Research Article

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Ethylene stimulation aggravates tapping panel dryness in partially affected *Hevea* trees

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Abstract

Commercially available ethylene stimulants are widely used in rubber plantations to enhance crop productivity irrespective of the physiological status of the trees. A sudden stress response was noticed in *Hevea* trees (clone RRII 105) immediately after a single application of ethephon as evidenced by the variations in several biochemical components such as hydrogen peroxide (H_2O_2), cyanide (CN), malondealdehyde (MDA), peroxidase (PX), β-cyanoalanine synthase (β-CAS) etc. in the laticiferous tissues of both healthy and tapping panel dryness (TPD) affected trees. Compared to normal healthy trees, ethephon treatment could enhance the levels of toxic compounds such as H_2O_2 and CN in the bark tissues of TPD affected trees. These toxic molecules are generally being removed from the tissue by the appropriate scavenging enzymes in the tissues of normal healthy trees. Whereas those trees which are experiencing partial panel dryness symptoms showed accumulation of these toxic compounds due to the inadequate levels of scavenging enzymes. Eventually accumulation of such toxic compounds leads to adverse physiological processes in the tissue, including incidence of TPD. Although stimulation enhanced the latex productivity of the plant, the present study demonstrates the deleterious effects of stimulant in the tapping panel of TPD affected trees. Usage of ethephon in trees that are partially affected with TPD resulted in further reduction of latex production and aggravation of TPD incidence.

Keywords: Ethephon, free radical scavenging, *Hevea brasiliensis*, tapping panel dryness

Introduction

Since the global requirement of natural rubber (NR) ... high, marked increase in crop productivity has been realized through adopting appropriate exploitation (tapping) system and incorporating effective methods of yield stimulation. In order to increase the latex production in *Hevea*, application of a wide range of chemical stimulants were experimented on the tree bark. Generally all these chemicals are ethylene generators. 2-chloro ethyl phosphonic acid, commercially known as "ethephon", is one of those chemicals frequently used in rubber plantations for yield enhancement. Ethephon application can increase the latex flow and thereby increase the total latex volume upon tapping (Ho and Paardekooper, 1965). The frequency of stimulant application can be regulated according to the type of clone and its inherent yield potential. However, frequent stimulation with ethylene compounds is considered as a "chemical damage" to the trees (Xu and Xiao, 1988), causing oxidative stress in the tissues eventually leading to tissue senescence (Chrestin, 1984; 1985; 1989; Das *et al.*, 2002, Krishnakumar and Jacob, 2003) resulting low crop productivity.

High intensity exploitations like high frequency tapping, over stimulation, long cuts and deep tapping etc. are known to cause several physiological disorders (Sethuraj, 1988; Jacob *et al.*, 1994). Tapping panel dryness (TPD) is one among such disorders and it is being considered as a metabolic or physiological syndrome affecting the laticiferous system. As TPD reduces the productivity of the trees (approximately 15 to 20 per cent yield decrease), panel dryness is being seriously viewed by the researchers. TPD incidence is noticed in almost all the rubber clones but its prevalence is much higher in high yielding *Hevea* clones.

Usage of ethephon is a general plantation practice and almost all the trees are being stimulated in the field for crop enhancement irrespective of the TPD status of the trees. This study mainly aims to understand the effect

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of stimulation on latex yield and the stress responses of trees with early symptoms of TPD.

Materials and Methods

The study was carried out in mature *Hevea brasiliensis* (clone RRII 105) planted during 1989 at Rubber Research Institute of India, Kottayam. The trees were being tapped for twelve years under ½ Sd/2 6d/7 tapping system. The trees were categorized into two groups namely healthy and TPD affected trees. The intensity of TPD incidence was determined by measuring the length of the dried area in the tapping panel. A group of ten trees showing the partial TPD incidence (30-60% dry tapping area) was selected for the present study. Another group of ten trees showing latex production throughout the tapping panel length was selected as healthy trees.

