Journal of Agricultural Sciences and Engineering
J. Agri. Sci. Eng.

Original Article: Cytogenetical Impact of Some Herbicides and Their Combinations on Maize (*Zea mays* L.) Seedling Root Tip Cells

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Citation Soliman M. El-Sagheer*, Sayed A. Ahmed, Gamal A. M. Abdu-Allah, Ahmed H. Ali , AbdEl-Mageed F. M. Ghanem. Cytogenetical Impact of Some Herbicides and Their Combinations on Maize (*Zea mays* L.) Seedling Root Tip Cells, Journal of Agricultural Sciences and Engineering, 2024, 6(1), 56-61

https://doi.org/10.48309/jase.2024.187128

ARTICLE INFO

Received: 2023-09-14

Accepted: 2023-12-24

Checked for Plagiarism: Yes.

Language Editor:

Fatimah Ramezani

Editor who approved publication:
Dr. Amin Baghizadeh

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Keywords:
Herbicides, Herbicide Combinations, *Zea mays*, Cytological Aberrations.

ABSTRACT

The extensive use of herbicides for associated weeds in maize fields may pose a threat through chromosomal aberrations in mitosis stages. In the present work, the effects of single herbicides and their combinations on germination, growth, mitotic activity, and mitotic aberrations were studied. The results indicated that the mitotic division rate was decreased in all treatments compared to the control. The most decrease in mitotic division was in (bromoxynil 20% + MCPA sodium 20%) and (bromoxynil 20% + MCPA sodium 20% + halosulfuron-methyl 75%). On the other hand, the most frequently occurred chromosomal aberrations were sticky metaphase and the observed aberrations were bridge, laggard and disturbed anaphase, and vacuolated nucleus. In general, treatments with these pesticides caused progressive changes in germination percentage and chromosomal aberrations in mitosis division for root tip cells in maize seedlings.

Introduction

Maize (*Zea mays* L.) is the third most important cereal crop grown worldwide, and has a significant impact in the global agricultural economy [1-4]. The total cultivated area of maize in Egypt in 2018 was about 2.5 million feddans [5]. Maize serve food for and feed to livestock, it can grow all year round [6, 7]. In general, mode of action of some herbicides depend on the effect on metabolism and therefore photosynthesis, lipids biosynthesis, pigments, and growth regulators inhibitors [8, 9]. Usage of herbicides in crop production may cause a risk to plants by causing cytological abnormal changes in cells [10, 11]. On the other hand, herbicide application for weed control may cause certain degree of phytotoxicity in maize plants [12]. Despite the fact that herbicides are present in some cases when crop seeds are germinated, studies on the impact of these herbicides have not been conclusively carried out [13]. Therefore, this study aimed to evaluate the effect of single and

combined herbicide formulations on maize seed germination based on germination percentage and some vegetative characteristics of seedling: radical and plumule lengths and mitotic stages abnormalities.

Materials and methods

This study was carried out in the Weed Science Laboratory of Plant Protection Department, Faculty of Agriculture, Assiut, Al-Azhar University, Egypt.

Maize grains

Single cross hybrid cultivar (Watanya 6) as a test plant was used. The seeds were obtained from Watanya Company for seed production, Giza, Egypt. Maize seeds used were surface sterilized using 5% sodium hypochlorite for five minutes, and then washed three times with sterilized distilled water to prevent contamination.

Herbicides

The common, trade names and rates of application to the herbicides used in experiments are presented in Table (1).

Seed germination and growing maize seedling

Fifteen seeds from each accession of maize were germinated on two cut sheets of Whatman filter paper moisten with distilled water in 15 cm inner diameter petri dishes and 5 ml were added daily in all treatments at 24 ± 1 °C in the laboratory, with three replicates and control. When radicles root germination with long (≥ 1 mm), the lengths of root and shoot were measured all tested treatments. At the end of the germination period, five randomly seedlings were taken from each Petri dish and measured. The observation and data collection were recorded for germination percentage ([14-16] as follow:

Germination percentage (GP) = (Number of germinated seeds / Total Number of seed tested) X100.

Radical and plumule length (cm).

