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Arginine and pyruvic acid and its effecttts on chlorophyll, carotenoids contents, insect infestations and growth of barly plants grown under salinity conditions

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Aiming to study the effect of arginine and pyruvic acid application on the amelioration of salt stress of barley plants, a pot experiments was conducted in the greenhouse of the National Research Centre in Dokkiu, Cairo Egypt. Salt concentration increases in water of irrigation induce adverse effect on barley plant height, and stem, leaves, spike and total dry weight. Arginine and pyrovic acid increased growth parameters but arginine effect on plant height and dry weight of leaves exceeded those from pyruvic acid>. The opposite was true for leaves area/main stem and stem, spikes and whole dry weight. Arginine and pyrovic acid increased growth parameters but arginine effect on plant height and dry weight of leaves exceeded those from pyruvic acid. The opposite was true for leaves area/main stem and stem, spikes and whole dry weight. Chl. and carotenoids increased and the opposite was true for chlorophyll b while chl.a+chl.b slightly affected when barley plants subjected to salt stress. Salt stress lowered chl.a:chl.b ratio and vice versa for ch.la+chl.b: carotenoids ratio. Arginine gave the highest values of Chl.a and Chl.a:Chl.b and under irrigation with fresh water but pyruvic acid gave it under saline condition. The vice versa was true for Chl.b and Chl.a+chl.b:carotenoids. However, the treating with pyruvic acid showed the highest effect under both irrigation treatments. The infestions with aphid decreased afier arginine and pyruvic acid under stress conditions.

Keywords: Barley (Hordium vulgare L.)-Salt stress- Arginine- Pyrovic acid-Growth-Dry matter-Chlorophyll-Carotenoid, *Rhopalosiphum padi, Rhopalosiphum maidis*

INTRODUCTION

Barley a member of family Graminey grown Manley in the new cultivated areas and its area in Egypt reached 93866 fed. (Fed=4200 m2) and 102000 tons in 2014 (Ministry of Agric. and land reclamation, Economic affairs sect.) the same obtained by Sabbour and Hussein (2016), Sabbour, (2013).

Barley (*Hordeum vulgare L.)* is a widely grown and highly adaptable winter cereal crop to adverse abiotic and biotic stresses that is used mainly for stock feed and the production of malt for the brewing industry. Because of barley's tolerance of salinity, by 1800 BC it had become the dominant crop in irrigated areas (Bowden, et al., 2010). Barley is considered as one from the important winter cereal crop in Egypt for feed and food especially in the new extension areas for its tolerant to moisture and salt stress.

Environmental stresses dramatically influence plant survival and its productivity. Different plant strategies are required for protection against transient stress (Hamada, 2001). Salinity reduced relative growth rate (RGR), leaf water potential, osmotic potential and fresh and dry weight at full turgor whereas it increased leaf sodium and chloride ions concentration and tissue rigidity. However, the adverse effect of salinity on plant growth was observed relatively late (after 15 d of salt treatment), suggesting that during the first period of treatment, the effect of salinity is osmotic rather than salt specific. Grain number per plant, and grain productivity (g plant–1) were more responsive to salinity than other assessed parameters. For sensitivity to salt stresses example, application of sodium chloride induced, in addition, an increase in Na+ content, while K+ content and K+/Na+ ratio decreased with increasing salt stress. More than growth and yield of reproductive parts, the essential oil (EO) yield decreased by 38.9% and 50.6% at 100 and 150 mM NaCl, respectively, compared with the control (0.77 ± 0.14%). Pulegone, menthone and iso-menthone were found to be the principal components of M. suaveolens subsp. timija EO. The relative abundance of menthone in particular was affected by salt stress. Based on the present results, it can be concluded that Mint timija is a salt sensitive crop and cannot be cultivated in salt-affected areas (Kasrati, et al., 2014). Arginine is one of the essential amino acids considered the main precursor of polyamines which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine (Bocherueu, 1999). Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and developmental processes in higher plants (Glastone and Kaur-sawhny, 1990). Polyamines are involved in the control of cell cycle, morphogenesis in phytochrome, cell division and plant hormone mediated process and the control of plant senescence, as well as the rwesponse of plants to various stress factors (Walters, 2000 and Abdel Monem, 2007). Arginine is a precursor for biosynthesis of polyamines, Agmatine and proline as well as the cell signaling molecules glutamine and nitric oxide (Liu et al., 2006).

