EDUCACIÓN TECNOLÓGICO NACIONAL DE MÉXICO
IPSUMTEC
ISSN: 2594 - 2905

EVALUATION OF THE SYNERGISTIC EFFECT OF CHITOSAN/ZINC OXIDE/MULTIWALL CARBON NANOTUBES/FRUCTANS ON TOMATO MORPHOMETRY (*SOLANUM LYCOPERSICUM*)

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https://doi.org/10.61117/ipsumtec.v8i2.422

Abstract -- Conventional agriculture presents multiple problems, such as leaching and chemical volatilization of the compounds used during crop development. Unfortunately, the uncontrolled abuse of these compounds has caused multiple environmental problems. This abuse has led to fertilization doses, even those that meet crop requirements, being insufficient for crop development. Therefore, the design of a formulation based on gelling compounds such as chitosan with zinc oxide (ZnO) nanoparticles, multi-walled carbon nanotubes (MWCNT), and fructans (Frut) was evaluated for use as a nanofertilizer. The overall results showed a synergistic effect between chitosan, MWCNT, and fructans, as well as remarkable morphometric values in plants treated with chitosan, MWCNT, and ZnO. The chitosan/ZnO/MWCNT/Frut formulation was expected to exhibit a strong synergy between them to enhance their original properties. However, a decrease in its effect was observed, possibly due to chemical interaction between the components, which did not provide good bioavailability for tomato plants. The treatment that showed the greatest synergistic effect in tomato plants (Solanum lycopersicum L., Moctezuma F1 variety) was reactive chitosan, MWCNT/frut, achieving a 90.6% increase in basal diameter, 76.5% in plant height, 81.25% in the number of leaves, 309.38% in fresh weight, 201.4% in dry weight, 14% in root length, and 212.69% in leaf area, compared to the control treatment.

Keywords—Chitosan, Nanofertilizer, Carbon Nanotubes, Agriculture, Fructans.

Abstract—Conventionally, agriculture involves multiple- problems, such- as the- - leaching- , and chemical volatilization of compounds used during crop development. Unfortunately, the uncontrolled abuse of these compounds has caused multiple environmental problems. This abuse has led to the fact that fertilization doses, even those that comply with crop requirements, can become insufficient for crop development. Therefore, the design of a formulation based on gelatinizing compounds such as chitosan with nanoparticles of zinc oxide (ZnO), multiwall carbon nanotubes (MWCNT), and fructans (Frut) was evaluated for its use as a nanofertilizer. Overall results showed a synergistic effect between chitosan, MWCNT, and fructans, as well as remarkable morphometric values in plants treated with chitosan, MWCNT, and ZnO. The chitosan/ZnO/MWCNT/Frut formulation was expected to have a great synergy between them in order to potentiate its original properties. However, there was a decline in its effect, which may be due to the chemical interaction between the components that could not provide good bioavailability for tomato plants. The treatment that registered the highest synergistic effect in tomato plants (Solanum lycopersicum L., variety Moctezuma F1) was reactive grade chitosan, MWCNT/frut, achieving an increase of 90.6% in basal diameter, 76.5% in plant height, 81.25% in number of sheets, 309.38% in fresh weight, 201.4% in dry weight, 14% in root length, and 212.69% in leaf area compared to the control treatment.

***Key words*–** Chitosan, Nano-fertilizer, Carbon Nanotubes, Agriculture, Fructans.

INTRODUCTION

Nanoparticles play an important role in agriculture, where their integration into everyday products is revolutionizing agricultural production, controlling plant diseases, improving crop quality, and increasing the yield of food crops [1]. They also help improve seed germination and act as antimicrobial agents [2]. Recently, there has been growing interest in exploring the potential of carbon nanotubes to influence plant development. This enthusiasm stems from their ability to penetrate plant cell walls, enabling smart delivery and nanotransport in agriculture [3]. In addition, carbon nanotubes show promise as fertilizers with a dual function: they serve as a source of slow-release nutrients and as plant growth stimulants [4, 5]. Research indicates that treating seeds with multi-walled carbon nanotubes (MWCNTs) can improve crop growth by increasing shoot and root elongation, biomass, and vigor indices after being absorbed through the roots and transported to the stem [6].

