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COMPARATIVE STUDY OF THE EFFECT OF PARAQUAT AND ATRAZINE ON SOIL MICROBIAL POPULATION AND CHEMICAL PROPERTIES

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Abstract
This study was aimed at assessing the microbial population and chemical properties of atrazine and paraquat treated soils. Three (3) different concentrations which involved 1.8 (low dose), 3.5 (recommended dose) and 7.0 (high dose) kg/ha; and 2.0 (low dose), 4.0 (recommended dose) and 8.0 (high dose) L/ha of atrazine and paraquat respectively were used. Soil with no herbicide application was used as control. The different concentrations of the herbicides were applied once using a knapsack sprayer. Soil samples were taken four (4) times at an interval of two weeks beginning from two weeks after the application of the herbicides. Fungal count, bacterial and actinomycetes populations, soil pH, organic carbon (OC) and organic matter (OM) were determined using standard procedures. The results showed that the fungal count and actinomycetes population were higher mostly at the 2nd week after treatment application (WAA), but lower at the 8th WAA especially in soil treated with the higher doses of both herbicides. The control soil samples on the contrary recorded lowest fungal count and actinomycetes population at 2nd WAA but, the highest at 6th and 8th WAA. The bacterial population was highest at the 8th WAA but, lowest at 2nd WAA in soils applied the different concentrations of atrazine and paraquat. The OC and OM were significantly lower at the 2nd WAA but, higher at other WAA; and similar observation was made in the control soil samples. The soil pH on the other hand was highest at 2nd WAA, but lowest at the 8th in soils applied the two herbicides. The study concluded that the effect of atrazine and paraquat has no significant difference on soil pH, OC, OM but, on the fungal count, actinomycetes and bacterial populations.

Keyword: Soil, chemical properties, Atrazine, Paraquat, Fungal Count, Microbial Population, Week after treatment (WAA)

Introduction

Soil microbial components are very important part of soil usually found at the topsoil where food sources are much than in the subsoil. In addition to their primary role of decomposing organic matter, they also aid in fixing nitrogen, detoxification of toxic chemicals, suppressing the multiplication of disease organisms and production of substances that could help in stimulating plant growth. They are also of direct beneficial to humans as some are the source of most antibiotics used in fighting diseases (Fred and Harold, 2010). Besides soil microbes, soil chemical property is another soil component that is of great important to soil fertility, plant growth and reproduction. For example, soil pH affects the availability of nutrients to plants and the activity of microorganisms in the soil (Rosen *et al*., 2014). Nutrient availability varies markedly according to pH. This is the main reason why pH is so critical for plant growth and reproduction (Foster *et al.,* 2016); Soil organic carbon is very useful in the improvement of the soil structure as higher soil organic carbon promotes soil structure, thus improving soil aeration and water drainage and retention, and reduces the risk of erosion and nutrient leaching (Anon, 2016); soil organic matter provides numerous benefits to the physical and chemical properties of soil and its capacity to provide regulatory ecosystem services (Brady and Weil, 1999).

Although, herbicides usage in agriculture has helped greatly in boosting crop yield, however, the increase in their usage in agricultural soils posed a great danger to most of these important soil components which include microorganisms (Stanley *et al*., 2013) and chemical properties due to their toxicity and slow biodegradation in soils. For instance, effect of herbicides like paraquat, glyphosate, 2,4-D amine and atrazine on soil bacterial population were reported by Adomako and Akyeampong (2016). The effect on the soil fungal population was also reported by the same authors as they observed absent of fungal species like: *Aspergillus niger, Trichoderma viride, Collectotrichum gloeosporioides, Aspergillus flavus, Mucor, Penicillium, Curvularia lunata* in all the soils treated with the mentioned herbicides which however, were all found in the control soil samples. Reduction in the population of bacteria, fungi and actinomycetes in soils treated with atrazine, primeextra, glyphosate and paraquat was also reported (Sebiomo *et al*., 2011). The use of herbicides like paraquat which is commonly known by trade name "gramaxon" and atrazine is very common among farmers in most part of Adamawa State, Nigeria. Therefore, this study was carried out to assess the effect of these two herbicides on the soil microbes and chemical properties in the region.

