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RESEARCH ARTICLE
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Potential Use of Sunn Hemp as Green Manure and of Biostimulant for Enhancement of Animal Feed Corn Crop and Fertilized Soil Properties

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ABSTRACT

Sunn hemp and biostimulants have been used for animal feed corn production in large fields. The sunn hemp crop is grown initially on rotation and the biostimulant is applied to the sunn hemp and corn plantation. In the current study, the field was divided into 4 experimental designs (No. 1–No. 4) with 5 data collection periods. The physical, chemical, and biological properties of the soil in each test plot were determined. The results showed that the soil with the highest organic matter was in field No.1 (1.23%) during corn growing and in field No. 2 (1.34%) during corn growing with biostimulant application at corn age 3 weeks. In addition, the soil in field No.2 had the highest total N (0.62 g.kg^{-1}) and the highest bacterial colonization (1.27×10^{7} CFU). The seed germination test showed that the biostimulant significantly increased the root length (from 1.6 to 9.1cm) and shoot length (from 2.9 to 5.2cm) of the corn seed after soaking for 17 h compared to the unsoaked seed. Corn physiology in fields No.1 and 2 had the significantly highest corn ear insertion height (71.6–73.9cm) compared to that in field No. 4 (51.1cm). In conclusion, sunn hemp performed well as a green manure and a source of organic matter. Furthermore, the biostimulant effectively increased the fertility of the soil by increasing total N and the corn ear insertion height during corn production. The cost-effectiveness was discussed of using sunn hemp with a biostimulant to improve the corn yield.

Keywords: Sunn hemp, Biostimulant, Animal feed corn, Soil property

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INTRODUCTION

Good agricultural practice involves reducing the use of chemicals to aminimal level by substituting with organic substances, supported by good agricultural management. Organic agriculture avoids the use of any chemicals in crop production, including fertilizers, herbicides, and pesticides, to prevent ecosystem damage and, in turn, protect the consumer. Organic agriculture is a technology based on ecological management supporting biodiversity, the ecological cycle, and biotic activity (Gomiero et al., 2011). The transformation period from intensive to organic agriculture can take 3–5 years with suitable management and the degradation of chemical residue in the agricultural field, which eventually increases the opportunity to export organically-grown agricultural products. Natural-based agriculture includes crop rotation for soil amendment using green manure from leguminous plants, such as sesbania pea, sunn hemp, jackbean, mungbean, and pigeon pea (Office of Soil Biotechnology, 2016). Recently, sunn hemp was substituted as green manure instead of urea, with a resultant increase in nitrogen use efficiency by Japonica rice (Khemtong et al., 2023). *Gliricidia sepium* leaf biomass used as natural fertilizer was reported to improve soil fertility for sweet corn (Aroonluk et al., 2020). These leguminous plants contain macronutrients—nitrogen (N, 2.34–2.87%), phosphorus (P, 0.25–0.54%), and potassium (K, 1.11–2.46%)—and micronutrients—calcium (Ca, 0.82–1.53%), magnesium (Mg, 1.74–2.04%), and sulfur (S, 0.48–2.27%) (Office of Soil Biotechnology, 2016). Organic fertilizer from agricultural residues, such as livestock manure, bone meal, rubber wood ash, and coffee bean shell, also contain N (0.93–4.0%, P (0.14–23.0%), and K (0.68–13.48%). Pests and insects can be repelled by plant extracts from some herbs, such as myrtle grass) *Acorus aclamus* Linn.(, neem (*Azadirachta indica* A. Juss.), citronella grass) *Cymbopogon nardus* Rendle.(, and *Kaffir lime*) *Citrus hystrix* DC.(. In addition, biocontrol from microbes, such as *Trichoderma viride* and *Bacillus subtilis*, can be used for antagonistic activity against plant pathogens, such as *Phytophthora palmivora*, *Sclerotium rolfsii*, *Fusarium moniliforme*, *Alternaria* sp., *Pestalotiopsis* sp., *Colectotrichum* sp., *Pythium* sp., and *Rhizoctonia* sp., that cause root rot, leaf wilting, leaf spotting, yellow leaves, and anthracnose in durian, orange pineapple, cassava, sugarcane, corn, rice, vegetables, and ornamental plants (Office of Soil Biotechnology, 2016). Pests' natural enemies, such as the lady beetle and green lacewing, can successfully control aphids (Baker et al., 2019). In addition, uses of plant growth-promoting rhizomicrobes (PGPRs) include: 1) nitrogen-fixing microbes such as rhizobium, azotobacter, mycobacterium, azospirillum ,and bacillus; 2) blue-green algae, such as anabaena, nostoc, tolypotrix, and anabaenopsis; 3) phosphate and potassium solubilizing microbes; and 4) sulfur-solubilizing microbes (Woitke and Schnitzler 2005; Office of Soil Biotechnology, 2016). PGPRs support crop growth via direct effects on growth promotion and via indirect effects on biocontrol. PGPRs are free-living microorganisms that can beneficially colonize the plant root surface and are sometimes endophytic. They are able to direct the suppression of plant pathogenetic diseases, by exclusion of beneficial or pathogenic microorganisms from the root surrounding, by competition, enhancement of the release of limited available nutrients from the soil matrix, by stimulation of host plant disease reaction, by induced systemic resistance (ISR) via direct plant-microbe interaction, and by the release of plant-growth regulating substances, such as plant hormones (Woitke and Schnitzler, 2005). However, PGPRs have limitations to their application in soil because successful root colonization is a prerequisite for their success. Many bacteria are weak competitors, so more work is needed to evaluate suitability (Woitke and Schnitzler, 2005).

