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I. Introduction

Corn is one of the most common grains grown worldwide, used both as food and forage. Corn seeds are rich in protein, sucrose, and various vitamins, minerals [1]. It ranks third in total world production after wheat and rice and is considered a staple food in many countries, especially in tropical and subtropical regions [2]. Seed sowing and germination of the seed under suitable conditions constitute the first and most important stage of plant production [3, 4]. Seeds and seedlings are very sensitive to physiological and environmental stress factors during germination, emergence, and early seedling.

Various seed applications have been developed to improve the quality of seeds and to minimize the risk of environmental pressure [5]. The effectiveness of the pretreatment method is determined by the osmotic processes, water potential, pretreatment agent and time, temperature, presence or absence of light, oxygen availability, initial seed quality and post-pretreatment drying factors [6]. Pre-sowing soaking, acid etching, growth regulators, vitamins, sowing as a gel after germination, holding in nutrients or osmotic solutions, coating and banding are some of the preliminary applications [7, 8, 9, 10, 11, 3]. Similarly, plant growth regulators and hormones increase the performance of crops with various seed pre-applications [12].

Gibberellins are widely used as a pre-treatment method because they are effective in eliminating seed and bud dormancy, controlling and stimulating seed germination [13]. Gibberellins are engaged in the stimulation of enzymes involved in the seed germination phase. However, in the next stage of germination, gibberellins are transported from the embryo to the endosperm and play a role in converting starch to sugar to provide the necessary energy by stimulating the α-amylase enzyme [7, 8].

Tocopherols are chemically lipophilic antioxidants belonging to the vitamin E family. They are naturally produced in green photosynthetic organisms [14]. Alpha-tocopherol is the compound with the highest antioxidant activity because it contains three methyl groups in its molecular structure. This antioxidant deactivates photosynthetic reactive oxygen species (O_2, H_2O_2, OH) and prevents the propagation of lipid peroxidation in thylakoid membranes [15]. Vitamin E (alpha-tocopherol) penetrates between cellular and organelle membranes and protects the membranes against lipid peroxidation by converting free radicals to less reactive compounds.

Ascorbic acid (AsA) is a cofactor for certain enzymes by protecting various physiological processes in a plan; Barth et al. work [16] on its help with the creation of signal generation and Farooq et al. [17] work on it as a phytohormone. AsA plays a role in photosynthesis, cell division, cell expansion, increase of antioxidants and hormone biosynthesis. As A affects cell division in plants and causes cell elongation, development and ageing, as well as vegetative reproduction [16].

This study comparatively demonstrated the effects of gibberellic acid, alpha-tocopherol and ascorbic acid on the germination of corn seeds. Upon reviewing the literature, numerous studies in which gibberellic acid, alpha-tocopherol and ascorbic acid were applied alone in plants have been found. However, there are no studies on gibberellic acid, alpha-tocopherol and ascorbic acid as a pre-application in the

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corn plant. For this reason, we think that the study preserves its original value.

II. Materials and Methods

In the research, the seeds of 71MAY82 varieties of maize (*Zea mays* L.) were used, and the seeds were obtained the Bursa MAY Agro seed company. Seed pre-applications in germination trials are:

1. C: Control
2. GA: GA_3 250ppm
3. E: Alpha-Tocopherol, 300 ppm (Vitamin E)
4. C: Ascorbic acid, 100 ppm (vitamin C)
5. G+E: GA+ Vitamin E
6. G+C: GA+Vitamin C
7. E+C: Vitamin E + Vitamin C
8. G+E+C: GA+Vitamin E +Vitamin C

Corn seeds were kept in de-ionized water (H_2O), gibberellic acid, tocopherol and ascorbic acid solutions at 20±1°C in dark conditions for 24 hours. After the waiting period, ten seeds were placed in each sterile petri dish, after which the water of the seeds was filtered, and two layers of blotting paper were placed. Germination tests were carried out in Petri dishes with a diameter of 10x2, between double-layered blotting papers, at 60% humidity and 25°C, on 3x10 seeds, with three replications, for seven days [18]. In pre-treated seeds, the initial viability of seeds was determined according to ISTA [18] rules and then taken to germination and emergence test.

a) Analyses

Physiological analysis of germinating plants: The fresh and dry weights and lengths of the radicle and plumula were calculated [19].

