

Safening corn seedling in sandy soil from certain herbicides injuries using naphthalic anhydride and its effect on oxidative enzymes

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ABSTRACT

Greenhouse trials were conducted to safe corn (*Zea mays*) and reduce injuries effect of imazamethabenz-methyl, EPTC and metolachlor at three different field rates using potassium salt of Naphthalic Anhydride (NA) at four concentrations (0.3, 3, 15 and 30 mM) as new method of seed application in sandy soil. NA succeeds to protect corn seedlings from herbicide injury even at 5 or 10 fold of the field rates of the herbicides. NA caused enhancement in both shoots and root length and fresh and dry weight. On the other hand, the use of NA as safener in sandy soil protects the corn seedling from herbicides injuries by reducing peroxidase and increasing polyphenol oxidase activities.

INTRODUCTION

Due to the new strategy of Ministry of Agriculture to protect the Egyptian environment from agrochemical hazards, many of the potent herbicides had been banned. So, weed now become a major problem, 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 injury 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 crop plant from herbicide damage without reducing the activity of this herbicides against the target weed species (Davies and Caseley, 1999). Herbicide safeners are compound of diverse chemical families which applied with herbicides to protect crops against their injury by improving selectivity (Abu-Qare and Duncan, 2002) without reducing the herbicidal potential.

The phenomenon of herbicide safeners was discovered in 1947 with the discovery of naphthalic anhydride (NA) and dichlormide (Davies and Caseley, 1999). NA safener enhance the tolerance of maize to chloroacetanilide, thiocarbamate, sulfonylurea and imidazolinon herbicide, seed treatment with NA increase corn tolerance to both pre-and post-emergence application of imidazolinon herbicide (Barrett, 1989 and Davies *et al.* 1998). For example 1% NA protect corn seedling from pre-emergence (PPE) and pre-planting incorporated into soil (PPI) imazethapyre phytotoxicity (Barrett, 1989; Shaner, 1991; Davies and Casely 1993 and 1995). Also 1% NA succeeded to reduce imazaquin toxicity in corn seedling (Barrett, 1989 and Milhomme, *et al.* 1991). In addition, NA could prevent corn injury from soil residues of imidazolinon herbicides remaining from use of previous year (Boldt and Barrett, 1991).

Metolachlor is a selective chloroacetamide herbicide used in several crops including corn to control annual weeds. Corn is usually tolerant to metolachlor; however, under certain unfavorable environmental conditions such as high application rates, inherent sensitivity of hybrids, cool temperature and high soil moisture before pre-emergence application of certain herbicides, and it can cause injury (Boldt and Barrett 1989; Foy and Witt, 1991; Viger *et al.*, 1991 and Hwang and Hatzios 2000). Viger *et al.*, (1991) reported that the only method which reducing corn injury by metolachlor is to use an antidote. Many safeners can be used to protect corn from metolachlor injury such as fenclorim, benoxacor and fluxofenim (Hwang and Hatzios 2000) and CGA-154281 and oxibetrinil (Foy and Witt 1988 and 1991 and Viger *et al.*, 1991; Fuerst *et al.*, 1993 and Irzyk and Fuerst 1993). Also, Naphthalic anhydride protects corn from metolachlor injury by stimulating the detoxification rate of this herbicide by enhancing GSH and GST's activities. (Griffin *et al.*, 1988, and Nemat-Alla and Hassan, 1998).

Naphthalic anhydride and dichlormide safeners can improve the tolerance of maize to thiocarbamate herbicides (Stephenson and Yaacoby, 1991). NA 0.5% protects corn against EPTC injury when applied as seed treatment (Ronald and George 1992) by blocking the enzyme cytochrome p₄₅₀ in maize (Barta and Duka, 1991a). MG-191 is a highly effective EPTC safener to maize (Jabalonkai, 1991; Hulesh, and Duka 1993 and Jabalonkai and Hatzios 1994). Also the ABT safener can protect maize from EPTC injury (Barta and Duka, 1991b).

