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Effect of Systemic Fungicides and Insecticides on Absorption Spectra, Chlorophyll and Phenolic Contents of *Vigna radiata* (L.) Wilczek

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Abstract: Effects of systemic fungicide (topsin-M) and insecticide (dimecron) on absorption spectra, chlorophyll and total phenolic content of *Vigna radiata* were examined. Results revealed significant ($P<0.001$) deviation on absorption spectra of both chlorophyll a and b. However, dimecron had greater effects as compared to control. Chlorophyll and phenolic contents were stimulated by the application of systemic fungicide and insecticide. Use of pesticides such as topsin-M and dimecron showed some harmful effects on plant at higher concentration.

Key word: Systemic fungicide, insecticide, absorption spectra, chlorophyll, phenols

Introduction

Topsin-M (systemic) are often classified under benzimidazole fungicides as they are based on thiourea. For its activity it is converted into benzimidazole ring and its fungicidal spectrum also resembles that of binomial and used against the control of diseases affecting legumes, cucurbit, malvaceous and solanaceous crops (Sobti, 1993). Topsin-M chemically is 3-methoxy carbonyl-2-thioureido benzene. Dimecron a systemic insecticide belongs to the organophosphate group that contains a.i. 500g/c.c phosphomidon used for the control of *Tryporyza, Chilo, Sesemia, Spodoptera, Plutella, Planococcus* and *Eulecanium* population (Siddiqui *et al.*, 1999).

Use of pesticides in the last few decades has revealed drastic changes in the environment and showed considerable bad effects on non target organisms (Mishra and Mani, 1994). Indiscriminate use of systemic fungicides and insecticides on fruits and vegetables causes direct and indirect problems (Mukerjee and Gopal, 1996; Sakul, 1995). Agrochemicals such as insecticides, fungicides, rodenticides and fertilizers could alter the various metabolic processes (Hartado, 1987). Application of organophosphate insecticides inhibit the seed germination and seedling growth of *Peniesetum americanum* L. (Siddiqui *et al.*, 1999). Like wise use of systemic fungicides produced chlorosis and irregular depression at the central and marginal portions of saffron leaf (Reyes, 1975), induced sharp decrease in cell division (Coman *et al.*, 1990) and inhibited seedling growth of pea (John *et al.*, 1975). Despite the facts, use of systemic agrochemicals are the need of present time, where population is increasing rapidly demanding great amount of foods. Extensive production of economically important crops is the remaining and only hope that would fulfill the basic requirements of the modern time. Present investigation was, therefore made to examine the comparative phytotoxicity produced by a systemic fungicide and insecticide. Effects were observed on light absorption of chlorophyll a and b, total chlorophyll and stress phenolic contents of *Vigna radiata*.

Materials and Methods

Collection, Sterilization and Treatments: Seeds of *V. radiata* (L.) Wilczek were obtained from National Institute of Agriculture and Biology Faisalabad, surface sterilized with 0.15% mercuric chloride for 10min and were then washed with distilled deionized water. Twenty-five seeds were sown in each plot of an area 2x2 sq. feet. Ten healthy seedlings were allowed to grow up to fruiting stage. Twenty-one days old seedlings were sprayed with systemic fungicide (topsin-M) and insecticide (dimecron) at a rate of 1000, 1500 and 2000ppm. The concentrations of both fungicide and insecticide were prepared in distilled water on the basis of a.i (active ingredient). Unsprayed plants served as controls. The average temperature 28-35°C and 45-55% relative humidity existed throughout the experimental periods. Leaf samples were collected randomly after one week of spray. Data sets were replicated thrice and subjected to factorial analysis of variance (FANOVA) followed by Duncan's multiple range test and correlation using SPSS program package (Zar, 1984).

Chlorophyll Determination: One gm fresh leaves were chopped and taken in test tube containing 90% ethanol and refluxed on a water bath at 75 °C for 30min. Optical density read at 663 and 645nm and total chlorophyll were calculated by Malchlalam and Zalik (1963) equation.

Absorption Spectrum of Chlorophyll a and b: Absorption spectrum of chlorophyll a and b were examined in ether solvent. Fifteen gm fresh leaves crushed in 50ml of 95% acetone with 0.05gm calcium carbonate. Centrifuged the whole set up at 2000rpm for 5 min. Forty ml of acetone extract was transferred to a separating funnel with 60ml pet. ether and 75ml distilled water. The set up was shaken in an electronic shaker for 10 min. Upper deep green layer was used to separate the pigments after repeated washing with 50ml distilled water. Forty ml of 90% methanol was added in washed pet. ether solution. Upper pet. ether layer extract was used to isolate chlorophyll a by mixing 15ml of 30% methanolic KOH and 30ml distilled water while, lower methanolic extract layer was used to separate chlorophyll b by adding 50ml pet. ether and discarding the lower methanol layer. The absorbance of chlorophyll a and b were measured at 380,400, 420, 440, 460, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700nm.

