OVSFRD Project Final Report 2022

Oxifertigation to improve soil health and sustain tomato yield and quality

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Introduction

Oxyfertigation is a promising approach to increase partial pressure of oxygen (formation and diffusion) in soil via mechanical or chemical means when irrigating or fertigating crops. Chemical oxyfertigation based on peroxides, especially dilute solutions of hydrogen peroxide (H_2O_2), can be a non-toxic, novel, and a holistic win-win approach to alleviate hypoxia and improve soil biological health for economic crop productivity under plasticulture. Once applied via irrigation or fertigation, the H_2O_2, upon decomposition, is expected to produce water and oxygen, and diffuse oxygen to aerate the rhizosphere due to its higher mobility.

As H_2O_2 is one of the most important signaling chemicals, we hypothesize that it will activate plant physiological and metabolic processes, induce salt and drought tolerance, enhance soil microbial activity and diversity, biocontrol services, and improve water- and nutrient-use efficiency to support economic crop productivity. Despite all these potential advantages, the routine use of H_2O_2 for horticultural crops is limited due to lack of consistent and valid results to help guide farmers.

Objective

The objective of our research was to evaluate the effects of variable concentrations of H_2O_2, as an enriched oxygen source, to aerate the rhizosphere under plasticulture to affect growth and fruit yield and quality of fresh-market tomatoes when compared to the control, and to disseminate the science-based knowledge to producers and educators.

Materials and Methods

Experimental site

This study was conducted at the Ohio State University South Centers at Piketon, Ohio (lat. 39.07° N, long. 83.01° W, elevation 578 feet). While the average monthly maximum air temp. of 32.2 ^{0}C was recorded highest in the month of August, it was lowest of less than 15.6 ^{0}C in the month of September of the year. Mean annual rainfall is 96.2 cm, with about 55% of the precipitation falling during the crop growing season (May to September). The highest monthly rainfall (15 cm) was recorded in July. The monthly relative humidity ranging between 79 to 93%, soil temperatures at 15 cm deep ranging between 3 to 30 ^{0}C, and solar radiation ranging between 9,980 to 43,000 KW/m^2.

The soil is a deep, nearly level and somewhat poorly drained Doles silt loam (Fine-silty, mixed, active, mesic Aeric Fragiaqualf) and had pH 6.0$\pm$0.3, total organic carbon 0.82$\pm$0.23%, total nitrogen 0.105$\pm$0.024%, bulk density 1.28$\pm$0.04 g/cm^3, and sand, silt, and clay 30$\pm$4, 55$\pm$2, and 15$\pm$2%, respectively at 0 to 20 cm depth.

Field experimental design

The field research trial was established using fresh market tomatoes using a two-factorial experiment in a completely randomized design (4 H_2O_2 rates x 2 growth stages) with four replications. The H_2O_2 treatments (control [0], 0.5, 1.0, & 2.0 ppm) were applied to the rhizosphere weekly from the maximum vegetative growth stage to the early flowering stage vs. the early flowering stage to the fruiting stage via subsurface drip fertigation. Each replicated plot was consisting of 5 plants with standard spacing and in rows provided with respective H_2O_2 treatments at different times under plasticulture.

Cultural practices

Tomato cultivar Sunbrite was seeded into 72 cell plug trays containing Metro Mix 360 soilless media on April 4, 2022. NPK at the rate of120 lbs. actual 19-19-19 was applied to the field prior to laying plastic. Plastic rows were 6 ft apart with tomato seedlings planted being spaced 1.5 ft apart in rows. Tomato seedlings were planted on raised beds using a waterwheel transplanter on May 24, 2022. Fungicides were applied following recommendations from the Midwest Vegetable Production Guide for Commercial Growers (ID-56).

All the H_2O_2 (3% strength) treatments was applied via the drip irrigation. The irrigation valves were shut off except for the H_2O_2 treatment that was being applied. Lines was pressurized then the H_2O_2 treatments was injected into the irrigation water, each treatment took 15 min to inject then was allowed to irrigate for an additional 15 min to purge the lines then the valve was shut off at each treatment. The header line was then uncapped to empty header line between each treatment.