Zinc oxide nanoparticles are effective in increasing food crop yield and growth [7], addressing zinc deficiency, and improving plant resilience to environmental stress [8]. Furthermore, research suggests that zinc oxide nanoparticles can boost food crop productivity and growth by improving seed germination and preventing disease [7]. On the other hand, chitosan can induce various responses in plants depending on its structure, concentration, species, and stage of development. The first study on the stimulating effects on pea (*Pisum sativum L.)* and tomato (*S. lycopersicum L.*) plants revealed their ability to improve defense mechanisms against abiotic and biotic stress. Chitosan supplementation also triggered an initial oxidative burst and the accumulation of hydrogen peroxide (H_2O_2) in various plants and plant cell cultures [9]. In recent years, fructans have attracted attention due to their unique chemical, physical, biochemical, and technological properties [10]. Specifically, the agricultural applications of fructans are related to their widely researched functions in mitigating biotic and abiotic stress [11]. In view of the above, our objective is to obtain valuable information on the synergistic effect of chitosan, zinc oxide (ZnO), fructans, and multi-walled carbon nanotubes (MWCNT) on promoting growth, root length, height, biomass, basal diameter, and leaf area of *S. lycopersicum* plants.

DEVELOPMENT

Materials

The tomato seed used was of the Moctezuma F1 variety. This variety has early production, excellent fruit firmness and shelf life, and high yield potential.

Two types of chitosan were used: reactive grade chitosan (low molecular weight), obtained from Sigma-Aldrich Mexico, and agricultural grade chitosan, obtained from Panvo Organic Pvt. Ltd. The fructans used were obtained from Hacienda ORO DE AGAVE, Mexico. The ZnO powders used for the experiment were synthesized in our laboratory using the homogeneous precipitation method. A more detailed description of this process is illustrated in Herrera's work [12].

The multi-walled carbon nanotubes (MWCNT) were synthesized, purified, and oxidized in our laboratory using the chemical vapor deposition (CVD) method and treated with acid concentrate, respectively. This process is described in more detail in the work of Román [13].

Preparation of the chitosan solution

The chitosan solution was prepared in a 1000 ml volumetric flask. Five grams of chitosan (agricultural grade and reagent grade, respectively), 990 ml of distilled water, and 10 ml of acetic acid were added. It was then shaken for approximately 90 minutes to homogenize.

and 10 ml of acetic acid were added. It was then stirred for approximately 90 min to homogenize.

Soil preparation and sterilization

The soil used for this experiment was prepared and sterilized as follows: in accordance with the International Rules for Seed Testing 2016 (ISTA Rules 2016), sterilization was carried out in two cycles, with a 12-hour interval between the first and second cycles. The soil was sterilized in an autoclave under the following working conditions: 121 °C and 15 kPa, with constant temperature and pressure during the process. Finally, the soil was dried in an oven at 40 °C for 3 days.

Seed Treatment Protocol

Tomato seeds (S. lycopersicum, Moctezuma F1 variety) were used in all experiments. The seeds were kept at room temperature until use. Before the experiment, they were surface sterilized by a 10-minute treatment with a hypochlorite solution (10% v/v) and rinsed three times with distilled water. The flotation method was used to determine seed viability prior to sowing. After sterilization, the seeds were treated with solutions of acetic acid, chitosan, and carbon nanotubes. In the control sample, the seeds were treated with distilled water. The concentrations of each component in the solutions used in the treatment are presented in Table 1.

Table 1. *Concentrations used for each component.*

Components	Concentration
Chitosan	0.5% solution
Multi-walled nanotubes	100 mgL
Zinc oxide	10.5 mgL
Fructans	5000 mgL

Experimental design and treatments

A completely randomized experimental design was used, with a 3x4 factorial arrangement and four replicates. Table 2 describes the experimental treatments. S. lycopersicum seeds were treated with each treatment by coating and placed at a constant temperature of 25 °C without lighting.

