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Table: 1, Figs : 7

Impact of soil sickness and post harvest residues on sugarcane metabolism and yield in prolonged cultivation

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ABSTRACT

The aim of this research was (i) to explore the influence of soil sickness, autotoxicity and allelopathic potential of post harvest residues on various physiological processes in ratoon cane and sugarcane yield and (ii) to identify the allelochemicals found in sugarcane post harvest residue of popular ratooning cultivars grown in Maharashtra state. Sugarcane post harvest residue of the investigated ratoon varieties were very rich in triterpenoids, steroids, flavonoids, pungent bitter essential oils and phenols, which may synergistically cause the negative impact on yield attributing parameters like millable cane height, number of internodes, number of canes per stool and cane weight. Primary and secondary metabolites present in post harvest residue also caused an adverse impact on metabolic processes of ratoon cane, leading to autotoxicity, causing reduction in number of tillers, resulting in a drastic decline in cane sprouting.

Keywords: Allelochemicals, sugarcane multiple ratooning, post harvest residues, yield.

INTRODUCTION

Multiple ratooning sugarcane is most profitable agricultural system for sugar industry and sugarcane growers in Maharashtra state, nevertheless, the drastic decline in cane yield in this system has discouraged the farmers. Maharashtra state is leading sugar producer in India and produced 90.8 million tons of sugar during 2007-2008 (2). The first crop (planted cane) and 1-5 multiple ratoons (2-8 years) are practiced (15), depending on variety and crop management practices (13). The productivity of sugarcane under multiple ratooning declines every year, due to reduction in plant stand (23) and this may be attributed to soil sickness and autotoxicity.

In India sugarcane is planted by planting stem cuttings (sugarcane sets with 1-3 eye buds), which produces primary and secondary shoots. Shoots become millable stalks with in 11 to 12 months after planting. The crop produces 5-6 t/ha post harvest residues, which are usually burnt in the field before raising of the next crop. Unburnt residues during decomposition releases several phytotoxic compounds (*p*-hydroxybenzoic acid, *p*-coumaric acid, 2,4-dihydroxybenzonazilone and benzonazinone) in soil, these are autotoxic to sprouting of new tillers in sugarcane and cause their wilting and growth

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inhibition. These allelochemicals from sugarcane residues, accumulate in the soil during the successive ratoons (8,19,26).

This research aimed (i) to determine the influence of soil sickness, autotoxicity and allelopathic potential of post harvest residues on various physiological processes in ratoon cane and sugarcane yield and (ii) to identify the allelochemicals found in sugarcane residues of popular ratooning cultivars grown in Maharashtra state.

MATERIALS AND METHODS

The third leaf from top of 25 sugarcane plants var. CoC 671 and Co 86032 were collected from 8-months old plants in farmers fields of medium (>9%) and high (<11%) sugar recovery zones. Leaf samples were collected from Plant crop, II, III, V and VI ratoon.

Physiological parameters: The collected leaves were washed with tap water and blotted to dry. The midrib was removed and the leaf was cut into 1.0 cm^2 pieces. From this composite sample, 1 g plant material was used to study physiological parameters. Standard protocols were used to quantify the photosynthetic pigments, proteins, total sugars and phenols (3,7,11,24). Yield parameters were recorded from randomly selected 25 canes. Total height was measured from base to the 4^{th} opened leaf from top of sugarcane plant, while millable stalk height was measured from the base to top most internode, number of internodes per cane, number of millable canes per stool and weight of stalks were also determined. The post harvest residues (old and dry leaves) of sugarcane cv. CoC 671 were collected from 8 month old crop at random at 6^{th} ratooning from the high and medium sugar recovery zones. These were used to determine the allelochemicals.

Allelochemicals detection (HPTLC): 0.5 g powdered samples of sugarcane post harvest residues were separately extracted with 10 mL methanol, sonicated for 10 min and kept overnight. After filtration, the methanolic extracts were subjected to TLC analysis using toluene: ethyl acetate (8:2) as mobile phase (9). TLC plates were observed with 10% solution of sulphuric acid in methanol or Liebermann-Burchard reagent and observed under UV light at 366 nm. Plates were also observed with anisaldehyde reagent and observed under UV light at 550 nm. Then, the plates were subjected to densitometric analysis using Scanner II, CAMAG CATS 3 software.

