BIOLOGIA PLANTARUM 35 (4): 567-576, 1993

A mathematical model to predict the tissue response to parthenin - an allelochemical

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Abstract

Parthenin - a sesquiterpene lactone from *Parthenium hysterophorus* L. is an allelochemical that prevents the germination of *Phaseolus aureus* Roxb. cv. ML-5 seeds. The response of the seed has been attributed to the inhibition of the respiratory electron transport ability of its embryo. It has been shown to depend directly not only upon concentration of parthenin, but simultaneously on the duration of exposure of the seeds to the chemical as well. A strong correlation exists between the quantum of the response and the product of the period of exposure and the concentration of parthenin. In order to predict the maximum possible germination ability of the seed exposed for a given period to a given concentration of parthenin, an expression $X = 10\,000\ Y^3 / [e^{Y/0.31}-1]$ was formulated from the equation $X = AY^3/[e^{Y/Y_0}-1]$, where X represents values of maximum respiratory activity, Y represents the product of concentration and time in units mg cm^{-3} h, A represents a dimensional constant. The trend and nature of response that is calculated on the basis of formulation coincides with that of measured response through spectrophotometry.

Introduction

Parthenium hysterophorus L. (*Asteraceae*) is a hazardous weed, well known for its deleterious effects on the environment and living beings including humans. It has many toxic principles besides sesquiterpene lactones. Parthenin - a sesquiterpene lactone constitutes a major component of plants in this genus. This compound is responsible for most of the harmful effects *viz.* allelopathic property (Kanchan and Jayachandra 1980, Kumari and Kohli 1987, Mersie and Singh 1988 and Rani 1990) and toxicity towards animals and human beings (Narasimhan *et al.* 1977, 1980) of the plant. The parthenin-caused effects of the plant includes inhibition of germination of seeds. For seeds to germinate, the embryo axis is required to elongate. The embryo elongation takes place at the expense of energy which is made available to the active meristematic cells through active respiratory metabolism. In other words, the of the cells as a respiratory function could be taken as a precise index response of action of

Received 4 February 1992, *accepted* 21 August 1992.

action of the allelopathic agent - parthenin. The seed germination depends directly on the concentration of the parthenin and period of exposure of the seed to it. This experiment was, therefore, designed to construct some empirical mathematical model fitting with the pattern of response.

Materials and methods

Extraction of parthenin: Parthenin was extracted from the dried leaf powder of *P. hysterophorus* L. by the method of Dominguez and Sierra (1970). Aqueous solution of parthenin in concentrations 0.01 % to 0.06 % (0.01 % increment) were prepared from the stock solution. For preparing the stock solutions, required amount of parthenin was dissolved in a few drops of ethanol and the final volume was made up with pure water.

Seed germination trial: Healthy, pure-line, viable [tested following ISTA (1976) rules] seeds of *Phaseolus aureus* Roxb. cv. ML-5 were procured from the Pulse Breeding section of Punjab Agricultural University, Ludhiana. The requisite number of seeds of *P. aureus* were soaked, separately for 8 h in 100 cm^3 solution of the respective concentration of parthenin. Soaking in water served as control. Embryos from the one quarter of the imbibed seeds, were recovered microsurgically and subjected to the determination of cell respiratory activity soon after 8 h. The rest of the water treated or parthenin treated seeds were placed to germinate on Whatman No. 40 filter paper discs underlined with a thin cotton wad, in glass Petri dishes in a seed germination cabinet maintained at 25 ± 1 °C. The substratum was properly moistened with the requisite amount of the respective solution of the treatment. Seeds subjected to germination were divided into three equal groups. Each group was removed from the substratum after 8, 16 or 40 h from the time of arranging them (seeds were arranged 8 h after the start of imbibition). The embryo part from these seeds was separated microsurgically under aseptic conditions. The extractions from the respective lots were pooled and subjected immediately to the calculation of % respiratory activity of the embryos with respect to control.

Determination of per cent respiratory activity of the embryos: Per cent respiratory activity of embryos of the sets of all concentrations soon after 8, 16, 24 or 48 h from the start of the treatment were determined using 2,3,5-triphenyl tetrazolium chloride following the method of Steponkus and Lanphear (1967). The % respiratory activity of embryos (with respect to control) of each set was calculated following the formula as given below:

$$\text{\% Respiratory activity} = \frac{\text{Absorbance of treated sample at 530 nm}}{\text{Absorbance of control at 530 nm}} \times 100$$

Ten replicates for each treatment and at each time including those of water treated control were used and the data were subjected to Duncan's Multiple Range Test, (DMRT, Duncan 1955).

