Effect of Soil Amended with Thin Stillage on Growth of Canola and Soil Properties in a Controlled Environment Study

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ABSTRACT

A growth chamber study was conducted to evaluate the potential use of thin stillage as a soil fertilizer amendment. Seven treatments; control, 3 thin stillage rates (100, 200, and 400 kg N ha^{-1}) and 3 urea rates (100, 200, and 400 kg N ha^{-1}) were used in a replicated growth chamber study conducted in the spring of 2008 at the University of Saskatchewan. Canola (*Brassica napus* L.) was grown on a nitrogen deficient Brown Chernozemic soil (Haverhill Association). Dry matter yield and nutrient concentrations in plant tissues were determined after 34 days of growth. Significant increases in canola dry matter yield and nutrient content were observed due to thin stillage application. The thin stillage had a beneficial effect on plant growth by enhancing plant biomass production and nutrient uptake. Applications up to 400 kg N ha^{-1} did not have a negative effect on canola biomass production. Land application of the residual thin stillage from ethanol production, may be an effective method to recycle nutrients to improve and maintain soil productivity, and increase plant nutrient availability and plant growth.

INTRODUCTION

During ethanol production, starch is converted to ethanol and the other constituents of the grain become co-products. Thin stillage, a liquid by-product of fermentation and distillation processes in ethanol production is one these co-products. Thin stillage can be used a nutrient- containing water source for livestock, but may have other potential uses as well. At present, there is a limited amount of information as to how thin stillage applied to soil might affect soil organic matter, nutrient availability, and biological activity. Ethanol production from sugarcane generates a co-product called vinasse. Vinasse has high levels of organic matter, potassium, sulfur and micronutrient content. Sugarcane production in Brazil and Australia generate large amounts of organic wastes including vinasse, and the vinasse has been land-applied as a source of plant nutrients over the past two decades (Benke 1998). The residual liquid (soluble) thin stillage fraction after distillation and separation contains the vitamins, minerals, fatty acids, fiber, yeast cells, enzymes, unfermented sugars, soluble amino acids, organic acids, nutrients, and proteins of the parent grain which are concentrated by removal of starch (Erickson and Klopfenstein 2005; Lardy 2003).

Thin stillage may have up to 7 per cent organic matter in the form of suspended and dissolved solids. Organic acids such as oxalate, citrate, and malate may also be present and assist in the solubilization of phosphorus and micronutrients, which improves their bioavailability and plant uptake (Vance et al. 2003; Fox 1995; Marschner 1995). Certain organic acids may be more efficient in limiting the adsorption of newly added phosphate than in desorbing naturally available soil phosphorus and play an important role in improving the availability of soil phosphate (Pypers et al. 2005; Jones et al. 2003). Thin stillage has high levels of potassium, calcium and organic matter in its chemical composition as well as moderate amounts of nitrogen, sulfur, phosphorus and micronutrients (Table 1) and could represent an alternative to chemical fertilizers to supply nutrients for plant growth and development (Sweeny and Graetz 1991). Organic compounds in the thin stillage are expected to be mineralized rapidly in the soil to release plant available inorganic nutrients and significantly increase the number of microbes in the soil and microbial activity. Such compounds include amino acids, organic acids, and sugars.

There is a need to consider all potential uses of thin stillage from ethanol production, with the aim towards recycling nutrients in the co-product. To date, no detailed studies have been conducted on the use of thin stillage as a potential soil amendment in western Canadian soils and how it affects plant growth and nutrient concentration. The objective of this study was to determine the effect of thin stillage application on the growth of canola and soil physical and chemical properties of a Brown Chernozemic soil under controlled environmental conditions.