The objective of the present study is to evaluate the efficacy of new application method of the naphthalic anhydride as safener to corn seeds against injury caused by imazamethabenz-methyl, EPTC and metolachlor at three different rates of application. The study is also dealing with the evaluation of this safener against oxidative enzymes and total phenols isolated from corn plants in the presence or absence of herbicides.

MATERIALS AND METHODS

In greenhouse, maize seeds (*Zea mays* var., Triple Hybrid 327) were soaked for two hours in potassium salt of naphthalic anhydride (KNA) at different concentrations (0, 0.3, 3.0, 15 and 30mM) in 0.02M K-phosphate buffer (pH6.5 – 7.2) (Frear, *et al.*, 1991). Five treated seeds were planted in 7cm diameter plastic pot contains sandy soil. Five replicates (pots) per each concentration were used. Three herbicides from different groups at three different rates 1, 5, 10 fold of field rate were applied PPE, imazamethabenz-methyl from imidazolinone, methyl (+)-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-m-toluate, and methyl (+)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-p-toluate, (Assert 25% FL, 850 ml/F.); EPTC, from-thiocarbamate; s-ethyl-dipropyl- thiocarbamate (Eptam 67.97% EC, 2L /F.), and metalachlor, from chloroacetanilide, 2-chloro-6-ethyl-N-(2-methoxy-1-methylethyl)acet-o-toluidine (Dual 96% EC, 1L/F.). The pots were irrigated when needed. The corn seeds were left to grow for 14 days after herbicides treatment. Shoot and root length, shoot and root fresh and dry weight, oxidative enzymes (Polyphenoloxidase, PPO; and peroxidase) and total phenol contents were measured.

Determination of peroxidase activity: Shoot and root sample (0.25 g/each) were homogenized using polytrone with phosphate buffer pH 6.0. The homogenates were centrifuged at 4000 rpm for 15 min. The enzyme activity were determined according to Fehrmaum and Diamond (1967) where the reaction mixture included 1ml crud enzyme, H₂O₂ (20%) and catechol. The enzyme activity was expressed as increase of optical density at 470 nm.

$$\% \text{ Activity} = [(\text{Absorbance of treated sample} / \text{Absorbance of untreated}) \times 100]$$

Determination of Poly Phenol Oxidase (PPO) activity : Shoot and root sample (0.25 g/each) were homogenized, using polytrone in borate buffer, pH 9.0. The homogenates were centrifuged at 4000 rpm for 15min. The enzyme activity was determined according to the method of Broesch (1954) where the reaction mixture consisted of 2ml buffer 1 ml *P*-amino benzoic acid and catechol. The colors were measured at 575 nm after one hour incubation in shaking water bath at 40°C.

$$\% \text{ Activity} = [(\text{Absorbance of treated Sample} / \text{Absorbance. of untreated sample}) \times 100]$$

Determination of Poly Phenolic Compounds, Total Phenols: According to the method of McGrath, *et al.*, (1982) which modified by Sabra (1993), samples of corn shoots or root were dried and ground to powder material. Total phenol were extracted from samples (0.25g/each) with acidic methanol and the extract stirred in shaker at 250 rpm for 30 min, and then, the stirred product were centrifuged at 4000 rpm for 10 min. Estimation of total phenol method depend on the color resulted from the reaction between folin ciocalteu reagent and hydroxyl group of phenolic compound under alkaline condition. The absorbance were measured at 660 nm Standard curve were made by tannic acid as standard polyphenolic compound.

$$\mu\text{g /g D. W.} = [(\text{Absorbance} / K) (1 / 0.25)].$$

All data were statistically analyzed using L.S.D. (0.05) to compare the means, according to Cohrt software Inc, (1986).