Results

The extinction values of water treated control show a gradual increase with respect to increasing period of germination from 8 to 48 h of imbibition (Fig. 1a). A strong direct correlation (r = + 0.957) between period of exposure and extinction value was observed. The embryos of seeds treated with 0.01 % parthenin showed nearly 40 % respiratory activity of that of untreated control at 8 h of inhibition (Fig. 1b). It registered an increase till 24 h stage followed by a decline at 48 h stage. Similar trend of respiratory activity in embryos could be seen in seeds treated with 0.02 % parthenin (Fig. 1b). The embryos of seeds treated with 0.03 % parthenin showed an

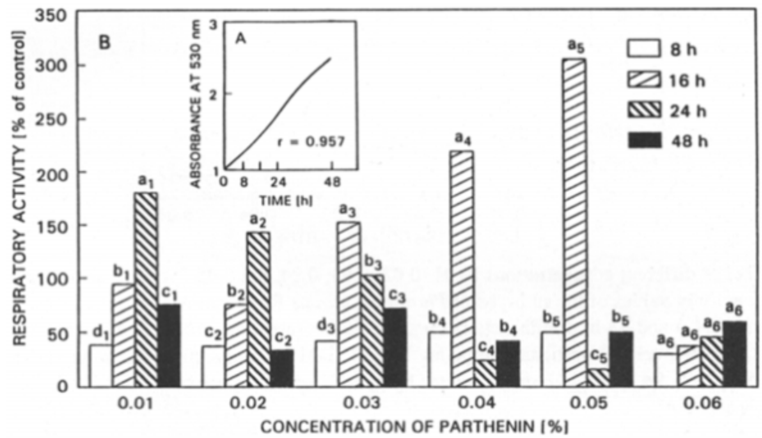

Fig. 1a. Extinction values of water treated control embryos of *Phaseolus aureus* Roxb. seeds at different time intervals. r = correlation coefficient.

Fig. 1b. Effect of different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06 %) of parthenin on per cent respiratory activity values (with respect to control) of embryos of *Phaseolus aureus* Roxb. seeds at different time intervals, *i.e.* 8 h, 16 h, 24 h and 48 h. Columns (representing mean values) having same superscript symbols do not differ significantly from each other at 5 % level of significance applying DMRT. DMRT was applied separately at every concentration, hence, the symbols have been differentiated by writing 1, 2, 3, 4, 5 or 6 representing six different concentrations.

increase in the value of respiratory activity till 16 h stage. However, beyond that a decline was noticed. With still higher concentration, *i.e.* 0.04 % or 0.05 % parthenin, a sharp increase in the values of respiratory activity was seen at 16 h stage. After 16 h stage *i.e.* at 24 and 48 h stages, these values were at the minimum. It is interesting to note that at still higher concentration of parthenin *i.e.* 0.06 %, no increase in the respiratory activity could be seen at any of the stages under test. It is evident from Fig. 2 that at 8 h stage of treatment, the values of respiratory activity at any of the treated cases remained nearly 50 % to that of control. The difference in the values under various concentrations of parthenin was statistically insignificant. However, at 16 h stage, a sharp increase in the values of cell survival was observed with increase in concentration of parthenin from 0.02 to 0.05 %. In the 24 h stage on the contrary, a steady decrease at every increase in concentration of parthenin from 0.01 to 0.05 %

of parthenin was visualized. At 48 h stage, the changes in the values of respiratory activity were not so sharp as in 16 h or 24 h stage.

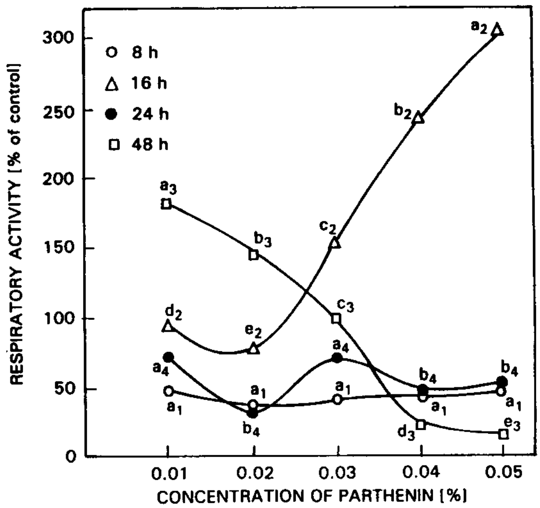

Fig. 2. Effect of different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 %) of parthenin on per cent respiratory activity values of the embryos of *Phaseolus aureus* Roxb. seeds at different time intervals, *i.e.* 8 h, 16 h, 24 h and 48 h. Similar letters on symbols (representing mean value) do not differ from each other at 5 % level of significance applying DMRT. DMRT was applied separately at each hour stage, *i.e.* 8 h, 16 h, 24 h and 48 h differentiated by 1, 2, 3 and 4, respectively.

