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Harmful effects of Fungicide Treatment on Wheat (*Triticum aestivum* L.) Seedlings

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Abstract

Fungicides, weedicides, insecticides etc. are all pesticides used for protection of plant. A fungicide is a group of pesticide that controls fungal disease by either inhibiting or killing the fungus that causes the disease. Fungicides are generally used for increasing yield of crop but they can induce biochemical changes in seedlings which may not be beneficial. Therefore the study was conducted to know the harmful effect of carbendazim which is a benziimidazole group fungicide on biochemical parameters of wheat seedlings. The biochemical parameters studied were protein, carbohydrate, total chlorophyll, chlorophyll a, chlorophyll b and alkaline protease activity. Five different concentration of fungicide were used viz. 500, 1000, 1500, 2000 and 2500 mg/l. Protein, carbohydrate, total chlorophyll, chlorophyll a, and chlorophyll b were found to decrease with increase in concentration of fungicide. However alkaline protease activity was increased with increase in concentration of fungicide. Maximum decrease in protein (– 62.49%), carbohydrate (– 62.5%), total chlorophyll (-57.44%), chlorophyll a (-47.89%) and chlorophyll b (-63.44%) and maximum increase in alkaline protease activity (152%) was obtained at highest studied concentration of fungicide i.e. 2500 mg/l. From the present study it was concluded that presence of fungicide exert harmful effect on the biochemical parameters of wheat seedlings.

Keywords: Fungicide -carbendazim, wheat seedlings, alkaline protease activity,

Introduction

Germination of seed and early seedling growth are considered the most critical phases for establishment of any species of plant[1]. Agriculture research till now has been primarily concerned how yield can be increased by the use of fertilizers, pesticides, irrigation etc. Pesticide form an essential part of the crop production technology that makes it possible for the farmer to feed the ever growing population[2]. Fungicides, herbicides, weedicides, insecticides etc. are all pesticides used in plant protection. Fungicide is is used to controls fungal disease by killing the fungus that causes the disease. Various pesticides are often employed during the maturation of seeds on the parent plant. Such treatment can influence the subsequent germinability of the seed crop. The external morphology of development is often marked by biochemical changes of seed reserves and enzymes of the internal tissues and is considered as markers of growth and development. During seed germination and seedling growth, the seed reserve gets hydrolyzed and a change in the cellular and organellar constituents such as proteins, lipids and carbohydrates takes place. However, the rate of change varies from crop to crop and species to species.

The effect of fungicide on the growth and metabolism of crop plants whether beneficial or detrimental is the subject of interest to the scientist since long ago. Hence the present study was aimed at a detail evaluation of the biochemical behavior during germination in wheat seedlings under influence of fungicide.

The fungicide used in the study was carbendazim having trade name Bavistin because i. This fungicide is used widely for agriculture, ii. It is broad spectrum fungicide therefore is applied to control wide range of fungal diseases.

Material and Methods

Seeds of wheat of uniform size were selected and surface sterilized with 0.1% solution of mercuric chloride for 5min to avoid any fungal growth, followed by washing for 4-5 times with distilled water[3]. The seeds were then placed in Petri dishes which were lined with Whatman No. 1 filter paper moistened with distilled water for 24 hours in dark for germination. The germinated seeds were then grown in presence of five different concentration of fungicide i.e. 500, 1000, 1500, 2000, 2500 mg/l for seven days. After seven days various biochemical parameters were estimated using seedlings and are as follows:

Biochemical parameters: Total protein: Total protein was estimated using biuret reagent[4]. The method is based on the principle that the amide groups of protein form a purple colour complex with copper ions in the presence of alkaline solution. The intensity of purple colour complex so produced is measured at 520 nm colorimetrically.

Total carbohydrate: Total carbohydrate was determined using anthrone reagent[5]. Carbohydrate from the seedling was extracted by boiling it with HCl. Then it was neutralized by adding sodium bicarbonate. Then the extract was used for

determination of carbohydrate by adding anthrone reagent and absorbance was measured at 630 nm.

Chlorophyl: Chlorophyll was estimated using 80% acetone and absorbance of the solution was read at 663 and 645 nm in colorimeter[6]. Total chlorophyll, chlorophyll a and chlorophyll b was calculated from the following formula-

Chlorophyll (a) in mg/g tissue= $12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$

Chlorophyll (b) in mg/g tissue= $22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$

Total chlorophyll (mg per g tissue) = $20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$

Alkaline protease: The tissue was extracted in phosphate buffer of molarity 0.05 and pH 8.0. The activity of enzyme was measured by determining the extent of hydrolysis of caesin as substrate followed by determination of unaltered caesin by Lowry's method of protein estimation. The specific activity was expressed as umoles of tyrosine equivalents liberated/ h/mg protein[7].

