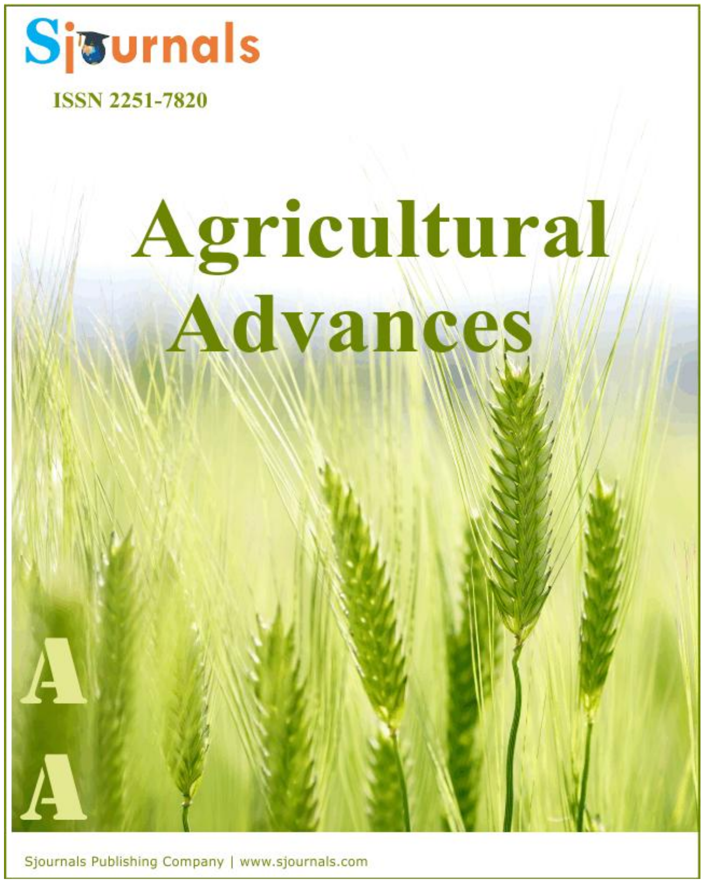
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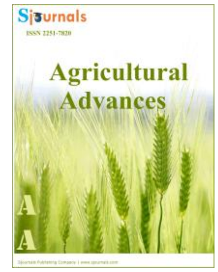

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Original article

Effects of humic acid and organic matter on quantitative yield and macro-element absorption in rice (*Oryza sativa L.*)

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This research intended to study the effects of various levels of humic acid and organic matter forming the substrate for rice seeds of the Tarom cultivar to germinate and for seedlings to grow. For this purpose, an experiment was conducted using split-plots base on randomized complete block design with three replications in the Research Field at Dasht-e Naz in Sari in 2015. Four mixed substrates of clay soil and organic matter (100% soil, 90% soil+10% organic matter, 80% soil+20% organic matter, 70%+ 30% organic matter) were applied as main plot, and four application levels of humic acid (2, 4, 6, and 8 ppt) as sub plot. Results showed that increases in the organic matter content of the substrate improved the seed yield, in which the maximum seed yield obtained in the mixture containing 10% organic matter (1485 g/2m^2) and in the treatment of applying humic acid at 6 ppm (1477 g/m^2). Moreover, the largest seed yield under the interaction effects of organic matter and humic acid (1800 g/2m^2) was achieved at the organic matter content of 10% and humic acid concentration of 6 ppt. Seed nitrogen concentration improved with increases in the organic matter content, with the highest seed nitrogen concentration (1.673%) observed under the interaction effects of 30% organic matter and humic acid at 6 ppt. In addition, when the organic matter content of the substrate was raised, seed phosphorous content improved, with the maximum seed phosphorous content (0.20%) obtained under the interaction effects of 20% organic matter and application of humic acid at 6 ppt.

1. Introduction

Disadvantages of chemical fertilizers and their high production cost have caused application of biofertilizers to be seriously considered. In sustainable agricultural systems, use of biofertilizers, especially in nutrient-poor soils, is of special importance for increasing production and for preserving soil quality. One of the major requirements in crop planning for obtaining crops of high yield and desirable quality is the evaluation of plant nutrition systems (Ayala, 2002).