Plant biostimulants and microbial-derived compounds are technological developments useful for agricultural sustainability. They are defined by EU Regulation 2019/1009 as products stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, and the availability of confined nutrients in the soil or rhizosphere. Plant biostimulants may be composed of substances or mixtures and microorganisms; therefore, they are classified as microbial or non-microbial plant biostimulants (Castigliore et al., 2021). Microbial-derived compounds are mostly secondary metabolites that are excreted by microorganisms in response to known and unknown stimuli, such as nutrient deficiency, competition for niche spaces, or even signals from a host plant. Such secondary metabolites may include hormones, volatile organic compounds (VOCs), enzymes, anti-microbial, and siderophores, which may serve a range of functions for the producer or microbe and receiver or another microbe or plant (Naamala and Smith, 2021). Microbial-derived compounds are involved in plant growth, the mitigation of biotic stress-related effects, the mitigation of abiotic stress-related effects, and the bioremediation of xenobiotic compounds. Xenobiotic compounds, such as organophosphates, aromatic hydrocarbons, phenols and heavy metals, are a major source of soil and environmental degradation in many parts of the world. However, their slow action and susceptibility to environmental conditions have been reported as potential limitations to the use of microbial cells in bioremediation. The direct use of compounds may address such limitations (Naamala and Smith, 2021).

In general, intensive corn production in a large field involves using some forms of chemical fertilizer, pesticide, and herbicide due to the limitations of farm management for a monocrop, and the need for pest and weed control on a large scale. Animal corn production involves planting agricultural products and their harvest, transportation, and conversion into animal feed. Corn seed is a promising energy source for animal feed due to its lipid and protein nutrition (Rahmani et al., 2022). However, as a monocrop plantation with the use chemicals for an extended period, it has led to soil infertility by creating adverse soil properties and accumulated chemical residues from pesticide and herbicide compounds and their degradates that then circulate in the human food chain. The introduction of organic substances through crop rotation and the use of biostimulants may effectively sustain soil fertility for long-term farming. Thus, the objectives of the present research were: 1) to clarify the potential use of sunn hemp as green manure; and 2) to evaluate the use of a biostimulant application for animal feed corn production in the field. The effects on soil were examined of sunn hemp and the biostimulant in terms of physical, chemical, and biological properties, as well as the effects of herbicide and its degradate residues on soil fertility. In addition, the effects of sunn hemp productivity, corn physiology, and corn seed germination were investigated in relation to the biostimulant application.

MATERIALS & METHODS

Experimental Design

The field experiment was conducted on approximately 6.4 rai or approximately 1 hectare (ha) of agricultural land managed by the Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-ok, Chonburi, Thailand, where the aim was to grow a sunn hemp crop on rotation for animal feed corn production. The area was divided into 4 fields with 2 replicates (Table 1 and Fig. 1). All field plots were planted with sunn hemp for crop rotation, with field No.2 being applied with the used biostimulant during the sunn hemp rotation (before the corn had been planted and during 3 weeks growing the corn). Fields No. 3 and 4 were applied with the biostimulant during 3 weeks and 6 weeks of the corn rotation, respectively (Table 1). The soil, biostimulant, and corn samples from each plot were collected in triplicate during 5 different periods; before planting the sunn hemp, during growing the sunn hemp, after harvest of the sunn hemp, after the corn crop had been planted and the application of the first biostimulant, and after the corn crop had been planted and the application of the second biostimulant. The soil samples used for determining biological properties were kept at 4 ºC, those for determining physico-chemical properties were kept at room temperature and the soil samples for determining the amounts of atrazine and propanil residues were kept at -20 ºC.

Fig. 1: Field plan of experiment: field No.1.1, 2.1, 3.1, and 4.1 are replicated with field No. 1.2, 2.2, 3.2, and 4.2, respectively.

Table 1: Biostimulant treatment during sunn hemp amendment and animal feed corn crop growth

Field No.	Sunn hemp	Biostimulant			
		Before corn (during sunn hemp growth)	Corn sowing	Corn at 3 weeks	Corn at 6 weeks
1.1	✓				
1.2	✓				
2.1	✓	✓		✓	
2.2	✓	✓		✓	
3.1	✓	✓	✓	✓	
3.2	✓	✓	✓	✓	
4.1	✓	✓	✓	✓	✓
4.2	✓	✓	✓	✓	✓

✓ indicated that each field no. had been applied sunn hemp for seedling, and biostimulant use along with corn plantation.

Soil Collection and Determination of Chemical, Physical and Biological Properties

Soil samples were taken from the experimental area (approximately 1 ha) in Bangpra district, Sriracha, Chonburi province, Thailand before, during, and after the sunn hemp rotation and subsequently, during the growth of a corn drop. The soil before conducting the experiment was determined for soil type (percentages of sand, silt, and clay) (Gee and Bauder, 1979), available phosphorus (P) (Bray and Kurtz, 1945), available potassium (K) (Helmke and Sparks, 1996), and available calcium and available magnesium (Ca and Mg) (Suarez, 1996). The soil chemical properties assessed during the experiment were: the lime requirement, total nitrogen (N) (Bremner 1996), total P, total K, and organic matter, with the lime requirement and organic matter determination following methods from Woodruff (1948) and Walkley and Black (1934). The soil physical properties assessed were pH and electrical conductivity (EC) following the methods from Thomas (1996). The soil biological properties estimated were total plate counts of bacterial and fungal colonies based on the standard agar method modified from Sofo et al. (2020), using nutrient agar (NA) for bacterial culture and potato dextrose agar (PDA) for fungal culture. Each soil sample (1 g) was added to 9 ml sterile distilled water (0.1% w/v), and homogenized for 15min. The suspension was decimally diluted (10^{-2} to 10^{-6}). An aliquot of 100 µl of each dilution was inoculated on each prepared medium and spread manually onto the agar surface. The numbers of colony-forming units (CFUs) were counted during incubation at 28±2°C for 24 h for bacteria and up to 5 days for fungi.

Sunn Hemp Sowing and Rotation

In total, 50 kg of sunn hemp seed was sown into approximately 8 rai or 1.3 ha of the field twice during the winter season (on 24 December 2022 and 5 January 2023). The field was watered every 2 days and the biostimulant was applied when the sunn hemp was aged around 1 month. After 43–55 days of sunn hemp growth, the soil samples were collected and determined for their chemical, physical, and biological properties. The sunn hemp biomass was plowed back into the soil after the plants had developed approximately 50% of the beanstalk. The plant was used as green manure and kept fermenting in the field for around a month before the corn was planted.

