Improving Barley (*Hardeum Vulgare* L.) Tolerant to Herbicides Injuries Using Two Methods of Safener Application

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ABSTRACT

In greenhouse, barley seeds (*Hardeum Vulgare* L) were treated with 1,8 naphthalic anhydride safener using two methods of application. The first method, seeds were coated with naphthalic anhydride (NA) by tow rates (0.5 and 1.0% w/w), and the second were soaking for two hours in potassium salt of naphthalic anhydride (KNA) at concentration 20 and 30 mM in K-phosphate buffer to compare the two methods against the toxic effect of post-emergence application of imazapic (oraban) and fenoxaprop-ethyl (furour) compared with fenoxaprop-*p*-ethyl which have safener in its formulation (puma super) at one field and one and half of field rate. All naphthalic anhydride concentration increase glutathione content, glutathione-s-tansferases activities and total chlorophyll determined 30 days after herbicides application. On the other hand all herbicide rates caused significant decrease in all determined parameters. Naphthalic anhydride succeeded to protect barley seedlings from herbicides injuries and the seedlings still alive even with 1.5 F of field rate. Fenoxaprop-*p*-ethyl (at the ready made formulation) with one field rate gave an increase in all parameter but the rate of increase were less than that with naphthalic anhydride and its safener failed to protect barley seedlings from its high rate.

INTRODUCTION

Due to the new strategy of the ministry of agriculture to protect the Egyptian environment from agrochemical hazard, many of the potent herbicides had been banded. Weed management in modern agriculture requires efficient weed control technologies that are safe to the crops. Recent efforts are thus aimed at protecting crop from herbicidal injuries by different methods including genetic engineering of herbicide –tolerant crop cultivars as well as herbicide safeners (El-Deeb, *et al*. 2002). Herbicide safeners selectively protect crops from herbicide damage without reducing the activity of these herbicides against the target weed species (Davies and Caseley, 1999). Herbicide safeners, also known a antidotes, they are a compounds of diverse chemical families which applied with herbicide to protect crops against their injuries by improving selectivity without reducing the herbicidal potential (Abu-Qare and Duncan, 2002, and Ying, *et.al.*2008)

The safeners were applied either as mixed formulation with the herbicides or as seed treatment. The most herbicide classes which could be applied with safeners were including: thiocarbamate, chloroacetamide, sulfonylurea, imidazolinones, and aryloxyphenoxy propionates.

Naphthalic anhydride (NA) is considered among the earlier herbicide safeners used in agriculture, NA when used as seed treatments it will be very effective and partially are completely protect cereal against injuries of various herbicides. Naphthalic anhydride is the most versatile safeners, it protect various crops against a wide range of herbicides and it capable of providing safening activity against post-emergence herbicides (Abu-Qare and Duncan, 2002).

Despite extensive research effort the protective mechanism of herbicide safeners is far from being completely understood. Several hypotheses have been advanced for the mechanism (s) of the protective action of herbicide safeners. Safeners protect crop plant from herbicide damage by reducing the ability of herbicide to reach and inhibit their target site. This may be achieved through safeners induce modification of herbicide target enzyme, accelerating herbicide metabolism and detoxification in these crops by oxidation or conjugation (with glucose, glutathione) and thus herbicide became less effective or immobile; increase the response of certain enzyme (e.g. glutathione–S tansferases isozymes) and increase the level of glutathione (Davies and Caseley, 1995 and Hall and Stephenson, 1995).

Conjugation of herbicide via the thiol function of reduced glutathione (GSH) (γ- glutamyl- cysteinyl-glycine,) is well established as one of the major detoxification and selectivity factor in plants (Lamourux *et. al.* 1991). In addition, the tripeptide glutathione may play a key role in the defence of plant against various environmental stress e.g. cold, heat drought, high light, fungal attack and herbicide.