Humic acid is one of the principal components of humic substances, has dark brown color, and contains various percentages of sulfur, nitrogen, phosphorous and of some microelements such as calcium, magnesium, copper, zinc, etc. Biochemically, humic acid is the active constituent of humus (Nasooti Miandoab et al., 2011). The most important property of humic acid is that it helps in the dissolution and release of fixed elements especially in alkaline soils on one hand and stores excess elements present in its environment and supplies them to plants when needed on the other hand. This leads to a balanced growth of plants. Foliar application of humic acid can enhance photosynthesis and increase tolerance to biotic and abiotic stresses including improved resistance to diseases. It also strengthens plant defensive systems through adding natural antioxidants and phenolic acid. Humic acid delays chlorosis in plants through improving absorption of magnesium and iron (Nasooti Miandoab et al., 2011). Researchers studied the effect of humic acid on several grasses and noticed that its application increased foliage of pasture plants (Hakan et al., 2011). They studied the effect of humic acid on growth of corn in calcareous soils under greenhouse conditions and found that various doses of humic acid spray had different and significant effects on crop dry matter, and had a positive and significant effect on absorption of copper, zinc, manganese, phosphorous, and sodium when utilized at the dose of 1%.

Adding organic matter to soil provides nutrients, increases cation exchange, and improves soil ventilation and moisture retention in addition to preserving greater quantities of water in the soil, which influences plant growth (Singer et al., 2007). The positive effect of applying humic acid to the soil and the effect of its foliar application on enhancing vegetative growth and on increasing foliage can be attributed to the greater development of the root system that results from the production of plant hormones. In addition to being a good source of micronutrients, humic acid helps in increasing release and absorption of fixed elements in the soil. Rice seedlings are sensitive to cold weather at the beginning of the growing season. Therefore, cold stress reduces growth of seedlings and, under severely cold conditions, may even result in their death. Moreover, studies have shown that compounds such as organic matter and humic acid increase tolerance of rice seedlings to cold. This research evaluated the possibility of increasing resistance of rice seedlings to cold weather at the beginning of the growing season in order to study whether it was possible to plant the crop earlier and also to reduce application rates of chemical elements and replace them with organic matter. The effects of applying various doses of humic acid and different rates of organic matter to the substrate for seed germination and seedling growth of the rice cultivar Tarom were studied in this research.

2. Materials and methods

This split plot experiment was conducted according to a randomized complete block design with three replications in a field in Dasht-e Naz in Sari in Mazandaran Province in 2015. Four mixed substrates of clay soil and organic matter (100% soil, 90% soil+10% organic matter, 80% soil+20% organic matter, and 70% soil +30% organic matter) were as the main plot and four levels of humic acid (2, 4, 6, and 8 ppt) as the sub plot. The treatments that were in the same substrate in the main field were compared and evaluated from the seedling to the harvest stages. Moreover, humic acid at the rate of 10 L/h and the base fertilizers at rates determined by the results of soil analysis and based on technical recommendations were applied to complete land preparation and provide the required nutrients. The measured traits included panicle length, number of seeds per panicle, length of flag leaf, number of fertile tillers, total number of tillers, 1000-seed weight, seed yield, biological yield, and seed and straw contents of nitrogen, phosphorous, and potassium. Analysis of the data was performed using SAS, and comparison of the means of the data was carried out by using Duncan's Multiple Range Test (DMRT) at the 1 and 5 percent levels.

3. Results and discussion

3.1. Panicle length

The analyze of variation of the data showed that the effects of organic matter and humic acid on panicle length were significant at the probability level of 1%, and the interaction effects of organic matter and humic acid were significant at the 5% level (Table 1). It was observed that the minimum panicle length (24 cm) was obtained when organic matter was not applied and humic acid concentration was 8 ppt. Moreover, the maximum panicle length (28 cm) was achieved under the interaction effects of organic matter at 20% and humic acid at 4 ppt (Table 2). Turkmen et al. (2005) observed that humic acid and organic matter application to the soil increased panicle length, stem diameter, stem length, dry matter, nutrient content, and yield of pepper plants. These results conform to those found in the present research.

