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The effects of pine wood vinegar on the germination, growth and photosynthetic characteristics of cucumber

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ABSTRACT- Wood vinegar is a substance, derived from cooling black carbon fire, which could be used instead of chemical materials in the agriculture industry as an organic compound. In order to study the effectiveness of pine wood vinegar on physiological and photosynthesis traits of cucumber, two experiments were conducted based on a completely randomized design with six treatments including 0, 1250, 2000, 2500, 3333 and 5000 mgL^{-1} of wood vinegar with four replications. The first experiment was designed in the laboratory in order to study the effect of pine wood vinegar priming on cucumber seeds and the second one was conducted in a greenhouse condition to investigate the effect of wood vinegar on seedlings of cucumber in Isfahan University of Technology. The highest flower number and yield in cucumber was observed in 2500 mgL^{-1} pine wood vinegar trearment. Photosynthesis in cucumber increased in 2000 mgL^{-1} pine wood vinegar trearment. The lowest transpiration in cucumber was 1.54 $mmolm^{-2}s^{-1}$ in 2000 mgL^{-1} pine wood vinegar treatment. 1250 mgL^{-1} wood vinegar treatment increased germination percentage and speeded up the germination process compare to control. The highest root length, volume and surface were observed at 1250 mgL^{-1} treatment. The concentration of nitrogen (5.5% DW) in the treatment of 3333 mgL^{-1} Pine wood vinegar, the potassium concentration in the treatment of 5000 mgL^{-1} and calcium and iron concentrattions in the treatment of 1250 mgL^{-1} were at the highest levels compared to other treatments. The highest amount of chlorophyll and photosynthesis was observed at 2000 mgL^{-1} treatment. The wood vinegar with 2500 mgL^{-1} pine wood vinegar concentration showed the highest flower and fruit yield in cucumber, but the best quality of fruit was produced by 2000 mgL^{-1} treatment.

INTRODUCTION

Pine wood vinegar is an organic product of distillation used in agriculture (Imanparast et al., 2009). Wood vinegar is used to improve soil quality, pests control, and as a plant growth regulator for increasing root, shoot, tubers, leaves, flower and fruit growth and yield (Mu et al., 2003, 2004; Burnett, 2013). Wood vinegar consists of 80-90 % of water and the rest consists of more than 200 organic compounds (Kim et al., 2008). The main components of WV are acetic acid and also organic acids, phenolic, alkane, alcohol and ester compounds (Jothityangkoon et al., 2008). The biologically active compound in pine wood vinegar is 3-methyl-2h-furo[2,3-c] pyran-2-one (Baxter et al., 1995; Commander et al., 2008). This compound is a karrikins and karrikinolide and is classified as a plant growth regulator (Baxter et al., 1995; Brown et al., 2003; Commander et al., 2008; Dixon et al., 1995; Flematti et al., 2004). Recently, wood vinegar has been wildly applied for various purposes such as medicinal, smoky aroma, food and platelet aggregation andanti-dermatophyte activity in pharmaceuticals (Amen-Chen et al., 2001). In particular, in organic agriculture, a great number of toxic-chemicals was replaced by wood vinegar, a natural product, which has been used to combat disease and pests, stimulate plant growth, improve the quality of fruit, accelerate the speed of plant seed germination and serve as herbicides (Loo et al., 2007). A great number of toxic-chemical in agriculture was replaced by wood vinegar, natural product, which has been used to promote growth and yield for field cultivation crops such as rice (*Oriza sativa*), sweet potato, *Ipomoea batatas*, sugar cane, *Saccharum officinarum*, melon, *Cucumis melo* (Koç, 2017). Effects of pine wood vinegar on inducing plant production are related to its thermal decomposition temperature (Mu et al., 2003, 2004). Yatagi et al. (1989)showed pine wood vinegar increased soil microorganism that helped roots absorb nutrients. Mungkunkamchao et al. (2013) reported pine wood vinegar could improve leaf surface area, fruit number and dry weight in cucumber.

