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Effect of plant extracts on seedling production of *Nicotiana tabacum* L var. "Corojo 2012" in Pinar del Rio, Cuba.

Efecto de extractos vegetales en la producción de plántulas de *Nicotiana tabacum* L var. "Corojo 2012" en Pinar del Río, Cuba.

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ABSTRACT

The research was carried out in traditional tobacco seedbeds on the farm "La Rosa", in February – March 2021 to evaluate the biostimulant effect of the combinations of extracts of *S. saman* + *A. vera* + *M. oleifera* on seedlings of *N. tabacum* var Corojo 2012. An experimental design of random blocks was used, with 13 treatments and three replications, indicators of plant height, number of leaves, internodes and nodes, stem diameter, root length, and root volume were evaluated. The T4 treatment presents the best results in plant height and internode distance indicators with 12.39 cm and 2.34 cm respectively. The best behavior in the number of leaves presented by T6 with 5.4 leaves. In radical volume, the treatments T5 and T9 are superior to the control by 54 %, and in the length of the root, the best results show T9 and T10 with values of 5.64 and 5.59 cm respectively, in turn, are superior to the control by 16 %. Combined extracts use shows a biostimulant effect on the different variables evaluated in seedlings of tobacco variety "Corojo 2012" in traditional seedbeds.

KEY WORDS: Extracts, biostimulant, *Aloe vera*, *Samanea saman*, *Moringa oleifera*.

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Revista Bio ciencias
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RESUMEN

La investigación se realizó en semilleros tradicionales de tabaco en la finca “La Rosa”, en los meses de febrero – marzo de 2021 con el objetivo de evaluar el efecto bioestimulante de las combinaciones de extractos de *S. saman* + *A. vera* + *M. oleifera* sobre plántulas de *N. tabacum* var Corojo 2012. Se utilizó un diseño experimental de bloques al azar, con 13 tratamientos y tres réplicas, se evaluaron los indicadores altura de las plantas, número de hojas, entrenudos y nudos, diámetro del tallo, largo de las raíces y volumen radical. El tratamiento T4 presenta los mejores resultados en los indicadores altura de la planta y distancia entrenudos con 12,39 cm y 2,34 cm respectivamente. El mejor comportamiento en el número de hojas lo presenta T6 con 5,4 hojas. En el volumen radical los tratamientos T5 y T9 son superiores al testigo en un 54 % y en la longitud de la raíz los mejores resultados los muestran T9 y T10 con valores de 5,64 y 5,59 cm respectivamente, estos a su vez son superiores al testigo en un 16 %. El empleo de extractos combinados muestra un efecto bioestimulante sobre las diferentes variables evaluadas en plántulas de tabaco variedad “Corojo 2012” en semilleros tradicionales.

PALABRAS CLAVE: Extractos, bioestimulante, *Aloe vera, Samanea saman, Moringa oleifera.*

Introduction

Cuban tobacco, the best in the world and one of Cuba’s traditional export products is consolidated in the world market and recognized for the unique quality of the leaves it produces, in terms of organoleptic properties (smell, color, flavor, and chemical composition of harvested leaves), ensuring a stable and secure market, both in its trade in leaf tobacco as well as in cigars and Habanos (Rodriguez, 2019; Jiménez Mariña, 2021). Therefore, it is of general interest to increase yields, in which biological control, biofertilizers, and biostimulants play an important role (Cruz & González, 2008). Currently, it is of great importance to research and find variants that allow us to develop a profitable and less environmentally polluting agriculture (Deming et al., 1989).

To achieve sustainable agricultural systems that are friendly to the environment and its main resources, biostimulant use is currently on the rise. When applied in small quantities, these compounds have a wide range of molecules capable of promoting plant growth. These positively affect plant growth and development by improving water absorption, root, and shoot growth, abiotic stress tolerance, protein content in plant tissues, and the activity of plant-connected enzymes (Puglisi et al., 2020).

Revista Bio ciencias
ISSN 2007-3380

Several plant species have an influence on crop growth and development due to their chemical composition for example, *A. vera* possesses a hormone capable of accelerating the formation and growth of new cells, enhanced by calcium, which is vital for cell osmosis (liquid exchange), and contributes to maintaining the cells' fragile internal and external equilibrium (Minond, 2011).

