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**PLANT SCIENCE**

# Effects of electromagnetic field on the nitrogen, protein and chlorophyll content and peroxidase enzyme activity in oil palm (*Elaeis guineensis* Jacq.) leaves

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**Abstract**

The effects of electromagnetic fields (EMF) from 275 kV high voltage transmission line on physiological, biochemical and antioxidant system changes in oil palm leaves were investigated under field conditions. Oil palm leaves were exposed to EMF from power lines at distances of 0, 8.8, 17.6, 26.4, 35.2, 44, 52.8 and 61.6 m away from the transmission lines for six months and seven years. The results showed that EMF exposure increased the content of chlorophyll a, b and the total chlorophyll in oil palm leaves planted nearer to the power lines (at 8.8 m) and decreased it with increasing distance from the lines. The protein banding profile of the oil palm exposed to electromagnetic field of different strength for seven years were insignificantly different. Oil palm exposed to the EMF for six months had three different protein banding patterns, which were different in the banding intensities of certain protein bands. The peroxidase enzyme activity (POX) of oil palm directly below the 275 kV power line was significantly ($p \leq 0.05$) higher. However, decreasing EMF strength signifying that exposure on oil palm for duration of both seven years and six months resulted in a stress related response. The POX activity bands from the oil palm exposed to EMF for both seven years and six months respectively showed that more banding intensity and number of bands were found in those planted nearer to the power lines with higher electromagnetic strength exposure.

*Key words*: *Elaeis guineensis* Jacq, Electromagnetic field, Oil palm, Protein marker

## Introduction

In many countries including Malaysia, thousands and hundreds of kilometres of electrical power line runs across highways and oil palm plantation lands. Electric distribution and transmission lines is the largest sources of electromagnetic field in the environment (Moon and Chung, 2000; Martinez et al., 2002). Therefore, it is inevitable for a large area like agricultural plantation to be free from electromagnetic field emitted from the power lines.

Although a number of studies have been done on the effects of electromagnetic filed on plants growth, very little is known about the specific physiological, biochemical and molecular changes that take place (Nucitelli, 1988; Karcz and Burdach, 1995; Liang et al., 2009). Oil palm *(Elaeis guineensis* Jacq.) is one of the major plantation crop grown extensively in Malaysia. With no visual differences observed in the oil palms, the biochemical analysis can provide an insight of how the plant is responding to the external stress, namely electromagnetic field.

Generally on occasions of environmental stress to plants, the present of visual symptoms signifies that the apparent damage to the plant is either irreversible or a valuable period of the vegetative growth has been lost which will be reflected in the final yield (Heimer et al., 1990; de Souza 2006; Liang et al., 2009). Therefore, biochemical studies are crucial as it provides information on the biochemical indicators of stress and plant physiology. The objective of the work is to

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determine the effects of EMF exposure from 275 KV high transmission line on physiological, biochemical and antioxidant system changes in oil palm leaves under field conditions. In an attempt to increase the understanding of the exposure of electromagnetic field on plants, this study was conducted to determine whether the exposure over a short-term period of several months and a long-term period of several years caused detrimental or encouraging biochemical changes in oil palm grown within the electrical environment of a 50 Hertz 275kV high voltage transmission line.

## Materials and Methods

### Plant materials

The oil palm (Elaeis guineensis var. tenera) grown in the vicinity of high voltage transmission lines were obtained from Guthrie Plantations, Sua Betong in Port Dickson, Malaysia. Six months old and seven years old oil palm grown at different distances from the power lines were exposed to different electromagnetic field strength for their duration of growth. Fully expanded leaf from the first frond of the oil palm plants were taken at 0, 8.8, 17.6, 26.4, 35.2, 44, 52.8, 61.6 m at ground level from the 275 kV high voltage transmission line for the biochemical studies. Ten leaf samples were randomly taken from each marked location at every 8.8 m from the 275 kV power line until 61.6 m away where the EMF were at ambient levels. Plants at 0 m referred to those planted directly below the line. The same leaf position was maintained for all the plants sampled to avoid variation during analysis.

### Electromagnetic exposure conditions

Magnetic field (mT) reading was taken at the marked distances using the extremely low frequency field strength meter. Electromagnetic field readings were taken three times at each marked positions to ensure consistent readings and the mean readings were used to compute the final data.

### Soluble protein and enzyme extraction

The harvested leaves were weighed and pulverized with liquid nitrogen before homogenizing with protein buffer using a chilled pestle and mortar at a ratio of 1:3 (g sample: mL extraction buffer). The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C. The pellet was discarded and crude soluble fraction was subjected to ammonium sulphate precipitation.

### Assay for soluble protein and soluble nitrogen content

Soluble protein content of the leaves obtained was determined according to the Coomassie Blue dye-binding method of Bradford (1976). Soluble nitrogen content of the leaves obtained was determined according to the Cadavid and Paladini (1964).

### Assay for Peroxidase enzyme activity (POX) enzyme activity

The enzyme extract used for the determination of peroxidase enzyme activity was prepared following the protein extraction method. Peroxidase activity (POX) (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 mM-1cm-1) from guaiacol using the method of Chance and Maehly (1955).

### Total chlorophyll and carotenoid content

The total chlorophyll and carotenoid of the sampled leaves were determined according to the method of Harborne (1984).

### Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)

The protein banding pattern of both six months and seven years old oil palm leaves planted at different distances from the 275 kV power lines were compared using SDS PAGE.

