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COMPARISON OF THE EFFECTS OF MANURE-BASED PRODUCT AND AMMONIUM NITRATE ON MAIZE (*ZEA MAYS* L.)

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Abstract

The storage and application of huge amounts of deep litter manure could be a serious issue generated by livestock systems. As broiler litter has high N and fiber content, pre-treatment is strongly recommended. Composting is one of the key method of pre-treatment. Due to Georgakakis and Krintas, 2000, Hosoya composting system is suitable for composting by-products with unfavourable properties such as poultry litter. The end-product (composted and pelletized poultry litter (CPPL)) is appropriate alternative to the substitute for chemical fertilizers, due to its pathogen-free, safe-to-use features and high organic content while it meets the requirements of the European Green Deal's goals.

In the frame of research maize (ZEA MAYS L.) plant test was carried out to get information about the effects of CPPL and ammonium nitrate (AN) fertilizer in pot experiment conditions. Measurements cover the following analyses: the length of the roots, stems, and seedlings, the dry matter content, germination percent, Vigour-index, and chlorophyll and carotenoid content.

On the whole, it can be stated that CPPL had higher stimulating impacts on measured parameters (root length, dry matter content, Vigour index) in comparison to AN with higher N content.

Key words: composted and pelletized poultry litter, ammonium nitrate, maize, pot experiment, European Green Deal

INTRODUCTION

Due to systems of animal husbandry have significant impacts on environment, which require increasing attention by environmental protection (Starme, 2011). Consequently, the sector also has impacts on climate change, nitrogen and phosphorus cycling, and biodiversity loss due to changes in land use patterns as well (Sala, 1995; Tilman et al., 2001; Margulis, 2003; Solomon et al., 2007; Rockstorm et al., 2009; Arima et al., 2011; Carpenter and Bennett, 2011; Bellarby et al., 2012).

The European Union had implemented the European Green Deal to manage environmental issues and support the birth of a climate neutral and sustainable Europe by 2050 (EC, 2019). In the frame of CAP, yearly quantity limits of chemical fertilizers application and prioritizing the use of organic fertilizers are key targets of the Green Deal.

In spite of huge quantity and easy accessible nutrients provided by chemical fertilizers (Scholl and Nieuwenhuis, 2004; Chen et al., 2007), number of environmental consequences have to be taken into consideration.

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Excessive use of fertilizers, increase the decomposition of soil organic matter, which resulting soil structure degradation, as well as polluting water bodies, generating leaching and acidification (Bíró et al., 1998; Adediran et al., 2004; Alimi et al., 2007).

Due to the Green Deal, the issue of manure management has become increasingly serious in recent years, with quickly growing livestock sectors such as broiler farming, which is likely to become even more important from a food perspective in the future (Kasule et al., 2014; Enahoro et al., 2018; Chia et al., 2019; Van Harn et al., 2019; Janković et al., 2020; Nalunga et al., 2021).

Unutilizable products in animal rearing, like deep litter and other organic materials (e.g. different types of compost, meat-, bone-, and feather meal, etc.), could play an important role in soil resource replenishment and could even be a suitable alternative to chemical fertilizers (Mézes et al., 2015; He et al., 2016, 2020; Gorliczay et al., 2021), thus farmed livestock also play a key role in soil fertility (Moyo and Swanepoel, 2010; Magnusson, 2016).

However, it is essential that organic manure have to be properly treated and disposed before application, as untreated manure is extremely harmful, containing numerous bacteria that can harm animals and humans, as well as contaminate food and cause epidemics. Many foodborne infections are associated to manure, either directly or indirectly (Garcia et al., 2010; Heredia and Garca, 2018).

Aerobic composting, a well-known and long-established progress for the disposal of organic wastes and by-products, as one alternative approach of manure management (Filep, 1999; Modderman, 2020).

Based on only few of literatures published the difficulties of composting poultry manure, due to its high fibre, nitrogen and moisture content. Based on Georgakakis and Krintas, 2000, the Hosoya system is a recommended solution for composting such less desirable by-products.

