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EFFECT OF FOLIAR APPLICATION OF ZINC AND MANGANESE ON YIELD OF PUMPKIN (*CUCURBITA PEPO* L.) UNDER TWO IRRIGATION PATTERNS

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ABSTRACT

The study was carried out to determine the response of pumpkin (*cucurbita pepo* L.) to zinc and *manganese* fertilizers at Agricultural Research Farm of Qazvin, Iran during 2011. The experimental design was a split-plot based on randomized complete blocks with four replications. Irrigation regimes (I_1–irrigation after 50 mm and I_2–Irrigation after 100 mm cumulative evaporation from class A pan) allotted to the main plots affected the pre-flowering stage. Subplots, which consisted of split plots, comprised of four foliar application levels (no spray application as control, Zn as $ZnSO_4$, Mn as $MnSo_4$ and Zn+Mn) of micronutrients at flowering stage. Seed number, seed yield, fruit yield and oil content of pumpkin showed significant response to foliar spray ($P<0.01$) and irrigation regimes ($P<0.05$).The highest seed yield (797 kg ha^{-1}) was obtained from foliar spray of Zn+Mn followed by irrigation patterns of I_2. Irrigation according to 50 mm evaporation, regardless of foliar application, resulted in maximum fruit yield. Clearly, reducing the irrigation interval led to increased fruit yield. Hence, foliar spray of Zn+Mn coupled with higher irrigation intervals (I_2) should be practised to obtain maximum seed number and seed yield from pumpkin in semi-arid region of Qazvin.

Key words: *Cucurbita pepo*, foliar application, regulated irrigation, seed yield

INTRODUCTION

Medicinal Pumpkin (*Cucurbita pepo* L. convar. *pepo* var. styriaca) is an important annual plant that belongs to the Cucurbitaceae family. It does not grow naturally in Iran, but recently has been planted and has been used as medicine in Iran [23]. The seeds of pumpkin contain medicinal raw materials that are used for producing pharmaceutical products such as peponen, pepostrin and gronfing to overcome prostatic hypertrophy and urinary tract irritation [4, 5, 17, 22, 32]. Pumpkin's seed oil is rich in unsaturated fatty acids including linoleic acid, oleic acid, and palmitic acid [22], phenolic compounds [31] and antioxidant vitamins, such as carotenoids and tocopherol [26]. Due to high omega-3 (6 and 9)-fatty acids [22], seeds and oil have been claimed to promote HIV/AIDS wellness [33].

Drought is one of the main limiting factors of plant growth throughout the world and is the most common environmental stress that has limited almost 25% of the world's agricultural lands. In spite of the extensive and comprehensive research that has been conducted on the effect of water stress on agricultural products, the behavior of medicinal plants in such conditions has not been adequately studied [12].Water deficit is frequently the primary factor for crop production under arid/semi-arid conditions [18]. It affects nearly all the plant growth processes. However, the stress response depends upon the intensity, rate and duration of exposure and the stage of crop growth [30].

Application of microelements fertilizers can enhance plants resistance to environmental stresses [6]. Foliar fertilization is an effective practice for the application of some micronutrients, since it uses low rates and the micronutrient does not directly contact the soil, avoiding losses through fixation [29]. Micronutrients application to leaves of growing crop will ensure better crop nutrition at anthesis and seed filling stage, which in turn may result in increased seed yield [1]. Foliar spray of different micronutrients has also been reported to be equally or more effective as soil application by different researchers [27]. On the other hand, one possible approach to minimize drought-induced crop losses is the foliar application of manganese, which plays several physiological and biochemical roles, i.e. chlorophyll formation, activation of enzymes, synthesis of proteins, carbohydrate metabolism and energy transfer. Zinc (Zn) is an essential trace element for every living organism. About 200 enzymes and transcription factors require Zn as a functional component [20]. Zinc is known to have an important role, either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes [16]. Manganese (Mn) is regarded as an activator of many different enzymatic reactions and takes part in photosynthesis; in other words, it activates decarboxylase and dehydrogenase and it is a constituent of complex PSII-protein, SOD and phosphatase [20]. Mclaughlin et al. [21] reported that Mn is a micronutrient element required by crops in trace amounts for amino acid synthesis, oxidation-reduction and chlorophyll formation. Deficiency of Mn induces inhibition of growth, chlorosis and necrosis, early leaf fall and low reutilization [20].