The tapping panel of the experimental trees (n=10) five each from both healthy and TPD affected group were scraped well (1" below the tapping cut) in order to remove the dead tissues and rubber particles from the panel. Commercially available Ethephon (2-chloro ethyl phosphonic acid) was diluted to 2.5% with coconut oil and mixed well. The compound was applied on the bark of the tapping panel 1" below the tapping cut using a brush. Single application of the stimulant was made in all the experimental trees. A set of unstimulated trees (n=10) five each from both healthy and TPD affected groups was maintained as control.

A set of physiological parameters and certain biochemical analysis were carried out in both the experimental and control trees before the application of ethephon (pre-treatment). The same parameters were analysed in both the experimental and control trees on the 3rd day after the stimulant application and subsequently for two times with an interval of 15 days (post-treatments). The details of the parameters analyzed were as follows.

Total latex yield in each tree was recorded by measuring the whole latex volume at weekly intervals from both the experimental and control trees during the experimental period. The latex yield was calculated and expressed as ml/tree/tap. TPD scoring was carried out at fortnightly interval by measuring the dry areas in the tapping panel and converted into percentage with respect to the total length of the tapping panel.

Bark samples of two centimeter square size were collected from the tapping panel of each tree belonging to experimental and control groups using a sharp chistle. The bark samples were brought to the laboratory in ice and stored at -80°C until use. The inner soft bark tissues from the bark samples were collected separately using a surgical knife and used for the following biochemical analysis.

The cyanide content in the soft bark tissues were determined by resorcinol picric acid method (Drochioin *et al.*, 2003). The hydrogen peroxide content in the soft bark tissue was determined using the commercially available Amplex red hydrogen peroxide/peroxidase assay kit procured from Molecular Probes, The Netherlands. The assay was carried out after the protocol described in the assay kit manual (Molecular probes, 2002). The Assay kit contained an Amplex red reagent (10-acetyl-3, 7-dihydroxyphenoxazine) in combination with horse radish peroxidase (HRP) which has been used for detecting hydrogen peroxide present in the tissue sample. β-Cyanoalanine synthase assay was done using the method of Hendrickson and Conn *et al.* (1968) and Urbanska *et al.* (2002). The peroxidase enzyme activity in the tissues was carried out after Guilbault (1976). Estimation of malondealdehyde in the soft bark tissue was carried out by the method after Heath and Packer (1968). Thin slice (approximately 0.5mm uniform thickness) of 150 mg fresh latecifer enriched soft bark tissue (adjacent to the cambium) was used for the measurement of bark respiration using a Clarke type oxygen electrode as described by Lambers *et al.* (1983) and modified by Annamalainathan *et al.* (1998).

Results and Discussion

In general, stimulation could enhance latex yield in larger volumes both in healthy and TPD affected trees. The maximum latex yield was obtained from trees on the 3rd day after stimulation and subsequently the late volume decreased towards the end of the experiment (35 day) (Fig. 1). However, the latex yield in trees that are affected with partial TPD showed comparatively less latex volume than that of the healthy trees due to stimulation. The increased latex volume observed in healthy trees which are treated with ethephon, started declining towards the end of one month after treatment, however, keeping a higher latex volume compared to unstimulated control trees (Fig. 1). Whereas in TPD trees, the stimulation induced latex production during the initial days but there was no marked difference in the latex yield when accounted for the whole experiment period.

The stimulation experiment conducted in healthy and TPD affected trees showed an increased incidence of TPD in ethephon treated trees. The trees that affected with partial TPD (30-60%) was attaining TPD incidence upto 60-100% at the end of the experiment (Fig. 2).

