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Effect of plant spacing and organic mulch on growth, yield and quality of natural sweetener plant Stevia and soil fertility in western Himalayas

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Abstract

The use of leaf mulch as a soil cover is effective in improving yield and soil fertility. The field experiment was conducted during 2010 and 2011 to study the effect of plant spacing (30 cm × 30 cm and 45 cm × 30 cm) and four mulches {pine needles (*Pinus roxburghii*), poplar leaf (*Populus deltoides*), silver oak (*Grevillea robusta*) tree leaf mulch and unmulched control} on growth, yield, quality of stevia and soil fertility. Weed count and dry weed weight was not affected by spacing levels during August and at the time of harvest, whereas, in unmulched plots it was significantly higher than mulched plots. Dry leaf yield, total dry biomass and leaf area index (LAI) were significantly higher in 30 cm × 30 cm spacing level and poplar leaf mulch. All the mulched plots significantly increased organic carbon (OC), available nitrogen (N), phosphorus (P) and potassium (K), bacterial and fungal population compared to unmulched plots. Rebaudioside-A content was higher in plots mulched with poplar leaves. Steviol glycosides were not significantly affected by different treatments. Soil biological activities were also enhanced by tree leaf mulches. Leaf mulch enhanced microbial biomass, relative to non-mulched soils, likely via improving C and water availability for soil microbes.

Keywords: Stevia; Organic mulch; Spacing; Growth; Yield; Steviol glycoside.

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a low calorie natural sweetener herb grown as a crop in many countries including Japan, China, India, Korea, USA, Canada, Mexico, Russia, Indonesia, Tanzania, Brazil, Paraguay, Canada and Argentina (Ramesh et al., 2006). The leaves of stevia have commercial importance due to the presence of non-caloric diterpenes and sweet glycosides, especially stevioside and rebaudioside - A which are ~300 times sweeter than sugar without any side-effects.The sweetening property of stevia and its extracts steviol glycoside is well renowned in humans (Kim et al., 2002; Mizutani and Tanaka, 2002; Geuns, 2004). Steviol glycoside content in stevia leaves greatly depends on the package of practices for cultivation of stevia (Kumar et al., 2012a; Kumar et al., 2013). Stevia is safe for use by both diabetics and hypoglycaemics (Singh and Rao, 2005) due to its low glycaemic index. Food Standards Australia and New Zealand (FSANZ, 2008) have approved the use of steviol glycosides as a food additive, while in the USA, the FDA has raised no objection regarding companies' self-affirmation of GRAS approval for rebaudioside - A, one of the Steviol glycosides. Stevia sweetener extracts have beneficial effect on human health including anti-hypertensive (Chan et al., 2000; Lee et al., 2001), anti-hyperglycemic (Jeppesen et al., 2000), anti-carcinogenic (Das et al., 1992; Brahmachari et al., 2011) and anti-human rotavirus activities (Takahashi et al., 2001).

Stevia can be grown as annual or perennial crop in temperate and tropical regions respectively. Since stevia crop is planted in the month of March under sub-temperate climate, it is heavily infested with weeds during rainy season (July-September) due to initial slow growth of the crop and wider row spaces (60 cm), which can minimize leaf yield. Kumar et al., (2012b), have reported that annual crop of stevia under mid hill conditions of North western Himalayas recorded significantly higher leaf dry biomass at a spacing of 45 cm × 10 cm. Narrow row spacing may also increase the competitive ability of a crop. Decreasing row spacing may also limit the period of time that weeds can compete with crops. The competitive advantage of narrow rows may also contribute to increased crop yield. Therefore, it seems that plant spacing could be used as a management tool for maximizing crop growth and yield.

Mulching is one of the important agronomic practice beneficial in conserving the soil moisture, suppressing the weeds, improving soil fertility (when organic mulch is used) and modifying the soil physical environment (Yoo-Jeong et al., 2003). Different types of mulches have been used to obtain good crop growth and yield in crops like banana (Ssali et al., 2003); vegetables (Richard et al., 2002); tomato (Rahman et al., 2006), pepper (Aiyelaagbe and Fawusi, 1986) and strawberry (Kumar and Dey, 2011). The effect of the surface mulch on the physical conditions of the soil surface layer is dependent on mulch type, quantity and structure (Teasdale and Mohler, 2000). Organic mulches have also been used for different medicinal and aromatic crops *viz.*, *Citronella java* (Singh et al., 2001); basil (Palada et al., 2000); mulberry (Shashidhar et al., 2009) and geranium (Ram et al., 2003). Moreover, living mulches can suppress weed growth by competing for light (Teasdale, 1993), water and nutrients (Mayer and Hartwig, 1986), as well as through the production of allelopathic compounds (White et al., 1989), which ultimately can result in reduced herbicide application.

During autumn season, large number of leaves shed from trees and shrubs are left to decompose to waste. These leaves could be utilized as mulch. This is a waste of resources and there is a need to encourage farmers to utilize such easily available resource to improve crop productivity and soil fertility in a sustainable manner. There is, however, a dearth of information on the influence of spacing and organic mulch on stevia. Therefore, field experiments were conducted to evaluate the effect of spacing and tree leaf mulches on the growth, yield, quality of stevia and soil fertility under western Himalayas.