The application of arginine significantly promoted the growth and increased the fresh and dry weights, different endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean plants (Nassar et al., 2003); in wheat Abd ElMonem, 2007) and El-Bassiouny et al., 2008). Moreover, Hassanein et al., (2008) recorded the positive role of arginine in alleviating the inhibition occurs as the result of exposing plants to stress. Pyruvic acid is prepared for entrance into the Krebs cycle by conversion to a two-carbon compound (acetyl group) followed by the addition of coenzyme A (CoA) to form acetyl coenzyme A (acetyl CoA). Indole acetic acid formation via indole-3-pyruvic acid (IPyA) and indole-3-acetaldehyde; Conversion of tryptophan into indole-3-acetaldoxyme and indole-3-acetonitrile (IAN); and indole acitic acid biosynthesis via indole-3-acetamide formation (IAM) (Zakharova et al., 1999). Application of organic acids or amino acids for amelioration of salt stress were reported in several studies: Bochrow, et al., (2001); Hussein et al., (2007); Khalid and Fawzi (2011); Hussein, et al., (2014) and Hussein and Alva (2014). *Rhopalosiphum padi, Rhopalosiphum maidis* considered a harmful pests attack many economic crops. In countries where it is abundant it is considered a pest as it damages cereals, legumes, perennial grasses, potatoes, vegetable crops, beet, sunflower, tobacco, hemp, flax and strawberry. Control measures may include deep and chemicals insecticides these pesticides, poison the Sabbour, 2003).

The goal of this work designed to investigate the effect of arginine and pyruvic acid on growth and chlorophyll and carotenoids contents in barley plants grown under salinity conditions.

MATERIALS AND METHODS

For study the response of barley plants to the nitrogen basics treatments and salinity, a pot experiment was conducted in the greenhouse of the National Research Center in the winter season of the 2010 / 2011. The physical and chemical properties of the used soil were shown in Table (1). Barley plants irrigated by 4000 ppm diluted seawater and tap water as a control and sprayed by 150 ppm of arginine and pyruvic acid. The control plants were sprayed by the same quantity of distilled water.

Barley (Hordeum *vulgare L.*) Were sown at Dec., 1. The treatments of the experiment 8 which was the combination of two salinity treatment and spraying treatments of arginine and pyruvic acid e in complete blocks design in six replicates. Calcium super phosphate (15.5% P2O5) and potassium sulphate (48.5% K2O) were added to the soil in the rate of 3.0 and 1.5 g/pot were broadcasted on the soil surface before sowing. Ammonium sulphate (20.5 % N) were added to the soil in the rate of 1.5 g/pot in two equal portion, the first wave was at three weeks and the second have was two weeks later. The treatments of arginine and pyruvic acid and distilled water treatments were applied at 21 and 35 days from sowing. Photosynthetic pigments were according to the methods described by von Wetistien, et al. (1957). All collected Data were subjected determined to the proper statistical analysis as described by Snedecor and Cochran (1982)

Rearing of aphids

R. Padi and R. maidis reared under laboratory conditions on barley at26 ± 2° C and 65 ± 5 RH.

The insecticidal activity of tested materials.

Experiment was designed to test the initial as well as the persistent effect of the tested silica and nano silica on aphids as cumulative mortality during successive intervals (24, 48, 96 and 168 h). Foam granules about 1cm in diameter were treated at time (zero time) with tested oils (2% conc.), dried and provided with heat sterilized bean seeds (100 g/each) fastened each with a string. Then all treatments were used immediately as non-choice test. The foam granules treated with the tested oils materials were mixed with bean seeds (2 g foam/100 g seeds) according to Abd El-Aziz (2001). A pair of newly emerged beetles was placed with treated or untreated broad seeds in glass jars (250 cc capacity) covered with muslin. The number of dead beetles in each jar was counted every day and the percentages of mortality were corrected using the Abbott formula (Abbott 1925). Repellency test the experiments were conducted in an arena based on the choice test (Abd El-Aziz and Ismail 2000). Disc of filter paper (Whatman No. 1) was treated with the tested oil at 1% concentration and placed in the cell A. While filter paper treated with distilled water and emulsifier only as control was placed in the cell B. Twenty newly emerged beetles were introduced into each arena. After 1, 2, 3, 4, 5, 6 and 7 days, the number of beetles present in the cells A and B was recorded. The percentages of repellency values were calculated using the equation:

$D = [1- (T/C)] x100$ (Lwande et al., 1985)

Where T and C represent the mean number of beetles in cells A and B treated and untreated, respectively.