Glutathione S- transferases (GSTs; EC2.5.1.18) are key enzymes catalyzing the detoxification of several herbicides in many plants. These enzymes are a diverse group of cytosolic enzymes found in all eukaryotes which catalyze the conjugation of synthetic electrophilic substrates with the tripeptide glutathione (γ- glutamyl – cysteinyl- glycine, GSH) (DeRidder, *et.al* (2002), Dengo and Hatzios (2002), Frova, 2003, Grundy, *et. al.* 2005 and Buono,*et.al.*2007). In addition these family of multifunction enzymes in plant and animals that are well

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know for their roles in detoxification of xenobiotics (Riechester *et.al.*1998) .

Fenoxaprop-*p*- ethyl is a selective herbicide with contact and systemic action, absorb by leaves and translocated acropetally to the roots, uses as post-emergence to control annual and perennial grass weed in broad leaves and (when applied with safeners it control annual and perennial grass weed in wheat, rye and barley, this herbicide is one of the fatty acid synthesis inhibitors due to the inhibition of acetyl CoA carboxylase (ACCase).

Mefenpyr-diethyl (a new foliar acting safener from the chemical group of pyrazolines) is described. World-wide studies showed that the application as safener for fenoxaprop-*P*-ethyl and iodosulfuron resulted in significant improvements of crop safety in winter wheat, winter rye, triticale, spring wheat, durum wheat and spring barley (Hacker, *et.al.*, 2000). This new safener can protect wheat and barley certain toxic herbicide, fenoxaprop-ethy and its active isomer (Scalla and Roulet, (2002) by increasing GST activity with CDNB as a substrate, and increase GSH and GSH peroxidase. After this safener was uptaken after application and translocated to leaves and roots resulting metabolization and detoxification of the herbicidal ingredients, without affecting metabolism of the herbicides in weeds. Mefenpyr-diethyl is the first foliar acting safener which can be used with herbicides with different modes of action(Hacker, *et.al.*, 2000). Also it could protect wheat and barley from iodosulfuron-methyl sodium (an inhibitor of acetolactate synthase) (Trabold, *et.al.*2000), pebulate (Baldwin,*et.al.* 2000)and metsulfuron (King, 2007).

Imazapic is an imidazolinon herbicide which is toxic to cereals, it used as post and pre- emergence to control wide range of annual and perennial weed in non – cropland area . During the 15 years the development of herbicide resistant crop has moved from transgenic resistant crop to glyphosate, and other herbicides to the development of new type of non- transgenic resistant crop to imidazolinon resistant crop (Alister and Kogan, 2005). Imidazolinon herbicide, like imazethapyre, imazapyr and imazapic, are characterized by their herbicidal effect at low doses, a wide spectrum of weed control and high soil persistence (Loux and Reese, 1993). These high soil persistence (may be 90 days or more) plus the sensitivity of certain crops, like *Beta vulgaris, Brassica rapa, Triticum aestivum* and *Hardeum Vulgare* L . However, in many studies barley showed loss in yield with imazapic and imazapyr (Shaw and Wixson 1991) and it reported as a sensitive crop to Imidazolinon herbicides, needing intercropping periods as long as 540 days after application in maize.

The aim of this studies are: a) compare the deference between the two methods of naphthalic anhydride application to the barley seeds against the post - emergence application of fenoxaprop- ethyl (furour), and compare these post- treatment to the ready made formulation from the same herbicide which has safeners (puma super), and b) try to protect barley from the toxic effect of imazapic to extended its used in barley.

