J. Plant Prot. and Path., Mansoura Univ., Vol. 3 (6): 517 - 532, 2012

EFFECT OF BISPYRIBAC-SODIUM ON *Oryza sativa, Echinochloa crus-galli* AND *Echinochloa colonum* UNDER EGYPTIAN ECOSYSTEM

Zein, A. A.[1] ; M. A. Ashry[1] ; M. F. El-Nady[2] and SH. M. Abdel-dayem[1]

1Dept. of Pesticides and 2 Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh Univ., Egypt

ABSTRACT

The objectives of this investigation were to study the effect of herbicide bispyribac-sodium at rate of 16 g a.i/fed on *Oryza sativ* (L.) cv. Sakha 104, *Echinochloa crus-galli* (L.) and *Echinochloa colonum* (L.) grown under Egyptian ecosystem (Nile delta ecosystem). Morpho-physiological changes were take into account were growth reduction, chlorophyll pigment content reduction and reduction of some anatomical leaf parameters in *O. sativa*, *E. crus-galli* and *E. colonum* treated with tested herbicide. Moreover, increasing in membrane integrity (permeability) and leaf water deficit and reducing in leaf water contents were obtained in all tested plants treated with bispyribac-sodium. Ultrastructural changes in tested plants after bispyribac-sodium foliar application were noticed as cytotoxic features (programmed cell death). Cytotoxic features were take into account were mesophyll cell plasmolysis, irregular, granular nucleus, reduction in thylakoid membranes in treated plants compared with untreated plants. The obtained results will improve our understanding to tested herbicide mode of action, which may be overcome or reduce the side effects on non-target plant (*O. sativa*).

Keywords: Membrane integrity, chlorophyll, cell plasmolysis, cytotoxic features, programmed cell death, thylakoid membrane, leaf water deficit.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important cereal crops in the world, providing staple food for nearly one half of the global population. With rapid increase of global population, much greater rice production is demanded, (Bao- Rang, 2004). Rice crop is facing weeds which may decrease the yield and change its quality. The most common rice weeds i.e. *Echinochloa colonum* (L.) Link and *Echinochloa crus-galli* (L.) Beauv. The first is a sever competitor of rice and is one of the world"s worst weeds. It is also an alternate host of the fungus *Pyricularia oryzae* which causes rice blast, the yellow stem borer (*Scirpophaga incertulas*) and of the viruses that produce hoja blanca disease and tungro disease of rice (Naples and Kessler 2005). The second rice weed is *Echinochloa crus-galli* (L.) Beauv is a type of wild grass considered also as one of the worst rice weeds. Yield losses caused by *Echinochloa* spp infestations in rice can be very severe and variable as far as the cultivar and the duration of competition are concerned,(Fischer *et al*. 1997). Heavy infestations can interfere with mechanical harvesting. Individual plants can produce up to 40,000 seeds per year. Water, birds, insects, machinery, and animal feet disperse it, but contaminated seed

is probably the most common dispersal method. More than 35% of grain yield in seeded rice was reduced by infestation with *E. crus-galli* (Naples and Kessler, 2005). Without weed control, yield losses have been estimated to range from 16% to 86% or even 100% (Zoschke 1990; Baltazar and De Datta 1992; Kropff 1993 and Hassan, *et .al* 2002). Weed problems are more complicated and serious in dry seeded than in other rice production systems because of simultaneous germination of crop and weed seeds (Hassan, *et .al* 2002). Potential yield loss caused by uncontrolled weed in Egyptian rice ranged from 4.42 to 7.6 t/ha, with an average yield loss of 6.67 t/ha (75%) (Hassan and Rao 1993, 1994).

Bispyribac-sodium is a pyrimidinyl carboxy herbicide, is effective to control many annual and perennial grasses, sedges, and broad-leaved weeds in rice fields throughout the world. The mode of action of this herbicide has been considered as inhibition of acetolactate synthase (ALS-ase) in the biosynthetic pathway of three branched-chain amino acids (Shimizu 1997). Plant death results from events occurring in response to ALS inhibition and low branched-chain amino acid production, but the actual sequence of phytotoxic processes is unclear.

There are not any ultrastructural studies in the literature that have used this herbicide to point out the target effects on *Echinochloa crus-galli* and *Echinochloa colonum* and the side effects on *Oryza sativa*. Therefore, this study attempted to identify bispyribac-sodium mode of action in morpho-physiological, anatomical and cytological levels, may improve our understanding in this respect .The results obtained from this study may be a way to avoid or reduce the side effects on *Oryza sativa* as the non-target plant.

MATERIALS AND METHODS

Whole plant bioassay

The work done in this study was carried out in greenhouse during 2010 and 2011 rice growing seasons. Experiments were conducted in order to study the biological and cytological effects of bispyribac-sodium with the trade name"Nominee (SL) 2%" on rice plants cv. Sakha 104 (non-target plants) and two different target rice-weeds (*E. colonum* and *E. crus-galli*).

The soil used in this experiment was fertilized with nitrogen at rate a 360 kg/h of urea fertilizer (contain 46% nitrogen). Super phosphate fertilizer (phosphorus 15%) was added at rate of 240 kg/ha before planting.

Germinated seeds of rice plants and the two different rice weeds were planted in 30x30 cm plastic pots filled with the before mentioned soil. Emerged seedlings were thinned to four uniform and equally distant-spaced plants per pot. These experiments were conducted at average daily temperatures ranging from 22 to 31°C and at a 16-h day length. Pots were immersed with water up to 4 cm above the soil surface. The tested herbicide, bispyribac-soduim, was applied as a single application using a hand sprayer at the 4-leaf to 1-tiller stage of growth of the tested weed. After forty-eight hour from treatment, the plants were irrigated and water was raised up to 4

cm above the soil surface (Osuna *et al*. 2002). Experiments were done in a completely randomized design with six replications.

Morphological parameters

Samples were taken at 7, 14 and 21 days from treatment to estimate plant height (cm), fresh and dry weights of rice plants cv. Sakha 104 and the two tested rice weeds (dried in an electric oven at 70^{o}C for 72 h till constant weight) g/plant. Leaf area (cm^{2}/plant) as affected by the treatments was estimated as the average of area of leaves of randomly taken fivee rice plants using LI-3100 area meter.

Chlorophyll measurements

Chlorophyll content of rice plants and the tested two rice weeds was determined after 7, 14 and 21 days from treatment with the tested herbicide at rate of 16 g a.i/fed. Chlorophyll A, B and total were determined in lamina using the spectrophotometer method described by Moran and Porath (1980).