RESULTS AND DISCUSSION

I- Effect on shoot and root length: All naphthalic anhydride (NA) concentrations caused significant increase in both shoot and root length (Figures 1 & 2). NA at 30nM gave the highest significant increase in both shoot and root lengths within herbicides concentrations. All herbicides concentration caused significant decrease in both shoot and root length in the absence of NA, except maize seedlings which treated with EPTC at field rate, gave 12.5% increase in root length. This finding may be due to the malformation as a toxic effect of the herbicide. NA succeeded to protect maize seedlings from phytotoxic effect. It reduced the percent of reduction

of both shoot and root lengths even when used 10 fold of field rate of the three herbicides. The results indicated that, imazamethabenz caused 75.82%

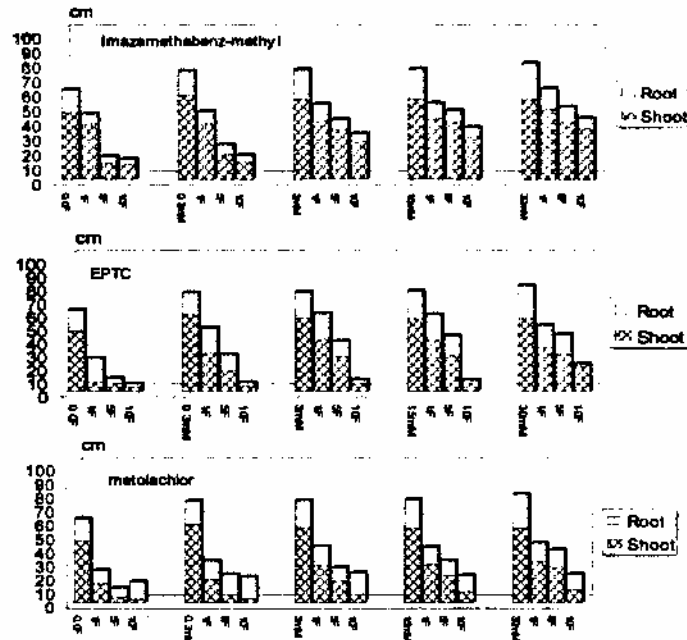


Figure 1. Effect of tested herbicides, naphthalic anhydride and their mixture on corn shoot and root length (cm) $LSD_{0.05} A*B$ for shoot = 2.63 and root = 2.71.

reduction in shoot length at the rate of 5F in the absence of NA, however, pre-treatment with 15 or 30mM of NA protect the seedling from that effect. The percentage of reduction became 14.28% in case of the treatment with 15 mM of NA, while 30 mM of NA caused 5.49% increase in shoot length. Also EPTC at 1, 5 and 10 F (fold of field rate) gave 84.62, 86.81 and 93.41% reduction in shoot length respectively, but when plants pre-treated with 30 mM (NA), these percentages of inhibition became 27.47, 38.46 and 58.24% in shoot length. Metolachlor follows the same trend, for example, 5 field rates reduced shoot length by 91.21%, but when plants pre-treated with 30 mM (NA), it reduced the shoot length by only 42.86% reduction. In addition, the effects of the three herbicides on root length followed the same trends, imazamethabenz, EPTC and metolachlor at 5 field rate gave 68.75, 75 and 56.25% decrease of root length respectively. In case of plants pre-treated with 30 mM of NA, the corresponding reduction became 12.5, 6.25 and 18.75%, respectively.

The effectiveness of herbicides or safener alone or their mixtures on corn seedlings clearly showed in Fig. 2, which represent the reduction in corn seedling due to different concentrations of different herbicides and how the safener protect the corn seedlings when their seeds were immersed in different concentrations of naphthalic anhydride.

