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Full Length Research Paper

Effects of electromagnetic field of 33 and 275 kV influences on physiological, biochemical and antioxidant system changes of leaf mustard (B*rassica chinensis*)

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The effects of electromagnetic fields (EMF) from 33 and 275 kV high voltage transmission line on biochemical and antioxidant system changes in mustard leaf (*Brassica chinensis*) were investigated under field condition. Mustard leaves were exposed to EMF from power lines at distances of 0, 3, 6, 9, 10, 12, 15, 18, 20, 21, 30, 40, 50 and 60 m away from the 33 kV power line and at 0, 10, 20, 30, 40, 50, 60 and 70 m away from the 275 kV transmission lines. The effects of EMF from 33 kV power lines on leaf mustard planted at different distances from the line showed that leaf mustard planted within 20 m from the line had significantly ($p< 0.05$) higher protein, soluble protein, soluble nitrogen and chlorophyll contents due to the higher EMF strength which decreased with increasing distance from the line. Higher EMF strength nearer to the 275 kV power line resulted in higher peroxidase enzymatic activity, and chlorophyll content. Protein electrophoretic profile obtained from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) analysis revealed no drastic alterations in the leaf mustard protein profiles. This suggests that electromagnetic field could be used as a tool to promote mustard growth via photosynthesis once the right EMF strength and duration of exposure has been established through future studies.

Key words: Mustard, electromagnetic field, biochemical markers.

INTRODUCTION

In Malaysia, power transmission lines cover a distance of 45,000 km with a right of way (ROW) of 40 m below resulting in an estimated 180,000 ha of vacant land, which can be utilized for agricultural purposes to increase land use. However, the thousands of kilometres of high-voltage transmission wires emit electromagnetic fields across agricultural land throughout the country.

Leaf mustard (*Brassica chinensis*) known as *sawi hijau* in Malaysia or choy sam belongs to Brassiceae tribe and Crucifereae family. *B. chinensis,* a 40-day short-term crop is among the most popular leafy vegetables in Asia. An important requirement for growing this vegetable is an open flat land with no shading or canopy effects from nearby trees due to its need of abundant sunlight. Therefore, many small-scale farmers grow leaf mustard on the empty land in the vicinity of high voltage transmission lines because transmission line clearance

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Abbreviations: EMF, Electromagnetic fields; **SDS PAGE**, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

land is free of any height vegetation. Small-scale farmers in Malaysia especially in Selayang have utilized the vacant land beneath high voltage transmission lines to grow leaf mustard (*B. chinensis*) and satisfactory yield have been obtained.

Most researchers reported that crop seeds treated with different magnetic fields could increase the rate of seed germination and seedling growth especially maize and fruits tree species (Dagoberto et al., 2002; Martinez et al., 2002; Socorro and Carbonell, 2002; Yan et al., 2009). In addition, Yan et al. (2009) promoted the regeneration efficiency of *in vitro* culture beach plum (*Prunus maritima*) from nodal segments treated with electromagnetic fields. They concluded that treatment of explants with a certain magnetic field strength could increase the number of regeneration sprouts and growth. Recently, Hajnorouzia et al. (2011) reported that an appropriate combination of geomagnetic and alternative magnetic fields promoted maize seedling growth by an excess production of reactive oxygen species.

Studies on suitability of vegetables beneath power lines and under the influence of electromagnetic field are rare and have not been carried out in Malaysia, particularly. Therefore, in an attempt to do an in-depth study on leaf mustard crops beneath power lines, some biochemical aspects and protein profile of leaf mustard planted beneath 33 and 275 kV transmission lines were investigated. Therefore, the study on the effects of electromagnetic field emitted from these wires on mustard plants will contribute new and important knowledge to the agricultural industry.

MATERIALS AND METHODS

Plant source

Leaf samples of leaf mustard (*B. chinensis*) variety with wide and non-branching green petioles were taken at different distances at ground level from 33 and 275 kV power lines. Samples were collected from a vegetable farm situated below high voltage transmission lines in Selayang, Malaysia. Leaves were harvested from 25 day old leaf mustard grown at different distances from power lines. Leaf samples were collected at marked positions every 3 and 10 m away from the line in three batches. The first batch was obtained from 0, 3, 6, 9, 12, 15, 18 and 21 m away from the 33 kV line. The second batch was obtained from 0, 10, 20, 30, 40, 50 and 60 m away from the 33 kV line and the third batch was obtained from 0, 10, 20, 30, 40, 50, 60 and 70 m (control) away from the 275 kV power line. The second youngest expanded leaves were collected from 10 plants per marked location from the power line. Leaves were numbered from shoot apex to base with leaf number one being the youngest fully expanded leaf. The same leaf position was maintained for all the plants sampled to avoid variation during analysis.

Electromagnetic field strength measurement

Electric field (kV/m) and magnetic field (mT) reading were taken at marked distances using the extremely low frequency field strength meter (Holaday Industries Company, US). Electromagnetic field readings were taken three times at each marked positions to ensure consistent readings and the mean reading was used to compute the final data.

Soluble protein content determination

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 the crude soluble fraction was subjected to ammonium sulfate precipitation.

Assay for soluble protein content

Soluble protein content of obtained leaves was determined according to coomassie blue dye-binding method of Bradford (1976).

Soluble nitrogen content determination

Soluble nitrogen content of obtained leaves was determined according to Cadavid and Paladini (1964).

Peroxidase enzyme activity determination

The enzyme extract used for the determination of peroxidase enzymatic activity was prepared following protein extraction method.

Assay for peroxidase activity (POX) enzyme activity

POX (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 $mM^{-1}cm^{-1}$) from guaiacol according to Chance and Maehly (1955). The POX reaction solution (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H_2O_2 and 20 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20 s. One unit POX was defined as an absorbance of 0.01 units per min.

Total chlorophyll and carotene content

Total chlorophyll and carotene of the sampled leaves were determined according to Harborne (1984).

Statistical analysis

All biochemical measurements were made on triplicate samples and each experiment was repeated thrice with independent extraction to ensure reproducibility of results. The data obtained was subjected to analysis of variance (ANOVA) of one-way and means between treatments were compared using Duncan multiple range test (DMRT) at a significance level of 0.05 using PC-SAS System version 6.12.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)

SDS PAGE protein banding patterns of leaf mustard planted at

Table 1. Electromagnetic field at different distances from the 33 kV power line in Selayang, Malaysia.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)
0	0.128^{a}	5.23^{a}
3	0.118^{b}	4.53^{b}
6	0.117^{b}	4.28^{c}
9	0.115^{bc}	4.12^{d}
10	0.113^{c}	3.98^{e}
12	0.109^{d}	3.80^{f}
15	0.106^{d}	3.63^{g}
18	0.102^{e}	3.44^{h}
20	0.096^{f}	3.23^{i}
21	0.088^{g}	2.95^{j}
30	0.084^{h}	2.43^{k}
40	0.078^{i}	1.92^{i}
50	0.072^{j}	1.61^{m}
60	0.071^{j}	1.25^{n}

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

Table 2. Electromagnetic field at different distances from the 275 kV power line in Selayang, Malaysia.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)
0	2.42^{a}	33.90^{a}
10	0.98^{b}	14.21^{b}
20	0.52^{c}	5.79^{c}
30	0.14^{d}	5.30^{c}
40	0.11^{d}	4.28^{d}
50	0.08^{d}	4.02^{d}
60	0.07^{d}	3.39^{e}
70	0.05^{d}	2.74^{f}

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

different distances and exposed to different electromagnetic field strength were compared using Bio-Rad Mini PROTEAN 3.