As far as we know, there are no reports on the use of pine wood vinegar on cucumber grown in the greenhouse condition. This research evaluated

cucumber seed germination in the lab experiment and cucumber growth in the greenhouse with pine wood vinegar.

MATERIALS AND METHODS

This research was arranged in a completely randomized design with four replications to evaluate the effects of pine wood vinegar on cucumber seed *Cucumis sativus* var. Super daminos germination and subsequently their physiological and photosynthetic characteristics in two separate experiments. Pine wood vinegar with concentrations of 0, 1250, 2000, 2500, 3333 or 5000 mgL^{-1} were used. The pine wood vinegar was used from Moein Zist Arya Company. In a laboratory experiment, pine wood vinegar priming was studied to evaluate the effects of these treatments on cucumber seed in 2014. Four-mL of each concentration of pine wood vinegar was added to Petri dishes with filter paper to which 25 seeds were placed. The covered Petri dishes were placed in an incubator at 25 °C and a 16/8 light/dark photoperiod. Germination percent and germination rate over time were determined (Etemadi et al., 2010).

The germination percentage (GP) and germination rate (GR) were calculated in the following way:

$$(\text{GP }\%) = \frac{g}{n} \times 100$$

As g is the number of germinated seeds and n is the total number of seeds.

GR=G1/1 + G2/2 +· · ·+ Gx/x

G1=Germination percentage × 100 at the first day after sowing, G2=Germination percentage × 100 at the second day after sowing.

A second experiment was conducted in a completely randomized design with four replications in greenhouse of the Isfahan University of Technology, at 30-35 °C and 30-35% relative humidity in the Spring of 2014. The 5-liter container with soil: perlite (50:50 V/V) was used. Irrigation of pots was used when plants needed irrigation. No fertilizer was used to see the direct effect of just wood vinegar on plants.

Chlorophyll content was measured with a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ) and, maximum photochemical quenching (Fv/Fm) was measured by chlorophyll florescence (model OS-30, Minolta Corp.). Photosynthesis rate, stomatal conductance, transpiration, photosynthetic active radiation, leaf temperature and intercellular CO_2 concentration were determined using portable gas exchange equipment (Li-Cor, Li-3000, Homburg, Germany). Root volume was measured using change in water volume (Haghighi et al., 2012). Shoots were excised from roots using a steel blade and root and shoot fresh weights were determined. Samples were placed in a forced air oven at 70 °C for 48 h and dry weights were determined. Three weeks after seedling emergence, antioxidant activity of cucumber leaves was determined following Koleva et al. (2002). Three mgs of fresh sample were put in 5 mL methanol stock and 1.4 mL of this solution was blended with 0.6 mL of 2,2-diphenyl-1-piccrylhydrazyl (DPPH) solution. After 30 min, absorbance of the solution was determined at 515 nm with a spectrophotometer (V-530, JASCO, Hitachi, Japan) against methanol as a blank. The 0.2 mM DPPH solution in methanol was used as a stock for determination of the free radical scavenging activity of the samples. Leaf surface was measured by leaf area (GA-5 model).

After ashing the plant material at 470 °C, an acid (HCl = 10cc, 2N) solution of the ash was prepared. Potassium concentration in the acid solution was determined using a flame photometer (model PEP7, Minolta Corp.). Phosphorous concentration was determined by the ammonium molybdatevanadate method (Wilde et al., 1972). Nitrogen concentration was determined by the macro-Kjeldhal method (model PEP7, Minolta Corp.) according to Wilde et al. (1972).

The data were subjected to a two-way ANOVA and means were separated with least significant difference (LSD) in Statistix (ver. 8, Tallahassee, FL). Figures were displayed using Excel.

REULTS AND DISCUSSION

The highest germination percentage was due to treatment with 1250 mgL^{-1} pine wood vinegar and the lowest was for the control (Fig. 1a). There were no significance differences among means except for 500 mgL^{-1}. The highest pine wood vinegar concentration inhibited germination rate (Fig. 1b).

