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Maize response to soil properties improved with beneficial microbes, humic acid and farmyard manure application

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Abstract

To avoid environmental pollution, the useful and safe disposal of farmyard manure (FYM) was assessed during a two-year (2017 and 2018) field experiment using beneficial microbes (BM): 25 and 50 L ha^{-1}, humic acid (HA): 3, 6 and 9 kg ha^{-1}, and FYM: 10, 15 and 20 Mg ha^{-1} compared with a control (without BM, HA or FYM). Doubling the amount of BM from 25 to 50 L ha^{-1} delayed phenological events of maize (*Zea mays* L.) by one day, decreased soil total nitrogen (N_{tot}) content (12.6%) but improved soil bulk density (BD) (1.25 Mg m^{-3}), electrical conductivity (EC) (1.90 dS m^{-1}) and mineral N (N_{min}) content (33.89 mg kg^{-1} soil). Increasing HA application from 3 to 9 kg ha^{-1}, physiological maturity of maize delayed by 2 days but increased soil EC (0.11 dS m^{-1}), BD (1.63%), N_{min} (12.94%) and decreased soil N_{tot} (34.42%) content. With the FYM level increasing from 10 to 20 Mg ha^{-1}, the phenological events were delayed, but soil properties, i.e., pH (−0.3 unit), EC (6.66%), BD (−5.83%), N_{min} (28.14%) and N_{tot} (30.16%) content, were improved. Soil properties like EC and N_{tot} and N_{min} contents showed significant positive correlation with maize leaf area, grain N content and grain yield. It was concluded that with addition of either 50 L ha^{-1} BM or 6–9 kg ha^{-1} HA, FYM mineralization increased, growth periods prolonged and soil fertility indices improved. Thus, for improving the N_{min} availability and prolonged maize phenological duration for maximizing productivity, 20 Mg ha^{-1} FYM should be applied in combination with 50 L ha^{-1} BM and/or 6 kg ha^{-1} HA.

Key words: decomposition, maize phenological duration, mineral nitrogen, soil properties.

Introduction

Farmyard manure (FYM) is a heterogeneous mixture of animal dung, urine and leftover materials of farm, i.e., bedding materials, residue of crops/trees/twigs and household sweepings materials that contain plant nutrients (Satyanarayana et al., 2002). Worldwide production of FYM is around 7 billion tons with around 3.30 million tons in Pakistan (Bhattacharyya et al., 2007). The storage of such huge amount of FYM can cause environmental problems (Jiang, Yan, 2010). Therefore, the safe disposal of this huge amount of FYM is a major concern for agronomists, soil scientists and environmentalists. One of the possible options for its safe and useful usage is the recycling of such huge amount of FYM for nutrient availability in farming practices. Though the FYM is widely used as agronomic management for nutrient availability by the famer specifically in low-income regions of the world (Khan et al., 2017; 2019); however, the bulky nature and usage in huge quantity of FYM hindered its widespread application (Muhammad et al., 2018). Similarly, the manure decomposition is slow and takes time to make the nutrients available for the plants. Thus, stimulating the FYM decomposition using soil amendment would enable the farmer to utilize the FYM more effectively with maximum benefits to soil and crop.

The benefits of FYM included enhancement in soil fertility and soil organic matter, microbiological activities (Cabilovski et al., 2014; Bello et al., 2020), improvement in soil structure for agricultural sustainability (Luo et al., 2018) as well as crop growth and production (Khan et al., 2017). FYM is a good source of nitrogen; thus, FYM application enhanced nutrient availability and plant production (Khan et al., 2015). The FYM application makes available nutrients upon decomposition, which improve crop growth and physical properties of soil (Luo et al., 2018). It is also well documented that FYM is useful for soil improvement (Khan et al., 2019), economic production and sustainable farming (Singh et al., 2015). However, the main problems of manure application included its composition, bulk application, handling and processing.

Maize is a widely distributed crop grown across the globe. It has a greater contribution toward the food stock in most developing countries, specifically it is second most important crop after wheat in Khyber Pakhtunkhwa and third after wheat (*Triticum aestivum* L.)

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and rice (*Oryza sativa* L.) in Pakistan (Ibrahim et al., 2020). Maize is used as food, feed and also as a raw material for commercial product preparation (Khan et al., 2017). In Khyber Pakhtunkhwa, the maize was planted on 0.474 million hectares having total production of 0.867 million tons with an average yield of 1827 kg ha^{-1} as compared to the average yield of 4716 kg ha^{-1} of Pakistan (Government of Pakistan, 2018; http://www.mnfsr.gov.pk/). Comparing the average maize grain yield of Pakistan with that of the world leading maize producing countries (USA, China, Brazil, etc.), the Pakistan yield is low due to poor soil fertility and inappropriate fertilisation. Being an exhaustive crop, maize needs more nutrients as compared to other cereal crops. Thus, to improve the manure decomposition and its impact on maize, soil amendments like beneficial microbes (BM) and humic acid were used.

Beneficial microorganisms are naturally occurring microbes, which are used as inoculants to enhance soil microbial activities ultimately, soil fertility and nutrient availability thus increasing crop yield and growth (Haji et al., 2014). Integration of BM inoculums with organic/inorganic fertilisers increase nutrient availability (Kurepin et al., 2014). Bacterial inoculation increases crop production through bacterial nitrogen fixation, nutrient uptake by solubilization and hormonal activities and thus has positive effect on nutrient availability. Soil quality, plant growth and crop yield increase with the application of beneficial microbes (Haji et al., 2014). Biostimulators increase atmospheric nitrogen fixation, supress pathogen, improve the organic residue decomposition and nutrient availability for plant growth (Kurepin et al., 2014).

Humic acid (HA) is a natural producer of carbon mostly found in lignite coal in Pakistan (Hai, Mir, 1998). The microbial humification of organic matter present in soil organic matter (SOM), sediments, peat, coal, water and soil form HA (Brannon, Sommers, 1985). The HA is considered as a major SOM source and is closely related to biological, physical and chemical characteristics of soil (Khattak, Muhammad, 2008). Enhancement in soil physico-chemical and biological condition is closely related to the presence of HA in the soil. Model HA polymer consists of 51–57% SOC, 0.70–1.65% N_{tot} and 0.25–0.94% P (Brannon, Sommers, 1985). HA increases soil fertility for better plant growth, development, nutrient accumulation and net economic income. The application of HA acts as chelating agent, which not only improves the nutrient availability but can also sustains the nutrients in the soil. It is reported that, if HA is applied with fertilisers, it can enhance the crop growth and development (Bharali et al., 2017).