Table 1. *Description of treatments.*

Nomenclature	Components
Control	Water
Q-R	Reactive grade chitosan
Q-A	Agricultural grade chitosan
Q-R/Fruit	Reactive grade chitosan + fructans
Q-R/MWCNT	Reagent grade chitosan + multi-walled carbon nanotubes
Q-R/ZnO	Reactive grade chitosan + zinc oxide
Q-A/Fruit	Agricultural grade chitosan + fructans
Q-A/MWCNT	Agricultural grade chitosan + multi-walled carbon nanotubes
Q-A/ZnO	Agricultural grade chitosan + zinc oxide
Q-R/MWCNT/Fruit	Reactive grade chitosan + multiple-walled carbon nanotubes + fructans
Q-R/MWCNT/ZnO	Reactive grade chitosan + multi-walled carbon nanotubes + zinc oxide zinc
Q-R/ZnO/Fruct	Reactive grade chitosan + zinc oxide + fructans
Q-A/MWCNT/Frut	Agricultural grade chitosan + multi-walled carbon nanotubes + fructans
Q-A/MWCNT/ZnO	Agricultural grade chitosan + multi-walled carbon nanotubes + zinc oxide
Q-A/ZnO/Frut	Agricultural grade chitosan + zinc oxide + fructans
Q-R/MWCNT/ZnO/Frut	Reagent grade chitosan + multi-walled carbon nanotubes + zinc oxide + fructans
Q-A/MWCNT/ZnO/Frut	Agricultural grade chitosan + multi-walled carbon nanotubes + zinc oxide + fructans

Measured parameters

The parameters measured were root length, height, basal diameter, fresh weight, dry weight, number of leaves, and leaf area of tomato plants.

Statistical analysis

The data obtained were subjected to analysis of variance. The significance of the differences was estimated and compared using the T-test with a significance level of $\alpha=0.05$ ($P \leq 0.05$). Finally, all statistical analyses were performed using SAS software version 9.4M8 (2023) and edited with OriginPro 9.

DISCUSSION AND ANALYSIS OF RESULTS

Analysis of Results

Biomass, plant growth, and morphology

Tomato seeds were treated with a series of different combinations of chitosan (reagent and agricultural grade), ZnO, multi-walled carbon nanotubes (MWCNT), and fructans (Frut) as a single or combined group. Morphometric values were higher in plants treated with Q-R/MWCNT/Frut, reaching remarkable values in both basal diameter (BD value of 1.7 cm) and plant height (PH value of 78 cm), followed by the Q-A/ZnO/Frut treatment (DB value of 1.21 cm and AP value of 75 cm) compared to the control (DB value of 0.91 cm and AP value of 44 cm). The data also reveal that the addition

Zinc oxide added to agricultural-grade chitosan shows significantly higher DB and AP values, registering 1.4 cm and 52 cm, respectively, compared to pure agricultural-grade chitosan (DB value of 1.1 cm and AP value of 44 cm). However, the addition of ZnO to reactive chitosan showed a decrease in both basal diameter and plant height (BD value of 0.83 cm and PH value of 52 cm) compared to the Q-R treatment performance (BD value of 1.19 cm and PH value of 55 cm)

ISSN: 2594 - 2905

(Figures 1b and c). Furthermore, statistical analysis shows that the Q-R treatment significantly improved ($P \leq 0.05$) root length (16 cm), compared to the control group (14 cm). However, a significant decrease was observed when incorporating fructans and zinc oxide to reactive chitosan. Root length was also significantly greater in the Q-R/MWCNT/Frut and Q-A/MWCNT/ZnO treatments, reaching 15.9 cm and 15.8 cm, respectively (Figure 1a).

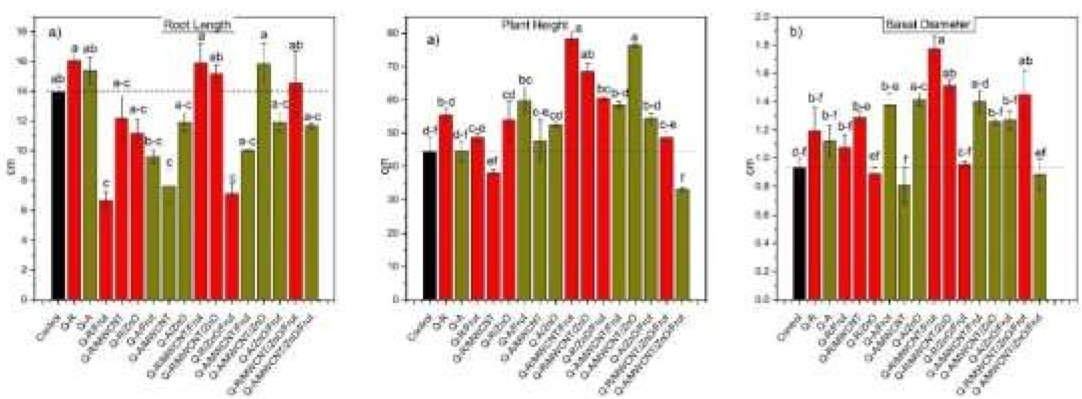

Figure 1. *Effects of various treatments on (a) root length, (b) plant height, and (c) basal diameter in tomato plants after seed coating. Each value represents the value of the triplicates, and each replicate consisted of four plant samples. Error bars represent ±SE (standard error).*