Materials and Methods

Experimental site

The study was conducted during 2010 and 2011 at the experimental farm of Institute of Himalayan Bioresource Technology (CSIR), Palampur (1325 m amsl, 32º 06´ 05” N, 76º 34´ 10” E), India. The soil of experimental field is clayey in texture, acidic in reaction (pH 5.64), electrical conductivity (0.102 m mhos cm^{-1}), high in organic carbon (1.4%), low in available nitrogen (188.9 kg ha^{-1}) and high in available phosphorus (26.5 kg ha^{-1}) and potassium (366.4 kg ha^{-1}).

Weather data

Palampur represents the subtemperate mid-hill region of North western Himalayas and endowed with mild summers (18.0-31.3 °C) and severe winters (3.3-13.2 °C). The average rainfall received is about 2500 mm per annum of which about 77% is received during June to September. Data on weather parameters *viz.*, maximum and minimum temperature, relative humidity, bright sunshine hours and rainfall for the crop season was recorded at the agro-meteorological observatory of CSK Himachal Pradesh Agricultural University, Palampur are shown in Figure 1. Maximum temperature ranged from 15.3 to 34.8 °C during 2010 and from 12.0 to 33.5 °C during 2011. Whereas, minimum temperature ranged from 4.9 to 21.3 °C during 2010 and 2.4 to 20.9 °C during 2011. During the crop growth season, mean relative humidity ranged from 42.3 to 95.9% in 2010 and 60.6 to 97.1% in 2011. Total rainfall received in the crop growth season was 2391.2 mm in 2010 and 2407.6 mm in 2011.

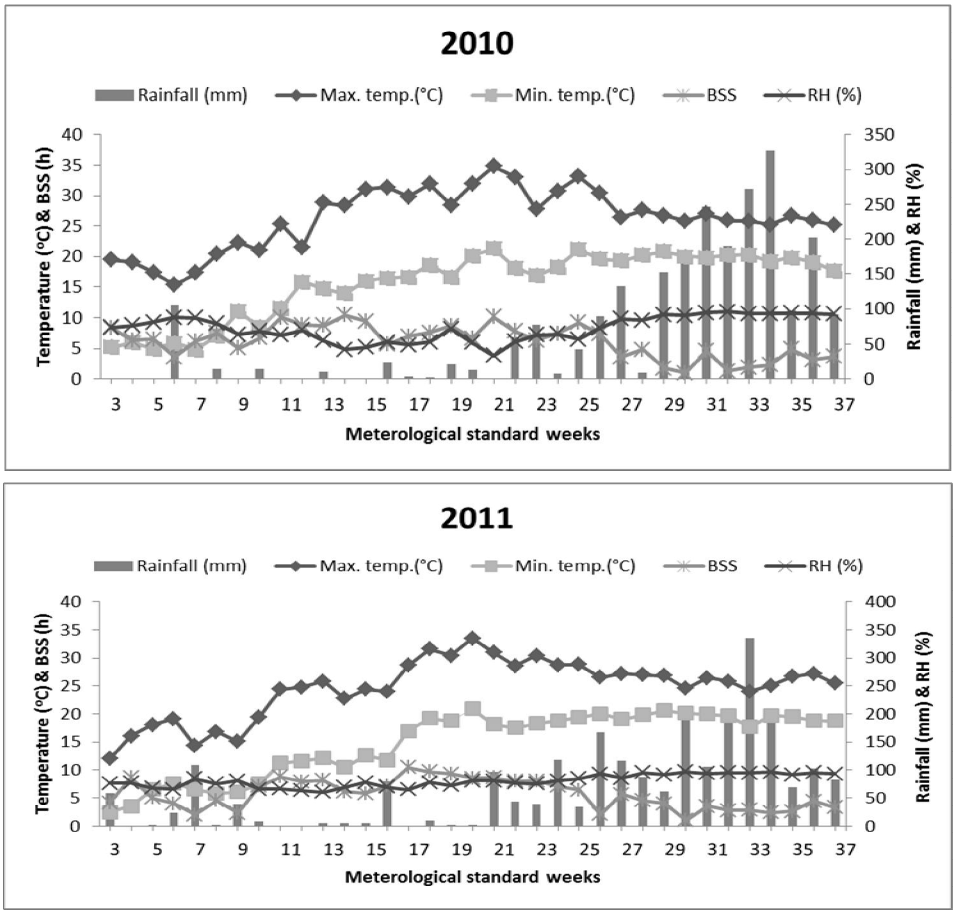

Figure 1. Weekly mean temperature (°C), bright sun shine hours (BSS), rainfall (mm) and RH (%) during the growing season at Palampur, India.