Growth and yield attributes of tomato

Rapid, non-destructive measurements of leaf chlorophyll (a measure of N uptake) was determined bi-weekly using the Minolta® SPAD-502 meter. At harvest, the number, size, and weight of fruits small, medium, large-sized and marketable yield of tomato were recorded. A sub-sample of tomatoes was processed to analyze for soluble solids (TSS) and brix, pH, and color index. *The TSS (°Brix)* concentration was determined by refractometry using an ATAGO® digital refractometer (Tokyo, Japan). Two drops of fruit juice were placed on the prism of the equipment surface, and the percentage of soluble solids was shown directly, expressed in terms of °Brix. The color measurement was performed at 10 points around the equatorial region on the tomato surface, using a Minolta® CR-200 Chroma meter (Minolta Camera Co., Osaka, Japan).

Sampling and analysis of tomato

Randomly selected fresh tomatoes at peak harvesting event were collected from each replication and processed to analyze for mineral nutritional quality of fruits. At last harvest, root, stem and leaf samples of tomato plants were collected separately. A portion of the

collected fruit, root, stem and leaf samples was oven-dried at 55±2 ^{0}C, ground with the Wiley Mill® grinder followed by sieving with a 125 µm mesh, and stored in sealable plastic bags until analysis.

A 1.0 g processed sample of tomato fruit, root, stem, and leaf was taken into a 50 mL Teflon tube and digested using a mixture of 10 mL of concentrated HNO_3 and 5 mL of 30% H_2O_2 (2: 1 ratio) at 185°C for 10 min using Anton Parr® Microwave Digestion System. After cooling, the digested aliquot was diluted with distilled deionized water, followed by filtration with a Whatman® filter paper. Nutrient concentration in the aliquot of fruit, root, stem, and leaf samples was determined in triplicates using Shimadzu® Inductively Coupled Plasma-Emission spectrometry (ICPE-9000).

The detection limits of nutrients and heavy metals such as B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, and Zn were 0.2, 0.5, 0.2, 0.3, 0.2, 0.3, 0.5, 0.5, 0.2, 0.3, 0.1 and 0.2 µg/L, respectively. After every 10 samples, a QC/QA sample prepared from certified standard solution, was analyzed to check the analytical quality with a relative standard deviation of QA/QC (5 to 8%). Analytical quality control was maintained by analyzing certified reference material NIST 1567b (wheat flour). Replicated analysis of the reference material showed a recovery of 94 ± 12%. Analytical precision as determined by QA/QC procedures, reagent blanks, and internal standards, was better than ±10%.

Soil and water analysis

Composite soils were collected, processed, and analyzed for pH, organic matter, total nitrogen, nutrient and heavy metals content (**Table 1**). The O_2 concentration at 0-to-15 cm depth at 5 cm intervals (soil between plants vs. rhizosphere) was determined using the Eijkelkamp® Oxymeter sensor. Soil microbial biomass was determined by rapid microwave irradiation and extraction method (Islam and Weil 1998). Water used for irrigation was analyzed for pH, nutrient and heavy metals contents (**Table 1**).

Table 1. Mineral nutrient and heavy metals contents of soil and water.

Element	**Soil**	**Water**
	(mg/kg)	(mg/L)
Phosphorus	349.0± 20.1	0.02±0.002
Sulfur	102.4±21.9	2.41±0.08
Calcium	1104.4±71.9	3.73±0.64
Magnesium	1431.1±264.3	10.5±0.93
Potassium	1834.1±351.1	4.8±0.31
Iron	17547.2±100.3	0.03±0.01
Manganese	671.1±69.0	1.77±0.53
Copper	31.8±6.3	<DI
Zinc	52.0±26.8	<DI
Molybdenum	0.94±1.0	<DI
Boron	1.67±1.16	0.01±0.002
Sodium	216.1±30.7	0.74±0.34
Cadmium	0.47±0.11	<DI
Chromium	27.9±9.4	<DI
Nickel	15.9±2.7	<DI

Eijkelkamp Soil Oxymeter

Prior to establish the field experiment, composite soil cores (2.54 cm internal dia.) at 0-20 cm depth were collected using the JMC® stainless-steel soil environmental probe lining with plastic tube and placed in sealable plastic bags for a short-term storage at 4 °C until analyzed. A portion of the field-moist soil was air-dried for a period of 15 days under shade at room temperature (~ 25 ^{0}C), ground by using an Agate mortar and pestle, and sieved through 2 mm mesh prior to analysis. Water was collected in sterilized plastic bottles and stored in refrigerator at 4°C before analysis.

