

Evaluation of adherents to coat maize seeds (*Zea mays* L.) with vegetable flours and their effect on germination

Ayala-Contreras, Carmen A.¹; Mancera-Rico, Arturo²; Sariñana-Aldaco, Oscar¹; Benavides-Mendoza, Adalberto²; Peralta-Manjarrez, Rocío M.²; Rivas-García, Tomas⁴; Sandoval-Rangel, Alberto^{2*}

¹ Secretaría de Ciencia, Humanidades, Tecnología e Innovación-Universidad Autónoma Agraria Antonio Narro. Buenavista, Saltillo, Coahuila, México. C. P. 25315.

² Universidad Autónoma Agraria Antonio Narro. Buenavista, Saltillo, Coahuila, México. C. P. 25315.

³ Centro de Investigación y de Estudios Avanzados- Saltillo. Ramos Arizpe, Coahuila, México. C. P. 25900.

⁴ Secretaría de Ciencia, Humanidades, Tecnología e Innovación-Universidad Autónoma Chapingo. Texcoco, Estado de México, México. C. P. 56230.

* Correspondence: asandovalr16@gmail.com

ABSTRACT

Objective: to evaluate the adhesion capacity of different materials in the coating of seeds with vegetable flours from broccoli shears as biostimulant extracts.

Design/Methodology/Approach: the percentage of seed coverage treated with different adherents was evaluated; commercial, polyterpene, carboxymethyl, starch and casein, previously standardized for application in doses of 1 mL in 100 seeds. The percentage of germination, growth and some biochemical variables of the seeds treated with adherents with and without vegetable flour was evaluated, with the application of 1.5 g in 100 seeds.

Results: the adherents with the highest coverage were commercial and polyterpene, with coverage percentages higher than 98%. Germination was not modified by the treatments. There were non-significant differences in the other variables in relation to treatments without HV. Except the application of HV that reduced plumule length by 7%, and fresh weight by 12%. In biochemical variables, the application of adherent and vegetable flours favored increasing in the content of phenols, total proteins and pigments.

Limitations/Implications of the study: in regard to adherents, similar results were found for most variables; this is, there were non-significant differences among adherents. Thus suggesting that some variations can be introduced in the composition or concentrations of adherents in order to amplify the test.

Findings/Conclusions: the information from this study can serve as a model for applying vegetable flours as bio-stimulant extracts in seeds or plants, with a sustainable approach.

Keywords: bio-stimulants, seed coating, germination, bio-compounds.

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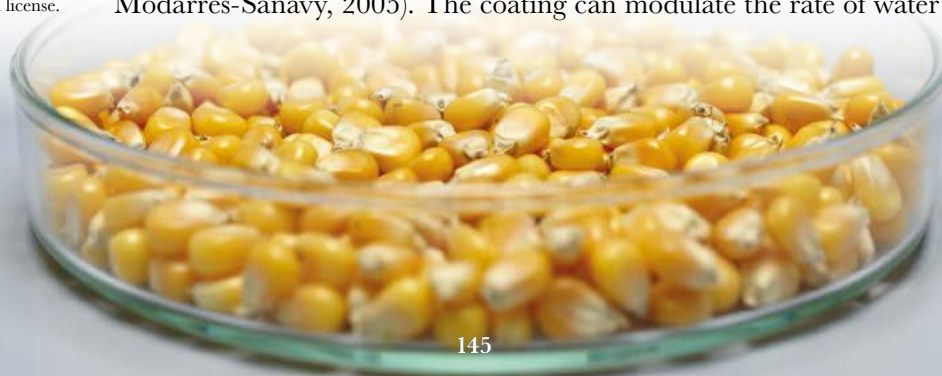
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INTRODUCTION

Seed coating is a technique that consists of covering the surface with materials that protect and improve the germination and initial development of the seedling (Ehsanfar & Modarres-Sanavy, 2005). The coating can modulate the rate of water absorption and gas



exchange; promote metabolic and enzymatic activation; protect the seed from pathogens; facilitate mechanical manipulation; and mainly, coating can act as a carrier of compounds that stimulate or protect the seedling (Su *et al.*, 2017). In addition, the size, shape, weight and texture of the seeds can be modified.

In seed coating techniques, a layer of adherent material is applied that acts as a vehicle for the adhesion of the materials. This allows incorporating not only nutrients and chemical protectants, but also bio-inputs of plant origin, such as extracts, vegetable flours, essential oils or beneficial microorganisms, in a direct and localized way (Piri *et al.*, 2019). The adhesion and homogeneous distribution on the seeds are determined by the physical and chemical characteristics of the adherent, as well as by its concentration. These adherents, depending on the purpose of the coating, are related to the improvement in water retention capacity; they are easy to degrade, provide adhesion strength and compatibility with nutrients and other additives (Montoya *et al.*, 2021).