3.2. Length of flag leaf

The results of analyze of variation indicated that the effects of organic matter on length of flag leaf were significant at the 1% probability level (Table 1). The minimum length of the flag leaf (26.33 cm) was obtained in the control treatment in which no organic matter was applied and humic acid concentration was 8 ppt. Furthermore, the maximum length of flag leaf (37 cm) was that of the treatment in which the application rate of the organic matter was 20% and humic acid concentration was 4 ppt (Table 2).

3.3. Number of tillers per hill

The results of analyze of variation revealed that the effects of organic matter and of humic acid on number of tillers per hill were significant at the 1 and 5% level of probability, respectively, but their interaction effects were not significant (Table 1). The minimum number of tillers per hill under the interaction effects of organic matter and humic acid (16.67) was obtained when the organic matter application rate was 20% and humic acid was 4 ppt, and the maximum (30.67) when no organic matter was added and humic acid concentration was 6 ppt (Table 2). Applying 50% of the chemical fertilizers at low levels of organic matter application could compensate for nutrient deficiency and improve plant growth including number of tillers per plant (Mahajan and Gupta, 2009). These results agree with those we found in our study. Osman et al. (2003) reported that application of NPK fertilizers together with 20 tons/h of poultry manure and the same rate of NPK fertilizers together with manure increased number of tillers per plant compared to the other treatments. They mentioned increased ability in the absorption of nitrogen, which plays an important role in cell division, together with improvement in soil physical characteristics after addition of the organic matter as the reasons for this increase. Kavitha and Subramanian (2007) noticed that application of compost together with NPK fertilizers significantly increased number of tillers per rice plant. They stated that the increase in available nutrients was the reason for the production of the maximum number of tillers per plant.

3.4. Number of fertile tillers

The effects of organic matter and humic acid on number of fertile tillers were significant at the 5 and 1% probability levels, respectively (Table 1), but their interaction effects were not significant and all the treatments were in the same class. Moreover, the maximum number of fertile tillers (30.67) was achieved in the treatment of applying organic matter at 20% and humic acid at 6 ppt (Table 2).

3.5. Number of seeds per panicle

ANOVA of the data demonstrated that the effects of organic matter, humic acid, and their interaction effects on number of seeds per panicle were significant at the 1% level (Table 1). Moreover, comparison of the means related to number of seeds per panicle showed that number of seeds per panicle significantly increased when the level of humic acid application was raised. The minimum number of seeds per panicle (116) was that of applying humic acid at 2 ppt and the maximum (134), which exhibited statistically significant differences with those of the other treatments, was that of the treatment in which humic acid concentration was 6 ppt (Table 2). The minimum number of seeds per panicle under the interaction effects of organic matter and humic acid (102) was obtained when no organic matter was applied and concentration of humic acid was 2 ppt, and the maximum (153) when organic matter was applied at 20% and humic acid concentration was 6 ppt (Table 2). Rezvantalab et al. (1998) noticed in their research that application of biofertilizers significantly increased number of seeds per row in corn (and their results conform to the findings in our study).

3.6. Seed yield

The analyze of variation of the data revealed that the effects of humic acid and organic matter on seed yield were significant at the 1 and 5% levels, respectively, but their interaction effects were not significant (Table 1). Raising the level of organic matter added to the substrate led to increases in seed yield, and seed yield in the treatments with organic matter application rates of zero and 20% were 1167.5 and 1345 g/3m^2, respectively. Moreover, the maximum seed yield (1485 g/3m^2) belonged to the treatment of applying 10% organic matter, while raising the application rate of organic matter to 30% reduced seed yield to its minimum of 1055 g/3m^2 (Table 2). Comparison of the means related to seed yield showed that seed yield improved with increases in the concentration of humic acid in the substrate. The largest seed yield (1447 g/3m^2) was achieved when humic acid was applied at 6 ppm, while seed yield declined to 1105, 1230, and 1240 g/3m^2 in the treatments that humic acid was applied at 2, 4, and 8 ppt, respectively (Table 2). Under the interaction effects of organic matter and humic acid, the lowest seed yield (820 g/3m^2) was obtained when no organic matter was applied and humic acid concentration was 2 ppt, while the highest seed yield (1800 g/3m^2) was achieved when the organic matter application rate was 20% and humic acid concentration 6 ppt (Table 2). Nardi et al. (2002) noticed that humic acid increased yield of crops through exerting its positive physiological influences including its effect on plant cell metabolism and by increasing leaf chlorophyll concentration. Their results are in line with those observed in our research. Delfine et al. (2005) reported that humic acid and organic matter application in wheat increased seed yield by 22%. Their results also conform to the findings of the present study.