Experimental detail

Nursery of stevia crop was raised during January 2010 in sand beds through seed. Three month old seedlings were transplanted in the field on April 24, 2010. The experiment consisted of two spacing levels *viz.*, 30 cm × 30 cm and 45 cm × 30 cm and four mulches *viz.*, pine needles (*Pinus roxburghii*) mulch, poplar leaf (*Populus deltoides*) mulch, silver oak (*Grevillea robusta*) tree leaf mulch and unmulched control. Each treatment was replicated three times in a randomized complete block design. After transplanting the crop in 2010, dry leaves of the three target tree mulches (5 Mg ha^{-1}) were uniformly spread on the soil surface in the inter-row spaces and second mulching was applied during April 2011. Stevia crop undergoes dormancy during winter months and regrowth of crop starts with rise in temperature during March under mid hill conditions of north western Himalayas. Well decomposed farmyard manure (FYM) @ 15 Mg ha^{-1} was incorporated into the soil before transplanting, every year in the month of March. A common fertilizer dose of @ 100:50:50 kg NPK ha^{-1} was applied to supplement the nutritional demand of stevia crop. Nitrogen was applied in two equal splits through urea (N: 46%), half dose at the time of transplanting and remaining half was applied 2 months after transplanting during 2010. During 2011, half nitrogen and full P and K was applied in the month of April and remaining half N was applied in the month of June 2011.

Growth and yield analysis

Biometric observations *viz*, plant height, plant spread, number of branches $plant^{-1}$, number of leaves $plant^{-1}$, leaf length, width, leaf area and chlorophyll concentration index (CCI) were recorded by selecting 10 plants $plot^{-1}$ at harvest. Plant height was measured from the ground level to tip of the top leaf. Plant spread was recorded in north-south and east-west directions. For estimation of dry matter accumulation, 5 plants were cut at ground level and partitioned into leaf, stem, root and the total dry matter was calculated. Crop was harvested from net plot area during October each year at 50% budding stage when the concentration of steviol glycoside is maximum (Kumar et al., 2012) and data was converted to per hectare. The stems were dried at 60 ^{o}C, whereas, leaf samples were dried at 40 ^{o}C in a hot air oven for 48 h. and data was taken when concordant values were obtained. Leaf area was measured by portable laser leaf area meter. Leaf area index (LAI) was calculated by dividing leaf area with ground area per plant. CCI was measured by CCM 200 (Opti sciences, Tyngsboro, USA).

Weed infestation data

Weed control assessment was conducted during August and at harvest in all the plots to determine the weed spectrum, density, weight of total weed biomass using fixed 50 cm × 50 cm quadrats. Collected weed samples were bulked counted and subsequently weighed. To determine dry weed weight, weeds were uprooted; roots were washed with tap water and dried at 70 ^{o}C in hot air oven until constant weight was achieved.

Steviol glycosides extraction

Plant material and chemical

Stevia leaves were collected from different treatments at harvest. Leaves were dried at 40 ºC in hot air oven for 48 h stevioside and rebaudioside-A were purchased from Chromadex, Life Technologies, India. All HPLC grade solvents (acetonitrile, water) were purchased from J.T. Baker (USA).

Preparation of sample solution

100 mg air dried powdered plant material (leaves) of *S. rebaudiana* macerated in MeOH (10 ml) for overnight and filtered. Plant material was re extracted with same solvent twice (5 ml each time) for 3 h each. The extractants were pooled together and concentrated up to dryness under reduced pressure. After defatting with *n*- hexane (2 ml) thrice and vacuum drying, extract was dissolved in 10 ml of HPLC grade ACN:H_2O (80:20) mobile phase, degassed for five-minutes and filtered through 0.45 µm filter. Filtrate was used for HPLC analysis.

Preparation of standard solution

Standard stock solutions (1 mg 2 ml^{-1}) of standards of stevioside and rebaudioside-A were prepared in HPLC-grade acetonitrile and water.

Equipment and chromatographic conditions

HPLC analysis was performed with Waters HPLC system Equipped with waters 1525 Binary HPLC pump, Waters 717 plus Autosampler, 996 PDA detector and Empower 2 software (version-4.01). All samples were filtered through 0.45 µm (Millipore) filters. Extracts of plant samples were separated on a LiChrosphere NH_2 100 column (250 mm × 4 mm × 5 µm particles) from E. Merck (Germany). The temperature of the column was set at 30 °C. Elution of standards and samples (20 µl) was performed. The mobile phase was pre-degassed acetonitrile-Water (80:20). The flow rate was 1 ml min^{-1}, the run time 30 min and the detection wavelength 205 nm. Identification of compounds was performed on the basis of the retention time, co injections and spectral matching with standards.

Soil analyses

Soil samples (0-15 cm depth) from experimental plots were collected twice, before transplantation and at the time of harvest. The samples were air dried and passed through 2 mm sieve. Soil textural class was determined by hydrometer method and soil pH was measured in a 1:2 soil: water suspension by glass electrode pH meter (Black, 1965). The organic carbon was determined by the Walkley and Black method and available N by macro Kjeldhal procedure (Black, 1965). Available P was extracted with 0.5 M sodium bicarbonate ($NaHCO_3$) and the amount was determined by spectrophotometry. Exchangeable K [1 N ammonium acetate (NH_4OAc) extractable] was determined by flame photometry (Black, 1965).