Ethanolic and aqueous extract of *M. oleifera* leaf is used as a biofomentation agent since it contributes to increase of nodules and weight in roots due to its content of phytohormones such as gibberellin and zeatin; it also reduces stress generated by excess NaCl and $Cd_{(2)}$, increases productivity due to its antioxidant activity in some crops (Rady et al., 2013; Howladar, 2014).

Oziel, (2014), stated that Moringa is currently being used as a natural fertilizer and organic biomass in nurseries and crops that are committed to the preservation of the environment.

According to Domínguez et al. (2017), *S. saman* tree of Pinar del Rio province presents in the cortex, flowers, leaves, and fruits secondary metabolites such as flavonoids, tannins, terpenoids, reducing sugars, mucilages, saponins, alkaloids, among others, which constitutes a source of research with respect to the obtaining of natural products. Additionally, Garcia et al. (2006) found total polyphenols, condensed tannins, hydrolyzable tannins, total alkaloids, saponins, and phytic phosphorus as tannic acid equivalent in the foliage of this species; this species also has high levels of potassium and phosphorus compared to other leguminous plants.

It has been demonstrated the positive effect that different biostimulants have in combination on plants, use of these constitutes an alternative for the achievement of an agriculture productive, sustainable, and more environmentally friendly where negative effects of excessive use of chemical products are reduced, given that in Pinar del Rio province the use of natural products in tobacco seedlings production of is infrequent and this is a crop in which high contents of agrochemicals are required to obtain them, this research aimed to evaluate the biostimulant effect of combined extracts of *S. saman* + *A. vera* + *M. oleifera* on seedlings of *N. tabacum* var Corojo 2012.

Material y Methods

Research area characteristics.

The research was carried out on farm "La Rosa" of the producer Eng. Yusbel Echevarría Olivera belongs to the Cooperative of Credits and Services (CCS) "Gervasio Hernández", located at km two of the Road to San Juan y Martínez, Pinar del Rio, this farm is adjacent to others of the same entity. The experimentation process was carried out in traditional tobacco seedbeds *Nicotiana tabacum* L var. ¨Corojo 2012¨ under the tunnel, in February - March 2021.

The soil in the experimental area was classified as Yellowish Ferrallitic Leached according to the New Version of Soil Classification of Cuba (Hernández-Jiménez et al., 2019).

Revista Bio ciencias
ISSN 2007-3380

Research setup.

The research was carried out in traditional seedbed conditions under a tunnel with a transparent polyethylene cover and sides with anti-aphid fabric. The soil was improved with organic matter at a rate of 2 kg m^{-2}. Soil preparation was carried out with a three-row plow pass, followed by a Creole tiller pass and the beds were made manually with dimensions of 18 m long by 1.20 m wide, height of 25 cm, and separation between beds of 40 cm. According to Espino et al. (2012), an area of 1 x 1.20 m was used for each treatment with a spacing of 0.50 m for a total experimental area of 21.6 m^2.

Seedbed services.

From sowing to seed germination, which occurred after 6 days, irrigation was carried out twice a day, in the morning and in the afternoon, to guarantee an adequate level of humidity in bed to allow seed germination. After germination, irrigation was done once a day, preferably in the morning. Weeds were eliminated systematically, maintaining extreme care to avoid damaging the small tobacco seedlings that had already germinated. Attention to seedbed was carried out following the criteria of Espino et al. (2012).

Extracts preparation.

The plant material of *S. saman* and *M. oleifera* comes from wild species close to the study area, young buds of adult plants were used for the preparation of the extract. *A. vera* material used came from plants more than two years old, which are grown by the producer. The study material was transferred to the research laboratory of the University of Pinar del Río and the samples were processed to obtain the extracts, this was done by maceration method following the criteria of García Luján et al. (2010).

Experimental design and treatments.

A randomized block experimental design was used, with 13 treatments and 3 replicates. Fifteen seedlings were taken for evaluation. The total number of seedlings sampled was 780.