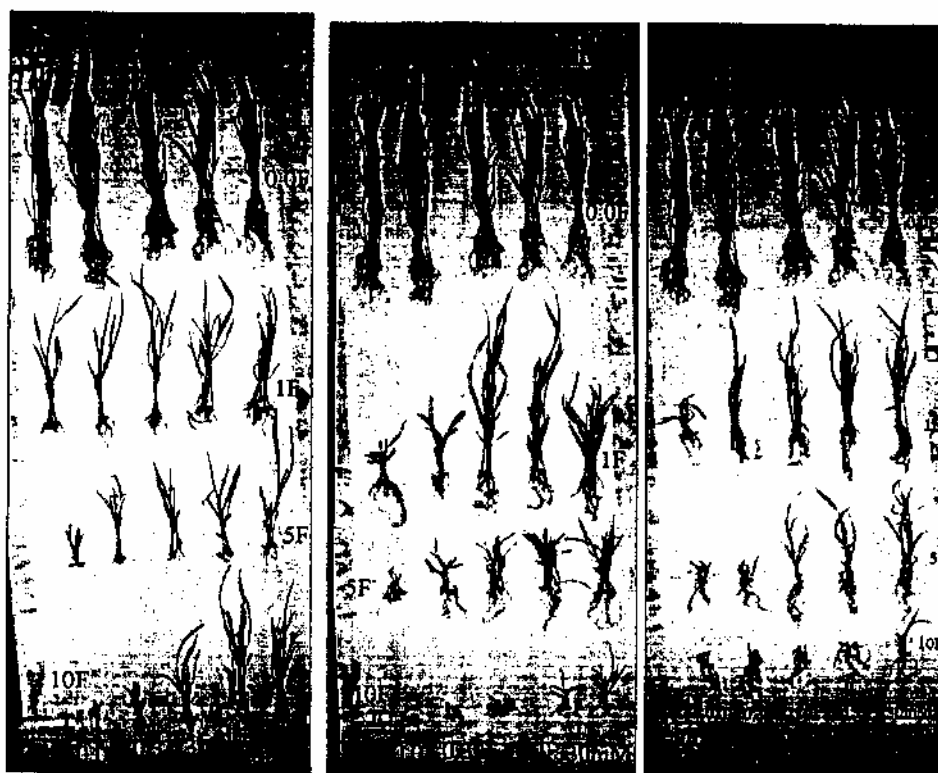


Figure 2. Effect of tested herbicides (I = Imazamethabenz - methyl, II= EPTC, III= Metolachlor) at 0.0, 1F, 5F, 10F; Naphthalic anhydride at (0.0, 0.3, 3, 15, 30 mM) and their mixtures on corn seedlings .

II- Effect on shoot and root fresh and dry weights: Tables (1 & 2), presented the effects on shoot and root fresh and dry weights. NA caused an increase or decrease in both shoot and root fresh and dry weight at all concentrations where the treatment with 0.3 mM of NA gave 27.38% increase in shoot fresh weight, 30 mM gave 140.46 and 92.25% increase in root fresh and dry weight, respectively. All herbicides at three application

31

Table (2): Effect of tested herbicides, naphthalic anhydride and their mixtures on root fresh and dry weights.

Treatments	Naphthalic anhydride concentrations (mM)									
	0		0.3		3		15		30	
	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight
0	0.3992	0.0516	0.6622	0.0728	0.8501	0.0737	0.8989	0.0869	0.9599	0.0992
Imazamethabenz										
1F	0.3568	0.0397	0.4561	0.0516	0.4971	0.0655	0.5207	0.0661	0.6308	0.0849
5F	0.2934	0.0543	0.3892	0.0599	0.4071	0.0756	0.6692	0.0860	0.7637	0.1128
10F	0.1658	0.0556	0.1965	0.0853	0.2159	0.0921	0.2698	0.1037	0.4841	0.1046
EPTC										
1F	0.8416	0.1229	0.9804	0.0711	0.7837	0.0854	0.8866	0.0853	0.5490	0.0849
5F	0.7151	0.0912	0.7059	0.0606	0.5956	0.0625	0.5550	0.0665	0.3551	0.0843
10F	0.4253	0.0719	0.5102	0.0301	0.6963	0.0303	0.1683	0.0368	0.1802	0.0301
Metolachlor										
1F	0.2469	0.0612	0.6717	0.0596	0.3963	0.0534	0.3896	0.0557	0.3957	0.0822
5F	0.3310	0.0610	0.6011	0.0640	0.3551	0.0710	0.3669	0.0707	0.3921	0.1152
10F	0.2754	0.0601	0.3219	0.0753	0.4037	0.0733	0.4458	0.0731	0.4541	0.1076
L.S.D _{0.05} (A*B) Fresh Weight = 0.112		L.S.D _{0.05} (A*B) Dry Weight = 0.009								

fold rates gave significant decrease in shoot fresh and dry weight. Imazamethabenz at 5 fold of field rate caused 67.07, 34.4; 26.5 and 23.06% decrease in both shoot and root fresh and dry weight, respectively, but when combined with naphthalic anhydride, it reduced the percentage of inhibition or turned it to significant increase. Treatment with 30 mM NA reduced the percent of inhibition to only 3.38% in shoot fresh weight, and increased its dry weight by 38.78%; and caused significant increase in root fresh and dry weight by 91.31 and 118.6%, respectively.