Data were subjected to statistical analysis of variance according to the method described by Gomez and Gomez (1984). Reduction % of chlorophyll content was calculated at the three sample dates.

Relative water content (RWC) and leaf water deficit (LWD):

Equal leaf discs (1cm) were cut from mature leaves, weighed to give the fresh weight (FW) floated on water for 6 hours until they reweighed (turgidity weight) and final oven dried at 70^{o} C for 72 hours to reach a constant weight. Relative water content and leaf water deficit (LWD) were calculated using the following formula as reported by Kalapos (1994):

$$RWC\ \% = \frac{(\text{Turgid weight} - \text{Fresh weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

$$LWD\ \% = 100 - RWC$$

Membrane integrity (permeability):

The absorption of the leakage of solutes across the cell membrane of tissues was determined at the ultraviolet wavelength 273 nm following the method of Leopold *et al* ., (1981).

Light microscopic test:

The leaf specimens including the midrib were taken from the second leaf on the plant tip. Specimens were taken on the 10th day after treatment. Specimens were fixed in 0.5% buffered glutaraldehyde in 0.1 M PBS, pH 7.4 at 40C for 2h. Specimens were washed three times with PBS (10 min. each) and post fixation in 1% Osmic acid for (30min), then dehydrated with ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol) each concentration for 30 min. infiltrated with acetone for 1 hour. In transmutation electron microscope (TEM), after dehydration samples were embedded in Araldite 502 resin. The resulted plastic molds were cut in the LEICA Ultracut UCT ultra-microtome, stained with 1% toleudine blue. Ten reading from 3 slides were examined with electric microscope (Lieca DM LS) with digital camera (Lieca DC 300), and then photographed. The histological manifestation was calculated using Lieca IM 1000 image manager software.

Lieca software was calibrated using 1 cm stage micrometer scaled at 100 µm increment (Leitz Wetzler, Germany 604364) at 4 and 10 X magnifications.

Electron microscopic test

The leaf specimens were taken from the second leaf from the plant tip. Specimens were taken on day 10th of treating. Specimens were fixed in 0.5% buffered glutaraldehyde in 0.1 M PBS, pH 7.4 at 40C for 2h. Specimens were washed three times with PBS (10 min. each) and post fixation in 1% Osmic acid for (30min). Washing three times with PBS (10 min. each), then dehydrated with ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol) each concentration for 30 min. infiltrated with acetone for 1 hour. In transmutation electron microscope (TEM), after dehydration samples were embedded in Araldite 502 resin (Spurr, 1969). The plastic molds were cut in the LEICA Ultracut UCT ultra-microtome, stained with 1% toleudine blue. After examination of semi-thin sections ultra-thin sections were cut, stained with uranyl acetate, then counter stained with lead citrate (Venable and Coggeshall, 1965) and examined and photographed using JEOL-JEM-100 SX electron microscope, Japan, electron microscope unit- Tanta university.

Statistical analysis

Data from the experiments were statistically analyzed using one-way repeated measurement analysis of variance. Duncan's multiple range tests was used to separate means using SAS software Version 6.12 (SAS Institute Inc., and Cary, USA).

RESULTS AND DISCUSSION

Results obtained in 2010 showed almost the same trend as those of 2011 season, so data of the first season for growth and physiological parameters and the second season to anatomical and cytological parameters were found enough to be presented. Without any exception foliar application with bispyribac-sodium at 16 g a. i/fed. have an undesirable effect on all tested plants. In general this effect of the used herbicides differs considerably in injury degrees, depending upon kind of plant.

Effect of tested herbicide on some growth parameters:

Data in Tables (1 and 2) showed that, the response of *O. sativa* and tested rice weeds (*E. crus-galli* and *E. colonum*) as a reduction in plant height (cm) and leaf area (cm2) of the treated plants relative to the control after 7, 14 and 21 days of treatment. Bispyribac-sodium foliar application had insignificant reducing effects on both plant height and leaf area in all tested plants. Foliar application of bispyribac-sodium at 16 g a. i/fed. induced a significantly reduction % in plant height and leaf area in *E. crus-galli* and *E. colonum.* Insignificantly reduction % in plant height and leaf area of *O. sativa* was recorded after foliar application with bispyribac-sodium. The obtained results indicated that, *E. crus-galli* was more sensitive to foliar application of bispyribac-sodium at 16 g a. i/fed. othan others, which achieved the highest plant height and leaf area reduction percentages. The reduction in plant height and leaf area may be attributed to the decrease in net photosynthetic

rates (photoinhibition) in plants due to the reduction in photosynthetic pigments (chlorophyll and carotenoid pigments). Moreover, this effect may be attributed to the results of massive and irreversible expansion of small daughter cells produced by meristematic divisions and growth inhibition is therefore related to the inhibition of cell expansions as well as reduced rates of new cell production may make additional contribution to the inhibition of growth. Moreover, this effect may be attributed to losses in tissue water content, which reduce turgor pressure in the cell, thereby inhibiting enlargement and division of cells causing a reduction in plant growth. These results related to the microscopic anatomical, cytological, relative water content, water deficit and membrane integrity under this study.

Table (1): Plant height (cm) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2010) at 7, 14 and 21 days after treatment (DAT).

Treatments	Plant height (cm)			Mean	Reduction %
	7 DAT	14 DAT	21 DAT		
***O. sativa* (control)**	29.45	30.10c	31.86c	30.47	0.00
***O. sativa* with +BS**	26.64	28.64c	30.92cd	28.33	5.70
***E. crus-galli* (control)**	31.80	51.53a	66.19a	49.84	0.00
***E. crus-galli* + BS**	27.83	12.53de	0.00e	13.45	73.01
***E. colonum* (control)**	30.16	39.52b	44.10b	36.65	0.00
***E. colonum* + BS**	26.33	12.03e	0.00e	12.79	65.11
LSD 5%	--	5.63	4.66		

Different letters in each column indicate significances by Duncan (1955) tests at *P* < 0.05.

Table (2): Leaf area (cm2) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2010) at 7, 14 and 21 days after treatment (DAT).

