

INFLUENCE OF THE HERBICIDE (THIOBENCARB) AND SITOSTEROL ON RICE PLANT (*Oryza sativa L.*).

Abd EL Wahed , M.S. A.; E. R. EL. Desoki and R.A. EL. Mergawi
Botany Department , National Research Centre Dokki, Cairo, Egypt .

ABSTRACT

Two pot experiments were conducted during two growing seasons, 2001-2002 at greenhouse of National Research Centre, Dokki, Cairo, Egypt to study the influence of Thiobencarb herbicide and Sitosterol on rice plant (*Oryza sativa L.*) cv. G177 . Thiobencarb herbicide concentrations (2,4 and 6 ml/l) were applied at 8 days from transplanting. Sitosterol concentrations (100 and 200 mg/l) were sprayed after 7 days from Thiobencarb application the main finding could be summarized as following :

Thiobencarb significantly decreased root and vegetative characteristics , yield, photosynthetic pigments of flag leaf , sugars , protein and indoles contents of all rice plant organs . However , it increased free amino acids and total phenol contents at leaf tube and grain ripe stages. Sitosterol application had stimulatory improvement the previous characters . Thiobencarb and Sitosterol combinations significantly improved root , vegetative and yield and its components compared with Thiobencarb treatment alone especially 2ml/l Thiobencarb and 200 mg/l Sitosterol . Sitosterol at 200 mg/l significantly improved all growth characters of root, vegetative and yield of rice plant . Also , these treatments significantly increased root soluble sugars , total phenols at leaf tube stage and decreased free amino acids content . There were significant increases in sugars , protein, free amino acids and total phenol contents of vegetative growth, photosynthetic pigments of flag leaf and grain biochemical contents at the physiological stages . Sitosterol at 200 mg/l with Thiobencarb levels was more effective on root and vegetative growth.

Keywords: Thiobencarb , Sitosterol , Root growth, Vegetative growth Biochemical constituents .

INTRODUCTION

Thiobencarb has become important tool in the light of reducing weeds and increasing the efficiency of other cultural practice. While, it causes injury to rice plant as inhibition of photosynthesis, respiration, reduce glutathione and the enzyme glutathione - S - Transferase levels (Fedtke, 1982). Thiobencarb was found to reduce the activity of ribonuclease at 72 h after treatment in rice seedling while Deoxyribonuclease activity is increased. Maximum activity of Deoxyribonuclease in rice was observed during the 96 h after treatment (Jitender *et al.*, 1995) . Thiobencarb phytotoxicity appeared as inhibition of germination, shoot and root growth of rice that was due to straight chain substitutes. There was an inhibition of alpha amylase biosynthesis in rice seeds also , Thiobencarb inhibited Indole - 3 - acetic acid and induced cell elongation (Chon and Guh, 1995; Chun *et al.*, 1995a and Chun *et al.*, 1995b). Thiobencarb phytotoxicity include inhibition of plant elongation and tillering stage in transplanted rice seedlings . So, the herbicide was more toxic to nursery stage seedlings than to young seedlings. Inhibition of leaf elongation was more pronounced and showed one leaf less in nursery stage seedlings . While in the root system, inhibition of elongation and

distortion of the basal portion of crown roots were observed after herbicide application, the number of crown roots decreased and their elongation was strongly inhibited in nursery stage seedling than in young seedling (Fujita 1996) Sitosterol was used to overcome these harmful effects of Thiobencarb on rice plant.

Sitosterol is the major compound of the steroids that are phytosterols (Deliu *et al.*, 1992). Brassinosteroid promoted root growth. Amylase activity was associated with root growth, root weight, root: shoot ratio and rooting ability were significantly increased with brassinosteroid application (Wang and Deng, 1992) and Fujii and Saka (2001). Additionally, rice lamina joint inclination increased with brassinosteroid concentration and exposure period. Etiolated lamina were more sensitive to Brassinosteroid than green lamina. The brassinosteroid induced inclination was accompanied by increased lamina fresh weight, total water content, free water content, proton extrusion and ethylene and decreased bound water content (Cao and Chen 1995). Brassinosteroid gave the greatest shoot height, seedling dry weight, chlorophyll concentration, root dehydrogenase activity and activity of alpha and beta amylase (Wang and Wang, 1997). So, brassinosteroid could stimulate a variety of physiological processes including changes in enzymatic activities, membrane potential, DNA, RNA and protein synthesis, photosynthetic activity and changes in the balance of the endogenous phytohormones (Mandava 1988 and Szekers and Konez, 1998). High leaf area index (LAI), increased accumulation of chlorophylls, higher soluble protein content and higher Nitrate reductase activity were evident in the treated rice plant with brassinosteroid resulting in increased biomass accumulation. Increased number of spikelets and reduction in spikelet sterility contributed to higher yield (Maibangsa *et al.*, 2000) Sitosterol had stimulatory effect on vascular differentiation, thickness of the upper epidermal layer, mesophyll tissue layer, photosynthetic pigments, growth and yield as well as chemical composition of wheat Abd El-Wahed *et al.* (2001).

Therefore, the objective of the present investigation is to attempt to overcome the harmful effect of the herbicide (Thiobencarb) on rice by adding Sitosterol.

MATERIALS AND METHODOS

The experiments were conducted to study the influence of Thiobencarb and Sitosterol effect on rice (*Oryza sativa L.*) during 2001 and 2002 seasons under green house condition at National Reseach Centre, Dokki, Cairo.

Herbicide: one herbicide was used in this work {Thiobencarb (Satum.) : S (4 - chlorobenzyl) N,N diethyl thiocarbamate} at three concentrations 2, 4 and 6 ml/l., Sitosterol at two concentrations (100 and 200 mg/l.) as well as control. Thiobencarb treatments were added after 8 days from rice transplanting and Sitosterol treatments were added after 7 days from Thiobencarb application. Grain of rice (*Oryza sativa L.*) cultivar G 177 were sown at 18 May in pots with clay soil. Rice seedlings were

transplanted after 30 days to permanent soil at 18 June in two seasons in pots No. 40 contained 5 hills (15 plants / pot) . The following treatments were applied :-

- 1- Control
- 2- Thiobencarb at 2ml/l .
- 3- Sitosterol at 100 mg/l
- 4- Thiobencarb at 2ml/l + Sitosterol at 100mg/l .
- 5- Thiobencarb at 2ml/l + Sitosterol at 200mg/l .
- 6 Thiobencarb at 4ml/l + Sitosterol at 100mg/l .
- 7- Thiobencarb at 4ml/l + Sitosterol at 200mg/l .
- 8- Thiobencarb at 6ml/l + Sitosterol at 100mg/l .
- 9- Thiobencarb at 6ml/l + Sitosterol at 200mg/l .