Lots of work have been already carried out on pumpkin regarding its growth, yield and yield attributes under different fertilizer, irrigation and pruning treatments [3, 13, 15], however the effects of foliar application of micronutrients attracted a little attention to this plant. As far as we are concerned, no information on the effect of micronutrients on pumpkin is available. The present study intends to evaluate the impacts of zinc and manganese foliar application on growth and yield of *cucrbita pepo* L. under different irrigation regimes.

MATERIALS AND METHODS

The present study was conducted at Agricultural Research Farm of Qazvin, Iran (latitude 36° 25´ N, longitude 49° 98´E) during the 2011growing season. This region has a semi-arid climate (270 mm mean annual rainfall, 55 to 65% relative humidity, and 15 to 25°C mean annual temperature). Soil samples were collected in compliance from the depths of 0 to 30 cm and 30 to 60 cm before application of fertilizer to study nutrient status of soil. This was done with a soil auger to obtain a compound sample for each depth finally. These samples were air dried, crushed and sieved through a 2 mm mesh prior to their analysis. Analysis work was carried out in the laboratory of the Qazvin Agricultural Research Centre. The soil pH was measured potentiometrically in a 1:2.5 soil – water suspension [28]**.** Twenty grams of soil samples were weighed, transferred into 100 ml beaker and then 50 ml of distilled water was added. Samples were agitated on a shaker for 2 h and by using a digital pH-meter (G91 pH meter, Metrohm, Swiss), after calibrating with buffer solutions of pH 4.0 and 7.0, three pH readings were taken by placing electrode in the supernatant of each sample suspension. The mean was recorded as sample pH. The soil texture was clay with a clay type montmorillonite. Physical and chemical composition of the soil is given in Table1.

Table 1. Physicochemical characteristics of the soil in trial area before planting pumpkin in 2011

Characteristics	0-30 cm	30-60 cm
Sand (%)	21	21
Silt (%)	33	30
Clay (%)	46	49
Textural class	Clay	Clay
Available Zn^{++} (mg kg^{-1})	1.02	0.8
Available Mn^{++} (mg kg^{-1})	4.28	5.56
Available P (mg kg^{-1})	12.7	12.7
Exchangeable K (cmol kg^{-1})	0.87	0.82
NO_3–N (mg kg^{-1})	5.7	5
NO_3–N + NH_4–N (mg kg^{-1})	7	6
Organic carbon (%)	0.73	0.65
pH (H_2O)	7.70	7.56
EC (dS m^{-1})	0.78	0.74

Notes. cmol kg^{-1}– *Centi*-mol *p*er *kg of* soil;
NO_3–N – Nitrate nitrogen; NH_4–N – Ammonium nitrogen; EC– Electric conductivity

The experimental units were designed as split plots based on randomized complete block design (RCBD) in three replications and eight treatment combinations. Soil-water regimes consisted of two irrigation patterns at pre-flowering stage (I_1–irrigation after 50 mm, and I_2–irrigation after 100 mm cumulative evaporation from class A pan) which allotted to the main plots. In each irrigation treatment, 100% of the evaporated water (208 m^3 ha^{-1}) entered the field. The amount of 500 l $plot^{-1}$ (0.05 mm $plot^{-1}$) water was utilized for the area of each experimental plot (Net plot size 6 m×4 m). In the mild stress condition (I_1), the number of irrigation applications and amount of irrigation water given over the total growing season were 8 times and 1664 m^3 ha^{-1}(166.4 mm) respectively; whereas under severe stress condition (I_2), they were 6 times and 1248 m^3 ha^{-1}(124.8 mm) respectively. Therefore, compared to the mild stress, in severe stress conditions (416 m^3 ha^{-1}) smaller amount of water was employed. Sub-plots were comprised of different foliar spray treatments: F_1–no spray application (control), F_2–0.3% Zn solution (3000 mg L^{-1}), F_3–0.3% Mn solution (3000 mg L^{-1}), and F_4–0.3% Zn+Mn spray (3000 mg L^{-1}). $ZnSO_4$ (35%) was applied as zinc source and $MnSO_4$ (31.8%) as manganese source. Foliar spray of Zn and Mn as per treatment was applied at flowering stages by means of a hand-held sprayer.