The highest shoot fresh weight was 2000 mgL^{-1} pine wood vinegar and fruit fresh weight was at the control (Fig. 2 a). The lowest shoot dry weight was seen in 1250, 2500 and 5000 mgL^{-1} pine wood vinegar treatments. The highest fresh and dry weights were for the control and 2000 mgL^{-1} pine wood vinegar (Fig. 2b).

The longest shoots were in the control treatment and the shortest for the 2500 mgL^{-1} pine wood vinegar treatment. Shoot length and node number were lowest in treatment with 2500 mgL^{-1} pine wood vinegar (Figs. 3 a, b). Root length increased in 1250 mgL^{-1} and fruit length increased in 2000 and 2500 and the highest flower and fruit numbers were seen in 2500 mgL^{-1}

Shoot diameter and leaf surface area were highest in treatment with 2000 mgL^{-1} pine wood vinegar (Figs. 4 a and b). Root volume (Fig. 5 a), root length (Fig. 3 a) and root surface (Fig. 4 b) increased when wood vinegar was used up to 2500 mgL^{-1} pine wood vinegar and decreased at 5000 mgL^{-1} pine wood vinegar.

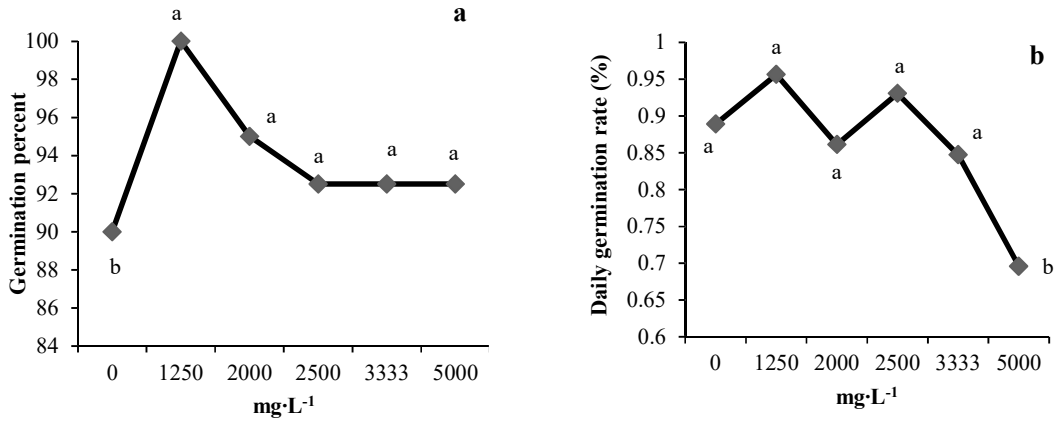

Fig.1. Effect of different concentrations of pine wood vinegar on cucumber seed germination percentage (a) and rate (b).