The results indicated that, EPTC and metolachlor followed the same trend, where EPTC at field rate caused 50.57 and 34.63% reduction in shoot fresh and dry weight respectively but combined with the lowest conc. of NA these percentage of inhibition became 12.05 and 1.87% for fresh and dry weigh respectively without significant different with untreated plants. Metolachlor at one and five field rate caused 60.3 and 69.25% reduction in shoot dry weight respectively, when combined with 30 mM NA, it caused 39.4 and 35.22% reduction respectively. In addition, EPTC alone caused significant increase in both root fresh and dry weight as a toxic effect of this herbicide, but when combined with (NA), this percentage of increase was decreased, for example 5F EPTC caused 76.74% increase but with 3 mM (NA), this percentage became 21.12%. Metolachlor at three field rates caused either increase in root dry weight or had no effect on fresh weight except 10 fold which caused 31.01% reduction in fresh weight, but when combined with NA, there was no significant differences with untreated plants.

III- Effect on oxidative enzymes:

1. Effect on peroxidase: Figure (3) showed the effect of the tested herbicides at the three application rates 1F, 5F, and 10F in absence and presence of NA on corn peroxidase activity in both shoot and root. NA alone caused significant reduction in the enzyme activity in both shoot and root systems, this observation was agreed with the finding of Hatzios (1989) Who found that, the safener reduced peroxidase activity in corn seedlings. In shoot, all herbicides caused significant reduction in the enzyme activity in absence of NA, but when plants pre-treated with NA, the percentage of reduction increased. For example, imazamethabenz at the field rate caused 29.36% reduction but when plants pre-treated with 0.3 mM NA, the percentage of reduction of peroxidase activity became 62.3%.

