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IMPACT OF MIXING GLYPHOSATE WITH MULTI ADDITIVES ON WEEDS CONTROL AND SOIL MICROORGANISM.

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ABSTRACT

Spray tank additives were used with glyphosate (IPA) through multi mixtures to increase its biological performance against intractable weeds in olive orchard. In addition to assessed the role of its combination on amino acids metabolism and soil microorganisms under greenhouse conditions. *Cynodon dactylon*, *Mesmbrayanthemum crystallinum and Solanum elaeagnifolium* are the dominant weed species respectively in olive orchard at north Sinai, El Arish, Egypt. Total weed frequency was affected greatly by glyphosate at micro rate 2.0 kg active ingredient ha^{-1} plus additives as compared with glyphosate at full rate 2.2 kg ai ha^{-1} without additives. An equal reduction in tyrosine aromatic amino acid was recorded after treated nutsedge leaves with glyphosate at full rate and glyphosate at micro rate with its additive mixture by 100%, also using additives increasing the level of reduction of phenylalanine acid when applying T_4 (Glyphosate at 2.0 ai ha^{-1} + glue + dioleate), T_5 (Glyphosate at 2.0 ai ha^{-1} + glue + glycerin)and T6 (Glyphosate at 2.0 ai ha^{-1} + glue +glycerin + dioleate) decreased phenylalanine acid reached, 85.05, 85.05and 89.655%, respectively as compared with the control (3 weak after treatment). Application of glyphosate at 2.2 kg ai ha^{-1} alone or at 2.0 kg ai ha^{-1} with some mixtures additives slightly affected total count of bacteria and spore forming fungi at 10 days after treatment (DAT) and caused slightly observed temporary effect in bacteria count at zero time to 10 days as compared with control. Adding glycerin as non ionic surfactant to glyphosate in the spray tank with other additives caused observed effect on total microbial count compared with others additive did not contain glycerin. The best control regime obtained when glyphosate at micro rate mixed with glue + dioleate (T_4) was applying at *C. dactylon* and *C. rotundus* and other annual weeds which have slightly effect on soil microorganisms was count, However the combination of glyphosate at micro rate +glue +glycerin + monoleate (T_7) was more suitable to intractable weeds such as *S. elaeagnifolium and* *M. crystallinum* with slightly effect on soil microorganism, Therefore, the improvement in glyphosate bio efficacy depends on additive types and weed species.

Keywords; Glyphosate, additives, spray tank, aromatic amino acid, weeds and microorganisms.

INTRODUCTON

Weeds around the olive (*Olea europaea*) tree trunk not only compete directly with tree growth for water and nutrients, but also provide a good habitat for field mice or voles and pathogenic microbes. So, weed control in olive orchards improves the growth and yield of established trees. Generally weeds are controlled between tree rows by mowing and a basal treatment of herbicide is applied around each tree or in a strip application down the tree row. On the other hand, using herbicides for olive weed control has several disadvantages specially when increasing the quantity for difficult or intractable weed control. Therefore, optimizing the usage of herbicides is important to achieve effective weed control, without a negative influence on the environment (Enflat *et al*., 1997). Also, adjuvants are added to a pesticide for enhance the activity of the herbicide, reduce leaching and improve the application characteristics (Foy and Pritchard 1996). Glyphosate is a non-selective, systemic herbicide that can control most annual and perennial plants and used in olives orchards at 4.3 kg/ha. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants (WSSA. 1994). Glyphosate interferes with the biosynthesis of aromatic amino acids, (Aubert *et al.,* 1997, Hassar and Rubin, 2003). Shikimic acid was found to be directly proportional to glyphosate application rates on wheat plant tissue (Ziora *et al.,* 2001). Enhancement of glyphosate herbicides were investigated by Kudsk and Mathiassen, (2004); Molin and Hirase, (2005); Shaner *et al*., (2006). Kraemer *et al.,* (2009). Weed management in olive orchards are often very important for olive plant growth and easy harvest. The present investigation throw light on the role of four chemical additives as a mixture with glyphosate on its herbicidal activity against difficult or intractable weed control behind olive tree, spray tank physicochemical parameter, amino acid metabolism and soil microorganisms.

MATERIALS AND METHODS

Chemicals

1--Round up (glyphosate IPA salt: N-(phosphonomethyl) Glycine isopropyl amine) was supplied by Monsanto Company.

2-Additives including:

2.1- Nonionic surfactant: Monoethylene glycol mono-oleate, Monoethylene glycol dioleate and Glycerin by 0.1% (V/V) supplied from Egyptian Company for Starch, Yeast and detergents, Alexandria and El-Gomhoria Medical Company.

2.2-Sticking agent (glue) by 1% (w/v) was supplied from El-Gomhoria Medical Company.

Treatments:

T_1: Glyphosate at the full rate (2.2 ai ha^{-1}) alone.

T_2: Glyphosate at micro rate (2.0 ai ha^{-1}) alone.