RESULTS AND DISCUSSION

Salt stress

Growth

Salt concentration increases in water of irrigation induce adverse effect on barley plant height, and stem, leaves, spike and total dry weight. This adverse effect may be due to the decreasing in moisture availability and toxic effect of Na+ and Cl-ions (Table 1).

Table (2) show that the effect of arginine and pyrovic acid on photosynthetic pigments of barley plants that increased after acid treatments the barley plant grown under salinity.

Table (3) show that the infestations with bot aphid significantly decreased after arginine and pyruvic acid treatments.

Figure 1 show that the weight loss of the barely significantly decreased after arginine and pyruvic acid.

Hessini, et al., (2014) reported that salinity reduced fresh and dry weight, relative growth rate (RGR), leaf water potential, and osmotic potential at full turgor whereas it increased leaf Na+ and Cl– concentration and tissue rigidity. However, the adverse effect of salinity on plant growth was observed relatively late (after 15 d of salt treatment), suggesting that during the first period of treatment, the effect of salinity is osmotic rather than salt specific. Grain number per plant, and grain productivity (g plant–1) were more responsive to salinity than other assessed parameters. Khatun, et al., (1995) indicated that salinity delaying flowering and spike emergence in barley. They added also that spike length and number of grains/spike were also reduced by salt treatment. However, Khan, et al., (1997) noticed that cereal crops tolerant of salts at germination stage. Dadashi, et al., (2008) confirmed these findings. The effect of sodium chloride (NaCl) concentrations (0.0, 60, 120, 240 mM) on growth, osmotic potential, chlorophyll content, protein content of (Vicia faba L.) seedlings was investigated. NaCl caused an increase in plant height with low and medium concentrations and a decrease with the highest concentration, in both measurement periods. No significant effect was observed in the number of leaves or leaf area with low concentration, while a decrease was noticed for each, with two higher concentrations and in both measurement periods. Salinity increased both fresh and dry weights of the shoot in the two measurement periods. Osmotic potential (O.P.) showed a significant decrease with the increase in concentrations, and in the duration of the stress periods (Abd El-Qados, 1999).

Table (1): Effect of arginine and pyrovic acid on growth of barley plants grown under salinity

Salinity	Chemcal treatment	Plant height	Leaves No /mean stem	Leaves area /mean stem	Dry weight			
					stem	leaves	spike	total
Fresh water	D.W	57.3	4.0	42.9	0.56	0.75	0.37	1.68
	Arginene	62.7	4.0	47.8	0.90	1.27	0.35	2.52
	Pyrovic A.	47.0	4.3	35.3	1.48	0.88	0.46	2.84
4000 ppm	D.W	51.0	4.3	40.8	0.41	0.45	0.27	1.13
	Arginene	38.0	4.3	46.9	1,04	0.98	0.31	2.33
	Pyrovic A.	46.0	4.0	29.6	0.96	0.76	0.35	2.07
Means of salinity	F.W	55.7	4.1	42.0	0.98	0.97	0.39	2.34
	400 ppm	45.0	4.2	39.1	0.80	0.73	0.31	1.84
Means of che.treat.	D.W	54.2	4.2	41.9	0.49	0.60	0.32	1.41
	Arginene	50.4	4.2	47.4	0.94	1.13	0.33	2.40
	Pyrovic A.	46.5	4.2	32.5	1.22	0.82	0.41	2.45
L.S.D at 5%	S	2.87	N.S	8.32	N.S	N.S	0.032	N.S
	C.T	0.525	N.S	10.2	0.596	0.27	0.036	0.670
	SxCT	N.S	N.S	N.S	N.S	N.S	N.S	N.S

F.W.: Fresh water D.W.: Distilled water S.: Salinity C.T,: Chemical treatment LSD: Least significant differences.