Isolation and enumeration of micro-organisms

Soil samples were collected from each plot at 0-15 cm depth with an auger. Soil samples from two locations of the net plots were taken and mixed to form a single sample. Isolation of micro-organisms from soil sample was done by using serial dilution agar plating method (Salwan et al., 2010). Ten fold serial dilutions of each sample were prepared in normal saline (0.85% NaCl) and 0.1 ml dilutions plated on trypticase soya agar for bacteria and Sabauraud dextrose agar medium for fungi were incubated at

28 °C for 24 and 48 h, respectively. The colony forming units were calculated by multiplying the dilution factor to find the number of cells spores^{-1} gram^{-1} of the sample.

Soil temperature

Soil temperature at 10 cm depth was measured with stainless steel Fisher brand bi-metal dial thermometers having a stem length of 20.3 cm, gauge diameter of 4.5 cm and accuracy of ±1.0% of dial range at any point of dial. Observations were recorded at alternate days at 02:00 pm (in mulched and unmulched plots).

Statistical analyses

The data was analysed by software SYSTAT-12 (Systat Software Inc., Chicago, Illinois, USA). When the ANOVA analysis found significant differences between treatments, Duncan's test was conducted to detect differences between individual treatment level means. All statistical analyses were performed at a 95% confidence level. The weed count and weed dry weight data were analysed after transforming the actual data (x) to square root (x+1).

Results

Soil temperature

Soil temperature was influenced by mulching. During summer from May to June, soil temperature was lower in poplar and silver oak tree leaf mulch compared to pine needle and unmulched control (Figure 2). These results confirm the efficacy of mulching in reducing high soil temperature extremes. The mean maximum temperature for pine needle (25.5 °C) was higher than poplar (23.6 °C) and silver oak (23.4 °C) mulch during May and it decreased until August which corresponds to the rainy season. However, differences in soil temperatures between the different mulches and control were not much in the month of September.

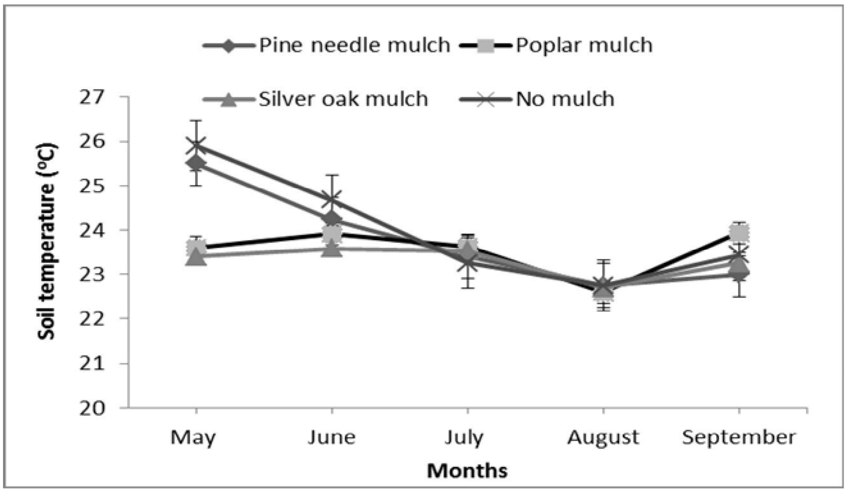

Figure 2. Effect of different organic mulches on soil temperature (^oC) at 10 cm depth (Mean data of two years).

Growth and development

Data presented in Table 1 showed that plant spacing had no significant effect on plant height, plant spread, number of leaves plant^{-1} during both years and number of branches and CCI during 2010. During 2011, number of branches plant^{-1} were significantly higher in 30 cm × 30 cm compared to 45 cm × 30 cm while reverse trend was observed for CCI. LAI was significantly higher when stevia plants were planted at 30 cm × 30 cm spacing compared to 45 cm × 30 cm during both the years.

Organic mulch could not bring any significant difference on plant height, plant spread and CCI in 2010 and on number of branches plant^{-1} in both years. However, during 2011 significantly longer plants were recorded in the plots which were mulched with poplar leaves and silver oak leaves compared to pine needle and control. Plots mulched with silver oak leaves recorded significantly higher plant spread in N-S or E-W directions as compared to pine needle and unmulched control but remained at par with poplar leaf mulch. Number of leaves plant^{-1} was significantly higher in poplar leaf mulch plots during both the years compared to pine needle and unmulched control. During 2011, CCI was significantly higher in the plots mulched with silver oak compared to other mulch treatments which remained at par with each other. Stevia plants mulched with poplar leaves recorded significantly higher LAI compared to pine needle mulch and control but remained at par with silver oak leaf mulch during both the years.

Table 1. Effect of spacing levels and organic mulch on growth of stevia.