Treatments	Leaf area (cm2)			Mean	Reduction %
	7 DAT	14 DAT	21 DAT		
***O. sativa* (control)**	10.27	38.74c	67.31c	38.8	0.00
***O. sativa* with +BS**	9.85	37.19c	66.37c	37.8	2.58
***E. crus-galli* (control)**	21.42	95.19a	160.41a	92.3	0.00
***E. crus-galli* + BS**	15.42	25.00d	0.00d	13.5	85.37
***E. colonum* (control)**	13.6	66.04b	98.60b	59.4	0.00
***E. colonum* + BS**	8.45	17.97de	0.00d	8.8	85.17
LSD 5%	--	9.33	12.80		

Different letters in each column indicate significances by Duncan (1955) tests at *P* < 0.05.

Concerning, fresh and dry weigh (g/plant)t parameters as affected by foliar application of bispyribac-sodium at rate of16 g a.i. /fed. data in Tables (3 and 4) showed that insignificant differences were obtained in these parameters after 7 days after application. The highest reduction % in fresh and dry weight were in *E. colonum* treated with bispyribac-sodium at rate of 16 g a. i/fed. The lowest fresh and dry weight reduction % was in treated rice plant. Treated all tested plants with bispyribac-sodium showed the same trend as those of plant height and leaf area parameters. The differences in

shoot fresh and dry weight of tested plants may be attributed to the different varietals tolerance to the applied herbicidal treatments as reported by Zhang and Webster, (2002). Reduction in fresh weight/ plant attributed to losses in plant water content. For dry weight, the reduction in dry weight per plant caused by herbicide application related with the reduction in photosynthetic pigments (chlorophylls), which led to reduce in photosynthesis rate.

Table (3): Plant fresh weight (g/plant) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2010) at 7, 14 and 21 days after treatment (DAT).

Treatments	Fresh weight (g/plant)			Mean	Reduction %
	7 DAT	14 DAT	21 DAT		
***O. sativa* (control)**	2.25	3.29c	4.46b	3.33	0.00
***O. sativa* with +BS**	1.96	3.15c	4.13b	3.08	7.51
***E. crus-galli* (control)**	3.51	5.52a	7.11a	5.38	0.00
***E. crus-galli* + BS**	2.77	2.16de	0.00c	1.64	69.45
***E. colonum* (control)**	2.79	4.63b	6.79a	4.71	0.00
***E. colonum* + BS**	1.83	1.99de	0.00c	1.27	72.68
LSD 5%	--	0.74	0.94		

Different letters in each column indicate significances by Duncan (1955) tests at *P* < 0.05.

Table (4): Plant dry weight (g/plant) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2010) at 7, 14 and 21 days after treatment (DAT).

Treatments	Dry weight (g/plant)			Mean	Reduction %
	7 DAT	14 DAT	21 DAT		
***O. sativa* (control)**	0.46cd	0.69c	1.44c	0.86	0.00
***O. sativa* with +BS**	0.41cd	0.63cd	1.39c	0.81	5.81
***E. crus-galli* (control)**	0.68a	1.84a	3.05a	1.86	0.00
***E. crus-galli* + BS**	0.46cd	0.55ef	0.00f	0.34	81.9
***E. colonum* (control)**	0.57ab	1.48b	2.20b	1.42	0.00
***E. colonum* + BS**	0.30e	0.39fg	0.00f	0.23	83.8
LSD 5%	0.11	0.16	0.11		

Different letters in each column indicate significances by Duncan (1955) tests at *P* < 0.05.

Effect of tested herbicide on chlorophyll pigment contents:

Chlorophyll content has been known as a typical parameter for evaluating the physiological conditions. Data in Fig. (1) indicated that, chlorophyll (chl.) pigment contents (chl. A, chl. B and total chl.) were reduced in all tested plants after bispyribac-sodium (16 g a. i/fed.) foliar application. Data in Fig. (1) showed that *E. crus-galli* and *E. colonum* recorded the highest values of chlorophyll content, while *O. sativa* (cv. Sakha 104) had the lowest values in the three sample dates. Concerning chlorophyll content as influenced by herbicide foliar application, the results revealed that, *E. crus-galli* treated with bispyribac- sodium at 16 g a.i /fed. achieved the highest chlorophyll contents reduction percentages (Fig.,2). This action was due to the chlorosis which resulted due to the loss of green pigmentation from the foliage due to the loss of chlorophyll; associated with ALS inhibitors,

photosynthesis inhibitors, and 5-enolpyruvyl shikimate-3-phosphate (EPSP) syntheses inhibitors.

