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Severity, incidence and distribution of Moroccan watermelon mosaic virus infecting cucurbits in selected counties in Kenya

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Moroccan watermelon mosaic virus (MWMV) is one of the potyvirus in the Potyviridae family that causes significant economic impacts in cucurbit species globally. The virus can infect these crops at every stage of development, with early infection resulting in significant harm or total yield loss. The virus is transmitted by several species of aphids and by mechanical means. The recent report of the virus in two counties in Kenya infecting pumpkin (*Cucurbita pepo* L) required urgent attention. Therefore, since the virus is known to infect several cucurbit species, determination of the incidence, severity and distribution of MWMV in the major cucurbits growing counties is important for the development of effective management strategies. A survey was conducted in the year 2024, covering a total of 123 cucurbit fields in seven sub-counties within three counties. The disease severity was determined using a scale of 0 to 4. While the disease incidence was determined by visually inspecting ten plants per field for MWMV symptoms. MWMV distribution was also determined by testing samples collected from the field using polymerase chain reaction (PCR). The highest disease severity recorded was 3.8 and the highest disease incidence was 100%. The highest number of positive samples were 12. Therefore, a detailed survey across the country is recommended to determine the extent of the virus spread.

Key words: Occurrence, disease, epidemiology, spread, farmer's awareness.

INTRODUCTION

Cucurbits are annual crops in the family Cucurbitaceae and are grown mainly for their fruits and vegetables (Stevenson et al., 2007; Tzortzakis et al., 2018). They are economically important crops grown for both domestic consumption, local and export markets (Woolhouse et al., 2005). The crops have nutritional and medicinal values that help in combating illnesses and maintaining good health (Tzortzakis et al., 2018). Fresh cucurbit fruits contain starch, sugars, protein, fats, and vitamin B complex. Cucurbit leaves can be cooked as vegetables and contains potassium, and vitamin B6 (Horticultural Crop Development Authority (HCDA), 2020; Kim et al., 2012).

Cucurbit production is however, challenged by several diseases especially viral diseases that lead to reduction in growth and productivity (Woolhouse et al., 2005). Some of these viruses that cause economic damage include; Cucumber vein yellowing virus (CVYV)

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(Ipomovirus), Watermelon mosaic virus (WMV) (potyvirus), Squash mosaic virus (SqMV) (comovirus), Zucchini yellowing mosaic virus (ZYMV) (potyvirus), Melon necrotic spot virus (MNSV) (gammacarmovirus), Papaya ringspot virus (PRSV) (potyvirus), Watermelon chlorotic stunt virus (WmCSV) (begomovirus), Moroccan watermelon mosaic virus (MWMV) (potyvirus), Squash leaf curl virus (SLCV) (begomovirus) among others (Radouane et al., 2021). Among these viruses, MWMV possess a serious threat to the production of cucurbit globally (Woolhouse et al., 2005). The virus was first reported in Morocco in 1974 (Fischer and Lockhart, 1974) and currently reported in many African countries and Mediterranean regions. The virus is transmitted by several species of aphid in a non-persistent manner (Lecoq et al., 2008, 2001). Mechanical transmission has also been reported (Mumo et al., 2022). MWMV causes substantial losses in cucurbit productions ranging from 60 to 100% (Ibaba et al., 2016; Chatzivassiliou et al., 2016). Early infection of the virus to the crop before fruits are formed may lead to partial or entire loss of yield (Miyao et al., 2020).

In Kenya, cucurbits are grown by small and large-scale farmers for domestic consumption and income generation (HCDA, 2020). However, MWMV was recently reported in Kenya in Meru and Kiambu counties infecting pumpkin crops (Mumo et al., 2022). The presence of the virus in major cucurbits growing regions in the county remains undocumented.

Therefore, documentation of the incidence, severity, and distribution of the virus in major cucurbits growing regions in the country is important for the development and implementation of effective management strategies.

