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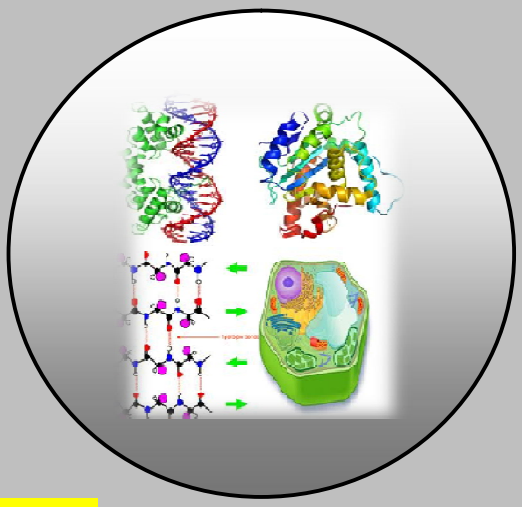

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**RESEARCH PAPER**

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# Studies on the effects of Chlorpyrifos on Germination and Photosynthetic Pigments in Green Gram (*Vigna radiata* L.) at Different Phenological Stages

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**ABSTRACT**

***Green gram (Vigna radiate (L.) Wilczek) is the ancient and well known leguminous crop of Asia which faces greater losses both quantitatively and qualitatively due to attack of several insect insects. Chloripyfos is one of the important pesticides which are used to protect the crop from these pests. It therefore seems important to test the changes that are occurring in this food crop under Chlorpyrifos treatments in order to identify the extent to which it tolerates the pesticide application thereby making it an economical food crop. The seeds were treated with different concentrations ranging from 50 ppm to 150 ppm of Chlorpyrifos in petriplates. The seedlings were taken out at an interval of eight days for the germination studies. For studies of photosynthetic pigments 20 day old seedlings were exposed to different concentrations ranging from 50 ppm to 150 ppm of Chlorpyrifos through foliar spray in the field condition. The seedlings were uprooted for analyses and observed at the pre flowering (5 days after treatment, DAT), flowering (10 DAT) and post flowering (20 DAT) stages for photosynthetic pigments namely (Chlorophyll) Chl a, Chl b, Total Chl, and (Carotenoid) Car content. Germination and amount of photosynthetic pigments increased at 50 ppm insecticidal treatment, when compared with control. Further increase in pesticide level had a negative impact upon aforesaid parameters. The data suggests that the application of Chlorpyrifos at lower concentration may be a useful tool to increase the seed quality as well as quantity in green gram plant, apart from their insecticidal properties.***

***Key words: Pesticide, Green gram, Stress, Germination, Photosynthetic Pigments.***

## INTRODUCTION

Pulses, also called grain legumes, are extensively grown in tropical regions of the world for centuries as a major protein rich crop bringing considerable improvement in human diet.

Legume *Vigna radiata* (L.) Wilczek which belongs to the family leguminosae, is commonly known as mungbean or green gram. The importance of green gram in Indian economy is hardly overemphasized due to its valuable and easily digestible protein (24%), fat (1.5%), calcium (0.124%), phosphorus (0.326%), iron (0.0073%) and vitamin B (Divyashri, 2006). It is the third most popular pulse crop cultivated throughout India. About 70 per cent of the world's production of green gram is in India wherein it is cultivated annually in an area of 3.29 million hectares with a total production and average productivity of 1.60 million tonnes and 485 kgs per hectare, respectively. The major green gram growing states are Orissa, Andhra Pradesh, Uttar Pradesh, West Bengal, Punjab, Haryana, Tamil Nadu, Maharashtra, Karnataka and Bihar.

Being a leguminous crop, poor establishment is often cited as a major constraint on mungbean production. Insect/pests are one of them which cause severe impairment to crop growth and yield. About 128 species of insects have been reported attacking the crop and damaging various parts of the seedlings which cause considerable loss. The most damaging insects/pests of mungbean recorded so far are bean fly (Ophiomyia phaseoli), white fly (Bemisiatabaci Genn.), thrips (Thripstabaci Lind.), pod borer (Helicoverpaarmigere) and grasshoppers which cause defoliation, as they consume foliage. They eat 30 to 100 mg of plant material (dry weight) each day (Siddiqui and Ahmed, 2006). These crop losses can be reduced by the application of pesticides. One such organophosphate pesticide, Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate), is extensively used in agriculture and forestry for its high activity against a broad spectrum of insect/pests. To the best of our knowledge, there is little information available so far about the effect of Chlorpyrifos especially on germination and photosynthetic pigments of legumes. Keeping in view the importance of leguminous crops, the present investigation was carried out to find out the optimum level of Chlorpyrifos for efficient germination and photosynthetic pigmentsin mungbean.

