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Research Article

PHYSIOLOGICAL ATTRIBUTES FOR YIELD DECLINE IN SUGARCANE UNDER MONOCULTURING

Hase C.P^{1*} and Ghayal N.A²

¹Department of Botany, BaburaojiGholap College, Sangvi, Pune- 411027, M.S., India

²Department of Botany, MES AbasahebGarware College, Karve Road, Pune, 411004, M.S., India

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ABSTRACT

Sugarcane is a wonderful industrial cash crop which is most efficient solar energy harvester and highly stress tolerant. It is planted by stem cuttings (setts) ratooning is the main practice for sugarcane cultivation throughout the world. The productivity of sugarcane under multi-ratooning is declining by 30-50 % every year, due to reduction in cane population per hectare. To find out concrete reasons for this decline an attempt was made to analyze the alterations in physiological, biochemical and enzymological parameters in ratoon cane plants, under monoculturing. To accomplish this objective sugarcane cultivar - Co 86032 from two different sites under ratooning was used. For all analyses standard physiological procedures were used. The results revealed that plant cane had higher contents of proteins and starch than successively increasing number of ratoons while total sugars increased in amount. There was also observed gradually increasing rate of accumulation of different antioxidants like proline and glycine betaine along with ratooning. It had stimulated the activities of antioxidant enzymes like SOD, POD and PPO in cultivar of both fields under monoculturing. The significant changes in physiological, biochemical and enzymological parameters in ratoon cane plants could be assigned to allelobiogenesis stress caused due to synergistic effects of allelochemicals produced during ratooning.

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INTRODUCTION

Sugarcane is an important industrial crop cultivated in tropical and subtropical areas of more than 121 different countries in the world, mainly for sugar and biofuel production. This cash crop is planted by stem cuttings (setts). Due to millable stalks raised within 12 to 18 months, 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.

In addition to *adsali*, pre-seasonal and *suru*, ratooning is the main practice for sugarcane cultivation which is highly eco - profitable to the farmers and sugar factories, because of low cost of cultivation and higher recovery. But the productivity of sugarcane under multi-ratooning goes on declining by almost 30-50 % every year, due to reduction in cane population (Sundara, 1999). Several factors like soil sickness, drastic changes in physiological, biochemical and enzymological parameters in ratoon cane plants, accumulation of phytotoxic allelochemicals in the soil, autotoxicity etc. are the main

reasons for this (Sampietro, 2006 a, b, 2007 a). 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.

The allelochemicals released through root system and sugarcane trash accumulate in the soil and generate very specific types of abiotic stress, known as allelobiogenesis (Dhumal, 2007). It adversely affects basic metabolic processes and enzymatic activities in sugarcane, grown under monoculturing. The intensity of this stress inhibits photosynthesis, carbohydrate metabolism, protein synthesis, respiration and secondary metabolic processes. The plants under stress generate reactive oxygen species (ROS), which cause cellular damage. To avoid this, plant produces various types of antioxidants like proline, glycine betaine, phenolics Muller *et al* (1989) etc. Proline acts as osmoprotectant, while phenolics accumulate in soil and affect various metabolic processes in cane plants.

It also shows stimulation in the activities of ROS scavenging enzymes like SOD, POD and PPO. Such biocatalysts protect the plants from stress injuries. Their level and changes in the

*Corresponding author: Hase C.P

Department of Botany, BaburaojiGholap College, Sangvi, Pune- 411027, M.S., India

activities indicate stress tolerance or susceptibility of the plants. The alterations in over all metabolic processes affect growth and yield. Considering all these aspects an attempt was made to determine the effects of physiological, biochemical and enzymological factors of the cane during multi-ratooning and understand the causes of yield reduction of the ratoon cane.