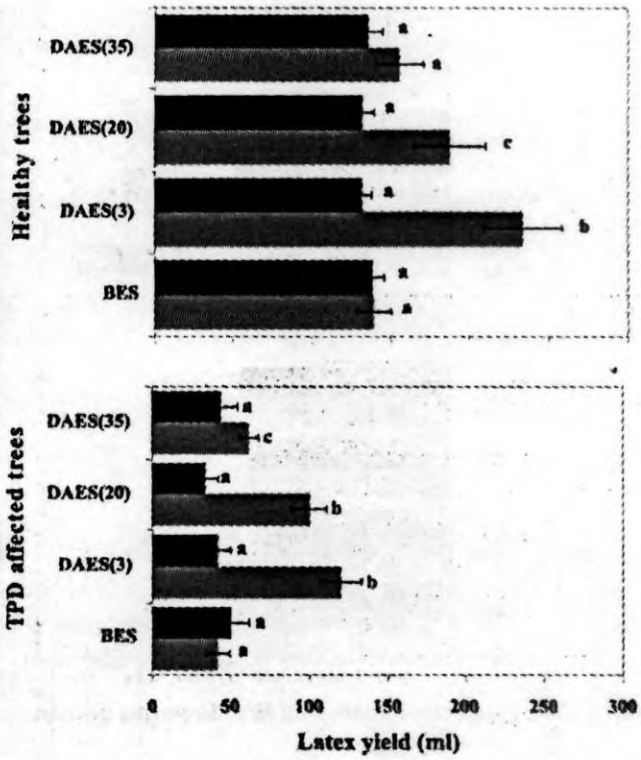

Fig. 1. Latex yield in healthy and TPD affected trees under unstimulated (■) and stimulated (▨) conditions

BES : Before ethephon stimulation
DAES(3) : 3 days after ethephon stimulation
DAES(20) : 20 days after ethephon stimulation
DAES(35) : 35 days after ethephon stimulation

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

Markedly, TPD incidence was low in healthy trees treated with ethephon. However, healthy trees showed 5-10% TPD incidence towards the end of the experiment (Fig. 2). The excessive drainage of latex leading to the depletion of metabolites in the laticiferous tissues has been suggested as the possibility for developing this disorder in *Hevea* trees (Lee and Hashim, 1989). TPD incidence was positively correlated with rubber yield and over-exploitation (Sethuraj, 1988; Premakumari *et al.*, 1991). It has been reported that metabolically active vigorous *Hevea* clones were generally more susceptible to TPD incidence (Sivakumaran *et al.*, 1988). Therefore, increasing intensities of tapping or chemical manifestation can increase the metabolic activity of the laticiferous tissues.

TPD affected tissue recorded higher levels of cyanide (CN) (Fig. 3). In stimulated healthy trees, the tissue CN level gradually increased from third day onwards and continued till the end of the experiment. The maximum tissue CN content was observed towards

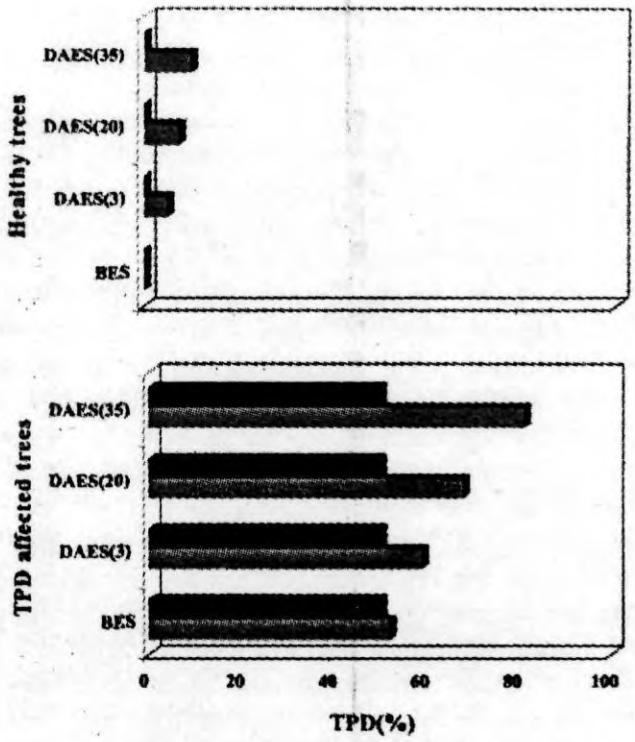

Fig. 2. Percentage of TPD in healthy and affected trees under unstimulated (■) and stimulated (▨) conditions

the end of the experiment (Fig. 3). Unlike in healthy trees, the TPD affected trees had higher levels of CN in the tissue before ethephon application and the tissue CN levels