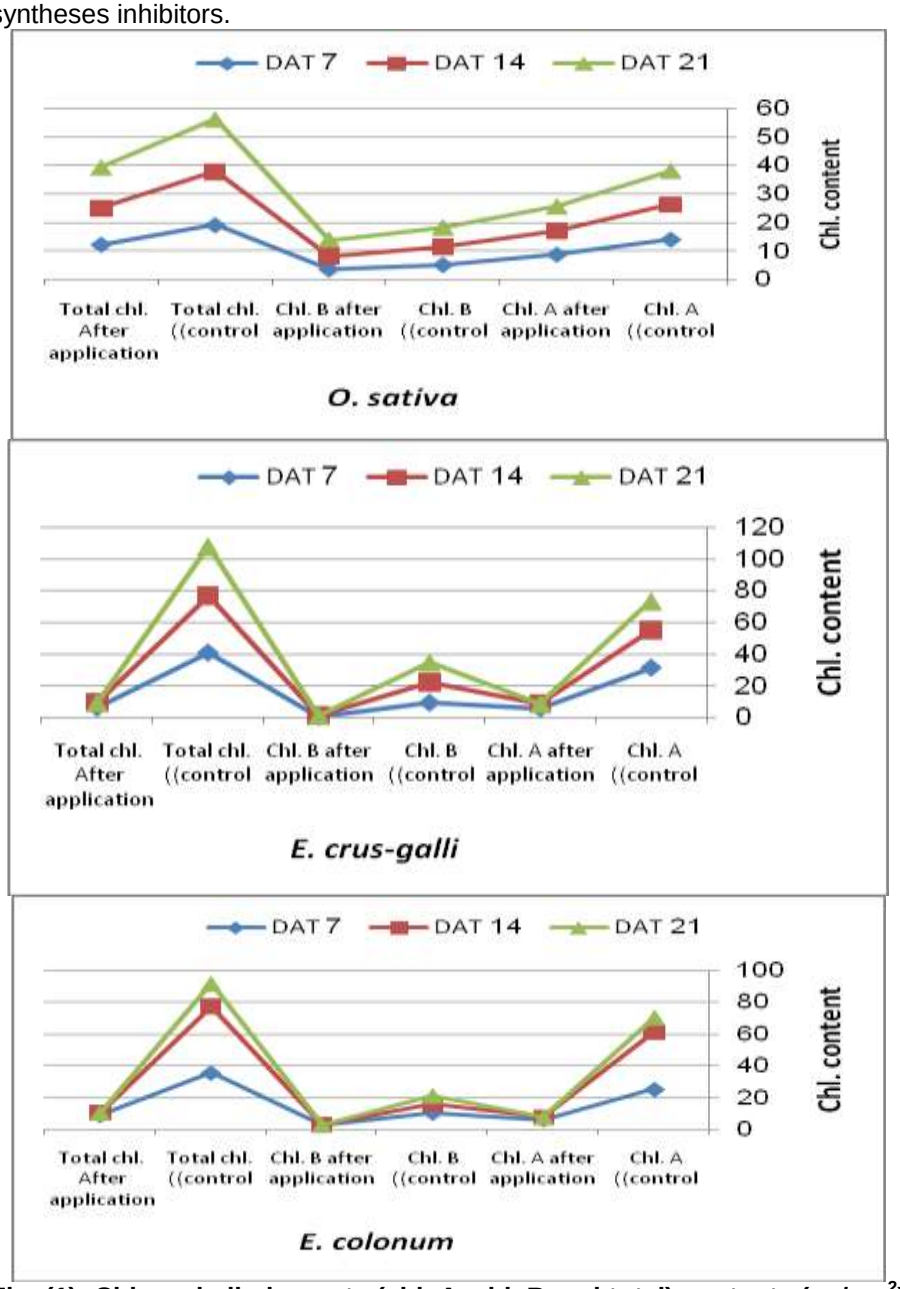

Fig. (1): Chlorophyll pigments (chl. A, chl. B and total) contents (µg/cm^2) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2010) at 7, 14 and 21 days after treatment (DAT).

The reduction in chlorophyll content of tested plants after foliar application of bispyribac-sodium is in agreement with the findings of Lycan and Hart (2005), who reported that application of bispyribacs-oduim as AIS-inhibitor for controlling different weeds leads to injury symptoms in the form of chlorosis (reduction in the chlorophyll content). Chlorophyll's reduction mechanism may be due to the enhanced activity of the chlorophyll degrading enzyme chlorophyllase and/or disruption of the fine structure of chloroplast, and instability of chloroplast or pigment-protein complex, which leads to oxidation and a decreased concentration of chlorophyll. Intermolecular weak electron-transfer interactions and intermolecular dispersive interactions mainly determine the toxicity of these ALS inhibitors mentioned by Ding *et al.* (2009).

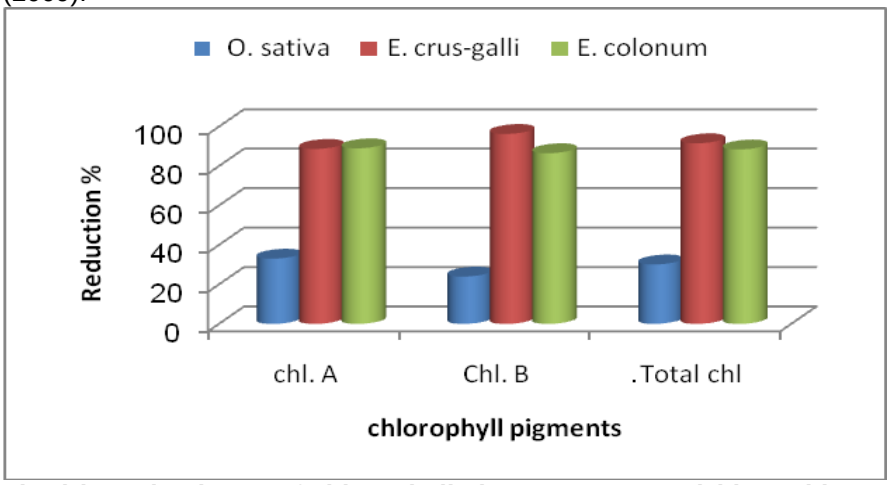

Fig. (2): Reduction % of chlorophyll pigment contents (chl. A, chl. B and total chl.) as affected by foliar application with bispyribac-sodium at rate of 16 g a. i/fed. *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during 2010 season.

Effect of tested herbicide on membrane integrity (permeability), relative water content (RWC) and leaf water deficit (LWD):

Cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Data in Fig. (3) showed that, bispyribac-sodium (16 g a. i/fed.) increased cell membrane integrity in all tested plants. The highest cell membrane integrity was recorded in *E. crus-galli* followed by *E. colonum*. Increasing in cell membrane integrity attributed to losses in plant fresh weight and relative water content (RWC).

For relative water content (RWC)% and leaf water deficit (LWD) % obtained results showed that, bispyribac-sodium at rate of 16 g a. i/fed foliar application of all tested plants led to reduce in RWC% and increase in LWD% relative to untreated plants. The lowest RWC% was in *E. colonum* treated with bispyribac-sodium. On the other hand the highest LWD % was treated *E. crus-galli* than other treatments (Fig., 4). Increasing in cell membrane integrity, water content and leaf water deficit led to cell plasmolysis.

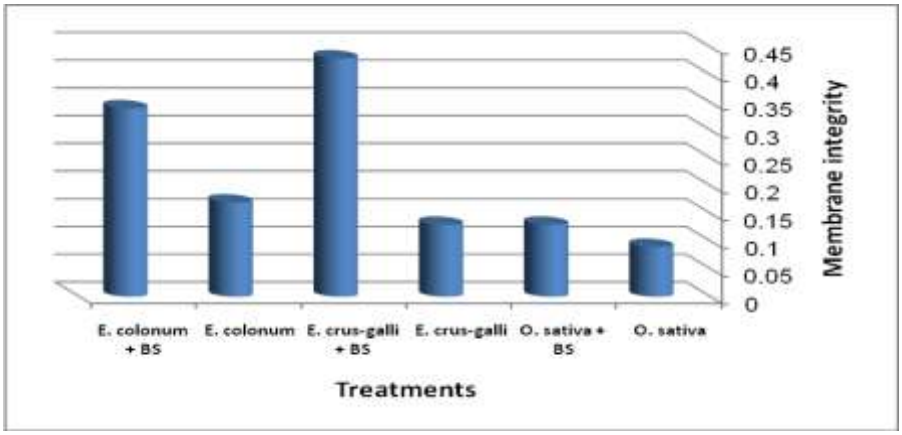

Fig. (3): Membrane integrity (selective permeability) as influenced by bispyribac-sodium at rate of16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2011) at 7, 14 and 21 days after treatment (DAT).

