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"Autotoxicity and Yield Decline in Sugarcane under Monocropping in Pune District, Maharashtra, India"

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Abstract: *Sugarcane is an industrial cash crop in India. Maharashtra is a leading state in sugar production. Monocropping is the most common practice in almost all the sugar producing countries of the world, including India. India is the largest producer of sugarcane as it is profitable crop.*

Present work deals the study of drastic reduction in cane and sugarcane yield during monocropping is attributed because of allelopathic potential of sugarcane trash (dried leaves), decomposing of trash, releasing many allelochemicals, exudation of phytotoxic chemicals from old roots of ratoon cane, soil sickness, alteration in enzyme activities and autotoxicity.

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I. Introduction

Sugarcane (Saccharum officinarum, L.)**:**

Sugarcane is an important industrial crop cultivated in tropical and subtropical areas of more than 121 different countries in the world. Mainly it is for sugar and biofuel production. This wonder cash crop is the most efficient solar energy harvester and highly stress tolerant, which is planted by stem cuttings (sugarcane setts) with one to three eye buds. These on germination develop in to millable stalks within 12 to 18 months. These canes are harvested at maturity and brought the factories for crushing, at the time of harvesting the millable canes are cleaned in the field due to which five to eight tones of trash (dried leaves) per hectare are left behind in the field. The utilization/ disposal of this trash is a great problem for the cane growers and hence either they cut it into small pieces and use as soil amendments to the second ratoon or they burn the whole trash in the field of self and start growing ratoon crop.

Monocropping of sugarcane:

Apart from adsali, pre-seasonal and suru, ratooning is the main practice for sugarcane cultivation throughout the world, including India, as it is highly economical and profitable to the farmers, as well as to the sugar factories, because of low cost of cultivation and higher recovery. Several factors like soil sickness, accumulation of phototoxic allelochemicals in the soil, autotoxicity etc. are the main reasons for this (Sampietro, 2006 c, d, 2007 c).

In ratoon crop, the decomposition of trash releases many phytotoxic secondary metabolites like p-hydroxy benzoic acid, p-courmaric acid, 2, 4, dihydroxy benzonazilone and benzonazinone, which affect root as well as shoot growth of new tillers and finally reduce the yield according to Gupta (1981). In general, the productivity of ratoon crops is very low as compared to plant cane (Mujumdar and Gangai, 2007). The perusal of information on monoculturing clearly indicated that autotoxicity is the main culprit of multi-ratooning of sugarcane.

Autotoxicity in sugarcane:

According to Chou and Lin (1976) autotoxicity during monoculturing of sugarcane was mainly due to allelopathy, which causes the decline in cane population and cane yield. Wang et al. (1984) suggested that the allelochemicals like ferulic, p-hydroxybenzoic, vanilllic, syringic and p-coumaric acids existing in the sugarcane leaves, leachates of straw and decomposing roots were responsible for autotoxicity. Similar was the explaination given by Sampietro et al. (2007 c). The autotoxicity is very well observed in the ratoon cane field, through creation of big gaps.

The practice of ratooning was mainly discouraged only because of this drawback. The autotoxic allelochemicals are responsible for 60% or greater mortality of tillers in ratoon cane, causing great loss to the farmers and sugar factories. As a result of this in the state of Maharashtra only first or at the most second ratoon is under taken.

Antioxidants and osmolytes:

Under biotic as well as abiotic stress conditions reactive oxygen species (ROS) or active oxygen species (AOS) are generated, which cause cellular damage and death of the plant itself. Amongst the varied types of mechanisms in plants for protection against ROS, the antioxidants and antioxidant enzymes work as very effective and efficient mechanism.

II. Material and Methods

1. Selection of sites – Pune District

i. A village Malegaon, from the area of Malegaon Sahakari Sakhar Karkhana, Limited, Malegaon Bk. (Shivnagar), Tal. Baramati, district Pune was selected.
ii. A village Ozar, from the area of Shri Vighnahar Sahakari Sakhar Karkhana, Limited, Nivritinagar, Shiroli, Tal. Junnar, district Pune was selected.