### Enzyme activity banding pattern

Enzyme activity banding pattern were done using native polyacrylamide gel electrophoresis (PAGE). Enzyme extracts was prepared according to the soluble protein content method and 10 µg proteins were loaded onto the gel wells. The native PAGE was run at 4ºC for approximately 4 h.

### Peroxidase enzyme activity bands

After the non-denaturing polyacrylamide gel electrophoresis was completed, the gels were stained for POX enzyme activity bands.

### Glutamate oxaloacetate transaminase enzyme activity bands

Similarity, native electrophoresis gels were stained for glutamate oxaloacetate transaminase.

### Statistical analysis

All biochemical measurements were made on triplicate samples and each experiment was repeated thrice (*n*=3) with independent extraction to ensure reproducibility of results. The data obtained were subjected to analysis of variance (ANOVA) of one-way and means between treatments were compared using Duncan Multiple Range Test (DMRT) at the significance level of 0.05.

## Results and Discussion

### Electromagnetic field (275 kV) from the seven years old oil palm plot

The electromagnetic field measured from the 275 kV transmission line in Port Dickson, Malaysia reduces with increasing distance as shown in Table

1. Electric and magnetic field range measured was from 0.0014 to 0.609 kV $m^{-1}$ and 0.3 to 1.39 mT and this indicates that different location of the power lines emits different electromagnetic field range as many factors may affect the readings such as the electricity load or demand at the time of measurement and the location of the power line in either an urban or rural area (Kuehn, 2001).

Table 1. Electromagnetic field at different distances from the 275 kV Power Line overlooking the seven years old oil palm in Port Dickson, Malaysia.

| Distance from Power Line (m) | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) |
|---|---|---|
| 0 | 60.90a | 1.39a |
| 8.8 | 0.87b | 1.15b |
| 17.6 | 0.17c | 0.99c |
| 26.4 | 0.16c | 0.81d |
| 35.2 | 0.14c | 0.62e |
| 44.0 | 0.15c | 0.41f |
| 52.8 | 0.17c | 0.28g |
| 61.6 | 0.14c | 0.30g |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

**Electromagnetic field from the six months old oil palm plot**

At a separate site where six months old oil palm were planted at different distances from the 275 kV power line, the electromagnetic field reading obtained were different from the first plot. Higher electric field range from 0.11 to 4.54 $kVm^{-1}$ and magnetic field range from 0.54 to 17.3 mT were obtained instead (Table 2). Although electromagnetic field from 275 kV transmission line were measured in a similar way previously, the readings obtained supported the fact that different location of the transmission line emits different range of electromagnetic field depending on the electricity load or demand. However, the inverse relationship between electromagnetic field and the distance from the source prevails where the electromagnetic field reading was found to reduce with increasing distance from the power line (Moulder, 1996).

Power line EMFs negatively and significantly influenced the soluble protein content in oil palm leaves. The palm planted nearer to power lines (0, 8.8, and 7.6 m) showed lower protein content than did those further away (26.4, 44.0, 52.8 and 61.6 m) (Table 3). The least protein content was reached for the palms grown beneath the 275 KV power line for seven years. Nemec and Meredith (1981) added that a general characteristic of plants subjected to stress is the increased level of free amino acids and reduced rates of protein synthesis which leads to decreased protein levels (Table 3).

Table 2. Electromagnetic field at different distances from the 275 kV Power Line overlooking the six months old oil palm in Port Dickson, Malaysia.

| Distance from Power Line (m) | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) |
|---|---|---|
| 0 | 4.54a | 17.3a |
| 8.8 | 0.82b | 3.12b |
| 17.6 | 0.52c | 1.44bc |
| 26.4 | 0.35cd | 1.24bc |
| 35.2 | 0.30cd | 0.87c |
| 44.0 | 0.22d | 0.63c |
| 52.8 | 0.14d | 0.57c |
| 61.6 | 0.11d | 0.54c |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

The low protein content of oil palm leaves grown directly below the line may be due to the seven years exposure to strong electromagnetic field. The long-term exposure to the high electromagnetic field may have broken the peptide bonds between peptides and polypeptides, thus reducing the formation of globular or functional protein and releasing more free amino acid or nitrogen containing compounds. Similarly, Shalaby and Al-Wakeel (1995) suggest that amino acids accumulation found in stress treated plants are possibly due to the increase in protein breakdown. Although the soluble nitrogen content in the oil palm leaves grown directly below the power line were the lowest, high soluble nitrogen content in an increasing order was found at 8.8, 26.4, 35.2 and 17.6 m distance. The soluble nitrogen content was significantly decreased at 44.0, 52.8 and 61.6 m distances without statistical differences in comparison with 0 m.

Hamada and Khulaef (1995) added that at these conditions, the increase in soluble proteins was at the expense of other amino acids and vice versa. The lack of anabolic processes such as protein synthesis as seen in Table 3 leads to ammonia accumulation, which are later sequestered into nitrogen containing compounds. Rabe (1990) reported that the accumulation of free amino acids might serve as a nitrogen source compound during periods of inhibited growth, thus suggesting that the high soluble nitrogen content observed in the oil palm grown between 8.8 m to 35.2 m may be undergoing some growth disruption.

