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ORIGINAL ARTICLE **OPEN ACCESS**

To Study the Impact of Pesticide Application on Specific Plant Physiological Processes

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ABSTRACT
Pesticides are often used in agriculture to protect human being from the insect vector of disease- causing pathogen to protect crop plant from competition. This study aim to evaluate the effect of chlorpyrifos on several metabolic and stress related parameters associated with pesticide resistance. The study was conducted at ICAR-Indian Institute of Soil Science, Bhopal (M.P.) during 2015-2016. The major objective of this study was to study the impact of different concentrations of pesticide chlorpyrifos viz. 0%, 0.25%, 0.75% and 1.25% on plant physiological characteristics of soybean and wheat. The impacts of foliar spray of pesticide on growth and metabolism of plants viz. Soybean, wheat and ashwagandha were studied under the control condition using chlorpyrifos. It was observed that the increased rate of pesticide treatment significantly decreased Physiological parameters i.e., rate of photosynthesis transpiration, were decreased with increased concentration of pesticide in soybean and wheat. However, the rate of photosynthesis was higher at higher concentration of pesticide in ashwagandha. The stomatal conductance was significantly increased at higher concentration of pesticide T_4 (1.25%) in soybean and wheat. However, the higher dose of reduced the stomatal conductance in ashwagandha.
Key words: *Oxidative stress, Pesticide, Soybean, wheat.*

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INTRODUCTION

The use of synthetic pesticides as crop protection chemicals has become the most accepted ecological weapon for assured crop production. With the restricted use of most of the organochlorine insecticides, the organophosphorus compounds are taking the major share of insecticide consumption in India [1]. Chlorpyrifos [*O,O*diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum organophosphate insecticide being used for more than a decade to control foliar insects that affect agricultural crops, to reduce pod damage [2-3], and subterranean termites . Chlorpyrifos produces hazardous effects on the environment when it is applied directly on plants or mixed with soil [4]. Manufacturing and consumption of pesticides worldwide have been increasing dramatically in crop production. In this process pesticide misuses become more and more serious, which has resulted in heavy environmental pollution and health risk of humans.

Soybean (*Glycine max L. Merrill*) is an important pulse as well as oilseed crop. It has become wonder crop of twentieth century and is often designated as "Golden bean."

Ashwagandha (*Withania somnifera*) known commonly as ashwagandha, Indian ginseng, poison gooseberry, or winter cherry, is a plant in the *Solanaceae* or nightshade family. Several other species in the genus *Withania* are morphologically similar. It is used as a herb in ayurvedic medicine. This species is a short, tender perennial shrub growing 35–75 cm (14–30 in) tall. Tomentose branches extend radially from a central stem. Leaves are dull green, elliptic, usually up to 10–12 cm long. The flowers are small, green and bell-shaped. The ripe fruit is orange-red. Ashwagandha 3rd important prioritized medicinal plant listed by NMPB is also known as Indian Ginseng. Ashwagandha is highly popular herb and widely used in lot of ayurvedic formulations, nutracitical product and other herbal product. The annual demand

of this herb was estimated to be 9127.5 tons per annum in the year 2005. Based on the trend the current demand of ashwagandha per annum would be around 12500 tons.

Wheat (Triticum spp.), the world's most widely cultivated crop, in 2000, world wheat production was approximately 572 million metric tons on 205 million hectares. Wheat is the staple food for about 40% of the world's population. Common bread Wheat (*T. aestivum,* L.) and durum Wheat (*T. durum* Desf.) make up 90% of the world's Wheat crop. Wheat is further classified as winter or spring, hard or soft, red or white, and by protein content.

MATERIALS AND METHODS

A pot experiment will be laid out in control condition in completely Randomized Block Design at Indian Institute of soil science, Bhopal. Lab work will be conducted in laboratory of Indian Institute of Soil Science.

Description of crop : Soybean Aswagandha and Wheat
Soybean genotype : JS 9305
Ashwagandha genotype :
Wheat genotype : HD 2987

Treatments:

Treatments	Treatment details
T_1	Control (No chloropyrifos)
T_2	Foliar application below normal (Low)-0.25%
T_3	Foliar application recommended dose (Medium)-0.75%
T_4	Foliar application supra-optimal dose (High)-1.25%

T_1 Control (no pesticide use)

T_2– The spray solution is prepared from stock solution. Take 0.25 ml of stock solution and make up the volume 100 ml. So 0.25% low chloropyrifos.

T_3- The spray solution is prepared from stock solution. Take 0.75 ml of stock solution and make up the volume 100 ml. So 0.75%medium chloropyrifos.