Table (2): Effect of arginine and pyrovic acid on photosynthetic pigments of barley plants grown under salinity

Salinity	Chem. treatment	Chl.a	Chl.b	Carotenoids	Chl.a+Chl.b	Chl.a: Chl.b	Chl.a+chl.b: Carotenoids
Fresh water	D.W	1.160	1.830	2.555	2.990	o.634	1.170
	Arginene	4.185	2.367	3.012	6.552	1.768	2.175
	Pyrovic	3.660	4.900	3.898	8.560	0.735	2.822
4000 Ppm	D.W	1.325	2.289	3.461	3.614	0.281	1.044
	Arginene	4.728	3.746	3.086	8.494	1.262	2.752
	Pyrovic	4.876	3.209	5.196	8.085	1.520	1.560
Means of salinity	F.W	3.002	3.323	3.155	6.325	0.900	2.004
	4000 ppm	3.643	3.081	3.914	6.724	1.182	1.718
Mean values of amino acids	D.W	1.243	2.060	3.008	3.303	0.600	1.098
	Arginene	4.457	3.057	3.049	7.514	1.458	2.464
	Pyrovic	4.268	4.055	4.547	8.323	1.053	1.030
	S	N.S	N.S	N.S	N.S	…….	…..
	C.T	2.24	1.29	N.S	1.49	……..	…..
LSD at 5%	SxCT	N.S	1.81	N.S	2.11	…….	….

Table (3): Effect of arginine and pyrovic acid on insect infestation under salinity

Salinity	Chem. treatment	% of target pest infestations *R. padi*	*R maidis*
Fresh water	D.W	87	89
	Arginene	4	7
	Pyrovic	3	6
4000 Ppm	D.W	66	64
	Arginene	1	3
	Pyrovic	2	3
Means of salinity	F.W	70	78
	4000 ppm	33	39
Mean values of amino acids	D.W	32	34
	Arginene	2	3
	Pyrovic	3	4
	S	N.S	N.S
	C.T	2.07	1.09
LSD at 5%	SxCT	N.S	1.71

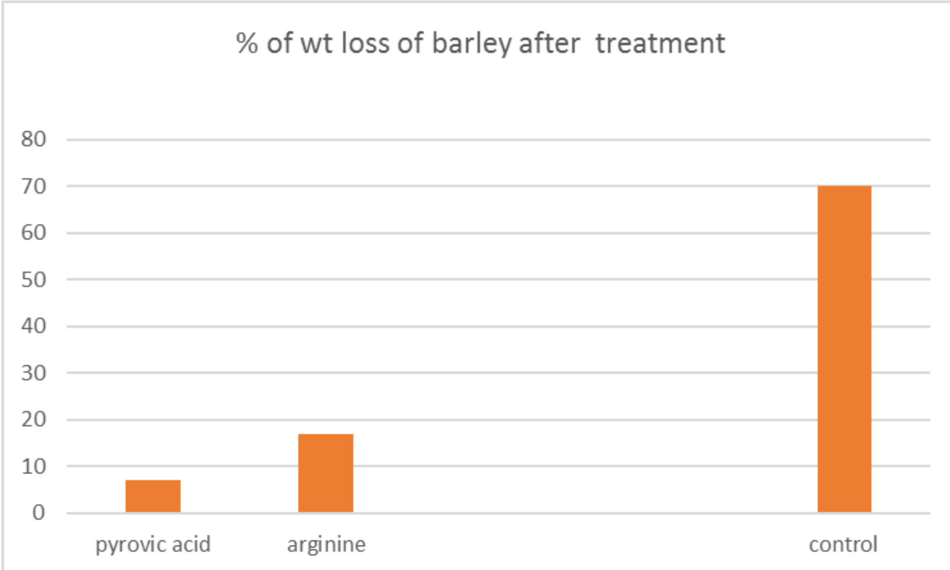

Figure. 1 weight loss of barley after treatments

Growth

Arginine and pyruvic acid increased growth parameters but arginine effect on plant height and dry weight of leaves exceeded those from pyruvic acid. The opposite was true for leaves area/main stem and stem, spikes and whole dry weight. Arginine as a precursor of polyamines which involved in many physiological processes activity such as cell cycle, cell division, morphogenesis in phytochrome and retarding cell senescence (Kuar-Sowhany, and Abd El-Moniem, et al., 2007). In conclusion it is shown for the first time that pre-treatment of hoscyamus plant with Arg increased their capability of Ni accumulation in aboveground organs by 1.5-2 times and reduced its toxic effects on chlorophyll content and oxidative parameters. It might be that arginine induced weakening Ni toxicity and its accelerated transport in plants (chelation) directly on through production of polyamines or NO (Nasaibi, et al., 2013). Organic acids play such as AsA, SA and and CA play an important role in plant metabolism as antioxidants and or as anti-microbial materials (Abd El-Aziz, et al., 2009, Abd El-Baky, et al., 2008 and Hussein, et al., 2007). Bochrow, et al., (2001) indicated that in the model expe riment, bre-treatment of seedling by indo;3-pyrovic acid 75 % growth reduction in untreated seedlings could be compensated completely after one week. Stawisck, et al., (2005) Indole-3-pyruvic acid (IPyA) is also found in plants (Cooney and Nonhebel, (1989); Tam and Normally, (1998), as is the weak auxin phenylacetic acid (PAA), but their physiological roles are unclear. A variety of indolic compounds and their conjugates occur in plant pathogenic and symbiotic microorganisms, but their roles are also largely unknown (Costacurta and Vanderleyden, 1995). The function of iaaL in Pseudomonas savastanoi may be related to pathogenesis, but the mechanism is presently not clear (Romano, et al., 1991 and Spena, et al., 1991). The same obtained by sabbour 2007, Sabbour and Hany 2007 a, b, Sabbour and Abd El Aziz (2010).

