THE EFFECTS OF SULTAN 70 WG ON STOMATAL FUNCTION

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ABSTRACT
Sultan 70 WG that belongs to Sulphonylurea (SU) herbicides was used in this study. Purpose of the study was to determine the effects of Sultan 70 WG which is one of the new herbicides in last five years on stomatal function. Both microscopically and biochemically parameters were measured as interested to stomata openning and closing. The effect of this herbicide was investigated as to the aperture of stomata of leaf tip and base compared. Chlorophyll and total protein amount, peroxidase activity (PO) were determined at the end of biochemical studies. Also guard cell protoplast isolation and purification were done and K^+ content of these cells were established. Length and fresh-dry weight analysis belonging to control and herbicide-applied plants were taken. The effects of Sultan 70 WG on leaves especially stomata were tried to be recognized with this work.

Introduction

Recently, use of pesticides for different aims in our life has increased. Some kind of pesticides are also used to kill weeds which have damaged cultural plants and these are named herbicides. Herbicides are chemicals that inhibit or interrupt normal plant growth and development. They are widely used in agriculture, industry and urban areas to control weeds. Herbicides kill plants in different ways. A herbicide must meet several requirements in order to be effective. It must 1) contact the target weed, 2) be absorbed by the weed, 3) move to the site of action in the weed, and 4) accumulate sufficient levels at the site of action to kill the plant. Herbicides may be classified according to selectivity (nonselectiv, grass control, broadleaf control, etc.), time of application (preplant incorporated, preemergence, or postemergence), translocation in the plant (contact or systemic), and mechanism of action. Herbicide selectivity may be based on herbicide placement, or differential spray retention, absorption, translocation, metabolism, or site exclusion of the herbicide in the plants.

Herbicides can work at various sites in plants. They generally interfere with a process essential for normal plant growth and development. Herbicides can be classifield by seven different mechanisms of action based on how they work and the injury symptoms they cause. Descriptions of each mechanism of action follow;1. Growth Regulators (Phenoxiex, Benzoic Acids, Pyridines), 2. Seedling Growth Ýnhibitors (Thiocarbamates, Acid Amides, Dinitroanilines), 3. Photosynthetic Inhibitors (Triazines, Phenylureas Uracils, Benzothia diazoles, Nitriles), 4. Amino Acid Synthesis Inhibitors (Sulfonylureas, Imidazolinones, Amino Acid Derivatives), 5. Lipid Synthesis Inhibitors (Aryloxyphenoxypropionates, Cyclohexanediones), 6. Cell Membrane Disrupters (Diphenylethers, Bipyridiliums), 7. Pigment Inhibitors (Isoxazolidinones) (1).

The primary kinds of herbicide formulations are: solution, soluble powder, emulsifiable concentrate, wettable powder, liquid flowable, dry flowables and water-dispersible granules, granules and pellets. Typically, pure herbicide molecules are of limited value to the end user. To give them practical value and make them usable, most herbicides are combined with appropriate solvents or surfactants to form a product called a *formulation*. Herbicides are available as formulations and rarely as the pure chemical. In addition, a given chemical may be formulated in a variety of differing formulations and sold under different trade names. Formulations vary according to the solubility of the herbicide active ingredient in water, oil and organic solvents, and the manner the formulation is applied (i.e., dispersed in a carrier such as water or applied as a dry formulation itself) (2).

Although herbicides have been used widespread, in the last a few years it has been expressed by crop scientists that weeds resistant to the herbicide have emerged (3).

In the last years, it is suggested that the using of pesticides and herbicides can minimize in the gardens and houses. In the gardens, instead of using chemical pesticides and herbicides to remove insect pests and weeds, consider the some alternatives. These; a) use natural predators to get rid of insects. For instance, ladybugs, several bird species and bats all eat insects. County extension services, nurseries or garden associations can offer tips on how to attract these beneficial predators to your garden, b) pull weeds manually, c) mulch the open spaces in your garden to reduce weed growth, d) Consider using biochemical pesticides such as pheromones and juvenile insect hormones if you must use pesticides to control insects (4).