Soil Preparation, Corn Sowing, and Corn Crop Growth

After plowing in the sunn hemp and allowing it to ferment as green manure, the field was shoveled for weed removal and ditches were dug for drainage prior to the rainy season. The corn seeds (303 variety) were soaked with the biostimulant for 30min before air-drying and sowing using a corn sowing machine pulled by a tractor. Approximately 1 ha was sown. In addition, 1,050g of the biostimulant was prepared by dissolving it in 125L of clean tap water for application over the 1ha. The seeds with (fields No. 3 and No. 4) and without (fields No. 1 and No. 2) biostimulant soaking were planted 20cm between the crop rows and 65cm apart in a row. After seed sowing, the soaked biostimulant was applied to fields No. 3 and No. 4 (Table 1). The corn seed germination was studied further in laboratory tests. In the field, chemical fertilizer was applied coupled with the corn sowing machine on the day of seed sowing (50g fertilizer: 10-5-15), at corn age of 1 month (100g fertilizer: 27-12-6 and 100g urea fertilizer: 46-0-0), and at corn age of 45 days (100g urea fertilizer: 46-0-0).

The corn seed germination test was conducted to clarify the performance of the biostimulant on seed germination promotion. The effect of biostimulant soaking time on seed germination for shoot and root lengths, seed dimension (width, length, and thickness), and 10-seed weight were also tested at unsoaked and at 30min and 17 h soaking times before air-drying and sowing. The corn seed not soaked in biostimulant was planted in fields No. 1 and No. 2, while the corn seed soaked for 30min was planted in fields No. 3 and No. 4 (Table 1) using a combine planter towed by a tractor with chemical fertilizer simultaneously. The corn seed soaked for 17 h was air-dried for 12 h, whereas the corn seed soaked for only 30min was air-dried for 2 h. The seed germination test was conducted using 9cm glass plates with 2 layers of Whatman filter paper that was filled with distilled water for investigating seed germination and growth (Jesus et al., 2016; Sooksawat et al., 2022).

Weed and Pest Removal and Determination of Herbicide Residue in Field Soil

After sowing the corn seed, chemical weed control was applied using an agricultural drone applying atrazine herbicide to control weed growth. At the corn age of 20 days, topramezone mixed with atrazine herbicide was applied again to control fast-growing weeds. Emamectin was used to control pests at corn plant ages of 1 month and 45 days. Soil samples in the field were collected and determined using HPLC for herbicide residues of atrazine and its degrades, namely 3,4-dichloroaniline (3,4-DCA) and 4,5-dichlorocetechol (4,5-DCC), and also for propanil, which has been historically used in the field. Each soil sample (2 g) was shaken in a rotary shaker at 180 rpm and 30ºC. Then, the sample was added with an equal volume of dichloromethane, dried, and redissolved in methanol before quantifying the herbicide residues based on HPLC. Chromatographic separation was carried out in a C18 column (250mm ×4.6mm, 5µm), Phenomenex at a UV wavelength of 210nm. Gradient methanol:0.1 %acetic acid (60:40) was used as the mobile phase .The temperature for each sample was 30ºC, with an injection volume of 10µL (Amadori et al., 2013).

Data Collection on Sunn Hemp Yield, Corn Yield and Corn Quality

The sunn hemp was grown and shoveled for use as green manure before planting corn. Physiological parameters were measured for the sunn hemp: the amount of growing plant/m^2, the height of the growing plant (cm), and the amount of seedling plant/m^2.

Weed control included herbicide and weed-cutting machine. At 2 weeks prior to harvesting the corn, weeds were removed using a cutting machine between the corn plant rows. At 100 days, the corn was harvested using a corn combine harvester. Corn leaves, stalks, and seed samples were measured. Corn productivity was analyzed using the corn plant height, ear insertion height, number of leaves, pod width, pod length, pod weight, pod weight without sheet, seed row number, seed number/row. The corn product quality was determined based on the moisture, protein, and lipid contents.

Statistical Analysis

All experimental data was analyzed using ANOVA in the SPSS program. Significant differences were tested at the 0.05 level using Tukey HSD's test. All results were shown as the mean and standard deviation from at least three replicate samples in all experiments.

RESULTS

Effect of Soil Amendment with Sunn Hemp and Biostimulant Treatment on Soil Fertility before Crop Planting

The field soil texture was determined before starting the experiment. The results showed that fields No. 1, No. 3, and No. 4 were predominantly loamy sand, whereas field No. 2 was sandy loam with a significantly high amount of clay soil (Table 2). More clay in the soil affects the soil water characteristics by increasing the water residue degree of saturation (Abd et al., 2020), facilitating absorbing and maintaining soil water in the field. All fields were fertile, with varied amounts of macronutrients and micronutrients, as shown in Table 2. The total N, availability of macronutrients, such as available P and K, and of micronutrients, such as Ca and Mg, differed among the 4 fields (Table 2). Field No. 1 had the highest available K (157.0mg/kg) compared to the other three field No. Field No. 2 had higher available P (42.6mg/kg) and available Mg (98.1mg/kg) compared to field No.4. Field No.4 contained the lowest amount of available P (6.4mg/kg) and available Mg (34.1mg/kg); thus, it may need soil amendment before planting a valuable crop dependent on a good production yield.

When using sunn hemp as a rotation crop for soil amendment, Table 3 shows the effects of using sunn hemp as a rotation crop for soil amendment and using the biostimulant on the chemical, physical, and biological soil properties among the 4 treatments (A, B, C, and D). Soil from plot A (without using biostimulant but growing sunn hemp)

Table 2: Soil type and macronutrient and micronutrient availability in experiment fields

Field No.	Sand	Silk	Clay	Soil type	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Available Ca (mg/kg)	Available Mg (mg/kg)
1	75.00±2.83	18.00±2.83	7.00±0.00a	Loamy sand	0.47±0.00ab	24.35±0.49c	157.00±4.24b	562.00±25.46	77.00±3.68bc
2	70.00±1.41	20.50±0.71	9.50±0.71b	Sandy loam	0.39±0.03ab	42.60±2.12d	130.00±4.24a	1229.50±709.23	98.05±15.49c
3	74.00±1.41	19.00±1.41	7.00±0.00a	Loamy sand	0.51±0.00ab	12.25±0.07b	118.50±9.19a	323.50±54.45	61.45±5.73ab
4	76.00±1.41	17.00±1.41	7.00±0.00a	Loamy sand	0.30±0.04a	6.39±0.15a	107.00±4.24a	176.00±25.46	34.10±0.14a

a, b, c, and d indicate significant (P<0.05) differences in columns (between fields).