The experiment was arranged as a complete randomized block design with three replications . Calcium superphosphate fertilizer (15-5% P₂O₅) was added to soil pretransplanting at 6 gm / pot . Nitrogen fertilizer at a rate of 24 gm as urea (46 % N) was also added at a rate of 24 gram/pot at three equal doses (21 , 30 and 39) days from transplanting .

Growth and yield measurements :

Growth measurements were taken at leaf tube stage (55 days after transplanting) and ripe grain stage (110 days from transplanting) , yield and its components were determined at late stage . Root measurements (length , fresh and dry weight) were also taken as well as vegetative growth measurements (plant height , fresh, dry weight and root : shoot ratio) and flag leaf area determined at milk stage , yield and its components (length , spikelet number , grain number , empty grain number/ panicle , grain yield / plant and 100 grain weight .

Biochemical constituents:

Photosynthetic pigments (chlorophyll a,b and carotenoids) were determined in fresh flag leaves according to Saric *et al.* (1967) . Root , top and grain samples were dried in a ventilated oven at 70 °C then finally ground in stainless steel mill to determine total, soluble and nonsoluble sugars according to Dubois *et al.* (1956) , protein percentage was calculated according to A.O.A.C. (1975) as well as free amino acids according to Plummer (1978) , total phenols according to Daniel and George (1972) and total indoles according to Glickmann and Dessaux (1995). Statistical analysis of data were carried out for analysis of variance as described of by Snedcor and Cochran (1980). L.S.D. at 5% level of probability was used to compare between means .

RESULTS AND DISCUSSION

Effect of herbicide and Sitosterol on rice plant :

In this study rice cv. Giza 177 was cultivated for two consecutive years 2001 and 2002 under the effect of the tested compounds i.e.

Thiobencarb and Sitosterol and the average of the two year values of all data were calculated .

Thiobencarb and Sitosterol effect of on roots :

Data presented in Table 1 show that Thiobencarb alone , Sitosterol alone, and Thiobencarb at high dose with Sitosterol at low dose caused significant decrease in mean values of root fresh and dry weight when compared with control and other combinations at all physiological stages. Thiobencarb at 2m/l with Sitosterol at 200mg/l gave pronounced effect for root dry weight at all stages. The other combinations were insignificantly affected compared with control. The highest root length was obtained by using Sitosterol alone. Thiobencarb alone and Thiobencarb at high dose with Sitosterol at two doses decreased the root length at grain ripe stage . These data showed that Sitosterol was more effective to avoid the effect of Thiobencarb on rice root growth characters. Our data are in agreement with those obtained by (Chon and Guh 1995 , Chun *et al.*, 1995 a) who reported that Thiobencarb application inhibited root growth of rice, Indole – 3 acetic acid and cell elongation . Thiobencarb phytotoxicity appeared in transplanted rice seedlings. While, the root system inhibition of elongation and distartion of the basal portion of crown root were observed after herbicide application (Fujita 1996) . On the other hand, Sitosterol is the major compound of the steroids that are phytosterols (Deliu *et al.*, 1992), that was related to auxin and cytokinin in *Euphoria characias cali* (Fernandes *et al.*, 1992) .

Table 1: Effect of thiobencarb and sitosterol on root characterstific

Treatments	Leaf tube stage		Grain ripe stage		
	Root fresh weight (gm)	Root dry weight (gm)	Root length (cm)	Root fresh weight (gm)	Root dry weight (gm)
Control	2.15	0.59	12.4	3.0	0.40
Thiobencarb2mL/L	1.21	0.18	9.5	1.7	0.42
Sitosterol 100 mg /L	1.73	0.32	14.5	2.5	0.43
Thiobencarb2mL + Stioosterol 100mg	2.21	0.58	11.2	2.3	0.57
Thiobencarb2mL + Stioosterol 200mg	2.80	0.77	11.3	2.4	0.71
Thiobencarb 4mL + Sitosterol 100mg	2.17	0.56	10.4	2.1	0.45
Thiobencarb2mL + Stioosterol 200 mg	2.57	0.60	10.9	2.5	0.51
Thiobencarb 6mL + Stioosterol100mg	1.63	0.46	9.9	1.7	0.40
Thiobencarb 6mL + Stioosterol200mg	2.10	0.60	10.0	2.5	0.59
L .S. D. at 5%	0.72	0.05	2.4	0.9	0.17

Thiobencarb and Sitosterol effect of on vegetative growth characteristics :-

It is evident from Table 2 that significant differences in rice growth characteristics of vegetative growth at leaf tub stage and ripe stages . At leaf tube stage , plant height, plant dry weight and root : shoot ratio gave significant differences between treatments . Sitosterol alone at 100mg/l gave the highest plant height at this stage while , Thiobencarb at 6 m/l with 100 mg/l Sitosterol and Thiobencarb alone at 2m/l gave the lowest plant height when compared with the other treatments and control .

Table 2: Effect of thiobencarb and sitosterol on rice plant characters.