Soil and water pH were determined using a glass electrode pH meter (Model 520A, Orion®, Boston, MA, USA). For nutrient and heavy metals analysis, a 1.0 g finely ground (<125 µm) soil sample was placed into a 50 mL Teflon tube and mixed with a mixture of 16 mL of conc. HNO_3 and HCl (1:3 ratio) at 185°C for 10 min using Anton Parr® Microwave Digestion System. After cooling, the digested aliquot was diluted with distilled deionized water followed by filtration with white ribbon filter paper (Macherey–Nagel®, Germany, 640 m, Ø 125 mm, Cat No. 203 210). Nutrient and heavy metals, such as B, Ca, Cu, Cr, Cd, Fe, K, Mg, Mn, Mo, Ni, P, S, and Zn were analyzed using Shimadzu® ICPE-900 spectrometry.

Water-use efficiency

Water-use efficiency (WUE) was calculated (Lavrenko et al. 2021) as the ratio of the marketable yield of tomato divided by the total volume of water available (soil moisture and irrigation) provided for growing tomatoes.

WUE (kg/m^3) = [Marketable yield of tomato (kg/ha)] / water used (m^3/ha)

Statistical analysis

Data on tomato yield and fruit quality parameters were processed for multivariate statistical analysis. Data were subjected to two-way analysis of variance procedure of the SAS® (SAS, 2010). Simple and interactive effects of predictor variables on dependent variables were separated by the Least Significant Difference test with a value of $p \leq 0.05$, unless otherwise mentioned. Graphs were prepared by using SigmaPlot®.

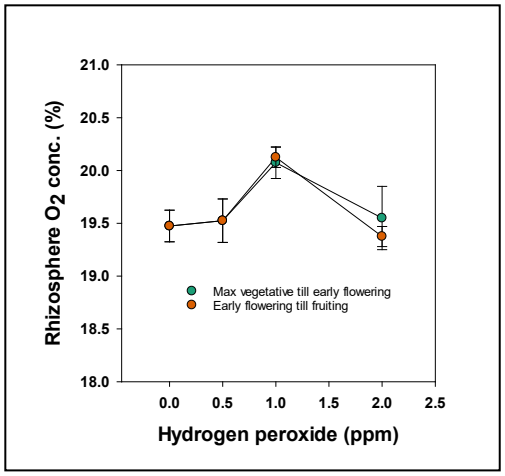
Results and Discussion

Rhizosphere oxygen concentration

Hydrogen peroxide application non-linearly increased tomato rhizosphere oxygen concentration, and its effect on soil oxygen concentration was more pronounced when applied at the rate of 1 ppm. Timing of application of H_2O_2 (from maximum vegetative growth to early flowering vs. early flowering till fruiting) did not vary significantly (**Fig. 1**).

Growth and yield of tomato

The SPAD reading, as measures of chlorophyll and nitrogen uptake by plants, did



not vary significantly neither by H_2O_2 treatments nor timing of application (**Fig. 2**). However, there was a significant interaction of H2O2 treatment and its application on leaf SPAD readings. In general, the leaf SPAD decreases non-linearly over time. Results suggests that mean leaf SPAD reading had to be maintained by around 65 to optimize growth and yield of tomato.