Table 3. Effects of seven years exposure to electromagnetic field at different distances from the 275 kV Power Line on the soluble protein and nitrogen content of oil palm.

| Distance from Power Line | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Soluble protein (mg/g fresh weight) | Soluble nitrogen x $10^{-2}$ (mmole/g fresh weight) |
|---|---|---|---|---|
| 0 | 60.90a | 1.39a | 5.5g | 1.67d |
| 8.8 | 0.87b | 1.15b | 12.2f | 2.60c |
| 17.6 | 0.17c | 0.99c | 35.9d | 3.03a |
| 26.4 | 0.16c | 0.81d | 45.2ab | 2.83b |
| 35.2 | 0.14c | 0.62e | 23.7e | 2.87ab |
| 44.0 | 0.15c | 0.41f | 40.7bc | 1.77d |
| 52.8 | 0.17c | 0.28g | 38.1cd | 1.70d |
| 61.6 | 0.14c | 0.30g | 46.1a | 1.73d |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

Barnett and Naylor (1966) explained that the increase in the total free amino acids and decrease in the soluble protein were due to the free amino acids which were preferentially synthesized and not readily incorporated into protein during stress period. Furthermore, the increment in soluble nitrogen content may be due to the presence of non-protein amino acids, which often increase under stress conditions. Rabe (1990) reported that the lack of feedback inhibition on nitrogen uptake and reduction during periods of reduced growth or protein synthesis causes ammonia-ammonium accumulation and preferential synthesis of specific amino-containing compounds. The preferential synthesis of amino acids normally incorporated into proteins resulted in reduced protein level and higher nitrogen-containing compounds in the cells.

**Six months electromagnetic field exposure**

The highest soluble protein content was obtained from the oil palm leaves planted at 17.6 m away from the line, with EMF strength of 0.52 kV $m^{-1}$ and 1.44 mT, and 26.4 m away from the line, with EMF strength of 0.35 kV $m^{-1}$ and 1.24 mT (Table 4). Similarity, the soluble nitrogen content was also higher at the two distances mentioned. Kordas et al. (1998) reported that intermediate strength magnetic field treatments resulted in a more pronounced effect when compared with high electromagnetic field strength treatment, which yields negligible difference.

The difference between the effects of EMF on the soluble protein and nitrogen content of the seven years and six months old oil palm may be due to the variation in the EMF strength and the duration of exposure on the crop. According to Lamattina et al. (1987), protein biosynthesis is a dynamic process that is vulnerable to changes in the environment. Protein breakdown in plant cells is important in determining developmental and adaptive phenomena where variations in degradation rates are a quick and primary strategy of cells under stress to regulate their protein mass.

**Peroxidase enzyme activity (POX): Seven years electromagnetic field exposure**

POX activity was significantly decreased with increasing distance from power lines, reaching the lowest values (99.61) at 61.6 m (Table 5). Oil palm grown nearer to the power line had significantly ($p \leq 0.05$) higher POX specific enzyme activity with the highest being directly under the line with $364.87 \times 10^2$ U $mg^{-1}$ protein compared to $0.51 \times 10^2$ U $mg^{-1}$ protein at 52.8 m away from the line. This result is correlated with those obtained for seven years in which higher protein content is reached. Thus, lower POX enzyme activities are correlated with higher protein content.

Table 4. Effects of six months exposure to electromagnetic field at different distances from the 275 kV Power Line on the soluble protein and nitrogen content of oil palm.

| Distance from Power Line | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Soluble protein (mg/g fresh weight) | Soluble nitrogen x $10^{-2}$ (mmole/g fresh weight) |
|---|---|---|---|---|
| 0 | 4.54a | 17.3a | 26.5d | 1.10d |
| 8.8 | 0.82b | 3.12b | 35.3c | 1.57c |
| 17.6 | 0.52c | 1.44bc | 68.0a | 2.37a |
| 26.4 | 0.35cd | 1.24bc | 65.2a | 2.47a |
| 35.2 | 0.30cd | 0.87c | 16.6e | 1.77b |
| 44.0 | 0.22d | 0.63c | 30.7d | 1.67bc |
| 52.8 | 0.14d | 0.57c | 46.5b | 1.57c |
| 61.6 | 0.11d | 0.54c | 18.0e | 1.70bc |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

Table 5. Effects of seven years exposure to electromagnetic field at different distances from the 275 kV Power Line on peroxidase enzyme activity of oil palm.

| Distance from Power Line | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Total enzyme activity x 10 (Units/mL) | Specific enzyme activity x $10^2$ (Units/mg protein) |
|---|---|---|---|---|
| 0 | 60.90a | 1.39a | 99.61a | 364.87a |
| 8.8 | 0.87b | 1.15b | 10.52b | 17.44b |
| 17.6 | 0.17b | 0.99c | 2.99e | 1.62e |
| 26.4 | 0.16c | 0.81d | 5.24d | 2.22e |
| 35.2 | 0.14c | 0.62e | 7.84c | 7.69d |
| 44.0 | 0.15c | 0.41f | 2.96e | 1.45e |
| 52.8 | 0.17c | 0.28g | 0.98f | 0.51e |
| 61.6 | 0.14c | 0.30g | 2.96e | 12.84c |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

Table 6. Effects of six months exposure to electromagnetic field at different distances from the 275 kV Power Line on peroxidase enzyme activity of oil palm.

| Distance from Power Line | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Total enzyme activity x 10 (Units/mL) | Specific enzyme activity x $10^2$ (Units/mg protein) |
|---|---|---|---|---|
| 0 | 4.54a | 17.3a | 208.3a | 19.92a |
| 8.8 | 0.82b | 3.12b | 58.7d | 4.13e |
| 17.6 | 0.52c | 1.44bc | 47.1e | 1.72f |
| 26.4 | 0.35cd | 1.24bc | 42.6f | 16.08b |
| 35.2 | 0.30cd | 0.87c | 70.4c | 10.62c |
| 44.0 | 0.22d | 0.63c | 49.1e | 3.97e |
| 52.8 | 0.14d | 0.57c | 77.1b | 4.14e |
| 61.6 | 0.11d | 0.54c | 35.0g | 4.78d |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