Treatment	Plant height (cm)		Branches $plant^{-1}$		Plant spread N-S (cm)		Plant spread E-W (cm)		Number of leaves $plant^{-1}$		CCI		LAI	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Spacing (cm)														
30×30	92.2	118.9	3.4	10.7	25.1	37.0	24.4	37.4	193.7	346.3	20.7	23.3	0.67	1.21
45×30	90.3	119.5	3.0	8.8	24.8	35.9	23.8	33.8	200.7	346.4	21.1	25.9	0.48	0.85
SEm±	3.2	1.8	0.2	0.6	0.89	1.12	1.1	1.27	13.1	20.7	0.98	0.8	0.03	0.06
LSD (P=0.05)	NS	NS	NS	1.88	NS	NS	NS	NS	NS	NS	NS	2.55	0.08	0.19
Organic mulch														
Pine needles	91.7	114.4	3.1	8.7	25.0	33.1	23.7	34.2	184.1	339.3	20.1	24.9	0.50	1.14
Poplar leaf	92.7	127.6	3.4	11.0	25.9	37.8	24.8	35.1	242.6	476.9	20.9	23.7	0.69	1.33
Silver oak leaf	94.0	123.3	3.0	10.2	24.3	41.9	25.4	40.6	210.5	355.9	22.0	28.5	0.64	1.09
No mulch	86.7	111.5	3.2	9.1	24.6	32.9	22.5	32.5	151.7	213.3	20.5	21.5	0.47	0.57
SEm±	4.6	2.5	0.3	0.9	1.3	1.6	1.6	1.8	18.6	29.3	1.38	1.18	0.04	0.08
LSD (P=0.05)	NS	7.6	NS	NS	NS	4.8	NS	5.5	56.3	89.0	NS	3.60	0.11	0.26

NS: Not significant, LSD: Least significant difference, CCI: Chlorophyll concentration index, LAI: Leaf area index.

Weed dynamics

Weed flora of the experimental plot consisted of annual grasses and broad leaves and perennial grass and sedges. Grassy weeds were predominant during rainy season (July- September), broad leaf constituted the major part of weed flora during winter October-November. Weed number and their dry weight during the second year were considerably lower than in the first year. Data presented in Table 2 revealed that during 2010, plant spacing could not bring any significant effect on weed count (number m^{-2}) and weed dry weight (g m^{-2}) in August and at the time of harvest. However, during 2011, weed dry weight in August and at harvest was higher under broader row spacing (45 cm × 30 cm) compared to narrow row spacing (30 cm × 30 cm).

Weed population and dry matter accumulation by weeds were significantly influenced by mulch treatments (Table 2). The magnitude of reduction in weed number and weed dry weight was much higher in plots which were mulched with poplar leaves, silver oak leaves than pine needle and unmulched control. Weed dry weight showed significant differences between the treatments. Plots mulched with poplar leaves recorded lower weed dry weight, whereas the unmulched plot recorded the highest weight of weeds.

Table 2. Effect of spacing levels and organic mulch on weed dynamics in stevia.

Treatment	Weed count (no. m^{-2}) August		Dry weed weight (g m^{-2}) August		Weed count (no m^{-2}) at harvest		Dry weed wt (g m^{-2}) at harvest	
	2010	2011	2010	2011	2010	2011	2010	2011
Spacing (cm)								
30×30	13.3	9.2	5.0	6.2	8.6	9.2	3.6	7.4
	(202.7)	(101.3)	(24.2)	(46.1)	(89.3)	(92.0)	(13.6)	(59.9)
45×30	13.3	9.8	4.8	5.4	9.2	9.8	4.1	8.7
	(258.0)	(116.7)	(26.0)	(34.7)	(105.5)	(102.7)	(18.2)	(80.0)
SEm±	0.4	0.6	0.2	0.3	0.5	0.5	0.3	0.4
LSD (P=0.05)	NS	NS	NS	0.8	NS	NS	NS	1.2
Organic mulch								
Pine needles	13.6	8.4	4.3	4.5	9.1	11.5	4.3	8.0
	(188.0)	(74.7)	(19.3)	(20.0)	(84.3)	(133.3)	(18.3)	(67.6)
Poplar leaf	6.7	6.5	3.7	4.7	4.9	7.1	2.3	5.3
	(46.7)	(42.7)	(13.1)	(21.6)	(24.0)	(53.3)	(4.6)	(30.8)
Silver oak leaf	8.7	7.0	5.3	3.9	6.3	6.9	3.3	7.9
	(88.0)	(50.7)	(28.5)	(15.3)	(41.3)	(48.0)	(10.9)	(63.9)
No mulch	24.2	16.3	6.2	10.2	15.3	12.4	5.4	10.8
	(598.7)	(268.0)	(39.6)	(104.7)	(240.0)	(154.7)	(29.7)	(117.5)
SEm±	0.6	0.8	0.3	0.4	0.7	0.7	0.4	0.6
LSD (P=0.05)	1.8	2.6	0.9	1.1	2.3	2.1	1.3	1.7

NS: Not significant, LSD: Least significant difference, Value in parentheses is the original mean.

Dry matter accumulation

Plant spacing could not bring any significant effect on dry matter accumulation in different plant parts *viz*., leaf, stem and roots during both the years (Table 3). However, organic mulches significantly affected dry matter accumulation in both years. The leaf, stem and root dry biomass (g plant^{-1}) were significantly higher in plots mulched with poplar leaves, except the stem dry biomass in 2010, when the effect of mulch was not significant. Root growth was not affected by spacing levels in both years. However, organic mulches significantly affected root volume in both years. During 2010 root volume was significantly higher in poplar leaf mulch as compared to other treatments, whereas, during 2011, plots mulched with silver oak leaves remained at par with poplar leaf mulch but recorded significantly higher root volume than other treatments.