T_4- The spray solution is prepared from stock solution. Take 1.25 ml of stock solution and make up the volume 100 ml. So 1.25% high chloropyrifos.

Physiological parameters were measured using standard procedures. The following parameters were studied in the present study.

Estimation of Gaseous Exchange Parameters

Gas exchange parameters viz. Photosynthesis rate (μMCO_2 m^{-2} s^{-1}), transpiration rate (MmH_2O m^{-2} s^{-1}) and stomatal conductance (μM m^{-2} s^{-1}) were recorded in the morning (9 to 11:30 AM) in the experimental plant leaves using Photosynthesis system (make: PP systems and model: CIRAS-3).

RESULT AND DISCUSSION

The physiological parameter *i.e.,* photosynthesis, transpiration rate stomatal conductance were recorded after one week of application of pesticide chlorpyrifos.

Photosynthesis rate (μ mole CO_2/ m^2/ sec)

The photosynthesis rate was measured in 3rd fully expended leaves from the top. The rate was highest in control and gradually decreased with increase concentration of treatment. The mean value was greater in control (8.32) in soybean. Whereas in wheat the average value of photosynthesis rate was highest at T_3 0.75% of chlorpyrifos (9.28) followed by control (8.53) and at T_1 0.25% (7.58) the minimum photosynthesis rate was observed in T_4 1.25% concentration of chlorpyrifos (6.58). The reverse trend of photosynthetic rate was observed in ashwagandha. The maximum rate of photosynthesis was observed at highest concentration of T_4 1.25% (3.78) followed by T_3 0.75% (3.23), T_2 0.25% (2.98) and minimum photosynthesis rate was observed in control (2.67). However, the result was non significant at 30 DAS (Table no 4.6 fig no.4.16-4.18)

Decreased photosynthetic efficiency of the high concentration Chlorpyrifos treated seedlings may decrease the production of dry matter. The studies of [2] 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^{-1}). The higher concentrations (200-300 mg l^{-1}) was inhibitory for photosynthesis rate stomatal conductance and transpiration rate. Similar results have been obtained by [5] in maize reported by [6] in pea and [7] in Citrus.

Table 1. Impact of pesticide on photosynthesis rate (µmoleCO_2/m^2/sec) of soybean, wheat and ashwagandha leaves at various growth phases *i.e.,* 30, 45 and 60 DAS and DAT.

Photosynthesis rate	DAS 30	DAS 45	DAS 60	Total	Mean
Soybean					
T_1- Control (No chlorpyrifos)	7.80	9.10	8.05	24.95	**8.32**
T_2- 0.25%(low)	8.20	5.90	8.35	22.45	**7.48**
T_3- 0.75% (medium)	7.05	4.10	9.65	20.80	**6.93**
T_4- 1.25%(High)	4.05	3.55	4.90	12.50	**4.17**
Mean	**6.78**	**5.66**	**7.74**		
CD(P=0.05)	1.00	0.86	1.30		
CD(P=0.01)	1.61	1.40	2.10		
SE(m±)	0.25	0.21	0.32		
C.V.	5.17	5.34	5.91		
Wheat					
T_1- Control (No chlorpyrifos)	7.85	11.15	6.60	25.60	**8.53**
T_2- 0.25%(low)	8.35	7.05	7.35	22.75	**7.58**
T_3- 0.75% (medium)	9.40	9.95	8.50	27.85	**9.28**
T_4- 1.25%(High)	5.15	7.25	7.35	19.75	**6.58**
Mean	**7.69**	**8.85**	**7.45**		
CD(P=0.05)	1.49	1.55	0.55		
CD(P=0.01)	NS	2.50	0.80		
SE(m±)	0.37	0.38	0.14		
C.V.	6.81	6.14	2.60		
Ashwagandha	**DAT 30**	**DAT 45**	**DAT 60**		
T_1- Control (No chlorpyrifos)	1.30	2.05	4.65	8.00	**2.67**
T_2- 0.25%(low)	1.80	1.20	5.95	8.95	**2.98**
T_3- 0.75% (medium)	1.20	5.00	3.50	9.70	**3.23**
T_4- 1.25%(High)	1.40	5.25	4.70	11.35	**3.78**
Mean	**1.43**	**3.38**	**4.70**		
CD(P=0.05)	0.35	0.62	0.82		
CD(P=0.01)	NS	1.00	1.32		
SE(m±)	0.09	0.15	0.20		
C.V.	8.60	6.46	6.11		