The results obtained signifies that the oil palm that were grown nearest to the power line and exposed to the high electromagnetic field for a long period of seven years yielded some stress response by increasing their anti-oxidative capacity via the increase in the POX enzyme level. Although the POX enzyme activity of oil palm leaves planted at the furthest distance of 61.6 m were higher than to those at 52.8 m, it may not be due to the effects from the electromagnetic field as the readings at the furthest distance have been deemed minimal and insignificant. Table 1 showed that the difference in the EMF strength was only $0.03\times10^{-2}$ kV $m^{-1}$ and 0.02 mT between the two distances, 52.8 and 61.6 m.

Same trend was observed in oil palms, which were grown under the influence of electromagnetic field for six months. A clear trend is showed within 17.6 m from the line where the POX enzyme activity decreases with increasing distance from the line (Table 6). Significantly ($p\leq0.05$) higher POX enzyme activity was observed in the crop planted directly below the line at 0 m. However, no trend could be observed in the POX enzyme activity of oil palm at 26.4 and further from the line. Perhaps the effects of electromagnetic field were not significant at the said distances in the oil palm plot.

Therefore, it can be concluded from Table 7 and Figure 4 that the electromagnetic field from the 275 kV transmission line, regardless of their period of exposure on the oil palm, both six months and seven years of exposure resulted in a stress-induced proteins response from the plants. Although a decreasing trend of total peroxidase enzyme activity and specific enzyme activity alongside the decreasing electric and magnetic field couldn't be establish, drastic increase in the peroxidase enzyme level especially in those grown directly below the line was significantly ($p\leq0.05$) observed. The enzymatic response to the electromagnetic field, a non-ionizing radiation is similar to the case of ionizing radiation as was reported by Adrain (1999) who suggests that the primary damage induced by ionizing radiation is the modification of the enzymatic repair processes.

Table 7. Effects of seven years exposure to electromagnetic field at different distances from the 275 kV Power Line on the chlorophyll and carotenoid content of oil palm.

| Treatments (kV/m) | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Chlorophyll a (ug/mL) | Chlorophyll b (ug/mL) | Chlorophyll a:b | Total Chlorophyll x $10^{-1}$ (mg/g fresh weight) | Carotenoid x $10^{-2}$ (mg/g fresh weight) |
|---|---|---|---|---|---|---|---|
| 0 | 60.90a | 1.39a | 11.0g | 8.2g | 1.34c | 2.89f | 6.78c |
| 8.8 | 0.87b | 1.15b | 22.0a | 22.3a | 0.99g | 6.64a | 4.97d |
| 17.6 | 0.17c | 0.99c | 19.2c | 15.0c | 1.29d | 5.13c | 8.28ab |
| 26.4 | 0.16c | 0.81d | 19.8b | 17.8b | 1.11f | 5.93b | 7.64bc |
| 35.2 | 0.14c | 0.62e | 16.1f | 10.6f | 1.52b | 3.99e | 6.71c |
| 44.0 | 0.15c | 0.41f | 10.5h | 4.8h | 2.17a | 2.28g | 7.92ab |
| 52.8 | 0.17c | 0.28g | 17.8d | 13.6e | 1.31cd | 4.70d | 7.88ab |
| 61.6 | 0.14c | 0.30g | 17.1e | 14.2d | 1.21e | 4.70d | 7.90a |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

**Total chlorophyll and carotenoid content: Seven years electromagnetic field exposure**

Table 7 showed the effects of electromagnetic field from 275 kV power line on the total chlorophyll and carotenoid content of oil palm grown at different distances from the line for seven years. Although the low chlorophyll content was obtained from the oil palm leaves planted directly below the line, the total chlorophyll content was significantly ($p<0.05$) highest in those planted at 8.8 m away from the line and the chlorophyll content seemed to be decreasing with increasing distance from the line. Generally, the trend could be seen from 8.8 m up till 44 m.

The significant ($p\leq0.05$) difference in chlorophyll content at 0 and 8.8 m may be due to the drastic difference in the electromagnetic field at the two distances where 0.609 kV $m^{-1}$ and 1.39 mT were measured at 0 m; and 0.087 kV $m^{-1}$ and 1.15 mT were measured at 8.8 m away from the 275 kV power line. Therefore, the electromagnetic field strength at 0 m may be too high especially over a seven year exposure period for encouraging increased chlorophyll development in the oil palm leaves. On the other hand, the electromagnetic field at 8.8 m may be optimal for the increased production of chlorophyll, thus as the electromagnetic field sway away from the optimal at 8.8 m with further distance from the line, so does the chlorophyll content.

Therefore, the results from Table 7 suggest that small doses of EMF at further distance of 8.8, 17.6 and 26.4 m from the 275 kV power lines may stimulate plant growth by increasing the chlorophyll formation in leaves while higher electromagnetic field directly below the power lines may be detrimental as indicated by the high peroxidase enzyme activity reported in Table 5.