Since, the parthenin caused effect is not only dependent upon concentration (*c*) but also upon duration of treatment (*t*), the product of these two basic variable (*ct*), when plotted against values of respiratory activity gave an areal type of curve (Fig. 3). This curve was obtained by joining maximum observed values of respiratory activity with respect to a particular *ct* value. The values of the respiratory activity of the embryo of seeds beyond the *ct* value of 1.2 mg cm^{-3} h was found to be always less than 100 (the value of that of water treated control). This value of respiratory activity gets stabilized at 40 % beyond 1.5 mg cm^{-3} h value of *ct*. For a particular value of *ct* below 0.8 mg cm^{-3} h in the graph mg cm^{-3} h, it decreases exponentially. In order to incorporate both these features, we tried to find a suitable function:

$$X = \frac{AY^3}{e^{Y/Y_0} - 1} \qquad (1)$$

(Justification for the use of this mathematical function has been given in Appendix 1)

X = maximum per cent respiratory activity value*

Y = product of concentration and time [mg cm^{-3} h]*

Y_0 = the threshold value of Y at which the value of respiratory activity would be maximum**

A = dimensional constant**

* Values known

**Values to be calculated

In order to find out the value of Y_0, the equation (1) was differentiated, equating the derivative to zero, we get:

$$\frac{dX}{dY} = \frac{3AY^2}{e^{Y/Y_0} - 1} - \frac{1}{Y_0} \times \frac{AY^3 \cdot e^{Y/Y_0}}{(e^{Y/Y_0} - 1)^2} = 0 \qquad (2)$$

Taking the 2[nd] term on right hand side and multiplying the both sides by $e^{Y/Y_0}-1$

$$3\,AY^2 = \frac{1}{Y_0} \times \frac{AY^3 \cdot e^{Y/Y_0}}{e^{Y/Y_0} - 1} \qquad (3)$$

Dividing both sides by AY^2

$$3 = \frac{Y}{Y_0} \times \frac{e^{Y/Y_0}}{e^{Y/Y_0} - 1} \qquad (4)$$

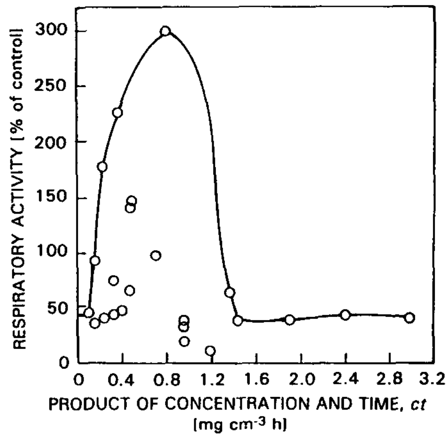

Fig. 3. Curve showing the behaviour of per cent respiratory activity values (with respect to control) of embryos of *Phaseolus aureus* seeds when plotted against the product of concentration and time interval of treatment (*ct* [mg cm^{-3} h]) of parthenin.

Dividing numerator and denominator by e^{Y/Y_0} and rearranging the term we get:

$$\frac{Y}{Y_0} = 3\,(1 - e^{-Y/Y_0})$$

This transcendental equation is satisfied with $\alpha = Y/Y_0$

(a) for $\alpha = 0$

(b) for $\alpha = 2.82$

The (a) value corresponds to the beginning of the function, *i.e.* $Y = 0$, whereas (b) gives us the desired value at which maximum of the function occurs. So

$$\alpha = 2.82$$

or

$$Y/Y_0 = 2.82$$

Using $Y = 0.8$ mg cm^{-3} h from the graph, at which maximum occurs

$Y_0 = 0.8 / 2.82 = 0.285$ mg cm^{-3} h

Now when the values of Y, X and Y_0 are known, the value of A can be calculated:
So taking

$$X = \frac{AY^3}{e^{Y/Y_0} - 1} \tag{5}$$

$$A = \frac{X\,(e^{Y/Y_0} - 1)}{Y^3} \tag{6}$$

Which yields A = 9147.8, for the values at maxima.
In order to calculate more appropriate value of A and Y_0, the equation (1) is reconsidered.