Enzymatic activity banding pattern

Enzymatic activity banding patterns were obtained using native PAGE. Enzyme extracts were prepared according to soluble protein content method and 10 µg proteins were loaded onto gel wells. Native PAGE was run at 4°C for approximately 4 h.

Peroxidase enzymatic activity bands

After the completion of non-denaturing polyacrylamide gel electrophoresis, the gels were stained for peroxidase enzymatic activity bands.

Glutamate oxaloacetate transaminase enzymatic activity bands

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

Protein and enzymatic activity banding evaluation

Protein-banding and enzymatic activity banding patterns were observed on a light box and photographed. Protein banding and enzymatic activity banding patterns were compared and assessed.

RESULTS AND DISCUSSION

Electromagnetic field from the 33 and 275 kV power lines

It can be concluded from Tables 1 and 2 that electromagnetic field range at different distances from the power lines differs according to the amount of electricity transmitted by the lines. A 33 kV power distribution line emits electromagnetic field in a smaller range of 0.07 to

Table 3. Effects of electromagnetic field at different distances from the 33 kV power line on soluble protein and nitrogen content of leaf mustard.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)	Soluble protein (mg/g fresh weight)	Soluble nitrogen x 10^{-1} (mmole/g fresh weight)
0	0.128[a]	5.23[a]	111.5[ef]	0.21[f]
3	0.118[b]	4.53[b]	117.6[bc]	0.22[e]
6	0.117[b]	4.28[c]	120.0[ab]	0.27[a]
9	0.115[bc]	4.12[d]	116.5[cd]	0.22[e]
10	0.113[c]	3.98[e]	114.0[de]	0.23[d]
12	0.109[d]	3.80[f]	117.5[bc]	0.23[d]
15	0.106[d]	3.63[g]	117.4[bc]	0.21[f]
18	0.102[e]	3.44[h]	120.6[a]	0.26[b]
20	0.096[f]	3.23[i]	105.3[h]	0.16[j]
21	0.088[g]	2.95[j]	107.7[gh]	0.24[c]
30	0.084[h]	2.43[k]	107.8[gh]	0.19[h]
40	0.078[i]	1.92[l]	107.5[gh]	0.19[h]
50	0.072[j]	1.61[m]	98.0[i]	0.17[i]
60	0.071[j]	1.25[n]	97.7[i]	0.17[i]

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

0.13 kV/m and magnetic field of 1.25 and 5.23 mT, while a 275 kV power transmission line which carries higher electricity load emits electromagnetic field in a higher range of 0.05 to 2.42 kV/m and magnetic field of 2.74 to 33.9 mT.

Therefore, a power line that carries more electricity radiates a higher electromagnetic field and the electromagnetic field decreases with increasing distance from the line. The observed result supports the inverse relationship between electromagnetic field and distance described by Horton and Goldberg (1995).

Soluble protein and soluble nitrogen content

B. chinensis below the 33 kV power line

Table 3 shows protein and soluble nitrogen content of leaf mustard (*B. chinensis*) that was grown at different distances from the 33 kV power line for 25 days and was therefore exposed to different strengths of electromagnetic field. Generally, it was found that protein and soluble nitrogen content decreases with increasing distance from the electromagnetic field source.

The highest protein content was found in the leaves of leaf mustard planted at 18 and 6 m from the power line, followed closely by those planted at 3, 12 and 15 m from the line. Beyond 20 m from the line, protein content of leaf mustard was found to decrease. Therefore, the results suggest that within 18 m from the power line where there is a higher electromagnetic field, higher protein content was found in the leaves of leaf mustard. Similarly, higher soluble nitrogen content was found in plants nearer to the power line compared to those 20 m and beyond. Furthermore, both protein and soluble nitrogen content were lowest at 30 m and further away from the power line.

Although the difference was not significantly large, it was enough to support the fact that leafy vegetables such as leaf mustard contained slightly higher protein and soluble nitrogen content when planted nearer to the power line which radiates electromagnetic field. The increase and decrease of protein content due to the interrupted synthesis of functional protein in the electromagnetic field treatments signifies that proteins were involved in the response of the plants to electromagnetic field, and the interaction was dependent on the electromagnetic field strength.

Brassica chinensis below the 275kV Power Line

Similar to the response of leaf mustard to electromagnetic field from 33 kV power line, Table 4 shows that the highest protein and soluble nitrogen content was found at 10, 20 and 40 m away from the 275 kV power line while the lowest was found in the leaf mustard planted at 60 and 70 m away from the line. Higher soluble nitrogen content was also found in the leaf mustard planted nearer to the power line, which was exposed to a higher electromagnetic field. This indicates that electromagnetic field may act as an environmental stress factor, as an increased nitrogen content was associated with stressed plants as reported by Mihailovic et al. (1992). However, Zhen and Leigh (1990) reported that growth increase is usually accompanied by an increase in inorganic nitrogen.

Therefore, this study suggests that higher protein and

Table 4. Effects of electromagnetic field at different distances from the 275 kV power line on soluble protein and nitrogen content of leaf mustard.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)	Soluble protein (mg/g fresh weight)	Soluble nitrogen × 10^{-1} (mmole/g fresh weight)
0	2.42[a]	33.90[a]	88.8[b]	0.12[d]
10	0.98[b]	14.21[b]	100.8[a]	0.20[a]
20	0.52[c]	5.79[c]	106.7[a]	0.17[b]
30	0.14[d]	5.30[c]	90.6[b]	0.15[c]
40	0.11[d]	4.28[d]	105.0[a]	0.18[b]
50	0.08[d]	4.02[d]	91.6[b]	0.10[e]
60	0.07[d]	3.39[e]	79.8[c]	0.12[d]
70	0.05[d]	2.74[f]	81.3[c]	0.09[e]