2. Selection of sugarcane cultivar – Co 86032

3. Selection of experimental ratoon cane field-

i. The selected ratoon field was belonging to farmer Mr. Rajendra Jadhav. The total area under ratooning was about one hectare and maximum frequency of ratooning was upto sixth ratoon.
ii. Second ratoon field was belonging to farmer Mr. Yogesh Anatrao Pokharkar. The total area under ratooning was about Six hectare and maximum frequency of ratooning was upto sixth ratoon.

4. Collection of green and dry leaf samples of sugarcane under monocropping. -

The randomly selected third leaf from top, which is physiologically active, was collected from twenty five different plants Co 86032 at the age of eight months, The composite leaf samples were representing plant cane, 2nd, 3rd, 5th and 6th ratoon.

5. Physiological, biochemical and antioxidants analysis of green and dry leaf samples of sugarcane under monocropping.

i. Total chlorophylls were estimated as per the method of Arnon (1949)
ii. The amounts of soluble proteins were estimated by employing Lowry et al. (1951) method.
iii. Total carbohydrates were determined according to the method of Thayumanavan and Sadasivam (1984)
iv. The proline content in composite leaf samples was determined by following the method of Bates et al. (1973).
v. Glycine betaine was estimated as per the method of Ishitani et al. (1993).
vi. The lipid peroxidation was measured in terms of malondialdehyde (MDA) by using Heath and Packer (1968) method.

III. Result

Metabolic activities attributes under monocropping: Photosynthetic pigments:

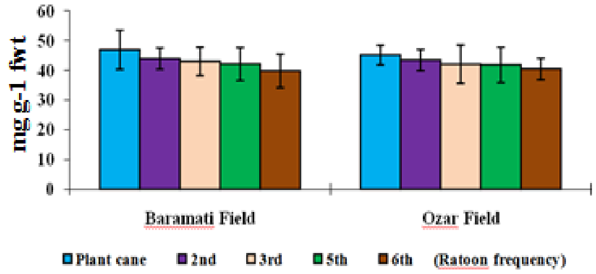
Table 1: Photosynthetic pigments in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monocropping.

Frequency of ratooning	**Total chlorophylls (mg g-1)**	
	Baramati field	**Ozar field**
Plant cane	3.47 ± 0.31	3.15± 0.28
2nd	3.20 ±0.48	2.88 ± 0.26
3rd	3.17± 0.44	2.78 ± 0.36
5th	2.81 ± 0.22	2.51 ± 0.15
6th	2.63 ±0.18	2.3 9 ±0.26
*LSD **0.05**	0.46	0.36
Significance	**	**

The results shown in Table 1 indicated that the range of reduction in total chlorophyll pigments, with respect to plant cane in Baramati field was in order of 6th ratoon (2.63) > 5th (2.81) > 3rd (3.17) > 2nd (3.20) > plant cane (3.47 mg g-1 fwt).While this range of reduction in Ozar field was in order of 6th ratoon (2.52) > 5th (2.71) > 3rd (2.88) > 2nd (3.10) > plant cane (3.20 mg g-1 fwt).

Proteins:

Fig 1: Effect of monocropping on protein content in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under mono-culturing.



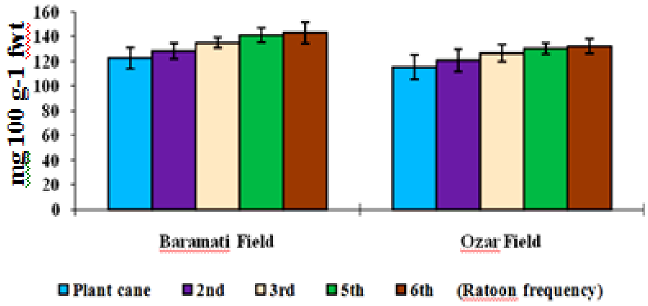
	Baramati Field	Ozar Field
LSD 0.05	3.405	--
Significanc e	**	ns

Column data are mean values (n=5) with error bars as standard deviation. '*', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.