T_3: Glyphosate at micro rate (2.0 ai ha^{-1}) + glue(1%W/V) + monoleate (0.1% V/V).

T_4: Glyphosate at micro rate (2.0 ai ha^{-1}) + glue (1%W/V) + dioleate (0.1% V/V).

T_5: Glyphosate at micro rate (2.0 ai ha^{-1}) + glue (1%W/V) + glycerin (0.1% V/V).

T_6: Glyphosate at micro rate (2.0 ai ha^{-1}) + glue (1%W/V) + glycerin (0.1% V/V) + Dioleate (0.1% V/V).

T_7: Glyphosate at micro rate (2.0 ai ha^{-1}) +glue (1%W/V) +glycerin (0.1% V/V) + Monoleate (0.1% V/V).

Abbreviations

Monoleate: Monoethylene glycol mono-oleate,

Dioleate: Monoethylene glycol di-oleate

DAT: Days after treatment.

WAT: Weak after treatment

ai: Active ingredient

Physical and chemical properties of glyphosate and multi mixtures additives in spray tank.

Physicochemical of spray tank properties were assessed such as pH, EC, viscosity and surface tension (Table.1): The solution (glyphosate additive mixtures) was shaked well to make homogeneous solution. The pH and EC (m mhos units) value were measured by pH/ISE meter, model 710A. Viscosity measured by Brookfield programmable DV-11+Viscometer: 60RPM where m/pouse is the unit of viscosity measurement (Anonymous, 1968) and surface tension (dyne/cm) was recorded according to Osipow (1964).

Dominant weed species in Egypt olive orchards.

The distribution of total numbers of weeds and species composition of the assemblage densities were determined around olive tress, where no weeding regimes were occurred throughout the experiment period. It is important to observe the distribution of total numbers of weeds and species composition and the dominant weed species in olive orchards included: broad leaves species: *Mesmbrayanthemum crystallinum, Solanum elaeagnifolium, Convolvulus arvensis*, *Phragmites Communis* and *Portulaca oleracea* L., while narrow leaves were *Cynodon dactylon L., Polypogon monspeliesis L., Setaria sp, Alhagi mourorum, Echinochloa colonum, Cyperus rotundus, Imperata cylindrical and Conyza aegptiace L.* The fore mentioned weed growth parameters significantly decreased in the order of increasing olive tree growing, total weed density (140 –150) plants/m^{-2} as well as in individual weed densities due to difference in growing densities of olive, *Cynodon dactylon,* was the dominant weed species with, mean density of 70.0 plants/m^{-2}. Followed by *Mesmbrayanthemum crystallinum* with density reached 30 plants/ m^{-2} . *Solanum elaeagnifolium* was the third with a density of 4 plants /m^{-2}. *Alhagi mourorum* recorded density ranged between 2 and 3 plants/m^{-2}**,** *Convolvulus arvensis* with density ranged between 1 and 2 plants/m^{-2}**,** other weeds were very rare and were found only in some location not all, where there is no weeding regime was occurred throughout the experimental period.

Glyphosate herbicide efficacy with and without multi additives on weeds in olives.

Under field conditions, experiment conducted at olive farm in North Sinai (El Arish) Egypt to evaluate the bio efficiency of glyphosate IPA (Round up) at full recommended rate 2.2 kg ai/ha (Faircloth *et al.,* 2004), glyphosate at micro rate 2.0 kg ai /ha with different additives to control difficult or intractable weed behind olive trees. Application of glyphosate was conducted at July 2010 by the direct spray on weeds at different stages and repeated in two locations under olive tree; weed fresh and dry weights were estimated after 4 weeks from the application for individual and total weeds. Assessments of survival weed plants/m^{-2} within each plot were determined as a frequency before and after treatments compared with the control. The experimental design was a complete block randomized design, with three replications and two concentrations from glyphosate and four additives.

Amino acids composition of *Cyperus rotundus* leaves by Amino Acid Analyzer

A Greenhouse study was conducted to evaluate the role of additives in amino acid composition of nutsedge (*Cyperus rotundus*) leaves by glyphosate herbicides. The plants of nutsedge were grown in pots experimental, five tubers of nutsedge were translocated in plastic pots containing a mixture of sand and clay (1:1) vol. Pots were placed in greenhouse and arranged in complete block randomized design with four replicates. Pots were gently and regularly irrigated at 3 day intervals with suitable amounts of water. Through 5-6 leaves stage plants were treated with glyphosate at the recommended rate 2.2 kg ai /ha and micro rate 2.0 kg ai /ha with and without additives in multi mixture for the control of (*C. rotundus*). Total amino acids were estimated after 3 weeks from herbicide application, acid hydrolysis was carried out according to the method of (Block *et al.,* 1958). A known weight of nutsedge (leaves) (0.5 gm) was transferred into a tube containing 10 ml of 6 N hydrochloric acid, the tube sealed and hydrolysis was continued for a period of 24 hours in an oven at $110C^0$. At the end of this period, hydrolsate was transferred quantitatively to a porcelain dish and the hydrochloric acid was then evaporated to dryness a 50-60 ^oC on a water bath. Distilled water 5 ml was added to the hydrolsate and then evaporated to dryness to remove the excess of hydrochloric acid and the final residue was dissolved in 10 ml of glass – distilled water added. The hydrolsate sample dried a second time. One ml of 0.2 N sodium citrate buffer a pH 2.2 was added and the samples stored frozen in a sealed vial until separation of the amino acids by Amino Acid Analyzer (Eppendorf – LC3000). The peak area and percentage of each amino acid were calculated by computer using software AXXIOM CHROMAOGRAPHY-727.