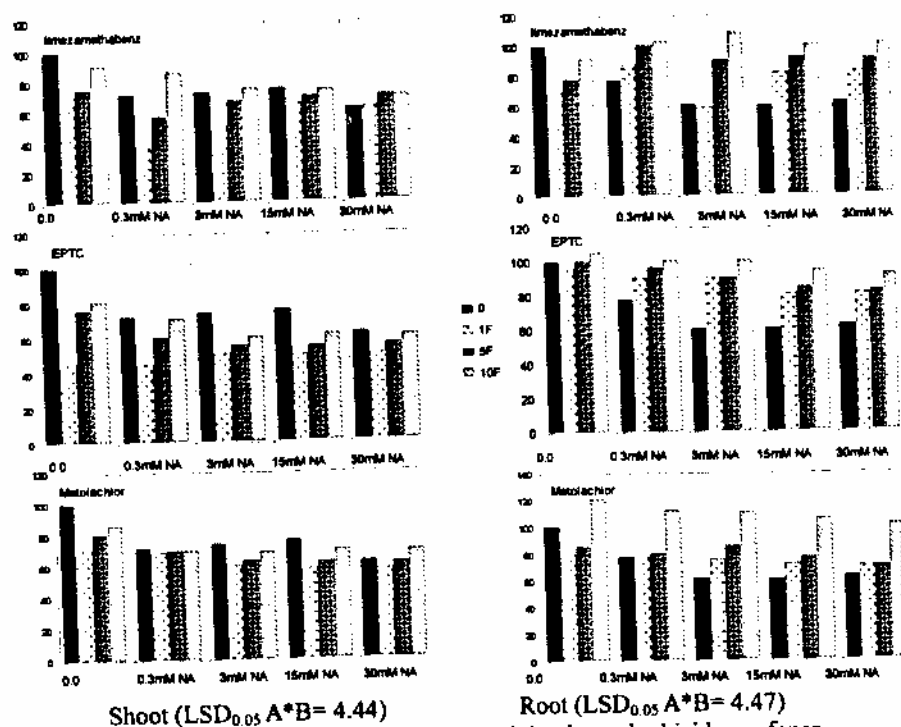


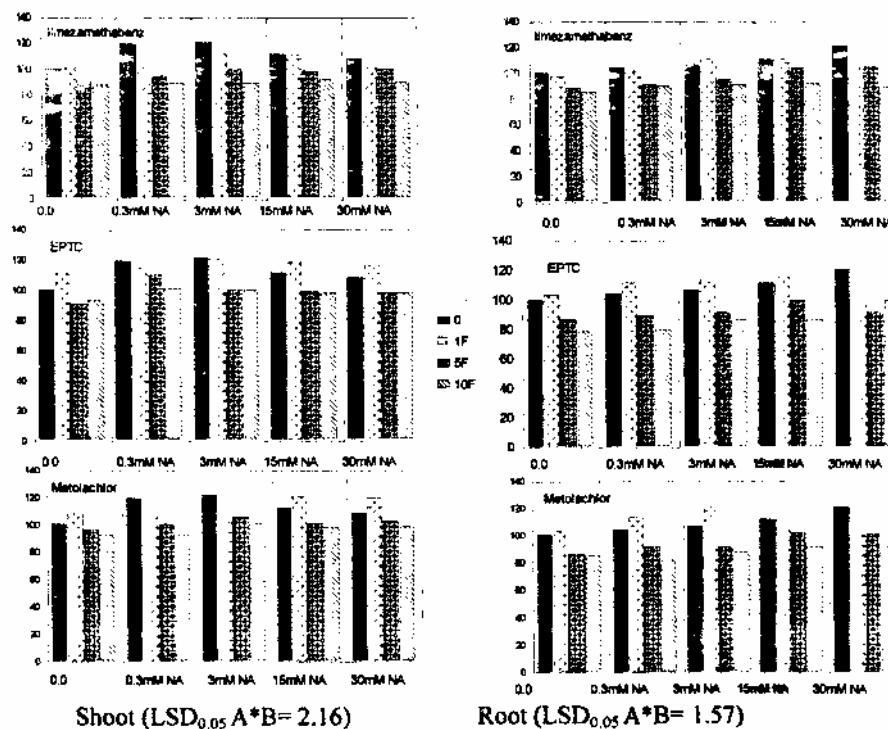
Figure 3. Affection of corn peroxidase activity due to herbicides, safener

Metolachlor at 5 field rate gave 19.11% decrease in shoot peroxidase activity, but when combined with 30 mM NA, it gave 39.4% reduction. Also, root peroxidase follow the same trend up to 5 field rate, but with 10 field rate, herbicide alone or with NA caused either significant increase or had no effect on the activity of peroxidase enzyme. For example, metolachlor at 10 field rate caused 20.21% increase in enzyme activity but with 15 mM NA it caused 5.54% increase.

The percent reduction in peroxidase activity increased at the low concentration of EPTC, but when combined with NA these percentages of reduction were increased. For example, 5 and 10 field rate caused 24.44 and 19.19% reduction in shoot peroxidase activity, respectively, but when combined with 30 mM NA it became 44.43 and 40%. In root, 10 field rate EPTC caused 5.15% activation in peroxidase activity but when plants pre-treated with 30 mM NA, it caused 9.05% reduction. This reduction counteracted the stimulatory effects of herbicide EPTC on the activity of

this enzyme in corn seedlings (Hatzios, 1989). Peroxidase metabolized EPTC to sulfoxide analog, which is more toxic than EPTC itself. Safener could prevent herbicide injury by inhibiting the metabolism of EPTC to its sulfoxide (Davies and Caseley, 1999). Thus, pre-treatment with safener caused inhibition in EPTC sulfoxidation in the presence of maize microsomal peroxidase.

2. Effect on polyphenol oxidase (PPO) activity and the total phenol contents: Figures (4 & 5) showed the following points, in general, all NA concentrations caused significant increase in the activity of PPO in both shoot and root. Vaughn *et al.* (1988) reported that, the safener NA stimulated the activity of polyphenol oxidase specially, chloroplast oxidase in corn. NA (3 mM) gave the highest increase in the activity of polyphenol oxidase in shoot by 21.5% while, 30 mM gave the highest increase in root by 20.51%, this increase is correlated with the content of total phenols. All NA conc. caused increase in its content, these increase either significant or without significant with untreated plants in both shoot and root.



