Evolution and Mechanism of Rice Weeds Resistance to Herbicides I- Resistance of *Echinochloa colonum* to Bispyribac-sodium Herbicide with Respect to its Effect on Chlorophyll Content.

By

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Abstract

The resistance evolution of *Echinochloa colonum* to bispyribac-soduim was monitored during 2005, 2006 and 2007 seasons under greenhouse conditions. Moreover, the resistance mechanism of this weed to bispyribac-soduim was investigated by determining the activity of ALSase and the determination of seeds and leaves protein in both susceptible and resistant biotypes of *E.colonum*. The results revealed that the *E. colonum* exhibit resistance to bispyribac-soduim after three seasons of selection pressure. The concentration of bispyribac-soduim that reduced the growth of treated *E. colonum* plants by 50% (GR_{50}) in the second and third seasons were 4.59 and 8.53 times higher than that required to obtain the same effect on the susceptible biotype (first season)of this weed. There were differences between the susceptible and resistant biotypes of *E. colonum* in protein content and acetolactate enzyme activity. This proved that the evolved resistance of *E. colonum* to bispyribac-soduim herbicide might be due to the alteration of the target site protein (acetolactate synthase enzyme) that made it insensitive to bispyribac-soduim. The effect of the tested herbicide on chlorophyll content was measured as this parameter indicates the physiological condition of the weed in common sense.

Keywords: weed, resistance, bispyribac-soduim, evolution

Introduction

Rice (*Oryza sativa*), is considered one of the most important crops worldwide, and is cultivated in all of the continents since it adapts itself to all climatic conditions. Under intensified rice cropping systems of today's agriculture, weeds have become a major pest attacking rice and

perhaps the major constraint for rice farming. Weeds are the cause of serious yield reduction problems in rice production worldwide. Losses caused by weeds vary from one country to another, depending on the predominant weed flora and on the control methods practiced by farmers. Two examples give an idea of the problem dimensions, i.e. in China, 10 million tones (Mt) of rice are lost annually due to weed competition (**Zhang, 2001**); such quantity of rice is sufficient to feed at least 56 million people for one year. In Sri Lanka, a country considered self-sufficient in rice, weeds are the major biotic stress in rice production and account for 30 to 40 percent of yield losses (**Anurddhika, 2001**).

E. colonum (L.) is a severe competitor worst weed. It is an alternate host of fungi, nematode, stem borer and viruses. Rice farmers in many parts of the world, particularly those establishing the crop by direct seeding, rely heavily on herbicides. With the repeated and intensive use of herbicides with the same mode of action, resistance to a wide array of useful herbicides has appeared in many key weed species of rice.

Generally, only a few herbicide modes of action are used in rice: acetolactate synthase (ALS) inhibitors, synthetic auxins, thiocarbamates, acetyl co-enzyme A carboxylase (ACCase) inhibitors and amides being among the most frequent groups. Under these conditions herbicide resistance has become a major and widespread problem in many areas, involving several chemical families **(Valverde, 2001).**

Weed resistance to herbicides is not a new phenomenon, but is somewhat less known and experienced than insecticides or fungicides resistance. The main direction of strategic research is to develop basic knowledge about herbicide resistance in rice weeds which lead to the development of integrated weed management strategies for rice with emphasis on crop interference.Therefore, this study attempted to monitor the development of the susceptible biotype of *E. colonum* to evolve resistance against bispyribac-sodium herbicide using a whole plant bioassay. To characterize the resistance mechanism of *E. colonum* against bispyribac-sodium by evaluating the effects of the tested herbicide on acetolactate synthase (ALSase) activity and by determination of the protein content in the resistant and susceptible plants of the tested weed. Also the effect of such herbicide on barnyard grass chlorophyll content was taken into consideration.

Materials and methods

1. Used herbicide:

Bispyribac-sodium herbicide (Nominee SL 2%), with a chemical name of: Sodium, 2, 6 bis (4, 6 dimethoxyprimidin-2-yloxy) benzoate according to IUPAC was used in this study.