Plant biomass was measured, and statistical analyses show higher yields in plants treated with Q-R/MWCNT/Fruit (fresh weight of 0.35 g and dry weight of 0.030 g), followed by treatment with Q-A/MWCNT/ZnO (fresh weight of 0.28 g and dry weight of 0.032 g). However, compared to the control treatment (fresh weight of 0.06 g and dry weight of 0.010 g), the Q-A/MWCNT/ZnO/Fruit combination caused a significant decrease in both fresh weight (0.05 g) and dry weight (0.005 g) (Figures 2a and b). Morphological parameters such as the number of leaves and leaf area were also measured, and the data reveal that the Q-A/MWCNT/ZnO treatment achieved the maximum values for both the number of leaves (6.5 leaves) and leaf area (65 cm^2), followed by the Q-A/Fruit treatment (6.2 leaves and a leaf area of 62 cm^2). However, leaf area and number of leaves were insignificantly affected by plants treated with Q-A/MWCNT/ZnO/Fruit compared to the control (Figs. 3a and b).

Discussions

The objective of this study was to reveal the interactive effects between chitosan (reactive grade and agricultural grade), ZnO, multi-walled carbon nanotubes (MWCNT), and fructans (Frut) as individual or combined groups on the growth of S. lycopersicum. Although there are few reports available that evaluate the synergistic effect between chitosan/zinc oxide and carbon nanotubes/zinc oxide [14], to date, there are no studies available that evaluate the interactive effects between chitosan, zinc oxide, and multi-walled carbon nanotubes, let alone the effects of fructans on plant growth. Therefore, the present study is a novel study of nanofertilizers from the proposal of chitosan, zinc oxide, multi-walled carbon nanotubes, and fructans.

ISSN: 2594 - 2905

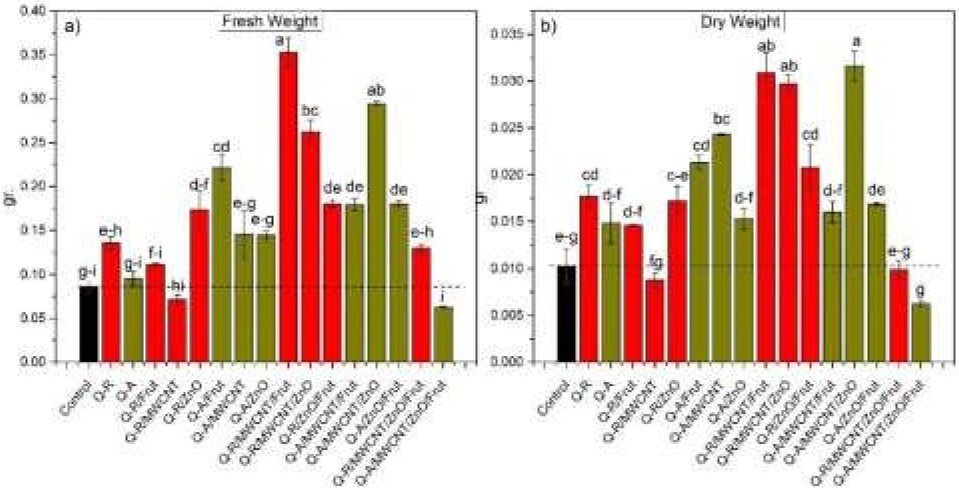

Figure 2. *Study of plant biomass under different treatments (a) Fresh weight, (b) Dry weight. Each value corresponds to the value of triplicates, and each replicate consisted of four plant samples. Error bars represent ±SE (standard error).*

Carbon nanotubes have been shown to have both positive and negative effects on the growth of seedlings of higher plant species. Carbon nanotubes reportedly increased the germination rate and improved the growth of tomato seedlings [15]. However, no impact of carbon nanotubes on the development and physiology of wheat and rapeseed plants was observed [16]. In another study, carbon nanotubes had a negative impact on the growth of A. thaliana [17]. Furthermore, carbon nanotubes have been shown to improve seed germination rates through various mechanisms. First, carbon nanotubes can improve water absorption and retention, thus creating a favorable microenvironment for germination [18]. Carbon nanotubes also stimulate plant hormonal activity, promoting the release of seed dormancy and accelerating germination [15]. In this study, we observed that most treatments, including carbon nanotubes, had a stimulating effect on basal diameter, height, number of leaves, leaf area, and plant biomass. However, in some cases, the inclusion of carbon nanotubes caused a decrease in root length, as observed in plants treated with Q-R/MWCNT and Q-A/MWCNT. All these discrepancies may be due to carbon nanotube agglomeration, root surface modifications, or species-specific responses [15, 19]. In addition, carbon nanotubes may increase the accumulation of contaminants, which could explain the greater synergistic effect.