Revista Bio ciencias
ISSN 2007-3380

Table 1. Treatments, way and time of application

	Treatments		Time of application after germination	Application form
T1	Aloe 6 % Moringa 6 %	A6 %+ M6 %	10; 17; 24 y 31 days	Foliate
T2	Aloe 6 % Moringa 4 %	A6 %+ M4 %	10; 17; 24 y 31 days	Foliate
T3	Aloe 8 % Moringa 4 %	A8 %+ M4 %	10; 17; 24 y 31 days	Foliate
T4	Aloe 8 % Moringa 6 %	A8 %+ M6 %	10; 17; 24 y 31 days	Foliate
T5	*S. saman* 10 % Aloe 6 % Moringa 4 %	S10 % + A6 %+ M4 %	10; 17; 24 y 31 days	Foliate
T6	*S. saman* 10 % Aloe 6 % Moringa 6 %	S10 % + A6 %+ M6 %	10; 17; 24 y 31 days	Foliate
T7	*S. saman* 10 % Aloe 8 Moringa 4 %	S10 % + A8 %+ M4 %	10; 17; 24 y 31 days	Foliate
T8	*S. saman* 10 % Aloe 8 % Moringa 6 %	S10 % + A8 %+ M6 %	10; 17; 24 y 31 days	Foliate
T9	*S. saman* 15 % Aloe 6 % Moringa 4 %	S15 % + A6 %+ M4 %	10; 17; 24 y 31 days	Foliate
T10	*S. saman* 15 % Aloe 6 % Moringa 6 %	S15 % + A6 %+ M6 %	10; 17; 24 y 31 days	Foliate
T11	*S. saman* 15 % Aloe 8 % Moringa 4 %	S15 % + A8 %+ M4 %	10; 17; 24 y 31 days	Foliate
T12	*S. saman* 15 % Aloe 8 % Moringa 6 %	S15 % + A8 %+ M6 %	10; 17; 24 y 31 days	Foliate
T13	Control	Chemical fertilization	Two days prior to planting and 20 days after planting	Bottom and Foliate

Source: Own elaboration

To the control was applied 375 g per square meter of complete formula N P K Mg (5-12-6-2,6), two days before planting 50 % of the fertilizer was applied and the rest 20 days after planting according to Espino et al. (2012) in the Technical Instructions for tobacco cultivation.

Indicators to evaluate in the research.

At the time of transplanting, 40 days after the plants were established in seedbed conditions, 15 plants per treatment were selected and three replicates were made. The seedlings were transferred to the research laboratory of the University of Pinar del Río for processing and evaluation of the following indicators:

Plant height (PH): It was measured from the basal part to the apex of the stem, using a 30 cm graduated ruler.

Revista Bio ciencias
ISSN 2007-3380

Leaves number (LN): It was determined by counting selected plants per treatment.

Distance between knots (Dist/K): It was determined by counting selected plants per treatment.

Knots number (KN): It was determined by counting selected plants per treatment.

Stem diameter (SD): The average was determined by several measurements with the caliper to stem of tobacco seedlings.

Root length (RL) (cm): It was determined by measuring the length of roots of the seedlings.

Root volume (RV) (cm^3): Roots were introduced in a graduated cylinder and the increase of water in the cylinder was taken as the volume.

Statistical analysis

The fit to a normal distribution was determined by the Kolmogorov-Smirnov Goodness of Fit test and Homogeneity of Variance by Bartlett's Tests. Where the data met the required criteria, it was processed by simple rank ANOVA, and Duncan's Multiple Range Test was used for comparison between means. Software SPSS v. 22 was used for statistical analysis.

Results and Discussion

Behavior of morphological indicators of *N. tabacum* seedlings with the application of combined extracts of *S. saman* + *A. vera* + *M. oleifera.*

Seedling quality results from the integration of numerous physiological and morphological characteristics that control the possibilities of seedling development and growth. It is one of the most important factors that plantation success determines. We agree with Almeida Zambrano (2010) in stating that the quality of plants is determined by their ability to survive under stressful conditions and predict vigorous growth after planting.

It can be seen from the data in Table 2 that for the plant height indicator, the T4 treatment (A8 % + A6 %) has the best result with 12.39 cm, showing significant differences over the control and other treatments. These results are superior to those obtained by Pérez-Martín et al. (2022) when using extracts of *A. vera* at 6 and 8 % and *S. saman* at 10 and 15 % in traditional tobacco seedbeds of the "Corojo 2012" variety, which shows the advantage of combined application of extracts due to the possible synergic and beneficial action between them. Moreover, these results are similar to those achieved by Rodriguez (2014) and Gonzalez et al. (2017) when using other biostimulants on tobacco seedlings.