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

Table 5. Effects of electromagnetic field at different distances from the 33 kV power line on POX enzyme activity of leaf mustard.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)	Total enzyme activity × 10^3 (units/ml)	Specific enzyme activity × 10^3 (units/mg protein)
0	0.128[a]	5.23[a]	1.20[b]	1.08[def]
3	0.118[b]	4.53[b]	0.54[c]	0.46[h]
6	0.117[b]	4.28[c]	0.23[c]	0.19[h]
9	0.115[bc]	4.12[d]	0.67[c]	0.58[gh]
10	0.113[c]	3.98[e]	1.52[b]	1.53[bcd]
12	0.109[d]	3.80[f]	0.69[c]	0.65[fgh]
15	0.106[d]	3.63[g]	0.41[c]	0.35[h]
18	0.102[e]	3.44[h]	0.34[c]	0.28[h]
20	0.096[f]	3.23[i]	1.56[b]	1.53[bcd]
21	0.088[g]	2.95[j]	1.21[b]	0.98[efg]
30	0.084[h]	2.43[k]	1.29[b]	1.29[cde]
40	0.078[i]	1.92[l]	2.18[a]	1.91[ab]
50	0.072[j]	1.61[m]	2.21[a]	2.14[a]
60	0.071[j]	1.25[n]	1.56[b]	1.58[bc]

Means with the same letter in columns do not show significant differences according to DMRT (p = 0.05). DMRT, Duncan multiple range test; POX, peroxidise.

soluble nitrogen content would be obtained from leaf mustard planted within 20 m from both the 33 and 275 kV power lines.

Peroxidase (POX) enzymatic activity

Brassica chinensis below the 33 kV power line

Table 5 shows the POX enzymatic activity of leaf mustard planted at different distances from the 33 kV power line and exposed to different strengths of electromagnetic field. There was no drastic increase in POX specific enzymatic activity of the leaf mustard that was planted nearer to the line signifying that the typical response of increased POX activity in stressed plants was not observed here. Instead, the leaf mustard planted at 3, 6, 12, 15 and 18 m away from the line yielded the highest protein content and had the least POX specific enzyme activity while the POX activity of the crops further away from the power line seemed to be higher.

Brassica chinensis below the 275 kV power line

In contrast to POX enzymatic activity obtained from leaf mustard planted at different distance from the 33 kV power line, Table 6 shows that POX specific enzymatic activity of leaf mustard planted at different distances from the 275 kV power line was higher nearer to the line. The highest POX specific enzymatic activity was found in the leaf mustard planted directly below the power line at 0 m

Table 6. Effects of electromagnetic field at different distances from the 275 kV Power Line on POX enzyme activity of leaf mustard.

Distance from power line (m)	Electric field (kV/m)	Magnetic field (mT)	Total enzyme activity (units/ml)	Specific enzyme activity (units/mg protein)
0	2.42^{a}	33.90^{a}	373.8^{a}	421.1^{a}
10	0.98^{b}	14.21^{b}	161.6^{b}	160.4^{b}
20	0.52^{c}	5.79^{c}	68.9^{c}	64.6^{c}
30	0.14^{d}	5.30^{c}	42.5^{d}	46.9^{d}
40	0.11^{d}	4.28^{d}	65.3^{c}	62.3^{c}
50	0.08^{d}	4.02^{d}	26.6^{e}	29.0^{f}
60	0.07^{d}	3.39^{e}	26.7^{e}	33.4^{e}
70	0.05^{d}	2.74^{f}	27.2^{e}	26.8^{f}

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

Table 7. Effects of electromagnetic field at different distances from the 33 kV power line on chlorophyll and carotene content of leaf mustard.

Treatment (kV/m)	Electric field (kV/m)	Magnetic field (mT)	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Chlorophyll a:b	Total chlorophyll × 10^{-1} (mg/g fresh weight)	Carotene × 10^{-1} (mg/g fresh weight)
0	0.128^{a}	5.23^{a}	10.5^{a}	14.0^{a}	0.75^{i}	3.81^{a}	0.73^{e}
3	0.118^{b}	4.53^{b}	9.4^{b}	8.9^{b}	1.06^{c}	2.83^{b}	0.90^{c}
6	0.117^{b}	4.28^{c}	5.7^{d}	6.8^{d}	0.85^{g}	1.90^{c}	1.02^{b}
9	0.115^{bc}	4.12^{d}	5.2^{ef}	5.8^{e}	0.90^{f}	1.72^{cd}	1.05^{ab}
10	0.113^{c}	3.98^{e}	4.5^{h}	3.8^{h}	1.19^{b}	1.76^{cd}	0.91^{c}
12	0.109^{d}	3.80^{f}	4.7^{gh}	5.1^{f}	0.92^{ef}	1.57^{de}	1.01^{b}
15	0.106^{d}	3.63^{g}	6.3^{c}	7.2^{c}	0.87^{g}	1.86^{c}	1.00^{c}
18	0.102^{e}	3.44^{h}	4.7^{gh}	4.5^{g}	1.06^{c}	1.46^{ef}	1.09^{a}
20	0.096^{f}	3.23^{i}	5.1^{efg}	5.5^{e}	0.93^{e}	1.57^{de}	1.05^{ab}
21	0.088^{g}	2.95^{j}	5.3^{de}	6.9^{cd}	0.77^{i}	1.78^{cd}	1.01^{b}
30	0.084^{h}	2.43^{k}	4.5^{h}	4.3^{g}	1.06^{c}	1.37^{ef}	0.62^{f}
40	0.078^{i}	1.92^{l}	3.1^{i}	3.8^{h}	0.81^{h}	1.09g^{h}	0.80^{d}
50	0.072^{j}	1.61^{m}	3.0^{i}	3.1^{i}	0.97^{d}	1.04^{h}	0.40^{h}
60	0.071^{j}	1.25^{n}	4.8^{fgh}	3.4^{i}	1.40^{a}	1.28^{fg}	0.49^{g}

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

and decreases with distance.

POX specific enzymatic activity of leaf samples directly below the line was 2.6 times higher than those obtained at 10 m away from the line. The high EMF directly below the power line may have stimulated this increase as plants respond to environmental stimuli by increasing their anti-oxidative enzymes to protect themselves against any further stress or damage (Polle and Pell, 1999). However, the stress response of increased POX level did not disrupt the production of protein and soluble nitrogen in leaf mustard.

Comparatively, the electromagnetic field emitted from 275 kV power line was much higher than that of 33 kV power line. Since leaf mustard is one of the most consumed leafy vegetables, any possible factor affecting taste or texture of the leaves will be of interest to consumers. The high POX activity observed in the leaves planted directly below the line may be an undesirable effect. According to Prabha and Patwardhan (1986), peroxidases have been shown to contribute to the deteriorative changes in flavour, texture, colour and nutritional value in processed fruits and vegetables. The high electromagnetic field from 275 kV power line may have stimulated the plant to increase its POX enzyme level as a response to high electromagnetic field.