DAS (Days after sowing**)**
DAT (Days after transplanting**)**

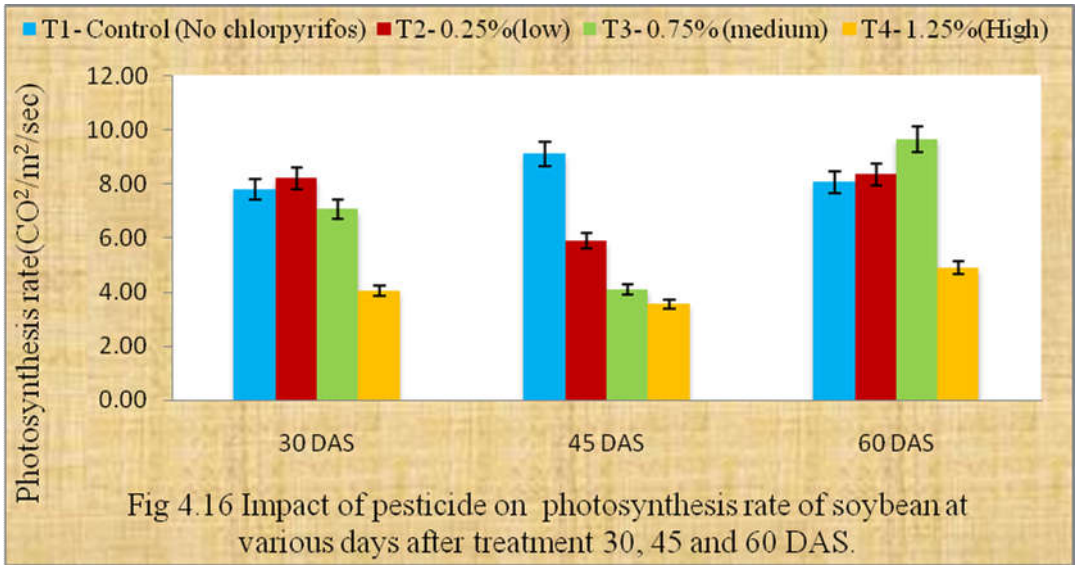

Fig 4.16 Impact of pesticide on photosynthesis rate of soybean at various days after treatment 30, 45 and 60 DAS.

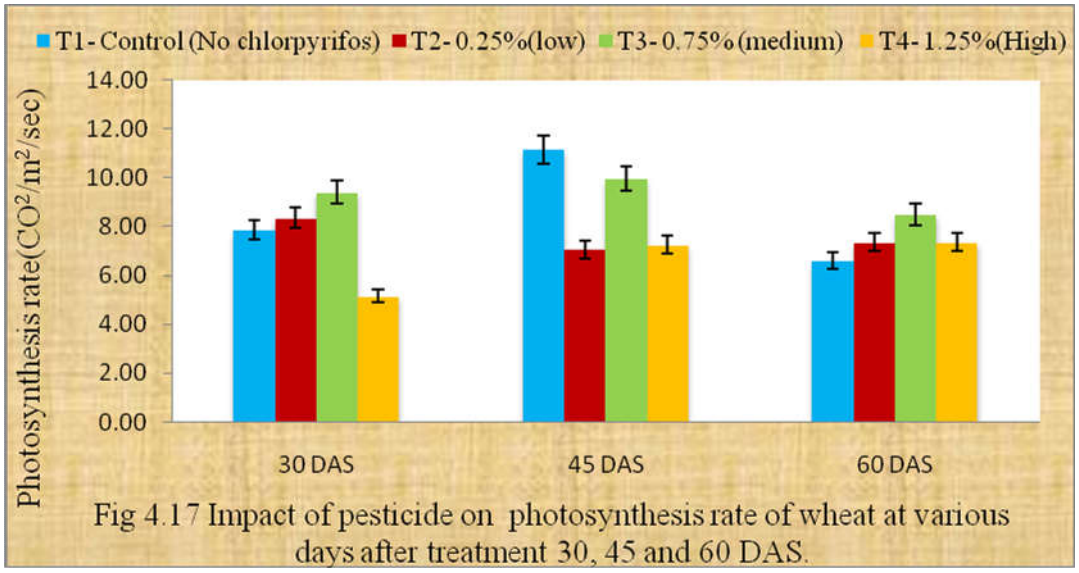

Fig 4.17 Impact of pesticide on photosynthesis rate of wheat at various days after treatment 30, 45 and 60 DAS.