Table 3: Effect of sunn hemp and biostimulant on chemical, physical, and biological properties of soil during sunn hemp rotation

Treatments	Organic matter (%)	pH	Total P (mg/kg)	Total K (mg/kg)	EC (dS/m)	Bacteria (CFU)	Fungi (CFU)
Sunn hemp without biostimulant (A)	1.39±0.09b	6.30±0.14	86.50±24.75b	36.00±15.56	0.15±0.01a	1.36 x 10^6a	1.17 x10^3
Non-sunn hemp and without biostimulant (B)	0.63±0.05a	5.95±0.07	14.50±0.71a	21.00±11.31	0.34±0.05b	1.28 x10^6a	1.00 x10^3
Sunn hemp with biostimulant (C)	0.88±0.23ab	6.25±0.21	28.50±19.09ab	17.00±1.41	0.07±0.01a	2.35 x10^6a	3.67 x10^3
Non-sunn hemp with biostimulant (D)	0.78±0.14a	6.15±0.07	9.00±4.24a	19.50±2.12	0.14±0.05a	7.87 x10^6b	5.00 x10^2

a and b indicate significant (P<0.05) differences in rows (between treatments). Non-sunn hemp soil sample was field soil without any sunn hemp growth. Soil sample collected on 17 February 2023.

contained the highest amounts of organic matter (1.4%), total N (0.54g/kg), and total P (86.5mg/kg). This result indicated that sunn hemp could improve soil fertility in terms of organic matter and macronutrients (N and P) in the soil. Notably, the soil without sunn hemp and without biostimulant had the highest EC value (0.34 dS/m) compared to the other treatments, perhaps because the high basal level of ionic forms of nutrients and substances present in the soil were not consumed by the plant and microorganisms. As expected, the soil containing the biostimulant but without growing sunn hemp (D) contained the highest number of bacteria (7.87×10^6 CFU, Table 3). Sunn hemp is a leguminous plant with possibly selective microorganisms colonized with the bacteria on the rhizomes, specifically nitrogen-fixing bacteria that possibly supported the succession of the microbial community in the soil (Maheshwari et al., 2020). The amounts of the total N in treatments A, B, C, and D were 0.54, 0.26, 0.26, and 0.31g/kg, respectively (data not shown). These values might indicate that the soil with only sunn hemp (A) could enhance the highest total N in the soil, possibly via its nitrogen-fixing ability, while adding biostimulant (C) could possibly consume this nutrient in the soil. The soils without sunn hemp (B and D) had low amounts of total N (0.26g/kg); hence, applying the biostimulant could enhance this nutrient in the soil (0.31g/kg), possibly via the activity of nitrogen-fixing microbes in response to the biostimulant application.

The biomass from sunn hemp growth was estimated to indicate its impact as green manure (Fig. 2). The sunn hemp was sown twice in two-week different periods to increase its biomass and the sunn hemp seedlings had germinated in different periods. The number of growing plants, the height of growing plants, and the number of seeding plants were compared among the 4 fields. Applying biostimulant during sunn hemp growth in fields No.2, No. 3, and No. 4 did not produce any significant differences in the number of growing plants (36.3–55.5plants/m^2), the height of growing plants (86.7–120.7cm), and the number of seeding plants (44.1-69.1plants/m^2) among the 4 fields. Thus, its biomass could be used for shoveling and fermentation as green manure in the field equitably for corn production.

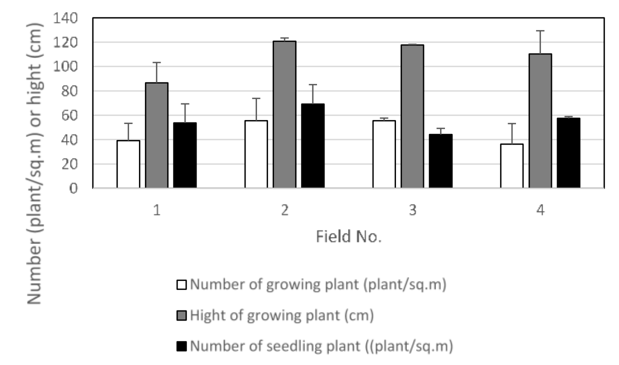

Fig. 2: Sunn hemp physiology in field experiments, where error bars indicate standard deviation.

Effect of Soil Amendment with Sunn Hemp and Biostimulant Treatment on Soil Fertility in Corn Plantation

The soil was collected at 5 different times: 1) before planting sunn hemp; 2) during sunn hemp growth; 3) after sunn hemp growth; 4) after corn planted with the first biostimulant application; and 5) after corn planted with the second biostimulant application. Table 4 shows the effects on soil fertility of the soil amendment with the sunn hemp and biostimulant treatments regarding corn growth. The soil from fields No. 1, No. 2, and No. 3 had pH values in the slightly acidic-to-neutral range (5.3–7.4); however, lime would be required to address the slight-to-high soil acidity (5.10–5.40) in the soil from field No.4 throughout the 5 periods of plant production, except for period 4 (after corn planted with the first biostimulant application (pH = 5.55). Thus, improvement of the soil acidity in field No.4 would require a lime application as high as 975–2,438 kg$CaCO_3$/ha to increase the pH for biological activity to improve the soil fertility and hence enhance crop yield (Table 4). The organic matter was significantly the highest in field No. 2 after growing the sunn hemp and after planting the corn with the second biostimulant application (1.3%) compared to the soil in field No. 4 before planting the sunn hemp (0.6%). The total N (0.62g/kg) and the bacterial count (1.27 x10^7 CFU) from the soil in field No.2 were the significantly highest, showing the same trend as after planting the corn with the second biostimulant application. This result suggested that the soil with the sunn hemp as green manure and using the biostimulant

