reduction in leaf width of the turfgrass. This research can assist in developing knowledge for having high quality lawns in urban landscapes of arid and semi-arid environments.

Keywords: Humic acid; Lawn; Mulch; Turfgrass quality; Vermicompost

1. Introduction

Lawns are principle elements in the development of urban landscapes. Therefore, achieving appropriate management strategies to improve their quality is necessary (Acosta-Martínez *et al*., 1999). Some major threats affecting turfgrass quality and management are abiotic stresses including drought (Siczek *et al*., 2015). While using chemical fertilizers and super absorbents has been suggested to mitigate such threats and to maximize the quality of turfgrasses especially in arid and semi-arid regions(Rabbani Kheirkhah and Kazemi, 2015), the excessive use of chemicals can cause such environmental problems as soil degradation and compaction and reduction in soil organic matter. Therefore, new strategies are required to mitigate the deleterious effects of long-term application of chemicals in agriculture and landscape development (Sun *et al*., 2015).

Soil mulching is a well-established technique for increasing the profitability of crops and the performance of landscapes in arid and semi-arid environments (Safari and Kazemi, 2016). Mulches improve soil physical conditions through increasing microorganism activities (Obalum and Obi, 2010). Plant growth, in response to soil deformation, can be influenced by organic mulches through the effect on soil water storage and conservation, soil temperature, topsoil aggregation, stability, and porosity. Moreover, the effect of mulches can prevent crust formation due to dissipating the energy of raindrops before they hit the soil surface (Siczek *et al*., 2015). Evaporation of water from a mulched soil decreases relative to bare soil, enabling greater water availability for beneficial plant transpiration. Soil mulching is also an efficient alternative to traditional methods of weed control because it prevents contamination of soil and groundwater by pesticides. Other

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advantages include protection against surface runoff and erosion, acceleration of crop maturity, and increased economic productivity of horticultural crops (Safari and Kazemi, 2016).

An important issue in turfgrass management is finding suitable mulches especially in planting, early growth, and before establishment stage. Currently, using animal manures and municipal waste compost can result in numerous environmental problems due to contamination from exterior sources, distribution of weed seeds, unpleasant smells, and spread of infectious and parasitic diseases. Therefore, finding suitable alternative mulches for planting stage to solve these problems and improve the quality of lawn is necessary (Kafi *et al*., 2009).

To date, the positive effects of compounds including vermicompost as a soil amender for different crops have been investigated (e.g. Atiyeh *et al*., 2000; Sohrabi Yourtchi *et al*., 2013). Using vermicompost as a soil amender may contribute to sustainable agro-ecosystems by reducing the dependency on inorganic fertilizers (Amossé *et al*., 2013). Vermicompost differs from conventional composts because it can improve soil physical structure, increase the presence of plant growth hormones and soil enzymes; and all these can result in better growth of plants. Traditional composts also have less agronomic values due to their high salinity and pathogen levels (Chaoui *et al*., 2003). Therefore, vermicompost appears to be more beneficial than animal manure compost due to its lower rate of soluble salts, higher cation exchange capacity (CEC), and more humic substances (Ngo *et al*., 2012).

Another developing strategy in turfgrass quality enhancement and management is using bio-stimulants such as humus compounds. HA is produced by extracting an organic matter source with an acidic solution (pH=2.0); any fraction that is insoluble below pH 2.0, but soluble above, is considered a HA (Liu *et al*., 1998). HA is one of the three fractions (HA, fulvic acid and humins) of humic substances (Zhang *et al*., 2003). HA can either be present in organic components such as vermicomposts or can be directly applied on plants foliage. Vermicomposts contain plant growth regulating materials including plant growth hormones and HAs. These components are probably responsible for most of the increased germination, growth, and yield of plants (Aguiar *et al*., 2013). HAs can also be used directly on plants at low concentrations given their well-known physiological effects (Aguiar *et al*., 2013). The positive effects of humic substances on the growth of plants in Poaceae family have been well documented (Chen and Aviad, 1990). Canellas *et al.* (2008) revealed that treating maize seedlings with HA caused significant changes in the root area, primary root length, number of lateral roots and their density and also increased plasma membrane H+-ATPase activity. Aşık *et al*. (2009), in a research on wheat plants grown in saline conditions, demonstrated that application of humus in soil increased N uptake and application of HA on foliage increased uptake of P, K, Mg, Na, Cu, and Zn. Hunter and Anders (2004) demonstrated that HA did not affect the nutritional status of the leaf tissues of *Agrostis stolonifera* (creeping bent grass), instead it had a positive effect on the root architecture and plant growth and the plant's resistance to drought.