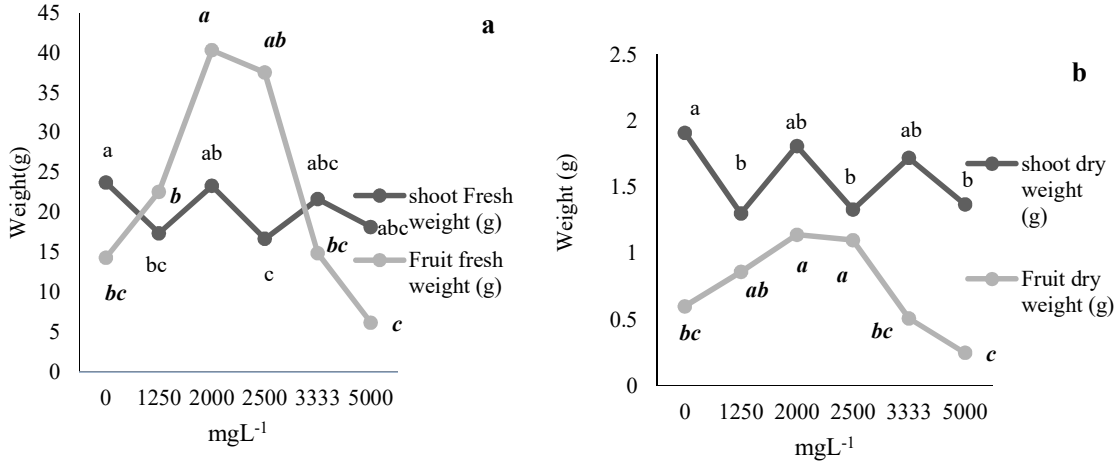

Fig. 2. Effect of different concentrations of pine wood vinegar on cucumber shoot and fruit fresh weight (a) and shoot and fruit dry weight (b)

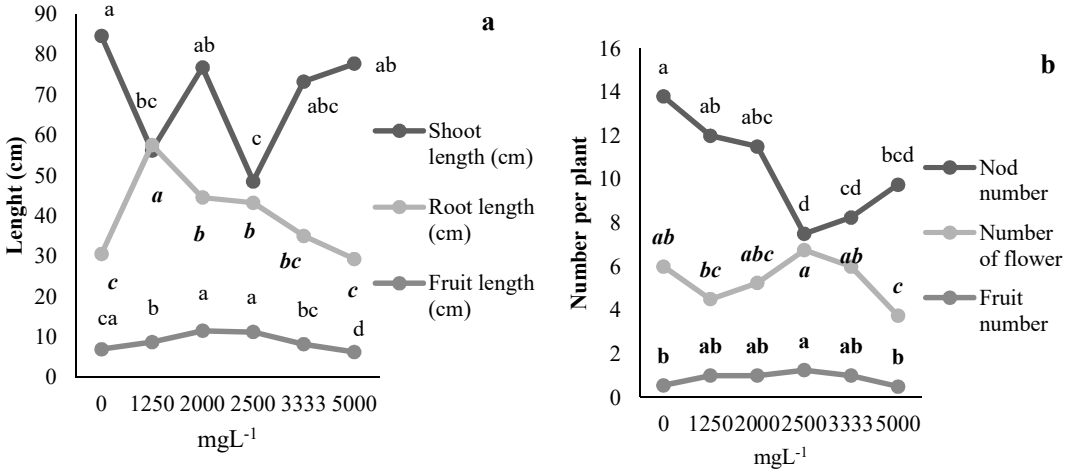

Fig.3. Effect of different concentrations of pine wood vinegar on cucumber shoot, root and fruit length (a) and nod, fruit and flower number (b)

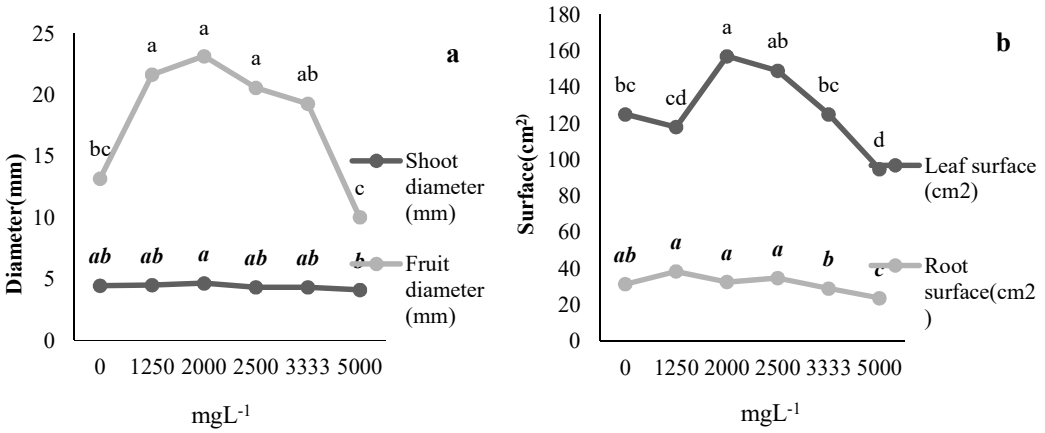

Fig. 4. Effect of different concentrations of pine wood vinegar on cucumber shoot and fruit diameter (a) and leaf and root surface (b)