Phenols Determination: Total phenols of leaf samples were extracted and estimated by the method of Swain and Hillis (1959). One gram samples were plunged into 2N HCl with the result that the tissues were killed immediately. The tissues were crushed with 10 ml of 2N HCl and material was taken in a tube and boiled for about half an hour on water bath. Then it was filtered and the filtrate was left over $CaCl_2$ in a desiccator at room temperature until dryness. Estimation was made in ethanol extract optical density read at 660nm against reagent blanks and quantity was expressed in Ug/gm fresh

weight using standard curve.

Results and Discussion

Absorption spectra of chlorophyll Aa@ and Ab@ were observed in leaves treated with systemic fungicide and insecticide (Fig. 1). Treatments showed significant ($F = 1234.56$, $P < 0.01$) deviation in the absorption pattern of both chlorophyll a and b. However, great variation was found in dimecron treated sample as compared to control and topsin-M. The results revealed some unusual absorption peaks at 400, 420, 460 and 560nm of topsin treated samples.

Application of systemic fungicide (topsin-M) and insecticide (dimecron) showed significant ($P < 0.001$) increase in total chlorophyll content with highly positive correlation ($r = 0.982$). However, maximum increase was recorded in plants when treated with topsin-M and dimecron at a rate of 2000ppm as compared to control (Fig. 2).

Stimulation in chlorophyll synthesis may lead to the unusual peaks of light absorption of chlorophyll a and b. Increase in chlorophyll content by the use of systemic fungicide has also been reported in *Triticum aestivum, Hibiscus esculentus* and *Capsicum annum* (Wangd and Waywood, 1959; Ahmed and Siddiqui, 1995) However, mechanism of increase in chlorophyll after the spray of systemic fungicide and insecticide has not been elucidated so far. Perhaps, It might be due to increase in number of grana and intergrana spaces (Klingensmith, 1961) NADP and NAD ratios (Godvary and Waywood, 1970) NADP and ATP Levels (Mishra and Waywood, 1968; Siddiqui, 1997) or it could be due to increased uptake of K^+, Mg^+ Ca^{++} and other ions (Dyar, 1968).

It has been reported that systemic fungicide (binomial) stimulates the synthesis of chlorophyll formation and showed a significant reduction of ozone damage in pinto bean using