Revista Bio ciencias
ISSN 2007-3380

Treatments T2, T4, and T6 have a stimulating effect on plant height indicators since they are superior to the control. Similar results were obtained by Dala et al. (2004) when they applied EcoMic®+FitoMas-E®+IHplus® in a combination way. Besides, results also coincide with those reported by different authors who have used the combination of biofertilizers and biostimulants in different crops, since these are capable of stimulating plant height with significant differences with respect to the rest of the treatments, and this combination improves plant response.

Table 2. Effect of combinations of *S. saman* + *A. vera* + *M. oleifera* on morph physiological indicators of *N. tabacum* seedlings.

	Treatments	PH (cm)	Sig.	LN	Sig	Dist/K (cm)	Sig	SD (mm)	Sig	KN	Sig
T1	A6 % M6 %	9.27	cd	4.20	c	1.44	de	3.37	a	5.20	de
T2	A6 %M4 %	11.20	b	3.60	d	1.71	c	3.13	ab	5.53	bcd
T3	A8 % M4 %	8.60	de	3.53	d	1.56	cd	3.09	ab	4.93	efg
T4	A8 % M6 %	12.39	a	4.67	bc	2.34	a	2.82	b	4.67	fg
T5	S10A6M4 %	7.77	e	4.87	ab	1.15	fg	3.39	a	5.33	cde
T6	S10A6M6 %	11.23	b	5.40	a	1.64	cd	3.32	a	6.07	a
T7	S10A8M4 %	9.83	c	5.00	ab	1.34	ef	3.27	a	5.93	ab
T8	S10A8M6 %	7.72	e	4.87	ab	1.19	f	3.12	ab	5.13	def
T9	S15A6M4 %	6.71	f	5.27	ab	0.96	g	3.02	ab	5.33	cde
T10	S15A6M6 %	9.77	c	5.20	ab	1.30	ef	3.45	a	5.93	ab
T11	S15A8M4 %	9.65	cd	5.27	ab	1.54	cd	3.28	a	4.53	g
T12	S15A8M6 %	9.97	c	4.93	ab	1.95	b	3.05	ab	4.00	h
T13	Control	9.20	cd	3.60	d	1.28	ef	3.16	ab	5.73	abc
	SE	1.872		0.521		0.700		0.285		0.385	

Lowercase letters in each column indicate significant differences, according to Duncan's test ($p \leq 0.05$). SE: Standard error Source: Own elaboration

In the number of leaves indicator, treatments T1, T2, T3, T4, and T13 (control) have significant differences with treatments T5 to T12, in the latter treatments, it is noted that although there are no significant differences between them, T6 (S10 % + A6 % + M6 %) treatment have

Revista Bio ciencias
ISSN 2007-3380

higher absolute value with 5.4 leaves. Pérez-Martín et al. (2022) when using simple extracts of *S. saman, A. vera,* and *M. oleifera* in tobacco seedlings variety "Corojo 2012" and Hernández and Jó (2022) when using different concentrations of moringa extract in tobacco seedlings variety "Corojo 2006" showed similar results.

In the distance between knots indicator, T4 (A8 % + A6 %) has the best result with 2.34 cm, showing significant differences over the control and other treatments. Treatments T2, T3, T6, T11 and T12 are superior to T13 (control) and to T5, T7, T8, T9, and T10 these latter being inferior to control.

In the stem diameter indicator, there are no significant differences, which makes this parameter more stable in its behavior in the face of the different treatments, results that coincide with Méndez et al. (2007), Terry-Alfonso et al. (2020) and Heredia-Moran (2021) in finding no variation among treatments, this indicator plays an important role in seedling quality, as it is closely related to NPK levels in the soil.

In number of knots, the best treatment was T6 with a value of 6.07 knots, which did not show significant differences with T7, T10, and T13, Hernández and Jó (2022) when applying moringa at 2 %, 3 %, and 4 % showed similar results where 5 knots obtained in 4 % moringa treatment.