3.7. Seed nitrogen

ANOVA of the data indicated that the effects of humic acid on seed nitrogen content were significant at the 1% probability level (table 3), while the interaction effects of organic matter and humic acid were not significant and all of these treatments belonged to the same class. It was also observed that the lowest seed nitrogen content (1.28) was that of the control treatment in which no organic matter was applied and humic acid concentration was 2 ppt, and the highest (1.67) that of the treatment in which application rate of organic matter was 30% and humic acid concentration was 6 ppt (Table 2).

3.8. Seed phosphorous

ANOVA of the data demonstrated that the effects of organic matter and humic acid on seed phosphorous content were significant at the 1% probability level, but that their interaction effects were not significant (Table 3). The minimum seed phosphorous content (0.14) was observed in the control treatment in which no organic matter was applied and humic acid concentration was 2 ppt and the maximum (0.20) in the treatment of applying organic matter at 30% and humic acid at 6ppt (Table 4). Turkmen et al. (2004) observed that applying organic matter and humic acid to soil increased macro- and micronutrient contents in the organs of tomato plants. Saran et al. (1999) attributed the 100% increase in phosphorous absorption caused by application of manure at 54t/ha to the improvement in soil structure and to better root distribution. These results agree with the findings of the present research. Mackowiak et al. (2001) reported that application of nitrogen at 120 kg/ha decreased phosphorous content of rice seeds, and these results are in agreement with those found in our research.

3.9. Seed potassium

ANOVA of the data showed that the effects of humic acid on seed potassium content were significant at the 1% probability level, while the effects of organic matter and the interaction effects of humic acid and organic matter were not significant (Table 3). The minimum seed potassium content (0.331) was that of the control treatment in which no organic matter was applied and humic acid concentration was 2 ppt and the maximum (0.428) that of the treatment in which organic matter was applied at 30% and humic acid concentration was 6 ppt

(Table 4). Turkmen et al. (2004) observed that application of humic acid and organic matter increased concentrations of macro- and micronutrients in the organs of tomato plants. Yanvinder-Singh et al. (2004) reported that adding manure to soil increased the ability of rice plants to absorb potassium, and their results conform to the findings of the present study.

3.10. Straw nitrogen

ANOVA of the data indicated that the effects of humic acid on straw nitrogen content were significant at the 1% probability level, but that the effects of organic matter and the interaction effects of organic matter and humic acid were not significant (Table 3). The minimum straw nitrogen content under the interaction effects of organic matter and humic acid (0.584) was obtained in the control treatment in which no organic matter was applied and humic acid concentration was 8 ppt, while the maximum (0.863) was achieved in the treatment of applying organic matter at 20% and humic acid at 6 ppt (Table 4). Timsina et al. (2002) noticed that the mean straw yield improved when the application rate of nitrogen fertilizer was raised.

Table 1
Analyses of variation (mean square) of yield and yield components of rice under the effects of organic matter and humic acid.

Source of variation	Degree of freedom	Panicle length	Length of flag leaf	Number of seeds per panicle	Number of fertile tillers	Number of tillers per hill	Seed yield
Replication	3	0.438^{ns}	22.39^{ns}	29.646^{ns}	3.81^{ns}	5.583^{ns}	158700.00^{ns}
Organic Matter (OM)	3	6.583^{**}	69.63^{**}	1429.944^{**}	84.55^{*}	125.39^{*}	433568.75^{*}
Error		0.188	7.09	122.84	22.20	28.39	5597.00
Humic Acid (HA)	3	4.472^{**}	13.52^{ns}	783.38^{**}	131.72^{**}	97.39^{**}	290368.75^{**}
Organic Matter x Humic Acid	9	1.713^{*}	10.07^{ns}	380.14^{**}	17.28^{ns}	24.37^{ns}	70935.42^{ns}
Error	15	0.611	7.42	129.29	18.82	20.27	42956.25
CV (%)		3.02	16.41	9.12	18.66	18.69	8.62

ns,** and * respectively non-significant and significant at 1% and 5% level.

