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Effect of Post-emergence Application of Dichlorophenoxy acetic acid (2,4-D) Herbicide on Growth and Development of three Weeds Associated with Maize plant growth

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ABSTRACT

Post emergence application of 2,4-Dichlorophenoxyacetic acid (2,4-D) as a herbicide even at low concentrations showed an effective control on three weeds (slender amaranth, goosefoot and prickly burweed) associated with maize growth. 2,4-D was applied at 0.0, 125, 250, 500 and 1000 ppm. 250 ppm 2,4-D treatment significantly decreased numbers and fresh weights of the three weeds by 50% approximately. At 1000 ppm 2,4-D, there were sever reductions in weeds numbers, fresh weights and photosynthetic pigments contents of the three weeds. Maize was undamaged by any tested 2,4-D treatment. Fresh weights and photosynthetic pigments contents of maize were favored significantly after application of 2,4-D on the associated weeds at all tested rates.

Key words: Maize, Weeds, Chemical control, Herbicides, Phenoxy herbicides, 2,4-Dichlorophenoxy acetic acid (2,4- D), Auxins.

Introduction

Maize (*Zea mays L.*) is the second most important cereal crop in Egypt in terms of total food production. It is grown for fodder as well as for grain. The grains of maize are used in a variety of ways by the human beings. Weeds are a major problem in crop production and drastically decrease crop yield. Therefore, weed control is an important management practice for maize production that should be carried out to ensure optimum grain yield. Yield losses due to weeds have been reported up to 35 % (Oerke, 2005; Dangwal, *et al.* 2010). Thus, in maize production, it is necessary to undertake control of weeds which cause losses of maize grain yield. The use of herbicides may reduce such losses, as herbicides may reduce the weed infestation provided that the herbicide-treated weeds are not herbicide resistant. Prior to the widespread use of chemical herbicides, cultural controls, such as altering soil pH, salinity or fertility levels were used to control weeds.

Weed is a common term for any wild plant, particularly an undesired plant, growing in cultivated ground, where it competes with crop plants for soil nutrients, light and water.

Slender amaranth (Amaranthus viridis), goosefoot (Chenopodium album) and prickly burweed **(***Xanthium spinosum*) are troublesome broad leaved weeds associate with maize growth (Malik *et al.* 2006). *Amaranthus viridis* is a cosmopolitan weed belong to family amaranthaceae, *Chenopodium album* is a fast-growing weed belong to family chenopodiaceae , whereas .*Xanthium spinosum* is a noxious weed in asteraceae family.

Most herbicides are applied as water-based sprays using ground equipment. Some herbicides are synthetic mimics of natural plant hormones. Herbicides are weed killers used to kill unwanted plants (Kellogg *et al.*, 2000). Malik *et al.* (2006) reported that herbicides proved effective in controlling weeds and produced relatively more weight of cobs of maize, number of grains/ cob, grain weight, biological yield and grain yield. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Herbicide use has undoubtedly contributed to crop yield increases and the efficiency of production (Andrew, 2012).

Some substantial benefits can be gained through the use of herbicides to manage unwanted vegetation. If herbicides are not used properly, damage may be caused to crop plants, especially if too large a dose is used, or if spraying occurs during a time when the crop species is sensitive to the herbicide. Unintended but economically important damage to crop plants is sometimes a consequence of the inappropriate use of herbicides (Robert, 1941 and Mehmeti, 2004). Herbicides have different modes of actions on enzymes (catalysts of biological reactions), this means that the target enzymes may no longer function properly, or at all. Herbicide distort enzymes molecules in some way, so component chemicals for the reaction accumulate and may be directly or indirectly damaging. Absence of the reaction's products will restrict growth, either through starvation of key building blocks or because the reaction makes chemicals which normally protect the plant (Santra and Baumann, 2008).