MATERIALS AND METHODS

Sampling sites and sampling procedure

Field surveys were conducted between the months of August and October in the year 2024 in three counties namely; Machakos County (Matungulu and Kangundo sub-counties), Kirinyaga County (Mwea East, Kirinyaga West, and Kirinyaga East sub-counties), and Embu County (Mbeere North and Embu West sub-counties). The counties and sub-counties within the counties were selected based on the presence of cucurbit species (HCDA, 2020). In the sub-counties, purposeful sampling was done based on the presence of the crop in the field. In the farmer fields, an imaginary line were drawn diagonally from both directions resulting into two transects (Sseruwagi et al., 2004). Representative plants were randomly selected during sampling along the X- shape transect in each field to reduced biases. A total of 123 cucurbits fields were surveyed.

Determination of incidence and severity of MWMV disease in farmers' fields

To determine the disease incidence, ten plants per field were visually examined for the presence of MWMV symptoms. The vigor of the plants was also inspected. Disease incidence was determined as a proportion of the plants exhibiting MWMV symptoms out of the ten plants inspected and expressed as a percentage (Ayo-John et al., 2014). The disease severity was calculated using the area or proportion of symptomatic plants. The scale of 0 to 4 (Ndunguru and Rajabu 2004) was used (Table 1). The presence of the virus was scored based on the amplification of the 613 bp fragment following the observation in the agarose gel.

Sample collection for determination of virus infection using PCR

Young leave samples were collected from 5 plants per field using sterile forceps. A total of seventy-seven symptomatic and forty-six asymptomatic plant leaf samples were collected using snowball sampling and an imaginary diagonally line drawn in each field (Sseruwagi et al., 2004). Asymptomatic samples were collected due to latent infection or early stage of infection but yet to development symptoms. The number of leave samples collected per field depended on the population and disease severity. The collected leave samples were preserved in 15ml sterile falcon tubes containing 10ml of RNA preservation solution (0.5M EDTA, sodium citrate, and Ammonium Sulphate; pH 5.2) to prevent RNA degradation. The samples in the falcon tubes were put in zip lock bags and clearly labelled before putting them in a cool box containing cold ice packs and transported to Jomo Kenyatta University of Agriculture and Technology laboratory and stored at 4ºC before RNA extraction.

RNA extraction

RNA was extracted from the collected leaf samples using a modified cetyltrimethylammonium bromide (CTAB) method (Lodhi et al., 1994). The CTAB buffer contained 2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, freshly prepared 1% sodium sulphite, and 2% polyvinylpyrrolidone in nuclease-free water. Briefly, 100 mg of leaf sample were homogenized in 2 mL extraction buffer, transferred in a 1.5 mL micro centrifuge tube and incubated at 65ºC for 15 min while mixing at 10-min intervals. The tubes were centrifuged at 10,000 rpm for 5 min, and the supernatants (700 µl) were transferred to new tubes. An equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 min. The aqueous layers were transferred into new tubes, and 700 µl of lithium chloride (stored at 4ºC) was added while the tubes were gently inverted. After overnight incubation at 4ºC, RNA was pelleted at 14, 000 rpm for 30 min at 4ºC.The supernatants were discarded, and the pellets re-suspended in 200 µl of TE buffer containing 1% of SDS.

Then, 100 µl of NaCl and 300 µl of ice-cold isopropanol were added, mixed gently, and incubated at -20ºC for 20 min. The samples were then centrifuged at 14,000 rpm for 10 minutes at 4ºC. The salts were removed and the pellets were washed with 500 µl of 70% (v/v) ethanol and then centrifuged for 4 min at 10,000 rpm. The ethanol was removed and the RNA pellets were air dried at room temperature for 15 min before resuspending in 50 µl of nuclease- free water. The integrity of the extracted RNA was assessed using agarose gel electrophoresis and visualized in UVP PhotoDoc-It™ imaging System.

The cDNA synthesis and PCR process

The cDNA was synthesized using LunaScript® RT SuperMix Kit (Biolabs) Briefly, a 25 µl reaction consisting of 4µl of 5X LunaScript RT SuperMix, 1µl of RNA sample and 20µl of nuclease-free water. The mixture was incubated at 55 º C for 10 min and at 95 º C for 1 min. The cDNA was stored at -25ºC before the PCR process.