MATERIALS AND METHODS

- Selection of sites**-Both the selected sites were from Pune District.
 - A cropfield from village Malegaon, Malegaon Bk. (Shivnagar), Tal. Baramati, District Pune.
 - A cropfield from village Ozar, Nivritinagar, Shirol, Tal. Junnar, District Pune
- Selection of sugarcane cultivar**-Co 86032
- Selection of experimental ratoon cane field**
 - The selected ratoon field had total area under ratooning about one hectare and maximum frequency of ratooning upto sixth ratoon.
 - Second ratoon field had more area under ratooning about six hectare and maximum frequency of ratooning upto sixth ratoon.
- 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 ratoons.
- Physiological and biochemical analysis of green and dry leaf samples of sugarcane under monocropping**.
 - Total chlorophylls were estimated as per the method of Arnon (1949)
 - The amounts of soluble proteins were estimated by employing Lowry *et al* (1951) method.
 - Total carbohydrates were determined according to the method of Thayumanavan and Sadasivam (1984)
 - The proline content in composite leaf samples was determined by following the method of Bates *et al*. (1973).
 - Glycine betaine was estimated as per the method of Ishitani *et al*. (1993).
 - The lipid peroxidation was measured in terms of malondialdehyde (MDA) by using Heath and Packer (1968) method.
 - The activity of SOD was determined according to method of Dhindsa *et al* (1981).
 - The activity of POX was assayed by using Vidyasekharan and Durairaj (1973) method.
 - The activity of PPO was assayed as per the method of Vidyasekharan and Durairaj (1973).
- Statistical analyses**-The data were summarized as the pooled means of three replicates each over two years with standard deviation as the measures of variability. LSD at $p = 0.05$ was used to test significant difference of results between the means. The statistical analysis was carried out by using Sigmastat 3.5 (SYSTAT) and Microsoft Excel 2007.

RESULTS AND DISCUSSION

Metabolic activities under monoculturing

Results on Photosynthetic pigments: 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).

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

Frequency of ratooning	Total chlorophylls (mg g ⁻¹)	
	Baramati field	Ozar field
Plant cane	3.47±0.31	3.15±0.28
2 nd	3.20±0.48	2.88±0.26
3 rd	3.17±0.44	2.78±0.36
5 th	2.81±0.22	2.51±0.15
6 th	2.63±0.18	2.39±0.26
LSD _{0.05}	0.46	0.36
Significance	**	**

^aData are mean values (n=5) followed by \pm standard deviation. **, *** and 'ns' represent significance at $p < 0.05$, $p < 0.01$ and non-significance, respectively.

Discussion on Photosynthetic pigments

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), Ervin and Wetzel (2000), Singh *et al* (2002), Einhellig (2002), Singh and Singh (2003) and Al-Wakeel *et al*. (2007) in oat, wheat, lentil, sorghum, finger millet, 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.

Results on 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. As explained by Sampietro and Vattuone (2006 c) the accumulation or reduction of different primary metabolites and organic constituents may give an idea about the intensity and level of their allelopathic nature.

According to Zeng *et al.* (2001) understanding of the various physiological and biochemical processes, leading to synthesis of different organic compounds help to understand the allelopathic interaction on recipient plants. Similar view was proposed by the pioneer allelopathy workers like Rice (1984), Einhellig (1986, 1995) and Seigler (1996). They highly emphasized the importance of physiological investigation in allelopathy.

Proteins: 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.

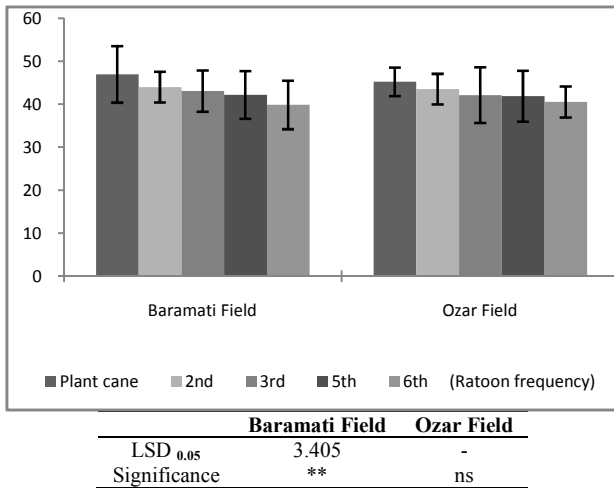


Fig 1 Effect of monoculturing on protein content in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