Soil of the study area contained less than 1% SOM, and thus the production efficiency was low to sustain continuous cropping (Ibrahim et al., 2020). Secondly, the synthetic fertiliser application undoubtedly increased crop production (Khan et al., 2018); however, its application is not sustainable and less profitable (Muhammad et al., 2018). However, if BM is applied along with organic fertiliser sources, it will further increase the decomposition and will be more profitable (Haji et al., 2014). In addition to BM, HA also acts as soil conditioner and has been reported to improve the soil fertility and, hence, crop production (Bharali et al., 2017). At present, an increasing trend for standard and quality agricultural production is needed in addition to quantity; thus, the organic fertilisers are used for ecological balance, low-cost cultivation, pollution free environment and quality food with no effect on human health (Khan et al., 2018).

Therefore, the current research was conducted with the aim of finding out the effect of beneficial microbes and humic acid in improving the mineral availability from FYM decomposition, soil fertility indices and their relationship with maize crop performance.

Materials and methods

Experimental site. A field experiment was conducted at Agronomy Research Farm (34° N, 71° E), the University of Agriculture Peshawar, north-west Pakistan in the summer season in 2017 and repeated in 2018. The physico-chemical properties of the experimental soil were determined before sowing in 2017 using standard protocols (detailed in Soil analysis section). The soil of experimental plot was silt loam having sand (20.16%), silt (70.21%) and clay (9.63%), classified as *Cambisol* (Siltinovic) according to WRB (2014) and as *Ustochrept* according to USDA (Soil Survey of Pakistan, 2007).

The initial physico-chemical properties before sowing measured on five randomly taken samples from a depth of 20 cm are given in Table 1. The soil of the experimental site was alkaline with pH 8.1, soil organic carbon (SOC) 0.53%, total nitrogen (N_{tot}) 0.03%, mineral nitrogen (N_{min}) 0.68 mg kg^{-1} soil, electrical conductivity (EC) 0.41 dS m^{-1}, EDTA extractable phosphorous (P) 3.87 mg kg^{-1}, EDTA extractable potassium (K) 112 mg kg^{-1} and soil bulk density (BD) 1.23 Mg m^{-3}.

During the last five years, the experimental plots were planted with maize in summer and wheat in winter seasons with a regular maize → wheat → maize rotation. The climatic data, i.e., temperature and rainfall of the study area, is provided in Figure 1.

Table 1. Physico-chemical properties of soil, farmyard manure (FYM) and humic acid (HA) on a dry weight basis

Properties	Unit	Soil	FYM	HA
Organic carbon	%	0.53	15.8	51.63
Total nitrogen (N_{tot})	g kg^{-1}	0.03	0.86	2.26
Mineral nitrogen (N_{min})	mg kg^{-1}	0.68	–	–
Acidity (pH)	–	8.1	6.9	7.21
Electrical conductivity (EC)	dS m^{-1}	0.41	3.7	2.13
Phosphorous*	g kg^{-1}	0.39	2.6	45.4
Potassium*	g kg^{-1}	11.2	7.1	73.2
Bulk density (BD)	Mg m^{-3}	1.12	0.69	0.78
Sand	%	20.16	–	–
Silt	%	70.21	–	–
Clay	%	9.63	–	–
Textural class	–	silt loam	–	–

* – in soil EDTA extractable

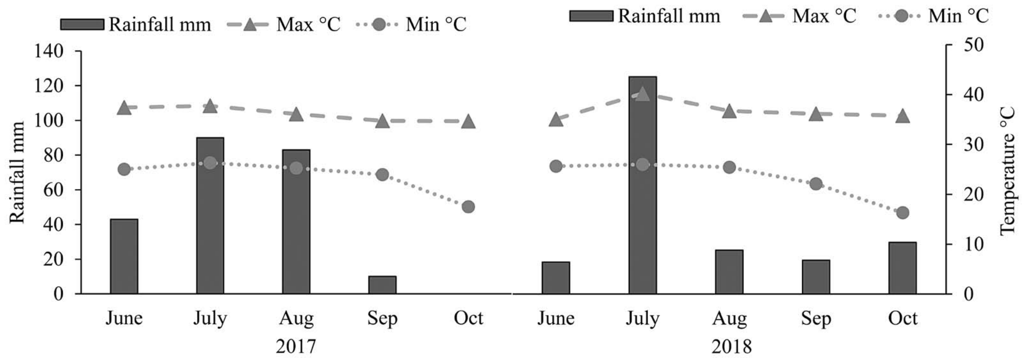


Figure 1. Rainfall and temperature maximum (Max °C) and minimum (Min °C) during 2017 and 2018

Materials and treatments. The experiment was conducted in a randomized complete block design (RCBD) with four replications. In the experiment, 3 factors: (1) beneficial microbes (BM), (2) humic acid (HA) and (30 farmyard manure (FYM) along with a control (without BM, HA or FYM), were used. The BM were applied as 25 or 50 L ha^{-1}, HA as 3, 6 or 9 kg ha^{-1} and FYM as 10, 15 or 20 Mg ha^{-1}. The FYM was collected from the Dairy Farm, the University of Agriculture Peshawar, Pakistan and was analysed for physico-chemical properties (Table 1) using standard protocol (detailed in Soil analysis section). The analysis indicated that FYM on a dry weight basis contained 15.80% SOC, 0.86% N_{tot}, pH 6.9, 3.7 dS m^{-1} EC, EDTA extractable 0.26% P and EDTA extractable 0.71% K. The HA was applied as Humic Plus 40%, a commercial product of Al-Hameed Chemicals (pvt) Ltd. (Pakistan), containing 51.63% SOC, 2.26% N_{tot}, pH 7.21, 2.13 dS m^{-1} EC, EDTA extractable 4.54% P and EDTA extractable 7.32% K. The BM were supplied as 1 litre pack from Bioaab, a commercial product manufactured by NFRDF-NGO and Faculty of Agriculture, University of Faisalabad, Pakistan. The Bioaab consisted of *Lactobacillus* sp. *Rhodopseudomonas* sp., *Actinomycetes*, *Cyanobacteria*, *Saccharomyces* sp. (yeast) and molasses as media. The Bioaab purchased from the market was converted to extended solution: 1 L molasses was mixed with 20 L water in a 30 L plastic cane and dissolved by shaking. Then 1 L of Bioaab was added to the cane having dissolved molasses in water, and the cane was tightly closed and stored at room temperature for three days. The gases were released once during 24 h by opening the lid of the cane, and the extended solution was used for field application.