The results recorded in Fig. 1 revealed that protein contents were significantly decreased in both the fields, with increase in number of multi-ratooning. The results are highly significant.

Total sugars:

Fig 2: Effect of monocropping on total sugars in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monocropping.



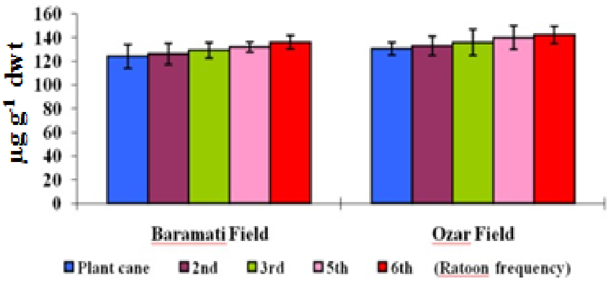
	Baramati Field	Ozar Field
LSD 0.05	9.90	10.40
Significance	**	*

#Column data are mean values (n=5) with error bars as standard deviation. '*', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.

The results explained in Fig. 2 clearly indicated significant and successive increase in total sugars in the leaves of cultivar of both fields as compared to plant cane. The increase in total sugars in ratoon was statistically significant at p<0.05.

Antioxidants and Osmolytes:
Proline, Glycine betaine and Lipid peroxidation:
Proline

Fig 3: Effect of monocropping on proline in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monocropping.



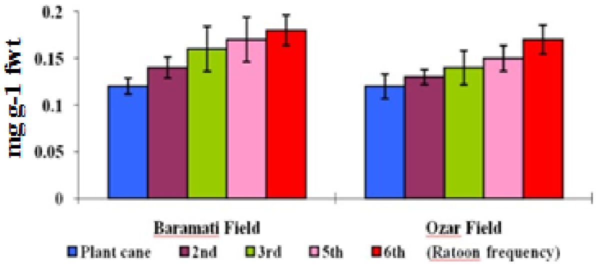
	Baramati Field	Ozar Field
LSD 0.05	--	--
Significance	ns	ns

#Column data are mean values (n=5) with error bars as standard deviation. '*', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.

The results presented in Fig. 3 indicated that proline was not significantly accumulated with increasing frequency of ratoon. But all the results are non significant.

Glycine betaine :

Fig 4: Effect of monocropping on glycine betaine in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monocropping.

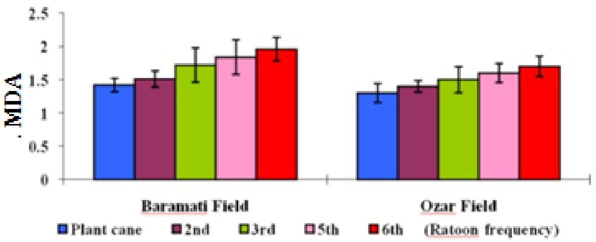


	Baramati Field	Ozar Field
LSD $_{0.05}$	0.024	0.019
Significance	**	**

Column data are mean values (n=5) with error bars as standard deviation. '*', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.
The results illustrated in Fig. 4 revealed that the range of increase in glycine betaine from Baramati field in plant cane, second, third, fifth and sixth ratoon was 0.11 to 0.16 and 0.12 to 0.18 (mg g-1 fwt) respectively. However the range in Ozar field was 0.12 to 0.16 and 0.12 to 0.17(mg g-1 fwt). All the results are statistically significant.

Lipid peroxidation :

Fig 6: Effect of **monocropping** on lipid peroxidation in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under **monocropping**



	Baramati Field	Ozar Field
LSD $_{0.05}$	0.26	0.20
Significance	**	**

#Column data are mean values (n=5) with error bars as standard deviation. '*', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.
The results depicted in Fig. 5 clearly showed that **monocropping** was responsible for increasing the lipid peroxidation (in terms of MDA content) in Co 86032 of both fields. The maximum value was noted in sixth ratoon as compared to other ratoons and plant cane. The results are statistically significant.