Relative water content: Fresh weights of radicle and plumula samples were measured in 6 [20]. They were kept in Petri dishes in dl-H_20 for 6 hours to become turgorized. Then, their turgorous state was measured. After drying at 70ºC for 72 hours, their dry weights were determined. Relative water contents are calculated as % (WW)/(TW-DW)×100 connection.

Germination times: Average germination time (AGT) was calculated with the formula (AGT) =∑Ti Ni/∑Ni. Ti: refers to the number of days after planting, Ni: refers to the number of seeds germinated on the day of observation [19].

Germination rate (%): 3x10 seeds were placed in Petri dishes and germinated in the air-conditioning room. The germination rate was calculated at the end of 7 days when germination was fixed, by proportioning sown and germinated seeds [21].

Germination index: The formula (GI)=∑(Gt/Tt) was used to calculate the germination index (GI). Gt: the number of seeds germinated on day t after sowing, Tt: the number of days after sowing [19].

Germination speed: The germination speed was calculated according to Ellis and Roberts [22]. Σn (Seeds germinated on the day of counting) x d (Day of counting) germination speed = Σn (Total number of germinated seeds).

Protein content: Protein determination in radicle and plumula samples of germinated seeds is made according to Bradford [23] method. 5 μL of samples homogenized with phosphate buffer is taken, 250 μL of Bradford's reagent is added to it, and it is mixed and kept at room temperature for 15 minutes. At the end of this period, the samples were recorded by reading their absorbance at 595 nm in the spectrophotometer.

Catalase enzyme activity: It was carried out according to the method of Bergmeyer [24]. The decrease in the content of H_2O_2 was determined by the decrease in maximum absorbance at 240 nm. The reaction mixture in quartz cuvettes with a final volume of 1 ml consists of 0.1 mM EDTA, 50 mM Na-phosphate buffer (pH: 7), dl-H_2O and 0.3% H_2O_2. The decrease in absorbance during the reaction was followed for 180 seconds. CAT activity was expressed as μmol H_2O_2 consumed per minute.

Results were evaluated according to the analysis of variance in the statistical program, SPSS (Statistical Package for Social Sciences, Version 22.0). Complementary statistics and significance statuses of the analysis results were presented in tables.

III. Results

a) Germination Rate, Germination Time, Germination Index and Germination speed

The effects of pre-applications (GA, vitamin E, vitamin C) on maize seeds (71MAY82) on germination rate, germination time, germination index and germination speed were found to be statistically significant at P <0.01 according to the results of analysis of variance (Table 1).

Table 1: The effects of preliminary applications (GA, vitamin E, vitamin C) on germination time, germination rate and germination index and speed in maize seeds (71MAY82)

	Aplication	Germination time (day)	Germination rate (%)	Germination index	Germination speed
Control	1	3.17 C	100 A	62.06 B	83.33 C
GA	2	3.07 G	96.67 B	63.14 A	90 A
E	3	3.10 F	96.67 B	62.03 C	86.67 B
C	4	3.11 E	93.33 C	59.78 D	83.33 C
GA+E	5	3.44 B	90 D	48.41 E	56.67 E
GA+C	6	3.13 D	53.33 G	33.84 H	46.67 F
E+C	7	3.05 H	66.67 F	43.97 F	63.33 D
GA+E+C	8	3.58 A	80 E	38.54 G	33.33 G
	F value	<0,0001	<0,0001	<0,0001	<0,0001
	LSD	4,8334	0,0071	4,367	7,0805

In terms of germination time, it was determined that corn seeds decreased in GA (3.07), E (3.10) and C (3.11) groups compared to control (3.17) groups. In addition, an increase in germination time was observed in GA+E (3.44), GA+C (3.13), GA+E+C (3.58) groups compared to GA application alone. Germination time in the C treated group increased compared to the E+C application. Based on these results, it can be said that E and C applications act in opposition to each other in terms of germination time.

The germination rate increased in the GA and E (96.67) groups compared to the C (93.33) group. While the germination rate decreased in GA+E+C (80) groups compared to GA+E (90) application, it increased compared to the groups treated with GA+C (53.33) and E+C (66.67). The lowest germination rate; was achieved with GA+C (53.33) application and C (93.3) application.

Compared to the GA (63.14) application, it was determined that there was a decrease in germination index in E (62.03) and C (59.78) applications. It was observed that there was a decrease in the GA+E(48.41), GA+C (33.84), GA+E+C (33.54) application group according to the GA application. The lowest germination rate and germination index were seen in GA+C and GA+E+C groups (Table 1). Pre-application of vitamin C showed a negative effect on the germination index.