2. Plant material:

Seeds of the weed *Echinihcloa colonum* supplied from Weed Research Department, Rice Research and Training Centre, Sakha, Kafr-El-Sheikh. Those seeds were cultivated in soil with the characteristics shown in Table (1)

3. Soil properties for greenhouse experiment:

The soil used in this study was collected from the upper 15 cm of the soil profile at the farm of Rice Research and Training Center Sakha, Kafr-El-Shiekh, Egypt. The collected soil was air dried ground to be homogenous. The properties of the used soil are presented in Table (1).

Table (1) the characteristics of the used soil.

PROPERTIES	AMOUNT
Sand	10.07%
Silt	51.52%
Clay	38.41%
Soil texture	Silty clay loam
pH	7.72
Electrical conductivity	0.52 mmhos/cm^3

4. Effect of bispyribac–sodium on fresh weight of the tested weed:

Dose–response experiments were conducted in the greenhouse to study the resistance development of the susceptible biotype (Obtained from Rice Weeds Research Department, Rice Research and Training Centre, Sakha, Kafr-EL-Sheik, Egypt.) of *Echinochloa colonum* to bispyribac-sodium. Germinated seeds of *E. colonum* were planted in 30x30 cm plastic pots filled with soil characterized as mentioned before. This experiment was conducted and determination of fresh weight was done 21 days after spraying according to Osuna et al., 2002. Some replicates of survived treated plants (*E. colonum*) at the highest concentration level of

bispyribac-soduim were kept to continue growing until giving seeds, and then the seeds were collected and kept for using in the second season. Those seeds were treated with gradually higher concentrations than that were used in the first season. The concentrations of bispyribac-soduim that was used in the selection pressure in the first season were 0.04, 0.38, 3.81, 19.04 and 38.08 gm ai ha^{-1}, while in the second season the concentrations were 3.81, 19.04, 38.08, 76.16, and 114.24 gm ai ha^{-1}. Finally the third season concentrations were 19.04, 38.08, 76.16,124.95 and 152.32 gm ai ha^{-1}.These experiments were conducted for three seasons (2005, 2006 and 2007) for monitoring the evolution of resistance in the tested weed (*E. colonum*) against the tested herbicide (bispyribac-soduim). Experiments were designed in a completely randomized design with six replications. Fresh weight data were expressed as percentage of untreated control. Data were pooled and fitted to a log–logistic regression model according to **Seefeldt *et al.* 1994** as follows:

$Y=c+\{(d-c)/[1+(x/g)^b]\}$,

where Y is the fresh weight of germinated seedling aboveground expressed as percentage of the untreated control, c and d are the coefficients corresponding to the lower and upper asymptotes, b is the slope of the line, g (GR_{50}) is the herbicide rate at the point of inflection halfway between the upper (d) and lower (c) asymptotes, and x (independent variable) is the herbicide dose. Regression analysis was conducted using the Sigma Plot statistical software version 10.0 **(Osuna *et al.*, 2002).** Herbicide rate to reduce plant growth by 50% relative to untreated control (GR_{50}) was calculated from the regression equation (1), while R/S ratios were calculated as the GR_{50} of the (R) accession divided by the GR_{50} of the (S) accession.

5. Chlorophyll measurements

Chlorophyll content has been known as typical parameter for evaluating the physiological condition in common sense **(Shim *et al.*, 2003)**. Therefore, chlorophyll content of the tested weed leaves was determined every five days after application with the tested herbicide till 20 days by chlorophyll meter model (Spad-502). The mean values were calculated from three replicates of each plant. The obtained data are presented in Figs.1, 2 and 3.