Phenolics detection (TLC): Shade dried post harvest residues samples of plant cane and ratoons (1 g) were homogenized in 10 mL methanol. The extracts were sonicated for 10 min at 70°C and then filtrated through Whatman # 1 filter paper. These samples were analyzed by TLC. 50 µl of each methanol extract was loaded on TLC plate which was developed in benzene: dioxane: acetic acid (90: 25: 4). Plates were sprayed with tetrazotized benzidine reagent and kept in an oven at 105°C for a few minutes. The R_f value of each spot was calculated (16).

Phenolic compounds phytotoxicity: Standards of 50 and 100 ppm concentrations were prepared for *p*-hydroxybenzoic, cinnamic and ferulic acids aqueous solutions. These stock solutions were stored in amber coloured bottles in refrigerator until use. 3-month old healthy plantlets of cv. Co 86032 (Vasant Dada Sugar Institute, Pune) were planted in perforated polyethylene bags (40 × 60 cm), filled with 4 kg soil and manure (3:1 ratio). Then, phenolic compounds solutions (50 and 100 ppm) were added to soil. Each treatment consisted of 9 plants including control (water). All treatments and control bags were maintained under uniform conditions in 5 replications. These bags were irrigated with 100 ml phenolics solution in root zone for 60 days. The plants were regularly irrigated during the experimental period (average 2 l per plant per week).

Growth parameters (plant height, number of leaves, leaf area and number of tillers per stool) were recorded at the end of experiment. Physiological parameters (photosynthetic pigments, proteins, total sugars and starch) were measured in the third leaf from top of sugarcane plant. Proline and total phenolic compounds were determined by standard protocols (4,7).

Statistical analysis

One way ANOVA was used to compare the mean values as influenced by time of cultivation. Fisher's LSD was applied as a post hoc test at $p<0.05$ to compare the mean differences. All the calculations were made by using (Sigma stat 3.5) and Microsoft Excel (Office 2000).

RESULTS AND DISCUSSION

Effect of prolonged cultivation on physiological parameters

Chlorophyll content in sugarcane cv. CoC 671 and Co 86032 declined in multiple ratooning over the years in high and medium sugar recovery zones (Table 1). This reduction may be due to phenolic compounds produced by the sugarcane plants, which damages the photosynthetic apparatus. Several other allelopathy workers had also claimed that the reduction in chlorophyll contents was due to the adverse impact of phenolic acids (applied externally or present in the plant residues). The phenolic compounds were inducing reduction in biosynthesis of porphyrin alone or chlorophyll pigments itself (28). Protein contents also decreased in sugarcane cultivars with prolonged time of continuous cultivation. The protein content decreased significantly in plant cane, second, third, fifth and sixth ratoon in both cultivars in high sugar recovery zone, however, there were no differences in medium sugar recovery zone. Reduction in protein content may be attributed to negative effects of some allelochemicals on enzymatic activities involved in protein synthesis and disruption in amino acid metabolism, hormonal metabolism and membrane perturbations (6,17,28). Total sugars in leaves increased in both cultivars in ratoon crops than plant cane (Table 1). The increase in total sugars was comparatively more in high recovery zone than in medium recovery zone.

The phenolic contents increased significantly in ratoon crops than plant cane in both cultivars from high and medium recovery zones (Fig. 1). In high sugar recovery zone, the increase in phenolics from plant cane to sixth ratoon was 4 mg g^{-1} of fresh weight in both cultivars CoC 671 and Co 86032, while in medium sugar recovery zone, the increase was 5 mg g^{-1} and 6 mg g^{-1} of fresh weight in CoC 671 and Co 86032, respectively. The

Table 1. Organic constituents in the leaves of sugarcane cv. CoC 671 and Co 86032 from high and medium sugar recovery zones under the influence of prolonged cultivation