The technology involves a three-phase system, including two phases of aerobic fermentation and one phase of final drying. A granulate with 80-85 % dry matter content is the end-product, with added benefits thanks to the heat treatment which removes toxic ammonia gases, weed seeds, and pathogenic bacterias (Gaál, 2011; Csiba and Fenyvesi, 2012; Hosoya & Co., 1996; Szabo, 2016).

This research covered a comparative analysis of the effects of composted and pelletized poultry litter (CPPL) by Hosoya composting system and ammonium nitrate (AN) fertilizer on maize. The hypothesis of the research was the following: whether a concentrated fertilizer is substituteable with a granulated broiler chicken manure based product with additives (CPPL).

MATERIAL AND METHOD

Soil analyses and plant tests are required to avoid and reduce the impact of excess nutrients on the soil and the environment. It contributes to a knowledge and experience based crop production management. Pot experiments provide an effective modelling opportunity as modelling technique for gaining insight into plant responses, as well as the status and changes in the soil-plant system nearby a controlled environment (Gibson et al., 1999; Poorter et al., 2012a; Poorter et al., 2012b; Nagy et al., 2020).

This method has the advantage of allowing a large range of treatment combinations to be set up at a minimal cost. Thanks to the size of pots in comparison to field level analyses, it provides a more accurate representation of the crop's status (Kawaletz et al., 2014).

According to several authors, the results obtained in the pot experiment can be successfully transferred to field conditions (Baraloto et al., 2006; Schumacher, 2007; Mangan et al., 2010; Kutasy et al., 2022).

The pot experiments took place in the Institute of Water and Environmental Management at the University of Debrecen's laboratory.

The soil used in the pot experiment

The soil used in the pot experiment was a sandy soil. Sandy soils are those that have not yet undergone humification, as well as the transformation and buildup of inorganic materials. Only sparse vegetation, which does not require a lot of organic matter, can colonise them. It has a high water absorption capacity and poor water holding capacity (Stefanovits et al., 1999).

Such soils are exceedingly water-stressed and particularly prone to both deflation and erosion. Table 1 summarises the parameters of the soil used.

The physical and chemical parameters of the soil were analysed at the Agricultural Laboratory Center (accredited laboratory by the National Accreditation Board of Hungary according to the standard from 1993) of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen.

The sandy soil was chosen for the experiment because of its limited nutrient supply capacity, so any changes in the soil-plant system are attributable to the products used.

The products used in the pot experiment

The manure-based product (CPPL) used in the pot experiment was tested at the Agricultural Laboratory Center, as was the soil. The parameters of the end product are summarised in Table 2.

According to the distributor, Baromfi-Coop Ltd., the CPPL product is supplemented with a natural ingredient, meat meal, which is high in amino acids and protein. Proteins and amino acids have been demonstrated to have an important function in plant life processes, influencing plant growth and yield positively.

The composition of ammonium nitrate fertilizer is 17 % ammonium nitrogen and 17 % nitrate nitrogen.

Table 1

Parameters of the sandy soil

Parameters	Measured value
pH (KCl)	6.5
Electrical conductivity (mS/cm)	0.215
Water soluble salts (w/w%)	0.05
Calcium carbonate (w/w%)	<0.100
Humus content (w/w%)	0.67
P_2O_5 (mg/kg)	131.2
K_2O (mg/kg)	177.96
NO_3^- (mg/kg)	7.42
V (mg/kg)	26.17
Zn (mg/kg)	49.96
Ni (mg/kg)	81.01
Cr (mg/kg)	222.13
Ca (mg/kg)	2081.88
K (mg/kg)	4821.24
Cl (mg/kg)	10016.97

Reference: Agricultural Laboratory Center, 2018.

Table 2

Parameters of the end product (CPPL)

Parameters	Measured value	Parameters	Measured value
Moisture content (w/w%)	12 ± 1.189	B content (mg/kg)	31.4 ± 1.155
Organic matter content (w/w%)	69 ± 4.785	Fe content (mg/kg)	545 ± 13.976
Humus content (w/w%)	51.84 ± 1.378	Mn content (mg/kg)	374 ± 14.230
N content (w/w%)	5.5 ± 0.606	Mo content (mg/kg)	3.66 ± 0.482
P_2O_5 content (w/w%)	3 ± 0.707	Zn content (mg/kg)	367 ± 39.438
K_2O content (w/w%)	2.5 ± 0.408	Cu content (mg/kg)	53.3 ± 1.811
Ca content (w/w%)	6 ± 0.770	pH	7.2 ± 0.532
Mg content (w/w%)	0.5 ± 0.264	Calorific value (J/g)	15 092 ± 151.391
S content (w/w%)	1 ± 0.236	C/N ratio	13/1

Reference: Agricultural Laboratory Center, 2018.