Total chlorophyll and carotene content

Brassica chinensis below the 33 kV power line

Table 7 shows the effects of electromagnetic field from the 33 kV power line on total chlorophyll and carotene of leaf mustard grown at different distances from the power

Table 8. Effects of electromagnetic field at different distances from the 275 kV power line on chlorophyll and carotene content of leaf mustard.

Treatment (kV/m)	Electric field (kV/m)	Magnetic field (mT)	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Chlorophyll a:b	Total chlorophyll × 10^{-1} (mg/g fresh weight)	Carotene × 10^{-1} (mg/g fresh weight)
0	2.42[a]	33.90[a]	6.5[b]	4.1[b]	1.59[a]	1.57[b]	0.68[e]
10	0.98[b]	14.21[b]	7.1[a]	5.0[a]	1.43[c]	1.81[a]	0.20[g]
20	0.52[c]	5.79[c]	5.8[c]	3.8[c]	1.53[b]	1.43[c]	0.55[f]
30	0.14[d]	5.30[c]	3.6[e]	2.9[g]	1.24[d]	0.96[e]	0.96[c]
40	0.11[d]	4.28[d]	3.6[e]	3.4[d]	1.05[h]	o.53[g]	1.00[a]
50	0.08[d]	4.02[d]	3.5[f]	3.1[f]	1.14[g]	0.97[e]	0.98[b]
60	0.07[d]	3.39[e]	3.8[d]	3.2[e]	1.18[f]	1.07[d]	1.00[a]
70	0.05[d]	2.47[f]	2.8[g]	2.3[h]	1.21[e]	0.78[f]	0.79[d]

line. The highest total chlorophyll was found in the leaf mustard grown directly below the line at 0 m, followed by that grown at 3, 6, 9 m and beyond. Generally, total chlorophyll content decreases with decreasing electro-magnetic field at increasing distance from the power line.

On a similar note to protein content, total chlorophyll content was higher nearer to the power line.

Therefore, the electromagnetic field from the 33 kV power line seemed to produce encouraging effects on the growth of leaf mustard by increasing its chlorophyll content. The increase in chlorophyll content was observed by Kordas et al. (1998) who reported that among the effects of magnetic field on growth and development are the increased in germination capacity, higher and greener plants with more dry matter compared to non-exposed plants. However, at the distance beyond 20 m, the low electromagnetic field may not have produce significant effects, thus resulting in a vague trend in total chlorophyll content after 20 m. No trend or specific relationship could be linked between carotene content of leaf mustard and distance from the power line.

Brassica chinensis below the 275 kV power line site

The effects of electromagnetic field from the 275 kV power line on leaf mustard grown at different distances from the line were encouraging (Table 8). Similar results are obtained where total chlorophyll was higher in leaf mustard grown within 20 m from the line and decreases at further distances. Furthermore, a higher chlorophyll a to b ratio was also observed in leaf mustard grown nearer to the power line and the ratio decreases with distance.

This suggests that electromagnetic field may have stimulated the production of more chlorophyll a compared to chlorophyll b, which is a desirable effect because chlorophyll a has a more important role in photosynthesis compared to the latter. However, the opposite pattern was observed on the total carotene content which was found higher in leaf mustard grown further away from the power line (Table 8).

SDS PAGE protein profile

Brassica chinensis below the 33 kV power line

Figure 1 shows SDS PAGE protein banding profile of leaf mustard grown at different distances from power lines and thus, exposed to different strength of electromagnetic field in the 25 days of growth. However, the 7.5% polyacrylamide gel used did not result in a satisfactory resolution and separation of protein bands due to large matrix pores.

Figure 2 shows the SDS PAGE protein banding profile of leaf mustard grown at different distances from the 33 kV power line in a 3 m interval. A reasonably high and better resolution and separation of protein bands was obtained when 12.5% polyacrylamide gel was used instead of the 7.5% (Figure 1). Insignificant differences were obtained with the treatments since the number and intensity of the major bands were found to appear consistently in all the treatments. However, the protein banding profile of leaf mustard can be characterized by a thick protein or polypeptide band with a molecular weight between 45 and 66.2 kDa, and at 14.4 kDa as seen in all the lanes.

In comparison, Figure 3 displays the same protein banding profile as Figure 2 except for the different staining solution employed. The protein banding profiles obtained in this study including Figure 2 were stained using coomassie brilliant blue G-250. However, the protein banding profile produced in Figure 3 was stained using coomassie brilliant blue R-250. Both figures showed that the highest resolution of bands was obtained using 12.5% polyacrylamide gel compared to Figure 1, which employed 7.5% polyacrylamide gel.

However, staining with coomassie brilliant blue G-250 was sufficient to provide a visibly clear protein-banding pattern in leaf mustard and coomassie brilliant blue R-250 which gives a deeper stain is not necessary. Furthermore, the deeply stained bands in Figure 3 may create some difficulty in identifying individual bands. Figure 4 shows the SDS PAGE protein banding profile of leaf mustard grown at different distances from the 33 kV

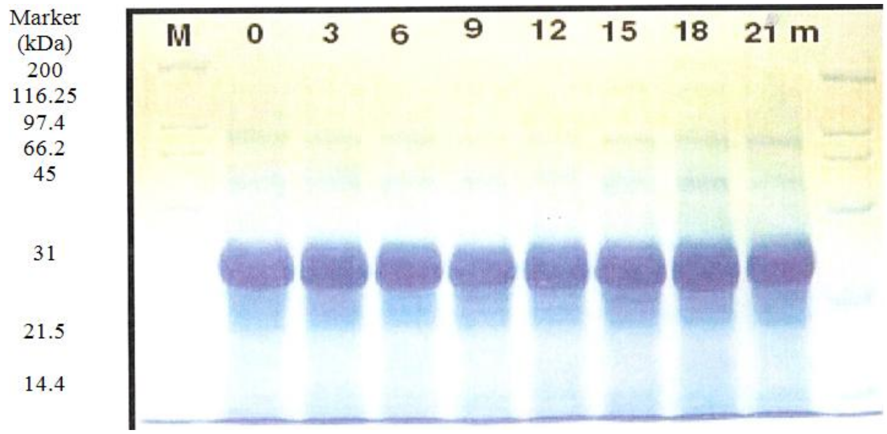


Figure 1. Effects of electromagnetic field at different distances of 3 m interval from the 33 kV power line on the 7.5% SDS PAGE protein banding profile of leaf mustard. Lane 1, Marker; lane 2, 0 m; lane 2, 3 m; lane 4, 6 m; lane 5, 9 m; lane 6, 12 m; lane 7, 15 m; lane 8, 18 m; lane 9, 21 m; lane 10, marker.