Ratoon Number	Photosynthetic pigments (mg g^{-1} f.w.)				Proteins (mg g^{-1} f.w.)				Total sugars (mg 100 g^{-1} f.w.)			
	High recovery zone		Medium recovery zone		High recovery zone		Medium recovery zone		High recovery zone		Medium recovery zone	
	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032
Plant cane	3.20 ±0.44	3.47 ±0.31	2.91 ±0.26	3.15 ±0.28	46.75 ±3.30	46.95 ±3.78	44.78 ±2.72	45.21 ±1.86	121.50 ±8.59	122.45 ±9.87	114.65 ±6.97	115.32 ±4.75
2nd	3.10 ±0.40	3.20 ±0.48	2.73 ±0.19	2.88 ±0.26	44.25 ±2.25	44.00 ±3.11	43.02 ±3.46	43.54 ±2.64	127.75 ±6.51	128.15 ±9.06	119.85 ±9.66	120.54 ±7.33
3rd	2.88 ±0.31	3.17 ±0.44	2.59 ±0.36	2.78 ±0.36	43.58 ±1.37	43.05 ±2.19	42.05 ±2.97	42.12 ±3.39	134.22 ±4.24	134.95 ±6.88	125.64 ±8.88	126.58 ±10.20
5th	2.71 ±0.21	2.81 ±0.22	2.3 8±0.31	2.51 ±0.15	42.50 ±1.75	42.15 ±1.33	41.17 ±2.09	41.87 ±2.96	140.50 ±5.79	141.14 ±4.46	129.73 ±6.61	130.25 ±9.20
6th	2.52 ±0.35	2.63 ±0.18	2.32 ±0.34	2.39 ±0.26	40.23 ±2.44	39.85 ±1.64	40.14 ±1.25	40.51 ±2.06	142.32 ±8.65	142.98 ±5.89	131.91 ±4.17	132.21 ±6.74
$LSD_{0.05}$	0.47	0.46	0.40	0.36	3.064	3.40	-	-	9.19	9.90	9.90	10.40
Significance	**	**	**	**	**	**	NS	NS	**	**	*	*

Data are mean values (n=5) followed by ±standard deviation. ‘*’, ‘**’ and ‘NS’ represent significance at $P<0.05$, and non-significance, respectively. HRZ: High recovery zone, MRZ: Medium recovery zone increase in phenolic compounds might be due to the induction of abiotic and allelopathic stresses (1,5,18,27). The phenolics leached from post harvest residues might be responsible for retarded growth, declined cane population, tiller mortality and finally the reduced cane yield during prolonged cultivation.

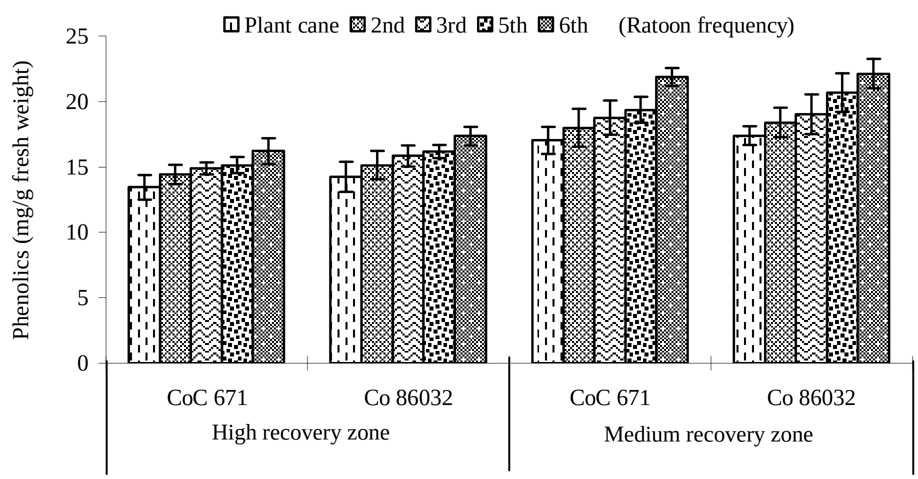


Figure 1. Effect of multiple ratooning on phenolic contents in fresh leaves of sugarcane cv. CoC 671 and Co 86032 from high and medium sugar recovery zones.

Yield attributes

Multi-ratooning reduced the yield attributes of the sugarcane cultivars. Height and weight of millable cane, number of canes per stool and number of internodes per cane were reduced with the increasing frequency of ratooning in both cultivars (Fig. 2). Millable cane height was reduced 109.26 cm and 116.47 cm in CoC 671 and Co 86032, respectively. The same trend was observed in number of internodes and millable canes per stool in both cultivars. The weight of millable cane was decreased by 9.30 % in 2nd ratoon, 37.20 % in 3rd ratoon, 45.11 % in 5th ratoon and 51.16 % in 6th ratoon of cv. CoC 671 over plant cane. Similar trend was observed in reduction of millable cane weight in Co 86032. The growth attributes per millable cane are important characters contributing to cane yield and their decline suggests the occurrence of soil sickness and autotoxicity. The yield decline in sugarcane has been attributed to phytotoxic substances like phenols released from the decomposition of post harvest residues of sugarcane in the soil (14).