Setting up the pot experiment

Before setting up the pot experiment, the soil was dried for 24 hours at 105°C in a Memmert-type drying oven to achieve a bacteriostatic effect, and then sieved to a 2 mm grain size fraction. For statistical purposes, the treatments were set up in four replicates.

The experiment was set up in 1 kg pots. After Chaminade, 1960, this method is known as the "small pot" method. The total dose to be applied to the field by the distributor was 1 and 1.5 t/ha (55 kg and 82.5 kg/ha N active substance), hence the applied doses for the 1 kg pots were 1.63 g and 2.43 g CPPL. These two doses are also applied by Szabó et al., 2019.

The experiment also included ammonium nitrate (AN) fertilizer to provide a more comprehensive comparison. Pure AN fertilizer has a 34 % nitrogen concentration. Similar to CPPL, AN fertilizer was applied in two doses. In accordance with the distributor's recommendation (which is 300 - 500 kg/ha), the AN was applied at a rate of 320 and 485 kg/ha (it is equal to ~110 and 165 kg/ha N active substance). For 1 kg of culture pot this represents 0.18 and 0.27 g AN fertilizer.

Considering the N active substance, twice as much N was applied with the fertilizer as with CPPL. The CPPL and the AN were applied at approximately the root depth. A control group was also set up in addition to the experiments with CPPL and AN. The environmental impacts of CPPL and AN (82,5 kg N/ha) production have also been assessed (Kiss et al., 2021).

According to most literature, 60-70 % of the minimum water capacity is usually the appropriate water capacity level for maize growth (Filep, 1999; Stefanovits et al., 1999).

According to this the sandy soil in the pots were irrigated to 70 % of the minimum water capacity. After the minimum water capacity level was determined, the maize seeds were sown (*Zea mays L.*; hybrid: Pioneer P023).

Methodology for the evaluation of the pot experiment

The experiment lasted 21 days. Firstly, by removing the soil, the wet biomass mass of the plants and the root and stem length (thus the length of the seedlings) were determined. The plant samples were dried in a drying oven at 105°C for 24 hours and the dry biomass mass was measured. The wet and dry biomass weights were used to determine the dry matter content of the plants.

Germination (%) was determined based on the formula of Bazrafshan et al., 2016:

$$\text{Germination \%} = 100 * \frac{\text{number of seeds germinated in treatment}}{\text{number of seeds germinated in the control}}.$$

The Vigour index is calculated by multiplying the germination % by the seedling length. The Vigour index is used to express the health of the seed and its germination capacity (Abdul-Baki and Anderson, 1973).

Chlorophyll and carotenoid content were determined using a Secoman Anthelie Light II UV-VIS spectrophotometer. The resulting values were converted to total chlorophyll and carotenoid content using the following formulae.

Total chlorophyll content based on the formula of Droppa et al., 2003:

$$\text{Chlorophyll (a+b) µg/g fresh weight} = (20.2*A644 + 8.02*A663)*V/w,$$

where:

A = absorbance,
V = volume of tissue extract (10 ml),
w = fresh weight of the wrecked tissue (grams).

The carotenoid content according to the formula of Lichenthaler and Wellburn, 1983, is as follows:

$$\text{Carotenoid µg/g fresh weight} = (1000*A_{470nm} – 3{,}27*(12.21*A_{663nm} – 2.81*A_{644nm}) – 104*(20.13*A_{644nm} – 5{,}03*A_{663nm}))/229*V/w.$$

Statistical evaluation

Statistical analysis of the data was performed using R software in the R Studio user environment. The normal distribution of the data was tested using Shapiro-Wilk test at 5 % significance level ($p = 0.05$). Where data were found to be normally distributed, Duncan's test was used to quantify statistical differences. When normality was not met for the groups, I used the Kruskal-Wallis test.