Recently, many regulatory agencies have expressed concern over high costs of managed turfgrasses because of high usage of pesticides, fertilizers and water, soaring mowing rates, and the problems associated with soil pathogens. For these reasons, increasing attention has been paid to the selection and propagation of native turfgrasses. Native species have demonstrated a variety of benefits, such as being adapted to the local climate and easy local availability (Simmons *et al*., 2011). Therefore, native turfgrasses from arid regions could be good candidates for low maintenance or xeric landscaping within arid and semi-arid regions. These species have evolved under the environmental extremes of a continental climate; nonetheless most have not been evaluated for suitability as managed turfs (Mintenko *et al*., 2002).

The major goal of this research was to evaluate the effects of foliar application of HA and use of some mulches on selected qualitative and quantitative characteristics of a native *Festuca arundinacea* (tall fescue) from Iran. This goal was defined to develop practical knowledge on the best mulch type and coverage for the lawn seeds in their planting stage, and the necessity to know how a biological fertilizer such as HA may influence the lawn quality factors.

2. Materials and Methods

2.1. Site Description and Experimental Design

This experiment was performed in experimental fields of the Department of Horticulture and Landscape, Faculty of Agriculture at Ferdowsi University of Mashhad, Iran (59º 38′ E and 36º 16 ′ N; elevation 989 m; mean annual rainfall 255.2 mm) during 2015.

The experimental design was factorial based on a randomized complete block design (RCBD) with three replications. HA (2 levels, 100 and 0 ml l^{-1} as control) and planting mulch types (4 levels including vermicompost, leaf compost, cow manure, and sand as the control) were used as factors.

2.2. Plants and Mulch Materials

The seeds of a local species of *Festuca arundinacea* were imported packages from Pakan Bazr, a local native seed company in Isfahan, Iran. This species was selected as the best representative of a local tall fescue in Iran, because it can be produced relatively easily at a commercial level within Iran and other countries. The seeds were sown in plots in a sandy loam texture soil in spring 2015. The seeds were covered with 2 cm mulch treatments including vermicompost, leaf compost, cow manure, or sand as a control treatment. Physicochemical properties of the used soil and planting mulches were measured by the following methods: soil texture was measured using hydrometric method and a soil texture triangle according to Bouyoucos (1962); organic carbon in soil and planting mulches was measured using Walkley-Black method (1934); total nitrogen in soil and planting mulches was determined by Micro-Kjeldahl method (Bremner, 1960); and the amount of phosphorus (P) in the soil and planting mulches was measured by the method developed by Bowman and Cole (1978). The amount of phosphorus was evaluated by sodium hydrogen carbonate ($NaHCO_3$) extraction in a spectrophotometer with a wavelength of 660 nm. The amount of potassium (K) was determined according to an adapted method from Poss *et al.* (1991). A 20 ml l^{-1} normal ammonium acetate solution was added to two grams of the soil and planting mulch, and the amount of K was determined after 24 hours by flame photometry. Electrical conductivity (EC) of the soil and mulch extracts were determined by an EC meter (Jenway 4510), and soil pH was measured by a pH meter (Meter Ohm 691).

Physicochemical properties of the used soil and planting mulches are listed in Table 1.

Table 1. Physicochemical properties of used soil and planting mulches

	Texture	pH	EC (dsm^{-1})	Organic Carbon (%)	N (ppm)	P (ppm)	K (ppm)	C/N Ratio
Soil	Sandy-Loam	7.60	3.28	0.88	417.00	1105.00	210.00	21.46
Vermicompost	-	8.26	2.66	15.20	13589.00	4200.00	8430.00	11.17
Cow Manure	-	8.11	3.93	23.40	18375.00	1.10	6.12	12.71
Leaf Compost	-	7.80	3.05	20.28	15458.00	0.31	23.25	13.16
Sand	-	8.60	0.56	0.13	84.00	0.102	9.58	16.25

2.3. Application of Humic Acid

After germination and emergence of seedlings of tall fescue, an irrigation program was established based on the field capacity of the soil. HA solution (Formula Humic Acid, Nature's Lawn and Garden, Inc.) was applied after complete establishment of the turfgrasses (approximately 90 days after sowing). HA was sprayed on foliage with a 100 milliliter per liter concentration level per month compared with the control treatments in which a solution was not applied. This treatment was continued for five months, i.e., the end of the experiment. The pH of this solution was 5.76.