6. Leaves protein determination:

Leaves of evolved resistant and susceptible biotypes of *E.colonum* weed exposed to bispyribac-sodium were collected for protein

determination. The collected leaves were stored in liquid nitrogen (-80C°) and transferred for determination at the Research Institute of Agricultural Genetics Engineering, Agriculture Research Centre, EL-Dokky, Egypt. The total protein was determined by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique according to the method described by **laemmli (1970).**

7. In Vivo assay of acetolactate synthase enzyme activity.

Dose–response experiments were conducted under greenhouse conditions to study the resistance mechanism of the evolved resistance biotype of the *E. colonum* to bispyribac-sodium. These experiments were conducted at the same conditions mentioned until the plants reached 4-leaves to 1-tiller stage of growth. Then bispyribac-sodium was applied at the rates of 0, 2.94, 14.7 and 29.4 µM which keep both the susceptible and resistant biotypes of *E. colonum* survived after herbicide application and not completely inhibit acetolactate formation by inhibiting the acetolatate synthase enzyme. The herbicide concentrations were selected based on the whole plant bioassay section and each concentration was replicated three times for each experiment. The herbicide was sprayed using hand sprayer. Eighty-four hours after bispyribac-soduim treatment; 1, 1- cyclopropanedicarboxylic acid (CPCA) which was obtained from Sigma Aldrich chemicals with purity of 97% was applied at 400 µM level to the biotype of *E. colonum*. The upper young leaves of the tested plants were collected after 2 days of CPCA application (the highest accumulation rate of acetolactate); stored in liquid nitrogen and transferred to the Plant Pathology Research Institute, Agriculture Research Centre, and EL-Dokey, Egypt for enzymes activity determination. Known amounts of youngest sliced leaves were added to a test tube containing 6 ml of %0.25 of Tween 20 (a nonionic detergent widely used in biochemical applications) and homogenized for 20 seconds using a polytron homogenizer. The homogenate was filtered through a layer of cheesecloth to remove extra cellular materials. For acetolactate decarboxylation to acetion, 50 µl of 6N H_2SO_4 was added to a 3-ml homogenate aliquot and incubated at 60°C for 20 minute to stop the reaction and allowed decarboxylation. One milliliter of freshly prepared solution of 1-naphthol and creatine (0.09 and 0.009 g/ml) dissolved in 2.5 N NaOH was added to the extracts and stirred for 10 seconds. Following incubation at 60°C for 30 min, color was allowed to develop at room temperature or at 37°C for maximum intensity (pink

color), then the aliquot was centrifuged at 10,000g for 5 min, and the absorbance at 530 nm of the supernatant was measured. Background absorbance was determined with untreated leaves as described previously. A standard curve of commercial acetion was used to quantify the enzyme reaction product. ALS specific activity ($\mu mol_a/mg/min$) was expressed as percentage of the untreated control. Data were pooled to the log–logistic model described before. The log-logistic analysis (**Seefeldt *et al.*, 199**) was used to calculate the herbicide concentrations that reduced enzyme activity by 50% and fitted (I_{50}) relative to untreated control as reported by **Lee and Owen, 2000**. R/S ratios were calculated by dividing the I_{50} value of the (R) accession by the I_{50} value of the (S) accession.