Fig. 1. Rhizosphere (soil-root) oxygen conc. in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

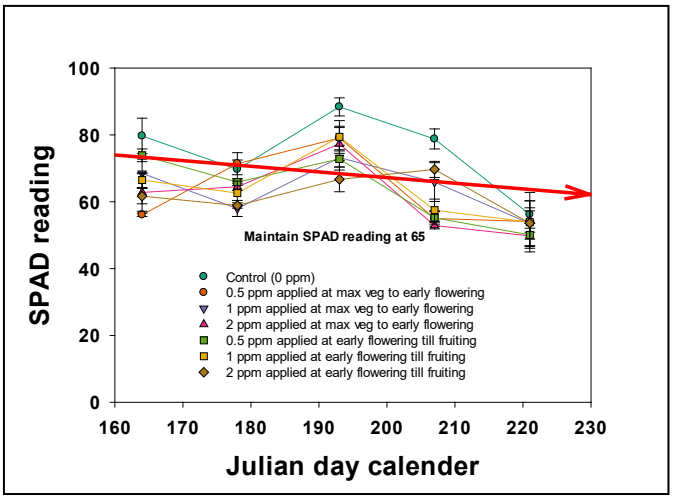


Fig. 2. Leaf SPAD reading in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

Results showed that total fruit yield of tomato was affected by H_2O_2 application (**Fig. 3 - 5**). Increasing concentration of H_2O_2 increased total fruit yield of tomato when applied at maximum vegetative growth to early flowing growth stages (**Fig. 3**). In contrast, increasing concentration of H_2O_2 treatments non-linearly increased the tomato fruit yield up to 1 ppm when applied at early flowering till fruiting growth stages. However, total fruit yield of tomato decreased when 2 ppm H_2O_2 was applied (**Fig. 3**). The small-size tomato yield was ranged from more than 6 tons (12,000 lbs.) per acre in the control treatment to a high of 10 tons (20,000 lbs.) per acre in the H_2O_2 treatments (**Fig. 4a**).

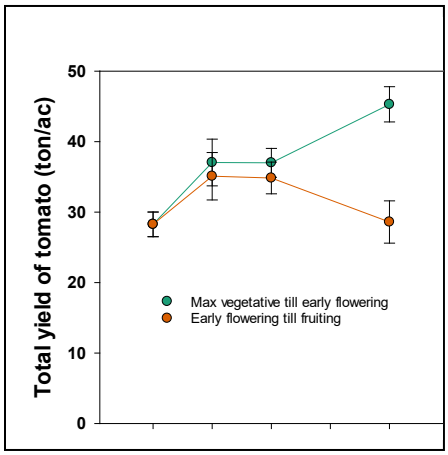


Fig. 3. Total yield of tomato fruits in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

Increasing H_2O_2 concentration increased small-size tomato fruit production when applied during maximum vegetative growth to early flowering stages. In contrast, a 2 ppm H_2O_2 concentration decreased small-size tomato production per acre. Likewise, medium-size tomato fruit yield per acre increased by H_2O_2 treatments, from a low of 10 tons (20,000 lbs.) per acre in the control to a high of 13 tons (26,000 lbs.) per acre in the H_2O_2 treatments (**Fig. 4b**). The 2 ppm H_2O_2 treatment

significantly increased the medium-size tomato fruits production by 15 tons (30,000 lbs.) pe acre, when applied during maximum vegetative growth to early flowering stages.

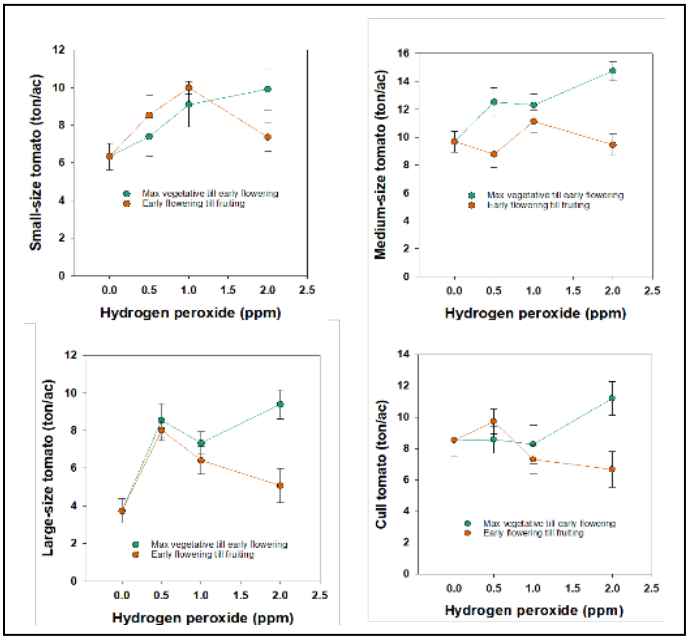


Fig. 4. Total yield of tomato fruits in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