2.4. Measured Factors

To evaluate total visual quality of the turfgrass, a visual scoring was done based on a 1–9 scale, as suggested in National Turfgrass Evaluation Program (NTEP) of the USA (Shearman and Morris, 2000). Score 1 indicated the weakest or the lowest value, 9 indicated the best or highest value, and of the score 6 or higher were considered as acceptable qualities.

Growth factors including height, leaf width, and fresh and dry weight of clipping parts of plants were also measured. Clipping of the turfgrasses were done every 14 days and plant heights were measured using a millimeter ruler before the clipping. To undertake this measurement, three random points in the plots were selected and then average heights of the turfgrasses in these three points were reported as height of the turfgrass. Leaf width was measured using a digital caliper. For measuring fresh weight, clipping was done at 5 centimeter height. Fresh weights of clipping parts of the lawn were measured with a digital scale (+/- 0.01 g, and dry weights were obtained following oven drying at about $70^{\circ C}$ until a constant weight was achieved (Kazemi *et al.*, 2011). Also, chlorophyll index was measured using a manual chlorophyll meter (SPAD 502, *Konica*- Minolta- Tokyo).

2.5. Statistical Analyses

The data were subjected to analyses of variance (ANOVA) using software package of JMP V.8. Comparisons of the means were conducted using least significant difference (LSD) tests. The significance of between-treatment means was tested at 0.01 and 0.05 levels of probability. The bar graphs were drawn utilizing a Microsoft Excel software package.

3. Results

According to the analysis of variance, interactions between HA and mulch was significant only for total quality of the turfgrass (P<0.01). The effect of HA was significant for height, fresh weight of clipping parts, total quality (P<0.01), and leaf width (P<0.05). Further, the effect of mulch was significant for height, fresh weight of clipping, total quality (P<0.01), and dry weight of clipping (P<0.05). The chlorophyll index trait was not significant neither as a simple effect of HA or mulch type, nor as an interaction effect between the humic. According to Fig.1, foliar application of HA significantly increased the height of turfgrass (Figure 1-A) and fresh weight of the clipping part of the plants (Figure1-C). Also, it was observed that different mulches had different effects on height and fresh weight of clipping of the turfgrass so that the plants covered with vermicompost had the highest height, and the seeds covered with sand had the shortest plants (Figure1-B). Positive effect of vermicompost on fresh weight of clipping part of the plants was also observed so that the most amount of fresh weight of clipping was obtained from the plants treated by vermicompost and there was not any significant difference among the plants treated by other three mulch types in terms of this factor (Figure1-D).