Table 4: Chemical, physical and biological properties of soil before and after growing sunn hemp and during corn rotation

Periods	Field No.	Lime requirement ($kgCaCO_3/ha$)	Organic matter (%)	pH	Total N (g/kg)	Total P (mg/kg)	Total K (mg/kg)	EC (dS/m)	Bacteria (CFU)	Fungi (CFU)
Before growing sunn hemp	1	0.00±0.00	0.79±0.02 abcde	6.45±0.07bcde	0.47±0.00ab	23.00±1.41ab	49.50±0.71	0.17±0.01cd	1.39 x10^6a	5.67 x10^2a
	2	0.00±0.00	0.87±0.00 abcde	6.65±0.21de	0.39±0.03ab	44.00±4.24bc	26.00±1.41	0.06±0.00ab	9.67 x10^5a	3.67 x10^3a
	3	0.00±0.00	0.82±0.06 abcde	5.80±0.42abcd	0.51±0.00ab	12.00±1.41a	18.50±0.71	0.07±0.00abc	5.60 x10^6ab	3.23 x10^3a
	4	975.00±1,378	0.55±0.00 ab	5.35±0.21ab	0.30±0.04a	4.50±0.71a	15.00±1.41	0.03±0.00ab	4.93 x10^5a	1.04 x10^4a
During sunn hemp plantation	1	0.00±0.00	1.17±0.33 bcde	6.05±0.07abcd	0.423±0.18ab	18.50±6.36a	44.00±5.66	0.22±0.10d	3.78 x10^6ab	4.56 x10^3a
	2	0.00±0.00	0.98±0.06 abcde	6.15±0.07abcd	0.33±0.06a	8.00±1.41a	18.00±1.41	0.08±0.01abc	4.92 x10^6ab	1.80 x10^4a
	3	487.50±689	0.91±0.13 abcde	5.35±0.21ab	0.41±0.13ab	16.00±14.14a	31.00±14.14	0.12±0.02bc	5.21 x10^6ab	3.73 x10^4a
	4	1,462.50±689	0.76±0.02 abcde	5.25±0.07ab	0.29±0.04a	10.00±0.00a	22.00±7.07	0.04±0.00ab	9.00 x10^6bc	1.28 x10^5b
After sunn hemp plantation	1	0.00±0.00	1.15±0.06 bcde	7.40±0.14e	0.46±0.07ab	24.50±3.54ab	64.00±35.36	0.17±0.04cd	5.07 x10^6ab	1.80 x10^3a
	2	0.00±0.00	1.29±0.46 de	6.60±0.14cde	0.46±0.04ab	11.50±4.95a	52.00±45.25	0.11±0.01bc	4.23 x10^6ab	3.90 x10^3a
	3	0.00±0.00	1.20±0.23 bcde	6.00±0.28abcd	0.30±0.06a	10.50±7.78a	34.50±19.09	0.11±0.02bc	4.30 x10^6ab	6.50 x10^3a
	4	975.00±1,378	0.80±0.07 abcde	5.40±0.14abc	0.42±0.06ab	14.50±0.71a	31.50±3.54	0.05±0.01ab	2.54 x10^6a	9.05 x10^3a
During corn rotation and 1st biostimulant	1	0.00±0.00	1.23±0.01 cde	6.15±0.21abcd	0.512±0.06ab	54.50±0.71c	108.00±45.25	0.00±0.00a	1.47 x10^7c	6.72 x10^3a
	2	0.00±0.00	0.70±0.08 abcde	5.80±0.14abcd	0.41±0.01ab	14.00±2.83a	36.50±21.92	0.00±0.00a	8.90 x10^6bc	6.35 x10^3a
	3	0.00±0.00	0.57±0.04 ab	5.70±0.14abcd	0.39±0.12ab	16.00±4.24a	22.50±9.19	0.00±0.00a	5.81 x10^6ab	7.65 x10^3a
	4	975.00±1,378	0.43±0.10 a	5.55±0.35abcd	0.29±0.01a	16.00±11.31a	25.50±3.54	0.00±0.00a	5.96 x10^6ab	3.80 x10^3a
After corn rotation and 2nd biostimulant	1	0.00±0.00	0.61±0.20 abc	6.35±0.07bcde	0.37±0.08ab	6.00±0.00a	25.00±8.49	0.00±0.00a	5.69 x10^6ab	1.35 x10^3a
	2	0.00±0.00	1.34±0.03 e	6.15±0.21abcd	0.62±0.01b	16.00±9.90a	124.50±120.92	0.00±0.00a	1.27 x10^7c	7.15 x10^3a
	3	1,462.50±2.068	0.63±0.21 abcd	5.25±1.06ab	0.44±0.04ab	6.50±0.71a	23.50±14.85	0.00±0.01a	3.78 x10^6ab	7.70 x10^3a
	4	2,437.50±689	0.68±0.18 abcde	5.10±0.14a	0.40±0.06ab	6.00±1.41a	14.50±2.12	0.00±0.02a	4.00 x10^6ab	4.75 x10^3a

a, b, c, d, and e indicate significant (P<0.05) differences in columns (between fields).

