MICROBIOLOGICAL CHARACTERISTICS AND EFFECT ON PLANTS OF THE ORGANIC FERTILIZER FROM VERMICOMPOST AND BAT GUANO

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Abstract
There is an increasing demand in the development of new and better types of organic plant fertilizers. The aim of the present study was to evaluate if the beneficial effect of vermicompost on plant growth and development could be further promoted by adding different amounts of bat guano using two model species under controlled conditions, as well as to assess the microbiological characteristics of bat guano and soil after its application. The study was performed at the Faculty of Biology, University of Latvia, during 2013 and 2014. The amount of bacteria was significantly lower in guano in comparison to vermicompost samples. No actinobacteria were present, but yeasts were found in the guano sample. Soil fungal populations after the application of organic fertilizer from vermicompost and bat guano were dominated by potentially plant growth promoting fungi *Trichoderma* and *Mortierella*. However, at increased guano concentration (300 g kg^{-1}) the proportion of potentially plant pathogenic fungi significantly increased. Addition of bat guano to vermicompost fertilizer significantly enhanced the positive effect of the fertilizer on growth and development of winter rye (*Secale cereale* L.) and potato (*Solanum tuberosum* L.) plants.
Key words: bat guano, microbiological diversity, organic fertilizer, plant growth, vermicompost.

Introduction

The contribution of organic farming in agricultural production in Europe and other parts of the world is continuously increasing. Organic fertilizers not only provide the necessary nutrients but also positively affect overall soil fertility, and can be efficiently used in conventional agriculture as a constituent of integrated fertilization systems (Saleque et al., 2004; Mottaghian et al., 2008). Therefore, there is an increasing demand in the development of new and better types of organic plant fertilizers. Vermicompost, produced by a corporate activity of earthworms and their associated microorganisms, represents a promising type of organic fertilizer. Biological aspects of vermicompost application as a rich source of plant-available minerals and efficient plant growth-promoting agent have been recently studied (Ievinsh, 2011; Grantina-Ievina et al., 2013; Karlsons et al., 2015). However, vermicompost preparations usually are not balanced in respect to their mineral nutrient composition (Srivastava et al., 2012; Karlsons et al., 2015). Consequently, other types of organic fertilizers can be used to improve the mineral balance of the resulting fertilizer mixes.

A possible candidate for organic fertilizer mixes can be bat guano, representing highly mineralized faeces of bats, certified for organic farming (Sridhar et al., 2006). In contrast to vermicompost, the studies evaluating the positive effect and possible mechanism of action of bat guano on plants have been extremely scarce until recently (Sridhar et al., 2006; Bhat et al., 2013; Shetty et al., 2013; Tasci et al., 2013; Almohammedi et al., 2014; Sothearen et al., 2014). There is no information available, though, on microbiological characteristics of bat guano-based fertilizers.

The aim of the present study was to evaluate if the beneficial effect of vermicompost on plant growth and development could be further promoted by adding different amounts of bat guano using two model species in controlled conditions, and to assess the microbiological characteristics of bat guano and soil after its application.

Materials and Methods

The study was performed at the Faculty of Biology, University of Latvia, during 2013 and 2014. Quantification of bacteria, *Escherichia coli* and coliforms, enterococci, cultivable filamentous fungi and yeasts, and potentially pathogenic fungi to humans and animals was performed as described previously (Grantina-Ievina et al., 2013). The number of actinobacteria was estimated on Luria-Bertani agar with the following content per 1 l: peptone 10 g (*Pastone*, BIO-RAD, France), yeast extract 5 g (*Pastone*, BIO-RAD, France), D-glucose 1 g (Penta, Czech Republic), NaCl 10 g (*Sodium Chloride pure p.a.*, *Chempur*, Poland), agar 20 g (*Agar Bios Special LL*, Biolife, Italy) (Hamaki et al., 2005). Potentially pathogenic bacteria and fungi were assessed only in guano and vermicompost samples. Genera of cultivable filamentous fungi were determined after 10 days of incubation on Rose Bengal and Mycosel agar, and after subculturing on Malt extract agar (Biolife Italiana S.r.l., Italy) according to the morphological characteristics and light microscopy results. The soil was analyzed only from the winter rye (*Secale cereale* L.) experiment.