Allelochemicals

The Plate 1 and 2 suggested that the major allelochemicals found in residues of cv. CoC 671 from high and medium recovery zones were terpenoids, steroids, triterpenoids, flavonoids and pungent, bitter essential oils (Figs. 3 and 4). The allelochemicals present in the post harvest residue of cv. CoC 671 were shown in the TLC

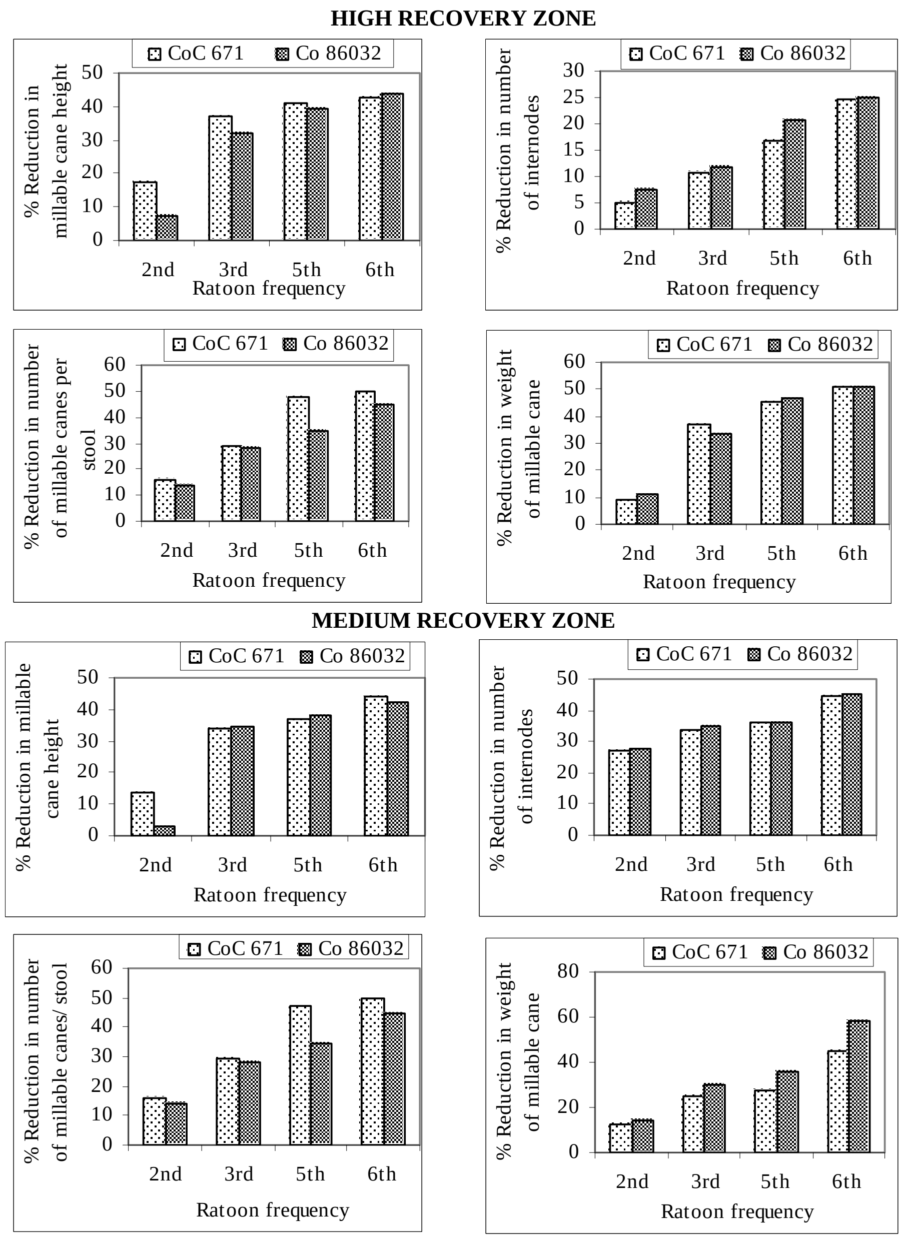
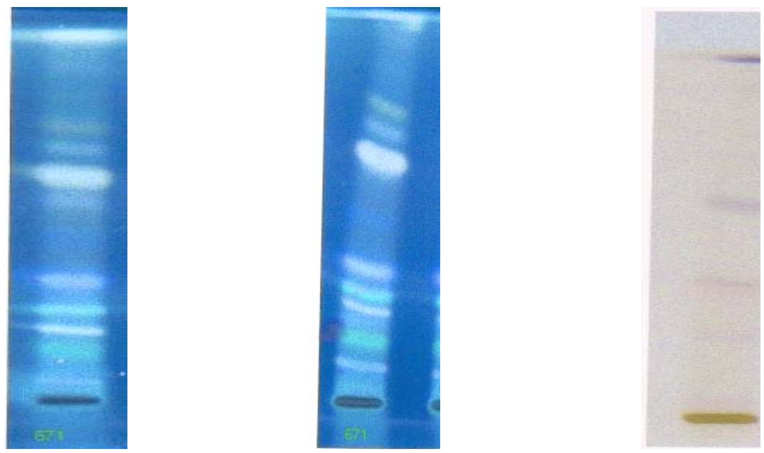


Figure 2. Yield parameters of sugarcane cv. CoC 671 and Co 86032 from high and medium recovery zones under the influence of prolonged cultivation.