In sustainable agriculture, vegetable flours and plant extracts are a source of bioactive compounds with biostimulant and biofungicidal potential (Sanfilippo & Patti, 2022). On the other hand, vegetable flours can also serve as soil improvers, biofertilizers, and a source of carbon (Arámbula-Castillo *et al.*, 2023). Species of the genus *Brassica*, such as broccoli (*Brassica oleracea* var. *italica*), contain secondary metabolites such as glucosinolates, isothiocyanates and phenols in their tissues, which have shown activity in stimulating plant growth, defending against pests and diseases, and improving nutrients absorption (Oulad El Majdoub *et al.*, 2020). In particular, flour obtained from dried and ground broccoli leaves represents a stable, easy-to-store and easy-to-apply form that retains a good part of these functional compounds (Waisen *et al.*, 2022; Peñas de la Corte, 2025).

That is why, the objective in this study was to evaluate the adhesion capacity of different materials in the coating of seeds with vegetable flours made of broccoli shears as biostimulant extracts.

MATERIALS AND METHODS

This study was done in the Department of Horticulture of the Autonomous Agrarian University Antonio Narro (UAAAN, Coahuila, Mexico), in the plant physiology laboratory. Seeds of the maize variety Tuxpeno were used; with various adherents in different concentration that were standardized before being applied to the seeds (Table 1). Distilled water was used as the zero control (T0) and a commercial adherent (pegoDel™, AgroDelta, Mexico) as a commercial control (COM). For the application of treatments, the indications on the label was used (Table 1).

Broccoli shears were used, leaves dried at ambient temperature, macerated and sifted in mesh number 100, in the form of vegetable flour (HV) as a biostimulant extract; of which we analyzed and characterized its biochemical composition (Table 2).

For conditioning, 200 maize seeds were used per treatment, previously disinfected with 5% sodium hypochlorite and dried in the environment. The application dose of the adherents was 1 mL in 100 seeds.

Table 1. Standardization of adherents.

Adherent	Concentration (%)	Temperature (°C)	Agitation (minutes)
T0	100	Ambiente	1
COM	100	Ambiente	1
P100	100	Ambiente	5
P75	75		
C0.5	0.5	45	20
C0.25	0.25		
AL	20	50	30
CS	20	45	40

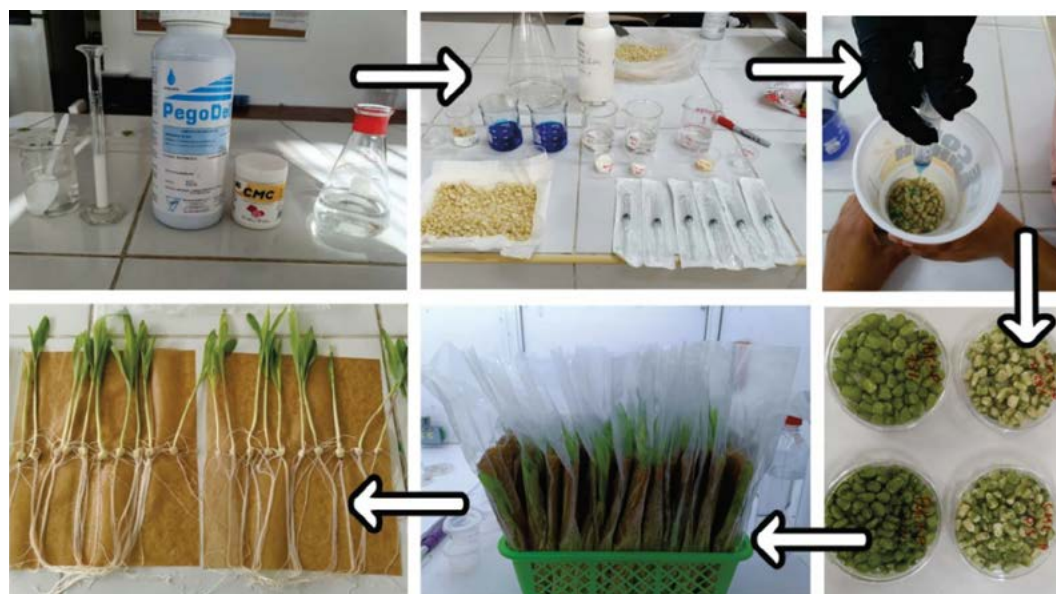
T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein).