Preparation of treatments and their application. The experimental plots of 4 × 4.5 m were prepared and separated with small bund (embarkment) of 5–7 cm height. The FYM was added to the soil as per proposed rates on a dry weight basis, whereas the quantified HA as per proposed rate was mixed with 1 kg soil per plot and sprinkled on the respective plots. The quantified extended Bioaab solution as per proposed rates was mixed with 1 L of distilled water per plot, well shaken before application and sprayed as saturated liquid on the soil to cover the entire plot area, where FYM and HA had been already applied. To incorporate all these treatments materials to a depth of 15 cm, after application of the treatments and one month before sowing the plots were ploughed single time with a common field cultivator. The application of BM, HA and FYM was carried out about one month before sowing each year. For proper and effective decomposition of treatments and to maintain plots at proper field capacity level before sowing of crop in each year, two irrigations: 1st after 10 days and 2nd after 20 days of treatments incorporation, were made.

Field operations and methodology. Field was ploughed two times using a common field cultivator after one month of treatments incorporation at proper field capacity level during each year for seed bed preparation. The field cultivator was followed by a rotavator in the respective plots for proper seed bed development. The plots were rebuilt and separated with 10–15 cm high and 30 cm wide bunds between two plots; however, the replication-to-replication distance was 1 m. Maize (*Zea mays* L., cultivar 'Azam') was sown at a seed rate of 30 kg ha^{-1} in plots of 18 m^{-2} having 6 rows of 4 m length and spaced 75 cm. Maize sowing was made on July 8, 2017 for the 1st year and July 7, 2018 for the 2nd year on the same plots with the same treatments. However, wheat was grown between two maize crops and provided with recommended amount of 120 kg ha^{-1} N and 90 kg ha^{-1} P as a gap crop. Basal dose of 90 kg ha^{-1} N_{min} was applied in three equal splits, i.e., ⅓ each at first, third and fourth irrigation, along with a basal dose of 60 kg ha^{-1} P at the time of sowing to all plots. Field was irrigated six times both in 2017 and 2018 as flood irrigation at critical stages: after emergence, three leaf stage, knee height, tasselling/silking, grain development and grain filling. Weeds were controlled manually by hoeing after 2nd irrigation, and no herbicides were used. However, shoot borers were controlled through Furadan (a.i. carbofuran 3%) applied at a rate of 20 kg ha^{-1} after 2nd irrigation, i.e., in three leaves stage.

Observations and measurements. Composite soil samples were collected from the experimental field (20 cm depth) before planting maize in 2017. However, three samples were made from 20 cm depth in each plot at tasselling stage (only for N_{min} determination) as well as after maize harvesting in 2017 and 2018, composited again (same established plots in 2017 and 2018), cleaned, ground to 2 mm mesh size and stored for determination of soil physico-chemical properties. Samples were also collected from FYM and HA and analysed for C, N, P and K content as well as pH and EC.

Phenological observations. Days to emergence, tasselling, silking and physiological maturity were counted as the days difference between sowing date and respective stage, when around 70% of plants attained the respective stage. Loss of green colour of leaves/cobs was considered criteria for physiological maturity.

Soil analysis. For determination of soil properties, soil samples made prior to experiment and/or after maize harvest were used. Soil water suspension

(1:5) was prepared by taking 10 g of dry soil and 50 ml of distilled water. The suspension was shaken for 1 h on mechanical shaker, left for 30 min and then filtered. The filtrate was used for determination of soil acidity (pH) following Mclean (1983) method using pH meter calibration with 4 and 7 buffers. Soil core No. 42 (100 cm^{-3}) was driven in soil to a depth of 20 cm after clearing the topsoil surface and carefully dug out to avoid compaction of soil block in soil core. The soil extended beyond the core side was removed carefully, the soil of the core was oven died at 105°C for 24 h and soil bulk density (BD) was calculated using formula:

BD = soil dry weight / volume of core.

The soil electrical conductivity (EC) was also determined using the same soil suspension with the help of soil EC meter (Rhoades, 1996).

The soil mineral nitrogen (N_{min}) content was determined following the steam distillation procedure as reported by Keeney and Nelson (1983). Briefly, 10 g of fresh soil was extracted with 100 ml of 1 M KCl solution by shaking on mechanical shaker for 1 h. The suspension was allowed to settle down until clear liquid supernatant and filtered. Thereafter, 0.2 g of MgO and Devarda's alloy were added to 15–20 ml of aliquot of soil solution in distillation apparatus, and the distillate was collected in beaker having 5 ml $B(OH)_3$ mixed indictor and titrated against 0.005 N HCl. The N_{min} content (mg g^{-1} soil) was calculated using formula:

$$N_{min} = \frac{(S–B) \times N \times 0.014 \times \text{extract total volume}}{\text{dry sample wt} \times \text{volume taken}} \times 10^6.$$

The soil total nitrogen (N_{tot}) content was determined as per the procedure of Bremner and Mulvaney (1983). Briefly, 0.5 g of soil and/or 0.2 of grain fodder was digested with digestion mixture and 3 ml H_2SO_4 at 350°C. The digest was used for soil N_{tot} using 40% NaOH and 4% $B(OH)_3$ solution in device Kjelflex K-360 (BUCHI Switzerland Ltd.) and titrated with 0.1 N HCl solution; N reading (in %) was noted directly from the tool of automatic titration Titrano plus (BUCHI Switzerland Ltd.) attached to Kjelflex K-360 Distillation Unit.

Statistical analysis. The data was analysed as per the procedure of RCBD (Jan et al., 2009). Means were compared using least significant difference (LSD) test at $P \leq 0.05$ upon significant *F*-test. The Pearson correlation analysis was carried out to understand the positive and negative relationship of soil properties with maize parameters. The statistical analysis was carried out using software *Statistix*, version 8.1 (Informer Technologies Inc.) and illustration by *Sigma Plot*, version 14 (Systat Software Inc., USA).