10% Methanol solution of H_2SO_4 | LB reagent | Anisaldehyde reagent

Allelochemicals detected with different reagents in cv. CoC 671 under sixth ratoon (HRZ)

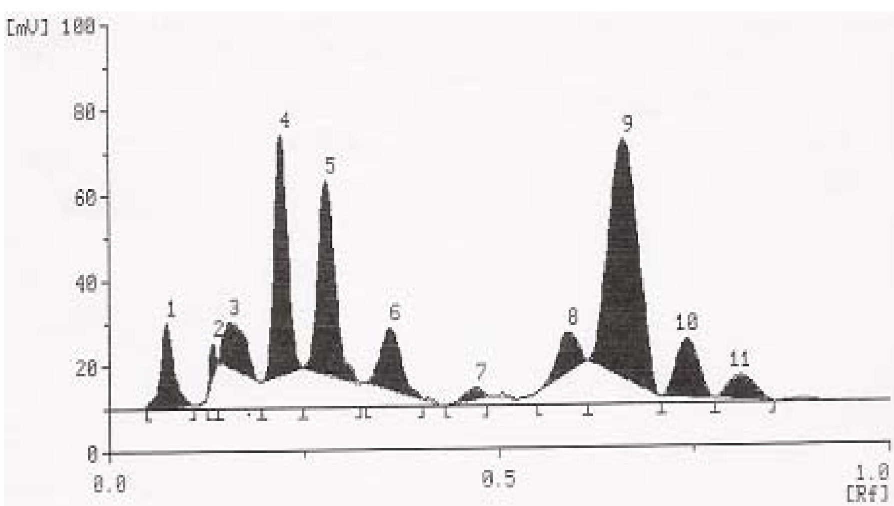
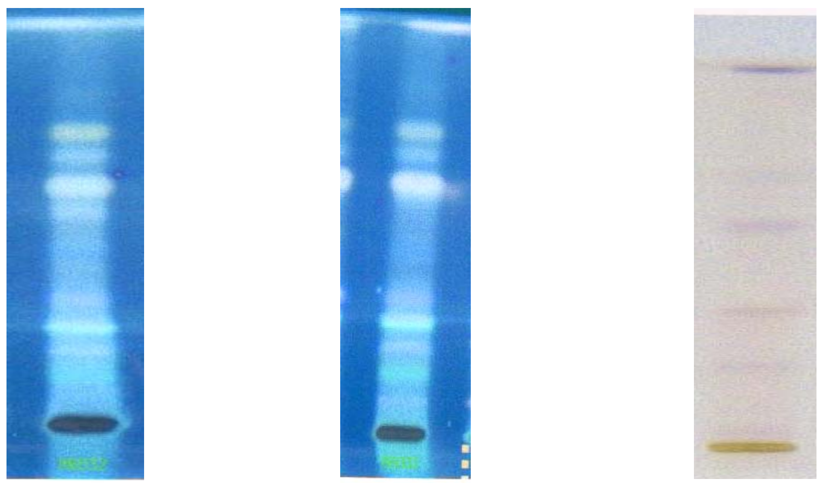


Figure 3. Number of peaks and AUC (Area under curve) of different allelochemicals detected in post harvest residues of sugarcane cv. CoC 671 under 6^{th} ratoon in high sugar recovery zone.



10% Methanol of solution H_2SO_4 | LB Reagent | Anisaldehyde Reagent

Allelochemicals detected with different reagents in cv. CoC 671 under 6th ratoon (MRZ)