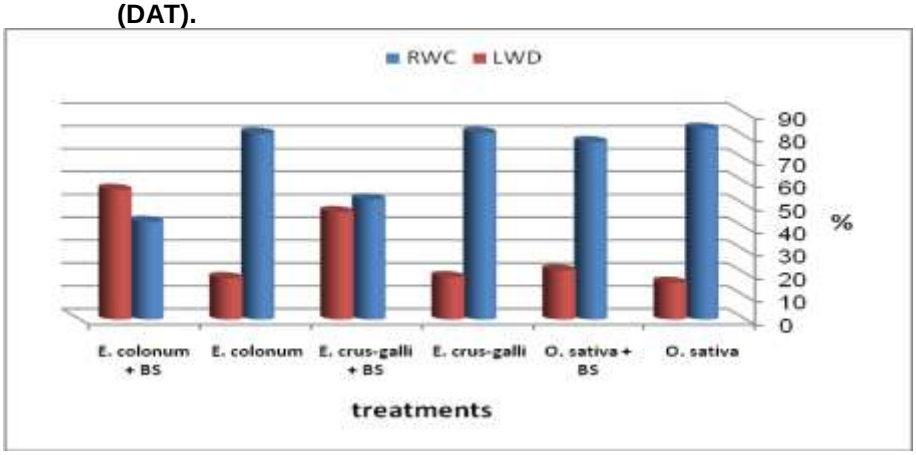

Fig. (4): Relative water content (RWC) % and leaf water deficit (LWD) % as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2011) at 7, 14 and 21 days after treatment (DAT).

Effect of tested herbicide on some leaf anatomical parameters:

The anatomical differences of tested plants treated with bispyribac-sodium at rate of16 g a.i/fed, with respect to lamina thickness (µm) , xylem vessels diameter (µm) and vascular bundle thickness (µm) were measured compared with untreated plants. *O. sativa* induced the highest leaf anatomical parameters compared with *E. crus-galli* and *E. colonum*. Bispyribac-sodium foliar application of tested plant resulted in reducing diameter of xylem vessels thickness of leaf lamina and midrib vascular bundle. The highest reduction values in leaf anatomical parameters were

achieved by treated *E. colonum* followed by *E. crus-galli*. The decrease in leaf lamina thickness may be attributed to inhibition of cell division and/or cell enlargement which subsequently may be due to the disruption in plasma membrane that mainly consists of protein and phospholipids. Therefore, any reduction in amino acids biosynthesis due to treated with bispyribac-sodium at rate of16 g a.i/fed application will affect the membrane formation and subsequently its functions. Therefore, the possible mechanism of this reduction in lamina thickness due to the inhibition of amino acids synthesis by bispyribac-sodium and penoxsulam that known as amino acids synthesis inhibitor which subsequently disrupt the plasma membrane formation subsequently its functions(Tranel and Wright 2002; Zhou *et al.* 2007). On the other hand, the reduce in diameter of xylem vessels and thickness of midrib vascular bundles lead to inhibition of transport of water, essential nutritional elements and photosynthesis production. Inhibitors of ALS can reduce transport of photosynthetic from source leaves to roots, resulting in root growth inhibition (Devine 1989; Devine *et al*. 1990; Shaner 1991).

Table (5): Some leaf anatomical parameters eg. leaf lamina, vascular bundle and xylem vessel diameters (µm) as influenced by bispyribac-sodium at rate of 16 g a. i/fed. foliar application on *O. sativa* (cv. Sakha 104), *E. crus-galli* and *E. colonum* during the first season (2011) at 7, 14 and 21 days after treatment (DAT).

Treatments	Leaf lamina thick. (µm)	Vascular bundle diameter (µm)	Xylem vessel diameter (µm)
O. sativa (control)	122.50a	55.00a	14.17a
O. sativa with +BS	71.67cd	36.67bc	6.67de
E. crus-galli (control)	119.17a	61.67a	14.17a
E. crus-galli + BS	77.50c	38.33bc	10.83b
E. colonum (control)	99.17b	40.83b	10.00bc
E. colonum + BS	47.50f	30.83c	6.67de

Different letters in each column indicate significances by Duncan (1955) tests at *P* < 0.05.

Effect of tested herbicide on cytological characters:

Data illustrated in Fig. 5 show the cytological difference in rice leaf treated with bispyribac-sodium at rate of 16 g a.i/fed as compared with untreated plants. Mesophyll cell plasmolysis (see arrows), irregular and granular nucleus was noticed in rice plants treated with bispyribac-sodium at rate of 16 g a.i/fed. No clear differences were found between chloroplasts in the treated and untreated rice plants. Mitochondria in the cells of treated rice plants appear smaller compared with untreated plants. Also, vesicles were found in cytoplasm of the cells which treated with bispyribac-sodium at rate of 16 g a.i/fed. Low number of plastid starch granules were detected in the cells treated with bispyribac-sodium at rate of 16 g a.i/fed in compared with control plants. Cell plasmolysis is the process in plant cells where the cytoplasm pulls away from the cell wall leaving gaps between pressures. Turgor pressure helps in maintaining the shape and form of the plant and holds the leaves in a flat by keeping the mesophyll cells turgid. The space between the

cell wall and the protoplast is occupied by the bathing solution as of the cell wall is permeable. Cell plasmolysis is a kind of defense mechanism against adverse (stress) conditions such as reduction in water absorption by root system and/or increasing in transpiration rate. The reduction in plastid starch granules number may be due to the reduction in photosynthesis rate. Small mitochondria in mesophyll cells of rice leaf treated with tested herbicide led to the reduction in aerobic metabolic functions. Also, vacuole fragmentation was found in mesophyll cells in rice leaves after foliar application with bispyribac-sodium at rate of 16 g a.i/fed. The cytological differences in rice plant treated with bispyribac-sodium occur as a result of the temporary sensitivity against the herbicide.