The greatest decrease in germination speed was in the GA+E+C (33.33) group compared to the GA (90) and E (86.67) groups. A decrease was observed in the group treated with E+C (63.33) compared to vitamin E application.

b) Radicle and Plumula fresh, Dry Weight and Length

The effect of pre-applications (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on the fresh, dry weights and lengths of the radicle and plumula was found to be statistically significant at P <0.01 according to the analysis of variance results (Table 2).

Table 2: The effects of preliminary applications (GA, vitamin E, vitamin C) on radicle and plumula lenght, dry and fresh weight in maize seeds (71 may 82).

Aplications		Radicle length (cm)	Plumula length (cm)	Fresh weight Radicle (mg)	Dry weight Radicle (mg)	Fresh weigth Plumula (mg)	Dry weight Plumula (mg)
Control	1	7.0 A	2.17 ab	76.24 A	8.49 A	84.60 A	10.22 A
GA	2	5.17 B	2.17 ab	48.27 D	5.56 B	70.30 D	6.17 E
E	3	3.83 B	2.17 ab	42.37 E	5.53 B	58.73 F	7.51 C
C	4	5.33 B	2.33 ab	61.44 B	5.17 C	62.33 E	8.15 B
GA+E	5	4.67 B	2.83 a	50.64 C	3.60 D	80.33 B	6.82 D
GA+C	6	1.83 C	2.00 ab	23.53 F	1.17 E	52.17 G	3.30 F
E+C	7	1.67 C	1.83 b	22.33 G	0.83 F	49.19 H	3.15 G
GA+E+C	8	1.83 C	2.33 ab	21.53 H	1.32 E	75.17 C	3.17 G
	F VALUE	<0,0001	0,4663	<0,0001	<0,0001	<0,0001	<0,0001
	LSD	1,5997	0,8833	0,7592	0,2024	0,7956	0,1179

A statistically significant decrease in radicular length was detected in the co-administration groups compared to the C treatment (Table 2). In addition, GA (5.17), E(3.83), C (5.33) according to application groups GA+E(4.67), GA+C(1.83) and GA+E+C(1.83). It was determined that there was a decrease in the application groups. On the other hand, E(2.17) application increased compared to GA+E (2.83), GA+E+C(2.33) and E+C(1.87) groups. Length measurements in the plumula were among the values in the GA+E(2.83) and E+C(1,83) application groups but were not found to be statistically significant.

The fresh weight of the radicle was decreased in the treatment groups compared to the control (76,24) group. In addition, a decrease was observed in the GA+C(23.53) and E+C(22.33), GA+E+C(21.53) application groups compared to the C(61.44) application (Table 2). In the dry weight of the radicle, compared to the E(5.53) and C(5.17) application groups, the greatest decrease was in the E+C (0.83) group.

If the plumula is on fresh weight, compared to the control (84.60) group, the highest decrease was observed with the E(58.73) application. If it is on dry weight, the highest decrease was observed with the application of GA (8.15 mg) compared to the control (10.22) group (Table 2). According to the GA application, an increase in GA+E fresh (80.33) and dry (6.82) weights was observed. It was determined that there was an increase in fresh weights in GA+E+C(75,17) application compared to GA+C(52,17) and E+C(49,19) application groups.

c) Relative Water Content (RWC)

The effect of pre-applications (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on the relative water content (RWC) was statistically significant at P <0.01 according to the results of the analysis of variance (Figure 1).

The relative water content of the radicle increased by 7.75% in group C compared to the control treatment. Compared to the applications performed alone, RWC increased by 26.14% in the GA+C group (Figure 1).