Each plot comprised two rows with 4 m spacing between them and each row had 16 plants with 40 cm space between plants. The pumpkin OF styriaca variety was used for testing. After the land preparation (such as plowing, disking and ridging), sowing was carried out on July 10^{th} in a well prepared seed bed. The cluster sowing method was applied: 3 seeds were placed into one hole of 4 cm depth. The plants were thinned to one at 4-6 true leaf stages. In order to control weeds, hand hoeing was carried out during the growth period. A urea fertilizer (CO $(NH_2)_2$; nitrogen content 46%) was used as a top-fertilizer at early flowering phase (100 kg ha^{-1}). In general, weather condition during all trial period was favorable for the growth and development of pumpkin var. styriaca. This might be attributed to the considerable rainfall increase in the year of the experiment, especially from August to October 2011(Table 2). All other recommended crop production practices were applied uniformly to all treatments.

Table 2. Meteorological values of trial site from July to October 2011 with respect to data pertaining to one year prior to *commencing the experiment*

Month	Year	Average temperature °C		Total rainfall mm
		minimum	maximum	
July	2010	38.83	18.10	0.6
	2011	36.93	17.21	0
August	2010	33.82	16.92	0.3
	2011	34.01	16.32	5.6
September	2010	30.70	13.76	0
	2011	29.73	11.92	15.3
October	2010	25.44	9.74	21.7
	2011	23.41	8.71	30.2

At physiological maturity stage, after discarding margins, eight random samples were hand harvested from each experimental unit and traits consisting of seed yield, fruit yield, seed number per fruit and oil content of seeds were measured, and their averages in terms of the said traits were considered. Obviously, when more than 75% of the fruits became yellowish orange in color and stem and leaves began to dry and the seeds became dark green and well rounded, manual harvesting was done at 12th October and 22th October (2011). Harvested ripen fruits were weighed (fresh weight).The seeds were manually extracted from the fruits and then naturally dried by sun light until constant weight. The sun-dried seeds were transferred to laboratory. All dried seeds were counted and weighed separately. The seed number was obtained by the average number of counted seeds per plot (8 plants). The seed yield and fruit yield was obtained through the following equations:

Seed yield (kg ha^{-1}):

$$\text{Mean dry weight of seeds per plot (8 plants)} \times 12500 \text{ (plants ha}^{-1}\text{)} \quad (1)$$

Fruit yield (t ha^{-1}):

$$\text{Mean fresh weight of fruits per plot (8 plants)} \times 12500 \text{ (plants ha}^{-1}\text{)} \quad (2)$$

Seeds from each treatment were powdered using electric grinders. Oil was extracted from 20 g of powder by 300 mL n-hexane solvent in a soxhlet *apparatus* at 60°C for 16 h. The solvent was removed from the extracted oil by rotary evaporator. The oil sample was dried in a vacuum oven kept at 60ºC for 30 min and then accurately weighed. The differences between containing and -empty beaker were recorded as oil amounts [7, 32].

Data were subjected to analysis of variance (*ANOVA*) using the SAS software package [25] and –variables with significant *F*-tests (P<0.01) were compared using Duncan's multiple range test (DMRT).Charts were drawn by means of MS Excel Ver. 11.

RESULTS AND DISCUSSION

Results of variance analysis showed that the effect of irrigation (P<0.05), foliar application (P<0.01) and their interaction (P<0.05) on seed number was significant (Table 3). The interaction effect of irrigation and foliar application on the seed number indicated that all the foliar application treatments showed no significant difference under the lower irrigation interval based on 50 mm evaporation (I_1). While, under irrigation treatment of I_2 (irrigation after 100 mm evaporation from class A pan), combined foliar application of Zn and Mn caused an increase in the seed number.