Soil from organic potato (*Solanum tuberosum* L.) field was used as a growth substrate in the present study. Analysis of total (extracted in aqua regia) and plant-available (extracted in 1 M HCl) concentrations of mineral nutrients was performed by standard methods at the Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia (Grantina-Ievina et al., 2014a; Karlsons et al., 2015). Vermicompost was produced from starchless potato pulp and grass by SIA GAHA, bat guano was produced in Madagascar and obtained from Guanomad Europe S.A. Basic characteristics of soil, vermicompost and bat guano used in the present experiments are shown in Table 1. The soil used for organic potato production had acidic pH and relatively high P content together with deficiency in N, K, Ca, Mg, S, Cu, and B. Comparison of plant-available nutrient concentrations in vermicompost and bat guano samples revealed that vermicompost had a higher amount of K and Mg, while bat guano was especially rich in P, Ca, S, Mn, Zn, Cu and Mo. On the downside, guano had a relatively high level of plant-available Na and Cl and extremely high total level of soluble salts as indicated by electrical conductivity of 53 mS cm^{-1}. The degree of mineralization was higher for bat guano (87.3%) in comparison to that of vermicompost (43.5%).

Organic fertilizer was prepared from vermicompost with different amounts (100, 200 and 300 g kg^{-1}) of bat guano, as regular (R) or pelleted (P). Growth substrate for treatments was prepared from soil adding different amounts of fertilizer and thoroughly mixed. For vegetation experiments, winter rye cv. 'Kier' (seed) and potato cv. 'Laimdota' (minitubers from tissue culture-propagated soil-grown plants) were used. Plants were grown in 8 × 8 × 12 cm plastic containers. For winter rye (13 treatments; Table 2), 9 seeds were sown in each container and after 2 weeks thinned to 4 uniform plants. The experiment lasted 5 weeks. Five containers per treatment were used. For potato, 17 treatments were used, consisting of control (soil only) and 1.25, 2.5, 3.75, 5 g of fertilizer per container with 0, 100, 200 and 300 g kg^{-1} of guano supplement for each dose. Tubers were planted individually, with 7 containers per treatment. The experiment lasted for 9 weeks. Substrate was adjusted to 65% water holding capacity and maintained throughout the study with deionized water. Containers were randomly arranged in a growth cabinet at 22/15 °C (day/night) temperature, relative humidity 60%. Illumination was provided by fluorescent lamps at 150 µmol m^{-2} s^{-1} of photon flux density of photosynthetically active radiation, 8/16 h dark/light cycle. Plant height was monitored throughout the experiment. At the end of the cultivation period, both fresh and dry mass of roots and shoots were measured.

Leaf chlorophyll content for winter rye plants was measured using a hand-held chlorophyll meter SPAD-502 (Konica-Minolta Corporation, Japan). For every treatment, the second leaf from five plants was measured with five readings along the leaf.

Table 1

Characteristics of vermicompost, bat guano and soil samples used in the present study in comparison to optimum level of minerals for cultivated plants (Osvalde, 2011)

Characteristic	Vermicompost	Bat guano	Soil plant-available[b]	Optimum for cultivated plants[b]		
	Total[a]	Plant-available[b]	Total[a]	Plant-available[b]		
N	20800	910	10600	882	76	120
P	4900	4578	59300	39240	447	60
K	12400	12400	3000	1250	120	150
Ca	16400	13100	133200	73500	720	800
Mg	5500	3650	3700	2120	70	50
S	3000	250	32500	2400	11	50
Fe	2940	775	6800	800	805	30
Mn	250	236	1840	1250	130	1.5
Zn	74	64	1080	650	4.95	1
Cu	11	4.5	280	10.5	2.0	0.5
Mo	1.9	0.04	–	0.98	0.07	0.02
B	14	7.4	–	6.2	0.2	0.2
Na	320	290	3300	2400	7.5	–
Cl	–	800	–	16750	0	–
Organic matter (%)	–	46.5	–	12.7	–	–
pH (units)	–	7.67	–	6.60	4.86	–
EC (mS cm^{-1})	–	24.8	–	53.1	–	–