In the pot experiment, we want to determine if there was a statistically verifiable difference between the effects of CPPL and AN fertilizer.

RESULTS AND DISCUSSION

Effect of treatments on growth parameters of maize (*Zea mays* L.)

At the end of the pot experiment, the root and stem lengths of the plants were measured and the sum of these was used to calculate the seedling length. At elimination of the experiment, the wet biomass mass of the plants was also measured on an analytical balance, and the dry biomass mass after drying, from which the dry matter content was calculated.

The mean values per treatment, with standard deviation and statistical group are shown in Table 3.

For ease of clarity, values where a significant increase over the control was observed at 5 % significance level are marked in green, and where a significant decrease was observed, values are marked in blue.

The data showed that none of the treatments had no effect on root growth, as the root lengths tested did not exceed the average of the control. The average root length of the control plant samples was 29.6 cm, which was approached by the CPPL treatment applied at 1.5 t/ha with an average root length of 28.27 cm. The shortest average root length, 22.66 cm, was measured with the higher rate of AN application. This treatment showed a significant reduction compared to the control.

Table 3

Compare the effect of CPPL and AN to the maize's (*Zea mays* L.) growth parameters

Treatments	Root (cm)	Steam (cm)	Seedling (cm)	Dry matter content (%)
Control	29.60^{a*} ± 11.69	25.00^{b} ± 6.29	54.60^{a} ± 16.69	18.80^{ab} ± 3.72
CPPL 1 t/ha	26.83^{ab} ± 7.98	31.56^{a**} ± 7.26	58.93^{a} ± 13.56	19.02^{a} ± 5.42
CPPL 1,5 t/ha	28.27^{ab} ± 5.98	32.74^{a} ± 6.90	61.01^{a} ± 11.31	18.59^{ab} ± 1.79
AN 320 kg/ha	26.86^{ab} ± 5.42	36.20^{a} ± 4.39	63.01^{a} ± 7.29	15.56^{b} ± 2.60
AN 500 kg/ha	22.66^{b***} ± 3.38	34.83^{a} ± 4.54	57.49^{a} ± 5.86	16.77^{b} ± 1.36

*Different letters indicate significant difference between treatments at $p < 0.05$ level, based on Duncan test or Kruskal-Wallis test. Where multiple letter indices are included, the separation of treatments is uncertain at 5 % significance level.
**Green colour: significant increase compared to control.
***Blue colour: significant decrease compared to control.

The effect of the treatments was clear when examining the stem length, with a significant increase in all treatments compared to the control. While the average root length of the control samples was 25 cm, the average root length of the treated plants exceeded 31 cm. However, no statistically verifiable difference between treatments was observed.

The longest stem lengths on average were observed with AN, with an average of 34.83 and 36.2 cm. This is probably due to the higher nitrogen content, as maize has the highest nitrogen demand of all the macroelements, which it was able to use as observed here.

The stem length of the treatments also resulted in a longer seedling length than the control. But the increase could not be statistically confirmed, with each seedling length neither significantly different from the control nor from each other.

The literatures suggests that maize (*Zea mays* L.) requires high amounts of nitrogen to increase dry matter content and yield (Berzsenyi, 2009; Cruscol et al., 2020; Ruiz et al., 2020). However, the dry matter content of plants treated with high N-content AN was lower than that of the control. The CPPL-treated plants showed a positive effect on biomass mass, especially in the case of 1 t/ha CPPL, but the difference wasn't clearly significant.

Effect of treatments on germination and viability of maize (*Zea mays* L.) seed

The germination percentage (%) was calculated from the number of germinated seeds compared to control. The Vigour-index was then calculated by multiplying the germination percentage by the previously calculated seedling length to give an indication of seed viability.

The mean values of germination percentage and Vigour-index per treatment, with standard deviation and statistical group are shown in Table 4.