PCR process was carried out using OneTaq 2XMaster Mix with Standard Buffer (New England Biolabs). Briefly, a 20µl PCR

Table 1. Scale used in rating MWMV disease severity in cucurbit plants during the field survey.

Scale	Symptoms expressed in cucurbit crops
0	No symptoms
1	1 to 25% Mild mosaic discoloration and mottling of leaves
2	26 to 50% Slight distortion, deformation, chlorosis, yellowing puckering, and dark green blisters on the leaves, mild stunted growth
3	51 to 75% Severe mosaic, distortion, deformation, chlorosis yellowing dark green blister, vein clearing on leaves and moderate stunted growth
4	More than 75% Leaves severely distorted, or leaf deformation and severe stunted growth

Source: Ndunguru and Rajabu (2004).

Table 2. The mean disease incidence, and severity of MWMV in selected Counties in Kenya.

County	Sub-county	Disease incidence (%)	Disease severity
Machakos	Matungulu	98.4	3.8
	Kangundo	100	3.7
Kirinyaga	Mwea East	81.7	2.7
	Kirinyaga East	100	2.7
	Kirinyaga West	62.5	2.2
Embu	Mbeere north	78.2	1.8
	Embu West	71.9	1.6
Mean		85.5	2.8
LSD (P = 0.05)		0.005	0.001

reaction consisted of 7.2 µl of nuclease-free water (New England Biolabs), 10 µl of OneTaq 2X Master Mix with Standard Buffer (New England Biolabs), 0.4 µl of 10 µM of forward and reverse primers, and 2 µl of cDNA. Primers used were 5 '-TGCTGTTGGTAGTGGCAAAT-3 'and 5 '-TTCTGTTCGCCCAACTTTA-3 (Macrogen), amplifying a 613 bp fragment for MWMV in the coat protein region. Both positive and negative controls were included in the reaction. The positive control constituted a sample infected with the virus from a previous study (Mumo et al., 2022), while the negative control contained nuclease-free water. The PCR reactions were run on a thermal cycler (BIO-RAD T100™) under the following cycling conditions: 3 min at 95ºC; 35 cycles of 30 s at 95ºC, 30 s at 55ºC and 1 min at 72ºC and a final extension at 72ºC for 5 min for the sets of primers according to the manufacturer instruction. The amplified PCR products, alongside the 1-kb plus DNA ladder, were run on 2% (wt/vol) agarose gels and visualized under a UV transilluminator.

Data analysis

Data on disease incidence and severity was analyzed through descriptive statistics (percentages and mean values) using Statistical Package for Social Scientists (SPSS). When determining disease severity, score 0 (no symptoms) were excluded.

RESULTS

Incidence and severity of Moroccan watermelon mosaic virus in selected counties in Kenya

The disease severity and incidence differed significantly between the sub-counties (P< 0.05). The highest disease severity reported was 3.8, 3.7, 2.7, 2.2, 1.8, and 1.6 in Matungulu sub-county, Kangundo sub-county, Mwea East and Kirinyaga East sub-counties, Kirinyaga West, Mbeere North, and Embu West respectively (Table 2). The mean disease incidence across the seven sub-counties reported was 85.5% with the highest of 100% in Kangundo and Kirinyaga East, followed by Matungulu 98.4%, Mwea East 81.7%, Mbeere North 78.2%, Embu West 71.9% and Kirinyaga West 62.5% (Table 2).