[a]Mineral concentration expressed as mg kg^{-1}

[b]Mineral concentration expressed as mg L^{-1}

Table 2

Experimental treatments used in the experiment with winter rye plants

Treatment No.	Type of fertilizer[a]	Vermi-compost[b]	Guano[b]	Dose[c]	Treatment No.	Type of fertilizer[a]	Vermi-compost[b]	Guano[b]	Dose[c]
1	–	–	–	–	8	R	800	200	2.5
2	R	1000	0	2.5	9	R	800	200	5
3	R	1000	0	5	10	P	800	200	5
4	P	1000	0	5	11	R	700	300	2.5
5	R	900	100	2.5	12	R	700	300	5
6	R	900	100	5	13	P	700	300	5
7	P	900	100	5	–	–	–	–	–

[a]R, regular; P, pelleted
[b]Amount of respective fertilizer expressed as g kg^{-1}
[c]Dose of fertilizer expressed as g per container

The significance of differences between means was determined by the Tukey-Kramer test at the $\alpha = 0.05$ level. Correlation analysis was performed with *Excel* (Microsoft, USA). Significance was evaluated at $p < 0.05$ level. Both Pearson correlation coefficients (r) and determination coefficients (R^2) were calculated.

Results and Discussion

The data about the microbiological content of bat guano and vermicompost samples used in the experiments are given in Table 3. The total amount of bacteria was significantly lower in guano in comparison to vermicompost samples, but the number of bacterial coliforms was higher in guano. *E. coli* and enterococci were not present in the analyzed guano and vermicompost samples. No actinobacteria that are characteristic component of organic matter-containing products were present in the guano sample. However, relatively high number of yeasts was present in guano.

In the winter rye experiment moderate and weak correlation, respectively, was estimated that the total number of bacteria increased by increased content of regular vermicompost ($r = 0.56$, $p > 0.05$) and smaller amount of guano ($r = 0.27$, $p > 0.05$) (Fig. 1). The highest number of actinobacteria was detected at 200 and 300 g kg^{-1} of guano [ranging from $(1.06 \pm 0.90) \times 10^5$ to $(1.18 \pm 1.07) \times 10^6$ *vs.* $(5.23 \pm 4.56) \times 10^4$ – $(5.20 \pm 2.71) \times 10^5$] that can be explained by the ability of these bacteria to tolerate high salt concentrations (Keshri et al., 2013), although the guano itself did not contain actinobacteria. The correlation coefficients (r) between the content of guano and actinobacteria were 0.47 in the regular vermicompost treatments and 0.49 in the pelleted vermicompost treatments, indicating moderate correlation. Statistically non-significant differences were observed regarding the total number of cultivable filamentous fungi and yeasts. In the treatments with 300 g kg^{-1} of guano, a higher number of potentially plant pathogenic fungi (members from genera *Verticillium*, *Acremonium*, *Fusarium*, *Thielaviopsis*, *Trichocladium*) was detected (on average 40%) in soil in comparison with the control

Table 3

Microbiological diversity of guano, vermicompost and soil samples, CFU g^{-1} dry mass