Materials and Methods

Study Area

The study was carried out at Modibbo Adama University of Technology, Yola in the open field of the Department of Plant Science botanical garden. The study area is located at the North Eastern part of Nigeria; and lies between Latitude 7^0 and 11^0 N of the equator and between Longitudes 10^0 and 14^0 E of the Greenwich meridian. The botanical garden

where the study was carried out has a sandy-loamy type of soil. The area has a tropical climate characterized by dry and wet seasons. It has an average annual rainfall of 759 mm (Adebayo, 199).

Experimental Design

Completely Randomized Block Design (CRBD) was used for the experiment. Each treatment concentration has one block which was replicated three times.

Concentrations of Herbicide Used for the Study

The herbicides used for the study were paraquat and atrazine. Three (3) different concentrations of each of the herbicides were prepared. The concentrations include: 1.8 (low dose), 3.5 (recommended dose) and 7.0 (high dose) kg/ha of atrazine; and 2.0 (low dose), 4.0 (recommended dose) and 8.0 (high dose) L/ha of paraquat. These concentrations were prepared in triplicates.

Soil Treatment

The soil was treated with the different concentrations of paraquat and atrazine prepared above using a 15 L knapsack sprayer. Polythene bag was used to cover the soil so as to avoid the drifting of the herbicide to the neighboring blocks, thus preventing contamination. The control blocks were not treated or applied any herbicide concentrations.

Soil Sample Collection

The top soil samples were collected at the depth of 0-5 cm from each treatment block into a sterilized polythene bag and were taken to the laboratory for isolation. The soil samples were then made free of large stones and plant debris using 2.0 mm mesh sieve and stored at 4^0 C before assessment. The soil sample collection was done four (4) times at an interval of two weeks beginning from the second week after the soil treatment.

Isolation and Enumeration of Soil Microorganisms

Media used for isolation

The media used for the isolation of bacteria, fungi and actinomycetes were nutrient agar, potato dextrose agar and starch casein agar respectively. These were prepared according to the manufacturer's instructions.

Isolation and enumeration of bacteria

The isolation and enumeration of the bacterial component of the soil was carried out using the method described by Johnson and Curl (1972).

Isolation and enumeration of fungi

The method described by Waksman (1922) was adopted in the isolation and enumeration of the fungal population of the soil.

Isolation of actinomycetes

Isolation of the actinomycetes were carried out using Arifuzzama *et al*. (2010) method.

Determination of Soil Chemical Properties

Determination of soil pH

The pH of soil sample was determined using pH meter 3150 Jenway model according to the method described by Onyeike and Osuji (2003).

Determination of soil organic matter and organic carbon

Organic carbon and organic matter in the soil were determined method described by Walkly and Black (1934).

Data Analysis

Data obtained especially from the soil chemical properties analysis were subjected to one way analysis of variance (ANOVA); and where there was significance difference, Duncan's Multiple Range Test (DMRT) was used to separate the means.

Results

Effect of Atrazine and Paraquat on Fungal Count at 2nd – 8th Weeks after Application (WAA)

The application of atrazine at different concentrations showed that at 2nd WAA the fungal count was higher especially in soils applied the lower and recommended doses of the atrazine with 4.00 and 4.00 x 10^5 cfu/mg respectively; while the lowest fungal count (3.00 x 10^5 cfu/mg) was recorded in soil applied the highest dose of the atrazine. The effect of the atrazine especially at the 4th WAA at all the concentrations determined was observed to negatively affect the fungi as lower fungal count was recorded especially in soil applied the highest concentration of atrazine. The number of the fungal species or count was observed to increase at the 6th and 8th WAA as a result of which the fungal count at those weeks was significantly similar to that of the 2nd WAA obviously in soils applied the highest dose of atrazine. The fungal count of soils paraquat were observed to be higher at the 2nd WAA with the lower, recommended and higher doses having 4.00, 3.00 and 4.00 x 10^5 cfu/mg respectively. However, except the soil applied the highest dose, the fungal count of soils applied the different doses of paraquat at especially 4th – 8th WAA were statistically significantly similar. The fungal count of soil applied the highest concentration of paraquat at the 4th WAA (1.00 x 10^5 cfu/mg) was the lowest, which however, was significantly similar to that of the 6th -8th WAA. The control soil recorded lower fungal count at the 2nd and 4th WAA (2.00 and 3.00 x 10^5cfu/mg respectively), but higher at the 6th and 8th WAA (5.00 and 5.00 x 10^5 cfu/mg respectively) (Table 1).