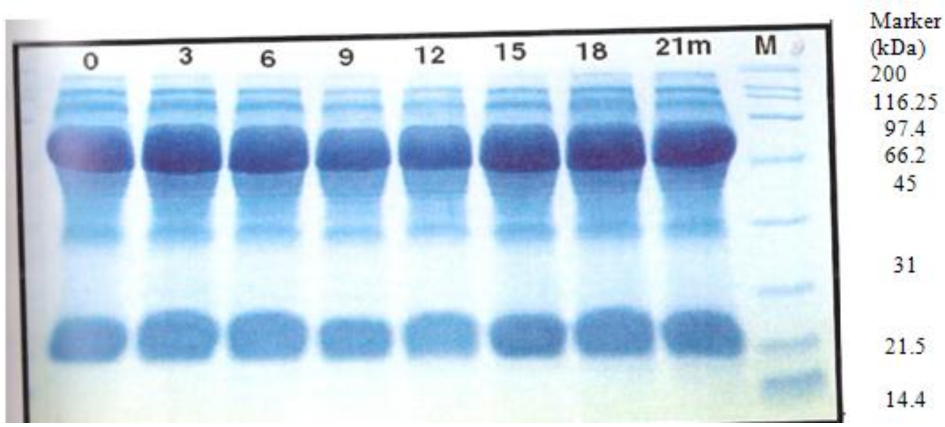


Figure 2. Effects of electromagnetic field at different distances of 3 m interval from the 33 kV power line on the 12.5% SDS PAGE protein banding profile of leaf mustard using Coomassie Brilliant Blue G-250 stain. Lane 1: 0 m, lane 2, 3 m; lane 3, 6 m; lane 4, 9 m; lane 5, 12 m; lane 6, 15m; lane 7, 18 m; lane 8, 21 m; lane 9, marker.

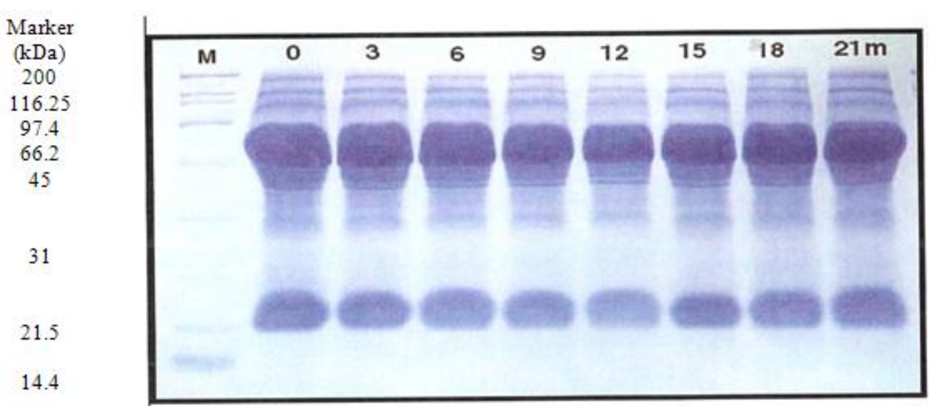


Figure 3. Effects of electromagnetic field at different distances of 3 m interval from the 33 kV power line on the 12.5% SDS PAGE protein banding profile of leaf mustard using Coomassie Brilliant Blue R-250 stain. Lane M, Marker; lane 1; 0 m; lane 2, 3 m; lane 3, 6 m; lane 4, 9 m; lane 5, 12 m; lane 6, 15 m; lane 7, 18 m; lane 8, 21 m.

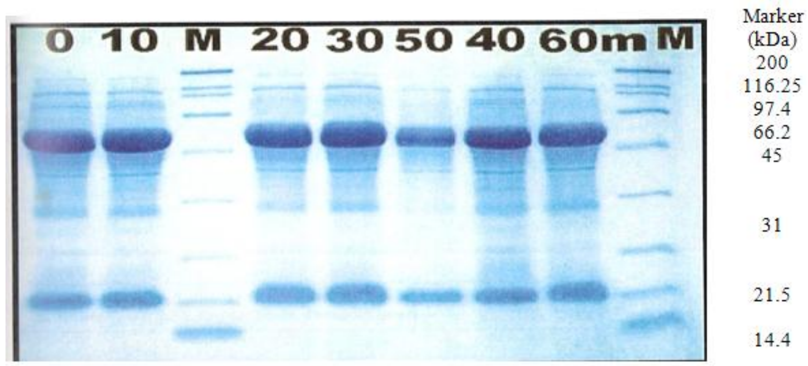


Figure 4. Effects of electromagnetic field at different distances of 10 m interval from the 33 kV power line on the 12.5% SDS PAGE protein banding profile of leaf mustard. Lane 1, 0 m; lane 2, 10 m; lane 3, marker; lane 4, 20 m; lane 5, 30 m; lane 6, 50 m; lane 7, 40 m; lane 8, 60 m; lane 9, marker.

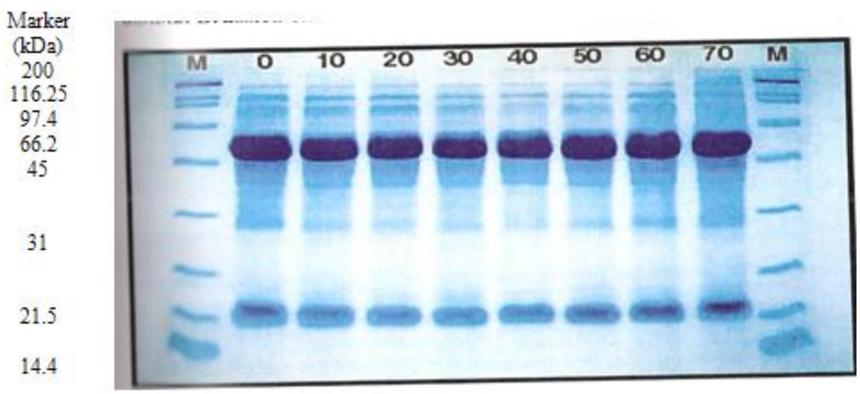


Figure 5. Effects of electromagnetic field at different distances of 10 m interval from the 275 kV power line on the 12.5% SDS PAGE protein banding profile of leaf mustard. Lane M, Marker; Lane 1,0 m; lane 2, 10 m; lane 3, 20 m; lane 4, 30 m; lane 5, 40 m; lane 6, 50 m; lane 7, 60 m; lane 8, 70 m; lane 9, marker.

power line in a 10 m interval. No quantitative differences were observed between the treatments except for the overall banding profile of lane 6 (50 m) which was greatly suppressed compared to the other lanes. Result obtained from the earlier biochemical analyses of this study show that the leaf mustard grown at 50 m had significantly ($p < 0.05$) lower protein, soluble nitrogen and chlorophyll content compared to other treatments but had the highest peroxidase specific enzymatic activity. Therefore, the suppressed protein banding profile obtained from leaf mustard grown at 50 m away from the power line may indicate an unfavourable effect marked by low protein and chlorophyll content and high peroxidase activity.