MATERIALS AND METHODS

In greenhouse, barley seeds (*Hardeum Vulgare* L) were treated with 1,8 naphthalic anhydride using two methods of application: first, seeds were coated with naphthalic anhydride (NA) at 0.5 and 1.0% w/w before planting by dusting pre- weighted seeds in closed container with the appropriate amount of the safeners and shaking to coat the seeds. The second one, seeds were soaked for two hours in potassium salt of naphthalic anhydride (KNA) at concentration (20 and 30 mM) prepared in 0.02M K-phosphate buffer (pH 6.5-7.2) (Frear *et.al.*, 1991 and Modified by Houssien and Sabra, 2005). Seeds were planted in 13 cm diameter plastic pot contains clay soil (41%clay, 22.2% silt and 36.1% sand). Herbicides were applied early post emergence at 3-4 leave stage at one and one and half fold of field râte. Herbicide fenoxaprop-ethyl (2[4-(6-chloro-2-bezoxazolyl-oxy-phenoxy-] propanoate) this is (furore 24% EC, the field rate 0.5L. / Feddan, the other formulation of this herbicide were (fenoxaprop -*p*- ethyl) 2[4-(6-chloro-1,3-bezoxazolyl-oxy-phenoxy-] propanoate+ safener (puma super 75% EW, 0.5L./Feddan) and imazapic, (RS)- 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) -5-methyl nicotinic acid (Oraban 10g/L at 2L /Faddan. Barley were left to grow 30 days after treatments. Glutathion -S-transferases activities at shoot and root of barley seedlings, glutathione content and chlorophyll a and b were determined at the end of the experiment.

Chlorophyll determination: Chlorophyll a, b and total chlorophyll as mg /g F.W. were determined in barley leaves according to Grodzinsky and Grodzinsky, (1973), and modified by Sabra (1993)

Glutathione content determination: barley root were extracted with 70% ethanol at 0°C, glutathione content (GSH contents as Ug GSH /gm F.W.) were determined spectrophotometry using DTNB (5, 5- di thio bis (2-nitrobenzoic acid) as a substrate in 0.1ml ethanol at 412nm according to Jabalankai and Hatzios (1991).

Glutathione-S-tansferases activities determination: According to Jabalankai and Hatzios (1991) and modified by (Houssien 1999), barley shoot and root were extracted by 0.1M phosphate buffer (PH 6.8), then specific activity (GST's specific activity as ((Umol

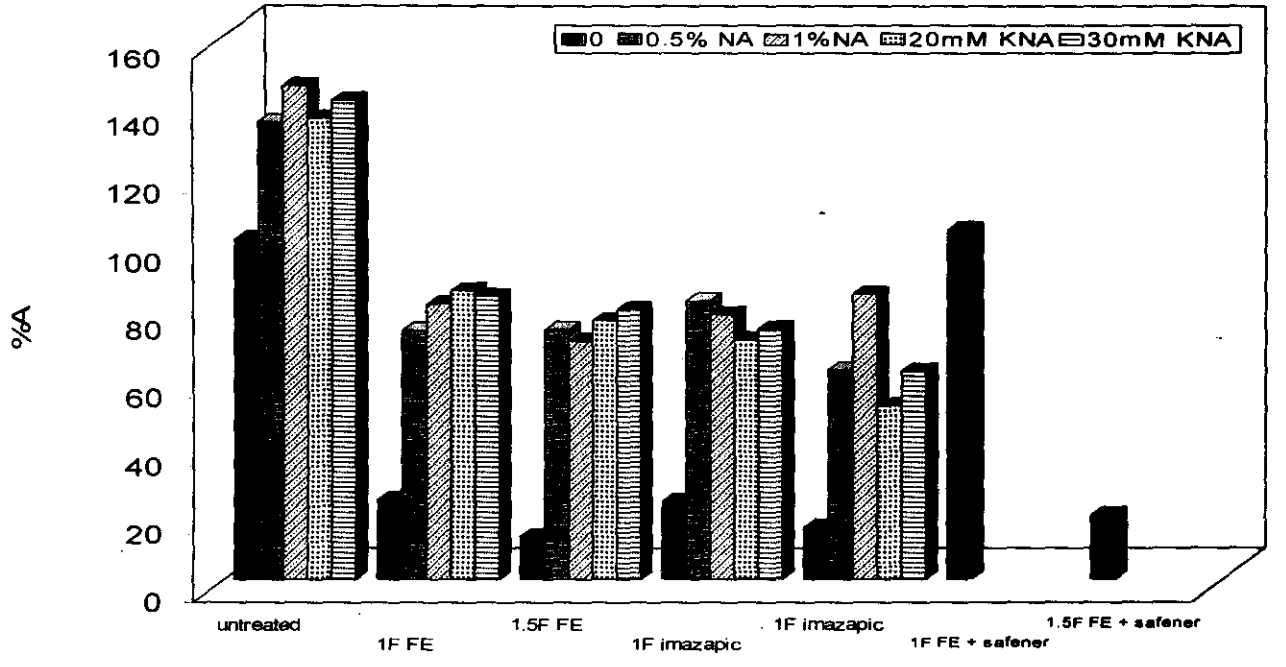
CDNB/min/mL/g F.W)) of the enzyme were determined by CDNB (1-chloro-2.4-Dinitrobenzen) as a substrate and reduced glutathione at 340nmm. The rate of non-enzyme conjugation was determined.