Table 2. Biochemical characterization of vegetable flour (HV) applied to seeds as biostimulant extracts.

Total Phenols mg GAE 100 g ⁻¹	Flavonoids mg CE 100 g ⁻¹	Glutathione mg 100 g ⁻¹	Antioxidant capacity mg AAE100 g ⁻¹	Total proteins mg AE 100 g ⁻¹ DW
501.157	7.040	498.134	40.44	882.928

GAEs: gallic acid equivalents; CE: catechin equivalents; AAE: azinobis acid equivalents; AE: albumin equivalents; DW: dry weight.

They were shaken for 1 minute and allowed to dry for 5 minutes. Then, 1.5 g of HV was added, to those treatments that included it; and mixed slowly with circular movements until homogenized (Figure 1).

**Figure 1.** Process of preparation, application and evaluation of treatments on maize seeds.

Seed coating variables

Coverage percentage. Photographs were taken with a (Nikon Coolpix L23) digital camera coupled to a (NOVA XTD 20) stereoscope. The coverage percentage (based on surface area) was determined using Image-Pro Plus version 6.0 (Media Cybernetics, Rockville, MD, USA).

Coating structure. A (JEOL JSM-IT510, InTouchScope) scanning electron microscope (SEM) was used with an integrated (Philips) digital camera. The samples were coated with Palladium by means of sputtering equipment (Sputter Coater LITH-SD80).

Physiological variables

Germination. Twenty-five seeds were sown on Anchor paper sheets with 4 replicates, placed in plastic bags in a semi-automatic germination chamber at a temperature of 28 °C for 7 days. The results are expressed as a germination percentage.

Length of plumule and radicle. The plumule was measured from the coleoptyl to the apex of the primary leaf with a digital vernier (Caliper, 0.001"). The radicle was measured from the junction with the coleoptyl to the tip of the taproot.

Fresh and dry matter. The endosperm was separated in 10 seedlings to assemble the experimental unit, with four replicates, they were weighed on an analytical balance (OHAUS). Then, those were dried in an oven (Mettler UF75) at a temperature of 75 °C for 72 hours, afterwards they were weighed.

Biochemical variables in seedlings

Frozen samples (Thermo Scientific-40086FA) and lyophilized samples (Labconco FreeZone) were used to determine biochemical variables.

Pigments. Determined as proposed by (Wellburn, 1994), 30 mg of the sample with 2000 μ L of pure methanol. Quantification was performed in a UV-Vis spectrophotometer (Genesis 10s Uv-Vis, Thermo Scientific, USA) at 666, 653 and 470 nm.

Phenols. Determined by colorimetry with the Folin Ciocalteu method proposed by Singleton *et al.* (1999); 10 mg of the sample with 1000 μ L of pure methanol for extraction. Absorbance was measured at 760 nm.

Glutathione. Determined according to Xue *et al.* (2001); DTNB (5,5-dithiobis-2 benzoic nitro acid) was used for the staining reaction. Absorbance was measured at 412 nm.

Flavonoids. Determined according to Zhishen *et al.* (1999); in a 100-mg sample with 2 mL of pure methanol for extraction. Absorbance was measured at 510 nm.

Total proteins. Determined by Bradford spectrophotometric; in 100 mg of the sample and 10 mg of polyvinylpyrrolidone (PVP) with 2 mL of 0.1 M phosphate buffer (pH 7-7.2). Absorbance was measured at 595 nm.

Antioxidant compounds. Determined according to Re *et al.* (1999), with the discoloration test of the radical cation ABTS [2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]. Absorbance was measured at 754 nm.

Experimental design

A completely randomized design with factorial arrangement was used. Factor 1 was the type of adherent and Factor 2, HV added or not-added. A Pearson correlation analysis was performed for physiological and growth variables, and a univariate analysis for the other variables. We arranged 10 seedlings as the experimental unit with four replicates per treatment. Once the assumptions of normality and homogeneity were met, an analysis of variance and the Fisher mean test ($p \leq 0.05$) were performed. All statistical procedures were performed with Statistica[®] v8.0 (TIBCO Software, Palo Alto, USA) and the graphs were generated with SigmaPlot[®] (v12.0).

RESULTS AND DISCUSSION

Seed coating with adherents and vegetable flour

The adherents that had the highest adherence capacity were those with vegetable flour (HV) that showed the highest coverage percentage on the seeds (Figure 2). The adherents Polyterpene, at concentrations 100% and 75%, and the commercial COM were the highest, with coverage percentages greater than 98% of the total surface area of the seed ($p \leq 0.05$). The adherents with the lowest coverage percentage were the zero control (9.44%), carboxymethyl in the two tested concentrations (5.56%), maize starch (9.90%) and casein (2.69%).