During use of herbicide for avoid or minimize herbicide carry-over; a) integrated weed management: Use a variety of seeding dates, crop selection and fertilizer placement to promote a vigorous competitive crop that has an advantage over weeds and helps to minimize carry-over, b) herbicide rotation with crop rotation: This is important to reduce the need to apply herbicides that may carry-over in the soil in successive years, c) selection of herbicides with minimum carry-over potential: Choose herbicides with little or no carry over given local soil and weather conditions, d) apply minimum rates of herbicides: The rate of herbicide should never be more than the amount required to achieve acceptable weed control, e) time of application: Early removal of weeds reduces competition and improves crop yield. The longer the herbicide is exposed to moisture and temperature, the lower the risk of carry-over, f) accurate application: Always read the label and follow instructions. Avoid sprayer overlap, g) grow a tolerant crop: When herbicide residue is detected or suspected, a tolerant crop should be grown, h) soil additives: Absorption of herbicide residue can be increased by the addition of absorbent material such as activated charcoal, ı) application of fertilizer: The addition of fertilizer enhances the growth of tolerant plants, which increases the uptake of herbicide from the soil (5).

SU herbicides are applied preplant incorporated, preemergence, and postemergence at doses of 0.5 to 6 ounces active ingredient per acre. This herbicide group provides selective control of wild garlic and Canada thistle in small grains; broadleaf weeds in soybeans; johnsongrass, shattercane, quackgrass and wirestem muhly in corn; and weeds in conifers, hardwoods and pastures. Several compounds are used for general vegetation control on non-crop sites. High soil pH greatly increases persistence since only biodegradation takes place at higher soil pHs.

At soil pHs below 6.8, chemical degradation occurs in addition to biodegradation and speeds inactivation. Sulfonylurea tolerant soybeans are available to farmers. Chlorimuron, chlorsulfuron, nicosulfuron, primisulfuron, thifensulfuron, tribenuron, sulfometuron, metsulfuron, halosulfuron, are in this group herbicides (6). Imazosulfuron is one of the SU herbicides developed for paddy rice and turf. Characteristics of imazosulfuron as a turf herbicide for the activity against weeds in turf and the effect on turfgrasses were investigated (7). In the study done with wine grapes (*Vitis vinifera* L.) and chlorsulfuron, leaf photosynthesis, stomatal resistance and growing up of wine grapes were investigated. According to this, decrease of photosynthesis and increase stomatal resistance were determined (8).

Sulphonylureas have a broad spectrum of selectivity and are used at low rates as soil-applied and postemergence treatments. Sulphonylurea herbicides are readily absorbed by both roots and foliage and translocated in both xylem and phloem. They can be used as soil-applied or foliar treatments. These herbicides block synthesis of the branch chain amino acids (leucine, isoleucine, and valine) that are essential in formation of new cells. Selectivity is based on differential metabolism and site exclusion (1).

Sultan 70 WG which was used in this research belongs to SU herbicides and its active ingredient is 70% Cyclosulfamuron. This herbicide has a type of granule formulation and dissolves in water. It is applied in the fields for spraying as 40gr/da and it has long time effect. Sultan 70 WG is easily absorbed from the roots, shoots and the leaves of weeds and transported to every part of them. It obstructs growing up weeds and at last kills causes them to wither after 3-4 weeks. It is used on cultured plants and rice it controlls weeds which are *Cyperus difformis*, *Alisma plantago*, *Juncus communis*, *Carex filiformis*, *Lindernia procumbens*. Sultan 70 WG is applied to rice when it has reached to the period of three leaves and applied to weeds which have their early period of 1-4 leaves (9).

Here, investigation of the effects of this herbicide on stomatal function was aimed.

Materials and Methods

Growth Conditions

Seeds (*Vicia faba* L., *Commelina communis* L. and *Zea mays* L.) were sown in soil-fertilizer-sand mixture as 2-2-1 proportional and plants were grown in environment cabinet giving 25 ± 1ºC, 12 hrs photoperiod and with a density of 6000 lux light for 3-4 weeks.