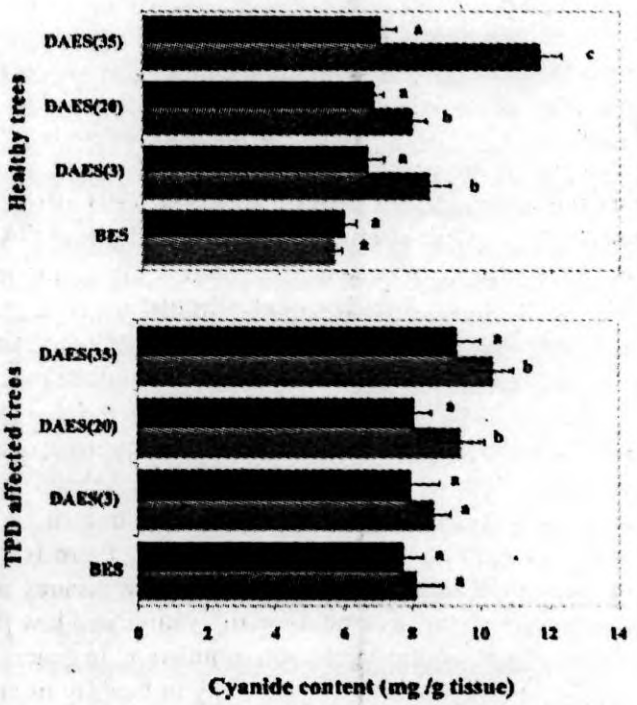

Fig. 3. Cyanide content in healthy and TPD affected trees under unstimulated (■) and stimulated (▨) conditions

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

increased further slightly due to stimulation (Fig. 3).

Cyanide is a co-product during the ethylene biosynthesis pathway. Etheridge *et al.* (2005) have reported ACC-oxidase dependent release of cyanide in the *Hevea* bark tissue during stimulation and proposed that cyanide can accelerate the development of several metabolic disorders. Production of more quantities of wound induced endogenous ethylene was reported in the bark tissues of TPD affected trees and a subsequently high content of cyanide (Krishnakumar and Jacob, 2003). Exogenous ethylene can induce a synergistic effect on endogenous ethylene production and the subsequent formation of its co-products in the tissue. Therefore, the possibility of generating cyanide in the tissue is likely more, particularly while stimulating trees with ethephon. The endogenous ethylene production in the TPD tree during stimulation was minimum compared to healthy trees since the TPD trees had already the maximum endogenous ethylene levels in their tissues. Therefore the cyanide content did not show much variation in TPD affected tissues (Krishnakumar *et al.* 2006). Presence of high cyanide content was reported in the bark tissues of TPD affected trees (Krishnakumar *et al.* 2006). Chrestin *et al.* (2004) proposed that prolonged stress in the tapping panel especially overexploitation through stimulation, may lead to accumulation of ACC oxidase-dependent cyanide production in the latex producing tissues.

β-CAS, an enzyme catalyzes the formation of β-cyanoalanine from L-cysteine and cyanide, widely seen in plants to detoxify the cyanide accumulation in tissues. This cyanide detoxifying enzyme was found more in the bark tissues of healthy trees compared to TPD affected trees (Fig. 4). While stimulating the healthy trees, β-CAS activity declined upon time and reached the minimum level towards the 35th day after stimulation (Fig. 4). Though the enzyme level was comparatively low in the TPD affected tissue, stimulation could not induce much change in its activity (Fig. 4). It was also noticed that the cyanide content increased gradually in healthy trees, over the time due to stimulation. The reason behind the reduced β-CAS activity in the healthy trees treated with ethephon is yet to be identified. However, there is an impaired cyanide metabolism in the bark tissues as evidenced from the accumulation of cyanide and low β-CAS activity in healthy trees upon stimulation. In general, stimulation affects the β-CAS activity in healthy tissue leading to the accumulation of cyanide in the bark tissue.