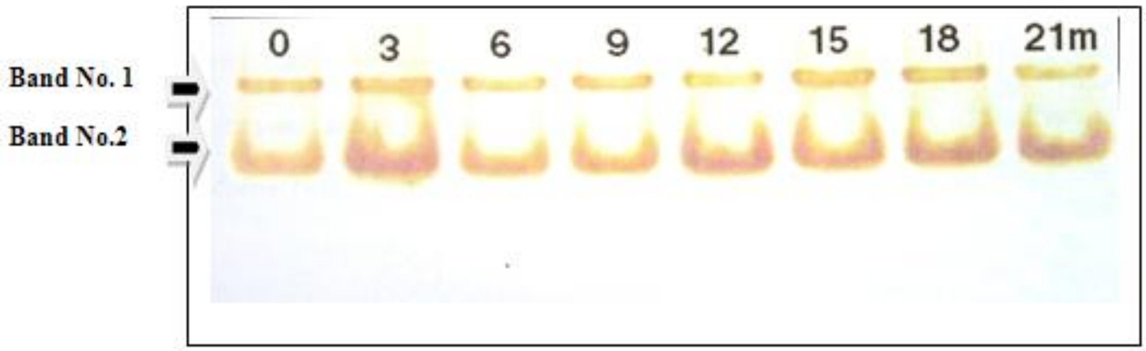
Brassica chinensis below the 275 kV power line

Figure 5 shows SDS PAGE protein-banding profile obtained from leaf mustard grown at different distances from the 275 kV power line at a 10 m interval. Quantitatively, insignificant differences were obtained between the treatments. Since no quantitative differences were observed on the protein profile of leaf mustard planted at different distances from the power line on SDS PAGE, no clear correlation could be concluded between protein bands and the electromagnetic field strength. According to Cooke (1984), protein composition is genetically determined; therefore, further genetic studies would be needed to investigate changes in the genetic level of plants exposed to electromagnetic field under power lines for 25 days.

Peroxidase enzymatic activity bands

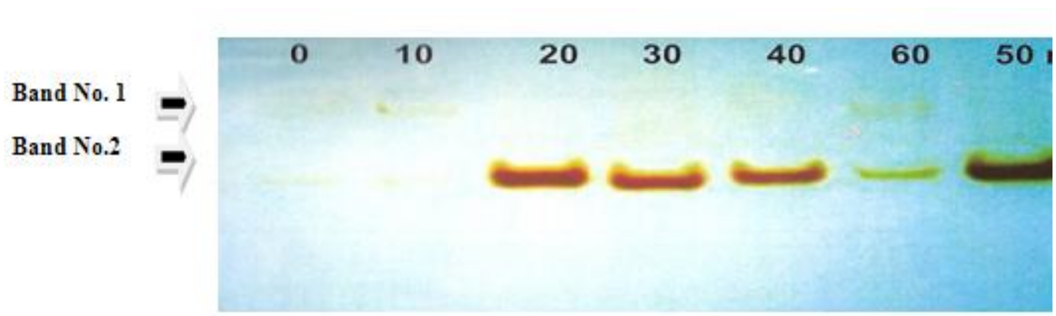
Brassica chinensis below the 33 kV power line

The peroxidase enzymatic activity bands of leaf mustard planted at different distances from the 33 kV power line at a 3 m interval using a 12.5% polyacrylamide gel and



Lane	1	2	3	4	5	6	7	8
DFPL (m)	0	3	6	9	12	15	18	21
Band No. 1	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Band No. 2	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

Figure 6. Peroxidase (POX) enzyme activity bands on 12.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances of 3 m intervals from the 33 kV power line.



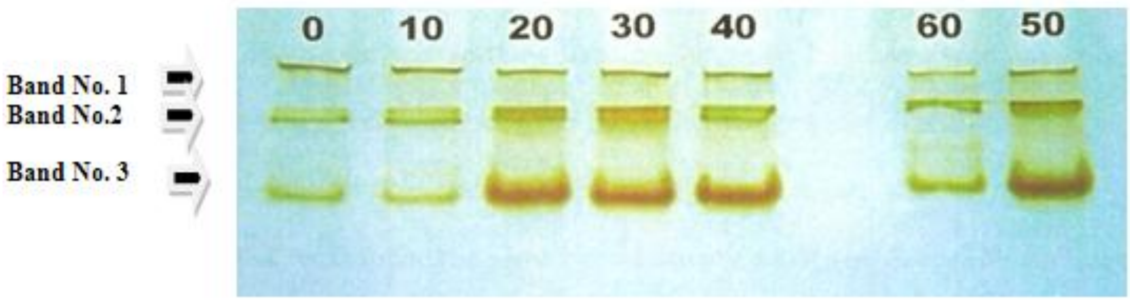
Lane	1	2	3	4	5	6	7
DFPL (m)	0	10	20	30	40	60	50
Band No. 1	0.13	0.13	-	-	-	0.13	-
Band No. 2	0.30	0.30	0.30	0.30	0.30	0.30	0.30

Figure 7. Peroxidase (POX) enzyme activity bands on 7.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances of 10 m intervals from the 33 kV power line.

corresponding R_f values are shown in Figure 6. A total of two enzymatic activity bands at R_f 0.02 and 0.16 were observed in all treatments. Therefore, no difference in the number of peroxidase enzymatic activity bands was obtained within 21 m from the 33 kV power line and no new enzymatic activity bands were induced by the exposure to electromagnetic field.

Peroxidase enzymatic activity banding patterns obtained were rather consistent for most of the samples except for some minor qualitative differences in the staining intensity of some peroxidase bands occupying the same position. The highest enzyme banding intensity was in lane 2 (3 m), followed by lane 5 (12 m), 7 (18 m) and 8 (21 m). However, the high peroxidase enzymatic activity intensity bands did not correlate with their corresponding peroxidase enzymatic activity in Table 3 which showed that all mentioned treatments had lower enzymatic activity than the control sample at 0 m except for 21 m which was insignificantly ($p > 0.05$) different. The band intensity although scored visually was found to be characteristic of electromagnetic field treated leaf mustard and not an artefact since several runs of different samples produced identical results on different gels. This was also observed by Kawaoka et al. (1994) who found increase activities of basic peroxidase isozyme of wounded leaves on isoelectric focusing patterns. Since the same amount of soluble proteins was loaded on gel, the intensity of enzyme activity bands reflects induction patterns of individual peroxidase isoforms (Sreenivasulu et al., 1999).