Treatments	leaf tube stage				Ripe grain stage				Root: Shoot ratio
	plant height (cm)	plant fresh weight (gm)	plant dry weight (gm)	Root: shoot ratio	plant height(cm)	plant fresh weight (gm)	plant dry weight (gm)	Number of tiller/ plant	
Control	59.1	9.0	1.6	0.39	80.8	15.8	4.5	2.5	0.13
Thiobencarb 2mL/L	55.5	10.6	1.5	0.13	79.0	8.3	4.4	2.5	0.11
Sitosterol 100 mg/L	78.1	9.7	1.6	0.21	82.5	10.9	3.2	2.3	0.14
Thiobencarb 2ml+Sitosterol 100	76.5	8.7	2.5	0.23	87.5	9.5	3.2	3.3	0.18
Thiobencarb 2ml + Sitosterol 200mg	66.8	9.8	2.8	0.27	91.3	17	4.8	3.5	0.15
Thiobencarb 4mL+ Sitosterol 100mg	63.3	9.5	2.1	0.17	85.3	9.1	2.4	3.3	0.16
Thiobencarb 2ml + Sitosterol 200mg	57.3	11.9	2.6	0.29	85.8	11.9	3.0	3.3	0.17
Thiobencarb 6mL+ Sitosterol 100mg	50.3	8.0	1.9	0.20	84.0	9.0	2.3	3.3	0.17
Thiobencarb 2ml + Sitosterol 200mg	64.0	9.4	2.3	0.29	95.0	9.6	2.8	3.8	0.21
L. S. D. at 5%	5.9	NS	0.9	0.11	NS	4.0	1.8	0.7	NS

Thiobencarb at 2ml/l with Sitosterol at 100 mg/l or 200mg/l gave the second rank at this stage . Plant dry weight showed significant variation between treatments . Thiobencarb with Sitosterol gave the heighest dry weight while , Thiobencarb alone , Sitosterol and control gave the lowest dry weight when compared with other treatments . The same trend was obtained with root : shoot ratio and plant fresh weight affected were insignificantly at this stage.

At ripe stage, plant fresh weight , plant dry weight and number of tillers / plant gave significant variation between treatments except plant height and shoot :root ratio .The highest number of tillers / plant was obtained by using Thiobencarb at 6ml/l with Sitosterol at 200mg/l and Thiobencarb at 2ml/l with 200mg/l Sitosterol. It appeared that Thiobencarb alone at 2ml/l phytotoxicity was continual at all developmental stages of rice . These results are in agreement with those obtained by (Chon and Guh 1995, Chun *et al.* 1995 a, Chun *et al.* 1995 b and Fujita 1996) who reported that Thiobencarb inhibited germination , shoot , root growth , plant elongation and tillering of rice plant . In this trend Mandava 1988 , Wang and Deng, 1992 , Cao and Chen 1995 , Abd EL Samie and EL Bially 1996, Szekers and Konez , 1998 found that increasing Brassionsteroid concentration promoted root : shoot ratio as well as rice lamina inclination .

Influence of Thiobencarb and Sitosterol on flag leaf characteristics :

The data in Table 3 showed that all flag leaf characters (fresh , dry weight and leaf area) were significantly increased with Thiobencarb and Sitosterol levels as foliar applications compared with control at all physiological stages except fresh weight at leaf tube stage. Flag leaf characteristics were significantly decreased with increasing Thiobencarb level. Thiobencarb at 2ml/l with 100 or 200 mg/l Sitosterol treatments were more effective on flag leaf characteristics in developmental stages.

Table 3: Effect of thiobencarb and sitosterol on flag leaf charaters.

Treatments	Leaf Tube Stage		Milk Stage	Ripe Grain Stage	
	Fresh weight (gm)	Dry weight (gm)	Flag Leaf area (cm)2	Fresh weight (gm)	Dry weight (gm)
Control	0.44	0.19	20.5	0.19	0.13
Thiobencarb 2mL / L	0.39	0.18	21.5	0.17	0.12
Sitosterol 100mg/L	0.43	0.18	23.3	0.16	0.11
Thiobencarb 2mL+Sitosterol /100mg	0.43	0.17	25.3	0.26	0.13
" " + " 200mg	0.44	0.17	27.3	0.36	0.15
" 4mL+ " 100mg	0.46	0.14	20.8	0.23	0.14
" " + " 200mg	0.35	0.20	21.9	0.27	0.15
" 6mL + " 100mg	0.35	0.14	22.3	0.18	0.09
" " + " 200mg	0.33	0.15	23.8	0.30	0.16
L.S.Dat 5%	NS	0.04	2.7	0.11	0.04

Sitosterol at 200 mg/l with Thiobencarb at 2 and 4ml/l gave significant stimulation for increasing resistance ability of rice plant to Thiobencarb

application. It appeared that Sitosterol concentrations were sufficient to avoid the phytotoxicity of Thiobencarb on rice plant. It could be due to its ability to overcome the inhibitory effect of the herbicide. These results were in agreement with those reported by Chon and Guh (1995), Chun *et al.* (1995) and Fujita (1996) who found that thiobencarb inhibited leaf elongation and straw yield. However, LAI and higher Nitrate reductase (NRase) activity were evident in the treated rice plant with brassinosteroid resulting in increased biomass accumulation (Maibangsa *et al.* 2000). On the other hand, Sitosterol had stimulatory effect on flag leaf structure of wheat (Abd EL. Wahed *et al.*, 2001).

Influence of Thiobencarb and Sitosterol on rice yield and its components:-

Thiobencarb and Sitosterol treatments on rice plant resulted in a substantial increase in grain weight / plant as reflected in increments in yield components recorded in Table 4. Significant increases in grains yield / plant were related to Thiobencarb with Sitosterol concentrations. From data in Table 4, it appeared that all characters except spikelets number / panicle and 100 grain weight were significantly affected by Thiobencarb, Sitosterol and their combinations especially Thiobencarb 2ml/l with 200mg /l Sitosterol which mostly gave the highest mean values in the number of grain / panicle and also in the grain weight /panicle as well as grain yield / plant.

Table 4: Effect of thiobencarb and sitosterol on yield and its component of rice plant.