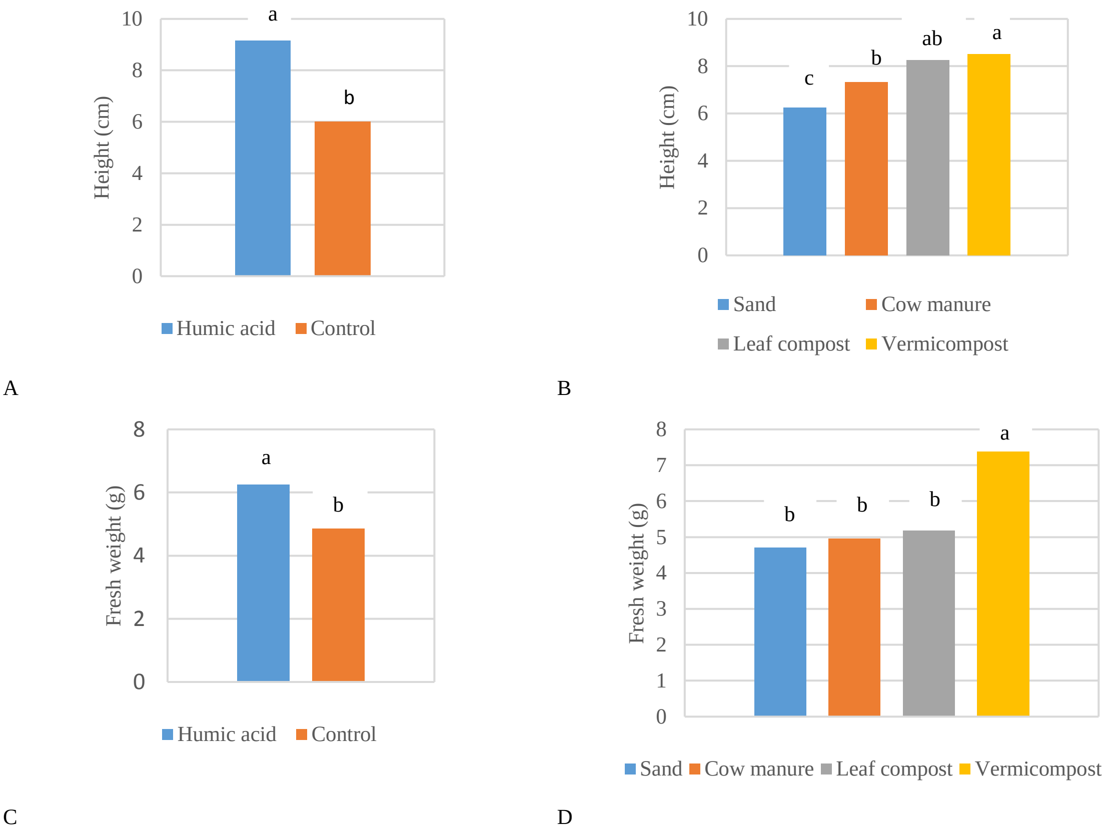

Fig. 1. A, C: The effect of humic acid compared with the control on height and fresh weight of tall fescue, B, D. the effect of four mulch types on height and fresh weight of clipping part of tall fescue. Same letters in each column indicates non-significant differences

Means of simple effect of mulch types on dry weight of clippings revealed that vermicompost significantly increased dry weight of clipping compared to other mulch types, though this increase was not significantly different from using leaf compost as a mulch type. Meanwhile, using sand and cow manure as mulch types were associated with the lowest amount of clipping part of the turfgrass (Figure2).

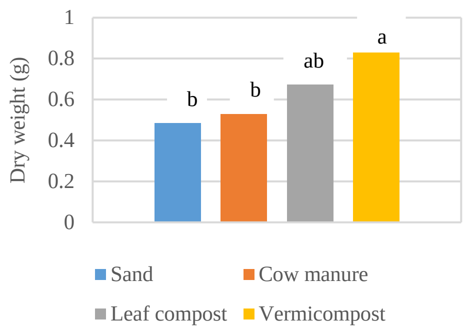

Fig. 2. The effect of four mulch types on dry weight of clipping part of tall fescue. Same letters in each column indicates non-significant differences

Means of simple effect of HA on leaf width showed that application of HA was associated with narrower leaves in the turfgrass. The leaves of the plants sprayed with HA were significantly different (narrower) from the leaves of the turfgrasses not treated with this substance (Figure3).

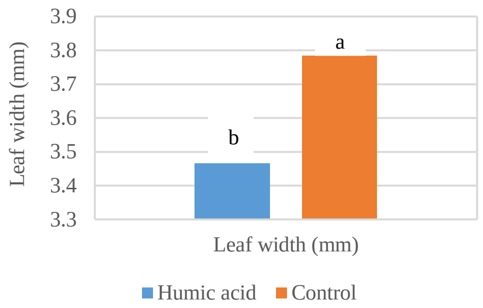

Fig. 3. The effect of humic acid on leaf width of tall fescue. Same letter in each column indicates non-significant differences

Means of interaction effects of HA and mulch types on the total quality of the turfgrass demonstrated that the total quality of the turfgrass was mainly positively affected by the application of vermicompost with or without a spray of HA. The next best total quality of the turfgrass was related to using leaf compost and then cow manure disregarding the foliage application of HA on the plants. The turfgrass with the poorest quality was related to using sand as the mulch. The turfgrass quality in this condition was, however, significantly higher when a foliar application of HA was combined with the application of this mulch type (Figure4).