Group of microorganisms	Guano	Regular vermicompost	Pelleted vermicompost	Soil
Bacteria	$(1.99 \pm 0.88) \times 10^6$	$(1.20 \pm 0.08) \times 10^7$	$(4.89 \pm 0.18) \times 10^7$	$(4.83 \pm 0.91) \times 10^6$
Coliforms	$(4.24 \pm 0.45) \times 10^4$	$(2.78 \pm 1.18) \times 10^4$	$(2.07 \pm 0.91) \times 10^4$	Not detected
Yeasts	$(6.64 \pm 0.33) \times 10^2$	0	0	0
Actinobacteria	0	$(1.39 \pm 0.79) \times 10^7$	$(1.06 \pm 0.07) \times 10^8$	$(1.77 \pm 0.23) \times 10^6$
Filamentous fungi	$(1.92 \pm 0.28) \times 10^3$	$(1.68 \pm 0.22) \times 10^5$	$(1.03 \pm 0.46) \times 10^5$	$(7.03 \pm 2.19) \times 10^4$
Represented filamentous fungi	*Penicillium* spp., *Mortierella* spp., *Mucor* spp.	*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Scopulariopsis brevicaulis*	*Pseudallescheria* spp., *Aspergillus* spp., *Acremonium* spp., *Sporothrix* spp., *Penicillium* spp., *Mortierella* spp., *Mucor* spp.	*Penicillium* spp., *Mortierella* spp., *Mucor* spp., *Paecilomyces* spp., *Trichoderma* spp.
Potentially human pathogenic fungi	0	$(9.04 \pm 1.80) \times 10^3$	$(3.62 \pm 0.86) \times 10^4$	Not detected
Represented potentially human pathogenic fungi	0	*S. brevicaulis*	*Aspergillus fumigatus*, *Geotrichum* spp., *S. brevicaulis*	Not detected

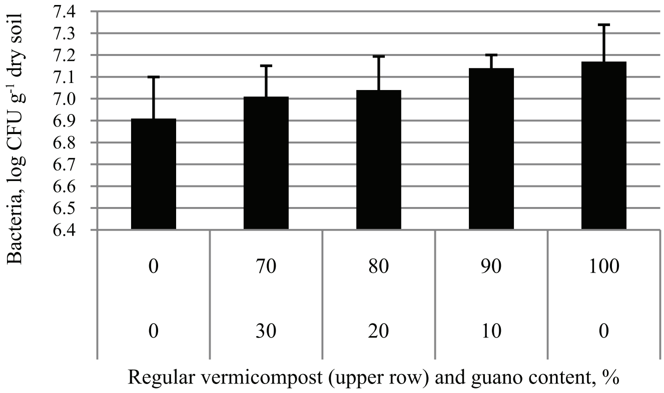

Figure 1. Relationship between the total number of bacteria in growth substrate and content of regular vermicompost and guano in the winter rye experiment.

Table 4

Amount of *Trichoderma* and *Mortierella* (%) from all filamentous fungi in the substrate of the winter rye experiment

Treatment[a]	*Trichoderma*	*Mortierella*	Total	Treatment[a]	*Trichoderma*	*Mortierella*	Total
1	31.34	9.47	40.81	8	40.69	38.63	79.32
2	62.31	0.56	62.88	9	63.76	33.07	96.83
3	45.97	40.59	86.56	10	60.07	6.31	66.39
4	33.00	9.92	42.92	11	10.98	18.67	29.65
5	43.33	56.68	100.00	12	51.77	21.52	73.29
6	85.64	12.31	97.95	13	45.59	3.08	48.67
7	45.18	22.01	67.19	–	–	–	–

[a]Treatments are the same as designated in Table 2

without any fertilizer (8%) and treatments with less guano content (5%) or only vermicompost (20%). However, in the microbiological analysis of guano only plant growth promoting and saprophytic fungi were detected (Table 3). In general, the soil fungal populations were dominated by potentially plant growth-promoting fungi *Trichoderma* and *Mortierella* from 16 to 100% of all fungi (Table 4), and the percentage of them was increased in the treatments with 1000, 900 and 800 g kg^{-1} of regular vermicompost in comparison to the pelleted vermicompost treatments and treatments with 30 g kg^{-1} of guano.