Treatments	Panicle length (cm)	Number of spikelets / panicle	Grain number/ Panicle	Empty grains / Panicle	Grain weight / Panicle (gm)	Grain yield /plant (gm)	100 - grain weight (gm)
Control	18.9	8.9	78.0	16.3	1.4	3.0	2.50
Thiobencarb 2mL / L	15.5	8.2	71.0	13.2	1.4	2.8	2.33
Sitosterol 100 mg/ L	19.1	9.6	96.3	23.9	1.8	3.9	2.52
Thiobencarb 2mL+ Sitosterol 100mg	18.5	9.9	90.9	7.4	2.2	3.7	2.48
" " + " 200mg	19.0	10.0	104.2	16.2	2.3	6.2	2.58
" 4mL+ " 100mg	16.9	8.7	83.0	14.7	1.7	3.7	2.39
" " + " 200mg	18.1	9.8	101.5	16.3	2.0	4.3	2.42
" 6mL + " 100mg	19.6	7.8	68.6	13.7	1.7	3.5	2.56
" " + " 200mg	18.0	9.1	89.9	15.6	1.8	3.7	2.71
L.S.D .at 5%	1.0	NS	20.0	6.6	0.5	1.6	NS

Although the decrease the mentioned characters with increasing Thiobencarb level. This effect could be due to the enhancement of root growth and criteria of vegetative growth of rice plant, panicle length, grain number /panicle, grain weight and panicle. These results are in accordance with Abd El Samie and El Bially, 1996 who found that panicle numbers / m².

grain number / panicle and panicle length were in rice cultivar as response to Thiobencarb application . On the other hand , Basudey *et al.* (1997) and Brar and Kolar (1997) reported that grain yield and its components in rice plant increased with thiobencarb application. On the other side, Abd EL - Wahed (2001) found that Sitosterol application was significantly increased spikelet number / spike ,1000 grain weight of wheat. So, plant yield and grains number /row in maize plant with Sitosterol treated significantly increased with increasing sitosterol concentrations (Abd EL - Wahed 2001).

Influence of Thiobencarb and Sitosterol on photosynthetic pigments :-

Significant differences between treatments in concentrations in mg of chl . a, chl .b and carotenoids of flag leaf were recorded at leaf tube stage and soft dough grain stage (Table 5). Sever reduction was found by Thiobencarb alone at 2ml/l and treatment with Thiobencarb at 6ml/l with 100mg/l Sitosterol. Thiobencarb application at 2ml/l with Sitosterol at 100 and 200mg/l gave significant increases of photosynthetic pigments at leaf tube stage compared at control treatment . At soft dough stage , The highest amount of chl.a were obtained by using Thiobencarb at 2,4 and 6 ml/l with 200 mg/l Sitosterol .These results showed that 200 mg/l of Sitosterol is able to reduced the sensitivity of rice plant to Thiobencarb phytotoxicity, which caused on increase in photosynthetic pigment contents of rice flag leaf

Table 5: Effect of thiobencarb and sitosterol on rice plant charaters.

Treatments	leaf tube stage			soft dough grain stage		
	chl.a	chl .b	carotenoids	chl .a	chl .b	carotenoids
	mg/gm					
Control	1.09	0.30	0.40	1.48	0.79	0.64
Thiobencarb 2mL / L	1.01	0.25	0.34	1.48	0.55	0.48
Sitosterol 100 mg/ L	1.15	0.36	0.38	1.50	0.48	0.51
Thiobencarb 2mL+ Sitosterol 100 mg/L	1.63	0.37	0.47	1.90	0.68	0.58
" " + " 200mg	1.83	0.48	0.60	2.16	0.80	0.64
" 4mL+ " 100mg	1.15	0.26	0.35	1.61	0.54	0.51
" " + " 200mg	1.33	0.37	0.48	2.08	0.74	0.60
" 6mL+ " 100mg	1.01	0.27	0.39	1.81	0.80	0.58
" " + " 200mg	1.18	0.38	0.48	2.34	0.85	0.62
L.S.D .at 5%	0.21	0.07	0.09	0.45	0.14	0.09

The other combinations did not differ statistically from control in the amount of chl.b and carotenoids . In this respect Fedtke, 1982 and Deka *et al.* (1996) mentioned that the harmful effect of Thiobencarb might be due to inhibition of long chain fatty acids and related to alcohols as well as alkanes, which reflected at reducing phospholipids synthesis . Whereas , the lipids are essential components in all contents of the cell . In addition , it conjugates with Sitosterol being the major compound of the steroids that are phytosterols (Deliu *et al.* 1992) .So, brassiosteroids could stimulate a variety of physiological processes including changes in enzymatic activities ,

membrane potential , DNA , RNA , protein synthesis , photosynthetic activity and changes in the balance of the endogenous phytohormones (Mandava , 1988 Szekers and Konez 1998).

Influence of Thiobencarb and Sitosterol on biochemical content of plant rice root:-

Rice plant root content of sugars were significantly variable in Table 6 . Thiobencarb treatment (2ml/l) gave higher root total sugar percentage than the other treatments in leaf tube and ripe grain physiological stages .High values of total sugar percentage were recorded by Thiobencarb alone at 2ml/l and Thiobencarb at 6ml/l with Sitosterol at 100mg/l at ripe grain stage . Thiobencarb concentrations with 100 mg/l Sitosterol significantly increased root total sugar percentage contents compared with control and other treatments .These results are in agreement with those obtained by (Chon and Guh 1995 and Chun *et al.*, 1995 b) who stated that Thiobencarb inhibited alpha amylase, that leads to accumulation of sugars in root and didn't translocate to plant organs . On the contrary, brassinosteroid gave the greatest activity of alpha and beta amylase (Wang and Deng ,1992 and Wang and Wang , 1997) . Also Abd EL Wahed (2001) found that root sugars content of maize significantly increased with Sitosterol leading to maize plant development .

Concerning , free amino acids , total indoles and total phenols were significantly affected with application of Thiobencarb , Sitosterol and their combinations at leaf tube and ripe grain stages as show in Table (6) . Free amino acids and total indoles contrasted in the two physiological stages . Whereas, it decreased in the first stage and increased in the second stage especially with the highest concentration of Thiobencarb with Sitosterol treatments compared with control .While , Thiobencarb at 2ml/l gave the highest phenols content in rice plant root . This effect led to increase resistance of rice plant to Thiobencarb herbicide with Sitosterol application . These results were in agreement with Jitender *et al.* (1995) who found that Thiobencarb herbicide reduced the activity ribnuclease and increased Deoxyribonucleas activity in rice seedling. Therefore, thiocarbamate herbicide cleavage occurs between the sulphur atom and ethyl group which led to produce phenols organic acid and amino acids metabolites(Fletcher and Kirkwood , 1982). While, brassinosteroid gave the highest dehydrogenase activity(Wang and Wang , 1997) and stimulate DNA and RNA (Mandava, 1988 and Szekers and Konez , 1998).