MATERIALS AND METHODS

Thin Stillage

Thin stillage samples were obtained from Pound-Maker Agventures ethanol plant (Lanigan, SK) and stored at 4°C before analysis. This thin stillage was analyzed for chemical properties by ALS Laboratory Group (Saskatoon, SK) using the methods outlined. A list of these properties and their concentration is presented in Table 1. The thin stillage contains about 50 lbs of total N/1000 gallons, similar to some liquid swine manure slurries. About 20% of the Total-N is comprised of immediately plant available ammonium. The Total-N to Total-P ratio is about 5:1, being wider than many manure sources (Schoenau and Davis 2006). . The N/S ratio is about 5:1, in line with relative requirements of canola for N and S (Grant and Bailey 1993). The thin stillage also contains many micro-nutrients necessary for plant growth and development. The thin stillage has an acidic pH of 3.8, which is likely due to the presence of organic acids. Thin stillage has very high water content (92%) and includes water soluble materials including protein, unconverted starch and sugars.

Table 1: Selected Physical and Chemical Characteristics of Thin Stillage†

Analysis	Result	Units	Analytical Method
NH4-N	9	lb/1000gal	SSSA (1996) 1133-1134
Total-N	47	lb/1000gal	CSSS (1993) 22.2-Titration
P	9	lb/1000gal	SSSA (1996) P.931
K	11	lb/1000gal	SSSA (1996) P.931
S	6	lb/1000gal	SSSA (1996) P.931
Na	4	lb/1000gal	SSSA (1996) P.931
Ca	2	lb/1000gal	SSSA (1996) P.931
Mg	4	lb/1000gal	SSSA (1996) P.931
Cu	<0.01	lb/1000gal	SSSA (1996) P.931
Fe	0.06	lb/1000gal	SSSA (1996) P.931
Mn	0.04	lb/1000gal	SSSA (1996) P.931
Zn	0.07	lb/1000gal	SSSA (1996) P.931
Total Solids	7.5	%	APHA 2540
% Moisture	92.5	%	
pH	3.8	pH	APHA 4500, 2510
EC	5160	$\mu S\ cm^{-1}$	APHA 4500, 2510

† Adapted from ALS Laboratory Group Analytical Report

Growth Chamber Study

For the growth chamber study, soil from the surface (0-15 cm) was collected from a wheat stubble field (Brown Chernozemic soil, Haverhill Association) near Central Butte, SK. The thin stillage used in the growth chamber study was collected and stored at 4°C. Three thin stillage application rates were used in the growth chamber study; 10.64, 21.28, and 42.55 g/pot, equivalent to 0, 200, 400 kg N ha^{-1} respectively (0, 50, 100, and 200 µg N g $soil^{-1}$). Three urea rates 0.108, 0.216, and 0.432 g/pot added to deionized water were used in the growth chamber study, equivalent to 0, 200, and 400 kg N ha^{-1} respectively. In each pot, 800 g of air-dried soil were weighed into a pot. The TS or urea were then spread on the surface of the soil, followed by addition of 200ml deionized water. An additional 100 g of soil was then added to cover the products and the soil was equilibrated for 4 days. Then 10 canola seeds were placed on the soil surface in each pot and an additional 100ml of water was added. The last 100g soil was added on the soil surface to bring the total soil weight to 1000 g for each pot. Soil moisture was kept at approximately 80% of field capacity in all the treatments. Each treatment was replicated four times in standard 15- cm pots with 1000 g of soil and kept in a growth chamber. Temperatures in the growth chamber were maintained at 22°C day and 13°C night, with an 18-h day length and 6-h night length.

Canola (*B. napus* cv. Invigor 5020) was used as the crop in this study. The experiment was designed using a randomized complete block design. Treatments were replicated four times in the growth chamber and were watered every second to third day with deionized water to field capacity. Pots were rearranged after each watering to minimize differential effects of temperature and lighting within the growth chamber. Plants were grown for 34 d, harvested from each pot, dried at 40°C for 4 d to a constant weight and weighed to determine dry matter yield.