Results and discussion

1. Evolution of *E. colonum* resistance to bispyribac-sodium on the basis of fresh weight reduction of treated plants:

Dose–response experiments were conducted on whole plants of *E.colonum* treated with bispyribac-soduim to monitor its resistance evolution against this herbicide within three seasons of selection pressure (2005, 2006 and 2007). The response of the tested weed against this herbicide was determined as reduction in the fresh weight of the treated plants as percent of control after 21 days of bispyribac-soduim treatment. Referencing to the results in Table (2), the herbicide rates of bispyribac-soduim which was required for 50% growth reduction were 0.52, 4.52 and 12.26 gm ai/fed. at the three seasons, respectively. The resistance ratio in Table (2) revealed that, the GR_{50} of *E. colonum* biotype in the second and third seasons were 8.63 and 23.40 times higher than that required to obtain the same effect on the susceptible biotype (first season). The resistance of *E. colonum* against bispyribac-soduim was achieved in the third season since the resistance ratio was higher than 10 folds (23.40). Therefore, the *E. colonum* weed exhibit resistance to bispyribac-soduim after three seasons of selection pressure (Table 2). The rate of resistance level was higher in the second season than the third season as shown in Table (2). Moreover, *Echinochloa* sp. evolved resistance against sulfonylurea herbicides (ALS inhibiting herbicides) as recorded **by Pratley *et al.*, (2002); Sattin and Zanin (2003); Sangakkara *et al.*, (2004); Castor and Alex (2006).** Also **Fader et al., 1994** reported that the resistance of *Echinochloa* spp to ALS –inhibitor herbicides appeared within 3-4 years of using this herbicides.**Sattin *et***

***al.*, (1999)** indicated that the resistance to rice weeds appeared after at least three years of using these ALS-inhibitor herbicides.

Table (2) Effect of bispyribac-soduim on the susceptible biotype of *E.colonum* at the three seasons (2005, 2006 and 2007) expressed as the rates of the herbicide required for 50% reduction of the aboveground biomass (GR_{50}) and estimated resistance ratio.

Herbicide	Seasons	GR_{50} gm ai/ha	b	c	d	R^2	R/S value	P
Bisbyribac sodium	S1	0.52	1.24	0.00	98.30	0.987		<0.001
	S2	4.52	3.81	0.00	100	0.99	8.63	<0.001
	S3	12.26	3.45	2.6	100	1.00	23.40	<0.05

C: The mean response (fresh weight as percent of control) at very high herbicide rate.

d: The mean response (fresh weight as percent of control) at zero herbicide rate.

b: Slope of the line

GR_{50}: Herbicide rate to reduce plant growth by 50% relative to untreated control

R^2: The coefficient of determination

R/S ratio: The GR_{50} of the tested biotype (the second and the third seasons) divided by the GR_{50} of the susceptible biotype (season 1).

P value: The probability of the obtained results

The chlorophyll content has been measured every 5 days after herbicide treatment till 20 days to evaluate the physiological conditions of the *E.colonum* during the period between bispyribac-soduim applications and weed harvesting. Figs. (1, 2 and 3) showed that, the chlorophyll content at each period was decreased with the increasing of bispyribac-soduim concentration and the rate of reduction in chlorophyll content was slower at the low herbicide concentration and then decreased faster with the increasing of herbicide concentration at the three tested seasons. Referring to the chlorophyll measuring time, the rate of reduction in chlorophyll content response differently with the increase of measuring time at all tested seasons. The reduction in chlorophyll content of *E.colonum* after foliar application of bispyribac-soduim with different concentrations agree with the findings of **Lycan and Hart**

;2005 who reported that the application of bispyribac-soduim as AlS-inhibitor for controlling different weeds leads to injury symptoms in the form of chlorosis (reduction in the chlorophyll content). Moreover, Willis et al., 2007 reported that the application of sulfonylurea herbicides (ALS inhibitors) for controlling weeds resulted in reduction of chlorophyll content of these weed.