Results

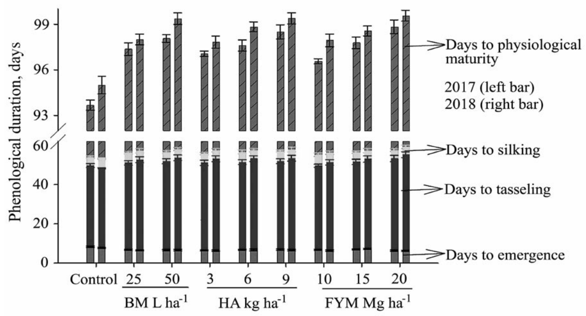
Phenological duration of maize crop. The application of BM had no effect on days to emergence but significantly delayed tasselling, silking and physiological maturity of maize by one day based on two years data (Table 2, Figure 2). Similarly, the HA application had no significant effect on days to emergence, tasselling and silking but delayed the physiological maturity of maize by one day both in 2017 and 2018. The application of 15 Mg ha^{-1} FYM delayed days to emergence (7 days) both in 2017 and 2018 as compared to the enhanced emergence (6 days) in plots having 20 Mg ha^{-1} FYM. However, the application of 20 Mg ha^{-1} FYM took more days to tasselling (53, 55 days), silking (58, 59 days) and physiological maturity (100, 100 days) of maize in 2017 and 2018, respectively. This indicates that doubling the amount of manure from 10 to 20 Mg ha^{-1} FYM delayed the tasselling in maize by 4 days, days to silking by 2 days, and physiological maturity by 2 days averaged over two years data.

Prolonged emergence (by 1 and 1 days), silking (by 2 and 5 days), tasselling (by 3 and 5 days) and physiological maturity (by 4 and 4 days) during 2017 and 2018, respectively was noted in treated plots compared to the control (Figure 2).

Table 2. Means square errors with significance of DTE (days to emergence), DTT (days to tasselling), DTS (days to silking) and DTM (days to physiological maturity) of maize and soil properties during 2017 and 2018

Source of variation	df	DTE	DTT	DTS	DTM	pH	EC	BD	N_{tot}	N_{min}
Year (Y)	1	0.32 ns	67.9**	30.54*	27.5 ns	0.01 ns	0.14*	0.006*	0.006 ns	65.47*
Reps (Y)	4	0.46	2.0	1.86 ns	0.5 ns	0.01 ns	0.01	0.001	0.001	5.58
Treatments	18	12.44**	129.3**	79.49**	78.1**	1.12**	0.45**	0.054**	0.798**	798.05**
C *vs* R	(1)	11.26**	63.7**	67.08**	85.1**	0.73**	0.30**	0.064**	0.599**	659.46**
BM	(1)	0.15 ns	25.0**	11.34*	29.0**	0.02 ns	0.18**	0.008**	0.062**	108.58**
HA	(2)	0.59 ns	3.1 ns	5.45 ns	20.3**	0.01 ns	0.12**	0.001 ns	0.468**	152.53**
FYM	(2)	7.34**	136.3**	62.95**	34.1**	1.15**	0.26**	0.039**	0.361**	605.83**
BM × HA	(2)	0.26 ns	2.1 ns	3.90 ns	0.5 ns	0.07*	0.01 ns	0.001 ns	0.007 ns	20.83*
BM × FYM	(2)	0.73 ns	1.2 ns	1.45 ns	0.1 ns	0.03 ns	0.03 ns	0.001 ns	0.010*	14.82*
HA × FYM	(4)	1.09 ns	1.8 ns	2.87 ns	1.1 ns	0.01 ns	0.01 ns	0.001 ns	0.008*	7.11 ns
BM × HA × FYM	(4)	0.93 ns	1.6 ns	0.26 ns	1.4 ns	0.02 ns	0.01 ns	0.001 ns	0.003 ns	2.42 ns
Y × treatments	18	0.54 ns	2.2 ns	1.15 ns	1.0 ns	0.02 ns	0.01 ns	0.001 ns	0.002 ns	2.01 ns
Y × C *vs* R	(1)	0.50 ns	13.1 ns	4.59 ns	0.2 ns	0.01 ns	0.01 ns	0.001 ns	0.001 ns	3.64 ns
Y × BM	(1)	0.59 ns	0.3 ns	1.56 ns	3.0*	0.01 ns	0.02 ns	0.003 ns	0.001 ns	1.56 ns
Y × HA	(2)	0.04 ns	0.6 ns	1.40 ns	0.5 ns	0.06*	0.01 ns	0.001 ns	0.006 ns	1.50 ns
Y × FYM	(2)	1.18 ns	0.7 ns	1.68 ns	1.2 ns	0.04 ns	0.01 ns	0.003 ns	0.004 ns	2.96 ns
Y × BM × HA	(2)	0.59 ns	1.0 ns	1.51 ns	0.3 ns	0.01 ns	0.01 ns	0.003 ns	0.004 ns	0.03 ns
Y × BM × FYM	(2)	0.23 ns	1.4 ns	0.40 ns	2.6*	0.01 ns	0.01 ns	0.001 ns	0.001 ns	3.14 ns
Y × HA × FYM	(4)	0.81 ns	2.2 ns	0.29 ns	0.3 ns	0.01 ns	0.01 ns	0.001 ns	0.001 ns	1.11 ns
Y × BM × HA × FYM	(4)	0.31 ns	2.4 ns	0.84 ns	1.1 ns	0.01 ns	0.01 ns	0.001 ns	0.001 ns	2.82 ns
Error	72	0.53	6.3	2.63	0.6	0.02	0.01	0.002 ns	0.003	4.58 ns
CV%		10.90	4.8	2.90	0.78	1.6	5.38	3.42	7.02	6.62

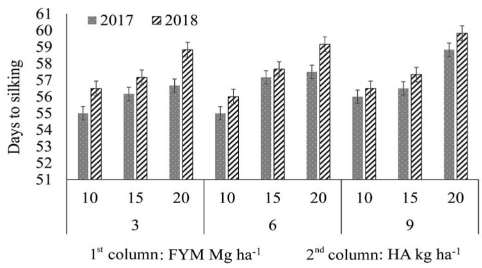
C *vs* R – control *vs* rest treatments, BM – beneficial microbes, HA – humic acid, FYM – farmyard manure; df – degree of freedom; EC – electrical conductivity, BD – bulk density; *, ** – significant at $p \leq 0.05$ and $p \leq 0.01$, ns – not significant



Note. The vertical bars denote standard errors of the mean.