Fungi from genus *Mortierella* are often found in different types of organic fertilizers, e.g., vermicompost from manure and plant residues (Anastasi et al., 2005) and conventional compost (Ryckeboer et al., 2003). Most importantly, *Mortierella* spp. produce arachidonic acid with potentially antagonistic activity against plant pathogenic fungi (Eroshin and Dedyukhina, 2002; Fakas et al., 2009; Grantina-Ievina et al., 2014). Also, *Trichoderma* spp. are well-known plant biocontrol agents (Cordier et al., 2006). On the other hand, increasing the proportion of bat guano up to 30% in the organic fertilizer composition significantly increased the number of potentially pathogenic plant fungi, evoking serious concerns in respect to plant protection. Evidently, as these fungi were not initially present in the guano samples, the use of organic fertilizers with a relatively high content of living microorganisms can lead to changes in soil microbiological diversity through the interaction with native microorganisms.

Growth and development of winter rye plants were significantly promoted by the addition of organic fertilizer to soil. Both fresh and dry mass formation in above-ground parts was enhanced 1.5 to 2.0 times by vermicompost application alone (Fig. 2). Addition of bat guano to the fertilizer further increased plant biomass production and dry matter accumulation up to 500 and 400%, respectively, in comparison to soil-only control plants. There was no significant difference between the application of regular vs. pelleted type of fertilizer. A positive effect of vermicompost and bat guano on physiological status of rye plants was indicated by an increase in leaf chlorophyll content (Fig. 3).

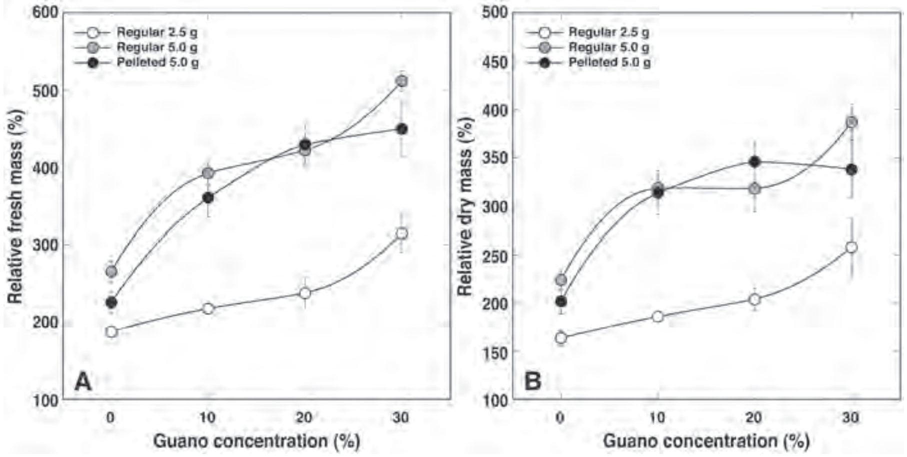

Figure 2. Effect of different concentrations of bat guano in vermicompost-based organic fertilizer on relative fresh (A) and dry (B) mass of shoots of 5-week-old winter rye plants. The data are means ±SE based on 5 replicates with 4 plants each for every concentration.

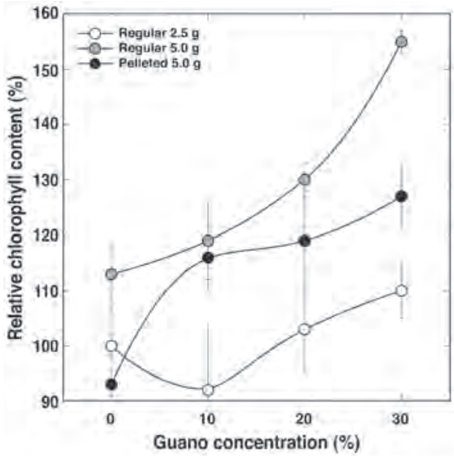

Figure 3. Effect of different concentrations of bat guano in vermicompost-based organic fertilizer on relative leaf chlorophyll content of 3-week-old winter rye plants. The data are means ±SE based on 5 replicates for every concentration.