Impact of glyphosate with and without additives on soil microorganism counts.

Sandy soil was collected from olive farm (none of previous treating with glyphosate) and treated with glyphosate (48%) at 2.2 kg ai /ha and micro rate 2.0 kg ai /ha with additives in multi mixture and compared with the untreated control. Samples were collected randomly from each treatment at daily intervals during trails period. Five g soil samples were taken from the top (0-10) centimeters at five replicates, stored at - 4 ^0C and subsequently used for microbiological analysis to 40 days from initial herbicide application. The dilution plate technique (Johnson *et al.,* 1959) was employed to enumerate the most important groups of soil fungi and bacteria. Potato dextrose agar media and nutrient agar (PDA) was used for the enumeration of fungi and bacteria respectively. Five petri dishes were incubated at 25 ±1for 5 days for fungi and at 30±1C for 24 h for bacteria (Parkinson, *et al.,* 1971). Data from triplicate readings were expressed as Colony Forming Units (CFU) /g soil.

Statistical Analyses: Data were analyzed by ANOVA according to Snedecor and Cochran (1990) and treatment means were compared by Duncan probability.

RESULTS AND DISCUSSION

The present study was conducted to control intractable weed behind olive trees by increasing glyphosate performance with some spray tank additives in multi mixture. The symptoms of glyphosate effect on weeds appeared after three weeks from spraying and consisted mainly of severe chlorosis and die. No olive tree injury was observed after glyphosate application alone or with its mixture additives.

Spray tank properties of glyphosate and multi mixtures additives.

Glyphosate at micro rate showed less value of EC and pH, while the contrary was recorded with viscosity and surface tension. As for the properties of glyphosate, additives in multi mixture clearly indicated the important role of chemical structure and nature of additive in changing of spray tank physicochemical properties. The reduction in EC values, viscosity and surface tension were clear in the mixture of glyphosate at micro rate with tested additives as compared with glyphosate at full or micro rates. It is clearly evidence that glyphosate and its tested additive mixtures are characterized with acidic pH properties. The surface tension, contact angle, and ^{14}C-glyphosate distribution were significantly affected by both the presence of different waxes on the plants and by the addition of surfactants to the glyphosate (Sharma and Singh, 2007).

Table (1): Effect of glyphosate with and without additives in physico-chemical properties of spray tank solution.

Treatment	EC (mmols/m)	pH	Viscosity (cm/pouse)	Surface tension dyne /cm
T_1	2.497^{e}	5.373^{b}	9.823^{c}	55.267^{d}
T_2	0.965^{a}	5.180^{a}	10.157^{d}	58.633^{e}
T_3	1.103^{b}	5.333^{b}	6.477^{b}	43.567^{c}
T_4	1.445^{d}	5.127^{a}	6.190^{a}	38.933^{b}
T_5	1.297^{c}	5.817^{d}	6.437^{b}	38.300^{b}
T_6	1.423^{d}	5.687^{c}	6.517^{b}	40.367^{c}
T_7	1.370^{cd}	5.827^{d}	7.693^{c}	35.733^{a}

Enhancing glyphosate activities with multi mixture additives on weed control in olives.

In general, the efficiency of glyphosate was known on bermuda grass *(Cynodon dactylon)* (Johnson, 1983), otherwise using additives increased glyphosate performance in C*. dactylon* control and reduced the lethal dose*.* Using glyphosate at full (T_1) and micro rate (T_2) without additives decreased *C. dactylon* fresh weight by 59.76% and 50.66%, respectively as compared with the control treatment. On the other side, Table (2) provide that adding the tested additives enhanced glyphosate activity as the four treatment (T_3, T_4,T_6 and T_7), which achieved the higher reduction in *C. dactylon* fresh weight than those obtained from the high or the low rates of glyphosate while the reduction ranged between 69.73 in T4 and 67.66% in T6 with no significant differences between the four treatment. T5 treatment also was superior over the two rates of glyphosate but with no significant difference with the high rate of glyphosate, it reduced C*. dactylon* fresh weight by 63.24% as compared with the untreated control treatment. The trend was observed when comparing between C*. dactylon* dry weights. In *C. dactylon* dry weight, the maximum inhibition had achieved from T_4 that control effectively and scored reduction in dry weight by 51.79%, however the lower reduction recorded from T_5 by 45.44%, than untreated control. Glyphosate without additive in T_1 and T_2 were reduced *C. dactylon* dry weight by 39.56% and 27.22%, respectively, compared with untreated check (Table.2).