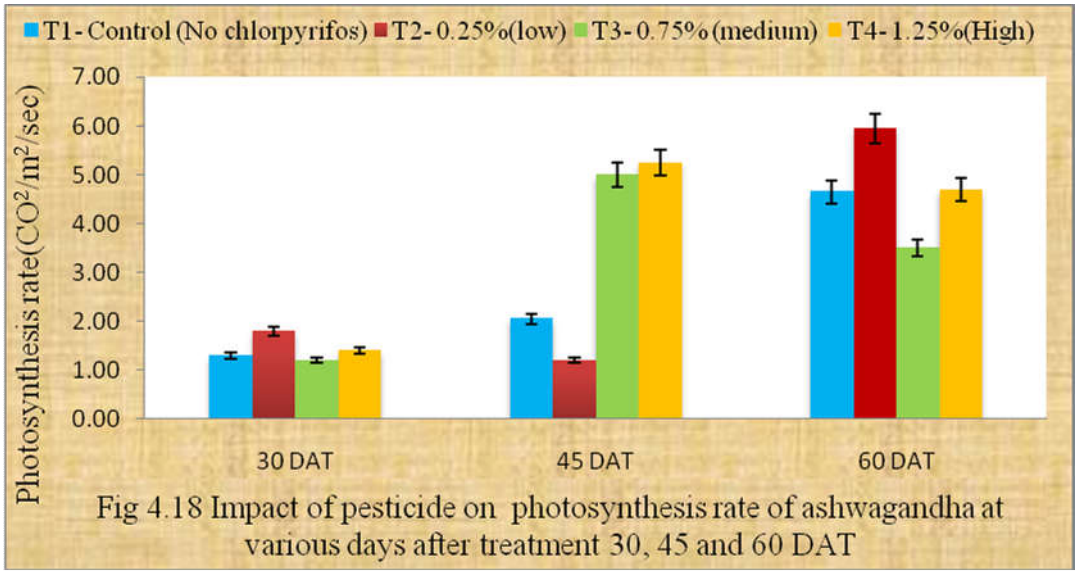

Fig 4.18 Impact of pesticide on photosynthesis rate of ashwagandha at various days after treatment 30, 45 and 60 DAT

Stomatal conductance (µmol /m²/sec)

Stomatal conductance was measured at 30, 45 and 60 DAS at various concentration of pesticide. It was observed that the pesticide have significant impact on this physiological parameter of gaseous exchange. The stomatal conductance was increase with increased level of pesticide in soybean and wheat. In soybean maximum stomatal conductance was observed at T_4 1.25% treated (111.33) over control (100.00). Whereas, in wheat value was maximum in treatment T_2 0.25% (3.31) followed by T_3 0.75% (3.09) and T_4 1.25% (2.86). in ashwagandha the maximum rate of stomatal conductance was observed at lower concentration of T_1 0.25 % (84.17) followed by T_4 1.25% (79.33) ,T_3 0.75% (76.17) and minimum rate of stomatal conductance was observed in control (66.67) (Table no. 4.7 fig 4.19-4. 21).

[8] also reported that the The phytotoxicities of nine pesticides at practical dosages on photosynthesis were investigated in cucumber (*Cucumis sativus* L.) Plants treated with paraquat showed the severest phytotoxic symptom with the highest reduction in net photosynthetic rate, stomatal conductance (Gs) and intercellular CO_2 concentration (Ci). Dias M. C. (2012) also confirmed the similar impact of these compounds on the photosynthetic apparatus.

Transpiration rate (µmol H_2O/m²/sec)

The transpiration rate was measured in 3rd fully expended leaves from the top. The rate was highest in higher concentration and decreased in control. The mean value was greater in T_4 1.25% (3.34) in soybean. Whereas in wheat the average value of transpiration rate was highest at T_2 0.25% of chlorpyrifos (3.31) followed by T_3 0.75% (3.09) and at control (3.03) and minimum rate of transpiration was observed at lower concentration of chlorpyrifos T_4 1.25% (2.86). In ashwagandha the maximum rate

of transpiration was observed at lower concentration of T_2 0.25% chlorpyrifos (3.24) followed by higher concentration of T_4 1.25% (2.30), T_2 0.75% (2.24) and minimum transpiration rate was observed in control (1.56) (Table no 4.8 fig no 4.22-4.24).

Transpiration is directly related to stomatal opening, which is essential for leaf intake of carbon dioxide for photosynthesis. Photosynthesis is a more general measurement of carbon dioxide intake and fixation (sugar production).

Table 2. Impact of pesticide on stomatal conductance (µmol/m²/sec) of soybean, wheat and ashwagandha leaves at various growth phases *i.e.,* 30, 45 and 60 DAS and DAT.