Cytological study

For cytological investigation, five radicals per each treatment were washed with distilled water and fixed in 1:3 glacial acetic acid: ethanol for 24 hours and stored in 70% ethanol at 4 °C until used. Treated and non-treated germinated fixed seeds were prepared using Feulgen squashing method [17]. Observations were done using objective X40, on Euromex light microscope and photographed used Canon kiss4 digital camera.

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS V.23 software. Data recorded were subjected to a one-way analysis of variance to explain differences between herbicide treatments by days. The mean value of treatment was compared with the least significant difference (LSD) at $p < 0.05$.

Results and Discussion

Seed germination and growing seedling conditions

Seed germination

The seeds were germinated in different concentrations of six herbicides such as (carfentrazon 1.5% + florasulam 0.5% + flurxypyr-methyl 14%), (tribenuron-methyl 16% + carfentrazon ethyl 12%), (bromoxynil 20% + MCPA sodium 20%), (halosulfuron-methyl 75%), (nicosulfuron 6%), and (foramsulfuron 2.25%).

Data are listed in Table (2). Germination percentages were decreased in all treatments in respect to the control. The least germination percentage (43.3%) was recorded in (Halosulfuron-methyl 75%) treatment followed by (bromoxynil 20% + MCPA Sodium 20%) with percentage (46.6%). On the other hand, (nicosulfuron 6%) and (foramsulfuron 2.25%) with (93.3%) showed slightly non-significant decrease in germination percentage compared control. In other trials, interaction of maize seed quality with herbicide treatments at the recommended rates was minor compared with poor seed quality [18]. The obtained results agree with previous studies on the effect of a fungicide on seed germination of monocot and dicot plants such as, *Zea mays* [19], *Triticum aestivum* [20], and *Vigna mungo* [21]. Decreasing of germination percentage indicate that Halosulfuron-methyl 75% and bromoxynil 20% + MCPA Sodium 20% have inhibitory or lethal effect on maize embryo.

Radical and plumule length

Radical and plumule lengths were decreased in all treatments compared to control. In root, the highest decrease was in (bromoxynil 20% + MCPA Sodium 20%) with (3.33) mm, while the lowest decrease was in (nicosulfuron 6%) and (foramsulfuron 2.25%) with (12.66) mm compared with (14) mm. in the control. In shoot, the highest decrease was in (bromoxynil 20% + MCPA Sodium 20%) with (8.33) mm, while the lowest decrease was in (nicosulfuron 6%) and (foramsulfuron 2.25%) with (34.33) mm compared with (35.8) mm in the control. The primary roots of maize seedlings were found to be affected at low concentrations of herbicides and thus delayed seedling growth as a result of premature root tip injury caused by the effect of herbicides [22].

Cytological study

All tested herbicide treatments decreased mitotic activity less than the control. The mitotic index in maize was decreased with increasing concentration of alachlor herbicide [23]. Chromosomal aberrations in mitotic division were observed as presented in Figure (1). The most aberrations were: sticky metaphase, bridge, laggard and disturbed anaphase, and vacuolated nucleus in (halosulfuron-methyl 75%), (bromoxynil 20% + MCPA sodium 20%), (carfentrazon 1.5% + florasulam 0.5% + flurxypyr-methyl 14%), (tribenuron-methyl 16% + carfentrazon ethyl 12%), (nicosulfuron 6%), and (foramsulfuron 2.25%) treatments, respectively. Sticky chromosome may be caused due to distort DNA's double helix structure and chromosome orientation [24]. Bridge, laggard and disturbed anaphase were observed. Bridge and laggard chromosome may be caused due to distorted of spindle fibers [25]. In general, treatment with the tested herbicides caused progressive changes in germination percentage and chromosomal aberrations in mitosis in root tip cells of maize seedlings. The effects of the tested herbicides on mitotic activity might be due to a decrease in the number of cells beginning DNA synthesis and subsequently a large number of cells lose their ability to undergo further division. Herbicides which disrupt cell division can inhibit cell division or mitosis.