[#]Column data are mean values (n=5) with error bars as \pm 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 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 decreased protein content noted in the present investigations can be interpreted on the same line. Baziramakenga *et al.* (1994) and Al-Wakeel *et al.* (2007) reported that residues of *Acacia* containing phenolic compounds inhibited the rate of DNA and RNA synthesis, leading to retarded protein synthesis. The phenolic compounds present in the sugarcane trash and root system might have acted in the same manner, inhibiting the synthesis of DNA and RNA and there by proteins. The reduced protein content may be due to this.

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 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 $p < 0.05$.

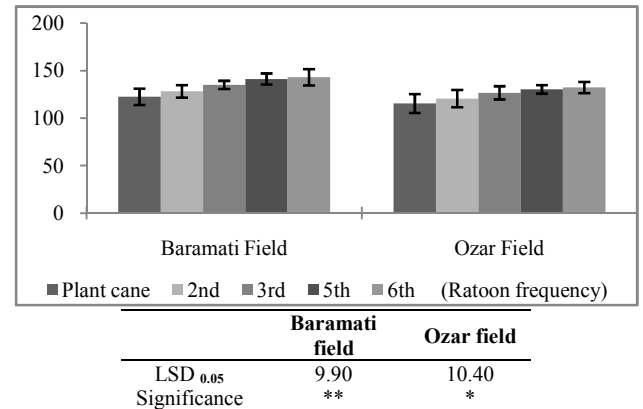


Fig 2 Effect of monoculturing on total sugars in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

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

Discussion on Organic constituents

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*, finger millet, 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.

Results on Effect of multi-ratooning on Antioxidants and osmolytes

Proline: 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: 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 ($\text{mg g}^{-1}\text{fw}$) respectively. However the range in Ozar field was 0.12 to 0.16 and 0.12 to 0.17($\text{mg g}^{-1}\text{fw}$). All the results are statistically significant.

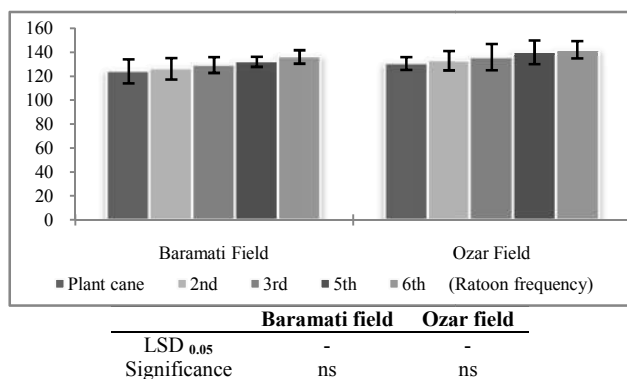


Fig 3 Effect of monoculturing on proline in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

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

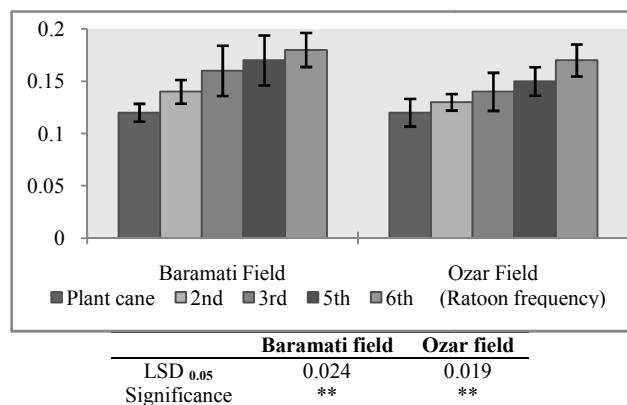


Fig 4 Effect of monoculturing on glycine betaine in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

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

Lipid peroxidation: The results depicted in Fig. 5 clearly showed that multi-ratooning 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.