## MATERIAL AND METHODS

### Experimental set up and treatment

The seeds of *Vigna radiata* (L.) R. Wilczek was procured from Pulse research Centre, Indian Agricultural Research Institute (IARI), New Delhi. Field experiments were conducted in the month of June 2011 at the experimental field, Najibabad. The individual plot size was 6 $m^2$ (4 × 1.5 m) having 4 rows with a row to row distance of 15 cm and plant to plant distance of 10 cm. The number of plants per $m^2$ was 15. Twenty days-old seedlings were subjected to foliar application of three levels of Chlorpyrifos [50%, emulsifiable concentrate, (E.C.)], ranging from 50 ppm to 150 pm, prepared by dissolving the required amount of pesticide in double distilled water. Seedlings were uprooted randomly at 5, 10 and 20 DAT that is at three developmental stages namely pre flowering, flowering and post flowering stages and then used for analysis of photosynthetic pigments.

### Seed Germination

Morphologically identical seeds of *Vigna radiata* were selected, sterilized with 0.1 per cent HgCl, rinsed thoroughly with distilled water and soaked in water for 12 hours.

After soaking 50 seeds of each lot were transferred to wet filter papers in petriplates. The filter paper of the first petriplate was made wet by using normal water(0 ppm), second was made wet by using 50ppm concentration of Chlorpyrifos ,third was made wet by using 100ppm concentration of Chlorpyrifos and fourth one was made wet by using 150ppm concentration of Chlorpyrifos. The radicle emergence (2 mm) was taken as criterion for germination. For seedling growth measurement, seedlings were allowed to grow in all the four concentrations i.e. 0 ppm, 50 ppm, 100 ppm and 150 ppm. Eight-day old seedlings were dissected into epicotlyl and hypocotyl and for growth measurement studies (Kumar 1994).

**Photosynthetic pigment**

Chlorophyll and Carotenoid were measured from the fresh leaf by the method of Hiscox and Israelstam (1979).

**Extraction:** The method involves the estimation of plant pigments without maceration. Leaves, kept on a moist filter paper in an icebox, were washed with cold distilled water. Leaf discs were taken from either side of the midrib at the intraveinal region for the determination of Chlorophyll and Carotenoid content. 100 mg of the chopped leaf material was taken in vials in triplicates containing 7 ml of dimethyl sulfoxide (DMSO). The vials were then kept in an oven at 65°C for 1 h for complete leaching of the pigments. Thereafter, the volume of DMSO was made up to 10 ml. The photosynthetic pigments were then measured immediately.

**Estimation:** 2 ml of the extract was transferred to a cuvette and the absorbance was read at 480, 510, 645 and 663 nm using a Beckman spectrophotometer (model DU 640, Fullerton, USA) against DMSO as a blank. Values of optical densities (ODs) were used to compute the Chl a, Chl b, total Chl and Car contents using the following formula given by Maclachlan and Zalik (1963) for Chl a, Duxbury and Yentsch (1956) for Chl b, Arnon (1949) for total Chl and Barnes et al. (1992) for Car contents:

$$\text{Chl 'a' (mg g}^{-1}\text{fr.wt.)} = \frac{[12.3(O.D_{663}) - 0.86\,(O.D_{645})]\ X V}{(D \times 1000 \times W)}$$

$$\text{Chl 'b' (mg g}^{-1}\text{fr.wt.)} = \frac{[19.3(O.D_{645}) - 3.60\,(O.D_{663})]\ X V}{(D \times 1000 \times W)}$$

$$\text{Total 'Chl' (mg}^{-1}\text{fr.wt.)} = \frac{[20.2(O.D_{645}) + 0.86\,(O.D_{663})]\ X V}{(D \times 1000 \times W)}$$

$$\text{Carotinoids (mg g}^{-1}\text{fr.wt.)} = \frac{[7.6(O.D_{480}) - 1.49\,(O.D_{510})]\ X V}{(D \times 1000 \times W)}$$

**Where, D = Distance travelled by the light path; W = Weight of the leaf material taken; V = Volume of the extract; OD = Optical density.**

**Statistical analysis**

The mean value of ten plants were calculated as represented in the results applying statistical analysis Standard Deviation (± S.D.) and't' test of significance at 5% level.