According to the activity of glyphosate on *Solanum elaeagnifolium* (4 WAT), the addition of additive to glyphosate decreased *S. elaeagnifolium* seedling biomass ranged from 32.73 to 60.45% (fresh weight) and 16.22 to 35.91% (dry weight), compared to untreated treatment. Assessment of T_1 and T_2 against *S. elaeagnifolium* causes a weak reduction reached, 21.82% and 10.91 % (fresh weight) and 10.42 and 8.11% (dry weight), respectively, as compared with the control treatment (Table. 2). Adding additive expended the efficacy in fresh weight as follow: treating with T_4 significantly inhibited fresh weight by 60.45%, followed by T_7 which gave a moderate reduction reached, 55.91%, while T_3 had achieved the least reduction in dry weight by 35.91% than untreated plot (Figure, 1). In this respect, silver leaf nightshade it is listed as a noxious weed in its native range (Americas) and as an invasive alien plant in many countries across the world (Mekki, 2007). (Eleftherohorinos *et al.,* 1993) indicated that silver leaf nightshade (*S. elaeagnifolium*) able to be controlled by glyphosate.

Table (2): Effect of glyphosate with and without multi mixture additives on weeds total biomass (gm/m^2).

Tested weeds	*C. dactyon*		*S. elaeagnifolium*		*M. crystallinum*		Total weeds species	
Treat ment	**Fresh weight**	**Dry weight**	**Fresh weight**	**Dry weight**	**Fresh weight**	**Dry weight**	**Fresh weight**	**Dry weight**
Control	174.47^{d}	57.80^{d}	14.67^{e}	8.63^{d}	446.83^{e}	87.03^{c}	943.83^{e}	349.20^{e}
T_1	70.20^{b}	34.93^{b}	11.47^{d}	7.73^{cd}	316.87^{d}	72.50^{b}	355.47^{c}	151.60^{c}
T_2	86.07^{c}	42.07^{c}	13.07^{e}	7.93^{c}	330.70^{c}	82.40^{c}	431.73^{d}	241.20^{d}
T_3	53.67^{a}	27.87^{a}	7.80^{abc}	6.33^{ab}	245.87^{b}	61.83^{a}	287.70^{a}	122.30^{a}
T_4	52.80^{a}	29.07^{a}	8.27^{bc}	5.93^{a}	228.60^{a}	64.53^{a}	283.23^{a}	120.50^{a}
T_5	64.13^{b}	31.53^{b}	9.87^{cd}	7.23^{bc}	304.13^{c}	70.50^{b}	328.83^{bc}	143.20^{b}
T_6	56.43^{a}	28.50^{a}	6.47^{ab}	5.53^{a}	228.60^{a}	58.33^{a}	297.70^{ab}	125.37^{a}
T_7	55.47^{a}	28.73^{a}	5.80^{a}	5.73^{a}	220.33^{a}	58.27^{a}	306.20^{ab}	138.43^{ab}

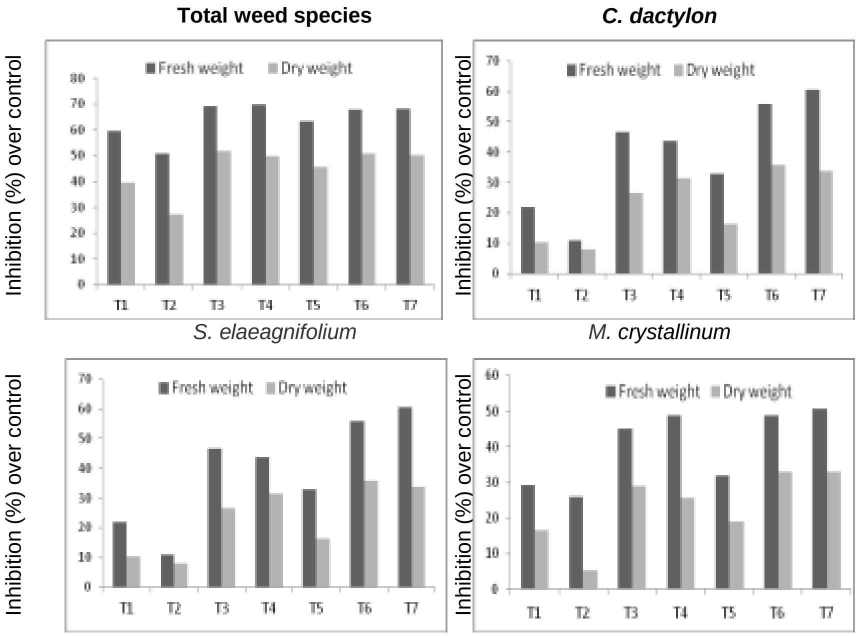

Figure 1: Reduction percentage on weed total biomass regardless of glyphosate with and without multi mixture additives.