Table 8. Effects of six months exposure to electromagnetic field at different distances from the 275 kV Power Line on the chlorophyll and carotenoid content of oil palm.

| Treatments (kV/m) | Electric Field x $10^{-2}$ (kV/m) | Magnetic Field (mT) | Chlorophyll a (ug/mL) | Chlorophyll b(ug/mL) | Chlorophyll a:b | Total Chlorophyll x $10^{-1}$ (mg/g fresh weight) | Carotenoid x $10^{-2}$ (mg/g fresh weight) |
|---|---|---|---|---|---|---|---|
| 0 | 4.54a | 17.3a | 12.6b | 11.4a | 1.11g | 3.61a | 8.81d |
| 8.8 | 0.82b | 3.12b | 10.4c | 10.0b | 1.05h | 3.06ab | 7.12e |
| 17.6 | 0.52c | 1.44bc | 12.5b | 9.0b | 1.39e | 3.22ab | 10.94b |
| 26.4 | 0.35cd | 1.24bc | 11.7b | 10.1b | 1.17f | 2.95b | 10.37bc |
| 35.2 | 0.30cd | 0.87c | 8.6d | 5.3f | 1.64c | 2.10c | 7.02e |
| 44.0 | 0.22d | 0.63c | 14.2a | 7.5de | 1.90a | 2.06c | 9.48cd |
| 52.8 | 0.14d | 0.57c | 12.0b | 7.9cd | 1.52d | 2.98b | 13.01a |
| 61.6 | 0.11d | 0.54c | 11.9b | 6.4ef | 1.87b | 2.74b | 10.38bc |

Means with the same letter in columns do not show significant differences according to DMRT (p=0.05).

Similarly to the trend observed on increasing chlorophyll content with increasing electromagnetic field in Table 7 from 8.8 to 44 m. Table 8 showed that the highest chlorophyll content was obtained from the oil palm grown at 0 m from the power line and the total chlorophyll content decreases with increasing distance from the line up till 44 m. Perhaps the total chlorophyll content in oil palm leaves at 0 m were not reduced as in Table 7 because the exposure period in Table 8 were only six months compared to the former with seven years.

However, in comparison with the EMF strength of 0.609 kV $m^{-1}$ and 1.39mT obtained from the 275 kV power line at the seven years old oil palm planting site, the EMF strength at the six months old oil palm planting site was much higher with 4.54 kV $m^{-1}$ and 17.3mT. The significantly ($p \leq 0.05$) lower chlorophyll a to b ration of oil palm planted at 8.8 m away from the power line may indicate the senescing effect of electromagnetic field on the crop. Nedunchezhian and Kulandaivelu (1995) reported a decrease in the chlorophyll a to b ratio during leaf senescence due to the relatively faster decrease of chlorophyll a than chlorophyll b. Total carotenoid content was higher at further distances from the power line in which low electromagnetic field radiation is received (Table 8).

**SDS PAGE protein banding pattern: Seven years electromagnetic field exposure**

Figure 1 showed the effects of seven years electromagnetic field exposure at different distances from the 275 kV power line on the 12.5% SDS PAGE protein banding profile of oil palm. Generally, SDS PAGE protein banding profile from oil palm leaves yield low-resolution bands due to the high amount of fibre in the leaves, polyphenols, certain lipids and nucleic acids that interfered with polypeptide resolution as was reported by Yuffa et al. (1994) although different extraction and purification steps were tried in order to extract the most protein from the oil palm leaves.

All the protein banding profiles of the seven years old oil palm at different distances from the 275 kV power line were insignificantly different quantitatively except for the enhanced intensity in bands from 35.2 and 61.6 m. Table 3 showed that the highest protein content was obtained from the leaves of oil palm planted at 61.6 m away from the line. Therefore, the enhanced banding intensity in lane 8 may be related to the high protein content originally contained in the leaf samples since the same amount of protein were loaded into each lane of the polyacrylamide gel.

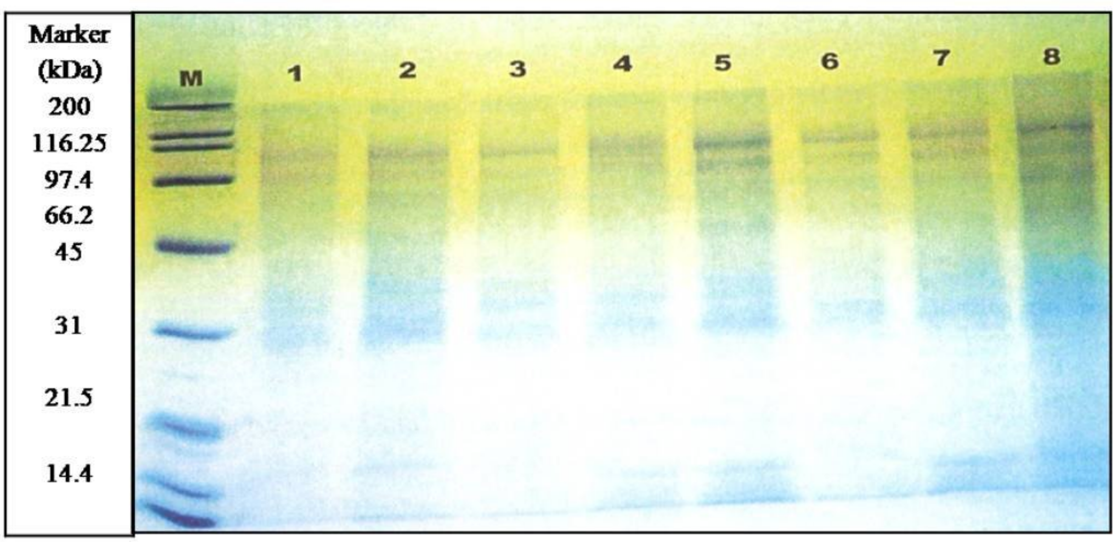



Figure 1. Effects of seven year electromagnetic field exposure at different distances from the 275 kV power line on the 12.5% SDS PAGE protein banding profile of oil palm. Lane M: Marker, Lane 1:0 m, Lane 2: 8.8 m, Lane 3: 17.6 m, Lane 4: 26.4 m, Lane 5: 35.2 m, Lane 6: 44m, Lane 7: 52.8 m, Lane 8: 61.6 m.