when corn was sown and again when it was age 3 weeks could enhance soil fertility in terms of total N and the number of bacterial colonies found in the soil. Notably, the soil from field No.1 had the highest EC values (0.17–0.22 dS/m) before, during, and after using the sunn hemp as a green manure crop, while the soil in the corn plantation with the first biostimulant application had the significantly highest levels of organic matter (1.2%), total P (54.5mg/kg), and bacterial colonies (1.47 x10^7 CFU). This might have been caused by the sunn hemp acting beneficially as green manure to increase the amount of organic matter and biological activity, resulting in improved soil fertility. During the growth of the sunn hemp, the soil from field No. 4 had the significantly highest colony levels of bacteria (9.00 x10^6 CFU) and fungi (1.28 x10^5 CFU). This may have been related to the initial low soil levels of pH (5.4, Table 4), total N (0.3g/kg, Table 4), available P (6.4mg/kg, Table 2), and Mg (34.1mg/kg, Table 2), so that soil microbial colonization increased after growing the sunn hemp and the biostimulant application. From the results, it could be concluded that using sunn hemp alone could improve soil fertility in terms of increased organic matter, total P, and bacterial colonization in the soil of field No. 1, whereas using sunn hemp and biostimulant application could improve soil fertility in terms of increased organic matter, total N, and bacterial colonization in the soil of field No. 2. Thus, suitable applications of biostimulant should be included in the treatment, as in field No.2, with biostimulant additions during the growth of the sunn hemp and at age 3 weeks for the corn crop (Table 1). In addition, the herbicides used for corn production (atrazine and its degradates, 3,4-DCA and 4,5-DCC, and the historically used propanil in the field soil) were not detected in any of the 4 fields at any of the 5 different periods of soil collection and analysis. The herbicide used in the study could be degraded biotically and abiotically in the environment.

Soil sample collection dates: before planting sunn hemp was on 26 December 2022; during sunn hemp rotation was on 6 February 2023; after sunn hemp plantation was on 20 March 2023; during corn rotation and the first biostimulant application was on 1 May 2023; and during corn rotation and the second biostimulant was on 2 June 2023. a, b, c, d, and e indicate significant (p < 0.05) differences among the 5 periods studied.

Effect of Biostimulant on Dimensions and Germination of Animal Feed Corn

The biostimulant-treated corn seed was tested for seed germination. The seed dimensions and 10-seed weight of unsoaked seed and seed soaked for 30min and 17 h are shown in Fig. 3. The results showed that the corn seed could absorb the biostimulant solution and increase its width significantly after soaking for 30min (8.5 mm) and for soaking for 17 h (9.4 mm). The 10-seed weight significantly increased with the soaking time with values for unsoaked seed (3.1 g), seed soaked for 30min (3.5 g), and seed soaked for 17 h (4.1g), as shown in Fig. 3. These results indicated that soaking for 30min affected seed absorption of the biostimulant solution and increased the seed weight. Soaking for a longer time (17 h) with drying for 1 day before the dimension and weight measurement might have allowed the biostimulant to activate seedling activity, thus increasing the seed width and weight.

During seeding for 7 days, the unsoaked corn seeds were measured for root and shoot lengths and for seedling growth. Fig. 4 shows the root and shoot lengths of the unsoaked corn seed and for the seed soaked for 30min and 17h in the biostimulant solution. The root length of the unsoaked corn seed did not significantly increase whereas its shoot length significantly increased from 2.9cm for 4 days to 5.0cm for 6 days (Fig. 4). The root length of the seed soaked for 30min and of the seed soaked for 17h significantly increased for 4 days (3.5 and 9.1cm, respectively) compared to the unsoaked seed (1.6cm). These results confirmed that the biostimulant soaking time had a more immediate effect on seed root length, with the longer the soaking time, the longer the germinated root. This might have been due to the biostimulant containing PGPR-generating plant supporter, including plant hormone, which enhanced root emergence and elongation. The shoot length after soaking for 30min had significantly increased at 4 days compared to the unsoaked seed (2.9cm); however, the shoot length of the seed soaked for 17h had significantly increased at 4 days

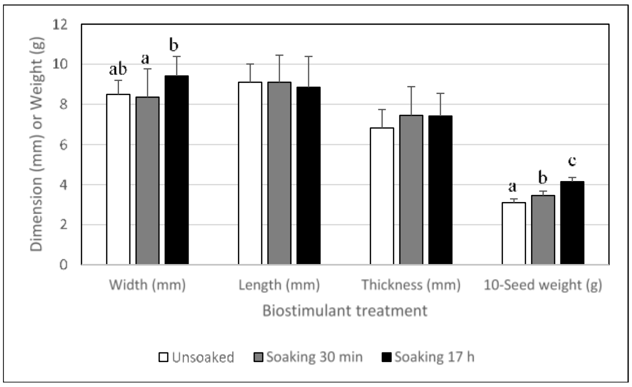

Fig. 3: Corn seed dimension and weight of corn seeds treated with biostimulant soaking, where a, b, and c indicate significant (P<0.05) differences among 3 soaking times for parameters and error bars indicate standard deviation.

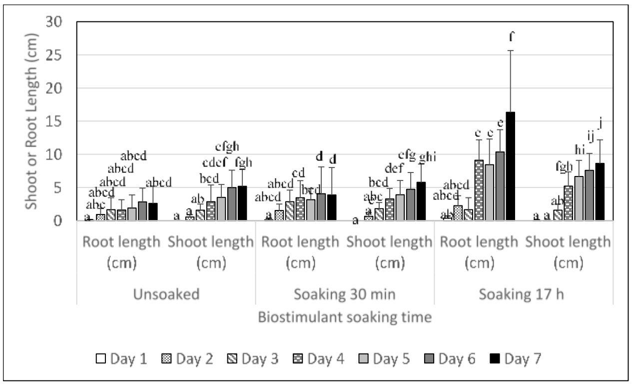

Fig. 4: Shoot and root length in corn seed germination test with 3 soaking times, where a, b, c, d, e, f, g, h, I, and j indicate significant (P<0.05) differences among shoot or root lengths of seeds from 3 soaking times and error bars indicate standard deviation.

(5.2cm) compared to the unsoaked seed and the seed soaked for 30min. Furthermore, the shoot length of the seed soaked for 17h had significantly increased at 4 days after germination and was the longest (8.6cm) at 7 days after germination. Thus, these results confirmed that the biostimulant shortened the period for seed germination and enhanced the subsequent root and shoot lengths of the corn seed. Soaking the corn seed for 30min should be applied prior to sowing and growing in the field rather than soaking for 17h, as the longer emerged roots could possibly be harmed and weakened by the sowing machine.