In general, the best performance was obtained in treatments T4 and T6 because they increased the indicators observed in comparison with the control. Calero-Hurtado et al. (2019) when applying combined biostimulants in dark tobacco variety 'Sancti Spíritus 2006' obtained similar results. Moreover, the results obtained by applying combined extracts are superior to those obtained by Pérez-Martín et al. (2022) when only using the same extracts.

Many authors attribute the adequate vigor manifested by plants to the contribution of biostimulating substances for growth, such as cytokinins, auxins, gibberellins, amino acids, and vitamins, which allows the acceleration of plant development, mainly through the fixation of atmospheric N_2, the production of phytohormones, enzymes and nutrients mineralization (Herve, 1994; Dala et al., 2004; Khan et al., 2009; Craigie, 2011; Sharma et al., 2013; Howladar, 2014; du Jardín, 2015; Del Ángel, 2017).

The components mentioned previously are present in different extracts combined in this study according to the literature review, where the presence of zeatin in extract of *M. oleifera*; amino acids, minerals, vitamins, hormones such as auxin and gibberellin, enzymes and minerals in *A. vera* extract and secondary metabolites such as flavonoids, tannins, terpenoids, reducing sugars, alkaloids in *S. saman* extract stand out for their high content of phosphorus and potassium.

Behavior of root volume and root length with *S. saman* + *A. vera* + *M. oleifera* extracts treatments in tobacco seedlings.

Revista Bio ciencias
ISSN 2007-3380

Racey (1985) points out that root volume is an attractive criterion for estimating plant quality and predicting plant performance in field after planting, as it can be measured in bare-root and covered-root plants by non-destructive methods.

In root volume, treatments T5 and T9 stand out with 0.3453 and 0.3067 cm^3, respectively. These treatments are 54% higher than the control, this result coincides with Pérez-Martín et al. (2022) who obtained results 45% higher than the control with the application of 6% *A. vera* and 10% *S. saman* extracts. Jo-García et al. (2020) also obtained results superior to control when using *A. vera* at 6 % in plantain seedlings.

Root length is a fundamental indicator of seedling quality as it contributes significantly to the resistance of seedlings to adverse factors such as winds, in addition to increasing plant exploration capacity.

The root system performs functions related to the absorption and transport of water and nutrients, it is also responsible for anchoring and supporting the plant, and it is capable of synthesizing hormones that regulate plant growth, mainly cytokinins (Intagri S.C, 2019).

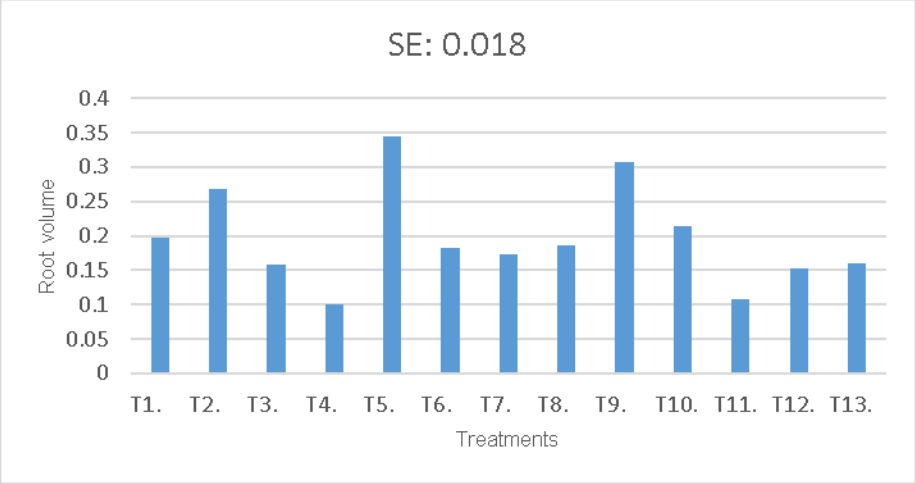

Figure 1. The behavior of the combined treatments of *A. vera* + *M. oleifera* + *S. saman* in the radical volume indicator in the seedlings of *N. tabacum*.