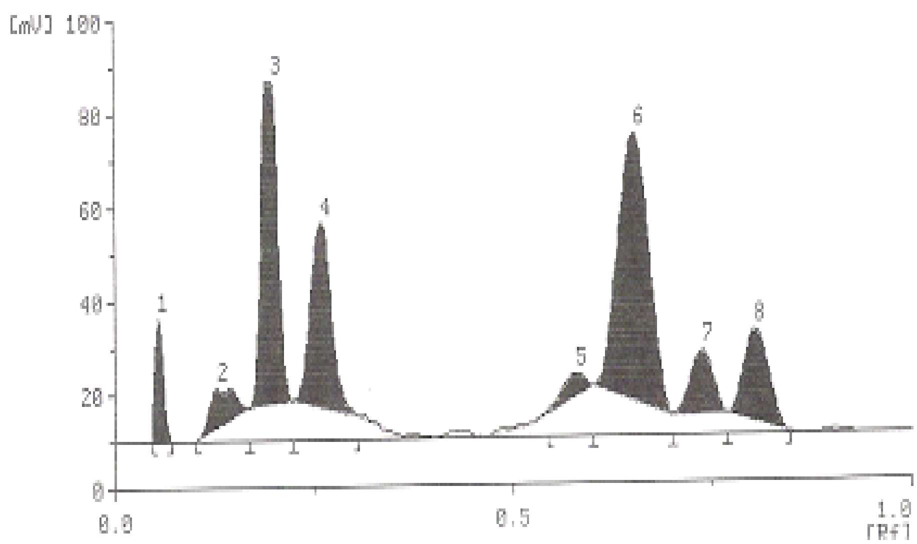
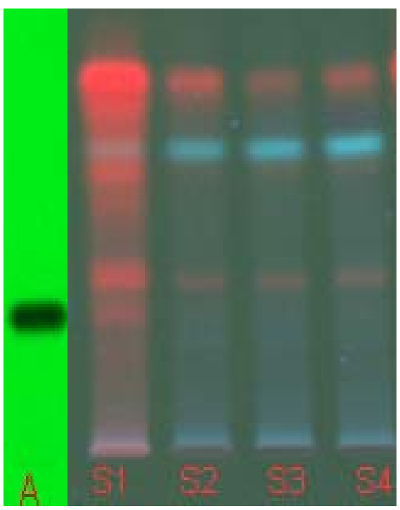


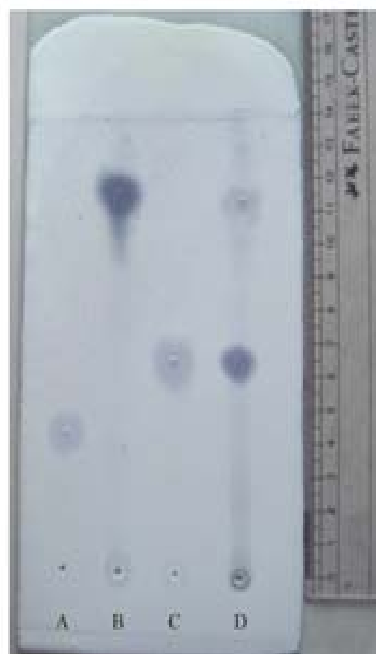
Figure 4. Number of peaks and AUC of different allelochemicals detected in the post harvest residues of sugarcane cv. CoC 671 under 6th ratoon in medium sugar recovery zone.

plates by the number of bands with different colours developed by 10% solution of sulphuric acid in methanol, Liebermann-Burchard reagent and anisaldehyde-sulphuric acid reagent. These results suggested that sugarcane residues contain allelochemicals other than phenolic compounds (25). These substances were also found in sugarcane post harvest residue (Fig. 5). Plate 3 showed that *p*-hydroxybenzoic acid, ferulic acid and other phenolic compounds were found in plant cane and in third, fifth and sixth ratoons (Fig. 7). The sugarcane allelopathy research workers (8,18,19,20,21,26) had also detected ferulic, vanillic and syringic acids, *p*-hydroxybenzoic and *p*-coumaric acids in sugarcane straw, senescent leaves and decomposing residues. These allelochemicals (i) modify the permeability of root cell membranes and decreases the energy metabolism, (ii) leading to inhibition in cell division, root hairs and branching and (iii) also adversely influences the nutrient uptake, protein synthesis, photosynthesis, respiration, enzyme activities, hormonal balance and water potential. All such alterations negatively influence the growth and yield attributes of ratoon cane under prolonged cultivation.