Mesembryanthemum crystallinum is a dicot weed in the Aizoaceae family **(**Tackholm, 1974), recorded in olive farm in North Sinai, Egypt which causes many harmful effects on olive growing. our result presented that *M. crystallinum* was slightly affected with glyphosate without additive such as applying T1 and T_2 that caused reduction reached, 29.09% and 25.99 % (fresh weight) and 16.70 and 5.32 % (dry weight), respectively, than the control (Table 2). In the other side, Better control regimes were obtained when the tested materials were added to glyphosate. Mixing glyphosate at the low rate with glue, glycerin and monoleate or dioleate (T_7 & T_6) gave the highest activity against *M. crystallinum* as they reduced the weed fresh biomass by 50.69% and 48.84%, respectively as compared with its control. In the other hands, the higher reduction in *M. crystallinum* dry weight from T_7 and T_6 were 35.91%and 32.98%, respectively than untreated control (Figure, 1). Concerning to total weed species, spraying glyphosate at the full rate (T_1) or at the low rate (T_2) caused significant reduction in fresh or dry weed biomass as compared with untreated control treatment. Adding the tested sticking and surfactants to glyphosate at low rate enhanced its weed control activity. Glyphosate at the full rate produced 62.33% reduction on total weed fresh weight as compared with 54.25 % reduction when glyphosate was treated alone at its low rate (T_2). The highest reduction in total weed biomass was achieved in T_3, T_4, T_6 and T_7 with no significant differences between them. They reduced fresh biomass in a range between 69.99 and 65.15%. Although T_5 treatment showed the least reduction between the tested mixtures, it showed significant differences with glyphosate treatment at low rate. In dry weight, the higher reduction effect had achieved from T_4 by 65.49%, followed by T_3 decreased total weed dry weight by 64.97%. Meanwhile, T_5 reduced dry weed biomass by 58.99% as compared with 30.92%, produced when glyphosate was spraying alone at low rate (T_2) (Figure,1). Result in table 1 gave good explanation of these results to the alteration in glyphosate spray tank physical and chemical properties by additive mixture can lead to variable glyphosate performance in weed control (Table.1). Addition of surfactant substances to the spray tank improved the activity of glyphosate against the target weeds than glyphosate alone. Also, surfactants enhance the retention and absorption of glyphosate by plants (Feng *et al.,* 2003).

Table (3): Weed frequency (plants/m2) after field application of glyphosate +additives (35 DAT).

	C. dactylon		***S. elaeagnifolium***		***M. crystallinum***		**Total weeds species**	
Treatment	**Pre spray**	**Post spray**	**Pre spray**	**Post spray**	**Pre spray**	**Post spray**	**Pre spray**	**Post spray**
Control	70.00	70.00^f	5.00	5.00^d	30.00	30.00^f	150.00	150.00^f
T1	80.00	9.00^d	3.00	3.00^c	27.00	27.00^e	138.00	18.00^c
T2	70.00	16.00^e	5.00	5.00^d	28.00	29.00^e	149.00	59.00^d
T3	70.00	7.00^b	4.00	2.00^b	30.00	13.00^d	139.00	7.00^b
T4	60.00	8.00^c	4.00	1.00^a	20.00	15.00^d	128.00	6.00^a
T5	70.00	6.00^a	3.00	1.00^a	20.00	9.00^c	149.00	8.00^b
T6	70.00	9.00^d	4.00	1.00^a	25.00	8.00^b	136.00	5.00^a
T7	70.00	9.00^d	4.00	1.00^a	20.00	7.00^a	131.00	8.00^b

Weed survival frequency was recorded after spraying glyphosate with and without its additive by 35 days. Glyphosate at micro rate with additive browned-out and showing the higher suppress in the target weeds faster than glyphosate at full (T_1) and micro rate (T_2) alone. Multi mixture additives were prolonged glyphosate inhibitory effect in total weed species *to* 35 days after treatment. Meanwhile there were no differences between T_6 and T_4 in the suppression of total weed species. Otherwise T_7 and T_3 caused the same frequency in weed species. Total weed species affected greatly from T_6 calculated by 96.6 % (reduction) than untreated control. However, glyphosate at the full rate alone decreased survival plants by 88% than the control. This results supported by Murray *et al.,* (1998) indicated that glyphosate+ Brown down treatment browned-out faster than the glyphosate+ pulse treatment (80 and 60% brown-out at 7 DAT, respectively), but there were no differences between them by 21 DAT (98% brown-out).

Role of glyphosate and its mixture on *Cyperus rotundus* amino acid content.