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Auxin is a plant growth regulator that controls cell enlargement, division and plant development through the plant life cycle. Over decades, the so-called auxin herbicides had resisted all efforts to elucidate their molecular interactions and the biochemical and physiological basis of their phytotoxicity (Cobb, 1992).

Auxin herbicides started a new era of weed control in modern crop production due to their systemic mobility in the plant and to their selective action, preferentially against dicot weeds in cereal crops (Cobb, 1992 and Grossmann, 2007).

Auxin herbicides mimic the action of the main auxin, indole-3-acetic acid (IAA) in higher plants (Cobb, 1992). However, they are long-lasting, particularly due to their higher stability in the plant, and, therefore, more effective than IAA. Auxins stimulate a variety of growth and developmental processes when present at low concentrations at the cellular sites of action. However, with increasing concentration and auxin activity in the tissue, growth is disturbed and the plant is lethally damaged (Grossmann, 2003).

At the present time, nobody really knows precisely how the auxin-like herbicides kill susceptible plants. As with most effects of plant hormones, it probably has a lot to do with the plant species. In general, grasses are much less sensitive to synthetic auxin herbicides than are dicots. That is, a much higher threshold level of auxin-based herbicide is required to elicit physiological responses in grasses versus the so-called "broadleaf" plants. So, for example, at the doses used to kill dandelions, grasses are largely unaffected (Cobb, 1992 and Grossmann 2003). Auxin-based herbicides are referred to as "selective" herbicides because they kill so-called "broadleaf" plants (dicots) but not grasses, hence, that's why they're popular herbicides with growers of lawns.

Phenoxy herbicides are structural and functional analogs of the natural auxin indole-3-acetic acid (IAA). Although they both look and act like auxins, plants cannot metabolize these phenoxy herbicides as they can with the natural auxin (IAA). Phenoxy herbicides work by binding to some or all of the same sites in the plant cells as naturally occurring IAA. While naturally occurring IAA concentrations in the cell are highly regulated, when phenoxy herbicides are applied, plant growth becomes deregulated. This deregulation causes twisting, thickening and elongation of leaves and stems and eventually leads to plant death as the plant cannot mobilize reserves, repair or function properly (Song, 2014).

The phytotoxic effect of phenoxy herbicides is basically caused by high concentrations of biosynthesized abscisic acid (ABA) which may lead to stomatal closure and inhibition of transpiration and CO_2 assimilation (Grossman, 2000). These effects are followed by foliar senescence with chloroplast damage and by the destruction of membrane and vascular system integrity and plant death (Fedtke and Duke, 2005).

2,4-D is a synthetic auxin, it is a member of the phenoxy family of herbicides. It is absorbed through the leaves and is translocated to the meristems of the plant. Uncontrolled, unsustainable growth ensues, causing stem curl-over, leaf withering, and eventual plant death. 2,4-D mimics the plant hormone auxin and sets off a complex series of events involving two other plant hormones that eventually lead to the death of susceptible plants. At hormonal levels, auxins affect plants but not people (Grossmann, 2007).

Spraying 2,4-D on susceptible plants kill them and it simply confuses the plant to death (Grossmann, 2007 and Song, 2014). 2,4-D is a common systemic herbicide used in the control of broadleaf weeds. It is one of the most widely used herbicides in the world. It was known to be a selective post emergence herbicide primarily used to weed control. The first publication of 2,4-D's herbicidal activity was reported by Hamner and Tukey (1944) and Quastel (1949). It revolutionized weed control, as it was the first compound that, at low doses, could selectively control dicots broadleaf plants, but not most monocots narrow leaf crops like wheat, maize (corn), rice, and similar cereal grass crops (Andrew *et al.,* 2010). 2,4-D generally has low toxicity for humans (Aylward, 2010).