Figure 2. Maize phenological duration in response to beneficial microbes (BM), humic acid (HA) and farmyard manure (FYM) during 2017 and 2018

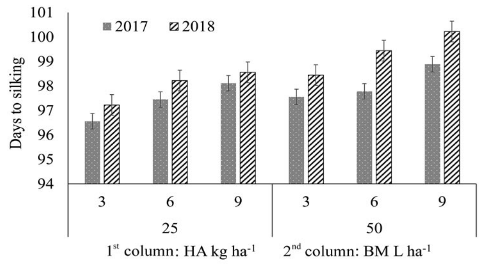
The interaction between year, HA and FYM (Table 2, Figure 3) indicated that, when 3 kg ha^{-1} HA was applied, increasing the FYM level delayed the days to silking of maize both in 2017 and 2018. However, with application of HA greater than 3 kg ha^{-1}, the increased FYM had no significant differences for days to silking.



Note. The vertical bars denote standard errors of the mean.

Figure 3. The interaction of days to silking of maize for farmyard manure (FYM) and humic acid (HA) during 2017 and 2018

The interactive response of year, BM and HA interaction (Table 2, Figure 4) showed that days to physiological maturity increased with increasing level of HA in 2017 and in 2018 across both levels of BM. However, the increases in physiological maturity in the 2nd year was prominent with 50 L ha^{-1} over 25 L ha^{-1} as compared to the 1st year of cultivation.



Note. The vertical bars denote standard errors of the mean.

Figure 4. The interaction of days to maturity of maize for humic acid (HA) and beneficial microbes (BM) during 2017 and 2018

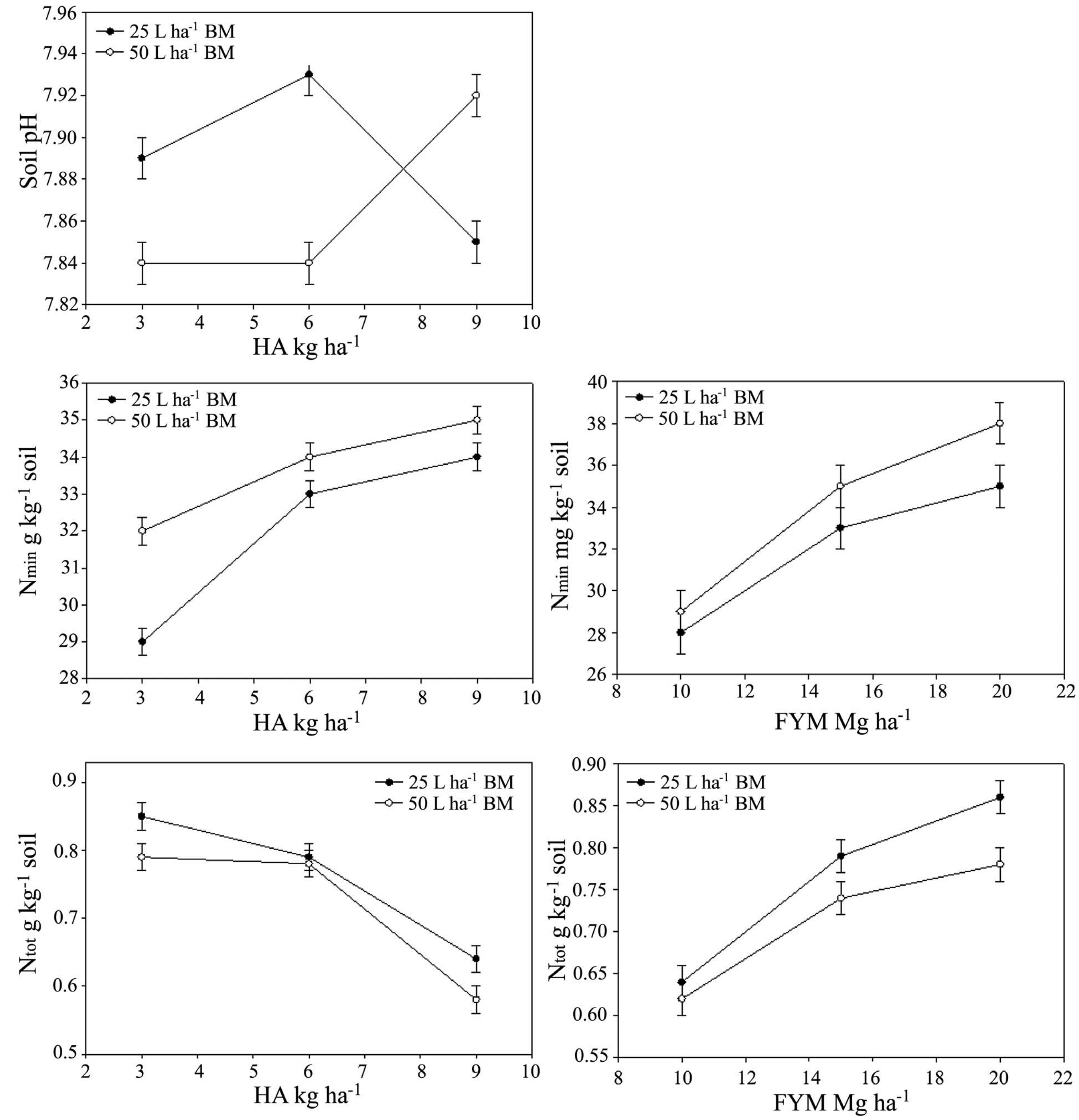
Soil acidity (pH) ranged from 7.7 (20 Mg ha^{-1} FYM) to 8.0 (10 Mg ha^{-1} FYM) both in 2017 and 2018. However, the differences for soil pH recorded in 2017 for 10 and 15 Mg ha^{-1} FYM were not significant (Tables 2 and 3). This indicates that increasing FYM from 10 to 20 Mg ha^{-1} the soil pH increased by 4% both in 2017 and 2018.

The treated plots had higher soil pH (7.9) than control plots (7.6). Interaction between BM and HA showed that 25 or 50 L ha^{-1} BM application had no effect with increasing the HA level from 3 to 6 kg ha^{-1} (Table 2, Figure 5). However, the 25 L ha^{-1} BM significantly lowered the soil pH compared to the 50 L ha^{-1} BM at 9 kg ha^{-1} HA application.