Table 3. Mean squares of tested treatments effect on seed number, seed yield, and fruit yield of pumpkin var. styriaca according to 2011 data

S.O.V	*df*	Seed number	Seed yield	Fruit yield	Oil content
R	2	17656.431 ^{ns}	66463.397 *	51.524 ^{ns}	3.444 ^{ns}
I	1	50133.815 *	235975.038 *	258.989 *	109.397 *
E_a	2	1205.792	2519.410	33.865	2.456
F	3	4052.024 **	9932.305 **	7.100 **	12.521 **
I × F	3	2157.519 *	5274.804 *	3.529 *	0.714 ^{ns}
E_b	12	536.561	972.482	0.855	0.618
CV (%)	–	7.68	4.89	4.63	1.93

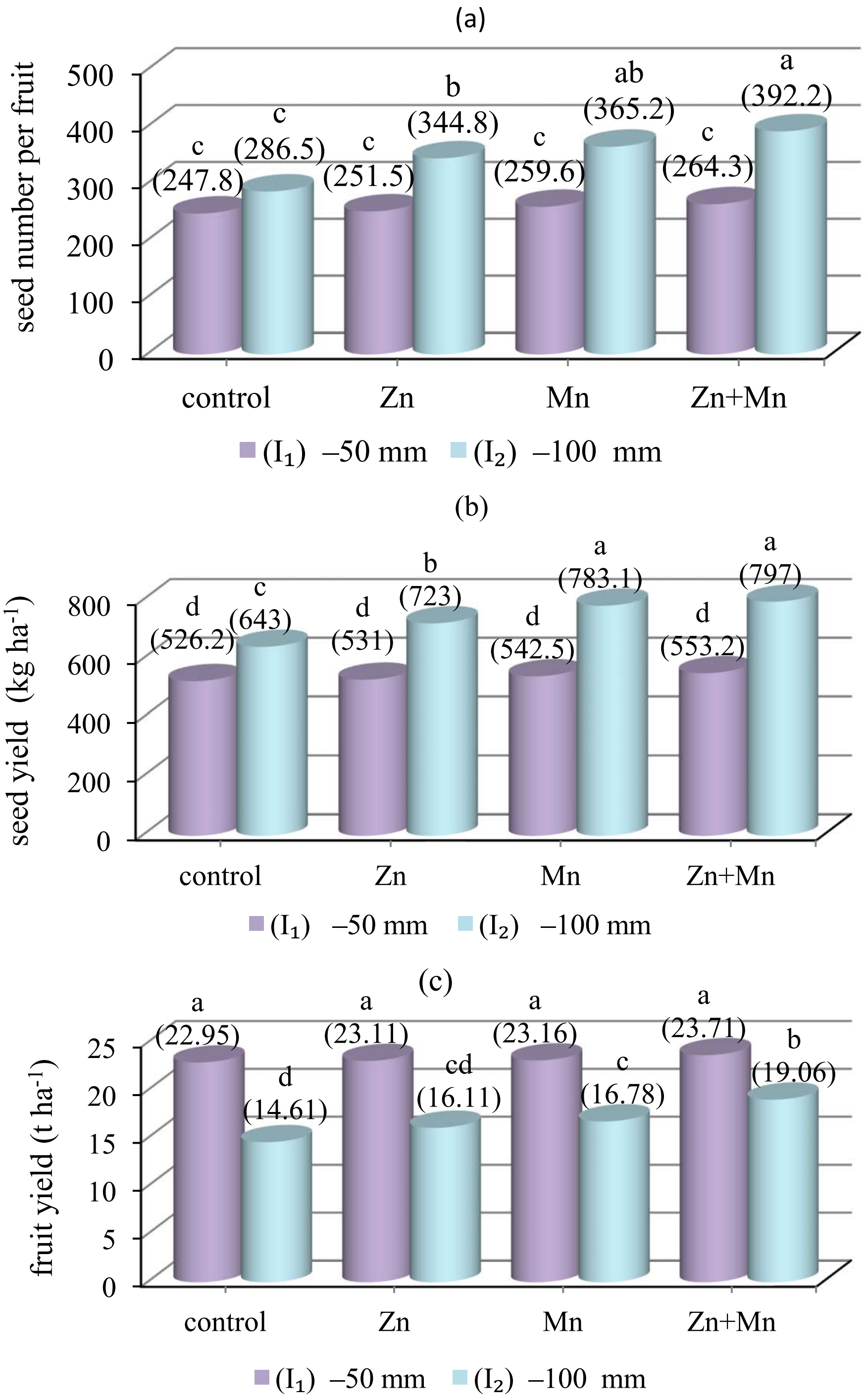
Notes. * – $P < 0.05$, ** – $P < 0.01$, ns – $P > 0.05$; S.O.V– Source of variance, *df* – degrees of freedom; R – Replication, I – Irrigation effect, F –Foliar application effect, I×F– represent interaction terms between the treatment factors