Productivity and Nutritive Value of Animal Feed Corn

Corn production, protection, and harvest were assessed in the experimental area at 100 days since sowing. Fig. 5 shows corn productivity and its nutrition status from plant production in the 4 fields. The results showed that for all 4 treatments, there were significant effects on plant height, number of leaves, pod length and width, pod with and without sheet weight, pod row number, seeds/row, 100-seed weight nor on the corn seed nutrition parameters of protein and fat percentages. However, the ear insertion height of the corn plants in fields No. 1 and No. 2 were significantly higher than field No. 4 (Fig. 5). The ear insertion height of the plant might have been affected by soil amendment with sunn hemp and the biostimulant treatment, which increased soil fertility for the corn plantation (Table 1 and 4). Sunn hemp biomass could maintain the EC in the soil of field No.1 at a high level (0.17–0.22 dS/m) during and after planting and amend the soil before the planted. After planting the corn, the soil contained high levels of organic matter (1.2%), total P (54.5mg/kg), and bacterial colonies (1.47 x10^7 CFU, Table 4). Applying biostimulant during the sunn hemp rotation and then again during the corn rotation once at age 3 weeks could improve the soil fertility through the high levels of organic matter (1.2%), total N (0.62g/kg), and bacterial colonies (1.27 x10^7 CFU, Table 4). Thus, the corn plantation had good growth and the highest corn ear insertion height in fields No. 1 and No. 2. The lowest pH in the soil in field No. 4 retarded organic matter (0.4%) and resulted in lower total N (0.3g/kg), which resulted in reduced soil fertility that might have affected the poorer corn production (Fig. 5).

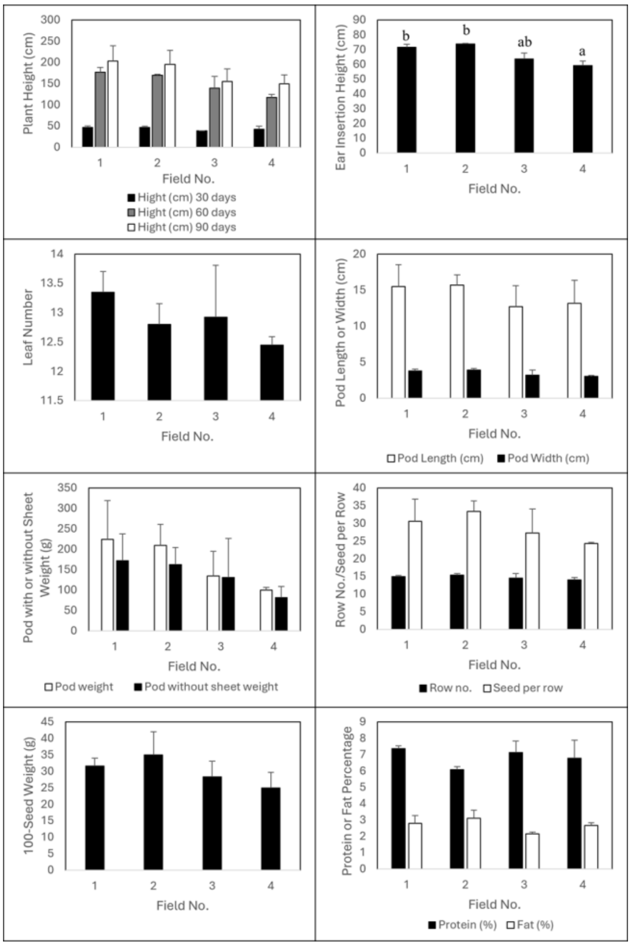

Fig. 5: Corn plant physiology in the field and corn nutrition (protein and fat percentage), where a and b indicate significant (p < 0.05) differences among 4 fields and error bars indicate standard deviation.

DISCUSSION

Field Soil Characteristics and Nutrient Availability Induced by Sunn Hemp and Biostimulant

Leguminous plants can be used as green manure, which increases the available organic matter in the soil, which in turn enhances soil fertility by amending the physical, chemical, and biological properties of the soil (Aroonluk et al., 2020; Khemtong et al., 2023). Nutrients from humus and organic matter improve the physical soil structure by increasing the water absorption capacity, and aggregation of the soil structure for water penetration. In addition, the nutrients improve soil chemical properties and increase the CEC, which induces the slow release of nutrients for plants and increases the soil buffer capacity. Nitrogen from sunn hemp (150 kg N/ha) increased Japonica rice biomass (3,763 kg/ha) more than from applying urea (2,725 kg/ha), resulting in high grain yields (900–1,167 kg/ha (Khemtong et al., 2023). *G. sepium*, leguminous leaf biomass after use as green manure for sweet corn growth at 8 weeks provided good soil properties with higher total N (0.083–0.106%), organic matter (1.66–2.12%), P (81–119 ppm), and Ca (522–866 ppm) compared to non-use (0.054%, 1.09%, 30 ppm, and 365 ppm, respectively) and intercropping (0.057%, 1.14%, 43 ppm, and 400 ppm, respectively (Aroonluk et al., 2020). Biologically, the increase in soil organic matter soil can increase microbial communities that affect soil activity by breaking down the inorganic forms that can then be used by plants, by fixing N from the air into the soil, by increasing the organic degradation rate and plant disease control, and by promoting plant growth with plant hormone (Woitke and Schnitzler, 2005; Office of Soil Biotechnology, 2016). The present study confirmed that using sunn hemp as green manure increased the amount of organic matter and nutrient P availability (Table 4). The application of the biostimulant supported and improved organic matter and nutrient N availability (Table 4). The use of the sunn hemp and biostimulant successfully colonized bacterial communities in the soil, possibly related to leguminous roots and direct PGPRs in the biostimulant, respectively. The results confirmed the potential of sunn hemp as green manure and the nitrogen-increasing ability of the biostimulant that was applied to the soil in field No.2. Thus, the corn planted in that field had the highest ear insertion height and tended to have greater growth compared to field No 4. The 4 periods of application of the biostimulant in the soil in field No.4 did not enhance corn production due to the unsuitable soil pH and the low nutrient N availability. Increasing the soil pH (>5.5) in field No.4 by adding lime (975–2,438 kg$CaCO_3$/ha) may make the soil more suitable for growing crops (Table 4). Animal-feed corn is usually grown in soils with a suitable soil pH in the range of 5.5–7.0 (Department of Livestock Development, 2020).