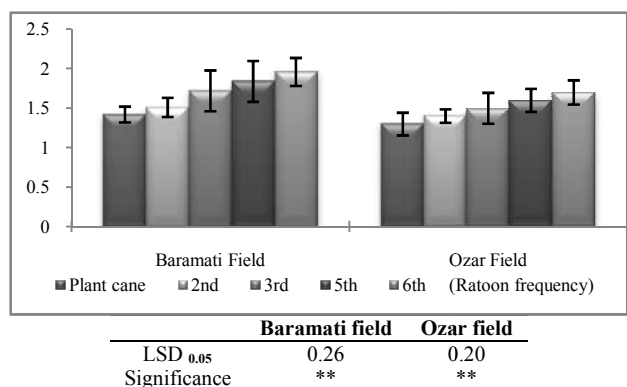


Fig 5 Effect of monoculturing on lipid peroxidation in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

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

Discussion on Effect of multi-ratooning on Antioxidants and osmolytes

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. Pawar (2004) noted significant increase in proline content due to the leachates of *Moringa*, *Melia*, *Parthenium* and *Glycine max* in sorghum seedlings.

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 cultivar 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. Same trend was detected by Zeng *et al.* (2001) in their studies on allelopathy in higher plants. They had also reported very high increase in lipid peroxidation in rape, rice and sorghum due to Secalonic acid F. Considering this it can be predicted that the enhanced lipid peroxidation might be due to different types of allelochemicals, accumulated in ratoon soil, which are released through root exudates and leached from trash and leaves.

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.

Results on ROS scavenging enzymes

The results explained in Fig. 6 clearly showed that the activity of enzyme SOD was significantly stimulated with increasing frequency of ratooning. All the results were highly significant. The significant results obtained for peroxidase activity illustrated in Fig. 7 revealed that there was very high stimulation in POD activity, which was directly correlating with the number of ratooning. Highest activity of peroxidase was recorded in sixth ratoon, which was followed by fifth, third and second ratoon as compared to plant cane.

The results presented in Fig. 8 indicated that polyphenol oxidase activity was significantly stimulated in Baramati field. The values of increased activity of PPO in that field was in the order of 6th > fifth > third > second ratoon > plant cane. All the results were highly significant.

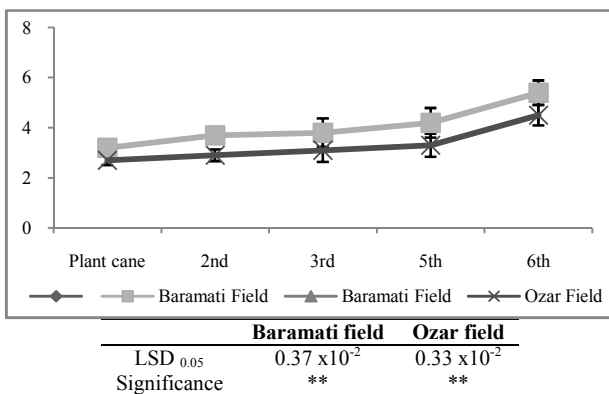


Fig 6 Effect of monoculturing on activity of super oxide dismutase (SOD) in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

#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.

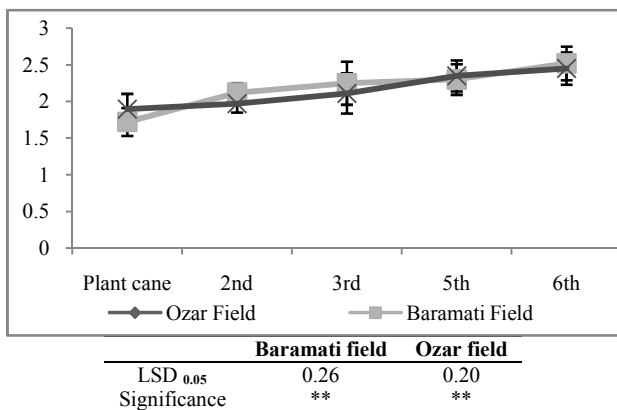


Fig 7 Effect of monoculturing on activity of peroxidase in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

#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.