Figure 7 shows peroxidise enzymatic activity bands and corresponding Rf values of leaf mustard grown at



Lane	1	2	3	4	5	6	7
DFPL (m)	0	10	20	30	40	60	70
Band No. 1	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Band No. 2	-	-	-	0.16	-	0.07	-
Band No. 3	0.14	0.14	0.14	0.19	0.14	0.14	0.14

Figure 8. Peroxidase (POX) enzyme activity bands on 12.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances of 10 m intervals from the 33 kV power line.

different distances from the 33 kV power line at a 10 m interval. The peroxidase enzymatic activity bands stained on a 7.5% polyacrylamide gel revealed a total of two enzyme activity bands at R_f 0.13 and R_f 0.30 in lane 1 (0 m), 2 (10 m) and 6 (60 m) but both bands were faint with low staining intensity. Lane 3 (20 m), 4 (30 m), 5 (40 m) and 7 (50 m) yielded only one peroxidase enzymatic activity band at R_f 0.30 which were high in staining intensity.

Comparatively with Figure 6 which shows an overall increase in banding intensity from 0 to 21 m away from the power line, Figure 7 shows that banding intensity increased from 20 m onwards. In comparison to results obtained in Figure 6, where there was no qualitative difference between the peroxidase enzymatic activity banding patterns from leaf samples within 21 m from the 33 kV power line, results from Figure 7 show that both qualitative and quantitative differences were obtained beyond a distance of 21 m from the power line.

Figure 8 shows peroxidase enzymatic activity bands separation and corresponding R_f values of leaf mustard planted at different distances from the 33 kV power line at 10 m intervals on a 12.5% polyacrylamide gel. The 12.5% polyacrylamide gel resulted in a better banding resolution. All leaf mustard from 0 to 50 m of distance yielded two peroxidase enzymatic activity bands at R_f 0.02 and 0.14 except for leaf mustard at 60 m, which had an additional enzyme activity band at R_f 0.07.

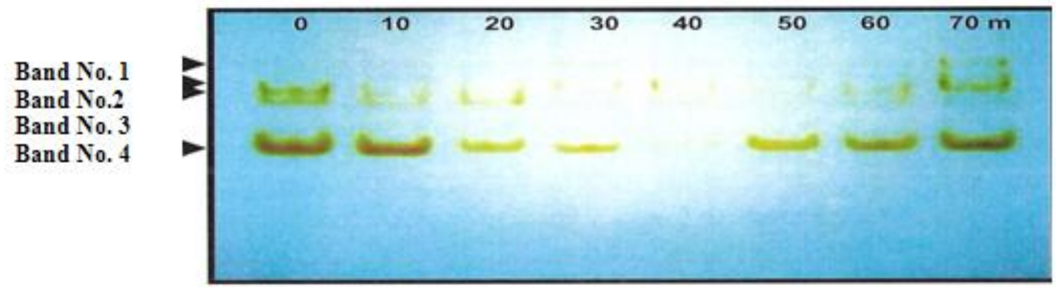
Similar to Figures 6 and 7, peroxidase enzymatic activity bands from leaf mustard nearer to the line at 0 and 10 m were less intense compared to those further away. Similarly, Table 5 shows that leaf mustard nearer to the 33 kV line had lower peroxidase enzymatic activity and enzymatic activity probably explain the low intensity of enzymatic activity bands obtained in lane 1 (0 m) and 2 (10 m). Kokkinakis and Brooks (1979) found that gel stained for peroxidase enzymatic activity bands revealed one major peroxidase band in tomato and more than 80% of peroxidase activity in tomato fruit was due to the single peroxidase enzymatic activity band.

Comparing the difference among leaf mustard peroxidase enzymatic activity bands obtained from 0 m to 50 m distance away from the power line, both Figures 6 and 7 show that the main enzymatic activity banding intensity increase with increasing distance from the power line, whereas leaf samples obtained nearer to the power lines had less intense enzyme activity bands. This observation was as reported by Castillo et al. (1984) who found the same band intensity pattern on Sebum album leaves exposed to ozone of different strength.

Brassica chinensis below the 275 kV power line

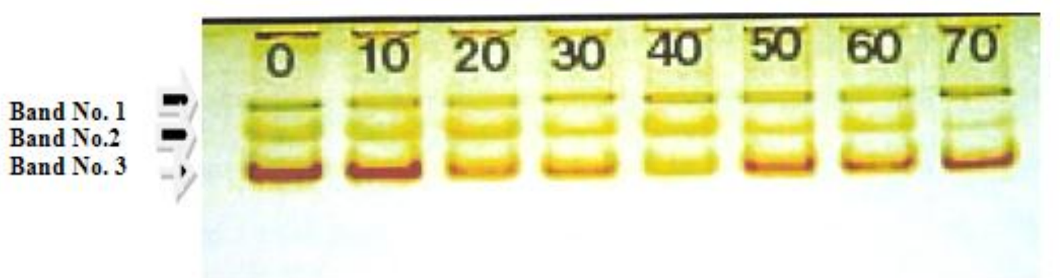
Figure 9 shows staining of peroxidase enzymatic activity bands of leaf mustard grown at different distances from the 275 kV power line on a 7.5% polyacrylamide gel. Enzymatic activity banding profile appears to be diffused due to the low polyacrylamide ration employed which caused bands to be incompletely separated.

Within lanes 1 (0 m), 2 (10 m) and 3 (20 m), a total of three enzymatic activity bands at R_f 0.16, 0.20 and 0.34 were observed with the highest intensity from lane 1, followed by 2 and 3 (Figure 9). Lanes 2 and 3 had a very faint band at R_f 0.16. In a similar pattern, Table 6 reveals that the highest peroxidase specific enzymatic activity was from leaf mustard grown at 0 m from the power line followed by 10 and 20 m. Figure 9 also reveals a very faint extra band at R_f 0.09 in lanes 4 (30 m), 5 (40 m), 6 (50 m), 7 (60 m) and 8 (70 m) resulting in a total of four enzymatic activity bands with the highest intensity in lane 8 followed by lanes 7, 6, 4 and 5.



Lane	1	2	3	4	5	6	7	8
DFPL (m)	0	10	20	30	40	50	60	70
Band No. 1	-	-	-	-	0.09	0.09	0.09	0.09
Band No. 2	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Band No. 3	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Band No. 4	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34

Figure 9. Peroxidase (POX) enzyme activity bands on 7.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances from the 275 kV power line.