All data were statistically analysis.

RESULTS AND DISCUSSION

I- Effect on glutathione content (GSH): both naphthalic anhydrides, (NA and KNA)caused significant increase in glutathione content extracted from root (figure, 1).

NA at 1% gave the highest increase in glutathione contents by 45.301% followed by 30mM KNA by 40.98% and 20mM which gave 35.52% increase. Fenoxaprop-*p*-ethyl+safeners (the ready made formulating puma super), caused 3.77% increase in glutathione content without significant deference with untreated barley. The cereals respond to safeners is an increase in the total of GSH pool, the significance of this response is unknown but suggested there would be sufficient GSH available for herbicide conjugation in seedlings after safeners treatment, (Hirase and Molin, 2001 and DeRidder *et.al.*, 2002). In addition, naphthalic anhydrides raised GSH content due to the increase of cystein synthase activity in treated seedlings (Mamdouh and Hassan 1998). (Fenoxaprop-ethyl (furour) at field rate and 1.5 fold of field rate, 1.5Fof field rate of imazapic and puma super at 1.5 F caused death to the plants after 30 days of treatments. All herbicide treatments alone caused the highest decrease in glutathione content which determined in decomposed root without significant deference. Fenoxaprop-ethyl at 1.5F gave 87.432% decrease followed by 1.5F imazapic which gave 84.754% decrease, while, 1F fenoxaprop-ethyl and 1F imazapic caused decrease by 76.557% and 77.158% respectively. Also, Fenoxaprop-*p*-ethyl + safeners (puma super) at 1.5F field rate caused 81.038% decreased. Naphthalic anhydride succeeds to protect



Treatments	0.0	0.5%NA	1%NA	20mM KNA	30MMKNA
0.0	64.211	86.383	93.298	87.018	90.526
1F FE	15.053	47.053	51.965	54.561	53.474
1.5F FE	8.070	47.193	45.018	48.912	50.982
1F imazapic	14.667	52.596	50.000	45.228	47.368
1.5F imazapic	9.789	39.579	53.895	32.737	39.298
1F FE + safeners	66.632				
1.5F FE + safeners	12.041				