Statistical Analysis: Laboratory characteristics of studied parameters were expressed as mean ± standard deviation (SD). ANOVA is used to compare these data between treated seedlings and control seedlings. P values < 0.05 were found to be significant.

Results and Discussion

Protein content of wheat seedling in control was 124.41 ± 5.83 mg/g. Protein content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 95.83 ± 3.81, 62.5 ± 4.33, 50.83 ± 2.75, 57.5 ± 1.5 and 46.66 ± 2.88 mg/g respectively as shown in table no.1. The % decrease in protein content at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was -22.97%, -49.76%, -51.90%, -53.78% and -62.49% respectively as compared to control. The highest % decrease in protein content was – 62.49% which corresponds to 2500 mg/l of fungicide as shown in table- 2.

As shown in table-1 carbohydrate content of wheat seedling in control was 14.4 ± 2.92 %mg Carbohydrate content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 10.9 ± 0.15, 9.4 ± 0.26, 8.36 ± 0.15, 5.9 ± 0.1 and 5.4 ± 0.2 %mg respectively. The % decrease in carbohydrate content at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was -24.3%, -34.72%, -41.94%, -59.02% and -62.5% respectively as compared to control. The highest % decrease in carbohydrate content was – 62.5% which corresponds to 2500 mg/l of fungicide.

Total chlorophyll content of wheat seedling in control was 0.47 ± 0.01 mg/g as shown in table-1.Total chlorophyll content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 0.35 ± 0.008, 0.30 ± 0.012, 0.27 ± 0.002, 0.23 ± 0.002 and 0.20 ± 0.006 mg/ g respectively. The % decrease in total chlorophyll content at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was -25.53%, -36.17%, -42.55%, -51.06% and -57.44% respectively as compared to control. Thus among the entire studied concentration highest % decrease (57.44%) in total chlorophyll was observed at 2500 mg/l concentration of fungicide.

Content of chlorophyll a wheat seedlings was found to be decreased at all the studied concentrations of fungicide as compared to control. Chlorophyll a content of wheat seedling in control was 0.19 ± 0.003 mg/g. As shown in table-1 chlorophyll a content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 0.147 ± 0.006, 0.167 ± 0.03, 0.12 ± 0.002, 0.111 ± 0.005 and 0.099 ± 0.0005 mg/ g respectively. The % decrease in chlorophyll a content at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was -22.63%, -12.10%, -36.84%, -41.57% and -47.89% respectively as compared to control. Thus among the entire studied concentration highest % decrease in chlorophyll a content i.e. 47.89% was observed at 2500 mg/l concentration of fungicide.

Chlorophyll b content of wheat seedlings was found to be decreased at all the studied concentrations of fungicide as compared to control as shown in table-1. Chlorophyll b content of wheat seedling in control was 0.279 ± 0.008 mg/g. Chlorophyll b content of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 0.205 ± 0.004, 0.179 ± 0.003, 0.155 ± 0.002, 0.12 ± 0.009 and 0.102 ± 0.004 mg/g respectively as shown in table-1. The % decrease in chlorophyll b content at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was -26.52%, -35.84%, -44.44%, -56.98% and -63.44% respectively as compared to control. Thus among the entire studied concentration highest % decrease in content of chlorophyll b (-63.44%) was observed at 2500 mg/l concentration of fungicide.

As shown in table- 1 alkaline protease activity of wheat seedlings at 500, 1000, 1500, 2000 and 2500 mg/l was found to be 0.250±0.042, 0.267±0.027, 0.274±0.019, 0.355±0.011 and 0.378±0.022 µmoles/h/mg protein respectively. The % increase in alkaline protease activity at 500, 1000, 1500, 2000 and 2500 mg/l concentration of fungicide was 66.66%, 78%, 82.66%, 136.66% and 152% respectively as compared to control. The highest % increase in alkaline protease activity was 152% which corresponds to 2500 mg/l of fungicide.

As seen in the results fungicide caused decrease in protein content which is significant for wheat seedlings. An osmotic shock caused by systemic fungicides results in the release of protein and loss of membrane transport ability in the cells of leaves. It has been suggested that the toxic substances which were produced by the use of systemic fungicides inhibits protein synthesis by binding to the larger ribosomal subunits and thus induces change in the enzymatic system [8]. The results of our study were in parallel with the work of some workers[9]. As the concentration of fungicide gets increased, amount of protein get gradually decreased[10]. The results of present study were

contradicted by work of some scientists [11,12]. The difference in result may be due to different species used.