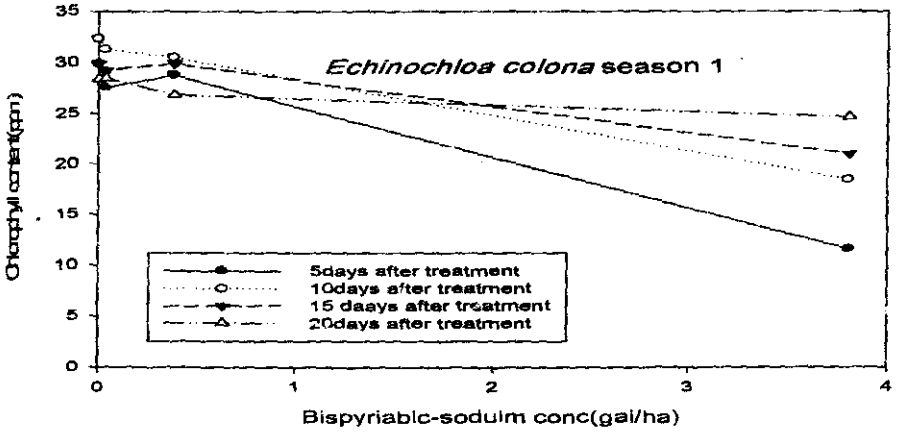

Fig. (1) Chlorophyll content of *E.colonum* leaves at 5, 10, 15 and 20 days after bispyribac-sodium application in the first season (2005).

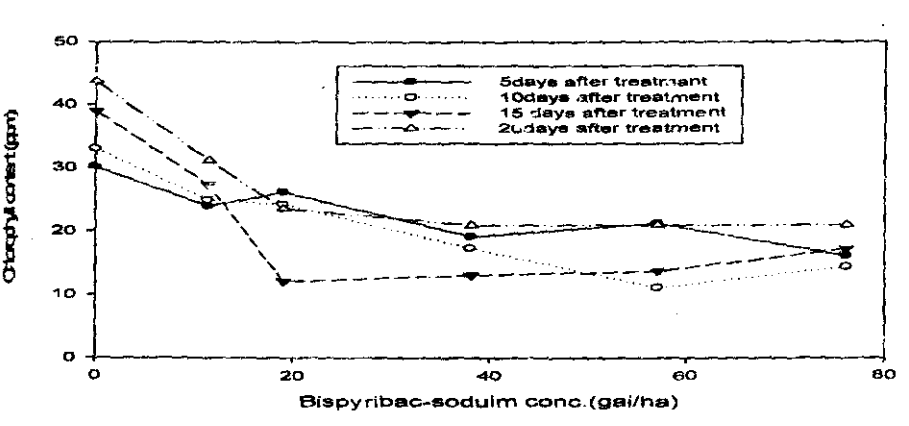

Fig. (2) Chlorophyll content of *E. colonum* leaves at 5, 10, 15 and 20 days after bispyribac-sodium application in the second season (2006).

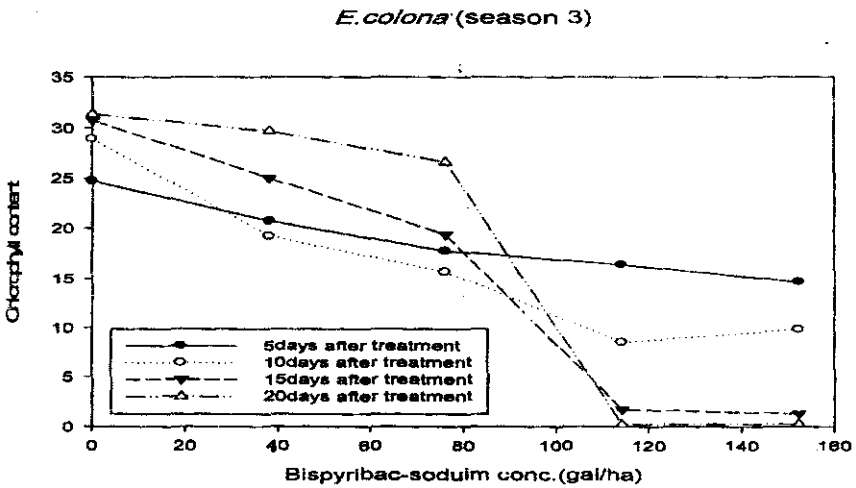

Fig. (3) Chlorophyll content of *E. colonum* leaves at 5, 10, 15 and 20 days after bispyribac-sodium application in the third season (2007).