Methodology

A pot experiment was carried out to examine the effect of foliar application of 2,4-Dichlorophenoxyacetic acid (2,4-D) as a herbicide on the growth of three weeds (slender amaranth, goosefoot *and* prickly burweed) which grow associated with maize plant. 2,4-D was applied at different concentrations (0.0, 125, 250, 500 and 1000 ppm). Maize and weeds were grown in pots (40 cm in diameter) of 15 kg of clay loamy soil. Fifteen seeds of each weed were sown with ten seeds of maize. 2,4-D was sprayed on weeds with a hand sprayer after 2 weeks of sowing and repeated after one week. Precautions were taken to avoid damage of maize plant. There were three replicates of each treatment of 2,4-D. Irrigation was done regularly to maintain soil field capacity. The effect of 2,4-D herbicide was measured after five weeks of sowing by counting of slender amaranth, goosefoot and prickly burweed numbers (plants/pot) and estimation of fresh weights of each weed (g/ 3plants). Also, fresh weight (g) of maize plant was estimated after 2,4-D application on the associated weeds. Chlorophyll a, b and carotenoids contents in the fresh leaves of weeds and maize (mg/g. F.W) were determined in samples according to Metzener *et al.*, (1965). Data were statistically analyzed by Snedecore and Cochran (1981) method.

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Results

Effect of 2,4-D application on numbers and fresh weights of slender amaranth, goosefoot and prickly burweed plants.

Tables (1 and 2) indicate that, 2,4-D herbicide controlled the number and the fresh weights of slender amaranth, goosefoot and prickly burweed plants even at low rates (125 ppm). 250 ppm 2,4-D treatment led to 50% reduction in plant numbers and plants fresh weights. Few plants of the slender amaranth, goosefoot *and* prickly burweed plants survived at 1000 ppm 2,4-D, but they were very small and damaged.

Table 1: Effect of 2,4-D application on numbers of slender amaranth, goosefoot and prickly burweed plants.

2,4-D (ppm)	Plant numbers/pot		
	Slender amaranth	goosefoot	Prickly burweed
0.0	15	15	15
125	13.6	12.3	13.3
250	11.3	9.6	10.6
500	9.3	7.3	9.3
1000	3.3	4.6	5.3
L.S.D. at 5%	0.51	0.92	0.82
L.S.D at 1%	1.30	1.83	1.73

Table 2: Effect of 2,4-D application on fresh weights of slender amaranth, goosefoot and prickly burweed plants.

2,4-D (ppm)	Fresh weights (g/ 3 plants)		
	Slender amaranth	goosefoot	Prickly burweed
0.0	5.56	6.01	5.09
125	3.78	5.13	3.42
250	2.23	3.88	2.33
500	1.78	2.03	1.58
1000	0.84	0.91	0.77
L.S.D. at 5%	0.063	0.067	0.059
L.S.D at 1%	0.081	0.091	0.078

Effect of 2,4-D application on photosynthetic pigment contents of slender amaranth, goosefoot and prickly burweed plants.

The data in tables (3,4,5) clarify that, chlorophyll a, b and carotenoids (mg/g.f.wt) contents of 2,4-D sprayed weeds were considerably diminished compared with untreated control weeds and the diminishing effect of 2,4-D on photosynthetic pigments contents was in concomitant trend with the growth parameters in response to 2,4-D treatments. Higher concentrations of 2,4-D seriously affected photosynthetic pigments contents of the three weeds. The lowest pigments contents were achieved by the highest concentration (1000 ppm) 2,4-D.

Table 3: Effect of 2,4-D treatments on chlorophyll "a" content (mg/g. fresh weight) in leaves of slender amaranth, goosefoot and prickly burweed.

2,4-D (ppm)	Chl. a (mg/g. f.wt)		
	Slender amaranth	goosefoot	Prickly burweed
0.0	3.753	3.971	2.953
125	3.013	3.123	2.222
250	2.345	2.133	1.791
500	1.326	1.544	0.987
1000	0.439	0.533	0.418
L.S.D. at 5%	0.035	0.052	0.035
L.S.D at 1%	0.057	0.083	0.046

Table 4: Effect of 2,4-D treatments on chlorophyll "b" content (mg/g fresh weight) in leaves of slender amaranth, goosefoot and prickly burweed.