Table 3. Soil acidity (pH), electrical conductivity (EC), bulk density (BD), total (N_{tot}) and mineral (N_{min}) nitrogen in response to beneficial microbes (BM, L ha^{-1}), humic acid (HA, kg ha^{-1}) and farmyard manure (FYM, Mg ha^{-1}) during 2017 and 2018

Treatment	pH	EC dS m^{-1}	BD Mg m^{-3}	N_{tot} g kg^{-1} soil	N_{min} mg kg^{-1} soil
			2017		
25 BM	7.9 a	1.80 b	1.23 b	0.80 a	31.00 b
50 BM	7.8 a	1.90 a	1.25 a	0.71 b	33.22 a
Significance	ns	*	*	*	**
3 HA	7.8 a	1.80 b	1.23	0.80 a	29.70 c
6 HA	7.9 a	1.83 a	1.24	0.80 a	32.64 b
9 HA	7.9 a	1.90 a	1.25	0.61 b	34.00 a
$LSD_{0.05}$	ns	*0.07*	Ns	*0.03*	*1.32*
10 FYM	8.0 a	1.70 b	1.26 b	0.63 c	27.70 c
15 FYM	8.0 a	1.83 a	1.23 a	0.75 b	33.33 b
20 FYM	7.7 b	1.90 a	1.22 a	0.81 a	35.20 a
$LSD_{0.05}$	*0.10*	*0.07*	*0.03*	*0.03*	*1.32*
Control	7.6 b	1.62 b	1.12 b	0.41 b	22.12 b
Treated plots	7.9 a	1.81 a	1.22 a	0.73 a	32.09 a
Significance	**	**	**	**	**
			2018		
25 BM	7.9 a	1.91 a	1.22 a	0.80 a	32.81 b
50 BM	7.9 a	1.90 a	1.23 b	0.72 b	34.60 a
Significance	ns	*	*	**	**
3 HA	7.9 a	1.82 b	1.22	0.84 a	31.53 b
6 HA	7.9 ab	1.90 a	1.24	0.80 b	34.44 a
9 HA	7.8 b	1.94 a	1.22	0.61 c	35.12 a
$LSD_{0.05}$	ns	*0.06*	Ns	*0.03*	*1.65*
10 FYM	8.0 a	1.80 b	1.27 c	0.62 c	29.00 b
15 FYM	7.9 b	1.90 a	1.23 b	0.80 b	34.64 b
20 FYM	7.7 c	2.00 a	1.18 a	0.84 a	37.50 a
$LSD_{0.05}$	*0.06*	*0.06*	*0.02*	*0.03*	*1.65*
Control	7.6 b	1.62 b	1.12 b	0.41 b	22.12 b
Treated plots	7.9 a	1.89 a	1.22 a	0.75 a	33.69 a
Significance	**	**	**	**	**

Note. Means of the same category followed by different letters are significantly different from each other at $p \leq 0.05$ using LSD test; *, ** – significant at $p \leq 0.05$ and $p \leq 0.01$, ns – not significant.



Note. The vertical bars are standard errors of the mean.

Figure 5. The interactive response of soil acidity (pH), mineral (N_{min}) and total (N_{tot}) nitrogen content for beneficial microbes (BM) and humic acid (HA) and BM and farmyard manure (FYM) interaction averaged over 2017 and 2018

Similarly, the interaction between year and HA showed that low level of HA was effective in controlling the soil pH in 2017, whereas in 2018, the high level of HA was more effective (Tables 2 and 3).

Soil bulk density (BD) was significantly lower in 2018 (1.20 Mg m^{-3}) as compared to 2017 (1.25 Mg m^{-3}) (Tables 2 and 3). The soil BD ranged from 1.25 to 1.23 Mg m^{-3} with increasing the BM from 25 to 50 L ha^{-1}; however, BM had no effect on soil BD during 2018. Similarly, the application of 20 Mg ha^{-1} FYM significantly lowered the soil BD over the low level of FYM. With the FYM level increasing from 10 to 20 Mg ha^{-1}, the soil BD significantly decreased from 1.26 to 1.22 Mg m^{-3} in 2017 and from 1.27 to 1.18 Mg m^{-3} in 2018. This showed that FYM had greater effect in 2018 as compared to 2017. This indicates that increasing FYM from 10 to 20 Mg ha^{-1} decreased soil BD by 3.28% in 2017 as compared to 7.63% in 2018. Fertilised plots had higher soil BD (1.22 Mg m^{-3}) than control plots (1.12 Mg m^{-3}).

Soil electrical conductivity (EC) indicates that the EC was improved in 2018 (1.89 dS m^{-1}) as compared to 2017 (1.81 dS m^{-1}) showing an increase of 5.55% (Tables 2 and 3). When the BM level doubled from 25 to 50 L ha^{-1}, the soil EC increased from 1.76 to 1.87 dS m^{-1} in 2017 and from 1.86 to 1.91 dS m^{-1} in 2018. The application of HA level increasing from 3 to 9 kg ha^{-1} soil EC significantly increased from 1.80 to 1.90 dS m^{-1} in 2017 and from 1.82 to 1.94 dS m^{-1} in 2018. Maximum soil EC was recorded with 20 Mg ha^{-1} FYM (1.90 and 2.00 dS m^{-1}) and minimum with 10 Mg ha^{-1} FYM (1.70

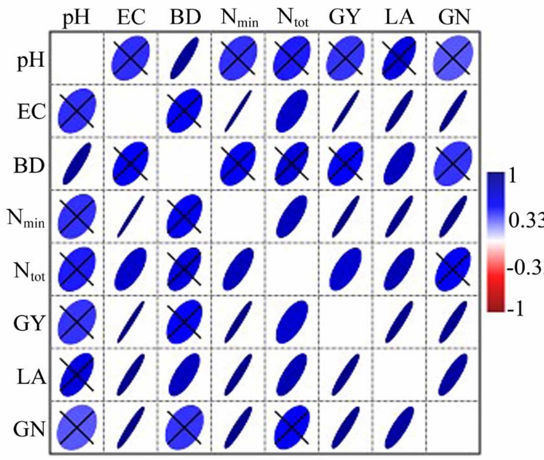
and 1.80 dS m^{-1}) during 2017 and 2018, respectively. The treated plots had higher soil EC (1.87 dS m^{-1}) than control plots (1.62 dS m^{-1}). Regarding year and BM interaction, the greater differences in soil EC was noted in 2017 as compared to 2018, when the BM level increased from 25 to 50 L ha^{-1} (Tables 2 and 3). The difference in EC values was 10 dS m^{-1} in 2017 and 9 dS m^{-1} in 2018.