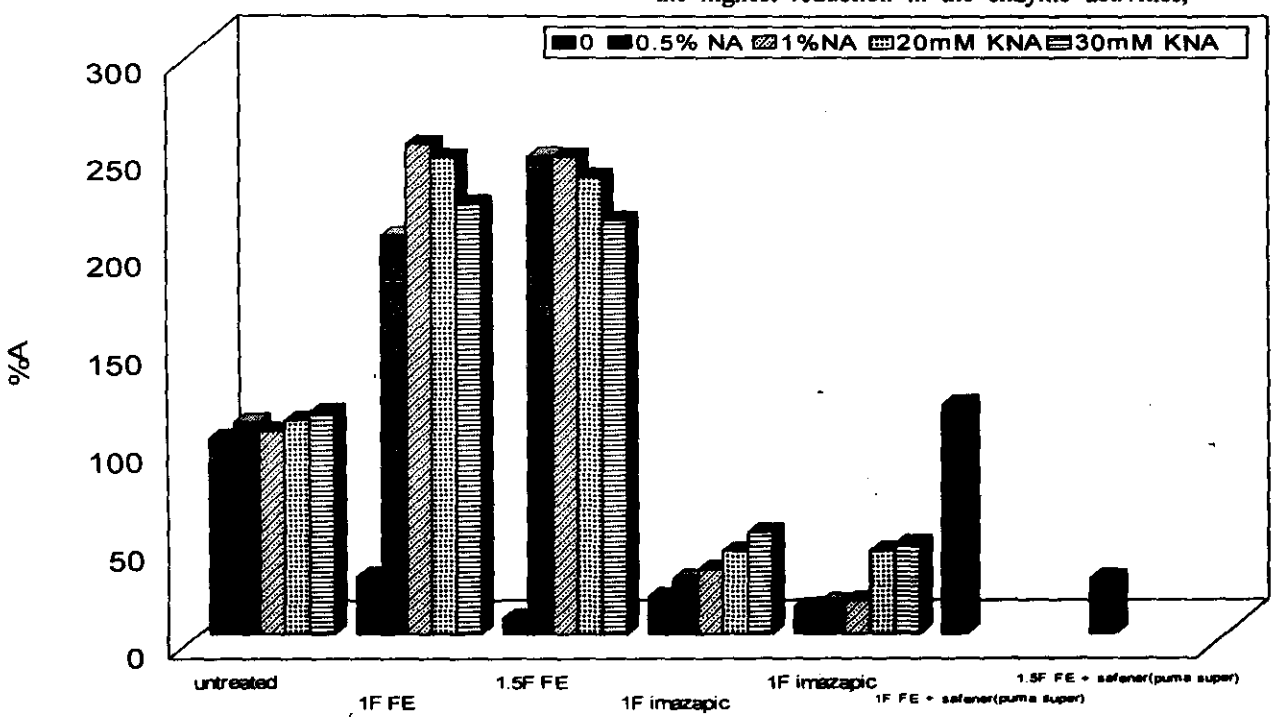
LSD (0.05) =5.036 Fenoxaprop-ethyl (FE)

Figure1. The effect of fenoxaprop-ethyl, imazapic, NA, KNA, and its combination on glutathione content as %A from control

barley seedlings from the injuries against two herbicide, all pre-treated plant either with NA or KNA still alive until the end of the experiment. There were no significant differences between the tow methods of naphthalic anhydride application at the glutathione content but all these combination reduced the reduction rate of glutathione content, for example, 1F fenoxaprop-ethyl gave 76.56% reduction but when combined with 20mM KNA, the percentage of reduction became 15.027%.NA at 1% succeed to protect barley even with 1.5F imazapic which gave 16.066% (imazapic at 1 and 1.5 F of field rate cause death to the barley plants.
(note: GSH which determined in herbicide treatment alone were in decomposed root which were separate from shoot in the pots).

II- Effect on glutathione-s-transferases activities in barley root:

Figure (2), showed that, both naphthalic anhydride alone (NA and KNA) gave an increase in glutathione –S-tansferases activities, 30mM KNA gave 13.25% increase, puma super, the formulated herbicide which have safeners gave 17.38% increase in these enzyme. This illustrated by many workers who mention that. safener increase the activity of one or more of the major herbicide detoxifying enzyme families, including GSTs and cytochrom p450-depend monooxygenases and glucosyltransferases, (Riechester, *et.al.*,1998). All herbicide treatments alone caused death of barley seedlings after 30 days from treatments, and they gave the highest reduction in the enzyme activities,



treatments	0.0	0.5%NA	1%NA	20mM KNA	30MKNA
0.0	603.56	654.22	624.89	658.67	683.56
1F FE	178.67	1231.11	1516.44	1474.67	1329.78
1.5F FE	48.89	1480	1477.33	1414.22	1280
1F imazapic	97.78	174.22	200.89	257.78	314.67
1.5F imazapic	84.44	96.89	102.2	258.67	379.11
1F FE + safener	708.44				
1.5F FE + safener	173.33				