The intensity of oxidative stress was severe in TPD trees as compared to healthy trees as evidenced through

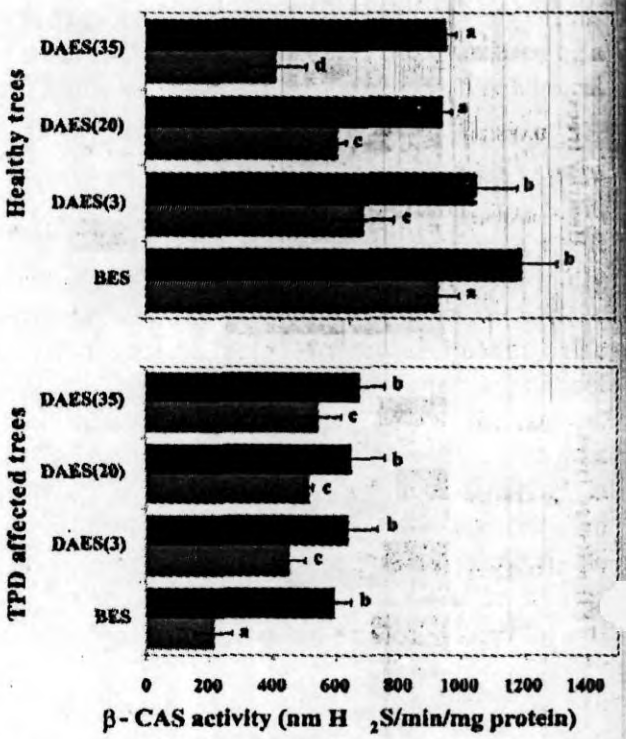

Fig. 4. β-CAS enzyme activity in healthy and TPD affected trees under unstimulated ( ) and stimulated ( ) conditions

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

the accumulation of H_2O_2 in the bark tissues of TPD affected trees (Fig. 5). In stimulated healthy trees, H_2O_2 production was more in the bark tissues than the unstimulated control. The maximum accumulation of H_2O_2 was noticed during the 20th day after stimulation which declined further towards the end of the experiment (Fig. 5). The H_2O_2 production in the bark tissues of TPD trees was at maximum on the 3rd day after stimulation and declined slightly towards the end of the experiment (Fig. 5).

High peroxidase enzyme activity was noticed in the bark tissues of TPD trees compared to healthy trees (Fig. 6). A steady decline in the peroxidase activity was noticed in stimulated healthy trees (Fig. 6). On the contrary, a gradual increase of the peroxidase activity was noticed in TPD affected trees up to 20th day and declined towards the 35th day (Fig. 6).

The H_2O_2 content and peroxidase activity noticed in the soft bark tissues of un-stimulated healthy trees are seems to be normal. Peroxidase enzyme activity appears to be capable of scavenging the H_2O_2 molecule produced in the healthy tissues thereby protecting the tissues from oxidative damage. But in TPD affected trees, peroxidase activity in the bark tissue seems to be inadequate to detoxify the H_2O_2 molecule produced that may lead to

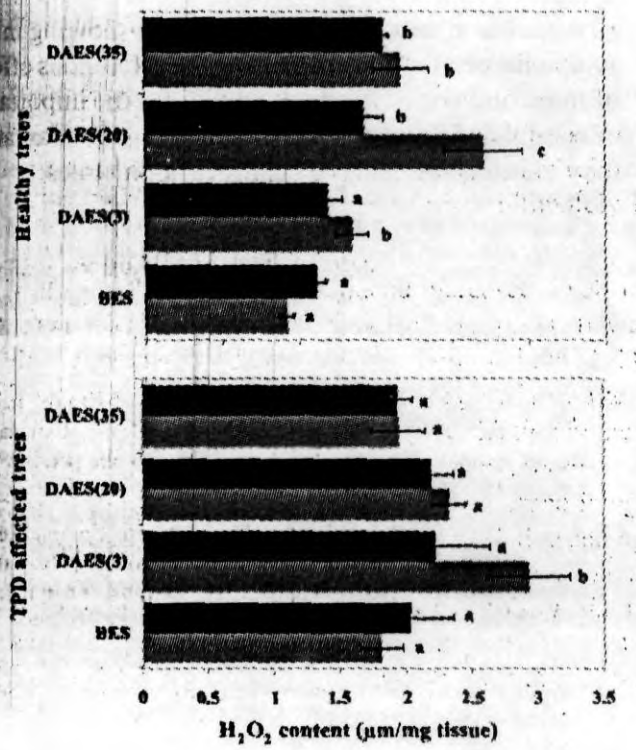

Fig. 5. H_2O_2 content in healthy and TPD affected trees under unstimulated ( ) and *stimulated ( ) conditions*