Effect of Thiobencarb and Sitosterol on biochemical contents of vegetative plants :-

Data in Table 7 revealed that Thiobencarb and Sitosterol treatments caused significant increases in all sugars content of vegetative rice plant at ripe grain stage . Sugar content increases were attributed to Thiobencarb at 2ml/l and 4ml/l with Sitosterol at 100and 200mg/l concentrations in the ripe grain stage , while, Thiobencarb application at 6ml/l with sitosterol at 100, 200 mg/l showed decrease in soluble sugar content when compared with the other combination .

Table 6: Effect of thiobencarb and sitosterol on biochemical content of root plant rice

Treatments	Leaf tube stage					Ripe grain stage %						
	Soluble sugars %	Non soluble sugars %	Total sugars %	Free amino acids%	Total indoles mg/gm	Total phenols mg/gm	Soluble sugars %	Non soluble sugars %	Total sugars	Free amino acids %	Total indoles mg/gm	Total phenols mg/gm
Control	1.6	12.2	13.8	2.64	3.87	7.0	5.9	17.1	23.0	3.0	3.4	3.6
Thiobencarb 2mL / L	2.9	23.8	26.7	2.59	3.88	8.6	4.2	34.5	39.7	3.6	3.0	6.1
Sitosterol 100 mg/L	3.9	15.9	19.8	2.67	4.04	6.4	5.5	18.9	24.4	4.6	2.9	5.6
Thiobencarb 2mL + Sitosterol 100 mg	3.8	19.5	23.3	1.75	3.70	8.3	7.1	20.0	27.1	3.7	3.2	4.2
Thiobencarb 2mL + Sitosterol 200mg	2.9	11.9	14.8	1.94	3.65	2.1	4.8	22.3	27.1	3.2	2.8	4.2
Thiobencarb 4mL + Sitosterol 100mg	2.6	17.7	20.3	1.56	3.76	8.1	4.7	27.3	32.0	3.3	2.9	1.8
Thiobencarb 2mL + Sitosterol 200mg	3.1	9.2	12.3	1.79	3.68	5.6	6.0	25.9	31.9	3.3	2.8	4.7
Thiobencarb 6mL + Sitosterol 100mg	2.3	18.4	20.7	1.95	3.87	6.7	5.0	34.3	39.3	5.3	3.1	3.2
Thiobencarb 2mL + Sitosterol 200mg	4.3	10.8	15.1	2.43	3.66	5.6	4.2	16.5	20.7	5.1	2.8	3.8
L. S. D. at 5%	0.6	1.4	1.3	0.8	NS	1.5	1.1	2.1	2.2	0.9	0.3	1.2

Table 7: Effect of thiobencarb and sitosterol on vegetative biochemical contents of rice plant

Treatments	Leaf tube stage						Ripe grain stage							
	Sugars %			Protein %	free amino acid mg/g	Total indoles phenols	Sugar %			Protein %	free amino acid mg/g	Total indoles phenols		
	Soluble	Nonsoluble	Total				Soluble	Non soluble	Total					
Control	5.7	18.1	23.9	4.3	6.8	3.6	2.3	4.1	18.6	22.7	2.9	5.0	4.53	3.73
Thiobencarb 2mL / L	4.8	19.8	24.6	3.9	6.3	6.0	3.7	2.2	23.0	25.2	3.0	5.6	3.87	4.19
Sitosterol 100 mg/L	7.0	17.7	24.6	4.8	8.1	5.4	2.6	3.8	22.0	25.8	2.6	5.1	3.91	2.00
Thiobencarb 2mL+Sitosterol 100mg / L	5.0	19.4	24.4	4.6	4.5	7.5	2.5	5.9	16.4	22.3	4.1	4.1	3.67	2.96
Thiobencarb 2mL + Sitosterol 200 mg / L	5.2	17.0	22.2	6.1	4.8	5.8	7.4	6.7	17.6	24.3	3.6	4.2	3.65	2.48
Thiobencarb 4mL + Sitosterol 100 mg / L	5.2	18.8	23.9	5.1	14.4	5.2	3.0	6.5	19.2	25.7	5.0	4.2	3.70	3.05
Thiobencarb 4mL + Sitosterol 200 mg / L	5.7	18.9	24.5	5.9	7.0	4.4	7.2	9.1	14.5	23.6	4.2	4.3	3.62	2.43
Thiobencarb 6mL + Sitosterol 100 mg / L	6.5	20.5	27.1	4.9	17.0	6.8	7.8	4.3	20.8	25.1	4.5	3.5	3.79	4.21
Thiobencarb 6mL + Sitosterol 200mg / L	7.1	16.0	23.1	5.0	13.5	7.0	7.2	4.4	19.5	23.9	4.8	6.2	3.77	4.21
L.S.D. at 5%	1.1	NS	NS	0.6	1.8	0.5	1.3	0.6	2.5	2.1	0.9	0.9	0.22	0.91

Concerning total protein and free amino acids, content of vegetative rice plant there was significant increase with increasing Thiobencarb and Sitosterol concentrations when compared with control. However total indole showed high values with Thiobencarb at 2ml/l with Sitosterol at 100mg/l, 6ml/l Thiobencarb with 100 or 200 mg/l Sitosterol and Thiobencarb alone at 2ml/l, while total phenols increased with 6ml/l Thiobencarb with 100mg/l or 200 mg/l Sitosterol, 4ml/l Thiobencarb with 200mg/l Sitosterol as well as treatment Thiobencarb at 2ml /l with 200mg/l Sitosterol at leaf tube stage. These results reflect the sensitivity of rice plant to Thiobencarb application in increasing soluble sugar, protein, total free amino acids, indoles and phenols in vegetative rice plant. These results confirm those reported by Chun *et al* (1995a) who found that thiocarbamate herbicides application led to accumulation of sugars. (Fletcher and Kirkwood, 1982) as the result to inhibit alpha amylase biosynthesis (Chun *et al.*, 1995 b). Also, protein content of vegetative rice plant was attributed with cultivars resistance to Thiobencarb application (Kim *et al.*, 1996). While, the total SH- content in tissue treated with thiocarbamate decrease (Fedtke, 1982).

