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Effect of plant growth regulators on control of saffron (*Crocus sativus* L.) corm dormancy

Somaye Amini[1] and Seyed Mahdi Ziaratnia[2]*

1, Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2, Department of Food Biotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

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***Corresponding author:**
Department of Food Biotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran.
E-mail: **m.ziaratnia@rifst.ac.ir**

ABSTRACT

Purpose: Saffron (*Crocus sativus* L.) is a valuable medicinal plant with a short flowering period. Its flowering management can be useful for the production of saffron in a farm or controlled condition. The objective of this study was to determine the effect of plant growth regulators (PGRs) on saffron corms sprouting. **Research method:** For this purpose, corms were treated with different PGRs including α-naphthaleneacetic acid (NAA) (0, 100, 200, 300 ppm), chlorocholine chloride (CCC) (0, 100, 200, 300 ppm) and gibberellic acid (GA_3) (0, 5, 10, 20 ppm) for two hours and incubated at 20-22 °C for eight weeks. **Findings:** The results revealed that the effect of PGRs on corms sprouting was significant. It has been shown that treatment of corms with auxin at all concentrations reduced sprouting compared to the control. To prolong dormancy for five weeks, NAA at 200 mg L^{-1} was the best choice. While for the fifth week onwards, the most effective treatment was NAA at 100 ppm. Treatment with GA_3 (20 ppm) has shown a stimulatory effect on corm sprouting. **Research limitations**: No limitations were founded. **Originality/Value:** Sprouting acceleration can keep flowering away from early autumn frosting in farm conditions, while prolongation of corms dormancy provides the possibility of harvesting saffron flowers in several times in a hydroponic system. The results of this study suggested two kinds of chemical for different purposes, inhibition and stimulation of sprouting of saffron corms that can be applicable for saffron hydroponic or farm production, respectively.

INTRODUCTION

Saffron *(Crocus sativus* L.) is a sterile triploid plant that vegetatively propagated by corms. Briefly, the phenology of saffron based on the growth of underground organs are consist of following stages including dormancy, flowering, formation and growth of replacement corms (daughter corms), plant senesces (leaf withering) and the beginning of dormancy again. Corm dormancy period itself consists of two stages, real dormancy (Early May to the middle of January) and pseudo dormancy (Mid-January to mid-October). In the real dormancy the physiological activity of the plant is at the lowest level, while in the pseudo dormancy, initiation and differentiation of the leaves and flower buds and finally growing leaves and flower are happened (Koocheki & Seyyedi, 2015).

The stigma of this valuable plant has been used since the ancient times for different purposes such as medicinal, aromatic, food additive, textile dyeing, etc. (Turhan et al., 2007). Relatively short flowering period, low average yield and high costs of production which is due to manually collection and drying of stigmas have made saffron as the most expensive spice in the world. For that issue, along with the farm production of saffron, many attempts have been started around the worlds to produce saffron under controlled conditions, like hydroponic systems due to increasing the saffron yield and reducing cost production (Maggio et al., 2006).

Generally, the growth of plants under controlled environmental conditions such as hydroponic system has less contamination to weed, soil, heavy metal, and pesticide. In addition, the multiple harvesting and extending the growing season are the other advantages of these systems (Hayden, 2006).

Although, the hydroponic system provides opportunities for improving quality, quantity, purity and consistency production on a commercial scale, one of the disadvantages of the hydroponic system is the high cost of its infrastructures and operating (Christie, 2014). As saffron flowering period takes around two months, this system can be benefited for production of saffron when multiple harvesting flowers applied or another plant replaced.

There are also some limitations on farm saffron production. According to the developed climate model, the temperature has been raised by 1.5-2.0 °C which resulted in postponing the flowering time to late December in the main saffron production regions in Khorasan province, which is the most important region of saffron production in Iran (Koocheki et al., 2010). On the other hand, Khorasan province is recently experiencing the confluence of flowering date with early fall cold that looks because of the flowering postpone. Exposure of saffron flowers to autumn early cold and frosting in saffron flowers causes a significant reduction in yield (Koocheki et al., 2010).