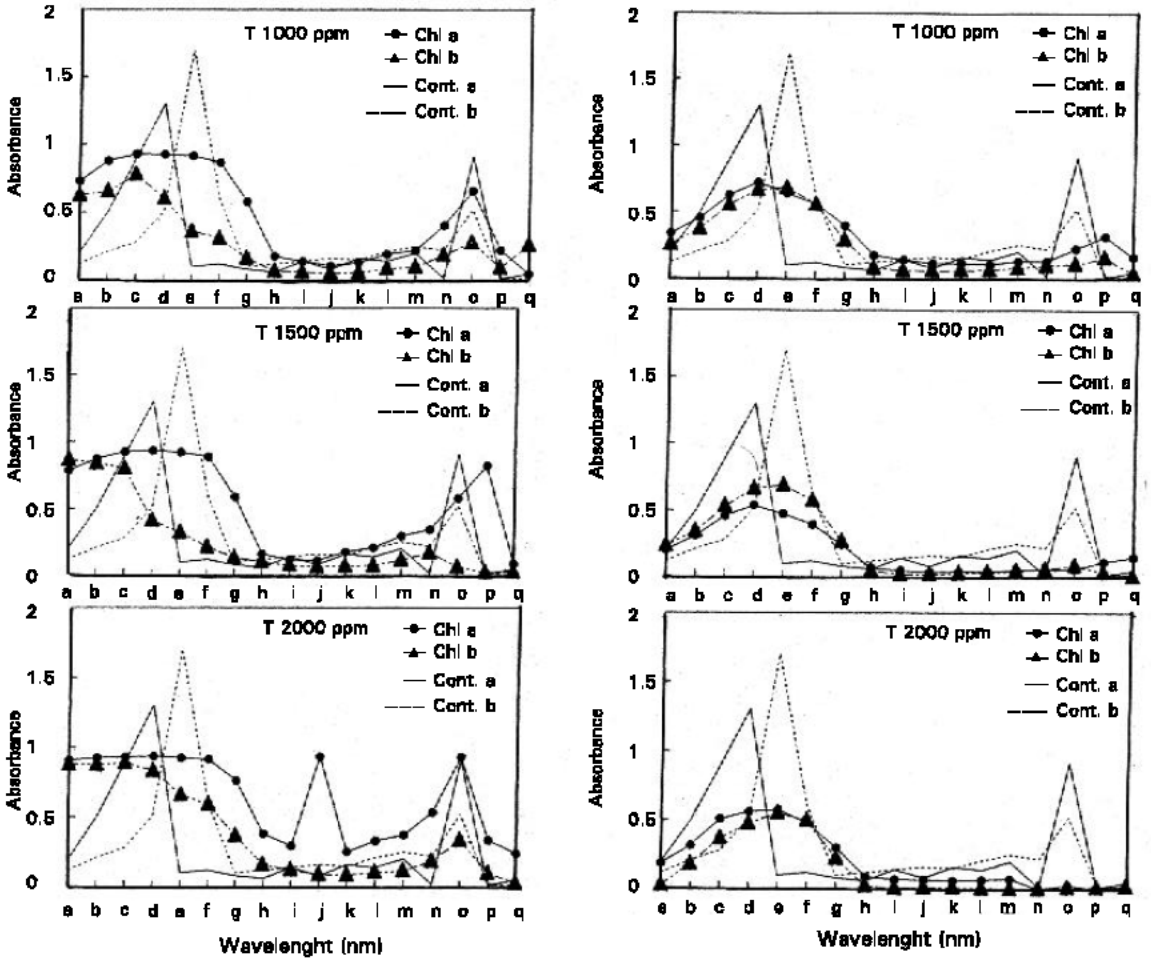

Fig. 1: Effect of topsin-M (T) and dimecron (D) on absorption spectrum of chlorophyll a and b of Vigna radiata (L.) Wilczek Symbol on x axis stand for: a = 380, b = 400, c = 420, d = 440, e = 460, f = 480, g = 500, h = 520 I = 540, J = 560, k = 580, l = 600, m = 620, n = 640, o = 660, p = 680, q = 700.

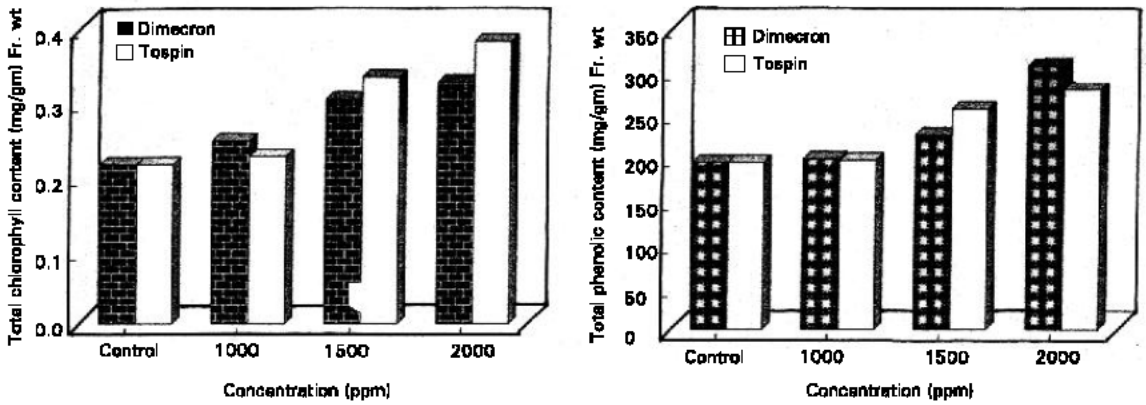

Fig. 2: Effect of systemic fungicide (Topsin-M) and insecticide (Dimecron) on total chlorophyll and phenolic contents of *Vigna radiata* (L.) Wilczek

great amount of ATPs (Pellissier *et al.*, 1971). Mechanism in the increase of chlorophyll and change of absorption pattern still need great deal of comprehensive research to elucidate the process in detail. Increase in total phenolic content was recorded in both treated samples (Fig. 2). However, maximum increase was measured in plants treated with dimecron at the rate of 2000ppm.

Spray of pesticides such as fungicides and insecticides caused significant stress on plants due to which toxin phenolic compound like flavone are formed (Reid *et al.*, 1992; Siddiqui *et al.*, 1999). Phytotoxins in the form of polyphenols are responsible for limiting cell division, nodulation, respiration, photosynthesis, disruption of cell membrane and reduction in total protein and carbohydrate contents of various plant species (Wilson, 1970; Bernestein and Ogata, 1966; Hafeez *et al.*, 1988; Siddiqui *et al.*, 1997). In the present study higher concentrations of both insecticide and fungicide were found more toxic than lesser concentration and control. A preferential use of pesticide at lesser concentration over higher could therefore be suggested for plant disease control.

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