Isolation and Incubation of Epidermal Strips

Two youngest fully-expanded leaves were harvested and abaxial leaf epidermis was peeled. The pieces of isolated strips from the plants were incubated for 3hrs at 25±1ºC in 5 cm diameter petri dishes containing 10 mol m^{-3} 2-[N-morpholino] ethane sulphonic acid (MES) buffer and 50 mol m^{-3} KCI (pH 6.15). At the same time, incubation medium was aerated with CO_2- free air (10). These conditions were prepared for promotion of stomatal opening.

Herbicide Treatments on Epidermal Pieces

Sultan 70 WG was applied on the open stomata of the epidermal pieces of *V.faba* (only the parts of leaf tip and bottom),*C.communis and Z.mays* leaves (three leaves from the top) for 3 hrs after their incubation. For the determination of the effects of Sultan 70 WG on closed stomata of *C.communis* leaves, Sultan 70 WG was applied on the epidermal pieces of *C.communis* leaves for 3hrs directly without stomatal openning process. For this process, the epidermal strips of *C.communis* leaves were isolated just after dark period.

Measurement of Stomatal Apertures
In following stage from incubation, stomatal apertures were measured with a Reichert microscope and immersion system.

Herbicide Treatments on C.communis Plants
Sultan 70 WG was sprayed on 3-4 week old *C.communis* plants. The concentrations of the herbicide were 5g/40 lt (9). 4 weeks later from this process, biochemical parameters were measured.

Length and Fresh-Dry Weight Analysis
4 weeks afterward from treatment of herbicide, leaf and shoot fresh-dry weights of control and herbicide-applied plants were determined. The length of plants were measured for get some idea about their vegetative growth.

GCP Isolation and Purification
GCP isolation and purification of the leaves of experiment and control plants of *C.communis* were occured (11). Firstly, lower epidermis was peeled from the first and second fully expanded leaves of herbicide-applied plants and control plants, floated cuticle uppermost on 10 mol m^{-3} MES/KOH ph 6.15 + 300 mol m^{-3} Mannitol buffer in glass petri dishes. After this process, they were transferred with a bent seeker to 9 cm Æ plastic petri dishes containing same buffer.Later on this buffer was removed with plastic syringe and the epidermal strips were refloated on10 mol m^{-3} MES / KOH ph 5.5 + 300 mol m^{-3} Mannitol + 2 % Cellulysin + 0.05 % Pectolyase and 0.5 % Bovin Serum Albumin (BSA) for 10 ml each petri dish. The petri dishes were placed in an incubation tank which was well illuminated and 30 ^{0}C for 1 ¼ h. At following process, the incubation medium was sucked off the petri dishes using a flame widened pipette and spun at 400g, 4 ^{0}C for 5 min. After centrifugation, the top layer of the supernatent (containing epidermal cells) was discarded and then the epidermal strips were resuspended in the supernatent, the pellet was discarded. The epidermal strips were incubated for a further 4,5 hours. The incubation medium was removed with a flame – rounded, widened pipette and spun at 100g, 4 ^{0}C for 5 min. The epidermal strips were washed in buffer and the dishes tapped whilst the incubation medium was spun. The supernatant was discarded and the pellet of the Guard Cell Protoplasts (GCPs) was resuspend in the washing solution and spun for 5 min at 100g, 4 ^{0}C. Last two steps were repeated two more times. The pellet was resuspended in 0.5 cm^3 buffer and floated on a 22.5 % , 45 % , 90 % percoll gradient and spun for 5 min at 100g, 4 ^{0}C. The layer of guard cell protoplasts was carefully removed and spun in experiment buffer, the pellet was resuspended in a little buffer. This was spun at 100 g, 4 ^{0}C for 5 min. and this last step was repeated two times.

Determination of K^+ Content
The extract of stomata guard cell which was prepared according to the step of GCP isolation and purification was used in this stage. K^+ levels in GCPs were investigated in emission mode using a AAS 680 Shimadzu atomic absorpsion spectrophotometer.