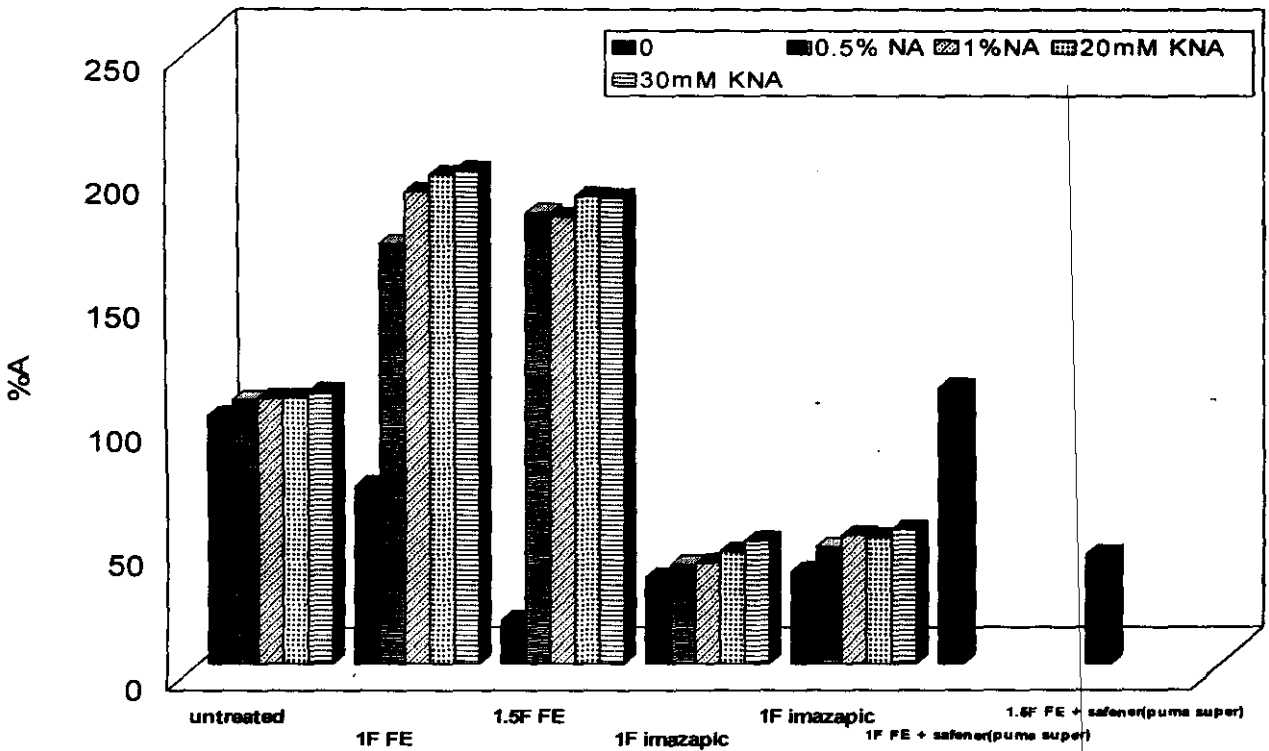
LSD(0.05)=38.964
FE= fenoxaprop-ethyl

Figure2. The effect of fenoxaprop-ethyl, imazapic, NA, KNA, and its combination on root glutathione –S-tansferases activities as %A from control