2. Mechanism of *E. colonum* resistance to bispyribac-sodium. (acetolactate synthase

Activity

2.1. Acetolactate synthase activity

The resistance mechanism of *E.colonum* weed against bispyribac-soduim was investigated by evaluating the concentration of bispyribac-soduim required to inhibit the acetolactate enzyme activity (the target site of this herbicide) by 50% (I_{50}) in susceptible and evolved resistant biotypes of this weed. The results in Table (3) showed that , the I_{50} value that was required to inhibit the acetolactate enzyme activity by 50% in evolved resistant biotype (154.7 µM) was much higher than the I_{50} value that induce the same effect in the susceptible biotype(16.36 µM) of *E.colonum*. The resistance ratio calculated as mentioned elsewhere revealed that, the I_{50} value of the evolved resistant biotype was 9.46 times higher than the same value for the susceptible biotype of *E.colonum* and this is imply that the acetolactate sysnthase enzyme in the evolved resistant biotype of *E.colonum* was less sensitive to bispyribac-sodium than the susceptible biotype. The resistance on the basis of whole plant bioassay correlated well with the reduction of acetolactate synthase sensitivity (target site) in *E.colonum* against bispyribac-soduim. The I_{50} values obtained in this study could differ from those obtained in other studies **(Brown;1990)** because of several factors including plant species

tested, ecotype, plant age, herbicide causing the resistance, and the level of resistance per season. Also, if other resistance mechanism is involved (in addition to modification of site of action) in a particular biotype tested, then significant differences can be observed in I_{50} values between biotypes from different location (**Thill *et al.*, 1991, Schmitzer *et al.*, 1993**). The respective R/S ratio of *E.colonum* against bispyribac-soduim on the basis of I_{50} (9.46) and the high resistance ratio in a whole plant bioassay technique for the same weed correlated well.

Table (3) Effect of bispyribac-soduim on acetolactate synthase enzyme activity of susceptible and resistant biotypes of *E.colonum* expressed as the rates of the herbicide required for 50% inhibition of acetolactate synthase enzyme activity and estimated resistance ratio.

Weed biotype	I_{50} Values (µM)	b	c	d	R^2	R/S	P value
Susceptible biotype	16.36	0.53	14	86	0.97		<0.05
Resistant biotype	154.7	1.04	26	100	0.99	9.46	<0.05

*Acetolactate synthase activity was expressed as the percentage activity relative to control treatment (without herbicide).

*Specific activity of acetolactate syanthase enzyme in control determined as µmol acetion/mg protein/min.

Leaves protein analysis

Leaves protein of susceptible and resistant biotypes of *E.colonum* to bispyribac-soduim herbicide was extracted and analyzed by SDS-PAGE technique to confirm the resistance mechanism of this weed to the above mentioned herbicide. The data in Table (4) showed that, there were four bands with molecular weights of 112.38, 87.50, 75.85, and 26.85 kDa were presented in the susceptible biotype (lane1) and absent in the resistant biotype (lane2) of *E.colonum*. On the other hand, another eight bands with molecular weights of 117.08, 90.12, 77.05, 62.76, 54.06, 28.98, 24.45 and 21.47 kDa were presented in the resistant biotype (lane2) and absent in the susceptible biotype (lane1) of *E.colonum*. In addition, there were some bands with molecular weights of 67, 65, 57, 43, 45, 37, 34, 33, 31, 30, 22, 18 and 17 kDa were presented with high density in the resistant biotype (lane 2) compared to the susceptible biotype (lane 1)of *E.colonum* (Table 4). Finally, the total bands in lane 2

(resistant biotype) were 23 compared to 20 bands detected in lane 1, the susceptible biotype of *E.colonum* to fenoxaprop-p-ethyl (Table 4).