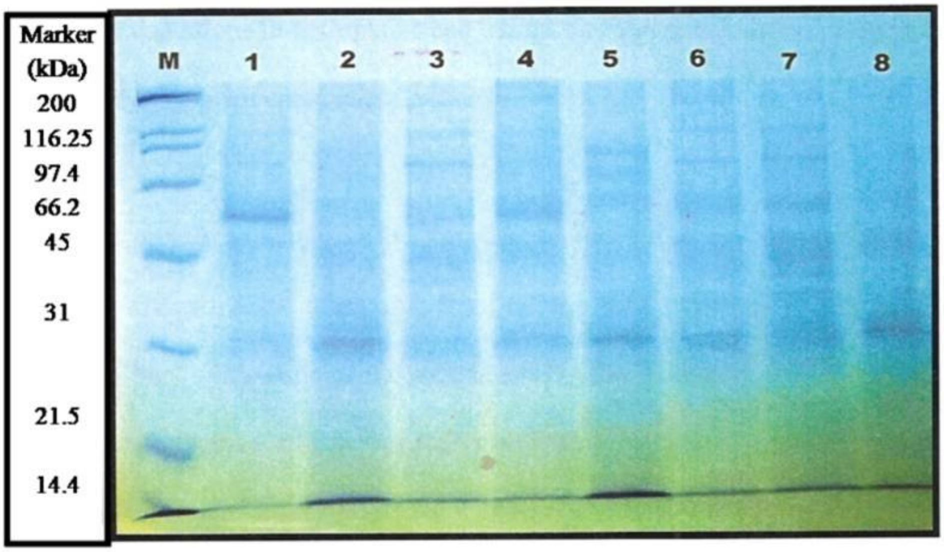



Figure 2. Effects of six months electromagnetic field exposure at different distances from the 275 kV power line on the 12.5% SDS PAGE protein banding profile of oil palm. Lane M: Marker, Lane 1:0 m, Lane 2: 8.8 m, Lane 3: 17.6 m, Lane 4: 26.4 m, Lane 5: 35.2 m, Lane 6: 44m, Lane 7: 52.8 m, Lane 8: 61.6 m.

**SDS PAGE protein banding pattern: Six months electromagnetic field exposure**

Figure 2 showed the effects of six months electromagnetic field exposure at different distances from the 275 kV power line on the 10% SDS PAGE protein banding profile of oil palm. Similarly, the overall resolution of bands was low due to the oxidation of high phenolic compounds in the oil palm grown at 0 m up till 61.6 m away from the 275 kV power line. At lane 1 (0 m), a significant protein band was observed at a molecular weight between 45 and 66.2 kDa. Similar protein banding pattern was also observed in lane 4 (26.4 m) except that the lane 4 had a less intense band at the molecular weight between 45 and 66.2 kDa but it had a more intense protein band at 31 kDa compared to lane 1 (0 m).

Oil palm planted 26.4 m away from the electromagnetic field source, which received and exposure of 0.35 kV $m^{-1}$ and 1.24 mT had a prominent increase in the relative amount of at least one polypeptide species at 31 kDa. This may be due to a consequence of a highly preferential synthesis of the polypeptide in the cells. The increase in the relative abundance of a particular polypeptide band detected by SDS PAGE may be either from an increase in the rate of synthesis or from the rate of synthesis being relatively unaffected while synthesis of other polypeptides are reduced. Webster (1980) reported that the usual increase in the relative amount and synthesis of at least one specific polypeptide in stressed plants signifies a common mechanism of plants dealing with environmental stress. The preferentially synthesized polypeptides are common responses to stress conditions in both plants and animals as a gene regulation attempt in higher eukaryotic cells experiencing stress.

A different protein banding profile from the above were observed in lane 2 (8.8 m), lane 5 (35.2 m) and 8 (61.6 m). All three of the lane's protein profile had a significant protein band with a molecular weight of 31 kDa. However, clearer protein banding profile was obtained at lane 5 (35.2 m). The third type of protein banding profile was obtained at lane 3 (17.6 m), lane 6 (44 m) and lane 7 (52.8 m) with three clear bands at a molecular weight between 116.25 and 200 kDa, at 116.25 kDa and between 66.2 and 97.4 kDa. The magnitude of the increase in band intensity or number could not be related with the magnitude of the electromagnetic field strength. This indicates that the response of plant to electromagnetic field treated plants was exclusive to certain EMF strength.