It seems that shortening the corm dormancy period can help the flowers to appear before cold starting and it may prevent frost damage. On the other hand, postponing the flowering by prolongation of the corm dormancy period can also be a reasonable way in flowering time management and in the following multi-flower harvesting can be achieved by breaking dormant corms in desired time in the hydroponic and greenhouse cultivation system. Therefore, any effort to shortening or prolongation of the dormancy period will result in the yield increment and reduction of costs in saffron production.

Application of plant growth regulators (PGRs) is a method to control flowering time in plants. Among different application methods of PGRs treatment such as pre-plant soaking, foliar application, and drenching; soaking of bulbs in solution of chemical compound is an efficient method for obtaining good results and also have advantages over other methods in terms of time consumption, labor cost, saving and using accurate dosage (Sajjad et al., 2015).

In addition to endogenous hormones, some exogenous growth regulators also effect on the various stages of plant growth and the dormancy by interactions between different inhibitory and promoting substances (Sharma et al., 2016). Although abscisic acid (ABA) is known as the sole plant hormone to prolong plants dormancy, scientists have recently discovered a coordinating network of auxin and ABA signaling in dormancy phenomenon. They showed that auxin acts on upstream of the major regulator of seed dormancy (Liu et al., 2013). Zheng et al. (2012) have investigated the effect of chlorocholine chloride (CCC) treatment in bulbs dormancy of *Lilium oriental*. Their research results confirmed the hypothesis that the (CCC) could improve the carbohydrate accumulation in bulbous plants by enhancing photosynthetic capacity and changing endogenous hormones such as GA and IAA contents. These researchers observed a slight delay of bud formation in plant treated with CCC (Zheng et al., 2012). So far, extensive researches have done on the role of gibberellin hormone in plants dormancy breaking and prolongation as well. They suggest that this hormone may play a role in the natural process of induces rapid emergence of sprouts. This compound can be used as an alternative to the cold requirement for breaking dormancy in plants (Langens-Gerrits et al., 2003). GA_3 has also been reported as a germination inhibitor in *Dioscorea sp.* (Girardin et al., 1998; Okagami & Tanno, 1993; Tschannen et al., 2003). For instance, Okagami and Tanno (1993) demonstrated that the application of gibberellic acid at the lowest concentrations (0.1-1 /1M) inhibited tuber sprouting of *Dioscorea Japonica*. They also observed more than 500 days delay in sprouting at 20 °C incubation by application of 100 M GA_3 (Okagami & Tanno, 1993).

There are a few reports on the effect of plant growth regulators on the sprouting of saffron corms (Aytekin, & Acikgoz, 2008; Hoseinifard et al., 2017). Therefore, this research has been tried to find out how different concentrations of selected plant growth regulators can effect on the saffron corm sprouting in case of shortening or prolongation of the dormancy period.

MATERIALS AND METHODS

The corms have been used in this research (10-15 grams) were harvested from an experimental farm at the Research Institute of Food Science and Technology (RIFST) in middle of June 2018, Mashhad, Khorasan Razavi province, Iran. After removing the outer covering shells of the corms, surface disinfection was done with Tebuconazole 2% DS fungicide. Then, the corms were immersed in different concentrations of plant regulators (purchased from Merck and Sigma companies) solution including α-Naphthaleneacetic acid (NAA) (0, 100, 200, 300 ppm), Chlorocholine chloride (CCC) (0, 100, 200, 300 ppm) and Gibberellic acid (GA_3) (0, 5, 10, 20 ppm) for two hours. In control, the disinfected corms were treated only with distilled water. The corms were transferred to a growth chamber (Conviron, Canada) after drying under air flow. The environmental conditions in the growth chamber were set up under relative humidity of 65% and temperature of 20-22 °C for two months in dark conditions (Molina et al., 2005). Sprouting happened when the temperature was dropped to 15 °C and relative humidity increased to 85% and the light adjusted to 18-6 light-dark cycle afterward. Recording data started when the first sprout was observed based on the number of sprouted corms over 8 weeks from 16th September.

This research was arranged in a factorial experiment with three factors including type of growth regulators (NAA, CCC, GA_3), concentrations at four levels (0, 100, 200, 300 ppm for NAA and CCC) and (0, 5, 10, 20 ppm for GA_3) and time of recording (eight times during eight weeks). Three replicates were considered for each treatment and each replication contained 26 corms.