Symptoms diversity and incidence of MWMV through PCR

Symptoms of MWMV observed in the fields included leaf

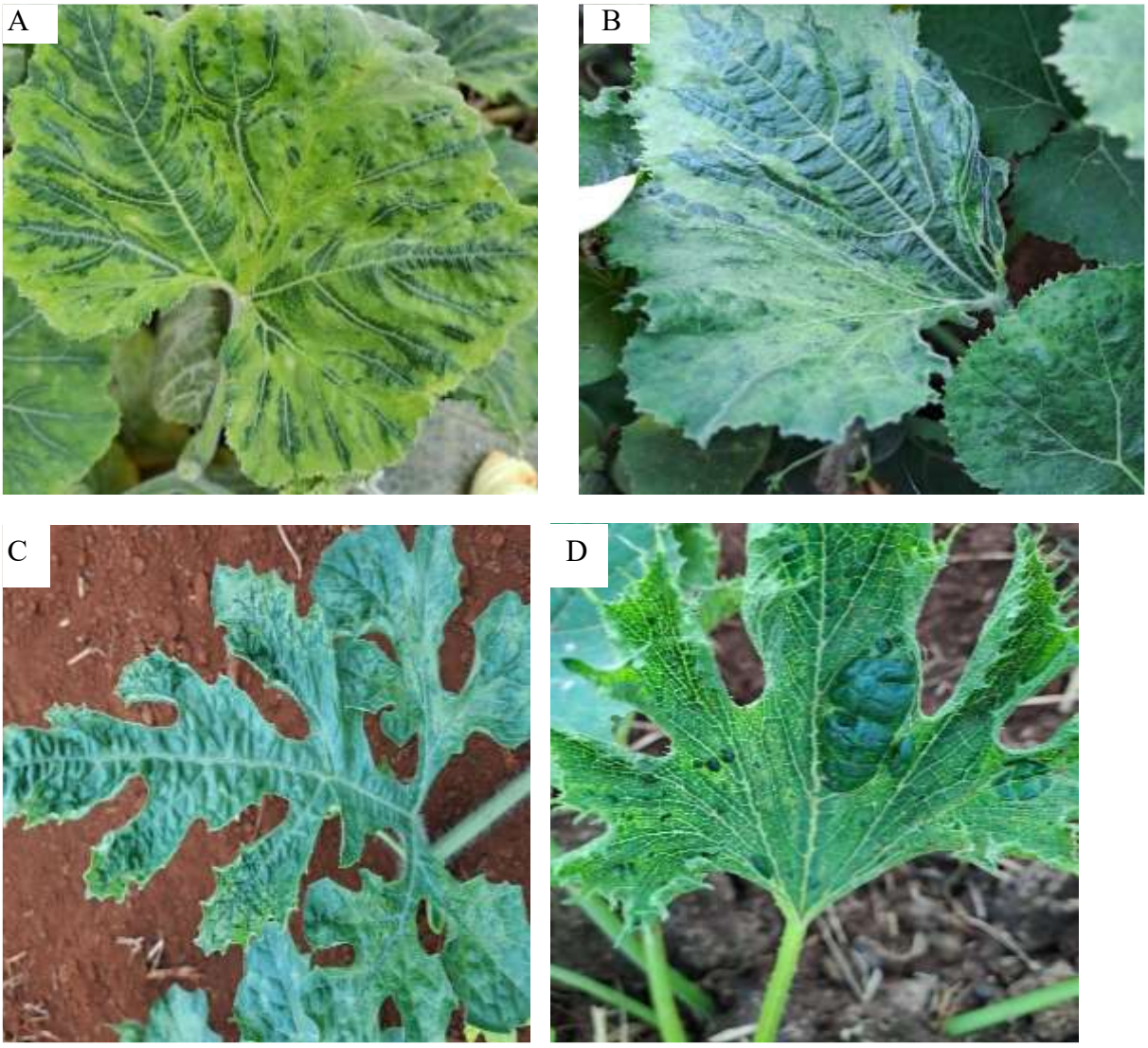

Figure 1. Moroccan watermelon mosaic virus disease symptoms observed in the field. A) leaf deformation, dark green blisters and vein clearing on pumpkin, B) Mosaic and leaf deformation on butter nut, C) mild mosaic, and leaf deformation on watermelon; D) leaf deformation, and dark green blister on zucchini.

deformation, dark green blisters of leaf, vein clearing; severity mosaic, mild mosaic, chlorosis and yellowing of leaf stunted growth, mottling and puckering of leaf (Figure 1). It was observed that the disease incidence was found throughout the seven sub-counties (Table 2). Machakos County (sub –county Kangundo) and Kirinyaga County (sub-county Kirinyaga East) obtained 100% disease incidence, followed by Matungulu sub-county (Machakos county) 98.4, 81% in Mwea East sub-county (Kirinyaga county), 78.2% in Mbeere North sub-county, 71.9% Embu West sub-county (Embu count), and 62.5% in Kirinyaga West sub-county (Kirinyaga county) respectively.