Allelochemicals detected with tetrazotized benzidine reagent

A: *p*-hydroxybenzoic acid
S1: Plant cane
S2: 3rd ratoon
S3: 5th ratoon
S4: 6th ratoon



A- Cinnamic acid (Std)
B- Ferulic acid (Std)
C- p-hydroxybenzoic acid (std)
D- Sugarcane post harvest residues

Phenolic compounds detected in trash of CoC 671 on TLC plate

Figure 5. Phenolic compounds detected in the post harvest residues of sugarcane after 3rd, 5th and 6th ratoon of cv. CoC 671

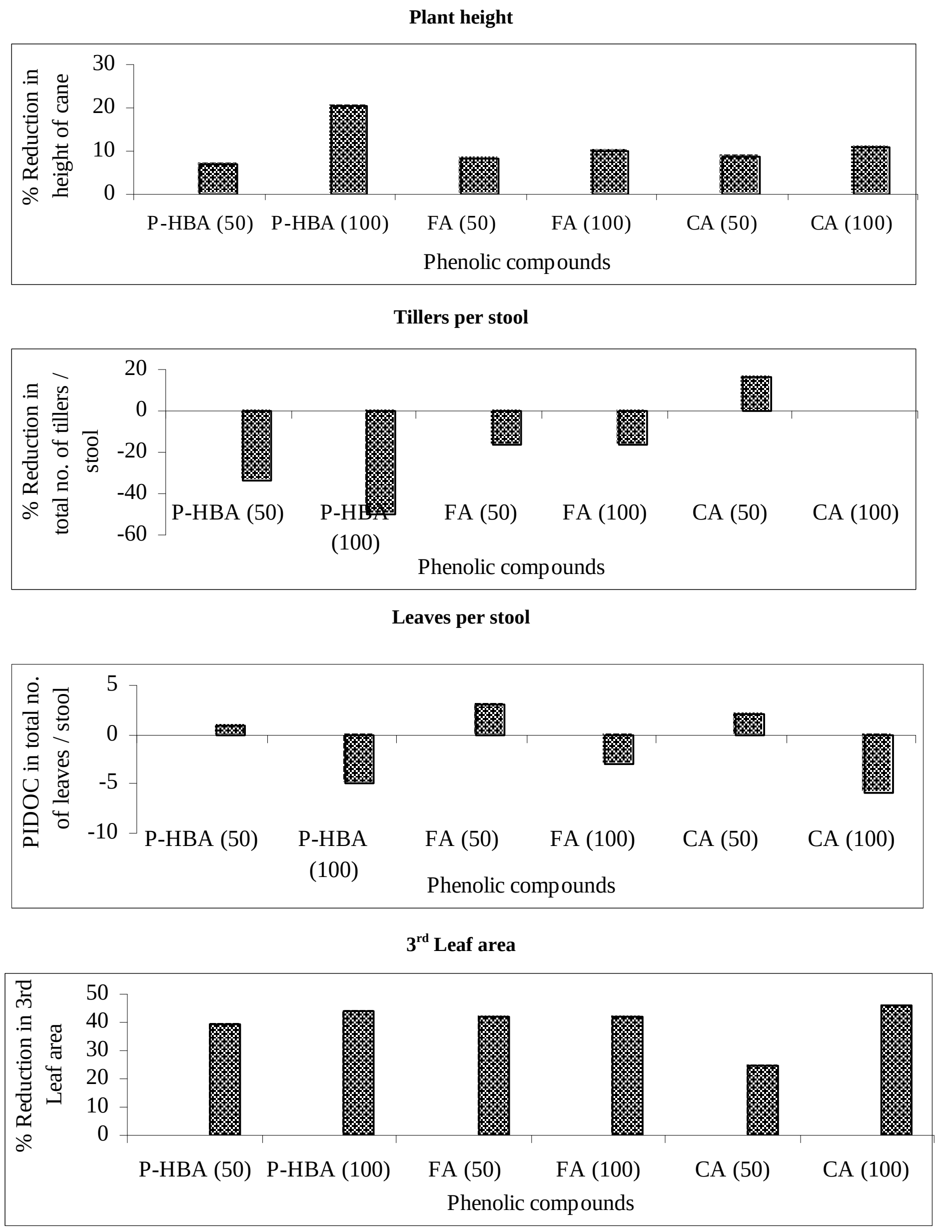


Figure 6. Effect of different phenolics applied to soil on growth parameters of sugarcane plantlets in cv. Co 86032, where P-BHA- P-hydroxybenzoic acid, FA- Ferulic acid, CA- Cinnamic acid