The influence of glyphosate at full rate and micro rate with and without additives on the amino acid content of *C. rotundus* (nutsedge) leaves was examined. Glyphosate inhibited *C. rotundus* growth and alter aromatic amino acid concentrations as well as change others amino acid levels in different trend. As for tyrosine aromatic amino acids, T_1 and T_2 caused inhibition in tyrosine acid by 100 and 81.1%, respectively than the control (3WAT). Otherwise, glyphosate at micro rate with its additive mixture also decreased tyrosine amino acid concentrations in nutsedge leaves by 100% than the control treatment. As for phenylalanine aromatic acid, T_1 and T_2 caused reduction in phenylalanine quantity calculated by 65.9% and 56.7%, respectively than the control (3 WAT). A significant result obtained from $T_{4,}$ $T_{5,}$ T_6 and T_7 in phenylalanine acid reached, 85.05, 85.05, 82.375, 89.655 and 72.605%, respectively compared with the control (3 WAT). Aromatic amino acid deficiency was apparently in treated nutsedge weeds as compared with untreated control (Table 4).

Table (4): Effect of glyphosate with and without additives on amino acids composition of nutsedge leaves after 3 weeks from application.

Treatment	Aspartic	Therionine	Serine	Glutamic	Proline	Glycine	Alanine	Valine
Control	0.450^{a}	0.227^{d}	0.199^{e}	0.515^{g}	0.248^{h}	0.236^{g}	0.275^{g}	0.324^{g}
T_1	0.060^{h}	0.001^{a}	0.004^{a}	0.012^{a}	0.052^{a}	0.052^{a}	0.057^{a}	0.043^{a}
T_2	0.312^{g}	0.225^{d}	0.170^{d}	0.323^{f}	0.182^{f}	0.198^{f}	0.138^{e}	0.242^{h}
T_3	0.183^{d}	0.074^{b}	0.067^{b}	0.191^{d}	0.060^{b}	0.107^{d}	0.106^{d}	0.092^{d}
T_4	0.200^{f}	0.074^{b}	0.066^{b}	0.211^{e}	0.073^{d}	0.115^{e}	0.107^{d}	0.097^{e}
T_5	0.170^{c}	0.070^{b}	0.066^{b}	0.173^{c}	0.088^{e}	0.097^{c}	0.101^{c}	0.082^{c}
T_6	0.106^{b}	0.369^{e}	0.328^{f}	0.101^{b}	0.436^{g}	0.061^{b}	0.063^{b}	0.052^{b}
T_7	0.251^{e}	0.111^{c}	0.101^{c}	0.275^{f}	0.064^{c}	0.135^{f}	0.151^{f}	0.135^{g}
Treatment	**Isoleucine**	**Histidine**	**Tyrosine**	**Phenyl alanine**	**Leucine**	**Lysine**	**Argenine**	**Ammonia**
Control	0.277^{f}	0.200^{f}	0.270^{c}	0.261^{g}	0.420^{f}	0.238^{g}	0.159^{g}	0.507^{f}
T_1	0.181^{e}	0.253^{h}	0.000^{a}	0.089^{e}	0.068^{a}	0.039^{a}	0.072^{e}	0.285^{a}
T_2	0.217^{d}	0.247^{g}	0.051^{b}	0.113^{f}	0.276^{e}	0.152^{f}	0.135^{f}	0.466^{d}
T_3	0.076^{c}	0.107^{c}	0.000^{a}	0.035^{a}	0.122^{c}	0.094^{c}	0.031^{c}	0.413^{c}
T_4	0.075^{c}	0.117^{d}	0.000^{a}	0.035^{a}	0.123^{c}	0.106^{e}	0.036^{d}	0.426^{c}
T_5	0.068^{b}	0.081^{b}	0.000^{a}	0.043^{b}	0.109^{b}	0.063^{b}	0.022^{b}	0.391^{b}
T_6	0.052^{a}	0.059^{a}	0.000^{a}	0.057^{c}	0.070^{a}	0.040^{a}	0.009^{a}	0.298^{a}
T_7	0.104^{d}	0.136^{e}	0.000^{a}	0.072^{d}	0.176^{d}	0.097^{c}	0.329^{h}	0.526^{g}

Data presented in (Table.4) indicated that $T_{1,}$ $T_{3,}$ T_4 and T_5 gives a better control to nutsedge than T_6 and T_7 which caused lower reduction value in total recorded aromatic amino acid (tyrosine and phenyl- alanine) as compared with untreated control. In this respect, accumulation of shikimic acid related to aromatic amino acids synthesis and quantity while not estimate in this study but the number of scientists in this estimate regardless of the presence of glyphosate: Glyphosate toxicity in nutsedge shoots was associated with the accumulation of shikimic acid so, there was increasing in many amino acid concentration whereas Argenine the highest accumulated acid, however a least amount of aspartic acid. Shikimic acid accumulated within the leaves 24 h after glyphosate application (Kim *et al*., 1995). Glyphosate at 33.5 mM increased total free amino acid concentration, and caused rapid accumulation of shikimic acid, (Wang and Wang, 2001). For common vetch, glyphosate applied at rates that selectively inhibited broomrape growth did not alter individual amino acid concentrations in the leaves, but generally increased amino acid levels at 0.18 kg ha-1 **(**Nandula *et al*., 2001). Also, Wang and Wang, (2001) indicated that tryptophan (aromatic amino acids) decreased quickly to 22% of control 3 days after treatment (DAT) and remained low afterwards.