Effect of Atrazine and Paraquat on Soil Bacterial Population at 2nd – 8th WAA

The statistically significantly highest bacterial count was recorded at the 8th (47.00), 6th (26.00 x 10^5 cfu/mg) and 4th (30.70 x 10^5cfu/mg) WAA in soils applied low,

recommended and highest doses of atrazine respectively. However, the significantly lowest bacterial count was recorded at the 4th (14.30 x 10^5 cfu/mg) for the low dose, and 2nd WAA for both recommended and higher doses with 19.40 and 12.30 x 10^5 cfu/mg respectively. In soils applied paraquat, the statistically significantly highest bacterial count was recorded at 4th (33.20), 6th (32.00) and 8th (90.00 x 10^5 cfu/mg) WAA in soils applied low, recommended and higher doses of paraquat respectively. The lower bacterial count in soils applied the lowest dose of paraquat was recorded at the 8th WAA (19.00 x 10^5 cfu/mg) which, however, was significantly similar to that of the 6th WAA (21.10 x 10^5 cfu/mg). The lowest bacterial count recorded at the 4th WAA (15.20 x 10^5 cfu/mg) in soil applied recommended dose was similar to that of the 2nd WAA (16.00 x 10^5 cfu/mg); while the lowest bacterial count (9.89 x 10^5 cfu/mg) in soil applied the highest paraquat dose was recorded at the 2nd WAA. The control soil sample was observed to decrease in its bacterial count with the advanced in week with the 2nd WAA having the statistically significantly highest bacterial count of 35.00 x 10^5 cfu/mg and the 8th WAA having the lowest count with 9.50 x 10^5 cfu/mg (Table 2).

The Effect of Atrazine and Paraquat on Actinomycetes Population at 2nd – 8th WAA

The statistically significantly highest actinomycetes population were recorded at the 2nd WAA in soils applied the low and recommended doses of atrazine with 2.50 and 4.50 x 10^6 cfu/mg respectively and at 4th WAA (9.80 x 10^6 cfu/mg) in soil applied the highest dose of atrazine. However, the statistically significantly lowest actinomycetes population was recorded at the 6th WAA (0.64 and 0.29 x 10^6 cfu/mg respectively) in soils applied the low and recommended doses of atrazine; and at the 8th WAA (0.13 x 10^6 cfu/mg) in soil applied the highest dose. The significantly highest actinomycetes population was recorded at the 2nd WAA (2.50 and 1.40 x 10^6 cfu/mg respectively) in soils applied the low and highest doses of paraquat; and at the 6th WAA (6.80 x 10^6 cfu/mg) in soil applied the recommended dose. The significantly lowest actinomycetes population in soil applied the recommended dose was recorded at the 2nd WAA (2.70 cfu/mg x 10^6); the lowest of soil applied low dose was at 4th WAA (0.55 cfu/mg x 10^6) which was significantly similar to that of the 8th WAA (0.82 cfu/mg x 10^6); while the lowest actinomycetes population of soil applied the highest dose of paraquat was at 4th WAA (0.12 x 10^6 cfu/mg) which was significantly similar to that of 6th WAA (0.17 x 10^6 cfu/mg). The control soil had the highest actinomycetes population at the 2nd WAA (3.48 x 10^6 cfu/mg) and the lowest (0.15 x 10^6 cfu/mg) was recorded at the 4th WAA (Table 3).

Effect of Atrazine and Paraquat on Soil Organic Carbon at 2nd – 8th WAA

The soil applied low, recommended and high doses of atrazine was observed to record the statistically significantly highest organic carbon at the 4th WAA with 1.07, 1.15 and 1.41 % respectively. The significantly lowest of organic carbon (OC) in soil applied the lowest dose of atrazine was recorded at the 6th WAA (0.42 %); and that of recommended and high doses were recorded both at the 2nd WAA with 0.41 and 0.38 % respectively.