Table 3. Effect of spacing levels and organic mulch on dry matter accumulation in different plant parts and root growth of stevia.

Treatment	Dry biomass (g plant^{-1})						Root volume (mm)	
	Leaf		Stem		Root			
	2010	2011	2010	2011	2010	2011	2010	2011
Spacing (cm)								
30×30	7.16	33.54	16.53	53.09	5.32	9.74	16.60	48.93
45×30	7.10	36.99	15.32	58.73	5.54	11.18	18.14	50.24
SEm±	0.37	1.76	1.19	2.60	0.38	0.55	0.92	3.38
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Organic mulch								
Pine needles	6.67	36.33	14.62	52.40	5.11	9.52	16.43	40.52
Poplar leaf	9.90	45.58	18.29	73.44	6.78	12.73	22.07	57.82
Silver oak leaf	7.39	39.69	18.22	63.36	5.57	10.61	17.13	57.00
No mulch	4.55	19.44	12.56	34.44	4.26	8.99	13.84	43.00
SEm±	0.53	2.49	1.69	3.70	0.54	0.78	1.31	4.78
LSD (P=0.05)	1.60	7.57	NS	11.23	1.65	2.39	3.96	14.52

NS: Not significant, LSD: Least significant difference.

Yield

Leaf is the main economic part in stevia plant so production of higher leaf biomass along with higher steviol glycosides is the main criterion for its performance. Significantly higher leaf, stem and total dry biomass (q ha^{-1})

were recorded when stevia plants were planted at 30 cm × 30 cm as compared to 45 cm × 30 cm during both the years (Table 4). Planting stevia at 30 cm × 30 cm recorded 50.9 and 35.6% significantly higher dry leaf biomass in 2010 and 2011, respectively, compared to 45 cm × 30 cm spacing. Leaf:stem ratio was not affected significantly by spacing levels (Table 4).

A perusal of data presented in Table 4 reveals that different mulches significantly affected leaf, stem and total biomass in both years. Stevia plants mulched with poplar leaves recorded significantly higher leaf, stem and total dry biomass compared to pine needle mulch and unmulched control but remained at par with silver oak leaf mulch. Similarly, though leaf:stem ratio was not affected significantly by mulch treatment but it was higher under poplar leaf mulch.

Leaf dry biomass of a plant was 118% higher in poplar leaf mulch as compared to unmulched control. Organic mulch treatments increased the leaf biomass yield of stevia significantly over unmulched control in both the seasons (Table 4). In 2011, the stevia mulched with poplar leaves recorded higher yield (128%) than the unmulched plots, 22% higher than the pine needle mulched plots and 15% higher than the plots mulched with silver oak leaves. Leaf: stem ratio was not affected significantly by different organic mulches.

Table 4. Effect of spacing levels and organic mulch on dry biomass (Mg ha^{-1}) of stevia.

Treatment	Dry leaf biomass (Mg ha^{-1})		Dry stem biomass (Mg ha^{-1})		Total biomass (Mg ha^{-1})		Leaf: stem ratio	
	2010	2011	2010	2011	2010	2011	2010	2011
Spacing (cm)								
30×30	0.64	2.09	1.47	3.31	2.11	5.40	0.44	0.64
45×30	0.42	1.54	0.91	2.45	1.33	3.99	0.49	0.63
SEm±	0.03	0.09	0.08	0.14	0.10	0.20	0.04	0.03
LSD (P=0.05)	0.08	0.28	0.23	0.42	0.29	0.61	NS	NS
Organic mulch								
Pine needles	0.48	1.90	1.09	2.68	1.58	4.59	0.47	0.70
Poplar leaf	0.72	2.32	1.36	3.77	2.09	6.09	0.60	0.62
Silver oak leaf	0.56	2.02	1.38	3.26	1.94	5.79	0.41	0.64
No mulch	0.35	1.02	0.92	1.79	1.27	2.81	0.39	0.57
SEm±	0.04	0.13	0.11	0.20	0.14	0.30	0.05	0.04
LSD (P=0.05)	0.12	0.40	0.33	0.60	0.41	0.87	NS	NS

NS: Not significant, LSD: Least significant difference.

Steviol glycoside concentration

Steviol glycoside concentration was not affected significantly by different treatments (Table 5). Stevioside (St) ranged from 7.0 to 8.6%, rebaudioside-A (Rb) from 0.8 to 3.5%, total (stevioside + rebaudioside-A) from 8.3 to 12.1% in different treatments. Rb/St ratio was higher in stevia plants mulched with poplar leaves and unmulched control. Stevia planted in control (no mulch) plot recorded higher steviol glycosides followed by those mulched with poplar leaves, silver oak leaves and pine needles. Rebaudioside - A content was higher in plots mulched with poplar leaves (Table 5). Rebaudioside is responsible for sweetness in stevia so higher Rb/St ratio is desirable.

Table 5. Effect of spacing levels and organic mulch on steviol glycoside concentration (%) of stevia after harvest during 2011.