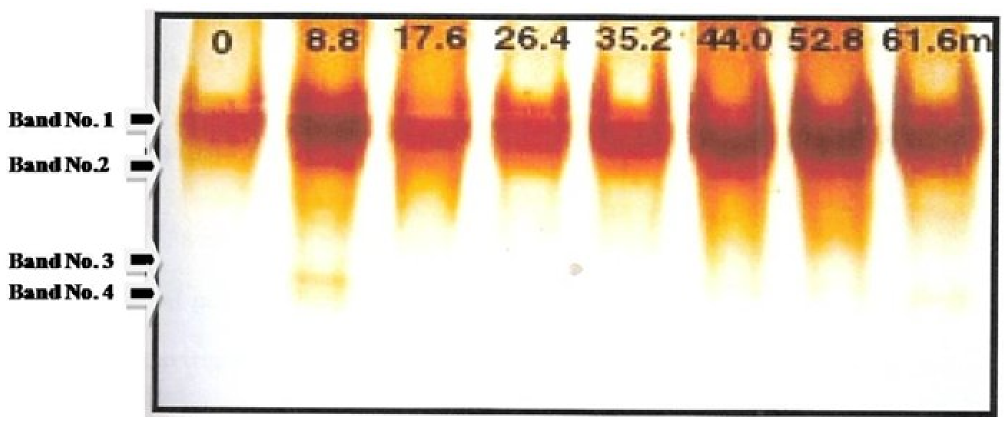
**Peroxidase enzyme activity bands: Seven years electromagnetic field exposure**

Figure 3 showed the effects of the seven years exposure to electromagnetic field on oil palm grown at different distances from the 275 kV power line. Most of the treatments had a total of two enzyme activity bands at $R_f$ 0.08 and 0.13 except for lane 7 (52.8 m) and lane 6 (44.0 m) which had a very faint extra enzyme activity band at $R_f$ 0.40 while lane 8 (61.6 m) and lane 2 (8.8 m) and additional two enzyme activity bands at $R_f$ 0.40 and 0.44 (Figure 3). The changes in peroxidase enzyme activity bands due to the exposure to EMF suggest the need for further genetic studies to determine the root cause of the changes.

The highest intensity of POX enzyme activity band at $R_f$ 0.40 were from lane 2 (8.8 m) while the highest intensity of POX enzyme activity band at $R_f$ 0.08 were from lane 7 (52.8 m), lane 6 (44.0 m), lane 2 (8.8 m) and lane 8 (61.6 m). The increase in the POX enzyme activity bands intensity may be related to the POX enzyme activity as Table 4 revealed that the highest POX specific enzyme activity was from the oil palm leaves grown at 0 m from the line followed by 8.8 m. Furthermore, Figure 3 showed that from 0 to 44 m away from the power line, the number of POX enzyme activity band decreases with increasing distance from the power line similarly to Table 5 which showed that the POX specific enzyme activity decreases with increasing distance from the power line, thus with decreasing electromagnetic field strength.

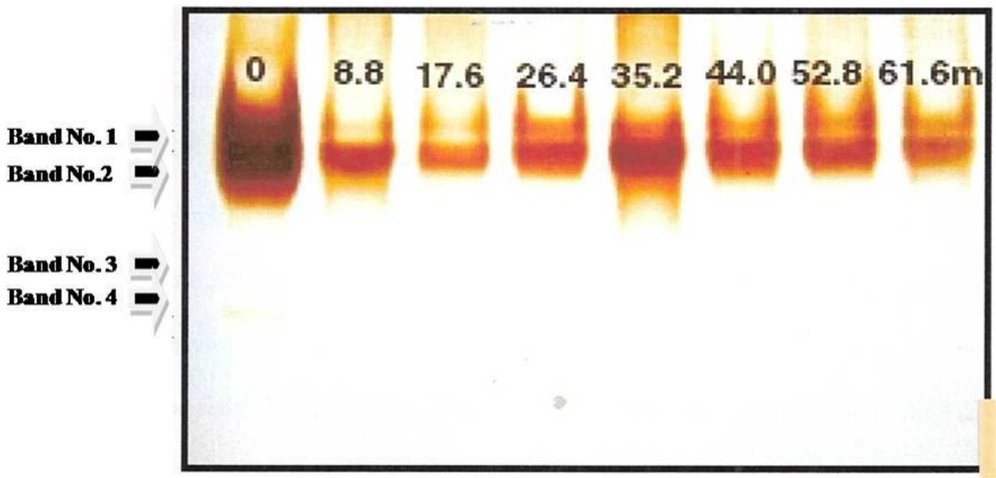
**Peroxidase enzyme activity bands: Six months electromagnetic field exposure**

Figure 4 showed the peroxidase enzyme activity bands and the corresponding $R_f$ values of oil palm grown at different distances from the 275 kV power line for six months. All the treatments had a total of two enzyme activity bands at $R_f$ 0.08 and 0.14 except for lane 1 (0 m) where the leaf samples were obtained from the oil palm planted directly below the 275 kV power line. Lane 1 (0 m) had two additional POX enzyme activity bands at $R_f$ 0.40 and 0.44. According to Yurenkova et al. (1995), the formation of new isozymes can be due to their biosynthesis *de novo*, or by transformation of active or inactive precursors into new molecular forms. Similarly to Figure 3 on the seven years electromagnetic field exposure on oil palm directly below the line, the six years electromagnetic field exposure on oil palm directly below the line also yielded a total of four POX enzyme activity bands and the number of enzyme activity bands decreases with increasing distance from the power line.