Stomatal conductance	DAS 30	DAS 45	DAS 60	Total	Mean
Soybean					
T_1- Control (No chlorpyrifos)	79.0	105.5	115.5	300.0	**100.0**
T_2- 0.25%(low)	98.0	116.0	116.5	330.5	**110.2**
T_3- 0.75% (medium)	100.5	120.0	96.0	316.5	**105.5**
T_4- 1.25%(High)	162.5	86.0	85.5	334.0	**111.3**
Mean	**110.0**	**106.9**	**103.4**		
CD(P=0.05)	15.2	16.0	18.9		
CD(P=0.01)	24.5	NS	NS		
SE(m±)	3.8	4.0	4.7		
C.V.	4.8	5.3	6.4		
Wheat					
T_1- Control (No chlorpyrifos)	2.94	3.60	2.55	9.09	**3.03**
T_2- 0.25%(low)	3.02	3.40	3.50	9.92	**3.31**
T_3- 0.75% (medium)	2.62	3.65	3.00	9.27	**3.09**
T_4- 1.25%(High)	2.07	3.50	3.00	8.57	**2.86**
Mean	**2.66**	**3.54**	**3.01**		
CD(P=0.05)	0.49	NS	0.58		
CD(P=0.01)	NS	NS	NS		
SE(m±)	0.12	0.14	0.14		
C.V.	6.39	5.74	6.74		
Ashwagandha	**DAT 30**	**DAT 45**	**DAT 60**		
T_1- Control (No chlorpyrifos)	36.0	57.0	107.0	200.0	**66.7**
T_2- 0.25%(low)	122.0	55.0	75.5	252.5	**84.2**
T_3- 0.75% (medium)	67.5	79.0	82.0	228.5	**76.2**
T_4- 1.25%(High)	132.0	52.5	53.5	238.0	**79.3**
Mean	**89.4**	**60.9**	**79.5**		
CD(P=0.05)	21.5	10.3	14.9		
CD(P=0.01)	34.8	16.7	24.0		
SE(m±)	5.3	2.6	3.7		
C.V.	8.5	6.0	6.6		

DAS (Days after sowing**)**
DAT (Days after transplanting**)**

Both processes are interrelated and are directly related to plant growth and productivity. The higher value of transpiration occurred on insecticide-free plants. Photosynthesis was also higher in the untreated check, suggesting that all insecticide treatments adversely influenced both stomatal opening and over all photosynthesis rates as reported by [10] similar result were reported by [6] observed a tendency towards a slight decrease in the rate of photosynthesis under the influence of a fungicidal agent in horse bean and pea. Further, the same author found a significant decline in the levels of photosynthesis and transpiration under the effect of foliar herbicide application. [9] Also confirmed the dimethoate causes a reduction in plant growth, photosynthesis pigments and photosynthetic activity of *Glycine max* L.

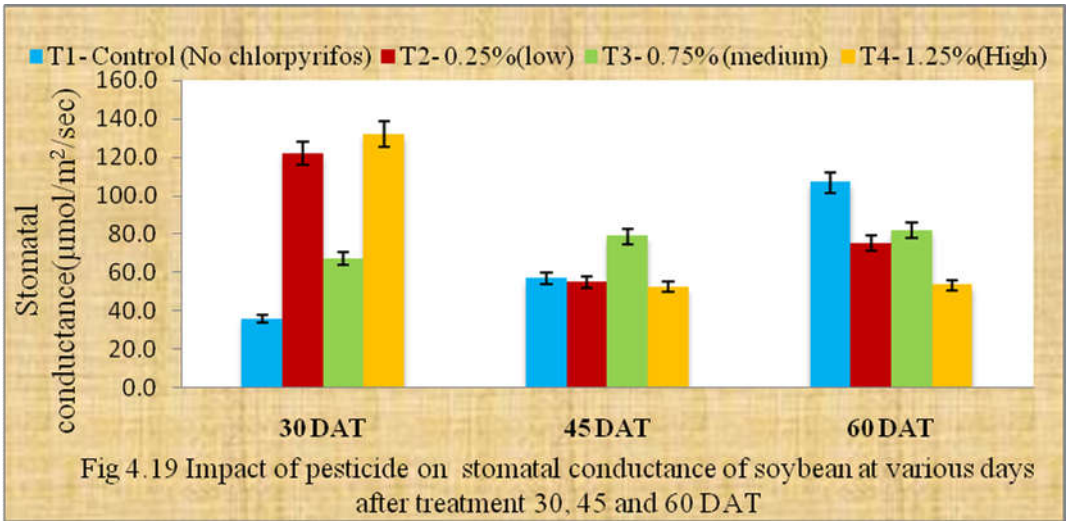

Fig 4.19 Impact of pesticide on stomatal conductance of soybean at various days after treatment 30, 45 and 60 DAT