for example, 1.5F fenoxaprop-ethyl gave 91.9% reduction followed by 1.5 F imazapic which gave 86.01% and 83.8% reduction for 1F imazapic, respectively, where is Fenoxaprop-*p*-ethyl + safeners (puma super) at 1.5 F and 1F fenoxaprop-ethyl (furour) gave 71.28% and 70.4% reduction, respectively. The highest increase in glutathione –S- tansferases activities were observed when barley seeds were pre-treated with NA or KNA at all concentration before treated with fenoxaprop-ethy at 1F and 1.5 F of field rate. 1% NA +1F fenoxaprop - ethyl gave the highest increase in the activities, it gave 151.2% increase followed by 0.5% NA + 1.5F fenoxaprop– ethyl which gave 145.21% increase, followed by 1% NA+1.5F fenoxaprop- ethyl and 20mM KNA+ 1.5F fenoxaprop- ethyl which gave 144.77 and 144.33 respectively. The two methods of naphthalic anhydride application protect barley from death which caused by fenoxaprop-ethyl. Fenoxaprop- ethyl + NA at two concentrations were increased the activities more than the ready made formulation which has safeners inside, NA is better than this safener. These results are agreed with DeRidder, *et.al.* 2002, so that, treatment of wheat and barley with naphthalic anhydride induce the activity of phi class Ta GST2-3 (enzyme isozymes) which are active in detoxifying fenoxaprop-ethyl). On the other hand, NA also protects plants from imazapic injuries but with decrease in the enzyme activities.

III- Effect on glutathione-s-transferases activities in barley shoot: -

Figure (3), showed the activities of glutathione –S-tansferases which extracted from barley shoots , these activities were following the same direction of that extracted from roots.



treatments	0.0	0.5%NA	1%NA	20mM KNA	30mMKNA
0.0	870.22	926.22	932.44	934.22	954.67
1F FE	244.44	1472	1656	1718.22	1733.33
1.5F FE	152.89	1582.22	1568.9	1640.80	1635.56
1F imazapic	303.11	346.67	352	392	431
1.5F imazapic	323.56	408.89	449.78	444.44	4700.22
1F FE + safener	968.00				
1.5F FE + safener	433.66				

Figure 3. The effect of fenoxaprop-ethyl, imazapic, NA, KNA, and its combination on shoot glutathione -S-tansferases activities as%A from control

All NA and KNA concentration gave small increase in the enzyme activities, where, 30mM KNA gave 9.7% whereas 20mM KNA and 1% NA gave 7.35% and 7.15% increase. This enhancement in GST's level were gained before, in cereal crops, safeners enhance the GST's activities (Davies and Casely, 1999). The lowest reduction had been obtained from herbicide alone; 1F fenoxaprop- ethyl gave 82.43% reduction followed by 1.5F and 1 F imazapic which gave 62.82% and 65.17% , reduction respectively without significant differences, compared with the other safeners [1.5 F fenoxaprop-*p*-ethyl + safeners] (puma super) gave 50% reduction. Naphthalic anhydride protects barley from death even with imazapic which when combined with Naphthalic anhydride caused reduction in the activity but the plant still alive.

VI- Effect on chlorophyll content: All naphthalic anhydride treatments caused increase in total chlorophyll due to its stimulation effect on ch a and ch b , for example 0.5% and 1% NA gave 25.401% and 26.413% increase in total chlorophyll due to high increase in cha which was 43.24% and 43.12% increase, whereas, only 4.09% and 6.45% increase in ch b followed by the ready made formulation of fenoxaprop-*p*-ethyl which contain safeners by 13.7% increase in total chlorophyll (9.6% increase in ch a and 18.5% increase in ch b). The herbicide treatment alone gave the highest reduction in total chlorophyll, these reduction also due to the highest reduction in ch a and ch b, for example, 1.5F imazapic gave 52.1% reduction in total chlorophyll (51.55% reduction in ch b and 52.56% reduction in ch a). Pre- treatments of barley seeds with naphthalic anhydride succeed to reduce the reduction rate in total chlorophyll, there was no significant deference betweed untreated plant and soaked plant in 20 and 30 mM KNA and plant were posted treatment with 1F fenoxaprop-ethyl and imazapic. All barley seedlings treated with fenoxaprop-ethyl at 1.5F of field rate when pre-treated either with NA or KNA caused small increase in ch a without significant different compared with untreated plant, (table (1).