2,4-D (ppm)	Chl. b (mg/g. f.wt)		
	Slender amaranth	goosefoot	Prickly burweed
0.0	2.53	2.83	2.31
125	1.66	2.06	1.62
250	1.01	1.33	1.01
500	0.71	0.51	0.81
1000	0.029	0.027	0.033
L.S.D. at 5%	0.033	0.017	0.055
L.S.D at 1%	0.047	0.029	0.071

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Table 5: Effect of 2,4-D treatments on carotenoids content (mg/g fresh weight) in leaves of slender amaranth, goosefoot and prickly burweed.

2,4-D (ppm)	Carotenoids (mg/g.f.wt)		
	Slender amaranth	goosefoot	Prickly burweed
0.0	1.75	1.49	1.56
125	1.05	1.07	1.09
250	0.96	0.90	0.72
500	0.71	0.57	0.44
1000	0.36	0.31	0.27
L.S.D. at 5%	0.021	0.019	0.051
L.S.D at 1%	0.041	0.028	0.073

Estimation of maize fresh weight (g) and photosynthetic pigments contents (mg/g. f.wt) of maize leaves after 2,4-D application on its associated weeds.

Table 6 elucidates that, maize fresh weights (g), chlorophyll a, b and carotenoids (mg/g.f.wt) contents of maize were significantly increased after application of 2,4-D on its associated weeds.

Table 6: Maize fresh weight (g), chlorophyll a, b and carotenoids contents (mg/g.f.wt) of maize leaves after 2,4-D application on its associated weeds.

2,4-D (ppm)	F.wt. (g)	Photosynthetic pigments (mg/g. f.wt)		
		Chl. a	Chl. b	Carotenoids
0.0	6.09	4.45	2.14	1.02
125	6.87	5.01	2.54	1.31
250	9.11	5.97	3.01	1.52
500	10.62	6.11	3.62	1.84
1000	11.23	7.62	4.33	2.120
L.S.D. at 5%	0. 55	0.035	0.052	0.035
L.S.D at 1%	0.82	0.057	0.083	0.046

Discussion

The primary mechanism of herbicide action is assumed to be a consequence effect of increasing the endogenous auxin concentrations to supraoptimal and extensive perception by auxin receptors (Dharmasiri *et al.* 2005 and Badescu, 2006). However, recent findings have provided important clues support the idea that plant death may occur as a result of a combination of factors. First off, one of the well-know effects of excess amounts of auxin on dicots is to cause them to overproduce the plant hormone ethylene. The excess production of ethylene may result in a number of plant responses, including epinasty and senescence. Chae (2005) reported that ethylene is a factor in defoliation caused by 2,4-D. Because plants can't break down 2,4-D, it's action persists. Another effect of excess ethylene production in response to 2,4-D is to stimulate the production of yet another plant hormone, abscisic acid (ABA). Auxin herbicides led to overproduction of ABA which consequently led to deleterious effects and contribute to eventual plant death (Grossmann, 2003).