Determination of Chlorophyll Amount
Chlorophyll amount of the stomata guard cells of *C.communis* was designated on fourth week after spraying Sultan 70 WG. Used Sultan 70 WG was diluted as 5gr / 40lt and 10gr / 40lt.
Chlorophyll pigment was extracted with acetone from epidermal strips of control and experiment leaves of *C.communis.* The epidermal strips which were peeled from abaxial leaf were weighed and crushed with some $CaCO_3$ powder 80 % acetone. This mixture was centrifuged for 10 minutes at 3000 g

and the volume of extract was measured. The absorbances of the supernatant were determined at 645 nm and 663 nm wave length, values of chlorophyll were calculated as mg chlorophyll/g.f.w. as concider the Arnon method (12).

Determination of Peroxidase Activity

For the determination of this parameter, the leaves of *C.communis* which were treated Sultan 70 WG (10 gr/40 lt) were used. The leaves of control and experiment plants of *C.communis* were extracted in 0.1 M, pH 7.0 and the tissue homogenates were centrifuged at 13 000 rpm for 30 minutes. Supernatants were processed with 15 mM guaiacol and 5 mM H_2O_2 in 0.1 M phosphate buffer, pH 7.7. Absorption value of colourful product in the extract was determined once in 10 seconds at 470 nm wave length for two minutes. The PO was expressed quantitatively, DA/g.f.w. min. By the spectral method according to Birecka et al. (13).

Determination of Total Protein Amount

The materials of this determination were the leaves *of C.communis.* Bradford's (1976) protein dye-binding method was applied for quantitative definition of total protein amount of control and herbicide-applied *C.communis* plants. Firstly, the leaves of control plants and herbicide-applied plants were homogenized in the 0.1 M, pH 7.0 phosphate buffer with the ratio of 100 mg fresh weight/ml. After this process, the extracts were centrifuged at 13 000 rpm for 30 minutes. Then, 0.1 ml of supernatant was taken and 5 ml of Comassie brillant-blue G-250 was added on top of this. Perfectly mixed extracts were placed in dark for 15 minutes and the absorbance of the protein at 595 nm, was measured spectrophotometricaly against blank. For the calculation of total protein amount was used BSA as a standart and the results were expressed as mg/ml (14).

Results and Discussion

In this research it was reported that Sultan 70 WG did not give a big damage to cultured plants. However it affected their some biochemical activities and caused decrease of their stomatal opening. When Sultan 70 WG applied for 3hrs on open stomata (after epidermal strips incubated in experiment buffer for 3hrs) of leaf tip and base of *V.faba* plants (a dicotyll plant) , it was seen that stomata closing became 26.1 % for tip and 2.6 % for base. This result showed that Sultan 70 WG has no effect on stomata of leaf base **(Fig. 1)**. Same treatment was repeated for the two monocotyll plant leaves (there is no discrimination of leaf tip and base), the levels of closing were 52.4 % for *C.communis* and 13.8 % for *Z.mays*. In here, attention is drawn to the fact that the closing percentage of stomata of *C.communis* is less than *Z.mays* **(Fig. 2)**. The effects of Sultan 70 WG on open stomata are wanted to be shown the processes in Fig.1 and Fig.2. Besides these processes, the effect of Sultan 70 WG on closed stomata of *C.communis* was investigated for 3hrs. For this application, the plants of *C.communis* were used as soon as their night period was over. It was seen that Sultan 70 WG has triggered 8.8 % less opening on closed experiment stomata than on control stomata **(Fig. 3)**.