Table 2
Mean comparison of interaction effects of organic matter (OM) and humic acid (HA) on rice yield and yield components.

Interaction effects	Seed Yield (kg.h)	Number of seeds per panicle	Number of fertile tillers	Number of tillers per hill	Length of flag leaf (cm)	Panicle length (cm)
OM_1HA_1	820.00^{f}	102.33^{f}	19.67^{a}	27.33	28.65^{bcde}	26.00^{cde}
OM_1HA_2	1170.0^{b-f}	137.33^{abc}	29.33^{a}	29.33	25.04^{ef}	26.00^{cde}
OM_1HA_3	1320.0^{bcd}	135.67^{abc}	30.67^{a}	30.67	27.54^{cdef}	26.67^{abc}
OM_1HA_4	1360.0^{bcd}	105.00^{f}	21.00^{a}	21.00	28.75^{bcde}	24.00^{f}
OM_2HA_1	1400.0^{bc}	119.67^{cdef}	20.67^{a}	21.00	28.87^{bcde}	25.33^{c-f}
OM_2HA_2	1530.0^{ab}	130.00^{bcd}	23.67^{a}	23.67	24.13^{f}	26.00^{cde}
OM_2HA_3	1800.0^{a}	129.00^{bcde}	28.00^{a}	30.33	28.67^{bcde}	26.33^{bcd}
OM_2HA_4	1210.0^{b-f}	106.67^{f}	23.67^{a}	23.67	27.16^{cdef}	25.33^{c-f}
OM_3HA_1	1220.0^{b-e}	129.33^{bcde}	20.00^{a}	20.00	28.61^{bcde}	25.33^{c-f}
OM_3HA_2	1370.0^{b-e}	133.33^{abcd}	26.33^{a}	26.67	33.41^{a}	28.00^{a}
OM_3HA_3	1460.0^{abc}	152.67^{a}	27.00^{a}	27.00	33.67^{a}	27.67^{ab}
OM_3HA_4	1330.0^{bcd}	147.67^{ab}	24.67^{a}	26.67	32.33^{ab}	26.67^{abc}
OM_4HA_1	980.0^{def}	122.67^{def}	18.00^{a}	18.33	31.06^{abc}	25.33^{cdef}
OM_4HA_2	850.0^{ef}	110.00^{ef}	16.67^{a}	16.67	30.69^{abcd}	24.67^{ef}
OM_4HA_3	1330.0^{bcd}	120.33^{cdef}	24.00^{a}	24.33	26.36^{def}	25.67^{cde}
OM_4HA_4	1060.0^{cdef}	123.00^{cdef}	18.67^{a}	18.67	27.32^{cdef}	25.00^{def}

Values within a column followed by same letter are not significantly different at Duncan (P ≤ 0.05).

3.11. Straw phosphorous

ANOVA of the data revealed that the effects of humic acid on straw phosphorous content were significant at the 1% probability level, the interaction effects of organic matter and humic acid were significant at the 5% probability level, but the effects of organic matter were not significant (Table 3). The minimum straw phosphorous content (0.032) was obtained in the control treatment in which no organic matter was applied and humic acid concentration was 2 ppt, while the maximum (0.077) was achieved when organic matter was applied at 20% and humic acid concentration was 6 ppt (Table 4).

3.12. Straw potassium

ANOVA of the data demonstrated that the effects of humic acid on straw potassium content were significant at the 1% probability level**,** while the effects of organic matter and the interaction effects of organic matter and humic acid were not significant (Table 3). The lowest potassium content of straw (0.150) was observed in the control treatment in which no organic matter was applied and humic acid concentration was 2 ppt and the highest (0.436) in the treatment of applying organic matter at 10% and humic acid at 6 ppt (Table 4).