Soil mineral nitrogen (N_{min}) content recorded at tasselling stage of maize was significantly higher (5%) during 2018 as compared to 2017 (Tables 2 and 3). Similarly, doubling the amount of BM from 25 to 50 L ha^{-1} the soil N_{min} content significantly increased by 7.16% (2017) and 5.45% (2018). Likewise, increasing the HA application from 3 to 9 kg ha^{-1} the N_{min} content increased from 29.70 to 34.00 mg kg^{-1} soil in 2017 and from 31.53 to 35.12 mg kg^{-1} soil in 2018. Similarly, when the FYM level increased from 10 to 20 Mg ha^{-1}, the soil N_{min} content increased from 27.70 to 35.20 mg kg^{-1} soil in 2017 and from 29.00 to 37.50 mg kg^{-1} soil in 2018 showing an increase of 27% (2017) and 29% (2018). The treated plots had higher soil N_{min} content (32.89 mg kg^{-1} soil) compared to control plots (22.12 mg kg^{-1} soil). Interaction between BM and HA showed that increasing the HA level from 3 to 6 the N_{min} availability sharply increased with 25 L ha^{-1} BM as compared to 50 L ha^{-1} BM, whereas with further increase in HA the increases in N_{min} availability were not sharp at both 25 and 50 L ha^{-1} BM applications (Table 2, Figure 5). Similarly, BM and FYM interaction showed that N_{min} availability was higher with 50 L ha^{-1} at high level (20 Mg ha^{-1}) of FYM as compared to either lower level of FYM and/or with 25 L ha^{-1} BM.

Soil total nitrogen (N_{tot}) content decreased with increasing BM application (Tables 2 and 3). Both in 2017 and 2018, the maximum soil N_{tot} content (0.80 and 0.80 g kg^{-1} soil) was observed with 25 L ha^{-1} BM, and minimum (0.71 and 0.72 g kg^{-1} soil) – with 50 L ha^{-1} BM, respectively. This decrease was quantified as 12.68% and 11% in 2017 and 2018. Soil N_{tot} content decreased from 0.84 to 0.61 g kg^{-1} soil in 2017 and from 0.80 to 0.61 mg kg^{-1} soil in 2018, when the HA level increased from 3 to 9 kg ha^{-1}, respectively. Unlikely, when the FYM increased from 10 to 20 Mg ha^{-1}, the soil N_{tot} content also increased from 0.63 to 0.81 g kg^{-1} soil in 2017 and to 0.62 to 0.84 g kg^{-1} soil in 2018. It was also noted that treated plots had higher soil N_{tot} content (0.74 g kg^{-1} soil) compared to control plots (0.41 g kg^{-1} soil). The data regarding BM and HA interaction showed that the application of 25 L ha^{-1} BM had significantly higher soil N_{tot} content across the level of HA as compared to 50 L ha^{-1} BM application (Table 2, Figure 5). However, in the case of both levels of BM, the soil N_{tot} content significantly decreased with increasing the level of HA from 3 to 9 kg ha^{-1}. Similarly, the BM and FYM interaction showed that with the level of FYM increasing from 10 to 20 Mg ha^{-1} the soil N_{tot} content increased with both levels of BM application. However, the application of 25 L ha^{-1} BM had higher soil N_{tot} content across the level of FYM compared to 50 L ha^{-1} BM level.

Maize production in response to soil properties. The Pearson correlation analysis of different soil fertility indices and maize leaf area, grain yield and grain N content is provided in Figure 6.

It was observed that grain yield positively correlated with soil EC and N_{min} and N_{tot} content. Similarly, leaf area showed positive correlation with soil EC, BD and N_{min} and N_{tot} content. However, grain N uptake was positively correlated with soil EC and N_{min} content. Among the soil fertility indices, significant positive correlations were observed among pH and BD, EC with soil N_{min} and N_{tot} as well as N_{min} and N_{tot} content. Among these relationships, the relationship among the N_{min} content and EC was stronger than all other. Likewise, the maize grain yield was positively related to leaf area and grain N content.



Note. GY – grain yield, LA – leaf area, GN – grain N; the crossed ellipse represents not significant correlations ($p \leq 0.05$).

Figure 6. Correlation matrix of soil fertility variables: acidity (pH), electrical conductivity (EC), bulk density (BD), mineral (N_{min}) and total (N_{tot}) nitrogen content and maize plant parameters averaged of all treatments during 2017 and 2018

Discussion

Phenological duration of maize. Days to tasselling, silking and physiological maturity in maize delayed with 50 L ha^{-1} compared to 25 L ha^{-1} BM. As a result of greater N_{min} availability in response to greater decomposition of manure in the presence of microbes (Khan et al., 2019), the delayed tasselling and silking was associated with higher vegetative growth of maize (Khan et al., 2014). The increased N_{min} content is directly related to the increase in vegetative growth of maize (Khan et al., 2014); hence, the crop phenological events delayed. Other possible mechanisms could be the improved moisture availability (Wang et al., 2013), soil condition (Singh et al., 2015), crop stand (Ibrahim, Khan, 2017) and root elongation. These findings are in agreement with those of earlier researchers (Haji et al., 2014), who obtained delayed phenological events in maize due to microbe addition. Increasing HA application three times (from 3 to 9 kg ha^{-1}) delayed the physiological maturity of maize by 2 days. As a result of chelating properties of HA, the delayed physiological maturity could be related to the improved soil properties (Mackowiak et al., 2001). The chelating properties of HA had enhanced the nutrients availability, which might have enhanced the vegetative period, thus might have delayed the physiological maturity in maize crop.

The application of 20 Mg ha^{-1} FYM decreased days to emergence but increased days to tasselling, silking and physiological maturity of maize. The earlier emergence of maize might be associated with enhanced permeability of soil (Jat et al., 2019), water storage capacity of soil and optimized soil temperature (Wang et al., 2013). These results are in line with the findings of Khan et al. (2009), who reported earlier days to emergence of maize due to the application of FYM as compared to control plots. Similarly, when the higher amount of FYM was added, N_{min} availability increased (Khan et al., 2019) as a result of increased decomposition of manure (Bowles et al., 2014). The direct addition of FYM is considered as addition of nutrients, which prolonged vegetative stage (Khan et al., 2014) and thus phenological events. The results agree with the data of Khan et al. (2009), who recorded delayed tasselling with 20 Mg ha^{-1} FYM.

Soil properties. The soil of the experimental site is low in SOM (Khan et al., 2019) thus providing FYM from external source provides a direct addition of nutrients (Khan et al., 2015). The BM increased the microbiological properties of soil (Odlare et al., 2008) and thus increased the decomposition of added manure (Ma et al., 2020). Similarly, the improvement in soil chelating properties by HA addition (Mackowiak et al., 2001) and the energy provision by FYM for microbes (Zhou et al., 2019) increased the nutrient availability (Khan et al., 2019), soil porosity and aeration (Guo et al., 2016) and soil health (Singh et al., 2015).