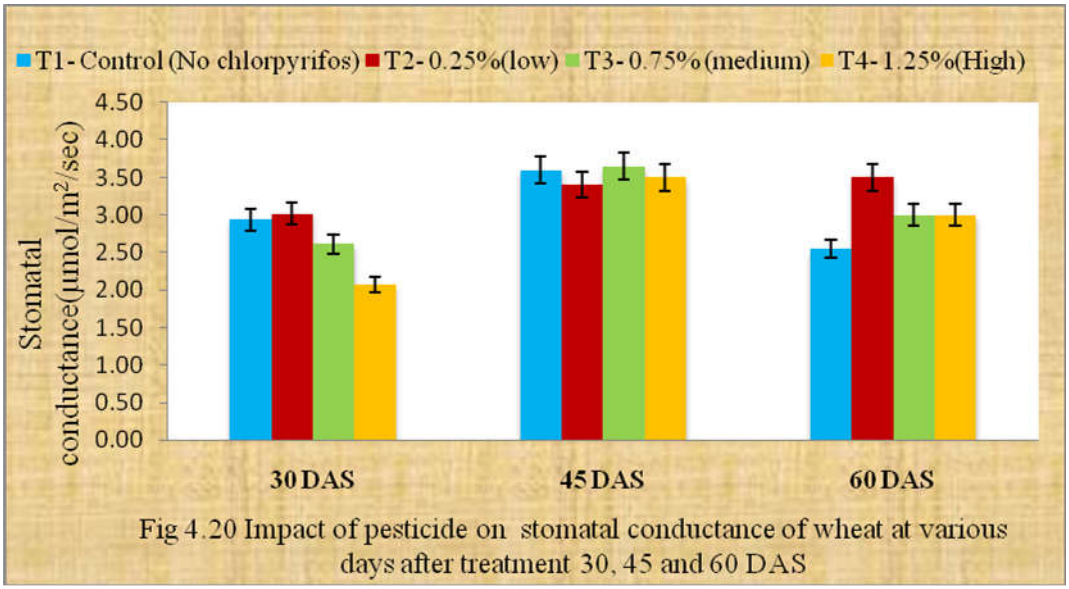

Fig 4.20 Impact of pesticide on stomatal conductance of wheat at various days after treatment 30, 45 and 60 DAS

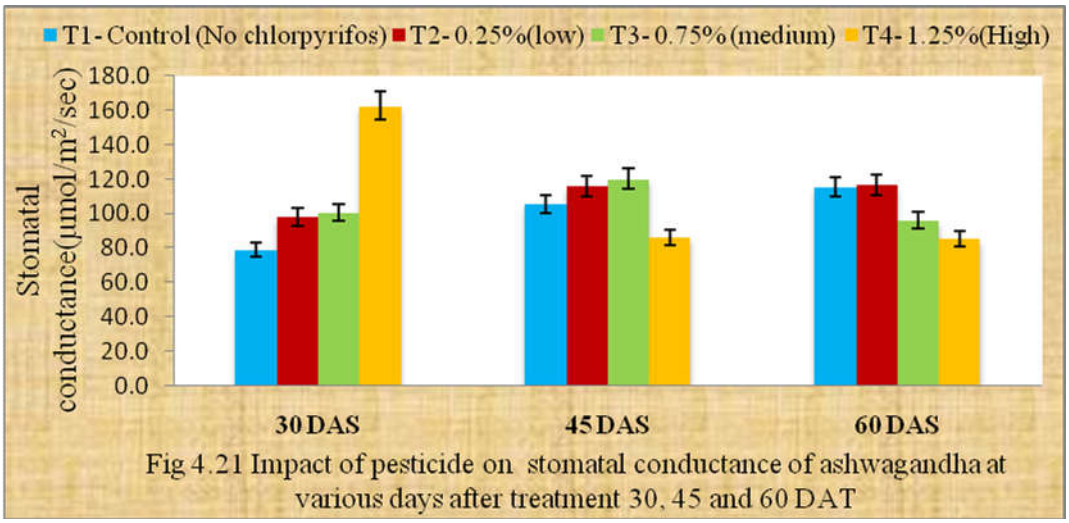

Fig 4.21 Impact of pesticide on stomatal conductance of ashwagandha at various days after treatment 30, 45 and 60 DAT

Table 3. Impact of pesticide on transpiration rate (µmol $H_2O/m^2/sec$) of soybean, wheat and ashwagandha leaves at various growth phases *i.e.,* 30, 45 and 60 DAS and DAT.