Statistical analysis

Data were analyzed in a split-plot in time under a completely randomized design with three replicates. JMP 11 software package was used for data analysis. To compare the differences between treatments Least Square (LS) Means student at a *P* value of less than 0.05 was considered.

RESULTS

The results of the variance analysis revealed that the effect of the PGRs on the percentage of corm sprouting was significant. According to the results presented in Figure 1, GA_3 hormone had a stimulatory effect on corm sprouting while; NAA and CCC compounds presented an inhibitory effect compared to the control. In an overview, NAA showed stronger inhibition than CCC.

The effect of different concentrations of NAA and CCC on dormancy prolongation of saffron corms was compared (Fig. 2), it was found that the lowest sprouting percentage (45.82%) was related to the concentration of 200 ppm of NAA. Unexpectedly, the concentration of 300 ppm of NAA showed the lowest inhibitory effect compared to 100 and 200 ppm, so that there is no statistically differences between 300 ppm NAA and the control.

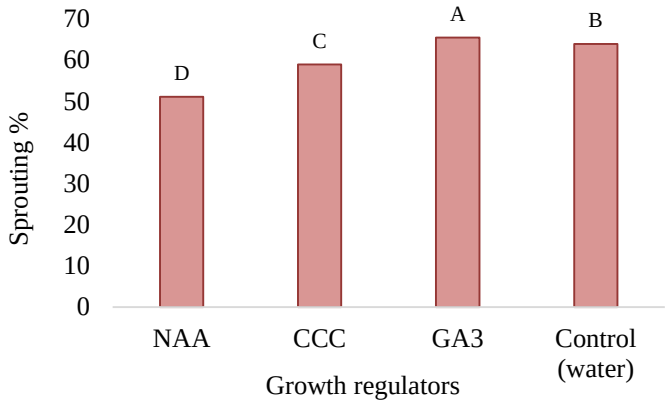

Fig. 1. The effect of different growth regulators on corm sprouting (p≤0.01)

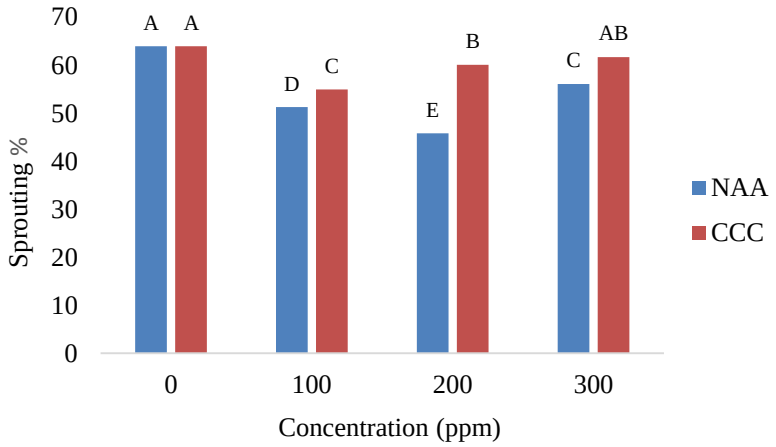

Fig. 2. Comparison of sprouting inhibitory effect in different concentrations of NAA and CCC (p≤0.01)