Large-size tomato production per acre ranged from a low of 4 tons (8,000 lbs.) per acre in the control to a high of 9 tons (18,000 lbs.) per acre in the 2 ppm H_2O_2 treatment, when applied during the maximum vegetative growth to early flowering growth stages (**Fig. 4c**). Increasing H_2O_2 concentration (1 to 2 ppm) decreased large-size tomatoes when applied at early flowering till fruiting. In general, cull tomato production decreased with an increase in H_2O_2 concentration when applied during early flowering till fruiting stages. When H_2O_2 was applied at 2 ppm during maximum vegetative growth to early flowering stages, it increased cull tomato production (**Fig. 4d**). However, percent cull tomato decreased with increasing in H_2O_2 concentration, regardless of application timing (**Fig. 5**).

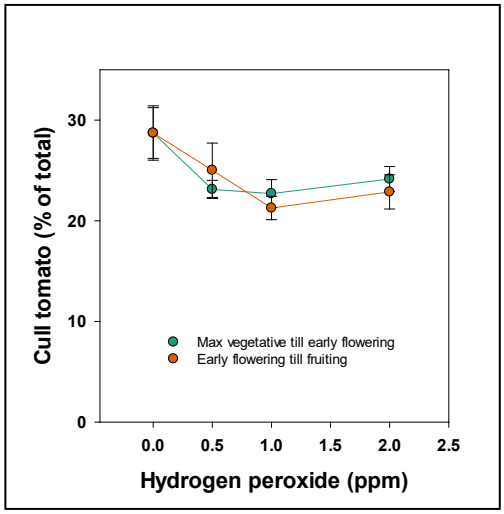


Fig. 5. Cull tomato production in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

Total marketable fruit yield of tomato per acre increased by H_2O_2 application (**Fig. 6a**). It ranged from a low of 20 tons (40,000 lbs.) in the control to a high of 32 tons (64,000 lbs.) per acre with 2 ppm H_2O_2 when applied during maximum vegetative to early flowering growth stages. However, increasing H_2O_2 treatments non-linearly increased total marketable tomato fruit yield. However, 2 ppm H_2O_2 treatment decreased marketable tomato fruit yield, when applied during early flowering till fruiting stages. An optimum rate of H_2O_2 application was 1 ppm for higher marketable tomato fruit yields (**Fig. 6a**).

Tomato fruit quality in terms of color index improved with an increase in concentration of H_2O_2 treatments (**Fig. 6b**). The effect was more pronounced when H_2O_2 treatments were applied during early flowering till fruiting stages compared with the maximum vegetative to early flowering stages. Application of H_2O_2 non-linearly affected the soluble solids (brix) content of tomato fruits when applied at both growth stages (**Fig. 6c**); however, when H_2O_2 was applied at 0.5 ppm during early flowering till fruiting stage,

the highest brix was obtained. Tomato fruit juice pH increased slightly with an increase in H_2O_2 concentration (**Fig. 6d**).