Out of the seven sub-counties surveyed, MWMV was detected in four sub-counties namely Matungulu, Mwea East, Mbeere north and Embu west (Table 3). MWMV was detected in 27 out of the 77 pumpkin samples tested. However, MWMV was not detected in other cucurbit plant samples tested including watermelon, zucchini, and butter nut. Presence of band size of 613 in the gel indicated presence of the virus in the sample (Figure 2). In terms of distribution of MWMV in Machakos County, 12 samples from Matungulu sub-county were positive for the virus. In Kangundo sub-county, no virus was detected. In Embu County, 8 and 3 samples from Embu west and Mbeere North sub-counties respectively tested positive. In Kirinyaga County, 4 samples from Mwea east sub-county were infected with the virus.

None of the samples collected from Kirinyaga east and Kirinyaga west tested positive for the virus infection. The

Table 3. Moroccan watermelon mosaic virus disease incidence (PCR) and symptoms diversity.

County	Sub-county	Cucurbit crop species	No. of samples collected	No. of symptomatic samples	No. of positive samples through PCR	Symptoms expressed
Machakos	Matungulu	Pumpkin	38	31	12	SG, LD, SM, LB, VC
	Kangundo	Pumpkin	10	5	0	LD, SM, LY, MM
Kirinyaga	Mwea East	Pumpkin	15	10	4	SG, LD, SM, LB
		Zucchini	1	1	0	LD, LB
		Watermelon	7	4	0	LD, MM
		Butternut	1	1	0	LD, SM
	Kirinyaga west	Pumpkin	16	8	0	LD, SM, LB, LM
	Kirinyaga east	Pumpkin	8	3	0	LD, LB, SM
Embu	Mbeere north	Pumpkin	15	8	3	SG, LD, SM,LB
	Embu west	Pumpkin	12	6	8	LD, LB, LM

*Symptom description: SG, stunted growth; LD, leaf distortion; SM, severe mosaic; LB, leaf blistering; MM, mild mosaic; VC, vein clearing; LY, leaf yellowing; LM, leaf Mottling, leaf puckering.

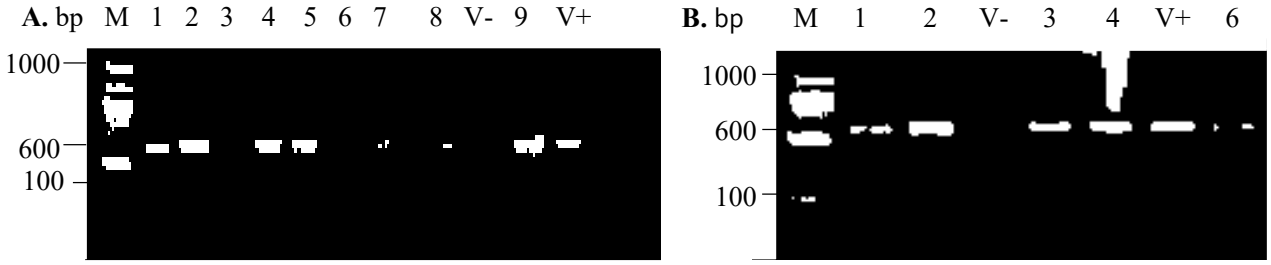

Figure 2. Gel electrophoresis for diagnostic studies of MWMV in collected leaf samples. A and B) bands of 613 bp indicate presence of MWMV. M indicate the O'GeneRuler ™ 1kb plus DNA ladder. +v is the positive control and –v is the negative control. Numbers in band A and B representative samples.

number of symptomatic samples varied with the highest obtained from Machakos County, Matungulu and Kangundo sub-counties 31 and 5 pumpkin samples respectively. In Kirinyaga County, Mwea East Sub County obtained 10 pumpkins, 4 watermelons, 1 butter and 1 zucchini symptomatic samples were collected. In Kirinyaga West, Kirinyaga East sub-counties 9 and 3 pumpkin samples were collected and in Embu county 8 and 6 pumpkin samples were collected from Mbeere North and Embu West sub-counties respectively (Table 3). Table 3 indicates asymptomatic and symptomatic samples of cucurbit species collected from the field per county and sub-counties to determine the present of MWMV through PCR process

DISCUSSION

Viral diseases rank second to fungal diseases in terms of economic losses, posing a significant threat to plant growth and development, which ultimately leads to decreased crop yields and quality, thereby impacting food security (Godfray et al., 2010).