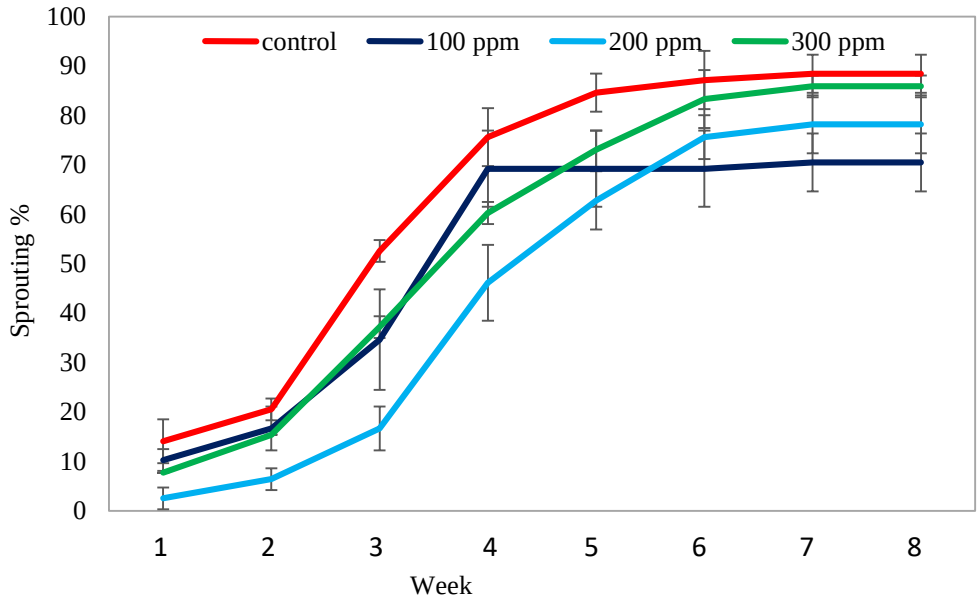

Fig. 3. The effect of different concentration of NAA on saffron corm sprouting during eight weeks ($p \leq 0.01$)

The effect of different concentrations of NAA on the sprouting of saffron corms over the eight weeks has been shown in Figure 3. Apparently, there are no significant differences among the different concentrations of NAA in the first week, but during the second to the fifth week; the inhibitory effect of NAA at 200 ppm was significantly higher than other treatments. In this concentration, the corm sprouting percentage was 62.81% while for the control it was 84.61%. But from fifth week onward, the sprouting percentage decreased down in 100 ppm even than 200 ppm (Fig. 3).

The effect of various concentrations of CCC on corm sprouting during the eight weeks was presented in Figure 4. Different concentrations of CCC did not show a significant effect on sprouting in the first and the second week. But in the third week, a significant effect observed among the different concentrations. In this period the maximum (52.65%) and the minimum (33.32%) of sprouting was found in the control and the treatment of 100 ppm, respectively. Although from the third to the fifth week, the most inhibition on sprouting was observed at a concentration of 100 ppm, there were no significant differences between the levels of 100 and 200 ppm. Since the sixth week onwards, CCC at a concentration of 100 ppm was showed the most inhibition than others. At this time, the percentage of sprouting in treated corms with 100 ppm of CCC was 74.35 while in the control it was 87.17%.

In fact, the results of this part of the study suggest that the percentage of sprouting at all tested concentrations of NAA and CCC was lower than the control. Treating of corms with 200 ppm of NAA was selected as the most effective concentration in order to make a five-week prolongation. While for more prolongation of dormancy, the most effective treatment can be attributed to NAA at the concentration of 100 ppm.