Chlorophyll and carotenoids

Salt stress

Data in Table (4) indicated that chl.a and carotenoids increased and the opposite was true for chlorophyll b while chla+ch.b slightly affected when barley plants subjected to salt stress. Salt stress lowered chla:chlb ratio and vice versa for chla+chlb: carotenoids ratio. Dawood and El-Awadi (2015) irrigated faba bean with saline water (3.85 and 7.68 d/Sm) more than fresh water as a control and found that salinity reduced photosynthetic pigments.

Hussein and Alva (2014) reported the depression in photosynthetic pigments in Radi, et al., (2013) related the adverse effect of salt stress to the chlorophyllase enzymes which responsible of chlorophyll degradation. However, Santons

(2004) mentioned that the degradation in chlorophyll as a result of salinity stress may be due to a decrease in the ALA (5-aminolinolic acid). Furthermore, some authors concluded that chlorophyll decreased related to Na ions increased as the major factor for this damage but the other support the finding (as in faba bean plants) of Cl more correlated with chlorophyll depression as in Echinacia purpurea or E. angestfolia (Sabra, et al., 2012). Salinity significantly reduced chlorophyll ‘a’ content in both measurement periods. It also significantly reduced chlorophyll ‘b’, total chl., and carotenoids contents after ten days of treatment. An increase was observed in the protein content in the two measurement periods due to the impact of salinity stress. A directly proportional relationship was found between protein content and the increase in salt concentrations in the first measurement period, while it was inversely proportional in the second (Abd El-Qados, 2011).

Arginine and pyruvic acid

The application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean and in wheat. Moreover, recorded the positive role of arginine in alleviating the inhibition occurs as in result of exposing plants to stress (Mohamed, et al., 2015). Sadak, et al., (2015) reported that spraying of faba bean plants with amino acid improved all fractions of photosynthetic pigments, especially in plants subjected to seawater stress in both seasons. However, amino acid treatments exerted stimulatory effects on photosynthetic pigments under both saline and non-saline (control) conditions. Amino acid treatments not only alleviated the inhibitory effect of salt stress but also in most cases induced an enhanced stimulating effect compared to the control plants.

Salt stress x Arginine and pyruvic acid.

Growth.

Amino acids is well known bio-stimulant which has positive effects on plant growth, yield and significantly mitigates the injuries caused by abiotic stresses. Application of amino acid mixture as foliar spray with different concentrations (500, 1000 or 1500 mg L-1) significantly improved dry weight and yield caused by the reduction induced by seawater stress. The highest level of amino acid of 1500 mg L-1 exerted the strongest effect in alleviating the harmful effect of seawater salinity stress (Kowalczyk and Zielony, 2008).