Discussion on ROS scavenging mechanism of enzymes

Any type of biotic or abiotic stress leads to generation of free radicals or active oxygen species (AOS) or reactive oxygen species (ROS), which initiates cellular damages and cause the death of stressed plants. But it is effectively and very efficiently prevented by the plants through ROS scavenging mechanisms i. e. antioxidant enzymes.

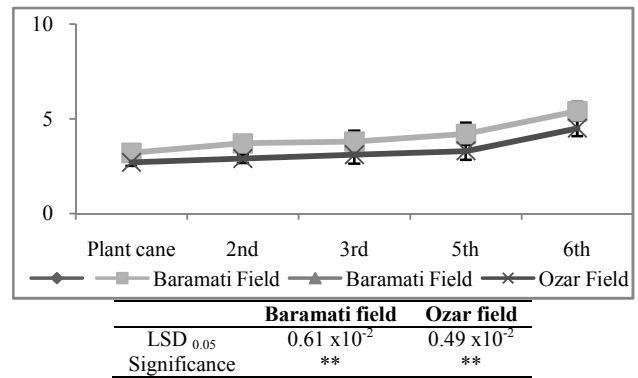


Fig 8 Effect of monoculturing on activity of polyphenol oxidase in the leaves of sugarcane cultivar Co 86032 from Baramati and Ozar fields under monoculturing.

#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 on activities of antioxidant enzymes in the leaves of sugarcane cultivar of both fields indicated significant stimulation in activities of POD, PPO and SOD. However, amongst these three enzymes stimulation in PPO activity was higher as compared to other enzymes. The stimulation in activity of POD was very low in both the cultivars as compared to SOD and PPO. The stimulation in PPO in Co 86032 of Baramati field was more than field of Ozar.

The enzymes like SOD, POD and PPO are known as the common antioxidant enzymes, because they scavenge the free radicals generated during stress conditions (Yim *et al.*, 1990, Lee *et al.*, 2003, Thippeswamy *et al.*, 2005, Joshi *et al.*, 2005, Deshmukh, 2005) and disparity in SOD and POD activities directly correlated with lipid peroxidation (Keppler and Novacky, 1987).

The research workers like Weir *et al* (2004) during their studies on biochemical and physiological mechanisms mediated by allelochemicals, indicated increase as well as decrease in SOD and POD activity. Yu *et al* (2003) claimed that the stimulation or inhibition in activities of POD and SOD was influenced by phenolic compounds and other allelochemicals. Similar explanations were given by Zeng *et al* (2001). They ascribed that secalonin acid F, significantly reduced the SOD and POD activity in several plants. Politycka (1996), Yu *et al* (2003) claimed that cinnamic acid had significant effects on the activities of POD and SOD scavenging the active oxygen species.

The work of Singh *et al* (2008) indicated that autotoxicity caused by *Lycopersicon esculentum* induced very high stimulation in catalase activity, but retardation of SOD and POD activities. The stimulation or inhibition was varying according to concentrations of root, stem and leaf leachates. They further explained that stimulated CAT activity might be due to increased photorespiratory activity under the influence of phytotoxins. The above workers had given clear cut message that alterations in activities of antioxidant enzymes and antioxidant compounds resulted in accumulation of toxic chemicals, which caused impaired metabolic activities and in turn inhibited the growth and development of the plants.

Similar were the results of present study, the positive changes in antioxidant compounds and enzymes under sugarcane

monoculturing might be responsible for inhibited growth and development of ratoon canes of both the varieties. These results were supported by Luo *et al.* (1995), Lin *et al.* (2001) and Luo (2005).

CONCLUSION

Sugarcane is the major cash crop, but multi-ratooning has become the major constrain for its growth. The soil sickness caused during ratoons mainly due to allelochemicals, released from trash, green leaves and old root systems of the canes. The generated abiotic stress and allelobiogenesis by allelochemicals under multi-ratooning might be acting as signal to affect photosynthesis in ratoon canes along with organic constituents and might trigger the synthesis and accumulation of antioxidants and antioxidant enzymes.

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