Soil Chemical Analysis

Soil samples from each pot were collected for analysis at the end of the experiment after the canola plants were harvested. The soil samples were air -dried and ground using a flail-type grinder to pass through a 2 mm sieve. Ground samples were then analyzed for 2 M KCL extractable NO_3-N and NH_4-N, Modified Kelowna (MK) extractable P and K, 0.01 M $CaCl_2$ extractable SO_4, DTPA extractable Cu, Cd, and Zn. All soil samples were analyzed for Total-N and -P by digest, organic carbon by automated combustion, and electrical conductivity (EC) and pH using a 1:2 soil : water extraction.

Plant Tissue Analysis

Aboveground plant biomass samples were collected and analyzed to determine the effect of thin stillage application on plant nutrient concentration. Plants were harvested after 34 d, and whole dried plant samples were collected and used to determine concentration of N, P, K, S, and the trace metals Cd and Zn via sulfuric acid-peroxide digest, followed by colorimetric or spectroscopic measurement in the digest. Plant element concentrations are shown in Table 2.

Table 2: Plant Tissue Nutrient Concentration, Plant Uptake, and Canola Biomass Yield 34d after Thin Stillage and Urea Application

Treatment kg N ha^{-1}	Dry Wt g/pot	Total-N	Total-P	Total-K μg/g	Total-S	Total-Zn	Total-Cd
Control	0.59	10859	3258	15553	5373	37	694
100 TS	1.84	8724	2419	13723	2910	24	243
200 TS	3.11	8099	2234	17235	2450	28	161
400 TS	4.97	9351	2210	21780	4483	21	176
100 Urea	2.22	7822	1344	13730	578	36	179
200 Urea	3.11	11609	831	13715	921	39	197
400 Urea	2.63	25208	922	13691	370	34	283
0.10 LSD	0.38	1576	193	1494	2162.6	12.8	88

Plant nutrient uptake was calculated by multiplying the yield by the concentration in the tissue and is expressed as milligrams of nutrient taken up per kilogram of soil. The results of plant nutrient uptake are shown in Table 3.

Statistical Analysis

Thin stillage amendment or urea fertilizer treatments were replicated four times within a randomized complete block design. Data on total biomass in the growth chamber study were subjected to analysis of variance using the General Linear Model (GLM) procedure of SAS software (SAS Inst., 1999). Variables that were analyzed included dry matter yield, NO_3-N, NH_4-N, TN, P, TP, S, K, TK, pH, EC, %OC, Cu, Cd, and Zn. Mean separation statistical comparisons were conducted using least significant difference (LSD) at $P \leq 0.10$ for comparing results between treatment means for both soil and plant variables.

RESULTS AND DISCUSSION

1. Growth Chamber Canola Yield and Plant Nutrient Uptake

Data from the growth chamber study indicate that thin stillage treatments significantly affected canola dry matter production (Table 3), and that plant growth was greatly increased when thin stillage was applied. The dry matter weight (canola biomass) showed a positive response to increasing rates of thin stillage and urea. All thin stillage treatments applied to the soil increased canola dry matter production significantly compared to the unfertilized, unamended control. At equivalent rates of applied N, the biomass yield of canola on thin stillage was similar to urea, especially at the low and medium application rates. The similar yields, N concentration and uptake in canola when equivalent rates of total N as thin stillage and urea were added indicate high availability of N contained in thin stillage, with greater than one half of the N added becoming available for uptake over the five week period. Even when plants were grown in pots treated with the equivalent of 400 kg N ha^{-1}, significant increases in canola dry matter yield were observed with the TS. The highest yield was obtained where thin stillage at 400 kg N ha^{-1} was applied. Some toxic effects of the high rate of urea were evident, with a lower yield at 400 kg N ha^{-1} compared to 200 kg N ha^{-1} of urea. This appears to be at least partly related to a nitrogen induced sulfur deficiency in the urea only treatments, as the sulfur concentrations in the plant ranged from 0.25%S to 0.45% S in the thin stillage treatments, while the S concentrations in the urea treatments were ten times lower: only 0.038%S to 0.058%S.