The results of present study demonstrated that carbohydrate contents decreased with increase in concentration of fungicide. Reduction in net CO_2 assimilation occurred due to changes in stomatal conductance and intercellular CO_2 concentration were reported in *Malus domestica* and *Cucumis sativus* after application of fungicide[13]. Use of systemic fungicides caused a significant decrease in protein and carbohydrate content as compared to the control[14].

Under the influence of fungicide, wheat seedlings exhibited decrease in the levels of photosynthetic pigments, namely Chlorophyll a, b and total chlorophyll with increase in concentration of fungicide, Pigment biosynthesis was found to be inhibited by the use of systemic fungicide like benomyl. This type of fungicide causes a considerable reduction in the chlorophyll a, chlorophyll b, carotenoids, and the total pigments content in the plants of *Helianthus annuus*[15]. The treatment of *Vitis vinifera* and *Nicotiana tabacum* with fungicides also decreases the chlorophyll and carotenoid content[16,17]. It was reported that chlorosis in leaf occurred after use of benomyl fungicide on *Impatiens walleriana, Cucumis sativus, Celosia plumose, Petunia hybrid,* and *Lycopersicon esculentum*[18]. Total chlorophyll content declined consistently with fungicides dose rates and application days[11].

Alkaline protease activity which is a marker for degradation of protein was get increased with increase in concentration of fungicide. The work of some researchers was not parallal to results of present study and showed decrease in protease activity by endosulfan treatment[10]. At higher concentration of fungicide the rapid growth of seedlings may be due increased availability of amino acids for protein synthesis. These amino acids may result from increased activity of alkaline protease enzyme. Thus, future investigation should be done in order to produce data which may be either in favour or against of the above fact.

Conclusion

Although the use of fungicide is considered good for increasing yield of crops but it adversely affects the biochemical parameters like protein, carbohydrate and chlorophyll. Therefore some other means should be used for increasing productivity or some fungicide should be designed which does not harm the crop adversely.

Table-1
Showing effect of fungicide on various biochemical parameters of wheat seedlings

Conc. of fungicide in mg/l	Total protein (mg/g)	Total carbohydrate (% mg)	Total chlorophyll (mg/g)	Chloro a (mg/g)	Chloro b (mg/g)	Alkaline protease (µmoles/h/mgprotein)
0	124.41±5.83	14.4±2.92	0.47±0.01	0.19±0.003	0.279±0.008	0.15±0.06
500	95.83±3.81**	10.9±0.15*	0.35±0.008**	0.147±0.006**	0.205±0.004**	0.250±0.042*
1000	62.5±4.33**	9.4±0.26*	0.30±0.012**	0.167±0.03*	0.179±0.003**	0.267±0.027*
1500	59.83±2.75**	8.36±0.15**	0.27±0.002**	0.12±0.002**	0.155±0.002**	0.274±0.019**
2000	57.5±1.5**	5.9±0.1**	0.23±0.002**	0.111±0.005**	0.12±0.009**	0.355±0.011**
2500	46.66±2.88**	5.4±0.2**	0.20±0.006**	0.099±0.0005**	0.102±0.004**	0.378±0.022**

*=Values are Significant ($p<0.05$),**= Values are very significant ($p<0.01$) and ns= not significant ($p>0.05$).

Table-2
Showing % increase/decrease in studied biochemical parameters of wheat seedlings under influence of fungicide

Conc. of fungicide in mg/l	Total protein	Total carbohydrate	Total chlorophyll	Chloro a	Chloro b	Alkaline protease activity
500	-22.97%	-24.3%	-25.53%	-22.63%	-26.52%	66.66%
1000	-49.76%	-34.72%	-36.17%	-12.10%	-35.84%	78%
1500	-51.90%	-41.94%	-42.55%	-36.84%	-44.44%	82.66%
2000	-53.78%	-59.02%	-51.06%	-41.57%	-56.98%	136.66%
2500	-62.49%	-62.5%	-57.44%	-47.89%	-63.44%	152%