The data about length and fresh-dry weight indicate that there are important decreases in experiment plants compared to control plants (measures belong to *C.communis*). According to these results, it was seen that the length of stem which Sultan 70 WG was sprayed was 42.7 % shorter than the stem of control plants **(Fig. 4a)**. Fresh weights of stems of experiment plants have showed 65.1 % decrease when compared with control plants. This decreasing ratio about dry weights of stem is 55.8 %. If we look at the fresh and dry weights of leaves; the decrease

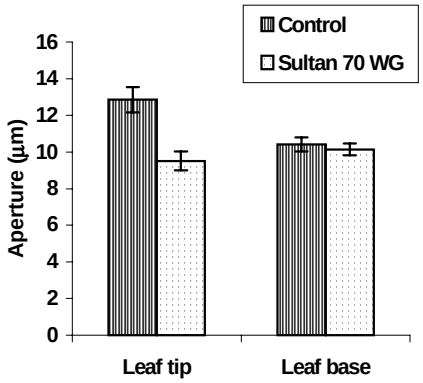

Fig. 1 : The effect of Sultan 70 WG on stomatal function of different parts of *V. faba* leaf.

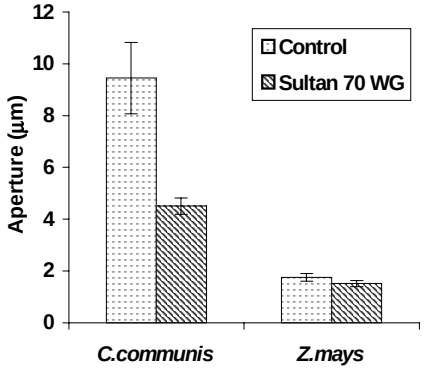

Fig. 2 The effect of Sultan 70 WG on open stomata of *C. communis* and *Z. mays.*

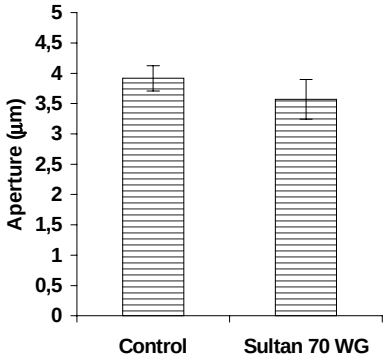

Fig. 3. The effects of Sultan 70 WG on closed stomata of *C. communis.*

of fresh weight belonging to experiment plants is 56.7 % and this ratio of dry weight data is 55.3 %. Therefore it was decided that Sultan 70 WG has triggered delay in the development of plants **(Fig. 4b)**.

The level of K^+ in GCPs has shown that there is rising 7.5 % compared to control. The value of K^+ in GCPs can also be expressed that stomata would be opened. But despite of this data stomata did not open for a big amount. Thus, it was assumed that K^+ has constituted its salts and it was not free in GCPs **(Fig. 5)**.

When the value of chlorophyll was checked, it indicated that the amount of chlorophyll (this data belongs to concentrated amount of herbicide as 10gr / 40lt) has diminished when compared with the other chlorophyll amount. If the control measurements were compared to experiment measurements, decrease of 11.5% has occurred about the application which has herbicide 5gr / 40lt but application which has herbicide 10gr / 40lt has increased up to 21.3 %. According to the literature, sometimes herbicides stimulate greening effects in plants (15). In this research, it was considered that greening effect has existed. **(Fig.6)**.

The activity of peroxidase enzyme increased as 48.9 % for the herbicide-applied plants which has contained herbicide 10gr / 40lt. to the control plants **(Fig. 7)**. It is known that the excess of peroxidase activity is a stress parameter. Concequently, it was thought that a stress which was revealed due to herbicide effect promoted the rise of peroxidase activity (16,17).

In opposition to peroxidase activity, the data about total protein amount indicate that Sultan 70 WG caused 8.3 % reduction in total protein amount of experiment plants (has herbicide as 5gr / 40lt) to the control plants. If this ratio was taken as twofold, the amount of protein would be 5.5 %. According to