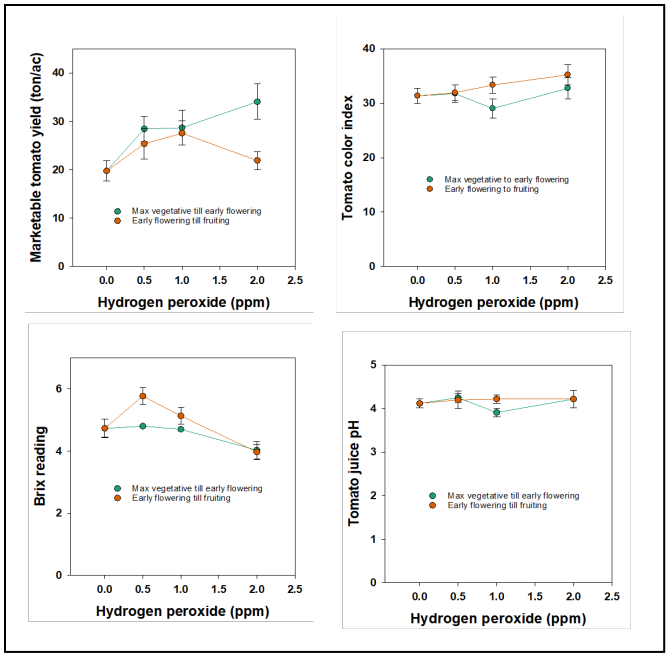


Fig. 6. Tomato fruit yield, fruit color index, soluble solids (brix), and tomato juice pH in response to H_2O_2 treatments applied in the rhizosphere at different growth stages.

Results showed that average weight of tomato fruits affected by H_2O_2 treatments (**Fig. 7**). Highest average tomato fruit weight (~ 9 oz) at 05. ppm H_2O_2 treatment. was Increasing the concentration of H_2O_2 treatments beyond 0.5 ppm decreased the average fruit weight of tomato. The effect was mor pronounced when H_2O_2 was applied during the early flowering till fruiting growth stages.

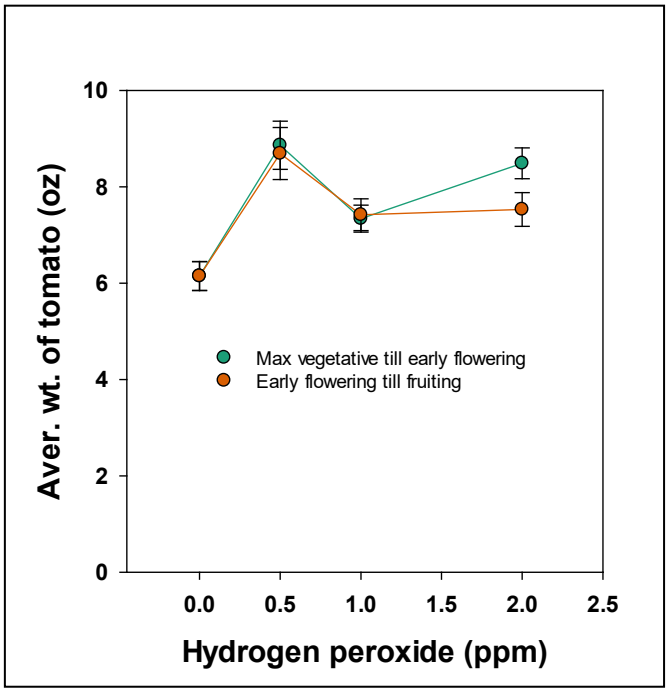


Fig. 7. Average weight of tomato fruit in response to H_2O_2 treatments applied in the rhizosphere at different growth stages.

Nutrient content of tomato

Both macro- and micronutrient concentration in tomato fruits affected by H_2O_2 application and its timing of application (**Table 2 - 3**).

Macronutrients, such as P, K, Ca, Mg and S content increased in tomato fruits with an increase in H_2O_2 content treatments (**Table 2**). While highest concentration of P (344 to 372 mg/kg) and Mg (147 to 154 mg/kg) was observed at 1 ppm H_2O_2 treatment, the K (1803 to 2037 mg/kg) and Ca (115 to 140 mg/kg) content was highest at 2 ppm H_2O_2 treatment, when compared to the control (276 and 108 mg/kg, respectively). Sulfur concentration (116 to 129 mg/kg) was higher in tomato fruits with an increase in H_2O_2 treatments (0.5 to 2 ppm), when compared to the control (89 mg/kg). The P, K, and Ca content was higher in tomato fruits when H_2O_2 was applied during maximum vegetative to early flowering growth stages.

Table 2. Macronutrient concentration in tomato fruits under different levels of hydrogen peroxide applied at maximum vegetative growth to early flowering growth stages and early flowing growth till fruiting growth stage (mean values were presented with standard deviation).