The fastest flower appearance was due to treatment with 2500 mgL^{-1} pine wood vinegar and the latest was due to treatment with 5000 mgL^{-1} pine wood vinegar and root volume improved in 1250 mgL^{-1} (Fig. 5b). Most of flowers and fruits were in 2500 mgL^{-1} pine wood vinegar. Fruit number, fruit diameter, fruit length, fruit fresh and dry weights increased with up to 2500 mgL^{-1} pine wood vinegar and decreased in 5000 mgL^{-1} pine wood vinegar (Figs. 1-4).

Chlorophyll a, greenness index and fluorescence chlorophyll (Fv/Fm) decreased due to treatment with 5000 mgL^{-1} pine wood vinegar, but chlorophyll b did not change in all other treatments (data are not shown). The highest Fv/Fm was due to treatment with 1250 and 2500 mgL^{-1} pine wood vinegar. Photosynthesis increased due to treatment reaching the highest values due to treatment with 2000 and 2500 mgL^{-1} pine wood vinegar. Internal CO_2 increased due to treatment with 2000 or 2500 mgL^{-1} pine wood vinegar. Transpiration increased by the highest level of wood vinegar application (5000 mgL^{-1}) (Table 1). Ion leakage decreased due to treatment with 2000 mgL^{-1} pine wood vinegar. The relative water content (RWC) did not change due to using wood vinegar (data are not shown). Nitrogen increased due to treatment with 3333 mgL^{-1} pine wood vinegar, but phosphorus decreased due to treatment. Potassium did not change with treatments. Magnesium decreased due to treatment with 3333 and 5000 mgL^{-1} pine wood vinegar. Calcium initially increased due to treatment and decreased at high levels of pine wood vinegar (Table 2). Iron decreased due to treatment with 2500 mgL^{-1} pine wood vinegar (Table 2). Different kinds of wood vinegar have the potential to improve agriculture with supplying plant growth and increasing its growth up to 70-80% (Nurhayati et al., 2005). There were some benefits reported for cucumber due to treatment with pine wood vinegar. Treatment exhibited the highest and the lowest relative water content and ion leakage, respectively. It has been suggested that after fires in forests, seed germination improved due to the fire smoke and its chemical changes (Brown et al., 2003; Commander et al., 2009).

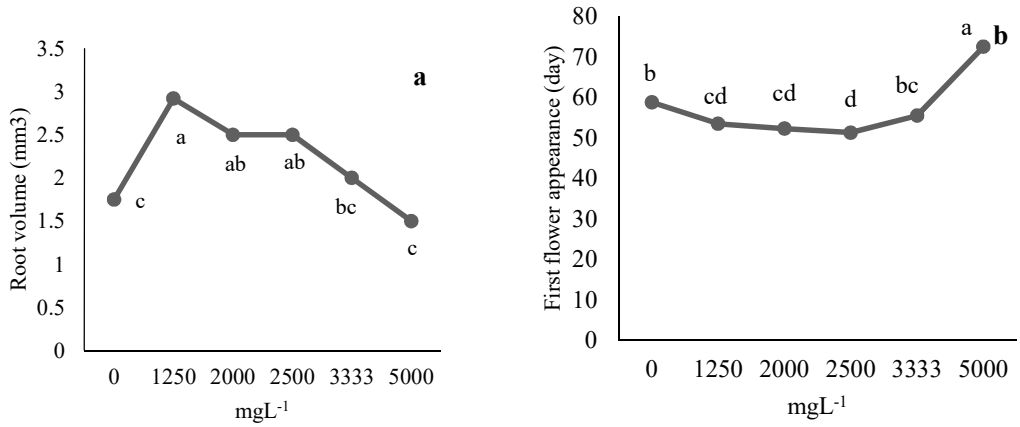

Fig. 5. Effect of different concentrations of pine wood vinegar on cucumber root volume (a) and first flower appearance (