The lowering of soil pH from 8.0 to 7.7 with increasing FYM from 10 to 20 Mg ha^{-1} might be due to organic acid presence in FYM (Odlare et al., 2008), which released H^+ on mineralization or buffering capacity of manure (Whalen et al., 2000). The lowered soil BD in response to greater amount of BM, HA and FYM might be associated with improved soil biological properties (Odlare et al., 2008) as a result of BM application or chelating properties of HA (Mackowiak et al., 2001) and the energy provision by FYM for microbes (Zhou et al., 2019). The BM, HA and FYM increased the soil EC values but not above 2 dS m^{-1}. The increased EC is due to the total soluble material of soil solution (Behera, Shukla, 2015) and available nutrients (Khan et al., 2019) as a result of microbially mediated decomposition of manure (Luo et al., 2018).

N_{min} availability. The addition of FYM at a rate of 20 Mg ha^{-1} increased the soil N_{tot} (30%) and N_{min} (28%) content compared with 10 Mg ha^{-1} FYM. The increased N_{tot} content might be due to the direct SOM addition that hold true as adding 20 Mg ha^{-1} FYM (having 0.86% N) can theoretically add 170 kg ha^{-1} N. However, when BM was doubled and HA was tripled, the soil N_{tot} content decreased by 12.7% and 34.4% with transformed increases in soil N_{min} content by 6.1% and 12.9%, respectively. It was also noted that HA application had a stronger impact on soil N_{tot} mineralization to N_{min} as compared to BM. This means that decreasing the soil N_{tot} content resulted in greater availability of N_{min} as a result of FYM decomposition. This increases in soil N_{min} content could be due to the fact that the soil of experimental site had lesser than 1% SOM (Ibrahim et al., 2020). Thus, even if microbes are added, they need carbon as source of energy (Bello et al., 2020), whereas the addition of HA (containing 30% SOC) provided carbon as source of energy (Mackowiak et al., 2001), and mineralization of soil N_{tot} increased the soil N_{min} availability. Increasing HA and BM levels improved the soil microbial activities (Bowles et al., 2014) that increased the soil N_{min} content and decreased the soil N_{tot}.

The current studies for improving soil N_{min} content could be related to a direct source of microbes improving the function of microbe by adding HA and a direct addition of FYM as a nutrient source. The increased soil N_{min} content might be due to increased residual effect of manure, improved moisture holding capacity and regulated temperature (Wang et al., 2013) as well as other physico-chemical properties of soil (Odlare et al., 2008). The results of our experiment are in agreement with the findings of earlier researchers (Muhammad et al., 2018; Khan et al., 2019).

Conclusions

1. Doubling the beneficial microbes (BM) amount delayed the maize phenological events (by 2 to 5 days) and improved soil mineral nitrogen (N_{min}) content (6.30%); however, the soil total nitrogen (N_{tot}) content decreased by 12.7%. The addition of humic acid (HA) had positive effect on N_{min} availability and delayed phenological observations. Similarly, increasing the farmyard manure (FYM) level from 10 to 20 Mg ha^{-1} the phenological observations delayed except for days to emergence.

2. Under both levels of BM, the soil N_{min} availability increased but soil N_{tot} content decreased with increasing HA and FYM levels.

3. Grain yield was positively correlated with soil EC, N_{min} and N_{tot} content, whereas leaf area was positively correlated with soil EC, BD, N_{min} and N_{tot} content. However, grain N uptake was positively correlated with soil EC and N_{min} content.

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Kukurūzų reakcija į dėl naudingųjų mikroorganizmų, huminių rūgščių ir tręšimo mėšlu pagerėjusias dirvožemio savybes

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Santrauka

Siekiant išvengti aplinkos taršos, dvejų metų (2017 ir 2018 m.) lauko eksperimento metu buvo vertintas gyvulių mėšlo naudojimas kartu su naudingaisiais mikroorganizmais (NM): 25 ir 50 L ha^{-1}, huminėmis rūgštimis (HR): 3, 6 ir 9 kg ha^{-1}, ir gyvulių mėšlu (GM): 10, 15 ir 20 Mg ha^{-1}, palyginus su kontroliniu variantu be šių priedų. Naudingųjų mikroorganizmų kiekį padidinus nuo 25 iki 50 L ha^{-1}, paprastojo kukurūzo (*Zea mays* L.) fenologiniai reiškiniai paankstėjo viena diena, dirvožemyje sumažėjo suminio azoto (N_{sum}) kiekis (12,6 %), tačiau pagerėjo dirvožemio tankis (1,89 Mg m^{-3}), elektrinis laidumas (1,90 dS m^{-1}) ir mineralinio N (N_{min}) kiekis (33,89 mg kg^{-1} dirvožemio). Huminių rūgščių kiekį padidinus nuo 3 iki 9 kg ha^{-1}, kukurūzų fiziologinė branda pailgėjo dviem dienomis, tačiau padidėjo dirvožemio elektrinis laidumas (0,11 dS m^{-1}), dirvožemio tankis (1,63 %), N_{min} (12,94 %) ir sumažėjo N_{sum} (34,42 %) kiekis dirvožemyje. Gyvulių mėšlo kiekį padidinus nuo 10 iki 20 Mg ha^{-1}, fenologiniai reiškiniai vėlavo, tačiau pagerėjo dirvožemio savybės, t. y. pH (−0,3 vieneto), EC (6,66 %), dirvožemio tankis (−5,83 %), N_{min} (28,14 %) ir N_{sum} (30,16 %) kiekis. Dirvožemio elektrinis laidumas ir N_{sum} bei N_{min} kiekis reikšmingai teigiamai koreliavo su kukurūzų lapų plotu, N kiekiu grūduose ir grūdų derliumi. Padaryta išvada, kad pridėjus 50 L ha^{-1} NM arba 6–9 kg ha^{-1} HR, padidėjo gyvulių mėšlo mineralizacija, pailgėjo kukurūzų augimo laikotarpis ir pagerėjo dirvožemio derlingumo rodikliai.
Taigi, siekiant pagerinti N_{min} pasisavinimą, pailginti kukurūzų fenologinę trukmę ir padidinti derlingumą, 20 Mg ha^{-1} GM reikėtų naudoti kartu su 50 L ha^{-1} NM ir/ar 6 kg ha^{-1} HR.

Reikšminiai žodžiai: dirvožemio savybės, kukurūzų fenologija, mineralinis azotas, skaidymas.