The differences between the susceptible and resistant biotypes of *E. colonum* either in the presence and absence or the density of leaves protein bands, implied that, there were different gene expression between the two biotypes of the weed, where some of them promoted, while novel proteins were induced. This is beside that the acetolactate synthase enzyme in the evolved resistant biotype of *E.colonum* was less sensitive to bispyribac-soduim than the susceptible biotype as mentioned before. These two reasons indicated that, the evolved resistance biotype of *E.colonum* weed against bispyribac-soduim herbicide is due to the alteration of the gene(s) target site (acetolactate synthase enzyme) that makes it insensitive to bispyribac-soduim herbicide compared with the susceptible biotype. This agree with the findings of **[Fischer *et al.*,2000]** who reported that the resistant weed biotypes may contain an action site with low herbicide affinity or may have evolved a high ability to detoxify herbicides.

The basis of resistance mechanism of *E.colonum* against bispyribac-sodium appears to be similar to that of other ALS-inhibitors (SU-herbicides). Since resistance to ALS- inhibitor herbicides had been linked to a reduced sensitivity of the target enzyme **[Hwang *et al.*, 2001; Shibuya *et al.*, 1999; Uchino and Watanabe, 2002]**. The resistance mechanism, as documented in many other species that evolved biotypes resistant to ALS inhibitors **[Osuna *et al.*,2002; Heap, 2004]** appears to be related to the decrease in sensitivity of the ALS enzyme **[Osuna *et al.*, 2002; Busi *et al.*, 2004**] caused by an alteration of the encoding gene **[Scarabel *et al.*, 2004]**.

Table (4) Molecular weight and density of protein in susceptible (1A), and resistant (2B) biotypes of *E. colonum* to bispryribac-sodium.

1A				2B			
Lane Number	Band Number	Mol. Wt. kDa	Average (OD)	Lane Number	Number	Mol. Wt. kDa	Average (OD)
1	1	112.38	102.33	2	1	117.084	78.723
1	2	87.501	80.072	2	2	90.124	57.379
1	3	75.85	80.254	2	3	77.053	66.282
1	4	67.466	90,426	2	4	68.279	74.792
1	5	65.277	88.367	2	5	65.002	71.988
1	6	57.448	97.154	2	6	62.762	69.54
1	7	48.343	82.613	2	7	57.039	86.455
1	8	45.775	76.323	2	8	54.067	70.745
1	9	37.945	93.538	2	9	47.889	62.864
1	10	34.563	94,45	2	10	45.177	56.832
1	11	33.646	99.106	2	11	37.495	71.384
1	12	30.581	80.692	2	12	34.276	75.097
1	13	30.162	68.431	2	13	33.168	80.858
1	14	26.852	71.6	2	14	31.434	51.856
1	15	25.448	52.786	2	15	30.26	49.47
1	16	23.655	61.225	2	16	28.982	41.112
1	17	22.461	56.775	2	17	25.929	28.25
1	18	19.783	60.324	2	18	24.453	21.667
1	19	18.804	77.56	2	19	22.576	31.351
1	20	17.84	54.504	2	20	21.474	30.55
				2	21	20.923	33.249
				2	22	18.878	29.122
				2	23	17.704	

Mol. Wt. = molecular weigh OD- optical density

Conclusion

This study indicated that *E.colonum* evolved resistance to bispyribac-soduim after three seasons of selection pressure. The resistance mechanism of *E.colonum* to bispyribac-soduim herbicide might be due to alteration in the target enzyme protein, acetolactate synthase (ALSase which made this enzyme insensitive to this herbicide.

Extensive studies on weeds resistance to herbicides and the possible solution to overcome this problem in Egypt are needed in this regard.