Table 1. The effect of pine wood vinegar on cucumber photosynthetic parameters

Wood vinegar concentration (mgL^{-1})	Chlorophyll a	Greenness (SPAD)	Fluorescence chlorophyll (Fv/Fm)	Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})	Internal CO_2 concentration (µmol mol^{-1})	Transpiration (mmol m^{-2} s^{-1})	Ion leakage (%)
0	24.31 [a]	50.05 [a]	0.033 [bc]	2.81 [e]	228. 75 [c]	2.13 [b]	54 [ab]
1250	20.95 [ab]	49.59 [a]	0.042 [a]	3.61 [c]	210.25 [c]	1.72 [c]	52.9 [ab]
2000	22.33 [ab]	48.81 [a]	0.041 [a]	6.95 [a]	362.75 [a]	1.54 [c]	45.5 [b]
2500	20.22 [ab]	47.8 [a]	0.029 [bc]	6.59 [a]	341 [a]	1.55 [c]	52.2 [ab]
3333	20.57 [ab]	42.11 [a]	0.035 [ab]	4.24 [c]	272.25 [b]	1.62 [c]	54.2 [ab]
5000	19.67 [b]	32.1 [b]	0.027 [c]	4.53 [c]	204 [c]	2.7 [a]	56.5 [a]

Within each column, means with different letters are significantly different at $P < 0.05$.

Table 2. The effect of pine wood vinegar on nutrient concentration of leaves

Wood vinegar concentration (mgL^{-1})	Nitrogen (% DW)	Phosphorous (% DW)	Potassium (% DW)	Magnesium (% DW)	Calcium (% DW)	Iron ($mgkg^{-1}$)
0	4.8 [c]	0.27 [a]	6.87 [a]	0.53 [a]	2.82 [b]	134.7 [a]
1250	5.18 [b]	0.19 [b]	6.7 [a]	0.5 [ab]	3.01 [a]	155.3 [a]
2000	5.05 [bc]	0.2 [b]	6.83 [a]	0.48 [ab]	2.89 [a]	116.7 [ab]
2500	5.08 [bc]	0.2 [b]	6.83 [a]	0.49 [ab]	2.78 [ab]	74.33 [b]
3333	5.5 [a]	0.19 [b]	6.64 [a]	0.46 [b]	2.62 [ab]	114.3 [ab]
5000	4.93 [bc]	0.23 [ab]	6.96 [a]	0.46 [b]	2.47 [b]	132 [ab]

Within each column, means with different letters are significantly different at $P < 0.05$.

The acidic pine wood vinegar works as a solvent causing softening of the seed coat to increase germination. Pine wood vinegar may act as a hormone, and the low molecule weight compound increase seed water absorption and seed germination rate (Commander et al., 2009; Flematti et al., 2004; Mu et al., 2004; Yatagai and Unrinin, 1989). This study indicated that root surface and root volume improved by wood vinegar as reported by Burnette (2013). Nutrient concentration was also improved as was reported by Yamato et al. (2006). Pine wood vinegar increased soil microorganism that helps roots absorb nutrients (Yatagi et al., 1989). The improving effect of wood vinegar on growth via increasing nutrient absorbtion was seen in celery and bamboo (Mu et al., 2003, 2004), especially when the wood vinegar was used in poor soil (Yamato et al., 2006). Wood vinegar with changing chemical properties of soil like PH, N and P contents and CEC could improve the plant growth (Yamato et al., 2006).