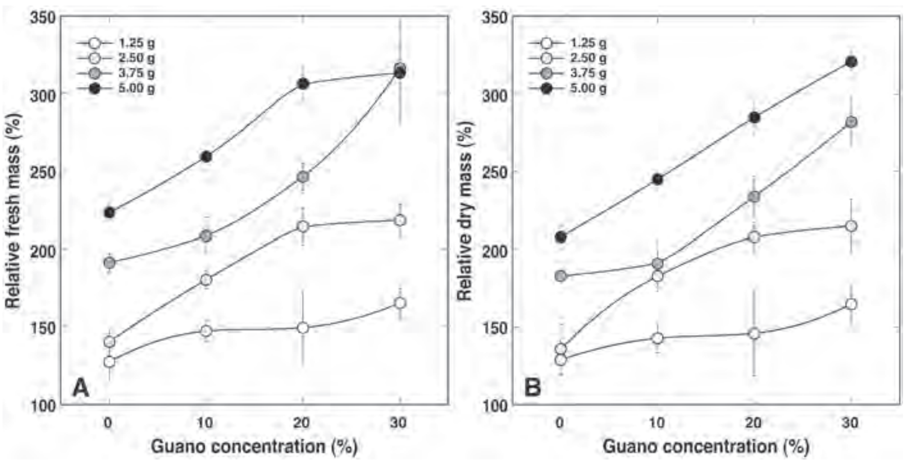

Figure 4. Effect of different concentrations of bat guano in vermicompost-based pelleted organic fertilizer on relative fresh (A) and dry (B) mass of shoots of 9-week-old potato plants. The data are means ±SE based on 7 replicates for every concentration.

A wider range of fertilizer doses (1.25 to 5.00 g per container, roughly corresponding to 2 to 10 t ha^{-1}) was assessed in the experiment with the potato minitubers. It is evident that the addition of guano had a more pronounced positive effect at higher doses of application (Fig. 4). However, an overall increase in accumulation of both fresh and dry mass by fertilizer supplement over unfertilized control was less evident than in the case of winter rye plants.

A clear beneficial effect of guano addition to vermicompost-based fertilizer was seen in the present study both for winter rye and potato plants under controlled conditions. This effect can be partially related to the increased amounts of plant-available mineral nutrients from guano. Similar positive effect in the conditions of limited soil nutrient availability was evident in the study with vermicompost (Karlsons et al., 2015). Plant growth-affecting activity of bat guano samples was not assessed in the present study. However, it is reasonable to suggest that a high microbiological activity in the guano has produced plant growth-promoting substances, leading to an additional positive effect on plant growth and development, similar to that found for other organic fertilizers, vermicompost (Karlsons et al., 2015) and freshwater sapropel (Grantina-Ievina et al., 2014a). Consequently, bat guano is a promising candidate for industrial organic fertilizer mixes.

Conclusions

1. Guano represents organic fertilizer with a high level of mineralization and significant content of living microorganisms, but microbiological diversity differed in comparison to vermicompost samples. The most characteristic was a lower total amount of bacteria, a higher number of coliforms, and the presence of yeasts.
2. Application of guano-containing fertilizer at 300 g kg^{-1} concentration significantly affected the microbiological diversity of the soil, increasing the amount of potentially plant pathogenic fungi. Therefore, microbiologically active organic fertilizers must be used with caution, as overall diversity can be altered due to microbial interaction. Treatment with regular vermicompost positively affected the percentage of potentially plant growth-promoting fungi *Trichoderma* and *Mortierella*.
3. Addition of bat guano to the organic fertilizer from vermicompost significantly increased the stimulating effect of vermicompost on plant growth and development of both model crops - winter rye and potato. This effect increased with an increasing proportion of guano indicating a higher concentration of plant-available minerals and, possibly, growth-promoting substances in the resulting fertilizer mix.

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