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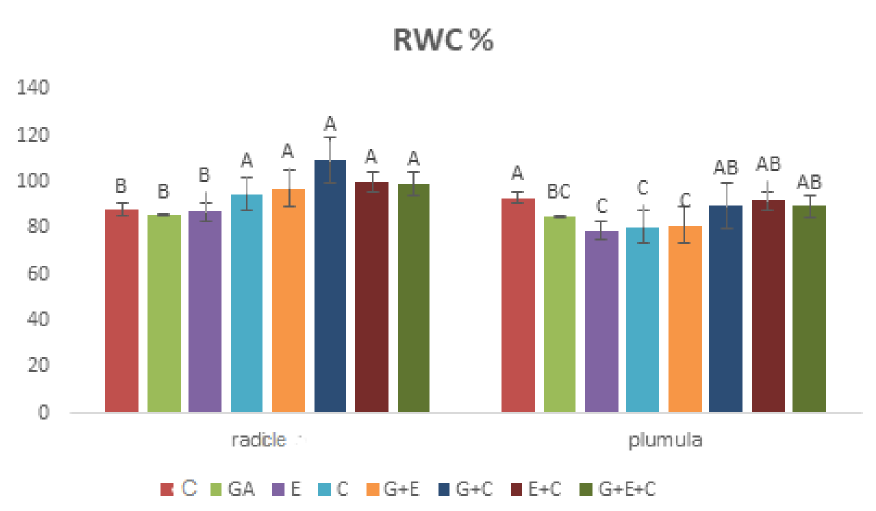

Figure 1: The effects of preliminary applications (GA, vitamin E, vitamin C) on relative water content (RWC) (%) in maize seeds (71may82)

In the relative water content of the plumula, a decrease of 7.225% was observed in GA+E applications compared to GA+C and GA+E+C applications. However, it was determined that there was a 3.13% increase in the GA applied groups compared to the G+E application (Figure 1). Compared to the E+C application, a decrease of 16.15% and C 14.14% was observed in the E applied groups, respectively.

d) Protein content

The effect of pre-applications (GA, vitamin E, vitamin C) on maize seeds (71May82) on protein content (mg) was found to be statistically significant at P <0.01 according to the results of the analysis of variance (Figure 2).

The content of protein increased in all treatments in the radicle compared to the control group. On the other hand, it was determined that there was an increase of approximately 1.36 times in the GA+C application compared to the GA and C application in the compass (Figure 2).

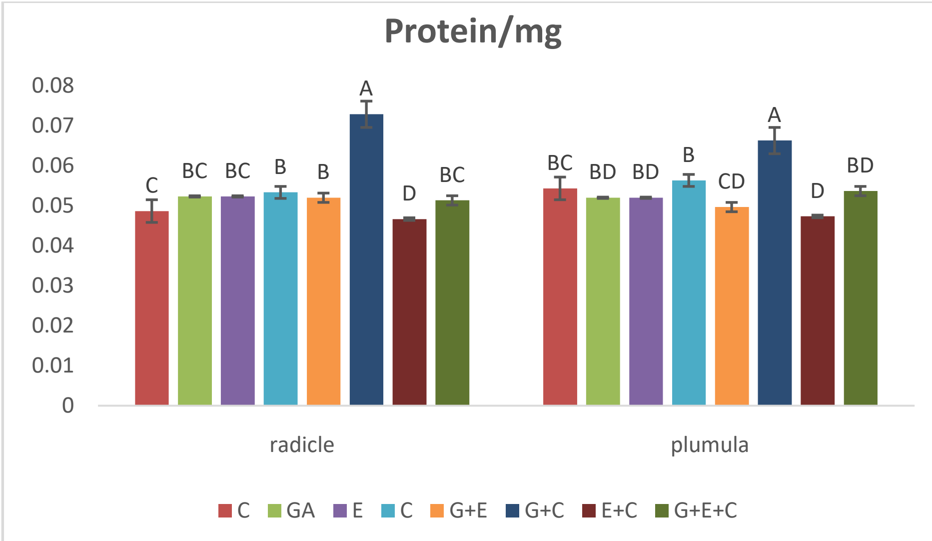

Figure 2: The effects of preliminary applications (GA, vitamin E, vitamin C) on protein content in maize seeds (71MAY82)

e) Catalase Enzyme Activity

The effect of pre-treatments (GA, vitamin E, vitamin C) on corn seeds (71MAY82) on CAT enzyme activity (unit/mg-1 protein) was found to be statistically significant at P <0.01 according to the results of analysis of variance (Figure 3).