Transpiration rate	DAS 30	DAS 45	DAS 60	Total	Mean
Soybean					
T_1- Control (No chlorpyrifos)	2.81	2.60	2.94	8.35	**2.78**
T_2- 0.25%(low)	3.50	2.93	3.02	9.45	**3.15**
T_3- 0.75% (medium)	3.60	3.15	2.62	9.36	**3.12**
T_4-1.25%(High)	5.17	2.59	2.26	10.01	**3.34**
Mean	**3.77**	**2.82**	**2.71**		
CD(P=0.05)	0.54	NS	0.42		
CD(P=0.01)	0.87	NS	NS		
SE(m±)	0.13	0.40	0.11		
C.V.	5.01	20.06	5.48		
Wheat					
T_1- Control (No chlorpyrifos)	2.94	3.60	2.55	9.09	**3.03**
T_2- 0.25%(low)	3.02	3.40	3.50	9.92	**3.31**
T_3- 0.75% (medium)	2.62	3.65	3.00	9.27	**3.09**
T_4-1.25%(High)	2.07	3.50	3.00	8.57	**2.86**
Mean	**2.66**	**3.54**	**3.01**		
CD(P=0.05)	0.49	NS	0.58		
CD(P=0.01)	NS	NS	NS		
SE(m±)	0.12	0.14	0.14		
C.V.	6.39	5.74	6.74		
Ashwagandha	**DAT 30**	**DAT 45**	**DAT 60**		
T_1- Control (No chlorpyrifos)	1.38	1.55	1.74	4.67	**1.56**
T_2- 0.25%(low)	4.32	3.95	1.46	9.72	**3.24**
T_3- 0.75% (medium)	2.40	2.85	1.47	6.71	**2.24**
T_4-1.25%(High)	4.80	1.05	1.05	6.90	**2.30**
Mean	**3.22**	**2.35**	**1.43**		
CD(P=0.05)	1.68	0.57	0.42		
CD(P=0.01)	NS	0.91	NS		
SE(m±)	0.42	0.14	0.11		
C.V.	18.28	8.45	10.41		

DAS (Days after sowing**)**
DAT (Days after transplanting**)**

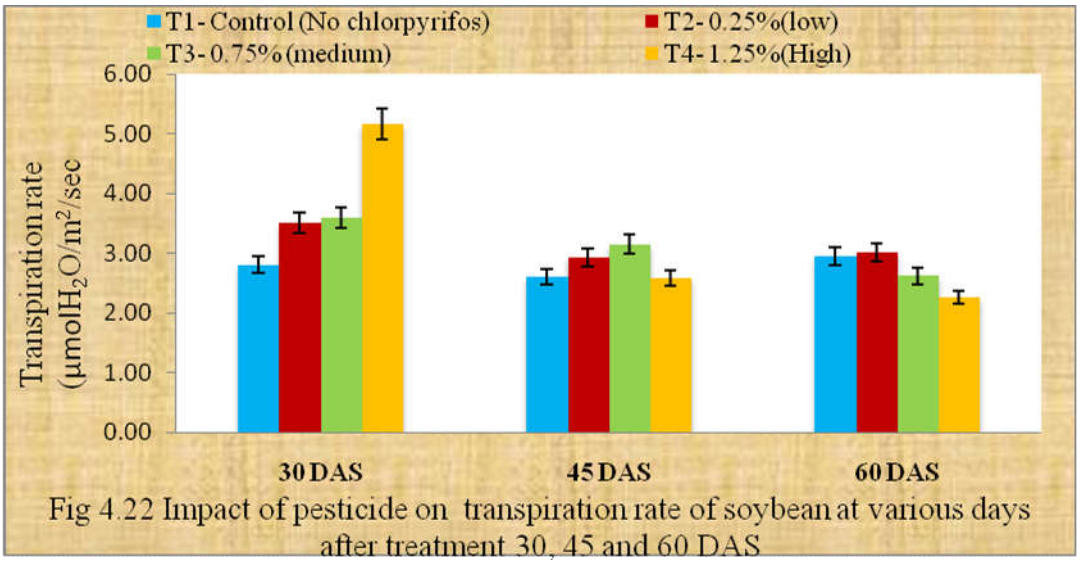

Fig 4.22 Impact of pesticide on transpiration rate of soybean at various days after treatment 30, 45 and 60 DAS