Table 4

Compare the effect of CPPL and AN to the maize's (*Zea mays* L.) seed germination and viability

Treatments	Germination %	Vigour-index
Control	100[a*]	5479.13[b] ± 1526.64
CPPL 1 t/ha	128.5[a] ± 16.49	8824.29[a**] ± 1663.82
CPPL 1,5 t/ha	121.4[a] ± 27.35	8155.71[a] ± 675.59
AN 320 kg/ha	128.5[a] ± 16.49	7672.14[ab] ± 1087.44
AN 500 kg/ha	128.5[a] ± 16.49	7102.86[ab] ± 1400.35

*Different letters indicate significant difference between treatments at $p < 0.05$ level, based on Duncan test or Kruskal-Wallis test. Where multiple letter indices are included, the separation of treatments is uncertain at 5 % significance level.
**Green colour: significant increase compared to control.

For germination percentage, plants germinated in control pots were used as a reference based on Bazrafshan et al., 2016. Accordingly, the number of seeds germinated in the control was 100 %. Compared to the control, almost all treatments had more seeds germinated, however, the difference was not significantly confirmed.

The Vigour-index showed that all treatments had higher values than the control, so the treatments had a positive effect on seed viability of maize (Zea mays L.). The highest values (8824.29) were obtained for CPPL applied at the lower dose. This treatment was also significantly different from the control (5479.13).

Effect of treatments on the chlorophyll and carotenoid content of maize (*Zea mays* L.)

Chlorophylls, which give photosynthetic plants their green color, characterize the physiological state of the plants, are related to the N content of the plants and determine biomass production. A- and B-chlorophylls are found in higher plants, as well as carotenoids (carotenes, xanthophylls) as supplementary pigments.

Allaga and Szántóné - Palánki, 1997, found that carotenoids, like chlorophylls, play a function in the absorption and transmission of photosynthetically active light.

According to the literatures, the essential elements required by the plant for healthy foliage are nitrogen, potassium, magnesium, and sulphur. Thus the chlorophyll, which is a nitrogen and magnesium-rich protein, is essential for successful photosynthesis.

Several authors have proven the link between nitrogen and chlorophyll content in leaves in maize, demonstrating that an increase in nitrogen content results in greater chlorophyll (Field and Mooney, 1986; Ercoli et al., 1993; Wullschegler, 1993; Bojović and Marković, 2009; Schlemmer et al., 2013).

The mean values of chlorophyll and carotenoid content per treatment, with standard deviation and statistical group are shown in Table 5. Based on the measured values, both the chlorophyll and carotenoid content of maize increased as a result of the treatments.

Table 5

Compare the effect of CPPL and AN to the maize's (*Zea mays* L.) chlorophyll and carotenoid content

Treatments	Chlorophyll content (µg/g)	Carotenoid content (µg/g)
Control	1457,34[b] ± 395,23	267,96[b] ± 65,65
CPPL 1 t/ha	1804,92[ab] ± 264,45	323,6[ab] ± 44,99
CPPL 1,5 t/ha	1851,51[ab] ± 543,39	328,93[ab] ± 92,59
AN 320 kg/ha	2163,13[a] ± 432,76	390,5[a] ± 71,83
AN 500 kg/ha	2006,72[ab] ± 177,26	342,49[ab] ± 26,77

*Different letters indicate significant difference between treatments at $p < 0.05$ level, based on Duncan test or Kruskal-Wallis test. Where multiple letter indices are included, the separation of treatments is uncertain at 5 % significance level.

**Green colour: significant increase compared to control.

Significant increases were observed for both parameters at the lower fertilizer doses. The AN with higher nitrogen content had the greatest effect on the chlorophyll and carotenoid contents of the plants. Especially, the 320 kg/ha AN treatment, which had a significant effect compared to the control treatment. Altough, the chlorophyll and carotenoid contents of the CPPL-treated plants also exceeded the control values, but no significant increase was clearly detected.

CONCLUSIONS

Most parameters (stem growth, germination percentage (%), Vigour-index, chlorophyll and carotenoid content) showed a stimulating impact on maize when compared to the control, even if the increase was not statistically detectable in all situations.

At the 5 % significance level, the differences between CPPL and AN fertilizer were not significant, and the separation of treatments was not evident. Despite the fact that the N applied with CPPL was half that of AN, higher values for root length, dry matter content, and Vigour-index were measured in the CPPL treatments.

Overall, despite the fact that half as much N was applied with CPPL as with AN fertilizer, the growth stimulating effect of the complex active ingredient manure-based fertilizer was as good as that of the concentrated active ingredient chemical fertilizer.

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