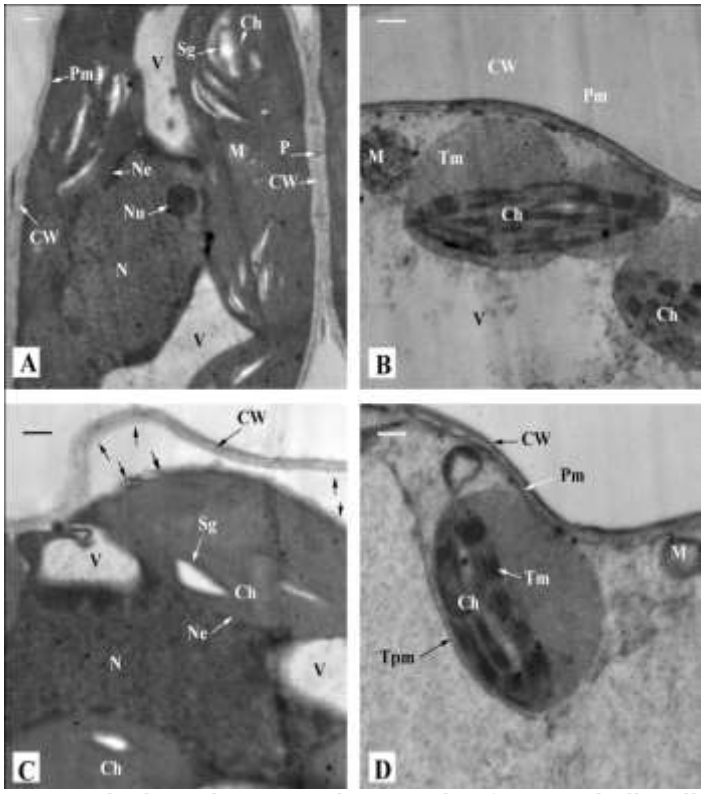

Fig. (5): Transmission electron micrograph of mesophyll cells of rice leaves showing cytological differences as effect of bispyribac-sodium at 16 g a.i/fed application, A and B: rice control C and D: rice with bispyribac-sodium at rate of 16 g a.i/fed., CW: cell wall, Ch: chloroplast, M: mitochondria, N: nucleus, Nu: nucleolus, Ne: nuclear envelope, V: vacuole, Pm: plasma membrane, Tm: thylakoid membrane, Sg: Starch grain, Tpm: Tonoplasm membrane, (Bars= 500 nm).

Data in Fig. 6 show that, the cytological responses of *E. crus-galli* plants to bispyribac-sodium at rate of 16 g a.i/fed foliar application. Fragmented vacuoles (small vacuoles), granular and irregular nucleus, thicken cell wall, as well as degenerated protoplasm were recorded as compared with the control. These cytological differences related to cytotoxic symptoms and programmed cell death (PCD) as responses of *E. crus-galli* to bispyribac-sodium at rate of 16 g a.i/fed application. Reduction in thylakoid membranes density was found in treated plants as compared with the untreated plants. The reduction in thylakoid membrane density linked to the reduction in chlorophyll contents which led to decrease in photosynthesis rate specially electron transport chain and photophosphorylation.

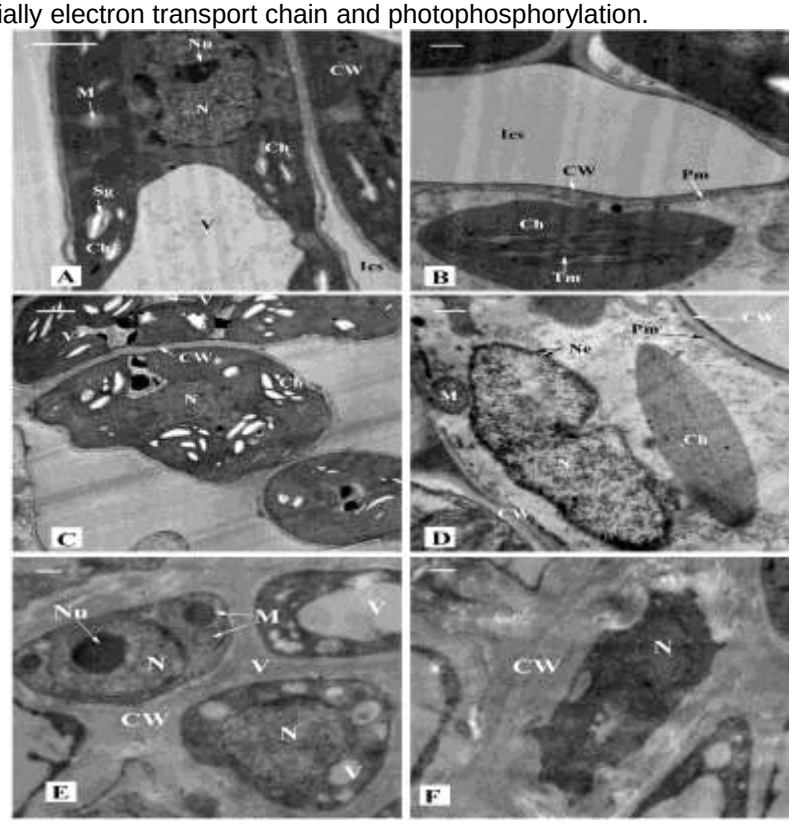

Fig. (6): Transmission electron micrograph of mesophyll cells of *Echinochloa crus-galli* as a results to bispyribac-sodium at 16 g a.i/fed application. A and B: *E. crus-galli* control C - F: *E. crus-gal*li with bispyribac-sodium at rate of 16 g a.i/fed., CW: cell wall, Ch: chloroplast, M: mitochondria, N: nucleus, Nu: nucleolus, Nv: nuclear envelope, V: vacuole, Pm: plasma membrane, Tm: thylakoid membrane, Sg: Starch grain, Tpm: Tonoplasm membrane, (Bars= A and C= 2 2µm, B, D, E and F=500 nm)

Concerning the cellular responses of *E. colonum* plants to bispyribac-sodium at rate of 16 g a.i/fed application, data in Fig. 7 showed that, mesophyll cells plasmolysis and vesicles were found between cell wall and plasma membrane (plasmalemasomes) and in vacuoles in compared with the untreated plants. In addition, separated cell walls in adjacent cells and it seem stained to be loosely (see arrows). Thylakoid membrane degenerated and intensive bodies (plastoglubules) were noticed in stroma (Fig., 7). Reduction in thylakoid membranes linked to chlorophyll contents and photosynthesis process.