On the other hand, thiocarbamates degradation produced organic acid and amino acid metabolites (Fletcher and Kirkwood, 1982). Also, phenolic compounds accumulate to a greater extent particularly at unfavorable conditions. Whereas, nonstructural carbohydrates and amino acid diversion model of secondary plant metabolism led to accumulation of phenolics stems from a decreased use of a common precursor (phenylalanine or tyrosine) for protein synthesis (Lambers, 1993).

Brassinolide treatment promoted ¹⁴C assimilation in leaf blades and subsequent translocation of assimilate to panicle of rice plant (Fujii *et al.* 1991). So, brassinosteroids induced an increase of invertase activity, alpha and beta amylase in rice plant (Adam *et al.*, 1991, Wang and Wang, 1997) as well as sterols were found to enhance nitrogen of wheat (Abd EL wahed *et al.* 2000) and nitrogenase enzyme activity of groundnut (Vidya and Seeta 1998).

Influence of Thiobencarb and Sitosterol on flag leaf sugar contents :-

A significant decrease in flag leaf nonsoluble sugar content was observed when compared with control as shown in Table 8 at the leaf tube stage. Soluble and total sugar contents were significantly affected with Thiobencarb and Sitosterol treatments, there was significant increases in flag leaf sugar contents (Nonsoluble and total sugar %) at ripe grain stage. However, soluble sugars content gave the opposite trend. The enhancement of sugar contents were attributed to the concentration of Thiobencarb and Sitosterol. Sitosterol treatment at 200mg/l treatment under Thiobencarb levels were more effective on the soluble and nonsoluble sugars but, 100mg/l Sitosterol had stimulatory effect on total sugar content in all stages of rice plant development. These results were in agreement with Chun *et al.*, 1995b who found that Thiobencarb inhibited alpha and beta amylase biosynthesis in rice plant. On the other hand Wang and Wang, 1997 gave the opposite trend whereas, brassinosteroid increased alpha and beta amylase activity in rice plant.

Table 8: Effect of thiobencarb and sitosterol on flag leaf sugars contents .

Treatments	Leaf tube stage			Ripe stage		
	soluble %	Non soluble%	Total %	Soluble %	Non soluble%	Total %
Control	9.0	17.2	26.2	9.7	16.6	26.3
Thiobencarb 2ml / L	7.2	17.6	24.8	8.9	18.1	27.0
Sitosterol 100mg/ L	9.3	16.0	25.3	7.6	18.1	26.2
Thiobencarb2mL/L+Sitosterol 100mg/ L	9.7	13.6	23.3	8.8	21.9	30.7
" " + " 200 mg	9.6	14.9	24.5	8.2	20.2	28.4
" 4ml + " 100	8.0	16.2	24.2	8.0	13.5	21.5
" " + " 200 mg	9.2	16.0	25.0	8.0	21.4	29.4
" 6ml + " 100 mg	9.4	14.6	25.4	7.8	23.4	31.0
" " + " 200mg	9.4	14.0	23.4	9.0	18.2	27.2
L .S. D.at 5%	NS	2.9	NS	0.5	3.9	4.0

Influence of Thiobencarb and Sitosterol on biochemical contents of rice grain :-

Data presented in Table 9 show that grain soluble, nonsoluble ,total sugars , protein percentage, and total indoles were significantly increased with Thiobencarb and Sitosterol application compared with control .The enhancement was attributed to Thiobencarb and Sitosterol concentration . Total sugars and nonsoluble sugars were in opposite direction with soluble sugars in this concentration .Whereas , Thiobencarb at 2,4 and 6 ml/l with 100 mg/l Sitosterol was effective than 200 mg/l Sitosterol . This showed that Sitosterol had a role on sugars translocation to grains . Increasing total protein percentage in rice grains were concomitant with 200mg/l Sitosterol with different Thiobencarb level applications. While, total phenolic compound was decreased in all treatments. But, total indoles gave opposite trend compared with control . It might be due to thiocarbamate herbicides effect on accumulation of sugars and quinone phenolic compound in the roots (Fletcher and Kirkwood , 1982). This effect was attributed to cultivar rice resistance to thiobencarb whereas, protein content of the tolerant cultivars were little affected than susceptible (Kim *et al.* 1996 , Abd El Samie and EL Bially (1996). It might be due to the effect of thiobencarb on the activity of ribonuclease and deoxyribonuclease (Jitender *et al.*, 1995). On the opposite, brassinolide increased starch accumulation in panicles and altered endogenous IAA (Fujii *et al.* 1991). So, Maibangra *et al.*, (2000) found increasing soluble protein content and higher NRase activity in the treated rice plants by brassinosteroid resulting in biomass that were correlated with carbohydrate and protein content (Abd EL Wahed *et al.*, 2000) .

Table 9: Effect of thiobencarb and sitosterol on biochemical contents of rice grain

Treatments	Sugars %			Total protein %	Free amino acids	Total indoles	Total phenols
	Soluble	Non soluble	Total				
Control	2.0	74.5	76.4	2.6	2.3	0.36	1.30
Thiobencarb 2ml / L	2.0	80.4	82.4	1.7	3.1	0.31	1.00
Sitosterol 100 mg/L	2.9	75.2	78.1	2.0	2.9	0.54	1.20
Thiobencarb 2ml+ Sitosterol 100mg/L	2.2	76.4	78.6	2.0	3.1	0.49	1.30
" " + " 200mg	3.6	72.0	75.6	2.6	3.3	0.41	1.40
" 4mL+ " 100mg	2.3	81.8	84.1	2.5	2.9	0.47	1.00
" " + " 200mg	2.5	76.0	78.5	3.4	3.1	0.45	1.20
" 6mL+ " 100mg	1.7	82.7	84.4	1.8	3.0	0.41	1.10
" 6mL+ " 200mg	2.1	79.7	81.8	2.9	3.0	0.33	1.30
L.S.D.at 5 %	0.5	5.0	5.0	0.2	NS	0.03	0.24