Table 3
Analyses of variation (mean square) of seed and straw nutrients concentration under the effects of organic matter and humic acid.

Source of variation	Degree of freedom	Straw potassium	Seed potassium	Straw phosphorus	Seed phosphorus	Straw nitrogen	Seed nitrogen
Replication	3	0.002^{ns}	0.002^{ns}	0.001^{ns}	0.005^{**}	0.001^{ns}	0.036^{ns}
Organic Matter (OM)	3	0.051^{ns}	0.001^{ns}	0.001^{ns}	0.001^{**}	0.031^{ns}	0.007^{ns}
Error		0.011	0.001	0.001	0.000	0.012	0.015
Humic Acid (HA)	3	0.025^{**}	0.005^{**}	0.001^{**}	0.002^{**}	0.033^{**}	0.113^{**}
Organic Matter x Humic Acid	9	0.002^{ns}	0.001ns	0.000^{*}	0.000^{ns}	0.003^{ns}	0.003^{ns}
Error	15	0.002	0.001	0.000	0.000	0.003	0.009
CV (%)		13.26	7.90	12.89	10.43	8.21	6.40

ns,** and * respectively non-significant and significant at 1% and 5% level.

Table 4
Mean comparison of interaction effects of organic matter (OM) and humic acid (HA) on seed and straw nutrients concentration.

Interaction effects	Straw potassium	Seed potassium	Straw phosphorus	Seed phosphorus	Straw nitrogen	Seed nitrogen
OM_1HA_1	0.150^{e}	0.331^{d}	0.032^{j}	0.144^{h}	0.599^{fg}	1.289^{a}
OM_1HA_2	0.222^{de}	0.387^{abcd}	0.039^{i}	0.150^{h}	$0.702^{b\text{–}g}$	1.482^{a}
OM_1HA_3	0.256^{cde}	0.421^{ab}	0.067^{bcde}	0.181^{cd}	0.775^{abc}	1.610^{a}
OM_1HA_4	0.247^{cde}	0.364^{abcd}	0.052^{h}	0.159^{g}	0.584^{g}	1.568^{a}
OM_2HA_1	0.209^{de}	0.381^{abcd}	0.036^{ij}	0.163^{g}	$0.645^{c\text{–}h}$	1.320^{a}
OM_2HA_2	0.347^{abc}	0.422^{ab}	0.058^{fgh}	0.187^{bc}	0.632^{efg}	1.491^{a}
OM_2HA_3	0.436^{a}	0.428^{a}	0.056^{gh}	0.194^{b}	0.795^{ab}	1.602^{a}
OM_2HA_4	0.352^{abc}	0.365^{abcd}	0.074^{ab}	0.165^{fg}	$0.681^{b\text{–}g}$	1.549^{a}
OM_3HA_1	0.313^{bcd}	0.341^{cd}	0.062^{efg}	0.165^{fg}	0.769^{abcd}	1.406^{a}
OM_3HA_2	0.426^{a}	0.390^{abcd}	0.066^{cde}	0.179^{de}	0.807^{ab}	1.422^{a}
OM_3HA_3	0.425^{a}	0.407^{abcd}	0.077^{a}	0.192^{b}	0.863^{a}	1.594^{a}
OM_3HA_4	0.400^{ab}	0.392^{abcd}	0.071^{abcd}	0.189^{b}	$0.756^{a\text{–}e}$	1.521^{a}
OM_4HA_1	0.352^{abc}	0.366^{abcd}	0.065^{def}	0.157^{g}	0.592^{fg}	1.387^{a}
OM_4HA_2	0.380^{ab}	0.344^{bcd}	0.072^{abcd}	0.172^{ef}	$0.719^{b\text{–}g}$	1.494^{a}
OM_4HA_3	0.435^{a}	0.413^{abc}	0.073^{abc}	0.202^{a}	0.742^{abcde}	1.673^{a}
OM_4HA_4	0.374^{ab}	0.393^{abcd}	0.055^{h}	0.178^{de}	0.641^{defg}	1.640^{a}

Values within a column followed by same letter are not significantly different at Duncan ($P \leq 0.05$).

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