



Tillage system and organic mulch influence leaf biomass, steviol glycoside yield and soil health under sub-temperate conditions



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ABSTRACT

Tillage system and organic mulch play important role to increase plant growth, soil organic carbon (SOC) and soil nitrogen (N) in sustainable agricultural production system. However, the short term (≤ 5 years) effects of tillage system and organic mulch on crop yield, SOC and N dynamics are often complex and inconsistent. Moreover, the effects of tillage system and levels of organic mulch on stevia (*Stevia rebaudiana*) are not documented, particularly in the western Himalayan region. Thus a field experiment was conducted with eight treatment combinations comprising two tillage practices (zero and conventional) and four levels of pine-needle mulch (no-mulch, 5 t ha^{-1} , 10 t ha^{-1} and 15 t ha^{-1}). The conventional tillage registered about 22 and 32% higher dry leaf yield compared with zero tillage practice in 2012 and 2013, respectively; however, remained statistically at par ($P \geq 0.05$) with zero tillage in 2014. The stevioside, Reb-A, and total steviol glycosides accumulations in leaf were not affected by the tillage system. The significantly ($P \leq 0.05$) higher available K was recorded with zero tilled soil. The effects of mulch were more pronounced, and the maximum leaf yield was recorded with the mulching at 15 t ha^{-1} but remained at par ($P \geq 0.05$) with the application of 10 t ha^{-1} . Moreover, available soil N and SOC were also higher with the mulching at 10 and 15 t ha^{-1} , respectively. Hence, the zero tillage is not effective for increasing crop productivity in short term; nevertheless, higher yield, SOC, and soil nitrogen can be achieved by the application of optimum level of organic mulch.

1. Introduction

The Incidence of type 2 diabetes is a major global health problem, and the prevalence of this disease in adults is higher in upper-middle and middle-income countries (Scully, 2012). India had 62.4 million people with type 2 diabetes in 2011, compared with 50.8 million in 2010 (Shetty, 2012). The main cause of increasing diabetes patient is more consumption of sugar-rich food. Thus, there is a pressing need to find out the low caloric natural sweeteners as sugar substitutes. Stevia (*Stevia rebaudiana* Bertoni), a perennial herb of Asteraceae family, is widely grown for its sweet-tasting and low-calorie diterpenoid steviol glycosides (SGs) content in its leaves. Thus, there is an immense scope for using the SGs in food products as a sugar substitute. Now, the global stevia market is rapidly increasing. In 2014, the global stevia consumption as food ingredient, was estimated at 5,100.6 t, and it is projected to reach 8,506.9 t by 2020 (Persistence Market Research, 2015). However, the leaf yield and concentration of SGs in leaf depend upon the growing conditions and agronomic practices (Tavarini and Angelini, 2013; Pal et al., 2013, 2015a,b).

The actual agricultural systems are concurrently damaging land, water, biodiversity, and climate on global scale (Foley et al., 2011). Thus, the main challenges in the agricultural sector are to feed burgeoning world population with reduced external inputs and minimal environmental impacts (Lobell et al., 2008; Foley et al., 2011; Tilman et al., 2011; Godfray and Garnett, 2014). However, raising level of atmospheric greenhouse gases (GHGs) threatens agricultural production's stability and productivity. Globally, agricultural activities contribute about 15% to the annual emission of CO_2 , N_2O , and CH_4 (FAO, 2008). It has also been reported that most of the agricultural lands in United States have lost 30–75% of their ancestor soil organic carbon (SOC) pool, which is about $30\text{--}40 \text{ t C ha}^{-1}$ (Lal et al., 2007).

Some agronomic management practices viz., method of tillage, residues management, crop rotation, and organic mulching, determine the quantity of OC retention in the soil (West and Post, 2002; Allmaras et al., 2004; Fuentes et al., 2009). Thus, conservation agriculture (CA) has been promoted in different parts of the world as an agricultural practice for soil SOC sequestration and reduction of greenhouse gases (Lal, 2004). The minimum soil disturbance, maintaining permanent soil

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cover, and diversifying of crop species are the main principles of CA. However, it is not possible to maintain permanent soil cover with crop residues in mono-cropping and hilly regions. The soil erosion in these regions is one of the major problems. In these situations, application of organic mulch from other sources and zero tillage practices may be contemplated as the means of addressing this issue. Zero or minimum tillage, as components of CA, are widely suggested to protect soil against erosion and degradation of structure (Petersen et al., 2011), increase soil organic matter, enhance sequestration of carbon (Six et al., 2000; West and Post 2002; Mangalassery et al., 2014), reduce GHG emissions (Mangalassery et al., 2014), improve biological activities (McCarthy et al., 1995; Helgason et al., 2010; Mangalassery et al., 2014), and increase total nitrogen in various soils (McCarty et al., 1995). Moreover, zero tillage has also been reported to reduce input costs for labour, fuel, tractors, and other equipment (Raper et al., 1994).

Mulching, another important agronomic practice, has direct influences on hydro-thermal regime of soils by moderating soil temperature, reducing soil water evaporation, suppressing the weeds by smothering action (Yoo-Jeong et al., 2003; Arora et al., 2011), improving soil fertility and modifying the soil physical environment (Yoo-Jeong et al., 2003), and improving nutrient cycling and organic matter build up over a period of several years (Holland, 2004). However, the effects of tillage method and application of crop residues on retention of SOC depend upon the types of soil, climatic condition, and cropping systems (Lal et al., 2007; Zhu et al., 2014). Thus, the effectiveness of zero/reduced tillage and mulching in terms of yield and SOC retention should be studied lucidly for different cropping systems and agro-climatic conditions. The effects of zero tillage and mulching on yield and soil health in stevia (*Stevia rebaudiana*)-fallow cropping system have not been studied in details under the western Himalayan region (sub-temperate condition).