Similar event was recorded in soil applied different doses of paraquat as the soils applied low, recommended and high doses of paraquat had the highest OC at the 4th WAA with 1.34, 1.12 and 1.12 % respectively. The highest OC recorded at the 4th WAA in soil applied the highest paraquat dose was significantly similar to that recorded at the 6th WAA (1.10 %). The statistically significantly lowest OC were recorded at the 2nd WAA in soils applied the low, recommended and high doses of paraquat with 0.38, 0.55 and 0.38 respectively. The control soil too recorded the lowest OC at the 2nd WAA with 0.95 % and the highest at the 8th WAA (1.43 %) which was not significantly different from that of 6th WAA (1.42 %) at p≤0.05 (Table 4).

Effect of Atrazine and Paraquat on Soil Organic Matter at 2nd – 8th WAA

The organic matter (OM) content of soil applied the low and recommended doses of atrazine were at 2nd – 8th WAA not statistically significantly different. However, the OM of soil applied the highest dose of atrazine at 2nd WAA (0.71 %) was the significantly lowest and the highest was recorded at the 4th WAA (1.93 %). The OM of soil applied the low, recommended and high doses of paraquat was statistically significantly highest at the 4th WAA with 1.93, 1.93 and 1.84 % respectively; and significantly lowest at the 2nd WAA with 0.65, 0.95 and 0.62 % respectively. The control soil too had the significantly lowest OM at the 2nd WAA (1.63 %) and the highest (2.46 %) at the 8th WAA (Table 5).

Effect of Atrazine and Paraquat on Soil pH at 2nd – 8th WAA

The pH of soil applied low, recommended and high doses of atrazine were significantly higher at the 2nd WAA with 7.49, 7.50 and 7.30 respectively. However, the pH at 2nd WAA of soil applied the recommended dose was significantly similar to that of the 6th WAA (7.50). The lowest pH of the soil applied the low and recommended doses of atrazine were recorded at the 8th WAA which was 6.64 and 6.49 respectively; while that of high dose was recorded at the 6th WAA with 6.02. The significantly highest soil pH in soil treated with low, recommended and high doses of paraquat were recorded at the 2nd WAA which were 7.39, 7.49 and 7.49 respectively. The lowest pH of soil applied the low and recommended doses were recorded at the 6th WAA which include 5.73 and 6.35 respectively. The lowest pH of soil applied the highest dose of paraquat was recorded at the 8th WAA (6.36). The control soil sample recorded the highest pH at the 2nd WAA (7.52); while the

Comparison of the Effect of Atrazine and Paraquat on Soil Microbial Population and Chemical Properties

Comparison of the effect of these two herbicides showed that the bacterial population and fungal count of soils applied atrazine were higher than they were in paraquat treated soils with 3.12 cfu/mg x 10^4 and 2.94 cfu/mg x 10^4 respectively; while in the case of the actinomycetes population, the paraquat treated soil had the highest with 3.39 cfu/mg x 10^5. The chemical properties (organic carbon, organic matter and pH) of the soils applied

atrazine were not statistically significantly different from that of paraquat treated soil (Table 7).

Discussion

The effect of atrazine and paraquat treatments on soil fungal count indicated a decrease in count of the fungi from the 2nd to 8th WAA for the both herbicides. Similar report was given by Stanley *et al.* (2013) who reported a decrease in the fungal count of the soil treated with butachlor, pyrazosulfuron and paraquat with the passage of time (from 7th to 28th day after treatment). The decrease of the fungal count observed in this study could be due to the adverse effect of the herbicides on the fungal component of the soil. This was attested by the fungal count of the control as the fungal count in 2nd week was the same as that of the 6th week. The duration of reversible impacts has been found to vary with rate of application, type of herbicide, place of application, stubble or plant, frequency of application (Gupat, 2011). Herbicide interference in fungal growth included suppression of spore germination, inhibition of the rate of linear extension of the mycelia, and abnormalities in growth habit in patterns of spore production.