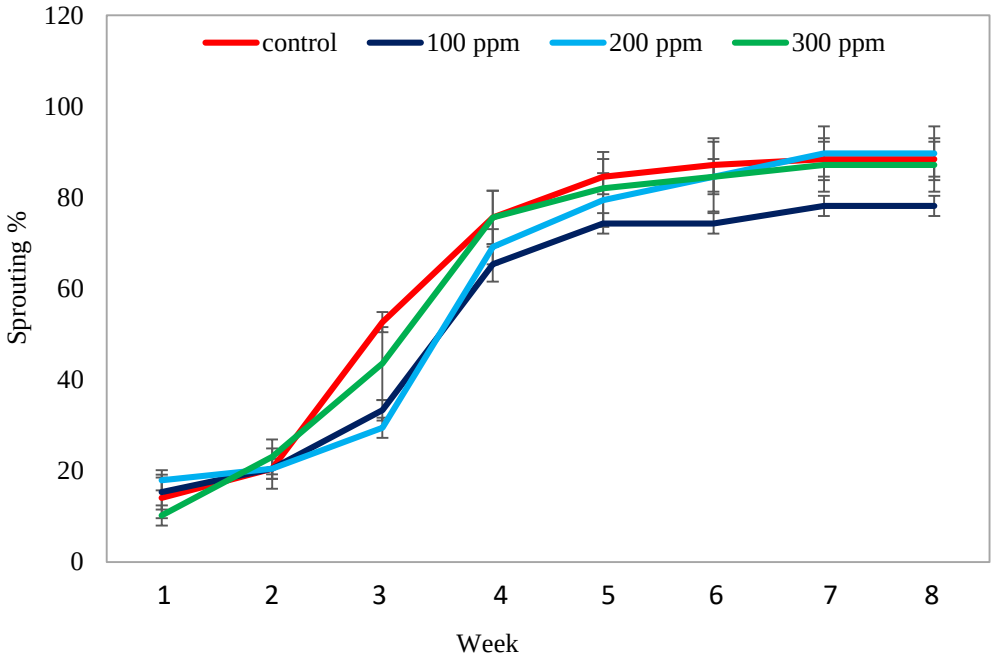

Fig. 4. The effect of different concentration of CCC on saffron corm sprouting during eight weeks ($p \leq 0.01$)

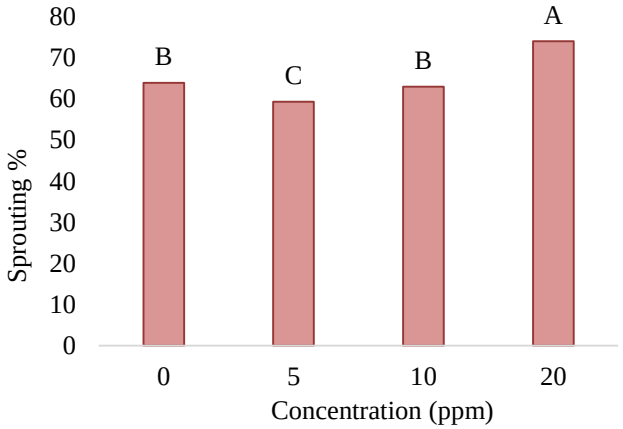

Fig. 5. Comparison of sprouting stimulatory effect in different concentrations of GA_3 ($p \leq 0.01$)

Based on the results presented in Figure 1, GA_3 has a stimulatory effect on saffron corm sprouting compared to the other PGRs tested in this study. The effect of different concentrations of GA_3 on corm sprouting is shown in Figure 5. Among studied levels (0, 5, 10 and 20 ppm), the highest concentration (20 ppm) showed the most stimulation (74.03% sprouting). There were no significant differences between the concentration of 10 ppm and the control. Contrary to our expectation, gibberellin at 5 ppm not only has no stimulation on corm sprouting but also showed an inhibitory effect on this characteristic.

Among the different concentrations of gibberellin on saffron corm sprouting, it was revealed that gibberellin did not play a role in sprouting stimulation during the first week (Fig. 6). In this time the highest sprouting (14.09%) was related to the control while sprouting percentage in treated corms with the maximum concentration of GA_3 was 12.81%. Notably, from the second week to the end of the experiment (especially to the fourth week) GA_3 at 20 ppm concentration was considered as the strongest sprouting stimulant (Fig. 6). In the fourth week, the highest (88.46%) and the lowest (75.63%) sprouting were related to GA_3 at 20 ppm concentration and control respectively.