REFERENCES

- Abdel Samie, F. and M. EL – Bially (1996). Azolla and chemical as well as manual weed control methods in two rice varieties. *Ann. of Agric. Sci. Mashtohor*, 34 (1):125-138.
- Abd EL Wahed, M.S.A. (2001). Sitosterol stimulation of root growth, yield and some biochemical constituents of maize. *J. Agric. Sci. Mansoura Univ.*, 26(5) :2563- 2577.
- Abd EL Wahed , M.S.A.;A. Amin and Z.A. Ali (2000). Effect of different concentrations of stigmasterol on growth, yield and its components of Maize plants. *Agric. Sci. Mansura Univ.*, 25(1) :201- 215.
- Abd EL Wahed , M.S. Z.A. Ali; M.S. Abd El Hady and S.M. Rashed (2001). Physiological and anatomical changes on wheat cultivars as affected by Sitosterol. *J. Agric. Sci. Mansoura Univ.*, 26 (8). 4823 – 4839
- Adam, G.; V. Marquardt; H. Varbrodt; C. Horhold and G. Adam (Ed) (1991); Aspects of synthesis and bioactivity of Brassinosteroids. *Brassinosteroids Chemistry, bioactivity and application* pp , 74-85. ACS Symposium Series No 474; .
- A.O.A.C. (1975). " Official Methods of Analysis of the Association of Analytical Chemists :14th. Ed. Washington, D. C., U.S.A.
- Basudey, B.; M. Manoranjan; B. Behera and M. Mishra (1997). Effect of nitrogen fertilization and weed management on weed growth, nitrogen uptake and productivity of rice and bean. *Annals of Agree Res* , 18 (2):214- 217.
- Brar , L. and J. Kolar (1997). Chemical control of *Caesulia axillaris* in transplanted rice(*Oryza sativa*). *Indian J. of Agron.*, 42 (1): 82 -85.
- Cao, H. and S. Chen (1995). Brassinosteroid induced rice lamina joint inclination and its relation to indole -3- acetic acid and ethylene. *Plant Growth Reg.*, 16 (2): 189-196.
- Chon, S. and J. Guh (1995). Effect of seeding depth on the growth, mesocotyl elongation and herbicidal response of rice and barnyardgrass. *Korean J. of Weeds Sci.*, 15 (1): 19-29.

- Chun, J.; C. Lee and S. Ma (1995a). Herbicidal activity of thiocarbamate herbicides and its effect on call division and elongation. *Korean J. of weed Sci.*, 15 (1): 46-53.
- Chun, J.; S. Ma and S. Kim (1995b). Effect of mixed herbicides on phototoxicity of azimsulfuron in rice and barnyardgrass. *Korean J. of Weed Sci.*, 15(3): 232-237.
- Danial, H.,D. and C.M. George (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *J. Am Soc. Hort. Sci.*, 17:651-654
- Deka, S.; A. Gogoi and D. Pathok (1996). Effect of herbicides on chlorophyll content and nitrate reductase activity in relation to grain protein yield and grain yield of rice. *Annals of Agric. ,Bio Research .*, 1(1-2):107-111.
- Deliu , C.; M.Tamas ; C. Dobrota and D. C. Munteanu (1992). Kinetics of Cellular growth and diosgenin biosynthesis in *Dioscorea caucasica* cell suspensions cultured in batch system. *Plant Sci.*, 85(1): 99-105 .
- Dubois , M.; K. S. Gilles ; J. Hamibition ; R . Rebers and F. Smith (1956). Colorimetric methods for determination of sugar and related substances. *Anal. Chem.*, 28: 350.- 356.
- Fedtko, C.(1982). Biochemistry and physiology of herbicide action. Springer-Verlage Berlin Heidelberg New York . P. 142-147.
- Fernandes , F. M.; J. M. Novais and M. S. S. Pais (1992). Hormonal control of triterpenols synthesis in *Euphorbia characias cali*. *Boiresource Techn.*, 39(1) : 31-37 .
- Fletcher ,W.W. and R.C. Kirkwood (1992). Biochemical Mechanisms of Action . *Herbicides and Plant Growth Regulators* , GRANADA, London , Toronto, Sydney , New York ,187- 312 .
- Fujii , S.; K. Hirai; H. Saka ; H.Cutler; T. Yokata and G. Adam (1991). Growth regulating action of brassinolide in rice plants *Brassinosteroids, Chemistry, Bioactivity and applications*, 306- 311; Acs symposium DC; USA.
- Fujii , S. and H. Saka (2001). The promotive effect of brassinolide on Lamina joint cell elongation , germination and seedling growth under low temperature stress in rice. (*Oryza sativa L.*). *Plant Production Science*, 4(3):210-214 .
- Fujita, S.(1996) .Effect of soil application of several herbicides on the growth of transplanted rice seedlings at the nursery stage . *Weed Res.*, Japan . 41(1):44-59 .
- Glickmann; E. and Y. Dessaux (1995). A critical examination of the specificity of the *salkowski reagent* for indolic compounds produced by phytophthogenic Bacteria . *Applied and Environmental Microbiology*. P. 793 – 796 .
- Kim , H. Y.; K. U. Kim ; D. Shin and K. Kim (1996). Protein patterns of rice(*Oryza sativa L.*) cultivars as affected by herbicide thiobencarb. *Korean J. of weed Sci* , 16(4):354-361 .