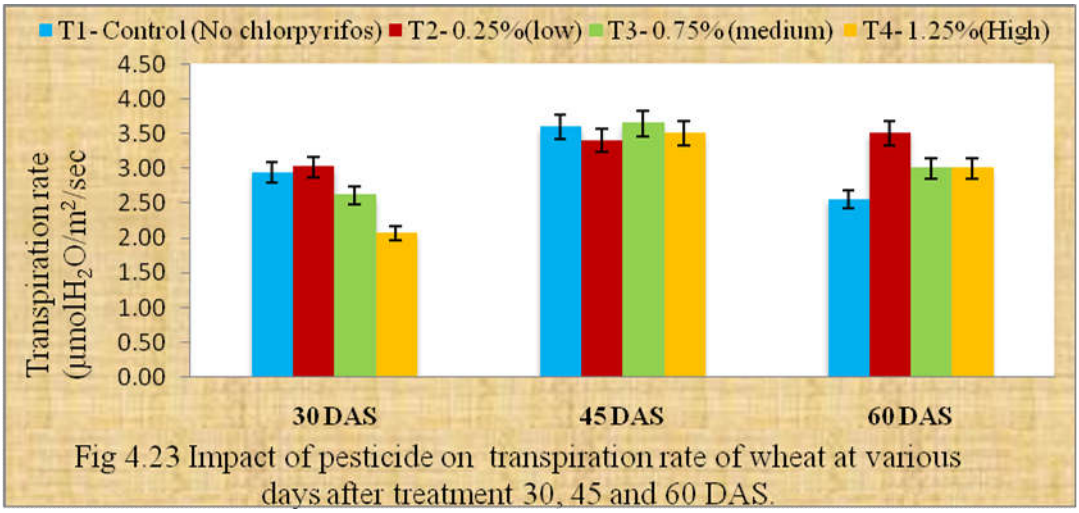

Fig 4.23 Impact of pesticide on transpiration rate of wheat at various days after treatment 30, 45 and 60 DAS.

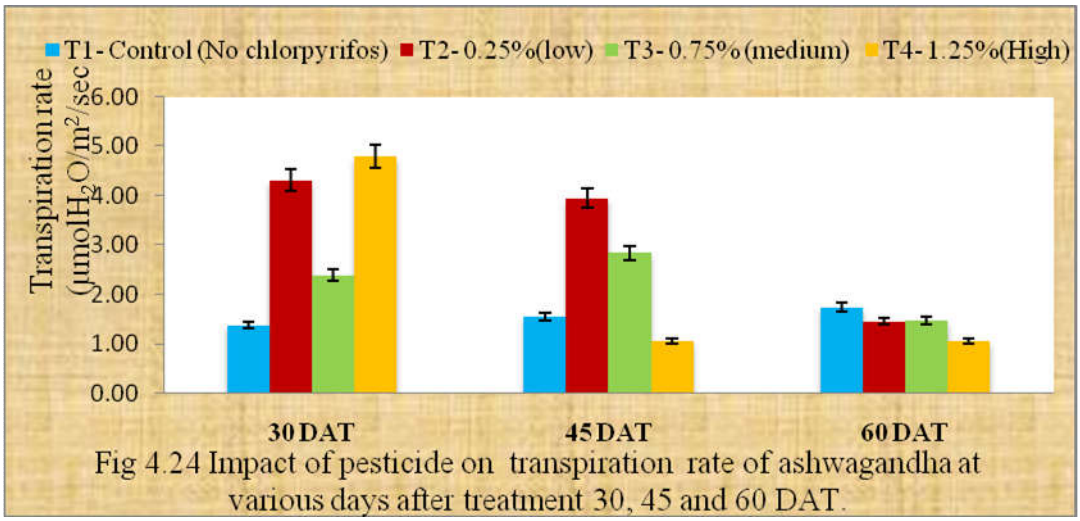

Fig 4.24 Impact of pesticide on transpiration rate of ashwagandha at various days after treatment 30, 45 and 60 DAT.

CONCLUSIONS

Chlorpyrifos is a hazardous and important pollutant of the environment. The EU Directive 2008/105/EC lists it as one of the priority water pollutant. Its presence in mainly detected by chemical but, since biological tests have general in importance in the last few years. Chlorpyrifos effect on several metabolic and stress related parameters *ie.,* morphological physiological and biochemicals. Therefore, the impact of oxidative stress was evaluated on these crop plants to find out the chemical stress tolerance and resistance mechanism. It was observed that as compared to soybean, wheat was found to be same tolerance against the oxidative stress. However, the ashwagandh was initially affected but later it increased its morphophysiological traits.

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