Source: Own elaboration

SE: Standard error

Revista Bio ciencias
ISSN 2007-3380

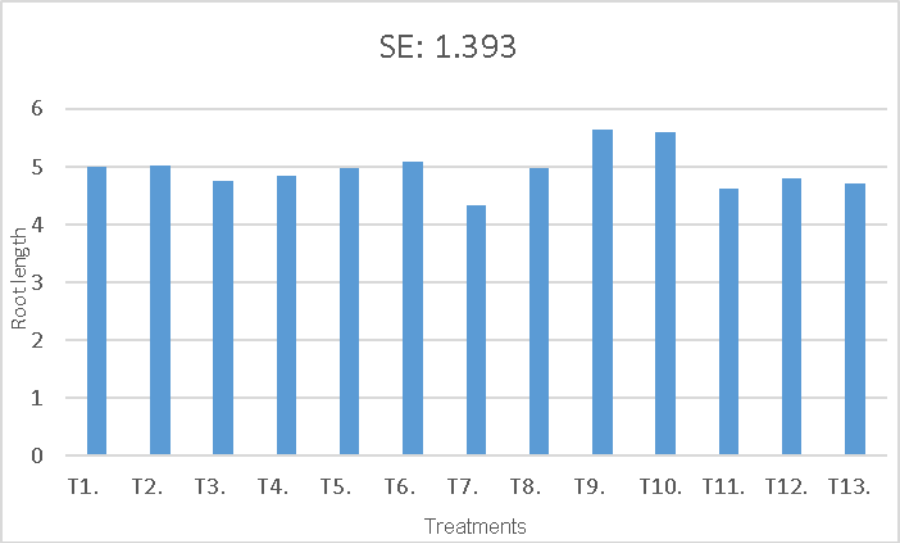

Figure 2. The behavior of treatments of *A. vera* + *M. oleifera* + *S. saman* in the long indicator of the root cm in seedlings of *N. tabacum*.

Source: Own elaboration.
SE: Standard error

In the root length indicator, no significant differences were observed between treatments, the best results were obtained with T9 (S15 %+ A6 %+ M4 %) followed by T10 (S15 %+ A6 %+ M6 %), with values of 5.64 and 5.59 cm respectively, these treatments are 16 % superior to control. We agree with Jo-García et al. (2020) who obtained the best results when applying *A. vera* at 4 and 6 % in banana vitroplants, besides, Hernandez and Jo (2022) obtained similar results to these with the use of moringa at 3 % and 4 %. These results are better than those obtained by Pérez-Martín et al. (2022) using simple extracts of *S. saman* at 10 and 15 % and *A. vera* at 6 and 8 %.

Alvarado-Aguayo and Munzón-Quintana (2020) using *A. vera* gel + yellow soil + rice use husk achieved 30 % greater root length than treatments containing a commercial rooting agent.

Rodríguez and Hechevarría (2004) evaluated the stimulating effect of different plant extracts, where *A. vera* gel extract was highlighted in relation to root formation, surpassing even the regulators traditionally used as controls, which demonstrates the possible presence of auxinic activity in it.

The increase of both root volume and root length, due to these extracts' use, in tobacco will favor the absorption of nutrients, better survival in the field at the time of transplanting, and better plant anchorage.

Revista Bio ciencias
ISSN 2007-3380

Conclusions

Treatments T4, T6, T9, and T10 show the best results in morphological variables evaluated, where they are superior to the control, evidencing a biostimulant effect on tobacco seedlings of the "Corojo 2012" variety. The combined use of plant extracts from *S. saman*, *A. vera*, and *M. oleifera* constitutes an alternative in tobacco seedling production.

Contribution of the authors

Conceptualization of the work, authors HVM, GMPM, MJG, RHG and JCMD; methodology development, authors HVM, GMPM, MJG and RHG; software management, HVM and RHG; experimental validation, authors HVM, GMPM, MJG and RHG; analysis of results, authors HVM, GMPM, MJG, RHG and JCMD. Experimental validation, HVM, GMPM, MJG and RHG; analysis of results, authors HVM, GMPM, MJG, RHG and JCMD; data management, authors HVM and RHG; writing and preparation of the manuscript, HVM and GMPM; writing, revising and editing, authors HVM and GMPM. All authors of this manuscript have read and accepted the published version of the manuscript.

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Conflict of interest

"The authors declare that they have no conflict of interest."

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