## RESULTS

It is borne out from the data presented in Table 1 that rate of germination and seedling growth was 20.51% faster in seeds treated with 50 ppm as compared to control seeds (0 ppm).

**Table 1. Influence of Different Concentrations of Chlorpyrifos on Seed Germination and Seedling Growth of *Vigna radiata L.***

| Parameters | Chlorpyrifos (ppm) | | | | Variation Over Control (%) | | |
|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 50 ppm (Values in +) | 100 ppm (Values in -) | 150 ppm (Values in -) |
| **Germination** | 78 | 94 | 58 | 42 | 20.51 | 25.64 | 46.15 |
| **Epicotyl** | | | | | | | |
| **Length (cm)** | 3.92±0.74 | 4.80±0.92 | 2.82±0.61 | 1.78±0.45 | 22.45 | 28.06 | 54.59 |
| **Fw (mg)** | 50.87±3.96 | 65.72±4.56 | 39.53±2.87 | 25.35±1.24 | 29.19 | 22.29 | 50.17 |
| **Dw (mg)** | 7.38±0.84 | 8.65±0.97 | 5.98±0.73 | 4.05±0.39 | 17.21 | 18.97 | 45.12 |
| **Hypocotyl** | | | | | | | |
| **Length (cm)** | 2.87±0.68 | 3.30*±0.82 | 2.09±0.51 | 1.78±0.34 | 14.98 | 27.18 | 37.98 |
| **Fw (mg)** | 100.34±5.45 | 120.64±6.72 | 80.00±4.49 | 65.40±3.64 | 20.23 | 20.27 | 34.82 |
| **Dw (mg)** | 12.10±4.59 | 15.00±5.26 | 9.42±3.86 | 6.95±1.98 | 23.97 | 22.15 | 42.40 |

**± Standard Deviation * Value Not Significantly Different At 5% Level From Respective Control**

**Table 2. Variation in Photosynthetic Pigments at Various Growth Stages of *Vigna radiate* L. Treated with Different Concentration of Chlorpyrifos.**

| Parameters/ Stages | Chlorpyrifos (ppm) | | | | Variation Over Control (%) | | |
|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 50 ppm (Values in +) | 100 ppm (Values in -) | 150 ppm (Values in -) |
| **(a) Chlorophyll 'a' (mg $g^{-1}$fr. wt.)** | | | | | | | |
| 5 DAT | 0.760±0.014 | 0.868±0.015 | 0.675±0.017 | 0.556±0.013 | 14.21 | 11.18 | 26.84 |
| 10 DAT | 1.500±0.023 | 1.636±0.025 | 1.454*±0.017 | 1.328±0.024 | 9.06 | 3.06 | 11.46 |
| 20 DAT | 0.595±0.025 | 0.746±0.035 | 0.462±0.026 | 0.375±0.023 | 25.37 | 22.35 | 36.97 |
| **(b) Chlorophyll 'b' (mg $g^{-1}$fr. wt.)** | | | | | | | |
| 5 DAT | 0.442±0.028 | 0.532±0.031 | 0.396±0.019 | 0.284±0.018 | 20.36 | 10.41 | 35.74 |
| 10 DAT | 0.570±0.014 | 0.696±0.009 | 0.432±0.012 | 0.312±0.027 | 22.10 | 24.21 | 45.26 |
| 20 DAT | 0.260±0.017 | 0.284±0.018 | 0.238*±0.22 | 0.184±0.017 | 9.23 | 8.46 | 29.23 |
| **(c) Total Chlorophyll (mg $g^{-1}$fr. wt.)** | | | | | | | |
| 5 DAT | 1.135±0.12 | 1.317±0.026 | 1.051*±0.008 | 0.862 ±0.025 | 16.04 | 7.40 | 24.05 |
| 10 DAT | 1.912±0.015 | 2.350±0.015 | 1.834*±0.013 | 1.648±0035 | 22.91 | 4.08 | 13.81 |
| 20 DAT | 0.732±0.020 | 0.873±0.017 | 0.670±0.019 | 0.688±0.016 | 19.26 | 8.47 | 19.67 |
| **(d) Carotenoid (mg $g^{-1}$fr. wt.)** | | | | | | | |
| 5 DAT | 0.495±0.017 | 0.538*±0.016 | 0.436±0.015 | 0.370±0.015 | 8.69 | 11.92 | 25.25 |
| 10 DAT | 0.562±0.028 | 0.698±0.027 | 0.496±0.024 | 0.462±0.014 | 24.20 | 11.74 | 17.79 |
| 20 DAT | 0.482±0.015 | 0.530±0.025 | 0.426±0.012 | 0.347±0.023 | 9.96 | 11.62 | 28.00 |