Pine wood vinegar could improve leaf surface area, fruit number and dry weight (Mungkunkamchao et al., 2013) as was the case with cucumber. This study indicated an increase in leaf surface and photosynthesis potential. Different kinds of wood vinegar have the potential to improve agriculture with supplying plant growth and increasing its growth up to 70-80% (Nurhayati et al., 2005).

preserves the seedling from different biotic stress and results in better growth (Nurhayati et al., 2005)

Flower induction in cucumber improved with 2500 $mgL^{-1}$of wood vinegar which may refer to the hormonal balance and hormone-like property of wood vinegar (Nurhayati et al., 2005). On the other hand, the best fruit quality was seen in a lower concentration of wood vinegar. Increasing fruit quality with wood vinegar was seen in tomato too (Mungkunkamchao et al., 2013) but another result can be reached according to which, wood vinegar in higher concentration is effective in vegetative growth and flower induction but it is more effective in fruit quality in lower concentrations in cucumber Conclusively, the best wood vinegar treatment for seed germination was 1250 mgL^{-1}, although 2000 mgL^{-1} pine wood vinegar had the best flower and fruit quality. In poor media, pine wood vinegar application could supply some nutrient for plant growth and enhance yield considering that wood vinegar would be toxic in high concentration; therefore, its use should be recommended according to the analysis of nutrient in media

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تاثیرات سرکه چوب کاج بر جوانه‌زنی، رشد و ویژگی‌های فتوسنتزی خیار

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واژه‌های کلیدی:
خیار
گلدهی
میوه
غلظت عناصر
عملکرد

چکیده سرکه چوب یک ماده مشتق شده از سوختن کربن سیاه است که می‌تواند جایگزین مواد شیمیایی در صنعت کشاورزی به‌عنوان یک ترکیب آلی گردد. به‌منظور مطالعه اثر کارآیی سرکه چوب کاج بر ویژگی‌های فیزیولوژیکی و فتوسنتزی خیار دو آزمایش براساس طرح کاملا تصادفی با شش تیمار شامل ۱۲۵۰، ۲۰۰۰، ۲۵۰۰، ۳۳۳۳ و ۵۰۰۰ میلی‌گرم در لیتر سرکه چوب با چهار تکرار انجام شد. اولین آزمایش در آزمایشگاه به‌منظور بررسی تاثیرات اولیه سرکه چوب بر روی بذر خیار (*Cucumis sativus* var. Super daminos) انجام شد و آزمایش دوم اثر سرکه چوب بر رشد گیاه خیار در گلخانه‌های تحقیقاتی دانشگاه صنعتی اصفهان انجام شد. بیشترین تعداد گل و عملکرد در خیار در تیمار ۲۵۰۰ میلی‌گرم در لیتر سرکه چوب مشاهده شد. فتوسنتز در خیار در تیمار ۲۰۰۰ میلی‌گرم در لیتر افزایش یافت. کمترین میزان تعرق (۱/۵۴ میلی‌مول برمترمربع بر ثانیه) در خیار در تیمار ۲۰۰۰ میلی گرم در لیتر سرکه چوب بدست امد. سرکه چوب ۱۲۵۰ میلی‌گرم در لیتر باعث افزایش درصد جوانه‌زنی و سرعت جوانه زنی آن در مقایسه با شاهد شد. بیشترین طول ریشه، حجم و سطح در تیمار ۱۲۵۰ میلی‌گرم بر لیتر مشاهده شد. غلظت نیتروژن (۵/۵ درصد وزن خشک) در تیمار ۳۳۳۳ میلی‌گرم در لیتر و غلظت پتاسیم در غلظت ۵۰۰۰ میلی‌گرم در لیتر، کلسیم و آهن در غلظت ۱۲۵۰ میلی‌گرم در لیتر در بالاترین سطح در مقایسه با سایر تیمارها بود. بیشترین مقدار کلروفیل و فتوسنتز در تیمار ۲۰۰۰ میلی‌گرم در لیتر مشاهده شد. تیمار ۲۵۰۰ میلی‌گرم در لیتر در خیار بیشترین عملکرد گل و میوه را نشان داد، اما بهترین و بالاترین کیفیت میوه با ۲۰۰۰ میلی‌گرم در لیتر حاصل شد.