$$X = \frac{AY^3}{e^{Y/Y_0} - 1}$$

or

$$e^{Y/Y_0} = \frac{AY^3}{X} + 1$$

Taking natural logarithm (ln)

$$\ln\,[e^{Y/Y_0}] = \ln\left(\frac{AY^3}{X} + 1\right)$$

$$\ln\left(\frac{AY^3}{X} + 1\right) = Y/Y_0$$

By drawing the plot between ln $[(AY^3/X) + 1]$ versus Y, the value of Y_0 can be calculated from the slope of the curve. Various curves were drawn by varying the values of A from 8 000 to 11 000 (the range 8 000 to 11 000 was selected keeping in view of the value of A, *i.e.* A = 9147.8 that was obtained from equation 6). At A = 10 000 best straight line curve was obtained. At this value of A = 10 000, the value of Y_0 can be calculated from the slope (Fig. 4). The slope yielded the value of Y_0 = 0.31. The equation can, therefore, be rewritten as:

$$X = \frac{10\,000\,Y^3}{e^{Y/0.31} - 1}$$

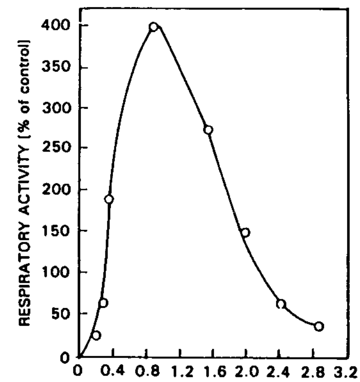

Fig. 4. Best straight line curve between product of concentration of parthenin and time interval represented by Y.
ln $(AY^3 / X) + 1$ is obtained at A = 10 000.
Y represents product of concentration of parthenin and time of treatment,
X represents per cent maximum respiratory activity value with respect to control, and
A represents dimensional constant value = 10 000.

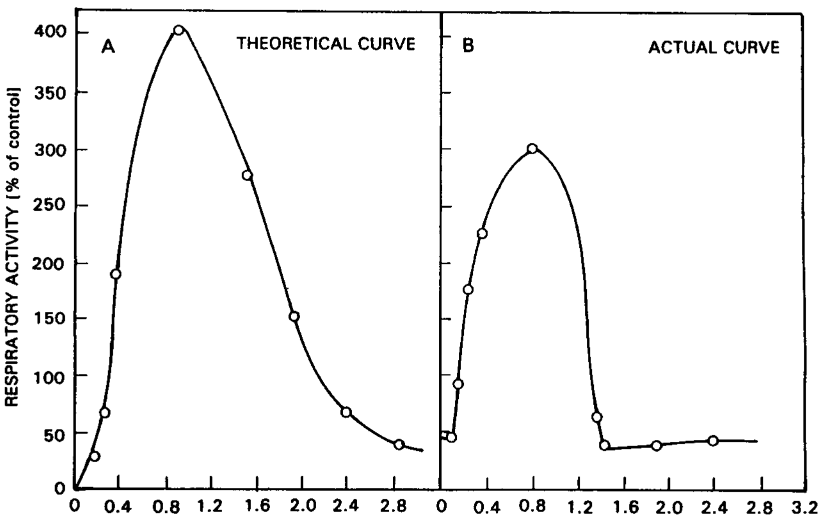

Fig. 5. Behaviour of curve obtained by plotting product of concentration of parthenin and time represented by ct. against calculated values of the per cent respiratory activity (Fig. 5a) or those obtained from the observed data (Fig. 5b).

When reciprocal values of X were calculated by substituting the values of Y in the above equation, same trend of the curve (Fig. 5) was obtained as was for the observed values of Y (Fig. 3). Upon putting the value of A, Y and Y_0 and calculating

X, it is seen that maximum respiratory activity is obtained after 16 h exposure of the seed embryos to the parthenin (Fig. 6).