From Figure 1a it was found that foliar spray of Zn + Mn had the highest seed number (392.2), while the lowest number of seeds was noticed in control (no spray) plots with irrigation patterns of I_1. *It should be reminded that hormonal activity in the growing fruits causes the fruit mesocarp to act as a stronger physiological sinks* than the other organs for photosynthetic materials [8]. Most likely under higher-frequency *irrigation* (shorter *irrigation intervals*), we would have *higher transmission rate of assimilates and dry matters to the* fruit mesocarp and ultimately excessive growth of fruit decreases the number of seeds per fruit [11]. However, Jahan et al. [19] stated that pumpkin is a sink-limited herb and there is no link between increase in fruit dimension and higher seed production.

During our investigation, analysis of variance showed that simple effects of irrigation (P<0.05), foliar application of micronutrients (P<0.01) and their interaction (P<0.05) were significant on the seed yield (Table 3). Data related to irrigation regimes and different types of foliar application (I×F) revealed that increasing irrigation interval before flowering stage surprisingly enhanced the seed yield due to reducing fruit diameter (Fig.1b). The data obtained from our study are in accordance with data of Al-Omran et al. [2], who found that full-irrigated condition expanded fruit dimension of pumpkin and even inhibited more seed generation. In contrast, Ghanbari et al. [14] stated that increasing irrigation interval decreased significantly the number of seeds per fruit and consequently seed yield per unit area. The mean values of foliar treatments under irrigation interval, based on 100 mm evaporation, showed that highest seed yield (797 kg ha^{-1}) was observed in plots sprayed with 0.3% Zn+Mn solution, followed by lowest value (526.2 kg ha^{-1}) in control (no spray) plots under lower irrigation interval (I_1). The possible reason might be more accumulation of dry matter in seeds with combined application of foliar spray. On the other hand, micronutrients increases photosynthesis rate, improves leaf area duration and increases seed yield consequently.

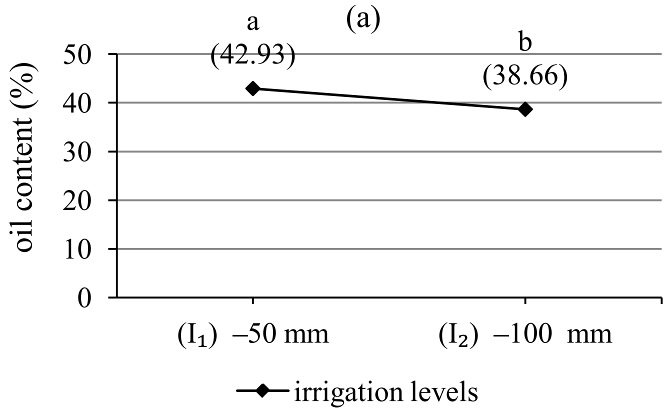
Fruit yield followed similar trends of other studied traits (I–irrigation effect, P<0.05; F–foliar application effect, P<0.01; I×F, P<0.05) except for seed oil content (Table 3). However, further irrigation caused more fruit yield as a result of higher drought matter transmission. Interaction of irrigation and foliar application of zinc and manganese showed that all foliar application treatments have not shown any statistical significance under irrigation after 50 mm evaporation (Fig.1c). Whereas, an appreciable increase in fruit yield occurred due to foliar application of Zn+Mn under irrigation after 100 mm evaporation (19.06 t ha^{-1}) as compared to higher fruit yield of 23.71 t ha^{-1} in treatment combination of Zn+Mn and lower irrigation intervals (I_1). In this regard, similar results were achieved by Erdem et al. [10] on watermelon, Rashidi & seyfi [24] on cantaloupe and Daneshian et al. [8] on pumpkin. They stated that an increase in irrigation intervals would result in a lesser fruit yield in a linear manner. Ertek et al. [11] claimed that increasing frequency of irrigation increased the fruit number and consequently fruit yield. He also reported that there was a significant correlation between fruit weight and irrigation frequency.