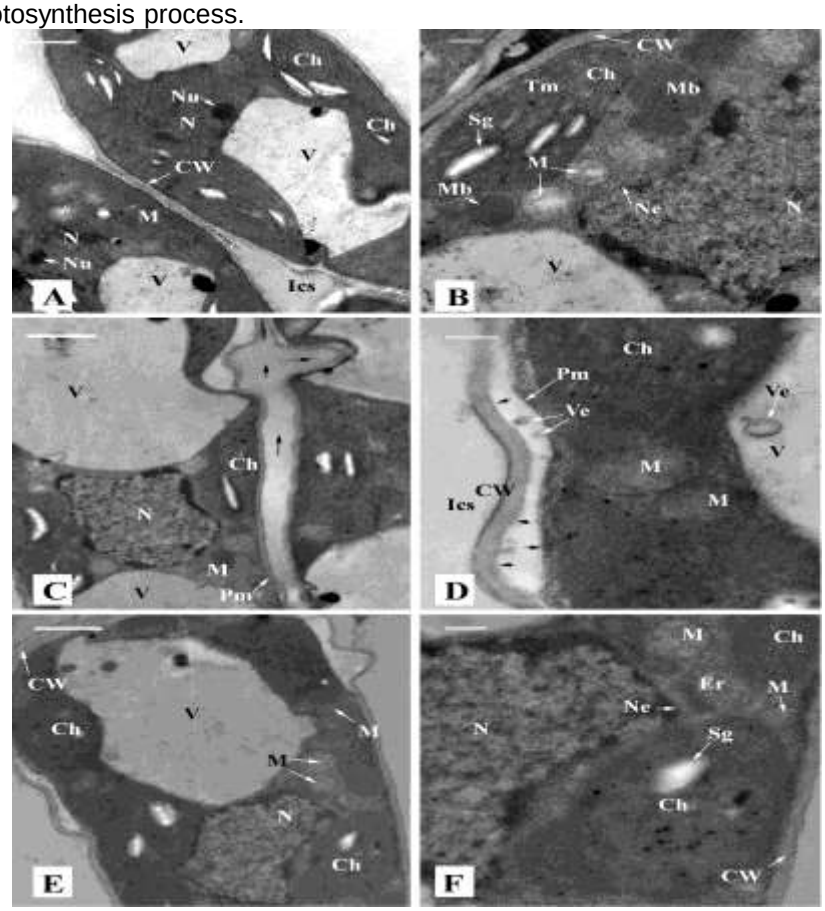

Fig. (7); Transmission electron micrograph of mesophyll cells of *Echinochloa colonum* as a responses to bispyribac-sodium at rate of 16 g a.i/fed. A and B: *E. colonum* (control), C - F: *E. colonum* with bispyribac-sodium at rate of 16 g a.i/fed., CW: cell wall, Ch: chloroplast, M: mitochondria, N: nucleus, Nu: nucleolus, Ne: nuclear envelope, V: vacuole, Pm: plasma membrane, Tm: thylakoid membrane, Sg: Starch grain, Ve: vesicles, Mb: microbodies, Er: endoplasmic reticulum, Ics: inter cellular spaces.(Bars: A, C and E =2 µm, B, D and F= 500 nm).

The mode of action has been considered as inhibition of acetolactate synthase (ALS-ase) in the biosynthetic pathway of three branched-chain amino acids (Shimizu 1997). In fact, it inhibit photosynthesis at pigment–protein complexes embedded within the thylakoid membrane system of chloroplasts essential to the capture of light energy. Lycan and Hart (2005) reported that application of bispyribac-soduim as AIS-inhibitor for controlling different weeds leads to injury symptoms in the form of chlorosis (reduction in the chlorophyll content). Chlorophyll's reduction mechanism may be due to the enhanced activity of the chlorophyll degrading enzyme chlorophyllase and/or disruption of the fine structure of chloroplast, and instability of chloroplast or pigment-protein complex, which leads to oxidation and a decreased concentration of chlorophyll. Bispyribac-sodium known as acetolactate synthase (ALS) inhibitor, is involved in biosynthesis of the branched – chain amino acids (Tranel and Wright 2002; Zhou *et al.* 2007). Therefore, the application of bispyribac-sodium against *E.colonum* should reduce biosynthesis of amino acids which subsequently inhibits protein synthesis, and growth, and finally causes cell and plant death (WSSA 2007). Also, the formation of protein, vital for cell division and membrane synthesis especially thylakoid membranes may be disrupted and growth retardation may be induced as a result of cell division inhibition and photosynthesis rate reduction. This in turn inhibits the production of the aromatic amino acid end products tryptophan, phenylalanine, and tyrosine. Without these essential amino acids, certain proteins cannot be produced and the plant dies (Zein, *et al*. 2010) reported that, leaf protein analysis showed significant differences between the susceptible and resistant biotypes of E. colonum in the number and the density of protein bands.

Conclusion:

Growth and physiological parameters of treated *Oryza sativa* cv. Sakha 104, *Echinochloa corus-galli* and *Echinochloa colonum* with bispyribac-sodium herbicide at rate of 16 g a.i/fed. were reduced relative to untreated plants. Moreover, cytotoxic features were take into account were mesophyll cell plasmolysis, irregular and granular nucleus and reduction thylakoid membrane and others in treated plants with tested herbicide, which will improve our understanding for the mode of action of bispyribac-sodium.

REFERENCES

Baltazar, A. M. and De Datta, S. K. (1992). Weed management in rice. Weed Abstracts, 41 (11), pp. 495- 497. (C.F. Computer research).

Bao-Rang, Lu .(2004).Gene flow form cultivated rice: Ecological Consequences. ISB. News Report, May. Fundan University. Shanghai, pp. 1-3. (C.F. Computer research).

Devine, M. D. (1989). Phloem translocation of herbicides. Rev. Weed Sci. 4:191-225.

Devine, M.D. Bestman, H.D. and Vanden Born, W.H.(1990). Physiological basis for the different phloem mobilities of chlorsulfuron and clopyralid. Weed Sci. 38:1-9.

Ding, G. Li, X. Zhang, F. Chen, J. Huang, L. and Qiao, X. (2009). Mechanism-based quantitative structure-activity relationships on toxicity of selected herbicides to Chlorella vulgaris and Raphidocelis subcapitata. Journal of Plant Production Research. 52(1). (2012).

Fischer, A., H. V. Ramirez and J. Lozano (1997). Suppression of junglerice [*Echinochloa colona* (L.) Link] by irrigated rice cultivars in Latin America. Agron. J. 521 (3): 516–521.

Gomez K.A., Gomez A.A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. Wiley, 680 pp.