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

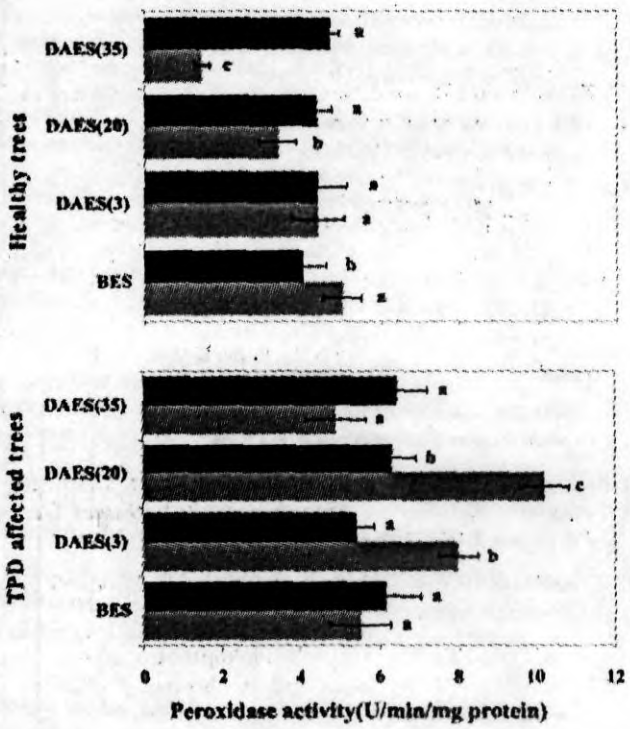

Fig. 6. Peroxidase activity in healthy and TPD affected trees under unstimulated ( ) and stimulated ( ) conditions

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

setting of oxidative stress in tissue. Ethephon application was reported to increase ROS generating activity and simultaneously decrease the level of scavengers (Chrestin, 1989; Das *et al.*, 1998; Krishnakumar and Jacob, 2003). Therefore, stimulation of rubber trees can cause more metabolic disorders ultimately leading to severe oxidative stress.

Stimulation induced increased levels of MDA in both healthy and TPD trees (Fig. 7). It was already reported that there was an increase in the MDA content in TPD affected bark tissue compared to the healthy tissue suggesting enhanced lipid peroxidation in the tissue as a result of ROS action (Krishnakumar *et al.*, 2006). It has been shown that the loss of membrane integrity is the final and irreversible phase of oxidative damage, closely linked to the peroxidation of membrane lipids leading to the accumulation of MDA (Bartoli *et al.*, 1995). The MDA accumulation in the stimulated trees can therefore be attributed to oxidative stress induced peroxidative damage.

An enhanced rate of tissue respiration was shown in bark tissues affected with TPD than tissues from healthy trees. Both the healthy and the TPD trees showed