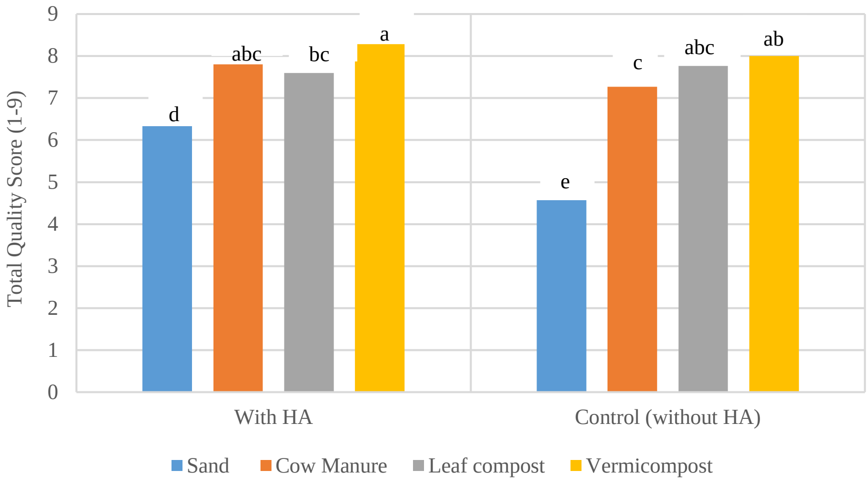

Fig. 4. The interaction effect between mulch types and Humic Acid (HA) on total visual quality score of tall fescue. Same letter in each column indicates non-significant differences

4. Discussion

According to the obtained results, among all the mulches utilized in this experiment, vermicompost had an enhanced influence on growth characteristics of *Festuca arundinacea*. This effect was even more profound when using foliar application of HA on the turfgrass. The major positive effect of HA was on leaf width which had a better performance than vermicompost in making the leaves finer. This effect might be because of foliar application of this substance. Narrower leaves might provide a positive feature through enhanced visual quality and also people's preference toward the tall fescue as this species is normally a wide leave turfgrass species. While not a clear record was found on previous publications in that foliar application of HA may reduce leave's width in turfgrasses, Ervin *et al.* (2008) reported that foliar application of HA on Kentucky bluegrass significantly increased root mass and root strength and, therefore, total growth of the plants. Further, HAs have been reported to enhance mineral nutrient uptake by plants by increasing permeability of membranes of the root cells (Valdrighi *et al*., 1996).

HAs are mainly obtained from geological or peat sources and it is noticeable that vermicompost is an environmentally friendly substitute for peat in ing media and is naturally enriched with high biologically active HA-like substances (Aguiar *et al*., 2013). In general, vermicomposting is the process by which worms are used to convert organic wastes into a humus-like substance called vermicompost (Komakech *et al*., 2016).

Since the beginning of the last century up to the present, humic substances have been studied as agents endowed with auxin-like activities (Jindo *et al*., 2012). The main impact of HAs on plant physiology includes promotion of root growth. The plasma membrane (PM) proton H^+-ATPase activity in root cells is induced by HAs isolated from vermicompost in the same way that exogenous auxin induces growth (Aguiar *et al*., 2013). Auxins induce PM H^+-ATPase activities in cell roots, which couple adenosine triphosphate (ATP) hydrolysis to H^+ transport across cell membranes. Consequently, the apoplast is further acidified; the cell walls are loosened and cells eventually elongate, thereby favoring an increase in acid root growth. At the same time, the activation of PM H^+-ATPase improves the uptake of plant nutrients by enhancing electrochemical proton gradient that drives ion transport across cell membranes via secondary transport systems. HA-like auxins induce PM H^+-ATPase synthesis and its activity (Jindo *et al*., 2012). In general, the effect of HA on plant physiology is recognized with regard to enhancement of root growth and nutrient uptake (Jindo *et al*., 2012). It is possible that enhancement in plants' growth observed in this study are a result of the application of HA or vermicompost and could be attributed, at least partially, to increased nutrient uptake by the plants (Atiyeh *et al*., 2002). Chen and Aviad (1990) also reported that growth and nutrient uptake of plants was attributed to the addition of humic substances. The improved uptake of mineral elements by the plants was potentially a result of more developed root system (Aşık *et al*., 2009). Humic substances may be absorbed by roots, and to a lesser extent, translocated to the shoots. They are also believed to influence plant growth directly by increasing absorption of ions, facilitating the distribution of heavy metals as chelates within the plant, and affecting metabolic reactions (Mylonas and Mccants, 1980). Released HAs from vermicompost play a beneficial role in improving macro-structural stability of weak aggregates of soils. This was attributed mainly to the formation of a hydrophobic coating around the aggregates that enabled them to resist slaking when they are in contact with water molecules (Mbagwu and Piccolo, 1989). Released HAs from vermicompost also result in avoiding degradation and erosion of soil, increased moisture retention, and promotion of plant growth (Gonzalez-Perez *et al*., 2006).