Individually, we found significant differences in irrigation (P<0.05) and foliar application (P<0.01) factors on oil content of seeds, but there were no interactions between irrigation and foliar application treatments regarding this trait (Table 3). Among the irrigation regimes, I_2 treatment (irrigation after 100 mm evaporation) reduced oil content of seeds as compared with Irrigation after 50 mm evaporation(I_1) (Fig.2a). Increasing oil content under higher irrigation frequency could be attributed to extended periods of plant growth, and finally late fruits maturity (about 10 days). Ghanbari et al. [14] in a study conducted in Mashhad (Iran) indicated that lower irrigation interval produced the highest seed oil yield of pumpkin. Experimental results revealed that foliar applications greatly enhanced the oil content (Fig.2b). The greatest improvement in seed oil content of pumpkin (44.12%) was obtained by combined application of both zinc and manganese (Zn+Mn). It was followed by foliar application of Mn. No foliar applications (Control) remained less effective on seed oil concentration. This observation is similar to that of Dehnavi & Sanavi [9] who found that foliar application increased the seed oil percentage in winter safflower (*Carthamus tinctorius* L.).

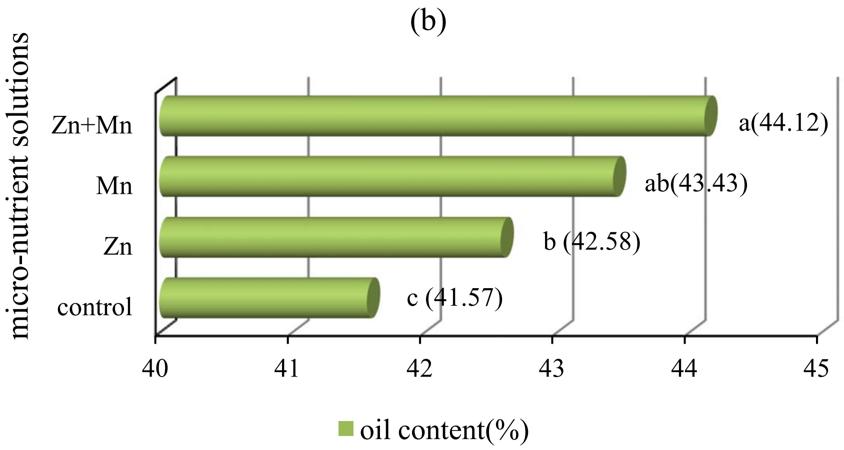


Notes. Bars not accompanied by the same letter are significantly different, for $P < 0.05$; I_1–irrigation after 50 mm evaporation from class A pan, I_2–irrigation after 100 mm evaporation from class A pan, control–no fertilizer, Zn–zinc sulfate ($ZnSO_4$), Mn–manganese sulfate ($MnSO_4$), Zn+Mn–mixture of zinc sulfate and manganese sulfate.

Fig.1a-b-c. Interaction effect of irrigation regimes and different foliar application of Zn and Mn on seed number (a), seed yield (b) and fruit yield (c) of pumpkin var. styriaca in 2011



Note. Points not accompanied by the same letter are significantly different, for $P < 0.05$.



Note. Bars not accompanied by the same letter are significantly different, for $P < 0.01$.

Fig.2a-b. Individual effects of Irrigation patterns (a) and different foliar application of Zn and Mn (b) on oil content of pumpkin var. styriaca in 2011

CONCLUSIONS

1. The results demonstrated that severe water stress (irrigation after 100 mm evaporation from class A pan) resulted in greater seed yield, seed number per fruit, and simultaneously declined the oil content and fruit yield as well.
2. It is probably possible to improve seed oil concentration of pumpkin to the desired levels by agronomic micronutrients (the oil content increased by a mixture of Zn+Mn which was followed by individual application of Mn).
3. Foliar application of zinc and manganese (Zn+Mn) generally was found to mitigate the adverse effects caused by severe water stress treatment especially in fruit yield. It could enhance the fruit yield more effectively compared to manganese.
4. However, further confirmation of the trends seen in this trial needs to be obtained before more specific recommendations can be made.

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