Arginine an individual of amino essential amino acids. Its importance comes from that it is the precursor of poly amines which converted to putrasine through the decarboxylation of arginine with arginine decarboxylase and polyamine play important roles in many physiological processes such as photosynthesis, protein building, enzymes activity and oxidative defense (Bouchurue, et al., 1999). Fore salinity, NaCl decreased leaf water contents and photosynthetic pigment levels, while the contents of α-to copherol and malondialdehyde increased, but with completely different kinetics. α-Tocopherol levels increased in a dose-dependent manner as stress progressed, while malondialdehyde levels increased at the highest dose (150 mM NaCl) but only during early phases of stress (Taieb, 2011). Concerning the role of arginine and pyruvic acid, these chemicals improved most of physiological processes and adverse the harmful effects of Na and Cl toxicity. Under salt stress, organic acids (OAs), Na+, K+ and Cl− were the main osmolytes in both roots and leaves. Under alkali stress, roots and leaves revealed different mechanisms of ion balance and osmotic regulation. Under alkali stress, in roots, OAs and Na+ were the main osmolytes, and the osmotic role of K+ was small; however, in leaves, OAs, Na+ and K+ all played important osmotic roles. The mechanisms governing ionic balance under both stresses were different. Under salt stress, the contribution of inorganic ions to keep ion balance was greater than that of OAs. However, under alkali stress, Cl−, H2PO4− and SO42− concentrations decreased, and tomato might have enhanced OA synthesis to compensate for the shortage of inorganic anions (Wang, et al., 2011). Plants are continuously subjected to environmental stress; with time they have evolved mechanisms to survive under stress. Plants are able to induce morphological and physiological mechanisms including the activation of different signaling pathways such as mitogen-activated protein kinase signaling, reactive oxygen species and redox signaling, as well as hormonal signaling under stress. The role of small RNAs is also of significance in plants under stress (Mirsnsari, 2014). Irrigation of faba bean plants with seawater levels of 3.13 and 6.25 dS m-1 led to significant reductions in shoot length, number of leaves per plant, fresh and dry weight of shoots, photosynthetic pigments, total carbohydrates, polysaccharides, nucleic acid DNA and RNA contents of faba bean leaves. Seawater salinity induced higher contents of Na+ and Cl- and decreased contents of K+, K+:Na+, Ca2+, Mg2+ and P3+ (Sadak, et al., 2015).

Chlorophyll and carotenoids

The interactive effect of arginine and pyruvic acid and salt stress on photosynthetic pigments of barley plants were presented in Table (6). Data clearly shown that arginine gave the highest values of Chl.a and Chl.a:Chl.b and under irrigation with fresh water but pyruvic acid gave it under saline condition. The vice versa was true for Chl.b and Chl.a+chl.b:carotenoids. However, the treating with pyruvic acid showed the highest effect under both irrigation treatments. The effect of arginine improving of chlorophyll may be related to its effect on enzymes and antioxidant activity (Niu, et al., 2012). Irrigation of faba bean plants with seawater levels of 3.13 and 6.25 dS m-1 led to significant reductions in shoot length, number of leaves per plant, fresh and dry weight of shoots, photosynthetic pigments, total carbohydrates, polysaccharides, nucleic acid DNA and RNA contents of faba bean leaves. Exogenous application of amino acids depressed these damages (Sadak, et al., 2015). They added that foliar spraying of faba bean plants with amino acid improved all fractions of photosynthetic pigments, especially in plants subjected to seawater stress in both seasons. However, amino acid treatments exerted stimulatory effects on hotosynthetic pigments under both saline and non-saline (control) conditions. Amino acid treatments not only alleviated the inhibitory effect of salt stress but also in most cases induced an enhanced stimulating effect compared to the control plants. Awad, et al., (2007) and Azooz (2009) confirmed these findings. Table 3 show that the infections of both target insects significantly decreased by *R. padi* and *R. maidis* in case of additions of Arginine and pyruvic acid. Figure show that the weight loss barley treated arginine and pyrovoc acid showed the weight loss decreasing under field condition and of both *R. maidis and R. Padi are* significantly decreased in the field*.*The same results obtained by Sabbour and shadia abd El azize (2010, 2016 and 2017,2018), Sabbour et al., (2012), Sabbour 2013 and 2014, Repellency test showmen in figures 3 and 4 which detected that nano silica gel is the most affected on the both target pests. The same obtained by Sabbour and Shurab (2018 a,b),and Sabbour and Solieman 2018.

CONCLUSION

From the above mentioned results, it could be concluded that salinity decreased growth and photosynthetic pigments on reverse, spraying pyruvic acid and arginine improved plant growth and photosynthetic pigments and causes a decreasing in aphids infestations. This intern indicated that application of these two acids via leaves help in improving the salt stress tolerant of barley plants and reduce insect infestation.

CONFLICT OF INTEREST

There is no any conflict of interest in our present work.

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AUTHOR CONTRIBUTIONS

Prof. Sabbour, M.M., share in putting the idea, designed the experiments, make the laboratory and store experiments share in statistical analysis and writing the research, reviewed the manuscript. Prof.Mohamed Moursy Hussein and Prof Sawsan El Fahem also, share in putting the idea, designed the experiments, make the laboratory and store experiments share in statistical analysis and writing the research, reviewed the manuscript. All authors read and approved the final version.

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