Influences of glyphosate with and without additives on soil microorganisms population.

Addition of glyphosate IPA to the soil at 2.2 kg ai ha^{-1} and glyphosate IPA 2.0 kg ai ha^{-1} with additives decreased total count of culture bacteria and fungi at 40 days after treatment. Total microbial numbers were recovered at (40 DAT) when plants treated with glyphosate alone or with its additives. Fungi was particularly sensitive than bacteria when treating with glyphosate; no fungal growth was detected from the treated soil and the spores lower limit than 10^3 CFU g^{-1}. Total count of fungi slightly affected by glyphosate at micro rate with additive (T_2 and T_4) than glyphosate at the full rate without additives (T_1) at (1 DAT). While there is no significant effect observed from other treatments (glyphosate with additive) on soil fungi as compared with the control treatment. In soil bacteria, the effect of T_5, T_6 and T_7 were prolonged from (1 to 10 DAT), while, glyphosate at micro rate without additive caused slightly effect on total count of bacteria. Details in (Table 5) indicated that adding glycerin to glyphosate in the spray tank with others additive caused observed effect in total microbial count compared with others additive did not contain glycerin especially in the first 10 days Our data not in the same trend with (Chakravarty and Chatarpaul, 1990). Glyphosate at 0.54 and 3.23 kg a.i ha^{-1} did not reduce soil microbial population or carbon dioxide evaluation in long term (6 months). However, there was a significant short term (2 months) effect of glyphosate on both fungal and bacteria counts at the 0.54kg ha^{-1} treatment.

Table (5): Soil microorganism populations as affected by glyphosate with and without multi mixtures additives.

Days after application	0	1	5	10	20	40
Fungi (X10^3 /g dry soil) CFU						
Control	7.67^a	7.33^a	7.67^a	7.33^a	7.33^a	7.33^a
T_1	7.33^a	6.67^b	7.67^a	7.00^a	7.00^a	7.33^a
T_2	6.67^a	6.33^b	7.33^a	7.33^a	6.67^a	6.00^a
T_3	7.67^a	7.00^a	7.33^a	7.00^a	7.00^a	7.33^a
T_4	7.67^a	6.33^b	7.33^a	7.00^a	7.33^a	7.67^a
T_5	7.67^a	7.33^a	7.33^a	7.67^a	7.00^a	6.00^a
T_6	7.37^a	7.00^a	7.33^a	7.33^a	6.67^a	5.00^a
T_7	6.67^a	7.33^a	7.33^a	7.00^a	7.33^a	6.00^a
Bacteria (X10^5 /g dry soil) CFU						
Control	6.33^a	5.00^a	5.00^a	5.67^a	5.50^a	5.33^a
T_1	5.33^c	5.33^b	5.00^a	5.00^b	5.00^a	5.30^a
T_2	5.50^b	5.00^a	5.00^a	5.00^b	5.00^a	5.00^a
T_3	5.67^b	5.00^a	5.00^a	5.67^a	5.00^a	5.33^a
T_4	5.33^c	5.33^b	5.00^a	5.67^a	5.50^a	5.33^a
T_5	5.60^b	5.00^a	5.50^b	5.00^b	5.00^a	4.70^a
T_6	5.50^{ab}	5.00^a	4.67^c	5.33^b	5.63^a	4.80^a
T_7	5.33^c	5.00^a	5.00^a	4.67^c	5.50^a	4.90^a