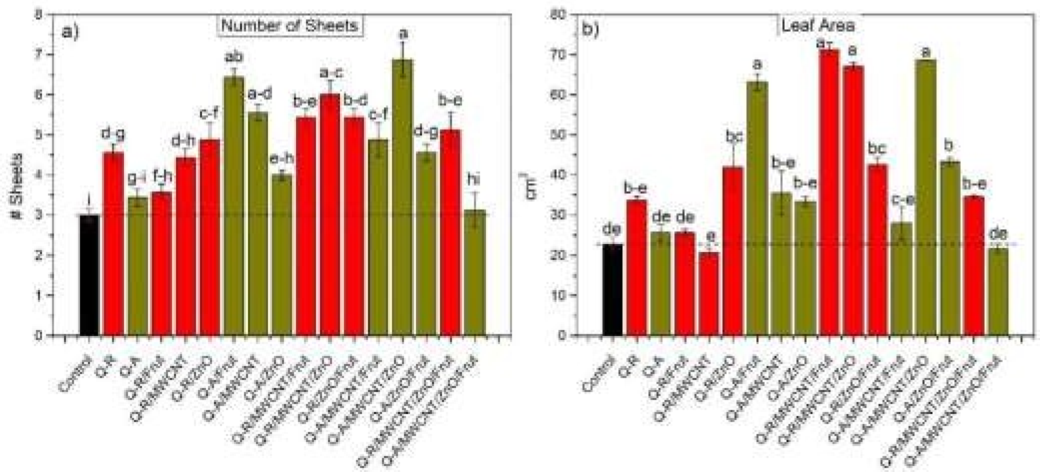

Figure 3. *Morphological study of plants under different treatments (a) Number of leaves and (b) Leaf area. Each value is the mean of triplicates, and each replicate consisted of four plant samples. Error bars represent ±SE (standard error).*

Similarly, chitosan has shown several properties that can be applied in horticultural crops, particularly as a plant root growth enhancer; however, these properties depend mainly on its molecular weight [20]. Some reports have emphasized the efficiency of low molecular weight chitosan in plant development. For example, when coated with low molecular weight chitosan, rapeseed (Brassica chinensis) exhibited favorable germination rate, seedling growth, and root length. Furthermore, Mahdavi and Rahimi [21] reported that the germination percentage, germination rate, and seedling vigor index were significantly affected by pretreating the seeds with chitosan. However, it has also been reported that the grade type of chitosan also plays a critical role in plant growth parameters. In this context, chitosan oligosaccharides are considered a broad-spectrum plant growth promoter; the application of agricultural-grade chitosan increased both the height (33.5%) of coffee seedlings and biomass yield (9.2%) [22].

Another study indicated that treatments with solutions of chitosan from grade agricultural significantly promoted significantly promoted the growth rate of plant height in Greek oregano; however, there was no significant effect on fresh matter or yield of the aerial parts of oregano [23]. With regard to reagent-grade chitosan, studies suggest that it has a significant growth-promoting effect on some parameters such as plant height, root length, number of roots, and stem diameter [24].

Our results showed better performance in plant growth parameters when using reactive-grade chitosan compared to treatments with agricultural-grade chitosan. For example, the treatment with reactive-grade chitosan showed a significant improvement in root length, plant length, and basal diameter. In addition, it showed a greater synergistic effect in combination with carbon nanotubes. This is because this type of chitosan undergoes a chemical modification process to increase its solubility and reactivity, allowing it to form covalent bonds with other compounds, such as nutrients and phytohormones, facilitating its absorption by plants. On the other hand, agricultural-grade chitosan is less processed and may contain impurities such as proteins and salts; these impurities can limit its biological activity and its effect in the early stages of plant growth. However, the potential of agricultural-grade chitosan as a root development stimulator, as well as for improving soil structure and the stages of development and production, should not be underestimated.