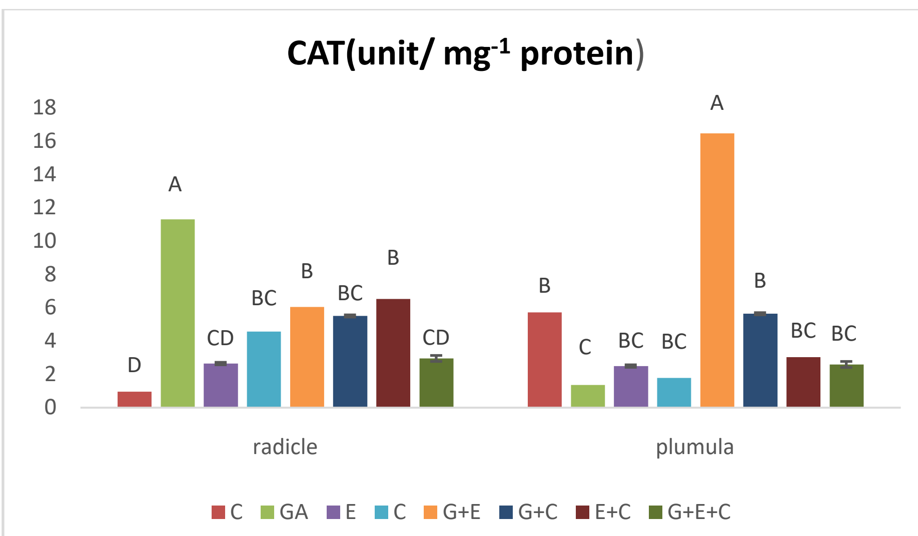

Figure 3: The effects of preliminary applications (GA, vitamin E, vitamin C) on CAT enzyme activity (unit mg^{-1} protein) in maize seeds (71MAY82)

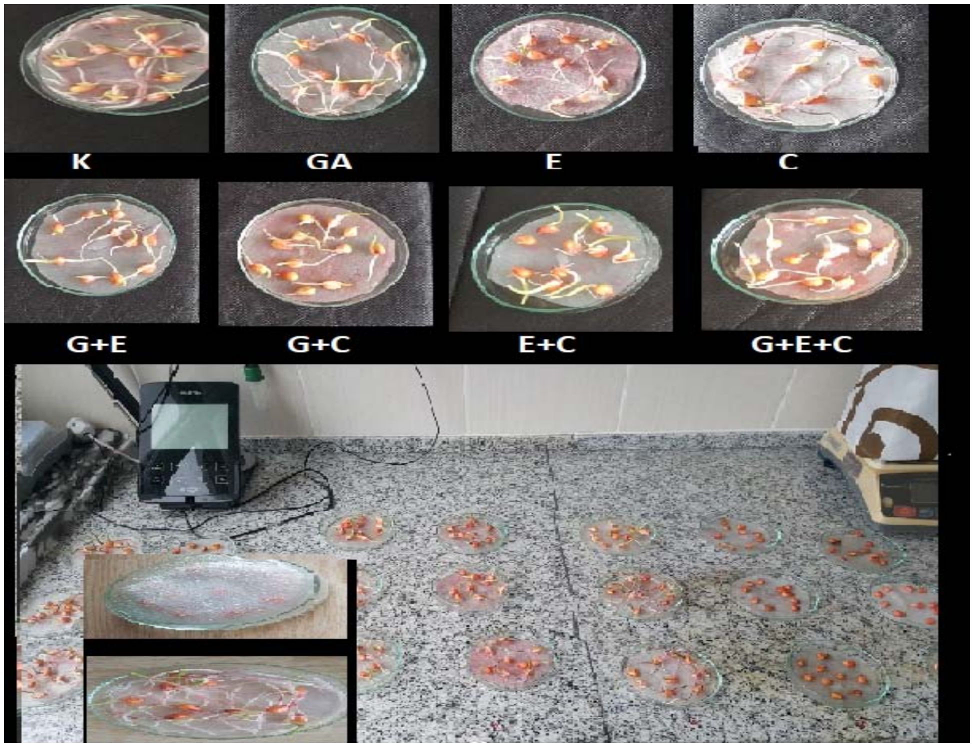

Photo 1: Maize seed spretreated with gibberellic acid, alphatocopherol and ascorbic acid SU Plant Physiology Laboratory

In the radicle, while CAT enzyme activity increased with the application of GA, it caused a decrease in plumula. It was determined that a 3.32-fold decrease in the radicle with the GA+E+C application compared to the GA application. In the plumula, a 12-fold increase was observed with the GA+E application compared to the GA application (Figure 3). E+C application increased CAT enzyme activity in the radicle compared to the E application.