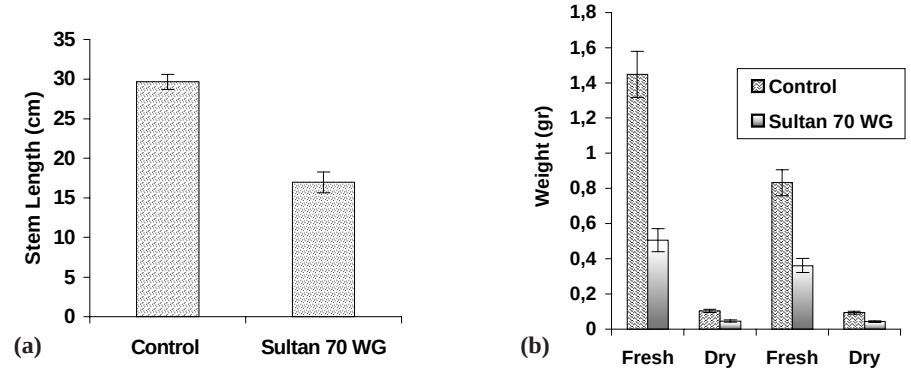

Fig. 4. The effect of Sultan 70 WG on length **(a)** and fresh-dry weight of leaf and shoot **(b)** of *C. communis* plants.

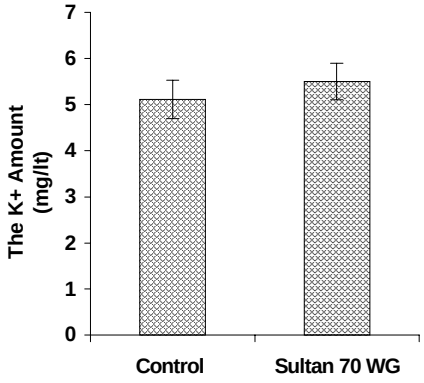

Fig. 5. The effect of Sultan 70 WG on the amount of K^+ of GCPs of *C. communis.*

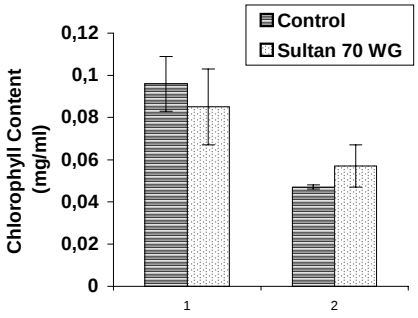

Fig. 6. The effect of Sultan 70 WG on the amount of chlorophyll of stoma guard cells of C. communis.

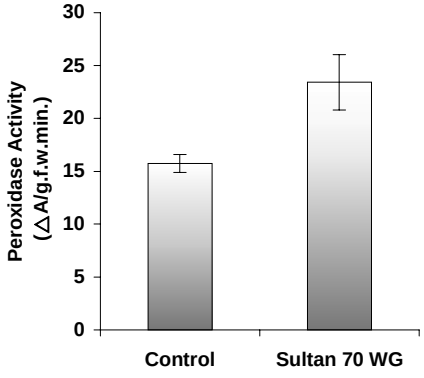

Fig. 7. The effect of Sultan 70 WG on peroxidase activity of *C. communis* leaves.

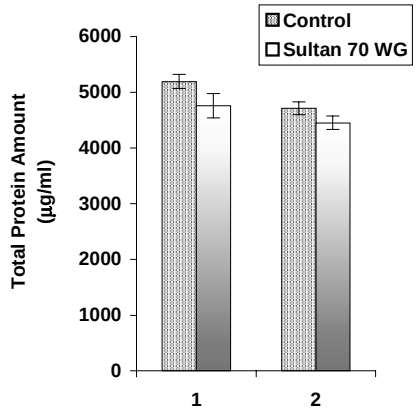

Fig. 8. The effect of Sultan 70 WG on leaf protein of *C. communis.*

these results it was seen that there is no significant difference for protein amount despite the usage of double amount of herbicide. SU herbicides cause inhibition of acetolactate synthase (ALS) enzyme [acetohydroxyacid synthase (AHAS)] which participates in syntheses of leucine, isoleucine and valine aminoacids (18, 19). The data presented that Sultan 70 WG hampered to protein synthesis for some amount in leaves due to the characteristic of sulfanylureas **(Fig. 8)**.
In conclusion, all of the results presented indicate that the plants have response to the effect of Sultan 70 WG by different metabolic ways, however plant death did not happen. Further investigation is required for understanding of other effects of Sultan 70 WG on different plant types.

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