IV. Discussion

Effect of Monocropping on photosynthetic pigments and organic constituents:

These results are in agreement with many allelopathy workers, who have also reported decrease in photosynthetic pigments in different plants due to allelopathic influence of different types of allelochemicals, released through leachates. The reduction in photosynthetic pigments like chlorophyll a, b and total chlorophylls was reported Padhy et al. (2000), and Al-Wakeel et al. (2007) in oat, wheat, lentil, sorghum, fingermillet, rice, peanut, black gram, mungbean, mustard and pea due to leaf extracts/leachates/residues of wheat, Rhamnus, Eucalyptus, Wedelia, Andrographis, Ficus, Ageratum, Acacia and Eucalyptus respectively.

The increase or decrease in chlorophyll pigments is also attributed to the allelopathic influence of monoculturing. The variety of allelochemicals and phytotoxic chemicals released through the sugarcane trash and exudates through its old root system and decomposing residues, might be adversely affecting the contents of photosynthetic pigments.

Organic constituents:

The organic constituents like amino acids, proteins, carbohydrates are the important indicators of metabolic status of crop plants, which are usually influenced by adverse environmental factors, stress conditions and allelochemicals. Blum (1996, 1998) had also proposed similar view and indicated that the studies on production of different primary and secondary metabolites help to explain the inhibition or stimulation of their biosynthetic pathways, due to different types of external factors including allelochemicals.

Proteins:

The results recorded in Fig. 1 revealed that protein contents were significantly decreased in both the cultivar of both fields with increase in the frequency of monoculturing from 2nd to 6th ratoon as compared to plant cane.

The above results corroborate with the finding of several workers like Al- Khatib and Abd-Elaah (1999), Singh and Singh (2003), Bhakat et al. (2005) and Thapar and Singh (2006) who recorded significant decrease in protein contents in various crop plants like mungbean, sorghum and horsegram due to higher concentrations of leaf extracts/leachates of Parthenium, Eucalyptus and Cassia. Similarly Prasad and Subhashini (1994) had clearly showed adverse impact of allelochemicals on protein synthesis.

The decline in leaf protein content in sugarcane cultivar of both fields under monoculturing might be responsible for the reduction in cane height and weight, as proteins are basic building blocks of plant body (Nelson and Michael, 2005). The reduction in protein content during ratooning might be due to its faster utilization for tiller development, as the number of tillers per stool increased significantly under ratooning. The inhibition of protein synthesis due to allelochemicals and phenolics present in the leaves and root exudates might be the main factor for the decrease in protein contents. The work of Schuab et al. (2001), Sukul and Chaudhuri (2001) was in agreement with the above findings.

Total sugars:

The results presented in Fig. 2 indicated that the total sugars were increased with sequential increase in frequency of ratooning. The increase was more in cultivar of Baramati field than Ozar field.

The results of the present study were in agreement with Sundara (1999), Tripathi et al. (2000), Padhy et al. (2000), Bhalerao et al. (2000), Singh and Singh (2003), Bhakat et al. (2005, 2006), Al-Wakeel et al. (2007). They had reported significant increase in total carbohydrates in different crops like sugarcane, Vigna, Capsicum, fingermillet, mungbean, horse gram, maize, pea etc. due to application of leaf extracts/leachates and residues of sugarcane, Acacia, Dalbergia, Parthenium and Eupatorium. Same may be true for the increased total sugar contents in the leaves of sugarcane cultivar of both fields. The long duration crop like sugarcane might be adding several types of allelochemicals and harvesting must be releasing different types of phenols, causing alteration in carbohydrate metabolism.

Effect of multi-ratooning on antioxidants and oxidant enzymes:

Proline:

According to Sampietro (2006 d) proline accumulation may indicate stress response to allelochemicals. Sampietro et al. (2007 a) also reported that leaching of allelochemicals from sugarcane straw caused increase in leaf proline content of Brassica and Sida species. Sampietro et al. (2007 b) further claimed that proline accumulation was regarded as a direct effect of organic molecules, released from straw leachates. All these clearly suggest that proline accumulation is a stress response induced by allelochemicals in sugarcane.