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الملخص العربى

تطور وميكانيكية المقاومه لحشائش الارز ضد مبيدات الحشائش
1-تطور وميكانيكية المقاومه لحشيشة ابوركبة ضد مبيد بيسبيريباك صوديوم مع الاخذ فى الاعتبار تاثيره على محتوى الكلورفيل

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تم دراسة تطور المقاومة لحشيشة ابوركبة والتى تعتبر من أهمْ الحشائش في حقول الأرز وذلك ضد مبيد "بيسبيريباك -صوديوم" داخل الصوبة الخاصة بمركز البحوث والتدريب في الأرز بسخا محافظة كفرالشيخ لمدة ثلاث مواسم زراعية (2005، 2006، 2007) حيث تم عمل ضغط انتخابي للحشيشة تحت الدراسة بواسطة المبيد المختبر. تم قياس تركيز الكلوروفيل في حشيشة ابوركبة بعد المعاملة بالمبيد سالف الذكر كل خمسة أيام ولمدة 20 يوم وذلك لمعرفة تأثير المبيد على الصفات الفسيولوجية للنبات بعد المعاملة. تم أيضا دراسة ميكانيكية مقاومة هذه الحشيشة لمبيد بيسبرابيك صوديوم عن طريق تقدير نشاط إنزيم "اسيتولاكتيت سينسيز" وتحليل بروتين الأوراق في كل من السلالة المقاومة والحساسة من كل الحشيشة تجاه مبيد "بيسبيريباك صوديوم". أظهرت دراسة تطور مقاومة حشيشة ابوركبة ضد مبيد " بيسبيريباك-صوديوم" باستخدام منحنيات الجرعة -الاستجابة أن الحشيشة أصبحت مقاومة للمبيد بعد ثلاث مواسم من الضغط الإنتخابي بهذا المبيد على أساس تقدير الانخفاض في الوزن الرطب في النباتات المعاملة مقارنة بالكنترول.معدلات مبيد " بيسبيريباك - صوديوم" اللازمة لخفض نمو حشيشة ابوركبة إلى النصف كانت(GR_{50}) 0.52 – 4.52- 12.25 جرام مادة فعالة/ هكتار على الترتيب للمواسم الثلاثة.أوضحت النتائج أن المحتوى الكلوروفيلى كان ينخفض مع زيادة تركيز المبيد على جميع فترات القياس ولكن المحتوى الكلوروفيلى في نباتات ابوركبة عند تركيز ثابت من المبيد أعطى استجابات مختلفة مع الوقت في كل موسم من المواسم الثلاثة.أوضحت النتائج أن التركيز من مبيد " بيسبيريباك صوديوم" المثبط ا 50% من نشاط إنزيم "اسيتولاكتيت سينسيز" في السلالة المقاومة(154.7ميكرومولر) كان أعلى بكثير منه في السلالة الحساسة من حشيشة ابوركبة (16.36ميكرومولر).حيث كانت قيمة I_{50} للسلالة المقاومة تعادل 9.46 مثل قيمة I_{50} للسلالة الحساسة من حشيشة ابوركبة للمبيد . أوضحت نتائج تحليل بروتين الأوراق في السلالة الحساسة والمقاومة من حشيشة ابوركبة ضد مبيد " بيسبيريباك صوديوم" وجود اختلافات بينها سواء في عدد الحزم الموجودة فى كل سلالة ووجود بعض حزم البروتين في سلاله واختفائها في الأخرى أو وجود حزم كثافتها أعلى في سلاله عن الأخرى و هذا يوضح وجود تعبيرات جينية في السلالة الحساسة تختلف عن الموجودة في السلالة المقاومة.اتضح ان الاختلاف بين السلالة الحساسة والاخرى المقاومة من حشيشة ابوركبة لمبيد " بيسبيريباك صوديوم" في حساسية إنزيم "اسيتولاكتيت سينسيز" وكذلك فى تحليل البروتين ربما يكون هو السبب في ظهور السلالة المقاومة من هذه الحشيشه لمبيد " بيسبير يباك صوديوم" حيث أن المقاومة تحدث نتيجة لتحور في بروتين إنزيم "اسيتولاكتيت سينسيز" (الهدف الحيوي لمبيد " بيسبيريباك- صوديوم")مما يجعله غير حساس للمبيد.