In the lower Himalayan region, the pine (*Pinus roxburghii*) is the large evergreen conifer and a principal species, which makes a carpet of dry needles on the forest floor under these trees during spring season. This huge biomass is generally burned. Nevertheless, there is an immense scope to utilize this natural resource in a form of mulch. Thus, the objectives of this study were to investigate the impact of tillage system and pine-needles mulching on growth, yield and secondary metabolites of stevia, SOC retention and available nutrient status in soil under sub-temperate conditions of the western Himalayan region.

2. Materials and methods

2.1. Experimental site, weather and soil characteristics

The study was conducted at CSIR-IHBT's experimental farm, situated in the sub-temperate condition of the western Himalayan region in India (32° 6' 47" N and 76° 33' 46" E). The altitude of the site is 1391 m from the mean sea-level. The station has an average annual temperature of 18 °C with 250 cm mean annual rainfall. The meteorological data (viz., maximum and minimum temperature, relative humidity, sunshine hours, and rainfall) during the experimental period is detailed in Fig. 1. The soil of experimental plots was silty clay in texture. Clay, silt and sand contents in the top 0–15 cm soil layer are 48.55, 41.15 and 10.30%, respectively. At the beginning of the experiment, soil pH was 6.11 (1:2.5), and organic carbon was 1.14%. Available nitrogen (N), phosphorus (P) and potassium (K) contents in the experimental soil were 235.5, 48.2 and 369.9 kg h⁻¹, respectively.

2.2. Plant material, treatments and crop management

The experiment was set up in 2012 and continued for three cropping seasons (2012–2014) with 24 plots. Eight treatment combinations comprising two levels of tillage (T₀ = zero tillage and T_C = conventional tillage) and four levels of organic mulch (M₀ = no

mulch as control, M₁ = dry pine-needle at 5 t ha⁻¹, M₂ = dry pine-needle at 10 t ha⁻¹ and M₃ = dry pine-needle at 15 t ha⁻¹) were evaluated. The chemically characterization of mulching material was done in terms of N, P and K content. The average N, P, and K content in the dry mulch material were 21.2, 3.17, and 8.73 mg g⁻¹, respectively. The treatments are not been changed till the end of the experiment. The split-plot design was used with three replications. The main plot factor was tillage, and four mulch levels were randomly allotted in sub-plots for each tillage treatment. In case of zero tillage treatment, soils were not tilled across the entire cropping seasons, and the plants were transplanted directly. Sixty-five to seventy-days-old stevia seedlings were transplanted at 2nd fortnight of March for all the cropping seasons. The square shape planting geometry was adopted with a spacing of 45 cm × 45 cm. The mulch was applied at 15 days after transplanting (DAT). The crop was fertilized with N, P₂O₅ and K₂O at the rate of 90, 60 and 50 kg ha⁻¹, respectively. Urea, single super phosphate and muriate of potash were used as sources of N, P and K, respectively. A half dose of N and full doses of P and K were applied at pre-planting, whereas the remaining half quantity of N was applied into two equal doses at 30 and 60th DAT as top dressing. In case of zero tillage treatment, fertilizers were mixed up with soil manually up to 5 cm depth with the help of small implement.

2.3. Growth and yield data

For growth data, five plants were randomly selected from each plot and uprooted from 0 to 25 cm soil layer. After separation of leaves from stems, leaf area of the plants under respective treatments was measured by a leaf-area meter (AM 300, ADC Bio-scientific Ltd., UK). Finally, the leaf area was converted into the leaf area index (LAI). Plant height and number of branches (primary and secondary) per plant of each treatment were also recorded. After recording the fresh weight of leaves, stems and roots, the samples were dried as representative samples at 70 ± 2 °C in a hot air oven until a constant weight was attained to calculate the percentage of dry matter (DM) accumulation. For estimation of leaf and stem yield, five another plants were harvested from the central portion of each plot at ground level. The leaves were separated from the stems, and fresh weight was recorded separately. Then the dry leaf and stem yield were calculated for each treatment by multiplying of respective factors, which were calculated from previous samples.

2.4. Determination of NPK in leaf and soil analysis

Spatial variations of N, P and K uptake under different treatments were investigated. Dry leaf samples of individual treatments were prepared with a grinder having a sieve spacing of 0.7 mm. For N estimation, concentrated H₂SO₄ and a catalyst mixture of potassium sulphate and copper sulphate (10:1) were used for digestion, whereas a mixture of concentrated H₂SO₄ and perchloric acid (5:1) was used for estimation of P and K. After that Kel Plus automatic nitrogen analyzer system, spectrophotometer (model T 90 + UV/vis, PG Instrument Ltd.) and flame photometer (model BWB XP, BWB technologies UK Ltd., UK) were used for the estimation of total N, P and K, respectively, as per standard procedure (Prasad et al., 2006).

After completion of three years of stevia-fallow cropping system, soil samples were collected from the surface layer (0–15 cm) to study the effects of different treatments on pH, organic carbon (OC) and nutritional (N, P and K) status of the soil. The pH of soil water suspension (1:2 w/v) was measured with the help of pH meter (model Eutech Instruments pH 510), whereas the soil OC was estimated as per standard dichromate oxidation method (Nelson and Sommers, 1982). A Kel Plus automatic nitrogen analyzer unit having automatic dilution and addition of boric acid, NaOH and KMnO₄ were used for estimation of available nitrogen. Available phosphorus (AP) was estimated by Bray and Kurtz P1 (Bray and Kurtz, 1945) method as the soil was acidic in

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