Discussion

From the results of this experiment, it is evident that the respiratory activity values of the embryos of *P. aureus* gradually increased with the passage of time. In the treated embryos, however, the trend of changes in the values of respiratory activity was different for different concentrations of parthenin. At lower concentration *i.e.* 0.01 and 0.02 %, the values of respiratory activity increased till 24 h stage, whereas at higher concentrations of 0.4 and 0.05 %, this increase was apparent only at 16 h stage. In other words, the higher dose of parthenin results in earlier and sharp increase in the respiratory activity. The respiratory activity is in fact depicted from the rate and excent at which 2,3,5-triphenyl tetrazolium salt traps the oxygen molecules releated through respiratory chain. It is apparent from the Fig. 6 that the maximum possible respiratory activity of the embryos in response to parthenin is achieved after 16 h exposure. It seems pertinent that the embryo cells tend to antagonise or resist the toxic effect of parthenin by stepping up the respiratory activity, but only to a limited degree of stimulus. When the stimulus crosses the threshold limit, the system fails to resist and thereby, shows less respiratory activity.

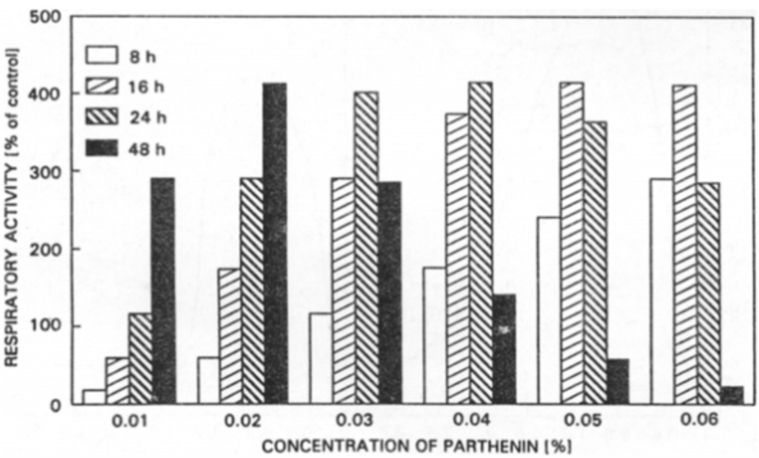

Fig. 6. Trends of % respiratory activity in embryos of *Phaseolus aureus* in response to different concentrations of parthenin calculated on the basis of the formulation.

It is primarily because of this reason that at higher concentration of 0.06 % parthenin, no increase in the respiratory activity could ever be noticed. The threshold level of stimulus could be achieved earlier with higher concentration of parthenin or after sometime, if the concentration of parthenin happens to be low. It is, therefore, logical to believe that both concentration of parthenin and duration of its contact in the system are important components of stimulus. The maximum possible response of embryo to a given concentration of parthenin could be predicated by substituting the value of Y (Y = product of concentration and time) in the mathematical formula:

$$X = 10\,000\, Y^3/[e^{Y/0.31}-1]$$

It is presumed that this formula may hold good even for other allelochemicals in the same system. In any case, for any allelochemical in the germinating system, such predictions are feasible through procedure outlined in this experiment.

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APPENDIX 1

The empirical mathematical function has been guessed based on the following observations:

a) The graph (Fig. 3) has a maximum at some central values, say at Y_{max}.

b) For $Y < Y_{max}$, the graph increase in a parabolic manner.

c) For $Y > Y_{max}$ the graph has a decreasing exponential behaviour.

We tried various kinds of mathematical function to obtain these trends. Finally the choice of the function.

$X = AY^3/[e^{Y/Y_0}-1]$ suited well for the purpose as:

(1) For low Y/Y_0, the denominator can be simplified to:

$$e^{Y/Y_0} - 1 = (1 + Y/Y_0 + \dots\dots\dots\dots) - 1 \rightarrow Y/Y_0 \text{ using}$$

$$e^x = \sum_n \frac{X^n}{n!}$$

Higher powers being small are ignored. Thus

$$X = \frac{AY^3}{e^{Y/Y_0} - 1} \rightarrow (AY_0)\, Y^2, \quad \textit{i.e.} \text{ parabolic behaviour.}$$

(2) For higher Y/Y_0, the exponential term dominates over unity in the denominator, thereby, reducing the function to

$$X = \frac{AY^3}{e^{Y/Y_0} - 1} \rightarrow AY^3 \,.\, e^{-Y/Y_0}$$

which represents an exponential decrease.

(3) So obviously, for some intermediate value of Y/Y_0, the function (1) has its maximum, which depends upon the choice of A sa well as Y_0.

Communicated by J. KREKULE