Zinc is an essential micronutrient for plants, and its availability can significantly influence their growth and biomass production. ZnO, as a source of zinc, plays a vital role in plant metabolism and development. It has been reported to increase fresh and dry weight in plants such as carrots, rice, and tomatoes [25, 26]. The beneficial effects of ZnO on biomass could be related to the positive effect of zinc on mineral balance and improved nutrient supply to the plant, thanks to its involvement in chlorophyll synthesis, enzyme activation, hormone regulation, nutrient absorption, and oxidative stress modulation. In line with previous findings, the results of this study indicated that most ZnO treatments improved the biomass of tomato plants, as well as their total height.

On the other hand, approximately 15% of flowering plants use fructans as reserve carbohydrates [27]. Various plant families store photosynthetic products such as fructans in their vacuoles, rather than starch in plastids. This adaptation facilitates their survival strategies in the habitat [28]. In this regard, Hendry and Wallace [29] recognized that fructans offer protection against plant stress. They suggested that regions characterized by arid temperatures and climates had an abundance of fructan-rich plants, while tropical areas lacked them entirely. Since then, fructans have been widely recognized for their ability to provide plants with tolerance to stress. biotic and abiotic [11, 30]. In this context, the present study revealed that the incorporation of fructans favored the plant's defenses. Specifically, the greatest response was obtained in plants treated with Q-R/MWCNT/Frut, which, as can be seen when analyzing the dry weight, this treatment recorded the highest amount of losses. This phenomenon could be due to the fact that plants release most of their reserves to survive under stressful conditions, and since fructans are widely recognized as a major source of reserves in plants, it is reasonable to suggest that fructans help plants regulate their dynamism to avoid extreme conditions. However, there is a tendency for fructans to negatively affect plant growth, as illustrated by the decrease in plant root length when treated with Q-A/Frut and Q-R/Frut. This limitation could be due to the fact that both are considered sources of energy and nutrient reserves, since fructans are mainly used by microorganisms in the soil, both restrict their beneficial actions on each other once they interact with the soil. However, a positive effect on plant growth can be achieved with the inclusion of carbon nanotubes since, in this case, chitosan and fructans act as a channel that promotes the efficient and balanced distribution of nutrients, allowing tomato seedlings to enhance their root vigor.

CONCLUSIONS

In this study, it was demonstrated that the chitosan/MWCNT/Frut and chitosan/MWCNT/ZnO combinations have a synergistic effect on seed germination, root establishment, nutrient uptake, and overall plant performance. The combined effects included improvements in growth parameters such as plant height, root length, biomass, leaf area, and basal plant diameter. However, in the chitosan/ZnO/MWCNT/Frut combination, the synergistic effect was reduced; this could be because the chemical interaction between the components does not provide good bioavailability for plants. Nevertheless, further research is needed to determine optimal concentrations, chemical interactions, release, application methods, and potential environmental impacts.

Future lines of work could focus on:

*Optimizing the concentrations and proportions of chitosan, MWCNT, ZnO, and Frut to maximize the synergistic effects found in plant growth variables. This requires designing controlled experimental studies to define the best formulation conditions and evaluate the physiological response in plants and the bioavailability of nutrients in different soils. In this way, standardized protocols could be developed for reproducible and efficient application in agricultural systems.

*In addition, it is important to establish a line of research to evaluate the environmental and toxicological effects of mixing these materials. These studies should consider the controlled release of compounds, the persistence and mobility of nanomaterials in the soil, and their interaction with the microbiota and the nutrient cycle. At the same time, it is advisable to develop field trials to verify the safety, efficacy, and sustainability of these formulations and thus develop innovative agricultural strategies with low environmental risk.

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Table of Contributing Roles

Roles	Author(s)
Conceptualization	Castellano-Castellanos Rigoberto S, Román-Doval Ramón. (Degree of contribution, equal)
Methodology	Castellano-Castellanos Rigoberto S, Cortés-Martínez Carlos I. (Degree of contribution, equal)
Formal analysis	Cortés-Martínez Carlos I., Lopez-Lopez Guillermo (Degree of contribution, equal)
Writing – original draft	Castellano-Castellanos Rigoberto S, Navarro-Ibarra Diana C. (Degree of contribution, equal)
Writing – review and editing	Cortés-Martínez Carlos I., Lopez-Lopez Guillermo, Román-Doval Ramón (Degree of contribution, equal)
Supervision	Lopez-Lopez Guillermo, Román-Doval Ramón (Equal contribution)
Project management	Román-Doval Ramón, Navarro-Ibarra Diana C. (Degree of contribution, equal)
Data visualization	Román-Doval Ramón