The high application rates (400 kg N ha^{-1}) of thin stillage did not have a harmful effect on crop growth, and the thin stillage appears to be an effective source of both nitrogen and sulfur, as suggested by its analysis. The increase in N uptake results from increasing N soil N availability and is evident in plant tissue samples having increased N concentration. Significantly higher plant P tissue concentration and P uptake in thin stillage compared to urea amended soil indicates that the thin stillage is an effective P source. Thin stillage application increased the K concentration and uptake in canola. Total Zn concentration and uptake in aboveground biomass was lower in thin stillage treatments when compared to urea. Although the thin stillage adds some Zn, the Zn uptake by the plant is reduced at the low rate. This is possibly related to the high P added, as high soil P can induce Zn deficiency. Total plant Cd concentration (data not shown) was highest in control and was reduced in thin stillage and urea applications, likely as a result of

Table 3: Properties of Canola Plants Grown with Thin Stillage (TS) and Urea Amended Soil

Treatment kg N ha⁻¹	Biomass g/pot	N-Uptake	P-Uptake	K-Uptake mg/kg	S-Uptake	Zn-Uptake μg/kg	Cd-Uptake
Control	0.59f	6.4f	1.92f	9.2f	3.17b	21.6c	409d
100 TS	1.84c	16.0c	4.43c	25.2c	5.34b	43.2c	446d
200 TS	3.11b	25.2d	6.94b	53.5b	7.64b	87.6ab	501cd
400 TS	4.97a	46.5b	10.9a	108.4a	22.29a	101.8ab	876a
100 Urea	2.22d	17.4e	2.98d	30.4de	1.28b	79.8b	397d
200 Urea	3.11b	36.1c	2.58de	42.7c	2.87b	119.8a	614c
400 Urea	2.63c	66.2a	2.42e	35.9d	0.97b	90.5ab	743b

Numbers in a column followed by the same letter are not significantly different at P<0.10

growth dilution in aboveground plant biomass. However, total uptake as calculated from the yield and Cd concentration, increased with addition of TS or urea as a result of growth response to amendment. The thin stillage does not appear to increase uptake potential of these metals in canola plant tissue compared to chemical fertilizer.

2. Residual Soil Nutrients

Similar to alfalfa powder and the DDG and WDG amendments, thin stillage added N, P, S, K, Ca, Mg, Fe, Na, Mn, Zn, Cu, and other elements, with potential to supplement plant growth if these elements are deficient in the soil. The soil used in these studies had low N availability and low P availability. After five weeks of canola growth, concentrations of soil Total-N were higher in the treatments that received the high rate of thin stillage or urea (Table 4).

Concentrations of Total-P were also increased slightly. The soil NO_3-N, and NH_4-N were all low with the exception of the high urea treatment, where residual nitrate levels were much higher, indicating residual urea N left at the end of the experiment. Extractable available P, K and S levels were slightly higher in the thin stillage treatments than the urea treatment, reflecting the contribution of the thin stillage to the available pools of these nutrients in the soil. The application of thin stillage enhanced canola P uptake and higher concentration of P in plant biomass compared to urea. The increase in plant available P and canola P uptake in the growth chamber is likely due to organic P mineralization and a reduction in P fixation in the soil. The concentrations of sulfate in the soil at the end of the experiment and especially in the plant tissue were increased by the thin stillage application. Although yield responses to S tend to be inconsistent, they can be significant in soils where S is limiting (Grant et al. 2004). Increased concentration of S in plant tissue could be attributed to the mineralization of organic S and oxidation of reduced S to plant available form. Soil organic carbon was slightly but significantly increased over the unamended control by application of thin stillage, suggesting that some of the organic carbon added as thin stillage is sequestered in the soil. However, the thin stillage organic matter content is low and is expected to decompose quickly (Gale et al. 2006).