Table 1. The effect of fenoxaprop-ethyl, imazapic, NA, KNA, and its combination on chlorophyll (a, b and total) as mg/g. fresh weight

Treatments				
Herbicide	**naphthalic anhydride**	**cha**	**Chb**	**total chlorophyll**
0	0	1.38	1.16	2.536
	0.5%NA	1.98	1.2	3.180
	1%NA	1.98	1.23	3.206
	20mM KNA	1.93	1.41	3.345
	30mM KNA	1.94	1.21	3.150
1F fenoxaprop- ethyl	0	0.99	0.66	1.645
	0.5%NA	1.4	0.54	1.936
	1%NA	1.52	0.82	2.347
	20mM KNA	1.59	0.77	2.361
	30mM KNA	1.64	0.91	2.555
1.5F fenoxaprop- ethyl	0	0.97	0.58	1.551
	0.5%NA	1.38	0.44	1.829
	1%NA	1.4	0.45	1.849
	20mM KNA	1.42	0.36	1.779
	30mM KNA	1.52	0.53	2.052
1F imazapic	0	0.78	0.63	1.418
	0.5%NA	1.19	1.01	2.201
	1%NA	1.14	1.17	2.309
	20mM KNA	1.14	1.36	2.498
	30mM KNA	1.36	1.19	2.546
1.5F imazapic	0	0.65	0.56	1.215
	0.5%NA	1.13	1.1	2.222
	1%NA	1.05	0.88	1.934
	20mM KNA	1.12	0.6	1.715
	30mM KNA	1.15	0.72	1.871
1F fenoxaprop-p- ethyl + safener	0	1.51	1.37	2.882
1.5F fenoxaprop-p- ethyl + safener	0	1	0.45	1.445

LSD for total (0.05) = 0.103

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الملخص العربي

زياده تحمل بادرات الشعير لسميه مبيدات الحشائش بإستخدام طريقتين مختلفتين فى المعامله بالترياق

أمل أحمد حسين

تم عمل تجربه فى الصوبه الزراعيه لزياده تحمل بادرات نبات الشعير و المترعه فى التربه الطينيه لمبيد الحشائش فينوكسابرب- إيثيل (فيورور) ومبيد الحشائش إيمازابيك (أورابان) وذلك بمعامله حبوب الشعير بطريقتين مختلفتين من الترياق نفثاليك انهيدريد، الأولى معامله الحبوب الجافه بالنفثاليك انهيدريد الجاف بتركيز (٠,٥% و ١% وزن/وزن)، و الطريقه الثانيه بنقع الحبوب فى محلول ملح البوتاسيوم من الترياق نفثاليك انهيدريد بتركيز(٢٠،٣٠ ميكرو مول)، وذلك قبل الزراعه و قد تم تطبيق المبيدات بعد الأنبثاق فى عمر ٣-٤ ورقات ومقارنه هذه المعاملات بمبيد (البوما سوبر) والمحتوى على المشابه الفعال للفينوكسابرب- إيثيل مضافا اليه ترياق داخل التجهيزه, كل المبيدات طبقت بالتركيز الحقلى و ١,٥ ضعف منه. و قد تم انهاء التجربه بعد ٣٠ يوم من المعامله. و لقد أحدثت تركيزات الترياق بمفردها زياده معنويه فى محتوى الجلوتاثيون و نشاط انزيم الجلوتاثيون-اس-ترانسفيراز وكذلك الكلوروفيل الكلى. وعلى النقيض من ذلك فقد أحدثت المبيدات بمفردها انخفاض معنوى فى كل الصفات المقدره. وقد نجح الترياق فى حمايه البادرات من التأثير السام الحادث من المبيدات وكذلك نجح فى حمايه البادرات من الموت خاصه مع التركيز ١,٥ ضعف الحقلى. مبيد البوما سوبر و الترياق داخل التجهيزه بالتركيز الحقلى أمكنه إحداث زياده فى كل الصفات المقدره، ولكن هذه الزياده بمعدل أقل منها فى حاله النفثاليك انهيدريد. فشل التريق الموجود فى البوما سوبر فى حمايه بادرات الشعير من التأثير السام للمبيد وذلك من التركيز ١,٥ ضعف الحقلى بينما نجح ال النفثاليك انهيدريد فى ذلك مع هذا المعدل المرتفع من التطبيق.