Among the treated mulches, the best total quality of the turfgrass was observed in vermicompost, and the lowest quality was related to sand as the control treatment. Potentially, the use of vermicompost improved physical, chemical, and biological characteristics of the soil and resulted in easier absorption of water and nutrition elements which are essential for vital synthetic and metabolic processes (Sohrabi Yourtchi *et al*., 2013). Compared to conventional composts, vermicompost has much finer structure and larger surface area providing strong absorbability and retention of nutrients. Nutrients such as nitrates, exchangeable phosphorus, potassium, calcium, and magnesium in vermicompost are present in readily available forms for plants to uptake. Additionally, vermicompost contains substances that stimulate and regulate plant growth (Zaller, 2007). Vermicompost is a finely fragmented peat-like material with excellent structure, porosity, aeration, drainage and also high moisture holding capacity. This organic substrate is especially interesting in tropical soils as an organic fertilizer because of its better stability, higher content of plant available nutrients (N, P, K), and more positive effect on plant growth than compost (Amossé *et al*., 2013). Vermicompost may have some reduced levels of microbial biomass due to the use of microbes as an energy source by earthworms (Chaoui *et al*., 2003). Atiyeh *et al*. (2002) extracted plant growth regulators such as indole acetic acid, gibberellins, and cytokinins from vermicomposts in aqueous solution and demonstrated that these can have significant effects on plant growth. Such substances may be relatively transient in soils.

Decomposition and subsequent nutrient release have important influences on the organic matter and nutrient budget of soil (Bayala *et al*., 2005). The elemental composition of the vermicompost and other used mulch materials suggests that the materials have potential as alternative plant nutrient sources (Chaoui *et al*., 2003). According to Table 1, it is observed that there is more balance between elements of NPK in vermicompost mulch, while in other three mulches, the amount of nitrogen is extremely more than phosphorus and potassium rates.

The low C/N ratio in vermicompost indicates that this material would be an effective source of N through rapid N mineralization reactions. The mulches of cow manure and leaf compost contained a higher level of soluble organic C than that in vermicompost. This might be an indicative of a lesser degree of decomposition and suggests that the material is still rich in unstable carbon compounds which can serve as an energy source for microbes (Chaoui *et al*., 2003). C/N ratio is a very important factor in vermicompost quality assessment as microorganisms will make benefit from available nitrogen in the soil for degradation of organic carbon which leads to lack of nitrogen in the soil. A C/N ratio of 20 is considered as a maturation vermicompost index (Alidadi *et al*., 2016).

According to Table 1, EC of cow manure and leaf compost is higher than that in vermicompost. Thus, it can be said that their application is able to potentially prevent seed germination and decline in the biological activity of soils; the lower EC of the vermicompost will also be more useful in fertility of the soils. In addition, the pH of vermicompost was higher. This can be due to the fact that HAs and ammonium ions (NH_4^+), which are products of organic matter degradation, are able to raise the amount of pH. Also, NH_4 production might be another cause of pH increase (Alidadi *et al*., 2016).

In this study, vermicompost appears to have supplied a rich source of nutrients and in fact the richest source of P and K among the studied mulches. Several studies have confirmed major positive effects of phosphorous and potassium fertilizers on early growth and establishment of plants, and even in facilitating uptake of other nutrients (Mallarino *et al*., 1999). The greater phosphorus availability by mulching may be attributed to increased activity of phosphorus solubilizing bacteria and fungi in soil (Gaur and Mukherjee, 1980) or by the virtue of phosphorous mineralization by bacterial and enzymatic activities, especially the phosphatase enzyme activity in an earthworm's gastro intestine (Alidadi *et al*., 2016). The second reason may be that mulching tends to increase the downward movement of phosphorus and may decrease the amount of phosphorus fixed by the soil (Gaur and Mukherjee, 1980).

5. Conclusion

With an overall review of the results of this experiment, it seems that spraying with HA at a concentration of 100 mll^{-1} would have positive influence on some quantitative and qualitative characteristics of *Festuca arundinacea* (tall fescue) turfgrass. Among the studied mulches, using vermicompost also provided better conditions for germination and early growth of the turfgrass by improvement in absorption of more nutrients, and hence, enhancements in the quality of turfgrass. Also, recycling urban wastes can potentially reduce biological pollutants within urban areas. Based on the results of this one-year experiment, foliar applications of HA and mulching with vermicompost provided evidence for enhancing urban turfgrass quality especially in arid and semi-arid environments. However, longer experiments are recommended to be able to widely suggest such application for sustainable urban turfgrass management in these regions.

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