Hassan, S. M. and Rao, A. N. (1993). Integrated weed management for sustainable rice. Production in Egypt. Int. Proc. Integrated weed management for sustainable Agriculture. Indian society of weed Science, Hisor. 18- 20 November. Vol. 1: 356- 363.

Hassan, S. M. and Rao, A. N. (1994). Weed impact analysis and management for sustainable Proceedings of the 5the EWRS Mediterranean Symposium on weed control in susbainable Agricultural in the Mediterranean Area. Perugia, Italy.

Hassan, S. M; Shebl, S. M and Abou El-Darag .(2002). Weed management in Rice. Annual Repot for 2001. In proceedings of the 6th National Rice Research and Development program workshop. Rice Research and training Center, Sakha, Kafr El-Shaikh. Egypt.

J. Pestic. Sci. 22: p. 254.

Kalapos, T. (1994).leaf water potential, leaf water deficit relationship for ten species of semiarid grassland community. Plant and Soil., 160:105-112

Kropff, M. J. (1993). Eco-physiological models for crop-weed competition. In Modeling crop-weed interaction, eds M.J.kropff and van Laar H. HX: X, pp. 25- 32 (C.F. Computer Research).

Leopold , A. G. Musgrave, M.E. and Williams ,K.M.(1981).leakage , resulting from leaf desiccation . Plant Physiol.,68:1222-1225

Lycan, D.W and Hart, S.E. (2005). Cool-season turfgrass response to bispyibac- sodium.Hortic. Sci. 40 (5): 1552–1555.

Moran, R. and Porath, D. (1980). Chlorophyll determination in intacttissues using N, N-Dimethyl formamide. Plant Physiol. 69: 1370-1381.

Naples, M.L. and P. J. A. Kessler(2005). Weeds of Rain Fed Lowland Rice Fields of Laos and Cambodia. MSc thesis, University of Leiden, Cambodia, 55 pp.

Osuna, M.D., F. Vidotto, A. J. Fischer, D. E. Bayer, R. DE Prado and A. Ferrero (2002). Cross-resistance to bispyribac-sodium and bensulfuron-methyl in Echinochloa phyllopogon and Cyperus difformis. Pestic. Biochem. Physiol. 73 (1): 9–17.

Shaner, D.L. (1991). Physiological effects of the imidazolinone herbicides. p. 129-138, In D.L. Shaner and S.L. O'Conner (Eds.). The Imidazolinone Herbicides. Boca Raton, Florida: CRC Press.

Shimizu, T. (1997). Action mechanism of pyrimidinyl carboxy herbicides. J. Pestic. Sci. 22: p. 254.

Spurr, A.R. (1969). Alow-vescosity resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31-43.

Tranel, P. J. and Wright ,T.R. (2002). Resistance of weeds to ALS-inhibiting herbicides: what have we learned. Weed Sci. 50: 700–712.

Venable, J. H. and R. Coggeshall (1965). A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 25, 407.

WSSA (Weed Science Society of America). (2007). Herbicide Handbook (W.K. Vencill, ed.). 9th ed. Lawrence, KS. 493 pp.

Zein, A. A. ABD-EL-Baky M. A. Hassan, S M. Derbalah1, A, S. and Hamza, A. M. (2010). Evolution and Mechanism of Rice Weeds Resistance to HerbicidesJ. Agric. Res. Kafer El-Sheikh Univ., 36(4)

Zhang, W and Webster, E. P. (2002). Shoot and root growth of rice (*Oryza sativa*) in response to V-10029. Weed Technology., 16 (4): 768- 772.

Zhou, G.Y; Liu, W.P; Zhang, Y.S and Liu, K.K.(2007). Action mechanisms of acetolactate synthase-inhibiting herbicides. Pestic. Bichem. Physiol. 89 (2): 89-96.

Zoschke, A. (1990). Yield loss in tropical rice as influenced by the competition of weed flora and the timing of its elimination. Pest management in rice,- Elsevier Science: London, pp. 301- 313.

تاثير مبيد الحشائش "بسبيريباك – صوديوم" علي نباتات الأرز وحشيشتي الدنيبة وأبو ركبة النامية تحت ظروف النظام البيئي المصري

أمين عبدالباقى زين[1] ،محمد على عشرى[1] ، محمد فتحي النادي[2] و شريف محمد عبدالدايم[1]

[1]قسم المبيدات، [2] قسم النبات الزراعي – كلية الزراعة جامعة كفر الشيخ- مصر

يهدف هذا البحث إلي دراسة تأثير مبيد البسبيريباك الصوديوم Bispyribac-Sodium بتركيز 16 جرام / فدان علي نبات الأرز صنف (L.) *Oryza sativa* سخا 104 وحشيشتي الدنيبة وأبو ركبة (L.) *Echinochloa crus-galli* و (L.) *Echinochloa colonum* النامية تحت ظروف النظام البيئي المصري (دلتا النيل) وتمثلت التغيرات المورفولوجية والفسيولوجية للنباتات المعاملة في خفض محتواها من صبغات الكلوروفيل وكذلك خفض بعض مقاييس النمو وكذا بعض القياسات التشريحية مقارنة بالنباتات الغير معاملة. كما حدث زيادة في نفاذية الأغشية الخلوية وكذلك زيادة في نقص محتوي الأوارق المائي كما حدث نقص في محتوي الاوراق النسبي من الماء. أيضا حدثت تغيرات سيتولوجية بالنباتات المعاملة وتمثلت في وجود تغيرات ناتجة للتسمم السيتولوجي فيما يعرف بالموت المبرمج للخلية وهي عبارة عن حدوث بلزمة لخلايا النسيج الوسطي بالأوراق (الميزوفيل) ووجود أنوية غير منتظمة الشكل وأكثر تحببا, كما حدث إختزال في أغشية الثيالاكويد بالكلوروبلاستيدات وغيرها من التغيرات مقارنة بالنباتات الغير معاملة. ومن المتوقع أن النتائج المتحصل عليها ستحسن وتعمق من فهمنا لميكانيكية عمل المبيد تحت الدراسة علي النباتات المختبرة والتي قد تمكننا من الحد أو التغلب علي التغيرات أو الآثار الجانبية علي نباتات الأرز.

قام بتحكيم البحث

أ.د / على على عبد الهادى — كلية الزراعة – جامعة المنصورة

أ.د / عطيه يوسف قريطم — كلية الزراعة – جامعة كفر الشيخ