Bacterial population was low for most of the atrazine and paraquat doses at the 2nd WAA, but increased at other weeks. Ayansina and Oso (2006) discovered that higher concentrations of herbicide treatments resulted in much lower microbial counts when compared to soils treated with recommended doses. This study agrees with the above statement because the recommended and high doses of both the atrazine and paraquat resulted in much lower bacterial counts when compared with the control and low dose of the herbicides. The effect of the herbicides on the bacterial population increases with the passage of time from the 2nd to 8th WAA. This contradicts the result of Stanley *et al.* (2013) who reported high bacteria count/population at the 2nd WAA of herbicides. This could be due to the fact that the bacteria were unable to temporarily mineralize and made use of the herbicides as energy.

Actinomycetes population decreases from 2nd to 8th weeks after application of atrazine and paraquat for atrazine low, recommended doses and paraquat low and high doses as well as the control. This could be influenced by the factors such as temperature, pH, organic carbon content, aeration and moisture content of the soils as reported by Arifuzzaman *et al.* (2010). However, for atrazine high dose and paraquat recommended dose, their effect increased the population of actinomycetes at 4th and 6th WAA respectively. Similar result was published by Stanley *et al.* (2013) who reported a significant increase in actinomycetes population from 7th to 28th day as a result of herbicide application.

Variation in the effect of atrazine and paraquat treated soils on organic carbon content was observed with respect to the doses of the herbicides and the weeks after application. Similar event was observed for the effect of atrazine and paraquat on the soil organic matter content as well as pH of the soils. This could be due to vigorous microbial activities in the soil (Greaves *et al.,* 1976). The capacity of the soil to hold positively

charged ions in an exchangeable form is important with paraquat and other herbicides that are positively charged. Soil pH is also of some importance. Adsorption increases with decreasing soil pH for ionizable pesticides such as atrazine, 2, 4-D etc., (Andrew and Pico, 2004). Paraquat binds rapidly and tightly to clay materials in soils and when adsorbed it is biologically inactive. It also binds to humus and other organic material; thus result in no, or very low soil residues or leaching into water sources.

Conclusion

The effect of atrazine and paraquat on soil pH, organic carbon and organic matter has no statistical significant difference. However, on the bacterial population and fungal count, paraquat has no effect than atrazine; while atrazine has more effect on actinomycetes than paraquat.

Recommendation

Preparation of atrazine and paraquat concentrations by farmers should be based on what the target organisms are as different concentration affect different soil microbial and chemical components.

Table 1: Effect of Atrazine and Paraquat on Soil Fungal Count at 2nd – 8th WAA

Fungal Count (cfu/mg x 10^5)							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	4.00^{a}	4.00^{a}	3.00^{a}	4.00^{a}	3.00^{a}	4.00^{a}	2.00^{b}
4th WAA	3.00^{ac}	2.00^{bc}	1.00^{bc}	3.00^{ac}	2.00^{a}	1.00^{b}	3.00^{b}
6th WAA	4.00^{a}	2.00^{bc}	3.00^{ac}	3.00^{ac}	2.00^{a}	2.00^{b}	5.00^{a}
8th WAA	2.00^{bc}	3.00^{ac}	3.00^{ad}	2.00^{bc}	3.00^{a}	2.00^{b}	5.00^{a}

Means with the same superscript(s) along the column are not significantly different at p≤0.05.

Key: ALD: Atrazine low dose; **ARD:** Atrazine recommended dose; **AHD:** Atrazine high dose; **PLD:** Paraquat low dose; **PRD:** Paraquat recommended dose; **PHD:** Paraquat high dose; **CON:** Control; **WAA**= Week After Application

Table 2: Effect of Atrazine and Paraquat on Soil Bacterial Population at 2nd – 8th WAA

Bacterial Population (cfu/mg x 10^5)							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	34.00^{b}	19.40^{c}	12.30^{c}	24.00^{b}	16.00^{c}	9.80^{d}	35.00^{a}
4th WAA	14.30^{d}	24.00^{ab}	30.70^{a}	33.20^{a}	15.20^{c}	12.30^{c}	16.40^{b}
6th WAA	28.70^{c}	26.60^{a}	25.10^{b}	21.40^{bc}	32.00^{a}	32.30^{b}	35.00^{a}
8th WAA	47.00^{a}	22.00^{b}	24.10^{b}	19.00^{c}	20.30^{b}	90.00^{a}	9.50^{c}

Means with the same superscript(s) along the column are not significantly different at p≤0.05.