IV. Discussion

The germination time of seeds varies depending on the amount of air and water in the germination medium, its temperature and the water absorption capacity of the seeds [21]. In this study, it was observed that there was a decrease in the GA treatment group compared to GA+E, GA+C, GA+E+C and control groups in corn seeds in terms of germination time. Similarly, Oral et al. [25] and Topcu et al. [26] revealed in their study that GA pre-application reduced the germination time. Accordingly, we think that the increase in germination rate observed in GA application compared to the co-treatment groups is related to the decrease in germination time. Depending on the results obtained in the study, GA and E application groups alone decreased the germination time compared to the combined application groups. The C application decreased it compared to the control group and increased it compared to the E+C application.

It has been demonstrated that seed pretreatments improve germination rate, speed and homogeneity [27]. The germination rate of corn seeds increased with GA and E application compared to C application (Table 1). It has been reported that the increase in germination rate in Arabidopsis mutant seeds treated with vitamin E is associated with a decrease in lipid peroxidation [28]. This result is in good agreement with our data. The greatest decrease in germination rate was with GA+C application compared to C application and co-treatments. Based on these results, we think that the applied vitamin C concentration (100ppm) negatively affects the germination rate.

In terms of germination index, GA application in corn seeds increased compared to GA+E, GA+C, GA+E+C application. In a study conducted by Yuonesi and Moradi [29], on wheat, it was stated that seeds subjected to GA pretreatment had a positive effect on plant growth. Similarly, it has been emphasized that GA applications increase productivity in plants [30]. This situation can be associated with an increase in germination rate and a decrease in germination time. The decrease observed in the groups where vitamin C was administered alone or together is associated with the decrease in germination rate. Similarly, the application of vitamin C to wheat seeds in high concentrations has been reduced germination [31].

In maize seeds, GA application increased fresh and dry weights and radicle length compared to the co-administered groups. Supporting this result, Kaya et al.

[32] found that pre-GA application to sugarcane seeds increased the length and weight values. In our study, radicular and plumula lengths increased with GA+E application compared to E application. This increase is in parallel with the increase in fresh and dry weight observed in the same groups.

Furthermore, while it causes a decrease in radicular lengths and weight values in GA+C and GA+E+C application groups compared to vitamin C application, an increase in RWC content was observed. In contrast, pre-application of vitamin C an increased root length and dry weight [17]. However, it can be said that an increase in plumula fresh weight observed in the groups treated together compared to the C application is due to the increase in RWC. This may be related to the fact that vitamin C has a positive effect on RWC, increasing the germination speed and rate. E+C application caused a decrease in all physiological parameters compared to the applications performed alone. We believe that this situation caused the negative effect of E+C application on seed germination by affecting the germination index and rate.

The content of protein constitutes the main nutritional source important during the development and maturation of seeds [33]. In our study, the protein content in the radicle and plumula increased with GA+C application than the control groups (Figure 2). We think that this result is related to the stimulation of enzyme activities. Mohsen et al. [34] showed that pre-treatment of *Viciafaba* seeds with vitamin C increased their protein content. Similarly, it has been determined that the application of 100 and 200 ppm vitamin C in oilseeds increases the activity of CAT and protein [35]. Accordingly, the increase in weight values of the radicle and plumula is associated with the increase in protein content.

In maize seeds, catalase enzyme activity increased with GA application in the radicle, decreasing in the plumula. In addition, we think that the increase observed in catalase enzyme activity with GA+E application in the radicle is due to GA application (Figure 2). Similarly, an increase in CAT enzyme activity is associated with limiting hydrogen peroxide production by preventing dehydration-related oxidative damage [36] and preserving lipid mobilization [33]. Moreover, Younesi and Moradi [29] observed an increase in catalase activity with pre-GA application to seeds of the Medicago sativa plant.

In our results, C application in the radicle increased catalase activity compared to control and co-administration decreasing it in plumula compared to control, causing no change compared to co-administrations. Thus, Dolatabadian and Sanavy Modarres [34] found that the application of vitamin C to sunflower seeds reduced catalase activity.

V. Conclusion

While it was observed that gibberellic acid and vitamin E applications on corn seeds had positive effects, it was determined that vitamin C caused negative effects depending on the applied concentration. Additionally, it was observed that the combined applications had a negative effect on the physiological and biochemical parameters of the radicle and plumula. Furthermore, we believe that the data obtained from this study will shed light on the applications to be made to increase the yield of maize seeds under stress.

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