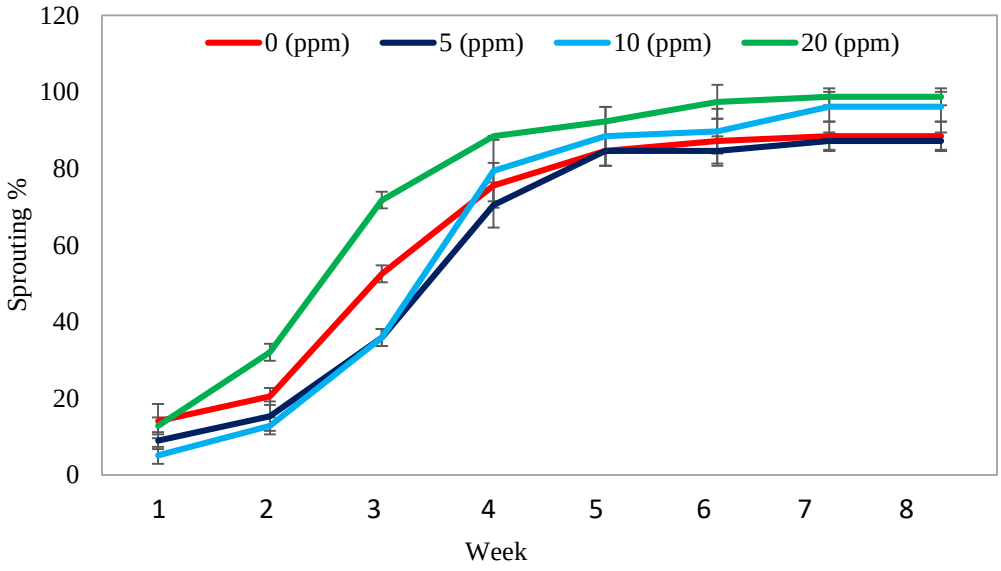

Fig. 6. The effect of different concentration of GA_3 on saffron corm sprouting during the eight weeks ($p \leq 0.01$)

DISCUSSION

In this study, the reaction of the dormancy period in corms of *C. sativus* in response to the different plant growth regulators and concentrations (NAA, CCC, and GA_3) was examined. Based on the results of this study, 1-naphthaleneacetic acid (NAA) especially at 100 and 200 ppm concentration was the most appropriate compound for delaying the sprouting and prolongation of dormancy period while GA_3 at 20 ppm caused dormancy breaking and stimulation of corm sprouting. Although there are no similar results in case of saffron corm sprouting, the results are confirmed by the findings from studies in other bulbous plants such as *Lilium*, in which the exogenous application of GA_3 (1 mg L^{-1}) for six weeks had significant positive effects on the dormancy breaking of in vitro bulblets (Mojtahedi et al., 2014). Padmalatha et al. (2013) also reported that GA_3 at a concentration of 150 ppm significantly increased the sprouting percentage of gladiolus corms over control and recorded a maximum number of sprouts per corm (Padmalatha et al., 2013). Many studies have been carried out to enhance the sprouting of multiple buds in *Gladiolus grandiflorus* L. through soaking of corms in a solution of plant growth regulators. They evaluated the effect of growth regulator on sprouting percentage, vegetative and floral variables. In this study, they soaked the corms in the solutions of gibberellic acid (GA_3), benzyladenine (BA) at different concentrations for 24 hr before planting. It was found that GA_3 hormone can influence on floral characteristics while benzyladenine increased multiple buds and production of corms (Sajjad et al., 2015).

Application of plant hormones to inhibit the growth of buds on various plants has also reported for many years (Thomas & Riker, 1945). Numerous reports demonstrated that plant growth retardants such as CCC have anti-GA effects (Wang & Xiao, 2009). Zheng et al. (2012) suggested that CCC treatment (300 mg L^{-1}) is an effective compound to promote carbohydrate accumulation in *Lilium oriental* bulbs by changing endogenous hormones. A slight delay of bud and anthesis formation was observed in CCC-treated plants (Zheng et al., 2012). Thomas and Riker (1945) found that sprouting of potatoes has been prevented for some time by treatments with the plant hormone, the methyl ester of alpha-naphthaleneacetic acid (Thomas & Riker, 1945). To test the hypothesis of growth regulators effect on root buds, researchers have examined different hormone types on the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula*). Their results showed that the BA, ABA, NAA, and paclobutrazol treatments compared to the other hormones inhibited root bud growth. NAA hormone inhibited growth at concentrations as low as 1 µM (Chao et al., 2006).

CONCLUSION

Based on the obtained results in this study, it can be concluded that PGRs have a significant role in the regulation of saffron corm sprouting. It means based on the type and concentration of PGRs, the corm dormancy can be broken or prolonged. Indeed NAA and CCC had an inhibitory, while GA_3 has a stimulatory effect on saffron corm sprouting. These results can be beneficial for a different purpose. For instance, in the production of saffron in a greenhouse system, prolong dormancy can be useful for regulation of corm storage to use them for multi flower harvesting in one platform. Therefore, chemicals like NAA and CCC could be recommended. In the case of farm saffron production, chemicals with stimulation of spouting can be applicable. Of course, additional work needs to find out which chemical at which concentration has the best effect on saffron sprouting and flowering in a specific condition.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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