Treatment	St	Rb	Total (St+Rb)	Rb/St
Spacing (cm)				
30×30	7.3	2.6	9.9	0.4
45×30	8.1	2.1	10.2	0.3
SEm±	0.6	0.1	0.5	4.0
Organic mulch				
Pine needle	7.5	0.8	8.3	0.1
Poplar	7.0	3.1	10.0	0.5
Silver oak	7.8	2.1	9.9	0.3
No mulch	8.6	3.5	12.1	0.5
SEm±	1.1	0.2	1.0	8.1

St: stevioside, Rb, rebaudioside-A.

Soil nutrient status and soil microflora

Spacing levels could not bring any significant effect on pH, organic carbon, nutrient availability status and soil bacteria in both the years. However, during 2011, plant spaced at 30 cm × 30 cm recorded significantly higher fungal population than those in plot with 45 cm × 30 cm plant spacing (Table 6).

Table 6. Effect of spacing levels and organic mulch on pH, organic carbon, available nutrients and microbial status of soil at harvest.

Treatment	pH	Organic Carbon (%)	C:N ratio	Available nutrients (kg ha^{-1})			Microbial count	
				Nitrogen	Phosphorus	Potassium	Bacteria (CFU×10^5 g^{-1})	Fungus (CFU×10^3 g^{-1})
Spacing (cm)								
30×30	5.78	1.67	21.1	218.05	27.16	400.92	7.31	13.69
45×30	5.80	1.66	21.3	213.42	28.41	413.34	7.14	9.39
SEm±	0.04	0.10	1.1	9.91	1.41	16.87	0.32	0.92
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	2.79
Organic mulch								
Pine needles	5.84	1.97	25.1	243.91	30.04	452.08	10.94	16.71
Poplar leaf	5.82	1.98	20.1	238.68	32.20	430.48	5.84	13.05
Silver oak leaf	5.73	1.79	22.6	224.05	29.18	400.18	6.91	10.10
No mulch	5.76	0.91	17.1	156.31	19.72	345.77	5.27	6.30
SEm±	0.07	0.14	1.6	14.02	1.99	23.86	0.46	1.30
LSD (P=0.05)	NS	0.41	4.7	42.52	6.04	72.39	1.31	3.95

NS: Not significant, LSD: Least significant difference.

All the mulched plots significantly increased organic carbon, available N, P and K as compared to unmulched plots (Table 6). Organic carbon was significantly higher in poplar leaf mulch compared to control but remained at par with other mulches. C:N ratio of unmulched plots was significantly lower than all the mulches which remained at par with each other. Available N and K were significantly higher in pine needle mulch compared to control but remained at par with poplar and silver oak mulch. Available P was significantly higher in poplar leaf mulch compared to control plots.

Significantly higher numbers of bacterial colonies were found in plots mulched with pine needle followed by those plots mulched with silver oak leaf as compared to other treatments (Table 6). Mulching with silver oak leaves was at par with poplar leaf mulch in terms of bacterial population.

Fungal population was significantly higher due to pine needle mulch, which was at par with poplar leaf mulch. Both these treatments provided significantly higher fungal population than the unmulched plot.

Discussion

Plant density affects canopy development, radiation interception, evaporation of water from soil under the crop, dry matter production, the development of fungal and viral diseases, weed competition and ultimately, the seed yield of a crop in the farming system (Lopez-Bellido et al., 2005). Planting stevia plants at 30 cm × 30 cm spacing recorded significantly higher branches plant^{-1} and LAI (Table 1) which resulted in higher leaf, stem and total dry biomass than 45 cm × 30 cm (Table 4). Higher weed count and weed dry weight (Table 2) under wider row spacing (45 cm × 30 cm) might have helped in reduction of leaf biomass as compared to narrow row spacing (30 cm × 30 cm). As plant densities decline, reduction in the number of plants per unit area is partially compensated by an accompanying increase in the productivity of each plant. Zhang et al. (2012), also reported similar results for oilseed rape.

Soil temperature under organic mulch was lower than unmulched control during summers (Figure 2). Mulches are known to increase the soil temperature since the sun's energy passes through the mulch and heats the air and soil beneath the mulch directly and then the heat is trapped by the ‘‘greenhouse effect’’ (Hu et al., 1995). If topsoil temperature is excessive, mulching can reduce temperature for more optimal germination and root development. Mulch reduces water evaporation from soil and help to maintain stable soil temperature (Ji and Unger, 2001; Kar and Kumar, 2007). Plant height was significantly higher in poplar leaf mulch (Table 1), which could be attributed to reduced leaching and movement of nutrients and more availability to the crop. Ossom and Matsenjwa (2007), reported similar results in field bean (*Phaseolus vulgaris* L.). Plant spread in N-S and E-W directions and CCI were significantly higher in silver oak leaf mulch, whereas, number of leaves plant^{-1} was significantly higher in poplar leaf mulch (Table 1). Weed count and weed dry biomass (Table 2) in August and at harvest were significantly higher in the control plots followed by the plots mulched with pine needle. Among organic mulches poplar and silver oak leaf mulch proved effective for weed management than the pine needle mulch. This may be due to the fact that poplar leaves and silver oak leaves have larger surface area which might have suppressed the growth of most of the annual weeds and resulted in lesser weed biomass. Earlier Kang et al. (1990), have reported that suppression of weeds is more effective in the presence of mulch that decomposes slowly. The dry weight of weeds in plots treated with mulches was less, which could be attributed to higher crop growth, as reflected in crop plant height, causing early canopy of crop and ultimately smothering of the weeds. In contrast, more weeds emerged in plot mulched with pine needle, which was perhaps due to improper coverage of soil surface as compared to poplar and silver oak leaf mulch. With increasing crop age the competitiveness of stevia against weeds increased with the overall increase in plant growth resulting in lower weed population and dry weight in the second year. Singh et al. (2001) and Sinkeviciene et al. (2009), also observed similar results for different crops.