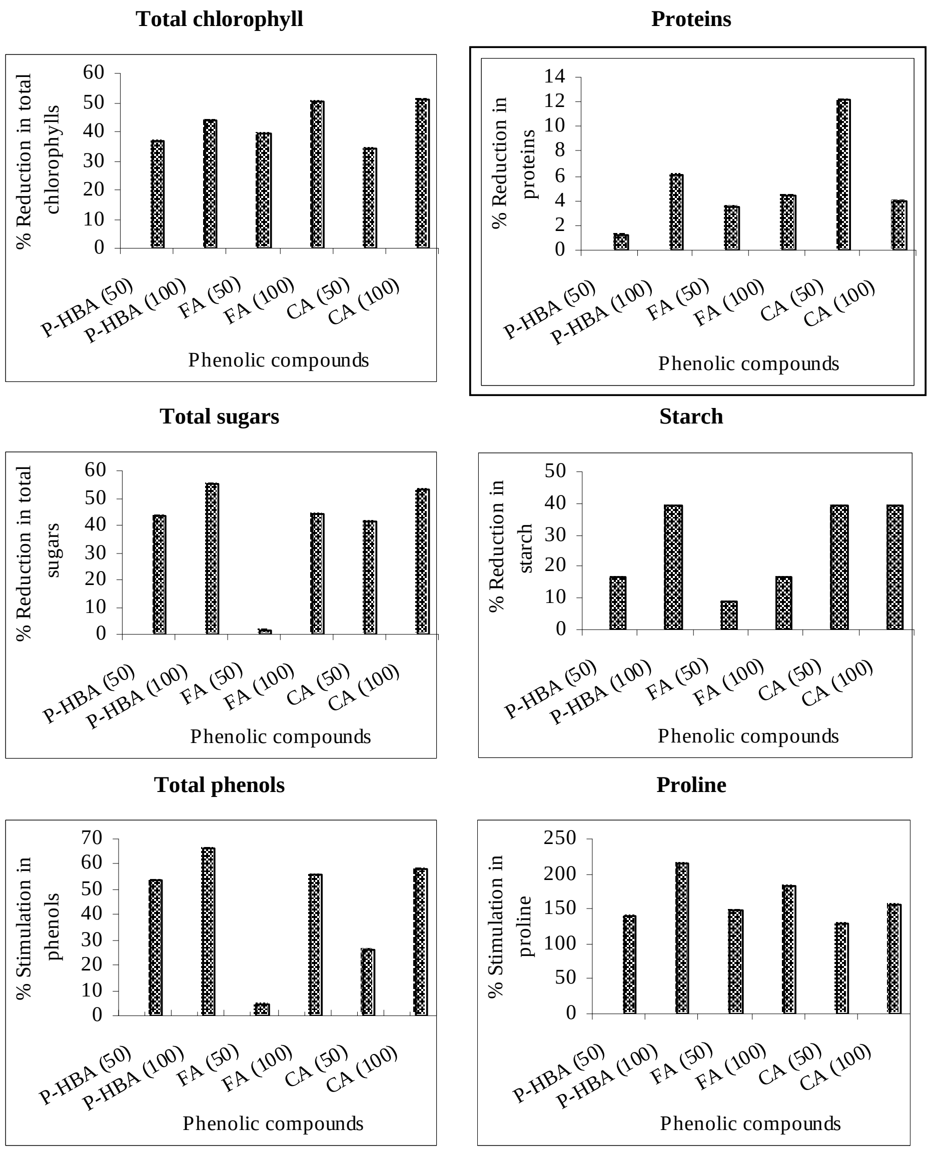


Figure 7. Effect of application to soil of different phenolics on organic constituents in the leaves of sugarcane plantlet in cv. Co 86032. Where P-BHA- P-hydroxybenzoic acid, FA- Ferulic acid, CA- Cinnamic acid.

Influence of phenolic compounds on growth and physiology of sugarcane plantlets

The *p*-hydroxybenzoic, ferulic and cinnamic acids, previously identified in the sugarcane post harvest residues were used to irrigated the plantlets of sugarcane cultivar Co 86032 at concentrations of 50 and 100 ppm. These compounds had negative effects on growth parameters (height of cane, number of tillers, number of leaves per stool and leaf area) (Fig. 6). Phenolic acids also decrease the photosynthetic pigments, proteins, total sugars and starch contents (Fig. 7). These results are in agreement with those obtained in monoculture of tomato (22). Contrarily, phenols and proline contents were increased significantly due to the treatments with phenolic acids.

Our results suggested that the autotoxicity in sugarcane during prolonged cultivation is mainly due to the allelochemicals found in the sugarcane post harvest residues. The allelochemicals released from the residues and exuded from old root system of ratoon canes might accumulate in the soil affecting sugarcane growth. These results are in agreement with many other workers (8,10,20,21,29). Allelochemicals like phenolic compounds accumulates in the soil under multiple ratooning and they decreased the cane yield, due to poor sprouting of new tillers and stunted growth of ratoon cane (12).

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