**± Standard Deviation * Value Not Significantly Different At 5% Level from Respective Control**

On the contrary the aforesaid parameters of the seeds treated with 100 ppm and 150 ppm showed significant decrease of 25.64% and 46.15% respectively. The length, fresh weight and dry weights of hypocotyl and epicotyl were increased in 50 ppm but decreased in 100 ppm and 150 ppm Chlorpyrifos treated seeds.

Likewise the photosynthetic pigments i.e. Chl a, Chl b, total Chl and Car content amplified at lower concentration (50 ppm) of Chlorpyrifos which touched maximum during the flowering stage (10 DAT) followed by pre- flowering (5 DAT) and post-flowering stages (20 DAT), respectively. Whereas higher concentrations (100 ppm and 150 ppm) of Chlorpyrifos treatment diminished the photosynthetic pigments and Carotenoid content of seedlings. The extent of reduction was 36.97, 29.23, 19.67 and 28.00 % under 150 ppm Chlorpyrifos solution at post flowering stage (20 DAT) over their respective controls (Table 2).

## DISCUSSION

Decreased germination in the pesticide treated seeds is mainly due to the toxic effect of Chlorpyrifos in high concentration (100 ppm and 150 ppm). Decreased photosynthetic efficiency of the high concentration Chlorpyrifos treated seedlings may decrease the production of dry matter. The studies of Kumar & Kumar (1993) indicate that the use of Metasystox on *Vicia faba* is promontory for seed germination and growth when used in lower concentrations (50-100 mg/l). The higher concentrations (200-300 mg/l) are, however, inhibitory for these parameters. Similar results have been obtained by Singh et al. (1970) in Citrus.

Photosynthesis is the ultimate physiological limitation to crop production. The photosynthetic capacity of individual leaves is one factor, which determines crop dry matter yield. It is also known that photosynthetic efficiency depends on leaf area, Chlorophyll contents and the stomatal response to environment. Some pesticide's mode of action interfere with photosynthesis, the content of Chl might reasonably be impacted by sub-lethal doses of those chemicals. For these reasons, Chl was examined as a parameter that may be a useful indicator of environmental exposure to the test pesticide. Reduction in Chl contents at high concentrations of Chlorpyrifos may be due to the inhibition of their biosynthesis or breakdown of pigments or their precursors. Another reason for reduced Chlorophyll is due to decrease in leaf area with increasing concentration of pesticides, which is in agreement with the study on inhibitory action on photosynthetic apparatus by pesticides on Chlorophyll of spinach (Sresen et al., 2000). Carotenoid is an important component of defense mechanism of plants which also acts as a natural defense against photodynamic damage caused to photosynthetic apparatus under pesticide stress condition. The enhanced level of Carotenoid with 50 ppm Chlorpyrifos (Table 2d) occurred to protect the plant against the photodynamic damage. High doses of Chlorpyrifos caused considerable reduction in Carotenoid content and this condition results in serious consequence on Chlorophyll and photosynthetic apparatus leading to reduced photosynthesis efficiency of the plant.

In the present study, it was observed that Chl a, Chl b, total Chl and Carotenoid contents in control as well as Chlorpyrifos treated plants were highest at flowering stage followed by pre flowering and post flowering stages. These observations are in accordance with the findings of Fu et al. (2000). They observed that Chl a, Chl b and Carotenoid contents reached maximum at 15 days after flowering and rapidly decreased after 33 days of flowering. At the post flowering stage, the decrease in the photosynthetic rate as compared to the flowering and pre flowering stages can be attributed to the fact that the ability to photosynthesize increases temporarily and then often, before maturity begins to decrease, which may be because of senescent leaves.

## CONCLUSION

The present study demonstrates the inhibitory effect on the germination and photosynthetic activity of *Vigna radiata* L. at the high dose but interestingly, low dose of Chlorpyrifos supported the plant's activity and concluded that the 50 ppm of Chlorpyrifos is beneficial for the germination and photosynthetic pigments of *Vigna radiata* L. plants.

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