H_2O_2 (ppm)	Application at tomato growth stages	P	K	Ca	Mg	S
				(mg/kg)		
Control		276±39	1363±12	69+8	108±14	89±11
0.5	Max. veg.- early flowering	351±42	1777±6	102±11	145±16	128±15
	Early flowering - till fruiting	327±52	1973+12	67±8	151±23	129±19
1.0	Max. veg.- early flowering	372±29	1471±14	137±12	147±12	116±12
	Early flowering - till fruiting	344±39	1813±6	93±9	154±16	119±12
2.0	Max. veg.- early flowering	315+35	2037±6	140±14	129±13	122±13
	Early flowering - till fruiting	325+36	1803±12	115±12	149±14	123±12

P=Phosphorus, K=Potassium, Ca=Calcium, Mg=Magnesium, S=Sulfur.

Both micro- and beneficial nutrients, such as Fe, Mn, Cu, Zn, Mo, B, and Na concentration in tomato fruits did not vary consistently in response to H_2O_2 treatment and its timing of application (**Table 3**). Among the micronutrients, the Fe (13.3 mg/kg), Zn (9.1 to 10.3 mg/kg) and Cu (10.9 to 11.5 mg/kg) concentration increased with an increase in H_2O_2 application, when compared to the control (12.3, 8.8 and 10.9 mg/kg, respectively).

Table 3. Micronutrient contents in tomato fruits under different levels of hydrogen peroxide applied at maximum vegetative to early flowering (MVEF) growth stages and early flowing till fruiting (EFF) growth stages (mean values were presented with standard deviation).

H_2O_2 (ppm)	Application time	Fe	Mn	Zn	Cu	Mo	B	Na
					(mg/kg)			
Control		12.3±0.8	8.4±0.1	8.8±0.1	10.9±0.0	5.2±0.2	13.0±0.7	80±0.6
0.5	MVEF	12.7±0.6	8.8±0.2	9.3±0.2	11.1±0.1	5.0±0.1	12.5±0.1	91±0.7
	EFF	12.3±0.7	8.8±0.2	9.4±0.3	10.9±0.1	5.0±0.1	12.9±0.2	80.7±0.5
1.0	MVEF	11.7±0.1	7.5±0.0	10.3±0.2	10.2±0.2	5.0±0.1	13.1±0.0	88.1±0.5
	EFF	13.7±0.7	9.1±0.2	9.3±0.2	11.0±0.1	5.1+0.1	12.5±0.1	84.6±0.4
2.0	MVEF	11.9±0.5	8.7±0.1	9.2±0.2	10.9±0.1	5.0±0.1	12.4±0.1	81.4±0.4
	EFF	13.3±0.6	8.9±0.1	9.1±0.2	11.5±0.2	5.0±0.1	12.6±0.1	87.8±0.6

Fe=Iron, Mn=Manganese, Zn=Zinc, Cu=Copper, Mo=Molybdenum, B=Boron, and Na=Sodium.

Water-use efficiency of tomato

Hydrogen peroxide treatments have shown beneficial effects to improve water-use efficiency of tomato (**Fig. 8**). Increasing concentration of H_2O_2 increased water-use efficiency 6.2 to 8.3 kg/m^3 water), when compared to the control (6 kg/m^3 water). The effect was more pronounced at 2 ppm H_2O_2 treatment when applied during maximum vegetative to early flowering growth stages, compared to early flowering till fruiting stages of tomato plants. Result showed that H_2O_2 applied at 2 ppm during early flowering till fruiting stages decreased water-use efficiency of tomato plants. Increased water-use efficiency of tomato was due to high production of tomato in response to the beneficial effects of H_2O_2 application to improve soil biological activity, optimize water and nutrient uptake, and greater soil aeration.