Table 3: The Effect of Atrazine and Paraquat on Actinomycetes Population at 2nd – 8th WAA

Actinomycete Population (cfu/mg x 10^6)							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	2.50^{a}	4.50^{a}	2.70^{b}	2.50^{a}	2.70^{d}	1.40^{a}	3.48^{a}
4th WAA	1.19^{c}	3.90^{b}	9.80^{a}	0.55^{c}	4.00^{c}	0.12^{c}	0.15^{c}
6th WAA	0.64^{d}	0.29^{d}	0.69^{c}	1.32^{b}	6.80^{a}	0.17^{c}	2.39^{b}
8th WAA	1.59^{b}	0.57^{c}	0.13^{d}	0.82^{c}	5.30^{b}	1.23^{b}	2.76^{b}

Means with the same superscript along the column are not significantly different at p≤0.05.

Table 4: The Effect of Atrazine and Paraquat on Soil Organic carbon at 2nd – 8th WAA

Soil Organic Carbon (%)							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	0.87^{c}	0.72^{d}	0.41^{c}	0.38^{d}	0.55^{d}	0.38^{c}	0.95^{c}
4th WAA	1.07^{a}	1.15^{a}	1.41^{a}	1.34^{a}	1.12^{a}	1.12^{a}	1.07^{b}
6th WAA	0.42^{d}	0.99^{c}	1.18^{b}	1.01^{b}	0.99^{c}	1.10^{a}	1.42^{a}
8th WAA	1.01^{b}	1.07^{b}	1.08^{b}	0.95^{c}	1.06^{b}	0.94^{b}	1.43^{a}

Means with the same superscript along the column are not significantly different at p≤0.05.

Table 5: The Effect of Atrazine and Paraquat on Soil Organic Matter at 2nd – 8th WAA

Soil Organic Matter (%)							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	1.50^{a}	1.32^{a}	0.71^{d}	0.65^{c}	0.95^{d}	0.62^{d}	1.63^{d}
4th WAA	1.84^{a}	1.98^{a}	2.43^{a}	1.93^{a}	1.93^{a}	1.84^{a}	2.33^{c}
6th WAA	1.75^{a}	2.02^{a}	1.74^{c}	1.62^{b}	1.89^{b}	1.57^{c}	2.44^{b}
8th WAA	1.77^{a}	1.84^{a}	1.86^{b}	1.63^{b}	1.82^{c}	1.62^{b}	2.46^{a}

Means with the same superscript along the column are not significantly different at p≤0.05.

Table 6: The Effect of Atrazine and Paraquat on Soil pH at 2nd - 8th WAA

Soil pH							
Week	**ALD**	**ARD**	**AHD**	**PLD**	**PRD**	**PHD**	**CON**
2nd WAA	7.49^{a}	7.50^{a}	7.30^{a}	7.39^{a}	7.49^{a}	7.49^{a}	7.52^{a}
4th WAA	6.82^{c}	6.83^{b}	6.81^{ab}	6.49^{b}	6.81^{b}	6.84^{b}	6.83^{b}
6th WAA	7.07^{b}	7.50^{a}	6.02^{c}	5.73^{d}	6.35^{c}	7.49^{a}	6.55^{c}
8th WAA	6.64^{d}	6.49^{bc}	6.51^{bc}	6.23^{bc}	6.94^{b}	6.36^{c}	6.74^{b}

Means with the same superscript(s) along the column are not significantly different at p≤0.05.

Table 7: Comparison of the Microbial Populations and Physicochemical Components of Atrazine and Paraquat Treated Soils

Herbicide	Bacteria Population (10^5 cfu/mg)	Fungal Count (10^5 cfu/mg)	Actinomycetes Population (10^6 cfu/mg)	pH	Organic Carbon (%)	Organic Matter (%)
Atrazine	3.12^a	2.94^a	2.37^b	6.99^a	1.06^a	1.86^a
Paraquat	1.95^b	3.06^b	3.39^a	6.89^a	1.02^a	1.67^a

Means with the same superscript along the column are not significantly different at p≤0.05.

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