In this study, there were no significant differences among treatments in soil residual zinc concentration. Studies have shown that the use of N fertilizer can increase plant Cd uptake (Mitchell et al. 2000), as was shown in the higher Cd concentration in plant tissue in urea treatments compared to thin stillage application. Residual soil extractable Cd concentrations were all low and generally not affected by treatment. It appears that the thin stillage does not appreciably alter the soil chemical or physical environment such that any increase in solubility and availability of indigenous heavy metals would occur. All treatments had similar soil pH values that were not significantly different from the control or the value for the soil at the start of the experiment. A pH of 8.0 suggests that this soil may have free $CaCO_3$ and has a higher buffering capacity against pH change. High pH can decrease the uptake of Cu, Fe, Mn, Zn and reduce the amount of exchangeable Al. Only the high rate of urea produced a small but significant increase in EC. In this study, after one application, the thin stillage does not appear to increase salinity in soil.

Table 4: Soil properties in thin stillage and urea amendment trial prior to application and after canola harvest

Treatment	NO3-N	NH4-N	P	K	SO4-S	Total-N	Total-P	pH	EC	OC	Cu	Zn	Cd
kg N/ha			µg/g			mg/g			mS/cm	%		µg/g	
Before amendment and seeding	2.4	6.7	4.2	587	12	1.17	0.5	8	0.3	1.9	0.5	4.3	0.05
After Harvest													
Control	2.2b	6.4ab	4.2c	594a	9.5bc	1.19b	0.46b	8.0a	0.25b	1.92b	0.47a	2.83a	0.057c
100 TS	2.1b	6.3ab	4.3c	605a	10.5ab	1.23a	0.49ab	8.0a	0.26b	1.95ab	0.39b	2.90a	0.067b
200 TS	2.0b	5.0cd	4.8b	599a	11.3a	1.22ab	0.44bc	8.0a	0.25b	1.99a	0.39b	2.97a	0.066b
400 TS	2.6b	6.2ab	5.9a	595a	10.9ab	1.26a	0.51a	8.0a	0.25b	1.96ab	0.49a	2.79a	0.057c
100 Urea	2.3b	5.5c	3.5d	586b	10.0ab	1.19b	0.43bc	8.1a	0.23c	1.91b	0.40b	2.98a	0.073a
200 Urea	2.5b	4.8d	3.7d	570bc	9.3c	1.21ab	0.41c	8.1a	0.22c	1.94ab	0.41b	2.95a	0.057c
400 Urea	20.6a	6.8ab	3.7d	566c	8.4c	1.26a	0.51a	8.0a	0.32a	1.90b	0.45ab	2.94a	0.056c

Numbers in a column followed by the same letter are not significantly different at P<0.10

CONCLUSION

In this study, canola had nearly equivalent dry matter yield response to the same rate of nitrogen added as thin stillage compared to urea at lower rates, and better response at higher rates. Availability of N in thin stillage appeared to be at least 50% of that in urea, suggesting that organic N compounds in thin stillage are rapidly mineralized in the soil. Canola took up more P, K and S when thin stillage was applied compared to canola grown with urea added. There were no significant impacts on metal uptake or residual metal availability in the soil, indicating that concerns about addition of trace metals or solubilization associated with thin stillage application are minimal. The results of this study demonstrate the potential of using thin stillage as an effective source of nutrients for plant growth, and improved management of soil nutrients. Like liquid swine manure, the amounts of organic carbon added directly to the soil are low compared to solid amendments. Still, there was a trend towards increased soil organic carbon in thin stillage amended soil. Also, like liquid swine manure, thin stillage has a low concentration of nutrients by volume when compared to chemical fertilizers, and application rates of one or two thousand gallons per acre may be required to provide crop N requirements (agronomic rates).

More detailed research is needed to better understand the effects of thin stillage use on a range of Saskatchewan soil types and crops, and its effects on available nutrients, especially any potential benefits in solubilization of P through the action of the organic acids contained within the thin stillage, and stimulation of microbial activity.

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