This report presents surveys conducted to determine the incidence, severity, and distribution of Moroccan watermelon mosaic virus disease affecting cucurbit crops in selected Counties in Kenya. The disease incidence and severity differed significantly between the sub-counties ($P < 0.05$).The increase disease incidence may be due to mono cropping of pumpkin on extensive fields for market purposes, potentially facilitating rapid disease dissemination due to the elevated host density and expansive cultivated area as observed during the survey in Machakos county (Kangundo sub-county) and Kirinyaga county (Kirinyaga East sub-county) (Kumar et al., 2010; Piper et al., 1996). Also, mono cropping enables rapid movement of vector between plants during their migratory flights while searching for an appropriate

host a condition that may exacerbate the high disease incidence and severity (Kumar et al., 2010).

Increase in the disease incidence could also be attributed to plant growth, which indicates that plant susceptibility to virus infection increases with age, as observed during the survey (Kone et al., 2017). This is in contrast to the cassava-ACMV pathosystem, which found that ageing cassava plants were less susceptible to virus infection (Fargette et al., 1993).Farmers' practices have a significant impact on viral infections in many crops; therefore, farming practices like weed presence, and volunteer cucurbit plants in and around the fields may be contributing to the rise in disease incidence (Jones, 2009; Anderson et al., 2004). Also, farmers limited knowledge regarding the disease and its occurrence as observed during the survey could attributed to the increase in the disease incidence (Abdurahman et al., 2017; Gorfu et al., 2012). Cropping seasons that overlap also influence disease incidence because the old maturing crop serves as a virus reservoir for the newly planted crop (Thresh, 2003).

The disease severity ranged from 3.8 high in Matungulu sub-county to 1.6 low in Embu west sub-county, this may be attributed to farmers' lack of understanding concerning the causes and mechanisms of the disease spread which may impact the severity (Abdurahman et al., 2017; Gorfu et al., 2012).

The extensive distribution of MWMV confirmed through the PCR process clearly indicates its significant presence in pumpkin. Also, several pumpkin samples obtained from Embu West sub-county exhibited no viral symptoms and were initially presumed to be virus-free; however, 2 out of 12 samples tested positive for infection, indicating a latent infection or maybe attributed to the plants being in the early stage of infection and yet to exhibit symptoms during the time of the survey (Kumar et al., 2010; Carrere et al., 1999).

In contrast, cucurbit crops such as butternut, zucchini and watermelon displayed MWMV symptoms yet the virus was not detected, additionally, pumpkin samples from Kirinyaga east and Kangundo sub counties displayed 100% disease symptoms while Kirinyaga west sub county exhibited 62.5% MWMV symptoms but the disease was not also detected. Similarly, the lower number of samples detected in Matungulu, Mwea east, and Mbeere north sub-counties as compared to symptomatic samples could be as a result of other virus or an existence of a variant that may not have been detected by the primer used (Barbedo, 2016; Ghoshal and Sanfacan, 2015; Bashir et al., 2006; Bos, 1999).

The virus was initially reported in two counties, Meru and Kiambu (Mumo et al., 2022). The current study has reported the virus in other counties including Machakos, Kirinyaga and Embu. This indicates that the virus could be widespread in other counties growing cucurbits but remains unreported. This calls for an extensive survey in the country to determine its spread.

Furthermore, the virus is predominantly reported in pumpkin crop species from both previous and current studies, although the other cucurbit crops are also host of the virus. Probably, the pumpkin crop species could be playing an important role in the spread of the virus which also needs to be determined. Additionally, mixed cropping should be avoided to reduce the level of the disease incidence and distribution since the virus can be transmitted mechanically from pumpkin to other cucurbit species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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