According to the results, the reducing effect of 2,4-D on slender amaranth, goosefoot *and* prickly burweed plants may be attributed to stimulation of ethylene production which trigger the overproduction of abscisic acid (ABA), causing its accumulation. Abdullah *et al.,* (2007) presented similar results and concluded that numbers and fresh weights of weeds were significantly decreased by 2,4-D application. Accumulated ABA in the shoot tissue is translocated within the plant and mediates events in the auxin herbicide syndrome, such as growth inhibition, tissue decay and plant death. The recent identification of receptors for auxin perception and the discovery of a new hormone interaction in signalling between auxin, ethylene and the upregulation of abscisic acid biosynthesis account for a large part of the repertoire of auxin-herbicide-mediated responses, which include growth inhibition, senescence and tissue decay in sensitive dicots. Stimulation of ethylene biosynthesis plays a key role in eliciting the herbicidal symptoms in sensitive plants (Grossmann, 2010). Auxin-induced abscisic acid (ABA) accumulation has been observed in a variety of dicot species. Also, induction of ABA and its accumulation was demonstrated for auxin herbicides from the different chemical classes of IAA in a variety of dicot species (Grossmann, 2003 and Kepinski *et al*., 2005)**.** Exemplified, auxins trigger de novo ABA biosynthesis in the highly-sensitive dicot cleavers (*Galium aparine*) (Hansen and Grossmann, 2000). Auxin herbicides and IAA are directly able to trigger activation of NCED *(*9-cis-epoxycarotenoid dioxygenase*) gene,* which in turn participate in ABA biosynthesis and accumulation (Hamner and Tukey 1944). In addition, NCED gene expression and activity appears to be stimulated by auxin-induced ethylene, leading to lasting ABA biosynthesis (Sterling and Hall, 1997) and Kraft *et al*.**, (**2007). Taylor *et al*., (2005) reported that, ABA biosynthesis is induced in the shoot tissue of Arabidopsis by increasing xanthophyll cleavage, leading to increased production of the ABA precursor, xanthoxin. Xanthoxin production which is a key regulated step in

the ABA pathway is catalyzed by the plastid enzyme NCED (9-cis-epoxycarotenoid dioxygenase), which is encoded by a family of NCED genes. NCED genes were assumed to be regulated by auxin treatment. Auxin treatment enhanced NCED precursor supply, hence, contribute consequently to ABA accumulation (Symonds *et al.,* 2005; Raghavan *et al.,* 2006). Walsh *et al*., (2006) and Grossmann, (2007) reported that, 2,4-D treated Arabidopsis showed that, expression of NCED genes are involved in biosynthesis and accumulation of abscisic acid.

The drastic effect of 2,4-D on photosynthetic pigments contents of the three weeds may led to the reduction in their fresh weights. The results are in consistence with the findings of Wrong and Chang (1988) who reported inhibition of growth, photosynthesis and chlorophyll a,b synthesis of the fresh water green alga *Chlamydomonas reinhardti* in the presence of high concentrations of the herbicide 2,4-D. Herbicide diverts the flow of sunlight energy which is captured by chlorophyll pigments and transferred as electrons through 'Photosystem I', resulting in the production of highly reactive free radicals which very quickly destroy cell membranes, spilling the contents, and appearing yellow and desiccated. Also blocks the transfer of energy through 'Photosystem II'. Herbicides may bind to a protein involved in the transfer chain, reducing its effectiveness. This causes some diversion of electrons interfere with an enzyme involved in producing chlorophyll and other large molecules important in photosynthesis. Without new chlorophyll leaves yellow and photosynthesis slows. However, unused building blocks of chlorophyll accumulate and react with oxygen to form reactive free radicals which are especially destructive to broadleaved species, prevents the production of leaf pigments called carotenoids (Powles and Preston, 2006).

The increasing of maize fresh weight and photosynthetic pigments content of maize leaves after 2,4-D application on its associated weeds may be attributed to the controlling of weed growth and decreasing competition between maize and its associated weeds on soil nutrients, water and light. Also the undamaging of maize may be attributed to its resistance. This agrees with Abdullah *et al.* (2007) who reported that, 2,4-D application on associated weeds strongly favored the aboveground biomass of maize crop. Also, these results were in are in conformity with the findings of Khan and Haq (2004), Bogdan (2007) and Dangwal, *et al.* (2010) who recorded a strong enhancement of photosynthetic pigments content (mg/g.f.wt) of maize crop after 2,4-D application on its associated weeds. Grasses are more resistant to such herbicides because of differences in leaf morphology, translocation of the herbicide inside the plant, and the ability to metabolize (breakdown) synthetic auxins (Mehmeti and Demaj, 2010).

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