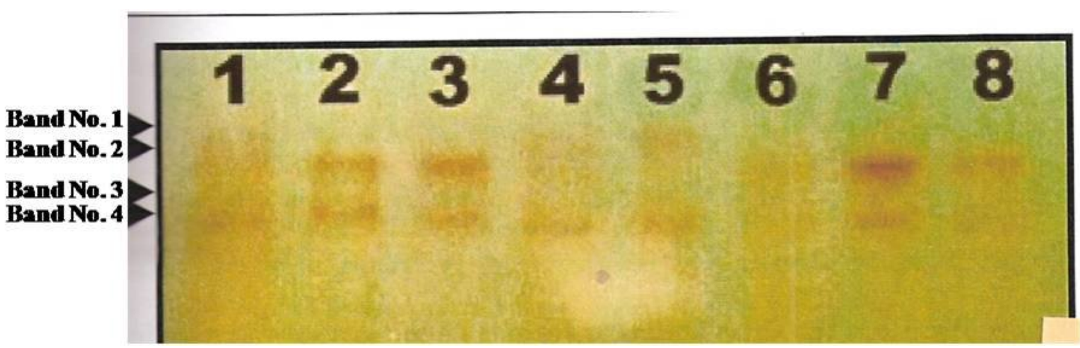

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| DFPL (m) | 0 | 8.8 | 17.6 | 26.4 | 35.2 | 44.0 | 52.8 | 61.6 |
| Band No. 1 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Band No. 2 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
| Band No. 3 | - | 0.40 | - | - | - | 0.40 | 0.40 | 0.40 |
| Band No. 4 | - | 0.44 | - | - | - | - | - | 0.44 |

Figure 3. Peroxidase (POX) enzyme activity bands on 7.5% polyacrylamide gel and the $R_f$ values of oil palm planted at different distances from the 275 kV power line for seven years. DFPL : Distance from the power line.




| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| DFPL (m) | 0 | 8.8 | 17.6 | 26.4 | 35.2 | 44.0 | 52.8 | 61.6 |
| Band No. 1 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Band No. 2 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Band No. 3 | 0.40 | - | - | - | - | - | - | - |
| Band No. 4 | 0.44 | - | - | - | - | - | - | - |

Figure 4. Peroxidase (POX) enzyme activity bands on 7.5% polyacrylamide gel and the $R_f$ values of oil palm planted at different distances from the 275 kV power line for six months. DFPL: Distance from the power line.




| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| DFPL (m) | 0 | 8.8 | 17.6 | 26.4 | 35.2 | 44.0 | 52.8 | 61.6 |
| Band No. 1 | - | - | - | - | 0.14 | - | - | - |
| Band No. 2 | - | - | - | 0.16 | - | 0.16 | - | 0.16 |
| Band No. 3 | 0.19 | 0.19 | 0.19 | 0.19 | - | - | 0.19 | - |
| Band No. 4 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |

Figure 5. Glutamate oxaloacetate transminase enzyme activity bands on 10% polyacrylamide gel and the Rf values of oil palm planted at different distances from the 275 kV power line for six months. DFPL: Distance from the power line.

Therefore, the different form of the POX enzyme activity bands obtained from the oil palm planted directly under the high voltage transmission line suggests that further study is needed to determine if the changes is due to the addition in the number of polymorphic bands corresponding to specific alleles in the peroxidase locus. Such genetic interpretation has been made several times in different plant species (Shields et al., 1983). Saker et al. (2000) reported that the reason behind the variation at the gene expression level obtained could be due to gene amplification, chromosomal irregularities, point mutation or alteration in DNA methylation. However, since only a small fraction of the bases in DNA have a coding or regulatory role, majority of mutations are silent and have no functional effect (King, 2000).

The peroxidase enzyme activity bands were most intense in lane 1 (0 m) followed by lane 5 (35.2 m). The increase in the abundance at $R_f$ 0.14 indicates the high POX activity in the leaves of the oil palm grown under the exposure of electromagnetic field. The least intense POX enzyme activity bands were observed in a lane 3 (17.6 m). The intensity of the POX enzyme activity bands correlate with the POX specific enzyme activity in Table 5 where the highest activity was found in treatment 0 m and the least was in treatment 17.6 m.

**Glutamate oxaloacetate transaminase enzyme activity bands: Six months electromagnetic field exposure**

Successful glutamate oxaloacetate transaminase enzyme activity band staining was obtained in the oil palm exposed to electromagnetic field for six months. Figure 5 showed that all the treatments resulted in a total of two glutamate oxaloacetate transaminase enzyme activity bands inclusive of a common band at $R_f$ 0.30. Lane 1 (0 m), 2 (8.8 m), 3 (17.6 m) and lane 7 (52.8 m) had two enzyme activity bands at $R_f$ 0.19 and $R_f$ 0.30. Lane 4 (26.4 m), 6 (44 m) and 8 (61.6 m) had a slightly smaller $R_f$ band number one of 0.16 instead of 0.19, and lane 5 (35.2 m) had an even smaller $R_f$ band number one with 0.14 (Figure 5). Higher intensity of the enzyme activity bands were observed in lane 7 (52.8 m), followed by lane 2 (8.8 m), lane 3 (17.6), and lane 1 (0 m). Lane 8 (61.6 m), lane 5 (35.2 m), lane 4 (26.4 m) and lane 6 (44 m) had less intense enzyme activity bands with lane 6 having the least. Although such observations could be made, no relationship could be established between the observations and EMF strength, thus, suggesting the need for a more in depth study in the future.

In the present study, the soluble protein content in oil palm planted beneath the 275 kV power line for both seven years and six months respectively were least directly below the line and increase with increasing distance when comparison were made up till 26.4 m away from the line. Similar trend was observe in both the oil palm exposed to EMF from 275 kV power line for seven years and six months, whereby the POX specific enzyme activity directly below the line was significantly ($p \leq 0.05$) higher and the enzyme activity decreases with decreasing EMF strength.

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