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# APPENDEX I

## Preparation of chitin hydrolysates (Rupiey,1964)

Chitin (polymer of GlcNAc) in the form of ground clam shells, was purchased from Sigma. It was ground further in a coffee grinder and sieving through a sieve No.100. The powdery chitin was washed extensively with water, 1N HCl, 2% $NaCO_3$, and 95% ethanol, by suction through a filter paper (Whatman#1) in a Buchner funnel, and allowed to dry. Chitin (20 gm) was dissolved in 200 ml cold concentrated HCl (Merck)and stirred at 4 °C by a magnetic stirrer, suspension was complete within 2 h. Hydrolysis was accomplished by maintaining the resulting solution at 40 °C for 2-3 h, conditions under which the maximum concentration of oligomers was obtained. The solution was subsequently cooled. and the pH brought to 1 with 50% NaOH. During the initial stage of neutralization, the temperature was kept below 20 °C. A precipitate of unhydrolyzed chitin and NaCl was removed by filtration. The hydrolysate was checked for its ability to inhibit agglutination of red cells by lectin, and 1% of chitin hydrolysates was prepared in 0.05 M sodium acetate buffer, pH 3.8 as eluant.

# APPENDIX II

## Statistical analysis of t-test

Table 3.8 Lectin distribution in root and leaf of rice (RD 7) seedlings under light and dark conditions.

| Time (day) | ng lectin/ mg protein | | | | Total lectin(ng)/100 plants | | | |
|---|---|---|---|---|---|---|---|---|
| | Light | | Dark | | Light | | Dark | |
| | leaf | root | leaf | root | leaf | root | leaf | root |
| 4 | 109±10* | 17±1 | 210±41* | 15±2 | 893±80* | 44±1 | 2628±522* | 58±6 |
| 5 | 40±1 | 14±1 | 127±12 | 19±1 | 461±9 | 32±2 | 1367±132 | 38±2 |
| 6 | 39±4 | 16±1 | 51±4 | 11±1 | 501±53 | 39±2 | 603±49 | 15±1 |
| 7 | 9±2 | 12±1 | 17±2 | 12±2 | 175±44 | 23±2 | 153±20 | 28±5 |

Values presented are mean of 6 measurements

From table of standard normal curve at 0.05 ; $Z_{\alpha/2}$ two side test = $\pm 1.96$

For total lectin in leaf of 4 day old seedling grown under dark and light condition.

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2) ) / \sqrt{(\delta_1^2/n_1) - (\delta_2^2/n_2)}$$

$$= (2628-893) / \sqrt{((522)^2/6) + ((80)^2/6)}$$

$$= 1735/215$$

$$= 8.07$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of total lectin in leaf grown under dark and light condition ($P < 0.004$)

For leaf lectin concentration (ng lectin/mg protein) of 4 day old seedling grown under dark and light condition.

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2) ) / \sqrt{(\delta_1^2/n_1) - (\delta_2^2/n_2)}$$

$$= (210-109) / \sqrt{((41)^2/6) + ((10)^2/6)}$$

$$= 101/17.2$$

$$= 5.87$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of total lectin concentration in leaf grown under dark and light condition ($P < 0.004$)

# APPENDIX III

## Statistical analysis of t-test

Table 3.10 Effect of $NH_4$ ion to lectin content in root and leaf. Rice seedlings grown in aqueous solution that contained $NH_4Cl$ 2 and 20 mM for 4 days under light condition, and lectin were measured by modified method.

| [$NH_4Cl$] (mM) | ng lectin/ mg protein | | Total lectin (ng/100 plants) | |
|---|---|---|---|---|
| | leaf | root | leaf | root |
| 0 | 228±61 | 31±7 | 2628±522 | 92±20 |
| 2 | 212±5 | 24±1 | 1681±57* | 102±4 |
| 20 | 173±1 * | 13±1 * | 1623±16* | 49±2 * |

Values presented are mean of 6 measurements

From table of standard normal curve at 0.05 ; $Z_{\alpha/2}$ two side test $= \pm 1.96$

For the total lectin in leaf at 0 and 2 mM $NH_4Cl$

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2) ) \sqrt{(\delta_1^2/n_1) - (\delta_2^2/n_2)}$$

$$= (2628-1681) \sqrt{((522)^2/6) + ((57)^2/6)}$$

$$= 947/214.3$$

$$= 4.42$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of total lectin in leaf grown under 0 and 2 mM $NH_4Cl$ ( $P < 0.004$)

For the total lectin in leaf at 0 and 20 mM $NH_4Cl$

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1-\bar{x}_2)-(\mu_1-\mu_2) ) / \sqrt{(\delta_1^2/n_1)-(\delta_2^2/n_2)}$$

$$= (2628-1623)/\sqrt{((522)^2/6)+((16)^2/6)}$$

$$= 1005/213$$

$$= 4.7$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of total lectin in leaf grown under 0 and 20 mM $NH_4Cl$ ( $P < 0.004$)

For the total lectin in root at 0 and 20 mM $NH_4Cl$

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1-\bar{x}_2)-(\mu_1-\mu_2) ) / \sqrt{(\delta_1^2/n_1)-(\delta_2^2/n_2)}$$

$$= (92-49)/\sqrt{((20)^2/6)+((2)^2/6)}$$

$$= 43/8.16$$

$$= 5.37$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of total lectin in root grown under 0 and 20 mM $NH_4Cl$ ( $P < 0.004$)

For lectin concentration (ng lectin/mg protein) in leaf at 0 and 20 mM $NH_4Cl$

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1-\bar{x}_2)-(u_1-u_2) ) / \sqrt{(\delta_1^2/n_1)-(\delta_2^2/n_2)}$$

$$= (228-173) / \sqrt{((61)^2/6)+((1)^2/6)}$$

$$= 55/24.89$$

$$= 2.21$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of lectin concentration in leaf grown under 0 and 20 mM $NH_4Cl$ ( $P < 0.02$)

For lectin concentration (ng lectin/mg protein) in root at 0 and 20 mM $NH_4Cl$

$n_1 = 6$ ; $n_2 = 6$

$$Z_c = ( (\bar{x}_1-\bar{x}_2)-(\mu_1-\mu_2) ) / \sqrt{(\delta_1^2/n_1)-(\delta_2^2/n_2)}$$

$$= (31-13)/ \sqrt{((7)^2/6)+((1)^2/6)}$$

$$= 18/2.88$$

$$= 6.25$$

$Z_c > Z_{\alpha/2}$

It was significant difference of the mean of lectin concentration in root grown under 0 and 20 mM $NH_4Cl$ ( $P < 0.004$)

# BIOGRAPHY

Mr. Srimake Chapongpang was born on August 4, 1961 in Samutsakorn and graduated with the B.Sc. in Biology from Faculty of Science, Ramkhamhaeng University in 1986. He has been working in the Poultry Disease Section, Division of Veterinary Service, Department of Livestock Development, Ministry of Agriculture and Cooperative since 1983.