Shoot ($LSD_{0.05} A*B = 2.16$) Root ($LSD_{0.05} A*B = 1.57$)
Figure 4. Affection of corn polyphenol oxidase (PPO) activity due to herbicides, Safener concentration and their mixtures.

Imazamethabenz at field rate, caused 2% reduction in PPO activity in root and had no effect on PPO in shoot, but when combined with NA at any conc., it caused significant increase in PPO in both shoot and root. For example, 1 field rate + 15 mM NA caused 10.12% and 10% increase in shoot and root respectively. EPTC and metolachlor at 5 and 10 field rate caused significant decrease in both shoot and root PPO activity. When combined with NA, it reduced the percent reduction or had no significant effect. This reduction leads to increase in total phenol in both shoot and root.

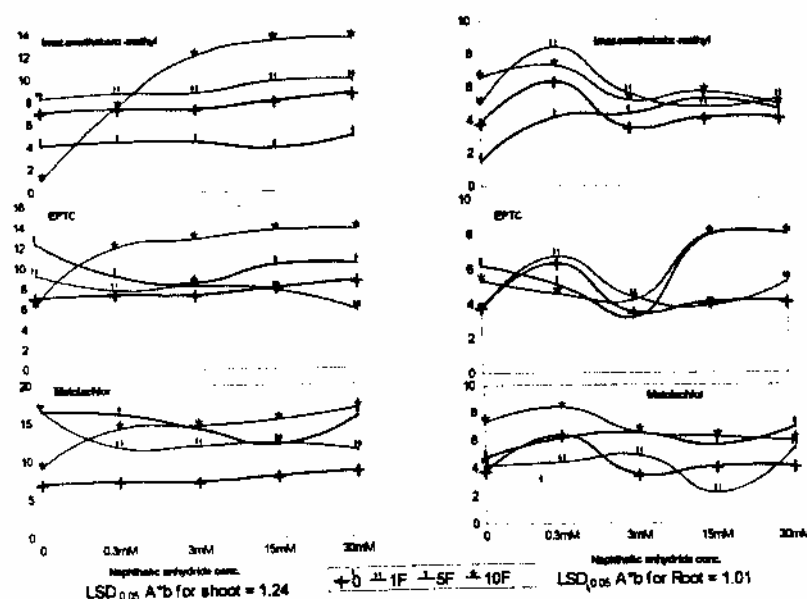


Figure 5. Effect of tested herbicides, safener, and their mixtures on polyphenol level in corn seedling.

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حماية البادرات الذرة في التربة الرملية من التأثير السام لبعض مبيدات الحشائش باستخدام
النفثالينك انهيدريد وتأثيره على أنزيمات الأكسدة

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تم عمل تجربة فى الصوبة الزراعية لحماية بادرات الذرة فى التربة الرملية من التأثير السام
لثلاثة من مبيدات الحشائش : إيمازاميثابنز-ميثل والابتام وميتولاكلور من ثلاث معدلات حقلية
وهى التركيز الحقلى وخمسة وعشرة أضعافه وذلك باستخدام طريقة جديدة للمعاملة بملح
البوتاسيوم من السرياق نفتالينك انهيدريد بأربعة تراكيزات مختلفة وهى 0.3, 3.0, 15, 30
ميكرومول ، وقد أحدثت هذه التراكيزات بمفردها زيادة فى طول المجموع الخضرى والجنرى
وكذلك فى وزنهما الرطب والجاف، كما نجح النفثالينك انهيدريد كنرياق فى حماية بادرات الذرة
من التأثيرات السامة لمبيدات الحشائش حتى خمسة وعشرة أضعاف التركيز الحقلى حيث أدت
إلى خفض نشاط أنزيم البيروكسيداز مع زيادة نشاط أنزيم البولى فينول أوكسيداز وكذلك محتوى
الفينولات.