Leaf, stem and root dry weight (g plant^{-1}) were significantly higher in plots mulched with poplar leaf mulch in both the years because poplar based agroforestry system improves aggregation of soil through high amount of organic matter in the form of leaf biomass (Gupta et al., 2009). Beneficial effects of mulching on growth and dry matter production have also been reported by Das (1999) in ginger, Gill et al. (1999) in turmeric, Shukla et al. (2000) in *Emblica officinalis* and by Joy et al. (2001a) and Joy et al. (2001b) in *Alpinia calcarata, Curcuma zedoaria* and *Kaempferia rotunda*. Root development and density was greatest under organic mulch compared to that under bare soil (Watson, 1988). In 2010, root volume (ml) was significantly higher in plots mulched with poplar leaves (Table 3). This may be due to the fact that organic mulches decompose under favourable water and temperature regimes, nutrients are released into the soil and become available for root uptake or microbial use (Chalker-Scott, 2007). Significantly higher roots were observed in mulched plots; organic mulches showed better root density possibly due to higher moisture retention during initial growth of crop.

Leaf, stem and total dry biomass of stevia were significantly higher in poplar leaf mulch as compared to unmulched control (Table 4). The variation in leaf dry matter production could be attributed to the variation in the number of leaves plant^{-1}. The number of leaves plant^{-1} at harvest was maximum in poplar leaf mulch followed by silver oak and pine needle mulch. Leaf area index (LAI) was also higher in poplar leaf mulch (Table 1). Weed population and weed dry weight was also significantly lesser in plots mulched with poplar leaves which contributed to higher leaf dry weight plant^{-1} and ultimately higher leaf yield production. Higher soil temperature accelerated the rates of leaf tip appearance and full leaf expansion, enabling the crop to rapidly attain maximum green leaf area index (Stone et al., 1999). Plant geometry helps in modifying the microclimate. Narrow row spacing results in higher leaf photosynthesis and suppresses weed growth due to smothering effect compared with wider row spacing (Dwyer et al., 1991).

Steviol glycoside concentration was not affected significantly by different treatments (Table 5). Rebaudioside-A concentration was higher in plots mulched with poplar mulch. Rebaudioside - A has sweetness of a good quality and a degree of sweetness of 1.3 to 1.5 time that of stevioside. So higher concentration of Rebaudioside - A is desirable character.

All the mulched plots significantly increased organic carbon, C:N ratio, available N, P and K as compared to unmulched plots (Table 6). Organic carbon was significantly higher in poplar leaf mulch compared to control but remained at par with other mulches. Available N and K was significantly higher in pine needle mulch compared to control but remained at par with poplar and silver oak mulch. Available P was significantly higher in poplar mulch compared to control plots. Microbial decomposition of organic materials with large amounts of labile organic nitrogen (N), such as biosolids or minimally composted animal manure, is expected to result in high rates of N mineralization (Forge et al., 2003).

Soil biological activity was also enhanced by organic mulches. The decomposition rate of tree leaf mulches followed the order silver oak > pine needle > poplar. The lowest decomposition rate in *P. deltoides* may be due to higher lignin ADF cellulose, calcium and lowest water soluble compounds and nitrogen content (Kaushal et al., 2012). The increase in bacterial colonies in mulched plots may be due to increasing organic carbon content of soil (Table 5), which is considered as one of the major constituents of food supply for bacteria (Shashidhar et al., 2009). The variation in microbial load of different organic mulches could be due to their different chemical composition and decomposition rates (Mukherjee et al., 1991; Wu et al., 1993). Changes in composition of microbial communities due to application of organic matter have also been reported by different researchers (Jha et al., 2004; Asari et al., 2008; Singh et al., 2011).

Conclusions

Incorporation of different organic mulches has not only controlled the weed population but also improved the soil-fertility after decomposition and protected the leaves of stevia from decaying due to muddy splash during rainy season. It has also contributed towards increase in organic carbon content of the soil and ultimately increased leaf biomass and quality of leaf. Over long run, it may be possible that consistent application of mulch may lead to continuous changes in microbial community structure and species diversity with enhancement in soil organic matter. Mulch will change the cation exchange capacity of the soil and lead to improved soil structure with increment in soil organic matter. It is evident from this study that application of poplar and silver oak leaf mulch are effective methods to improve soil nutrient availability and that stevia plants are very responsive to these mulches. Both these mulches affected soil properties and created more favourable environment for roots, which resulted in enhanced plant growth and yield.

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