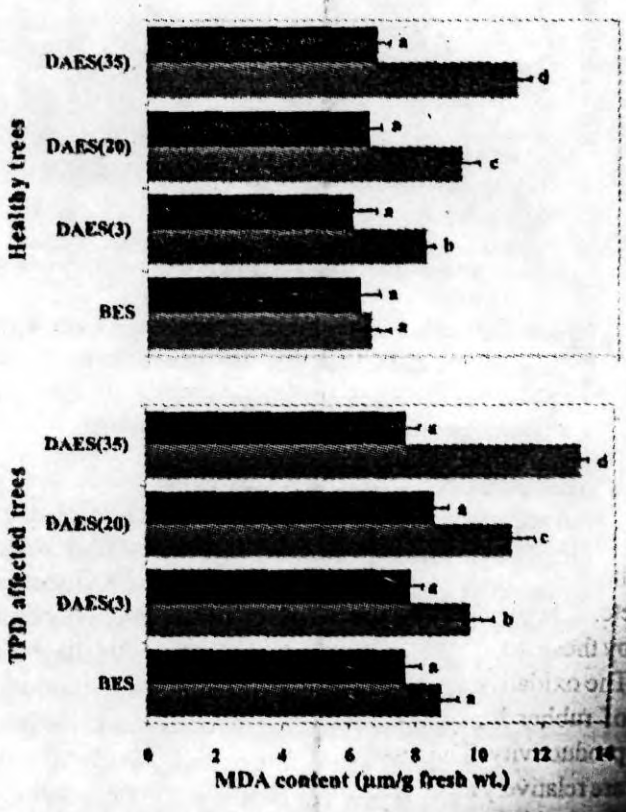

Fig. 7. MDA content in healthy and TPD affected trees under unstimulated ( ) and stimulated ( ) conditions.

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

enhanced rate of tissue respiration when stimulated with ethephon (Fig. 8). Generally tapping induces the rate of respiration in bark tissues of *Hevea* (Annamalainathan *et al.*, 1998). It was found that the respiratory rate in the tissue could go up under stress conditions as a result of the enhanced cyanide resistant pathway operated in the tissue. The accumulation of cyanide in the bark tissues of stimulated trees was evident in the present study and therefore it is obvious to operate high rates of cyanide resistant respiration in the tissue. As a result of this, the total respiration was higher in stimulated trees.

The application of ethylene stimulants on the bark tissues of *Hevea* for enhancing productivity may lead to accelerated endogenous ethylene production in the tissue.

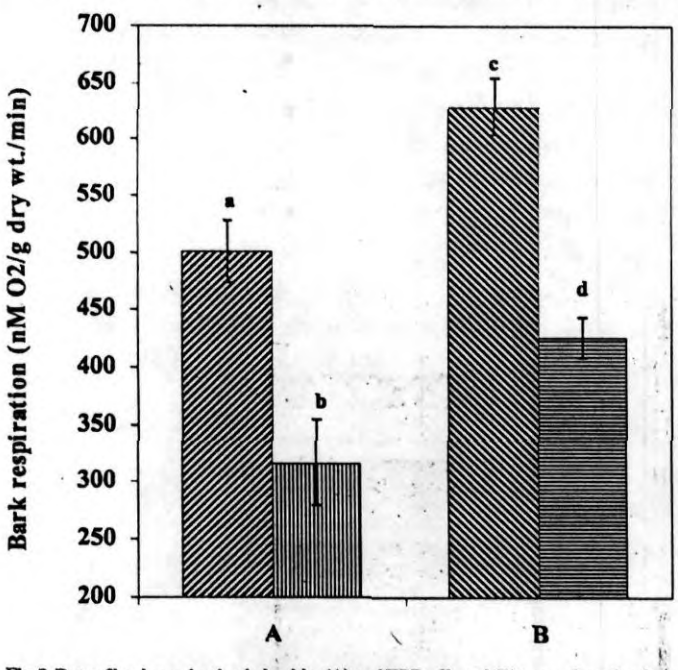

Fig. 8. Rate of bark respiration in healthy (A) and TPD affected (B) trees after ethephon treatment.

Stimulated healthy trees | Unstimulated healthy trees
Stimulated TPD affected trees | Unstimulated TPD affected trees

[SE shown; Chart bars with same alphabets are not significantly different and those with different alphabets are significantly different at $P \leq 0.05$]

This enhanced ethylene levels in the tissue may lead to the accumulation of toxic biomolecules like ROS, cyanide etc. The physiological and biochemical changes caused by these toxic molecules can induce metabolic disorders. The oxidative stress can ultimately lead to the inhibition of rubber biosynthesis and thereby limiting the crop productivity. The tissues adjacent to the tapping panel are relatively more sensitive to oxidative stress leading to the incidence of TPD. In general, occurrence of TPD incidence is a common phenomenon in rubber plantations. Therefore, application of stimulants to enhance latex productivity on such trees is not recommended. It is not advisable to stimulate the trees those are showing initial symptoms of TPD. Characterizing the deleterious effects of stimulation on rubber trees necessitate the importance of careful and judicious use of ethephon in a plantation for yield enhancement, especially in young trees.

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