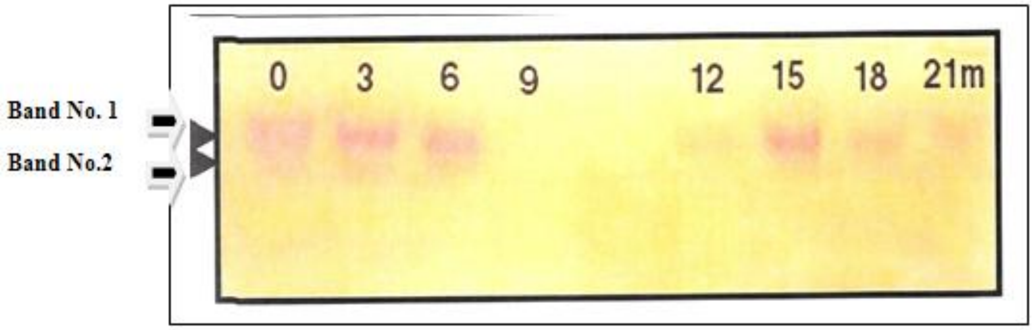
Lane	1	2	3	4	5	6	7	8
DFPL (m)	0	10	20	30	40	50	60	70
Band No. 1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Band No. 2	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Band No. 3	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Figure 10. Peroxidase (POX) enzyme activity bands on 12.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances from the 275 kV power line.

In an attempt to obtain a better resolution of peroxidase enzymatic activity bands, the same samples were stained on a 12.5% polyacrylamide gel as shown in Figure 10. The enzymatic activity banding profile obtained from the 12.5% polyacrylamide gel supported the fact that a higher polyacrylamide ratio with smaller gel matrix pore results in a better resolution banding profile.

A total of three peroxidase enzymatic activity bands at R_f 0.01, 0.06 and 0.15 were observed with insignificant differences quantitatively (Figure 10). However, the highest intensity was observed in lanes 1 (0 m) and 2 (10 m), which correspondingly had the highest peroxidase specific enzymatic activity as shown in Table 6. In contrast to the peroxidase enzymatic activity bands staining obtained from leaf mustard planted at different distances from the 33 kV power line as shown in Figures 6 and 7 which showed an increasing banding intensity with increasing distance from the power line, Figures 9 and 10 showed a decreasing intensity with increasing distance from the 275 kV power line.

Higher banding intensity was observed in leaf mustard at 0 and 10 m away from the 275 kV line while lower banding intensity was observed in leaf mustard planted at 0 and 10 m away from the 33 kV power line. This difference in response may be due to the electromagnetic field strength emitted form the 33 and 275 kV power line where the latter produced a much higher electromagnetic field strength which may result in the increase in



Lane	1	2	3	4	5	6	7	8
DFPL (m)	0	3	6	9	12	15	18	21
Band No. 1	0.16	0.16	0.16	-	0.16	0.16	0.16	0.16
Band No. 2	0.20	0.20	0.20	-	-	-	-	-

Figure 11. Glutamate oxaloacetate transminase enzyme activity bands on 12.5% polyacrylamide gel and the R_f values of leaf mustard planted at different distances of 3 m intervals from the 33 kV power line.

peroxidase enzymatic activity banding expression and enzyme activity.

Glutamate oxaloacetate transaminase (GOT) enzymatic activity bands

Successful glutamate oxaloacetate transaminase enzymatic activity banding profile was obtained from leaf mustard that was grown at different distances from the 33 kV power line in a 3 m interval. Figure 11 shows the enzymatic activity bands stained on a 12.5% polyacrylamide gel and corresponding R_f values. Lanes 1 (0 m), 2 (3 m) and 3 (6 m) revealed a total of two enzymatic activity bands at R_f 0.16 and 0.20 with the highest intensity in lane 1, followed by 2 and 3.

The GOT enzymatic activity banding pattern on Figure 11 shows that lane 6 (12 m), 7 (15 m), 8 (18 m) and 9 (21 m) yielded one enzymatic activity band with the highest intensity in lane 7, followed by lanes 8, 9 and 6. The results on Table 8 suggests that the intensity of glutamate oxaloacetate transaminase enzymatic activity bands may be correlated with total chlorophyll content of leaf mustard as the highest total chlorophyll content was found at distance 0 m, followed by 3, 6 and 15 m which coincide with the enzyme activity banding intensities in Figure 11.

According to Shalaby and Al-Wakeel (1995), glutamate oxaloacetate transaminase enzymatic activities are sensitive and decrease during stress as reduction in GOT leads to accumulation of glutamate and a decrease in aspartate level often seen in plant-related stress. Another observation from Figure 11 is that no glutamate oxaloacetate transaminase enzymatic activitiy band was detected at lane 4 which correspond to leaf mustard at 9 m away from the power line. The suppression or deletion of GOT enzymatic activity bands from the leaf mustard planted at 9 m away from the line suggests that GOT, one of the key enzymes in nitrogen metabolism in leaf mustard, is sensitive to the changes induced by electromagnetic field. However, the significance of the electromagnetic field of 0.115 kV/m and 4.12 mT at 9 m and the mechanism involved for the interaction could not be establish.

In comparison to Figure 8, which shows no difference in the number of peroxidase enzymatic activity bands obtained from leaf mustard planted at 3 m intervals from the 33 kV power line, the glutamate oxaloacetate transaminase enzymatic activity bands exhibited a more distinct difference between the treatments. This is supported by Shalaby and Al-Wakeel (1995) who reported that the staining activities of GOT were more sensitive than peroxidase, therefore, the effect of electromagnetic field on the GOT enzymatic activity bands was more pronounced.

Conclusion

The results obtained show that higher protein, soluble nitrogen, chlorophyll content and peroxidase enzyme activity were obtained from leaf mustard planted within 20 m from the 275 kV power line, thus exposed to higher electromagnetic field. The increase in total chlorophyll content and chlorophyll a to b ratio signifies a desirable effect of electromagnetic field from 275 kV power line on increased plant growth via photosynthesis.

The analysis of protein elctrophoretic profile and peroxidase enzymatic activity bands revealed no drastic changes in the protein and peroxidise banding profile of leaf mustard grown under the influence of electromagnetic field. Increased peroxidase enzymatic activity and the minor difference in the staining intensities of peroxidase enzymatic activity bands indicate the sensitive response of the plant towards external electromagnetic field stimuli. Similarly in the case of leaf mustard planted at different distances away from the 33 kV power line, higher protein and soluble nitrogen content was found in leaf mustard planted within 20 m from the line and decreases with increasing distance. Chlorophyll content was also higher in leaf mustard exposed to higher EMF and decreases with increasing distance from the line.

The wide range of results obtained from the effects of electromagnetic field from different power lines sources on leaf mustard suggest that the effects of electromagnetic field on crop are dynamic, as many factors play a role in the amount of electromagnetic field received by the crop. However, a common resemblance in the results obtained shows that it is unlikely for extremely damage or protein banding changes to plant tissues or cells to appear, instead, there are possibilities of accelerated growth to emerge due to increased chlorophyll and protein content.

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