The aforementioned results concluded that each of the tested chemicals, i.e. glyphosate herbicides, additives and their multi mixtures having its own physical and chemical properties such as EC, pH, viscosity and surface tension. Additives when mixed with glyphosate in the spray tank caused changing in different properties, but in various levels according to the type of additive and its chemical natural which affected glyphosate activity. Addition of surface active agent to glyphosate prolonged its activity against *S. elaeagnifolium*, *M. crystallinum* and *Convolvulus arvensis* but in fluctuated response sometime complete death and sometime only yellow leaves and stopped weed growth as well as no observed effect on some treatment as compared with glyphosate without additives. Using most additives increased glyphosate biological activity against *C. dactylon, P. monspeliesis, Setaria sp, E. colonum, P. Communis, C. rotundus, I. cylindrical and C. aegptiace L*. However, there no observed damage was observed in *A. mourorum* after treated with glyphosate with or without additives. The weeds biomass dry weight reduced significantly when treated with glyphosate with additives ranged from 5.54 to 20.51% (total weed biomass), 9.73 to 20.23 % (*C. dactylon*) and 8.11 to 35.91 % (*S. elaeagnifolium*) as compared with glyphosate at the full rate without additives. Glyphosate with additives had a prolonged inhibition effect on *S. elaeagnifolium* and *I. cylindrical* than glyphosate alone. Our results in the same line with (Ruiter 1996; Sundaram *et al.,* 1996; Cabanne *et al.,* 1998; Nelson *et al.,* 2002) they reported that the synergistic effects of different adjuvants on the herbicidal activity and performance of glyphosate herbicide. These data provide reliable estimates of compatibility specially when conducted under the field conditions. By comparing the obtained results in both tyrosine and phenyl alanine aromatic amino acid, as for tyrosine glyphosate at the micro rate with additives and glyphosate at the full rate alone caused complete inhibition. However in phenyl alanine, all mixture occurred observed synergistic effect to glyphosate, otherwise the highest synergistic effect came from adding glue plus monoleate and glue plus dioleate (T_3 and T_4) respectively. The field applications rate of glyphosate have little or no affect on soil microbial communities (Basse *et al.,* 1990). Therefore, the addition of adjuvant to glyphosate reduced the necessary application rate and improved the bio efficacy of glyphosate. Finally treating with T_4 (Glyphosate at micro rate + glue + dioleate achieved a good control regime in *C. dactylon and C. rotundus* and other annual weeds without any effect in soil microorganism count. However multi additives such as T_7 (Glyphosate at micro rate +glue +glycerin + monoleate) more suitable to intractable weeds *S. elaeagnifolium* and *M. crystallinum* with slightly temporary effect in soil microorganism, so the improvement in bio efficacy depends on additive type and plant species.

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تـأثير خلـط الجليفوسـات مـع المـواد الإضـافية علـى مكافحـة الحشـائش و المحتـوى الميكروبي للتربة

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استـهدفت الدراسـة تحسـين الأداء البيولـوجي لمبيـد الجليفوسـات (ايزوبروبيـل امـين) علـى الحشائش العنيد ة في بساتين الزيتون من خلال إضافة أكثر من مادة إضافية لسائل الرش ، بالإضافة لدراسة تأثير هذه المخاليط على ميتـابولزم الأحمـاض الأمينيـة و أعداد الكائنـات الدقيقـة فـي التربـة. لـوحظ إن النجيــل *Cynodon dactylon* و الغســول *Mesmbrayanthemum crystallinum* وعنب الديب *Solanum elaeagnifolium* كانـت أكثـر الحشـائش سيادة في بساتين الزيتون في منطقة العريش شمال سيناء. أثرت المعاملات في معدل التعداد الكلى للحشائش قبل وبعد المعاملة عند استخدام مخاليط المواد الاضافية مع الجليفوسات بمعدل 2.0 كيلوجرام مادة فعالة عن استخدام الجليفوسات بمعدل 2.2 كيلوجرام مادة فعالة بدون مواد إضافية.أظهرت جميع المعاملات تثبيط كامل للحامض الامينى الاروماتى التروسين فى أوراق السعد بنسبة 100% بعد 3 أسابيع من المعاملة ، بينما زاد مستوى الانخفاض فـى الحـامض الامينـى الارومـاتى الفنيل ألأنين عند استخدام المواد الإضـافية مع الجليفوسـات عن المبيد بدون مـواد إضـافية كمـا فـي المعـاملات T6(**جليفوسات مع الغراء و الجلسرين**), T5(**جليفوسـات مـع الغـراء و الجلسـرين و الـداى اوليـت**) , T4(**جليفوسات مع الغراء و الداى اوليت**)أحدثت انخفـاض بنسبة 89.65 و 85.05 و85.05 % بالمقارنـة بـالكنترول. تـأثر تعـداد الميكروبـات قلـيلا وبشـكل مؤقـت وضـعيف عنـد اسـتخدام الجليفوسات منفردا او مع بعض المواد الإضـافية عند بدايـة المعاملـة وحتـى 10 ايـام .اتضـح مـن الدراسـة ان الخلـط مـع الجلسـرين مسـئول عـن الانخفـاض فـى أعـداد الميكروبـات. كماأوضـحت الدراسة إن أفضل نظام مكافحة لحشائش النجيل و السعد و الحشـائش الحوليـة هو خلـط الجليفوسـات مـع الغـراء والـداى اوليـت حيـث كـان لـة تـأثير ضـعيف علـى كثافـة الكائنـات الدقيقـة.بينما خلـط الجليفوسـات علـى المعـدل الصـغير مـع الغـراء و الجلسـرين و المونـواوليـت كـان أفضـل فـى مكافحـة الحشائش العنيدة مثل الغسول و عنب الديب بينما كان لهذا الخلط تأثير طفيف على أعداد الكائنـات الحية، لذا فتحسين الكفاءة البيولوجية يعتمد على نوع المادة الإضافية و نوع الحشيشة.

قام بتحكيم البحث

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