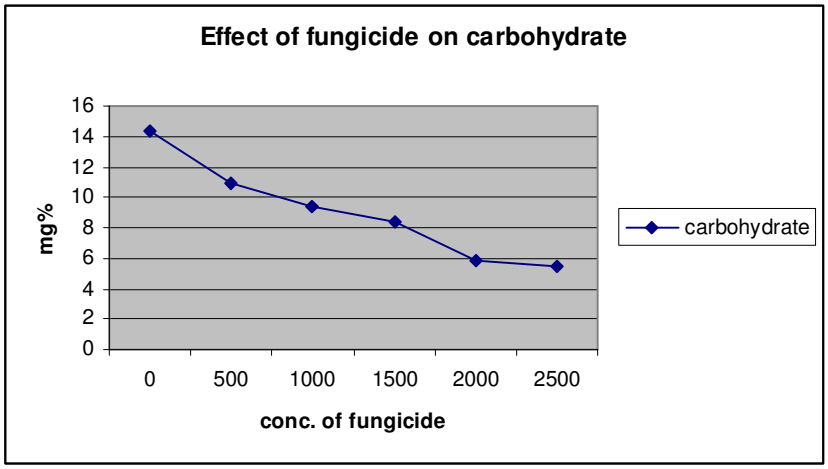

Figure-1
Showing effect of fungicide on carbohydrate content of wheat seedlings

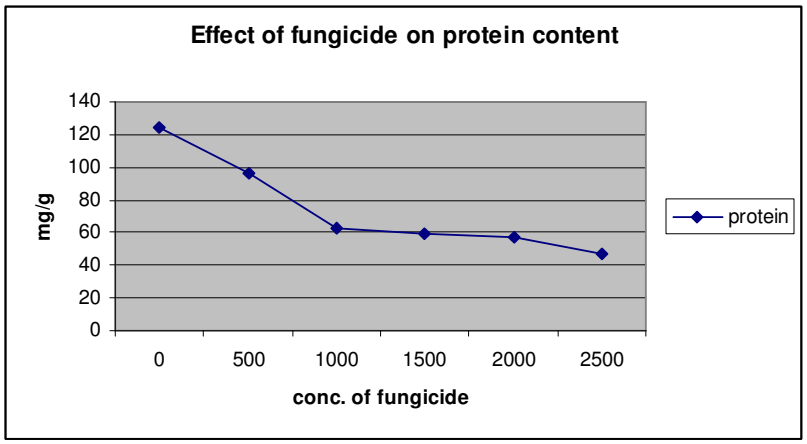

Figure-2
Showing effect of fungicide on protein content of wheat seedlings

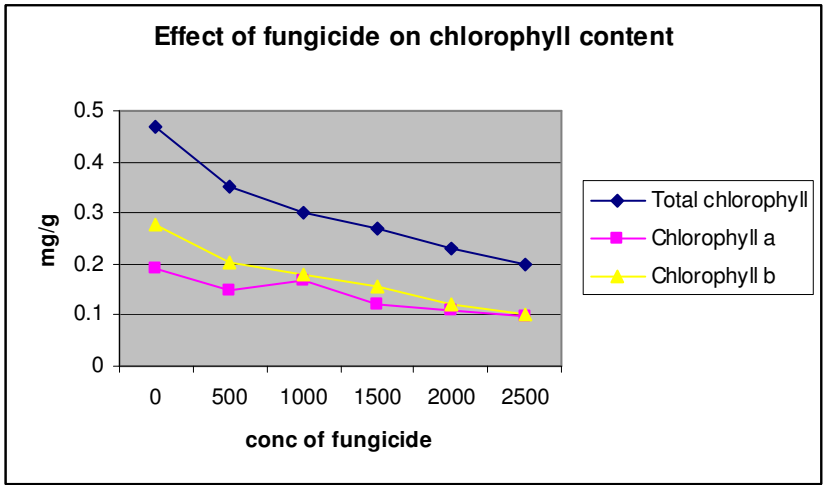

Figure-3
Showing effect of fungicide on total chlorophyll, chlorophyll a and chlorophyll b of wheat seedlings

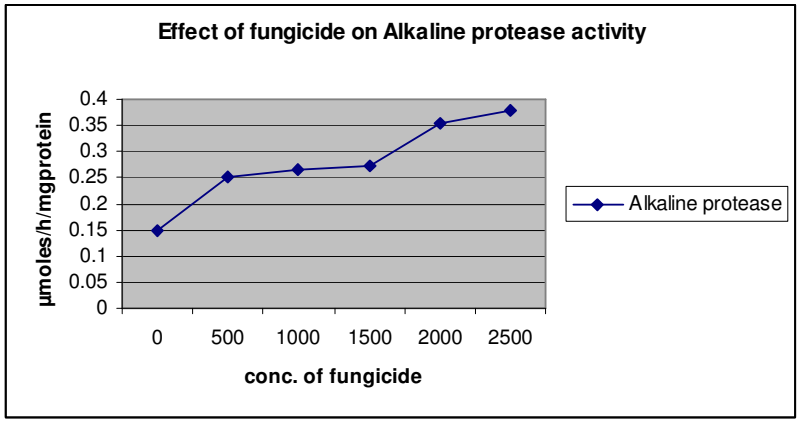

Figure-4
Showing effect of fungicide on alkaline protease activity of wheat seedlings

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