Table 1. Common, trade names, rate/ feddan, and importers/ distributors of the used herbicides

Common name	Trade name	Rate/ Fed	Importers and Distributors
(Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-meptyl 14%)	Frosty 16% SE	300 ml	Starchem for pesticides
(Tribenuron methyl 16% + Carfentrazon ethyl 12%)	Foldex 28 % WP	35 g	
(Bromoxynil 20% + MCPA sodium 20%)	Rondo 40% SP	600 g	
(Halosulfuron-methel 75%)	Inpul 75% WG	20 g	
(Nicosulfuron 6%)	Active 6% SC	400 ml	Mecca for agricultural development
(Foramsulfuron 2.25%)	Equip 2.25% OD	750 ml	Cairo chemicals

Table 2. Germination (%), root and shoot length to maize seedling

Treatments	Germination%	Root Length (mm)	Shoot Length (mm)
(carfentrazon 1.5% +florasulam 0.5% + fluroxypyr-meptyl 14%)	80±a	12.33±a	27.66±b
(tribenuron methyl 16% +carfentrazon 12%)	80±a	9.33±b	22.66±c
(bromoxynil 20% + MCPA sodium 20%)	46.66±b	3.33±d	8.33±e
(halosulfuron-methel 75%)	43.33± b	5.66±c	15±b
(nicosulfuron 6%)	93.33±a	12.66±a	34.3±3a
(foramsulfuron 2.25%)	93.33±a	14 ±a	34.33±a
Control	96.66±a	14.66±a	35.33±a
LSD	15.60	2.21	2.37

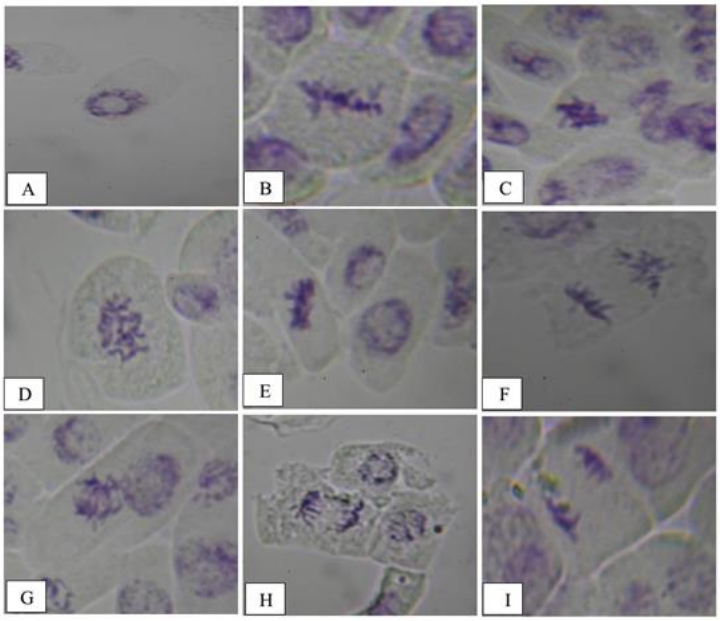

Figure 1. Chromosomal aberrations and nuclear anomalies by single and combinations of herbicides in root tip cells of maize: A: vacuolated nucleus, B: sticky and laggard chromosome in metaphase, C: disturbed metaphase, D: C-metaphase, E: diagonal metaphase, F: disturbed anaphase, G: bridge and laggard in anaphase, H: bridge in anaphase, and I: diagonal telophase.

Conclusion

To sum up, the cytological changes induced by herbicides are expressed by structural changes in chromosomes and chromatids, called chromosomal aberrations, such as breaks, deletions, inversions, gaps, translocations, rings and other disturbances stickiness, clumping and erosion, as well as mitotic index decreased proportionally with herbicide with increasing exposure time [26]. Many abnormal mitotic figures were observed in all mitotic phases [27]. Accordingly, it is concluded that, (Halosulfuron-methyl 75%) followed by (bromoxynil 20% + MCPA Sodium 20%) affected seed germination, radical and plumule length and have genotoxic effect to *Zea mays*. Therefore, it is recommended to use these types of herbicides with maize crop because these may reduce productivity or cause undesirable mutations.

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