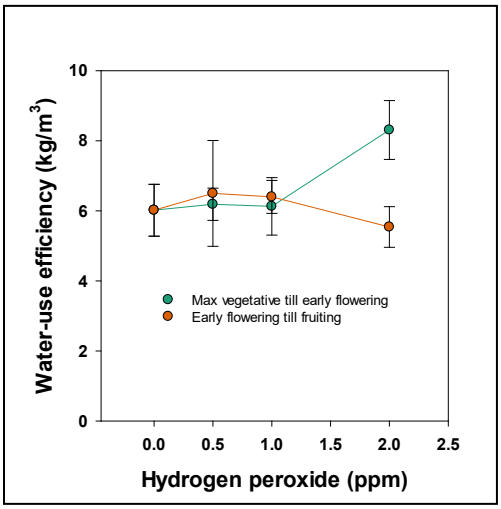


Fig. 8. Water-use efficiency of tomato in response to H_2O_2 treatments applied in the rhizosphere at different growth stages.

Soil biology

Soil biology especially total soil microbial biomass increased in response to H_2O_2 treatments (**Fig. 9**). Soil microbial biomass increased from 1.76 mg/kg under control to 216, 231 and 287 mg/kg under 0.5, 1 and 2 ppm H_2O_2 treatments, respectively.

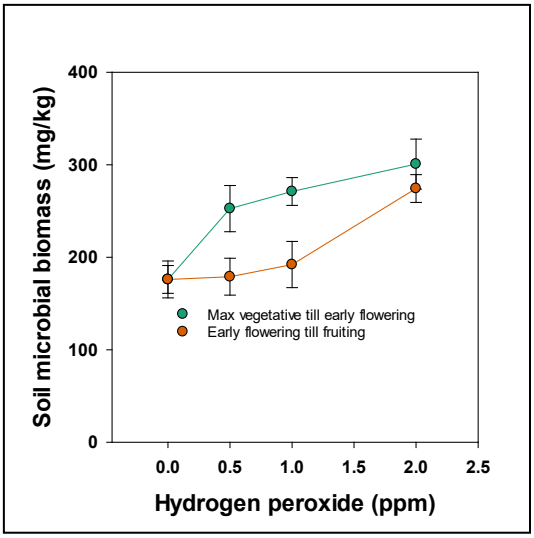


Fig. 9. Soil microbial mass in response to H_2O_2 treatments applied in the rhizosphere at different growth stages of tomato.

Soil microbial biomass increased pronouncedly when H_2O_2 was applied during the maximum vegetative to early flowering growth stages compared to H_2O_2 applied during early flowering till fruiting stages. Increased microbial biomass content in response to H_2O_2 treatments was due to greater soil aeration, higher labile carbon and nitrogen availability for microbes, and greater biodiversity.

Extension outreach activities

A tomato field day/night was organized in August 2022 to demonstrate our research to the growers. A presentation was delivered to the participants, who attended the field night. At Farm Science Review, another presentation on tomato production and health benefits was delivered at Specialty Crops small farm presentation. A radio/TV

telecast (including YouTube video) on our project was conducted to disseminate evidence-based knowledge to educators and OVSFRDP farmers, especially young, minority, and future farmers.

A news article has been published in CFAES OSU South Centers Connections 2022 Achievements issue related to our project on fresh market tomato production. (southcenters.osu.edu/newsletter/connections-newsletter/winter-2022-achievements-edition).

A radio/TV telecast and YouTube video (South Centers Chat with Tom Worley. Arif Rahman - Benefits of Tomatoes - YouTube) on our project was conducted to disseminate evidence-based knowledge to educators and OVSFRDP farmers, especially young, minority, and future farmers.

Three educational, institutional facility and research tours were conducted for clientele in 2022.

References

Islam K.R. and R.R. Weil (1998) Microwave irradiation of soil for the routine measurement of microbial biomass C. Biology and Fertility of Soils. 27:408-416.

Lavrenko, S.O., Lavrenko, N.M., Maksymov, D.O., Maksymov, M.V., Didenko, N.O., and Islam, K.R. (2021) Variable tillage depth and chemical fertilization impact on irrigated common beans and soil physical properties. Soil & Tillage Research https://doi.org/10.1016/j.still.2021.105024

SAS Institute (2010). SAS Statistics Users' Guide R. 9.4, SAS Institute. Cary, NC, USA.