- Jitender , K.; P. Jai ; J. Kumar and J. Prakash (1995). Influence of thiobencarb and butachlor on ribonuclease and deoxyribonuclease activity in seedlings of rice (*Oryza sativa*) and watergrass (*Echinochloa crus-galli*). Indian J. of Agric. Sci., 65(1):17- 19.
- Lambers, H. (1993). Rising CO₂ secondary plant metabolism, plant herbivore interactions and litter decomposition theoretical considerations . Vegetation, (104 , 105) : 263-271 .
- Maibangsa , S.; M. Thangaraj and M. Praba (2000). Physiologicl approaches for improving yield of rice under low irradiance. Madras Agric. J., 86(13):74-77 .
- Mandava , N. B. (1988). Plant growth promoting brassinosteroids . Annu. Rev Plant Mol., 39:23-52.
- Plummer , D.T. (1978). An inroduction to practical Biochemistry 2nd Ed.p.144. Mc Graw – Hill Boak company (UK) limited London. New York . ST louis. San Francisco . Auckland . Bogota Guatemala . Hamburg Johannesbug .Lisbon.Madrid .Mexico . Monterial. New Delhi . Panama. Paris. San Juan . Sao Paulo . Singapore. Sydney. Tokyo. Tarnto
- Saric, M; R. Kastrori ;R.Curici T. Cupina and I.Geric (1967). "Chlorophyll Determination"Unovon Saud Praktikumis Fiziologize Biliaka – Beograd , Haucna Anjiga ,pp.215.
- Snedecor, G.W. and W.G. Cochran (1980). Statistical methods. 6th Ed. Iowa, State Press, Ames Iowa , USA.
- Szekers, M. and C. konez (1998). Biochemical and genetic analysis of brassinosteroid metabolism and function in Arabidopsis .Plant Physiol. Biochem ., 36: 145 – 155.
- Vidya, V.B and R.R. Seeta (1998).Effect of brassinosteroids on nodulation and nitrogenase activity in ground nut. (*Arachis hypogaed L*) .Plant Regulation , 28:165- 167 .
- Wang, S. and R. Deng (1992). Effect of brassinosteroid on root metabolism in rice . J. of Southwest Agric . Univ, 14(2).177-181.
- Wang, S. and S. Wang (1997). Influence of brassinosteroid on rice seedling growth .International Rice Research Notes , 22(1) 20-21 .

تأثير مبيد الحشائش الثيوبينكارب والسيتوستيرول على نبات الأرز
محمد سلامة احمد عيد الواحد و إبراهيم رشدي الهجرسي الدسوقي
ورجب عيد المحسن المرجاوى
قسم النبات - المركز القومي للبحوث - القاهرة - مصر

أجريت هذه الدراسة بصوبة المركز القومي للبحوث في الموسمين ٢٠٠١ و ٢٠٠٢ بهدف دراسة تأثير مبيد الحشائش الثيوبينكارب و السيتوستيرول على نبات الأرز (صنف جيزة ١٧٧) وكانت المعاملات كالتالي:

- ١- معاملة الكنترول .
 - ٢- ثيوبينكارب بتركيز ٢ ملليتر / لتر .
 - ٣- سيتوستيرول بتركيز ١٠٠ ملجرام / لتر .
 - ٤- ثيوبينكارب بتركيز ٢ ملليتر / لتر + ١٠٠ ملجرام سيتوستيرول / لتر .
 - ٥- ثيوبينكارب بتركيز ٢ ملليتر / لتر + ٢٠٠ ملجرام سيتوستيرول / لتر .
 - ٦- ثيوبينكارب بتركيز ٤ ملليتر / لتر + ١٠٠ ملجرام سيتوستيرول / لتر .
 - ٧- ثيوبينكارب بتركيز ٤ ملليتر / لتر + ٢٠٠ ملجرام سيتوستيرول / لتر .
 - ٨- ثيوبينكارب بتركيز ٦ ملليتر / لتر + ١٠٠ ملجرام سيتوستيرول / لتر .
 - ٩- ثيوبينكارب بتركيز ٦ ملليتر / لتر + ٢٠٠ ملجرام سيتوستيرول / لتر .
- أضيف مبيد الحشائش الثيوبينكارب بتركيزات (٦،٤،٢ مل / لتر) بعد ٨ أيام من نقل الشتلات في الأرض المستديرة كما أضيف السيتوستيرول بتركيزات (٢٠٠،١٠٠ مجم / لتر) بعد ٧ أيام من إضافة وتم تنفيذ التجارب في تصميم القطاعات كاملة العشوائية في ثلاثة مكررات المبيد وكانت أهم النتائج كما يلي :-
- ١- أدت إضافة مبيد الحشائش ثيوبينكارب إلى انخفاض معنوي في كل من صفات نمو الجذر والوزن العنق والجفاف وطول الجذر و صفات المجموع الخضري (طول النبات ، الوزن الجاف للنبات ونسبة وزن الجذور إلى الوزن الخضري) في مرحلة الورقة الأنبوبية لنبات الأرز والوزن العنق والجفاف وعدد الاضطاء ونسبة وزن الجذور إلى الوزن الخضري من مرحلة النضج بينما كان تأثير المبيد غير معنوي على عدد الاضطاء في مرحلة الورقة الانبوييه ، وارتفاع النبات ونسبة وزن الجذور إلى الوزن الخضري في مرحلة النضج .
 - ٢- أدت إضافة السيتوستيرول على النباتات المعاملة بمبيد الحشائش ثيوبينكارب إلى زيادة كل من صفات نمو الجذر والنمو الخضري ومحصول الحبوب وتحسين المكونات الكيماوية لها مما أدى إلى انتظام نمو النبات .
 - ٣- أوضحت النتائج أن هناك تأثيرا معنويا لإضافة مبيد الثيوبينكارب بالتركيزات المختلفة مع السيتوستيرول بتركيز ٢٠٠،١٠٠ مجم / لتر مما كان له اثر كبير في تحسين صفات نمو الجذور والنمو الخضري وورقة العلم ومحصول الحبوب / نبات بمقارنته بمعاملة نبات الأرز بالمبيد منفردا .
 - ٤- كان التركيز ٢٠٠ مجم / لتر من السيتوستيرول أكثر فاعلية في مقاومة تأثير المبيد ويظهر ذلك في زيادة نمو الجذور والنمو الخضري وورقة العلم والمحصول للنبات عن التركيز ١٠٠ مجم / لتر من السيتوستيرول . كما أدى إلى انخفاض مكونات أجزاء النبات من الفينولات الكلية وزيادة الايتولات وزيادة المكونات الكيماوية مثل السكريات الكلية وانغير ذاتية والبروتين ، الأحماض الأمينية و الصبغات النباتية بورقة العلم و صفات النمو لورقة العلم في مراحل نمو النبات المختلفة .
- من النتائج السابقة يمكن الاستنتاج بأن معاملة نباتات الأرز بتركيز ٢٠٠ مجم / لتر من السيتوستيرول هي افضل المعاملات في مقاومة تأثير مبيد الحشائش الثيوبينكارب الضار لنباتات الأرز