Thus an estimation of free proline could serve as a valuable indicator of stress resistance in cane varieties under multi-ratooning.

Glycine betaine:

The results of present investigation on glycine betaine (Fig. 4) had shown maximum accumulation in sugarcane cultivar, grown under different frequencies of ratooning, in both fields. But the increase of GB was more in cutivar of Baramati field than Ozar field.

Similar results were reported by Rathinasabapathi et al. (1997), Yang et al. (2002), Deshmukh and Dhumal (2005) and Hase et al. (2006 b) in sorghum, mungbean, chillies and sugarcane during different abiotic stress conditions. Yang (1990) reported that almost all the cereal crops including sorghum accumulate GB under stress except rice and its levels vary both among and within species. Wood et al. (1996) also reported that sorghum genotypes differ in the accumulation of GB under stress conditions. The results of present investigation are in agreement with above findings.

Lipid peroxidation:

The results explained in Fig. 5 clearly revealed that increased lipid peroxidation (in terms of MDA content) was observed with increasing frequency of ratooning in the cultivar both fields. The maximum lipid peroxidation was reported in sixth ratoon as compared to other ratoons and plant cane.

The findings of Sampietro et al. (2007 a) also indicated increase in lipid peroxidation of sugarcane due to different types of allelochemicals present in its straw. The results of present study are in agreement with them. During multi- ratooning the quantity of sugarcane trash is added into the soil at the end of every harvesting, which caused the increase in the level of different allelochemicals, which had influenced the lipid peroxidation.

The autotoxicity and death of tillers in multi-ratooning of sugarcane may be attributed to increased LPO as suggested by the above workers. The decline in cane population per hectare is the major cause of yield reduction in ratoon sugarcane, which is due to tiller mortality under multi-ratooning.

V. Conclusion

Sugarcane is the major cash crop in the state of Maharashtra, but multiratooning has became the major constrain for the growth of this second largest industry in India. Monoculturing of sugarcane is a routine practice in India and even in the state of Maharashtra. It is highly profitable, but soil sickness and autotoxicity are response for the drastic reduction in cane yield, which discouraged the farmers to under take ratoon cultivation. As a result of this the area under ratoon is comparatively less than plant cane.

The soil sickness in ratoon was mainly due to allelochemicals, released from trash, green leaves and old root system (Gupta, 1981). These caused severe changes in the physical, physiological, biological and enzymological properties of soil, leading to autotoxicity, which is playing a key role in the crucial issue of yield reduction.

Many research workers like Putnam and Duke (1978), Fisher (1979) and Inderjit and Weiner (2001) have claimed that allelochemicals change the growth patterns, physiology of ratoon cane plant and inhibit the growth leading to reduction.

The leaves of plant cane had higher contents of proteins and starch than successively increasing number of ratoons (from 2^{nd} to 6^{th}). However there was considerable increase in total sugars as compared to plant cane in the cultivar of both fields. This reduction in both organic constituents might be due to the retardation in biosynthetic processes or enhanced degradation, caused by the synergistic effects of different allelochemicals released through trash leachates or its decomposition and root exudation. The increase in total sugars might be due to stimulated degradation of starch or allelobiogenesis stress caused by various allelochemicals.

The allelobiogenesis (stress developed due to allelochemicals) had also lead to significant accumulation of different antioxidants like proline and glycine betaine which enhanced with increasing number of ratoons cultivated. These play a major role in scavenging of ROS and also act as osmoprotectants or compatible solutes. The generated abiotic stress and allelobiogenesis by allelochemicals under multi-ratooning might be acting as signal to trigger the synthesis and accumulation of above mentioned antioxidants.
The abiotic stress in ratoon sugarcane crop was confirmed by increased lipid peroxidation in cultivar of both fields.

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