Biostimulant Promotion of Plant Growth

The biostimulant in the present study clearly activated corn seed germination (Fig. 3 and 4). The biostimulant included PGPRs, microbial-derived compounds, and phytohormone and plant-derived compounds (Thome et al., 2023). The PGPR mix, containing bacteria, yeast, mycorrhiza, and Trichoderma-beneficial species enhanced the leaf anatomy, photosynthetic activity, and plant growth of soybean in a hydroponically closed nutrient film technique (NFT). Paradiso et al., (2017) reported that PGPR root inoculation had positive effects by enhancing leaf photosynthesis, plant growth, and seed production of soybean grown hydroponically. Some PGPRs (*Pseudomonas* spp., *Bacillus* spp., and endomycorrhizal fungi) have been successfully tested in hydroponically grown vegetables (such as tomato, cucumber, and lettuce), with positive effects on plant growth, yield, and quality (Moncada et al., 2020). In the present study, the soil in fields No. 1 and No. 2 had suitable media for promoting growth as demonstrated by the high corn ear insertion height (Fig. 5). The corn production among the 4 fields clearly showed the effect of plant growth promotion, with soil fertility in fields No. 1 and No. 2 promoted through the higher levels of bacterial colonization (Table 4) and after biostimulant application which possibly was supportive of microbial colonization. Thus, the soil in field No. 2 had a significantly higher level of total N to support corn production. Soil fertility based on the measured physical, chemical, and biological properties strongly affected corn productivity (Table 4 and Fig. 5). The soil fertility as measured by microbial diversity may be linked to a variety of plant- and environmental-specific factors which may include differences in the original indigenous microbial population of each crop and in root exudate composition, as well as in environmental conditions, including bacterial mobility and chemo-attraction, adherence and anchoring capacity, temperature, plant developmental stage, and nutrient fertilization (Table 3) (Sheridan et al., 2017). Recently, Thome et al. (2023) reported the ability of biostimulants (in terms of phytohormone and nicotinamide) to increase maize growth and yield, where both biostimulants synergically affected the first ear insertion height and the number of rows per ear. Their results showed the same trend as reported in the present study.

Agricultural Management to Increase Corn Production, Productivity and Quality

The nutrient content in green manure that can be utilized as chemical fertilizer can be determined using the nutrient content (N, P, and K), which costs in the range of USD 95–185/ha (Office of Soil Biotechnology, 2016). Sunn hemp could be estimated the value of nutrient content to chemical fertilizer N, P, K as USD 157/ha (Office of Soil Biotechnology, 2016). The present study has revealed the potential use of crop rotation and biostimulant application during sunn hemp plantation, and during 3-week corn plantation (Fig. 5). Adding more biostimulant during week 6 in the corn rotation did not enhance corn production (Fig. 5). Corn seed soaking before sowing improved seed germination and increased the shoot and root lengths of the seed that germinated. However, soaking the seed for 17 h should not be used for growing in the field as the longer roots may damaged by the sowing machine and negatively impact crop production. The corn plants germinating from soaked seed may be expected to have a gain of 3 days over unsoaked seed (Fig. 4). The corn used in this study (303 variety) is usually harvested after growing for approximately 110–120 days. The present study accelerated the harvest to within 100 days. The total corn yield from the study area (1,520 kg) could be improved not only by pre-planting a sunn hemp and using biostimulant application, but also by good agricultural management, such as ensuring sufficient watering, sufficient fertilizer, and effective and timely pest and weed control produce a uniform plant crop for harvesting with a combine harvester machine. Yield losses could result due to insufficient watering and fertilizing, excessive pest damage and weed growth, resulting in heterogeneous, small corn pods that are not suited to mechanized harvesting. Soil used for growing animal feed corn should contain more than 1% organic matter, more than 10mg/kg P, and more than 60mg/kg K (Department of Livestock Development, 2020). Initially, before growing the sunn hemp crop, all 4 fields had low levels of organic matter (<1%) and low bacterial colonization (Table 4). Growing sunn hemp and using a biostimulant application enhanced the organic matter and nutrient availability in fields No. 1 and No. 2. Crop production management in fields No. 3 and No. 4 could benefit from adding sufficient P and K, with regard to corn plant consumption for growth and corn pod development. Seasonal and weather factors during corn harvest can affect corn yield quality. Excessive or regular precipitation increases the moisture content of the corn yield, so keeping it below 14% is necessary to reduce processing energy consumption and improve cost efficiencies in post-harvest management.

Conclusion

Recently, sustainable agriculture has focused on using natural-based technology through biodiversity management. Sunn hemp, a leguminous plant with nitrogen-fixing ability, is a promising low-cost green manure. Plant biostimulants are a new generation of available products that may be useful in successfully implementing agricultural sustainability policies. The present study showed that sunn hemp, considered solely as green manure, could increase the organic matter in the soil; furthermore, when incorporated with biostimulant application, the organic matter and total N in the soil were increased. The biostimulant solution clearly increased seed width and seed weight following soaking for either 30min or 17 h. The biostimulant promoted seed germination by increasing root and shoot lengths after soaking for 17 h. The application of the biostimulant in the 4 different plots of the sunn hemp and corn crops resulted in fields No. 1 and No. 2 (with biostimulant) being able to support the growth of the corn crop, with the highest corn ear insertion height compared to fields No. 3 and No. 4. It can be concluded that sunn hemp is a promising green manure and a potential source of organic matter. In addition, the biostimulant used in the present study could act synergistically with the field soil (field No.2), thus increasing total N, an essential macronutrient for corn production. Finally, the recommendation biostimulant could be substituted for chemical fertilizer, during the sunn hemp rotation and at age 3 weeks in the corn rotation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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