The comparison is shown of an uncoated seed with seeds treated with adherents and HV (Figure 3). There, the statistical results of coverage percentage discussed above (Figure 2) are visually confirmed. This information is of great importance since it allows us to make a selection of the adherents that can be used most effectively, when conditioning seeds with products in the form of flours or powders. Using the correct adherent in seed conditioning minimizes product losses during application, which makes the process more efficient and reduces costs (Job *et al.*, 2018).

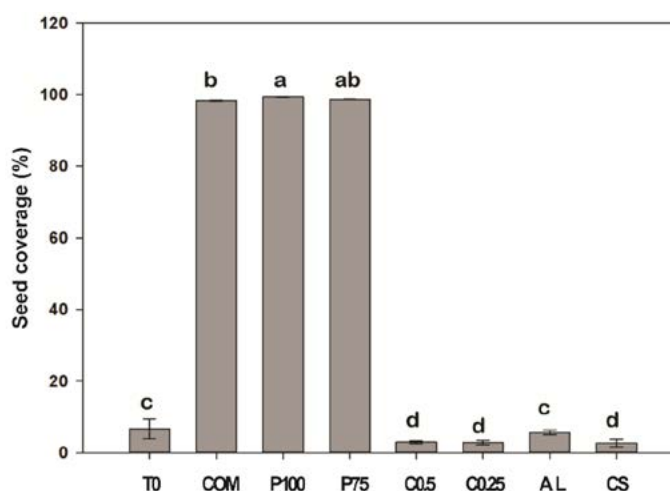


Figure 2. Coverage percentage of maize seeds conditioned with the different adherents and vegetable flour (HV). Different letters indicate statistical difference (Fisher, $p \leq 0.05$). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein).

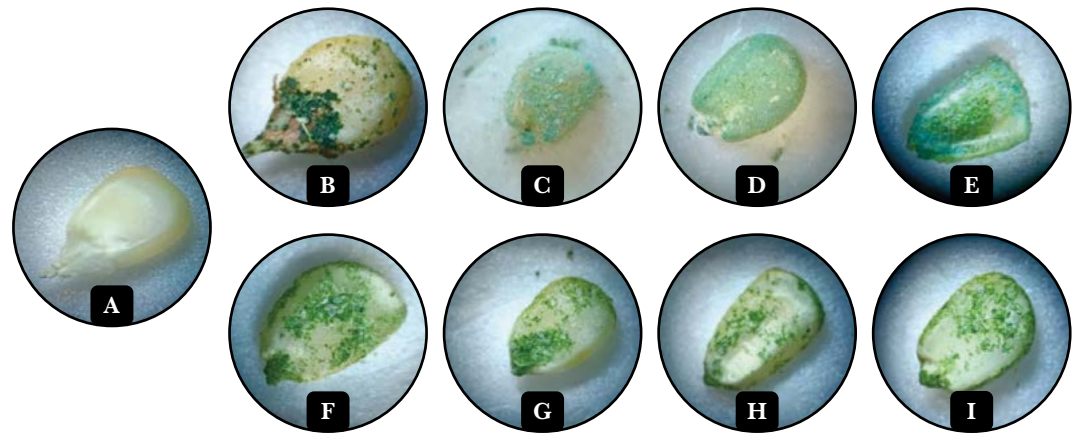


Figure 3. Maize seeds conditioned with the different adherents and vegetable flour (HV). A: unconditioned seed; B: zero control (water); C: commercial adherent; D: 100% polyterpene; E: 75% polyterpene; F: 0.5% carboxymethyl; G: carboxymethyl 0.25%, H: maize starch; I: Casein.

Figure 4 shows the images captured under a scanning electron microscope (SEM) of the surface of the seeds coated with the different adherents and their distribution. The commercial adherent was the most homogeneous (Figure 4A), followed by the polyterpene in its two concentrations (Figure 4B). Those were different from carboxymethyl-cellulose, maize starch, and casein (Figure 4C, Figure 4D and Figure 4E), which showed a low presence of the adherent.

Although the seed surface distribution of the polyterpene was visibly lower compared to the commercial adherent, the latter was statistically lower than the 100% polyterpene, but equal to the 75% polyterpene concentration. The characteristics that must be met may be related to physical processes, such as fixing the material of interest without it being detached during handling or storage, it must not be toxic so that physiological processes are carried out properly, it must not react with the compounds of interest that are applied, so that they do not interfere with biostimulant activity (Zhang *et al.*, 2022).

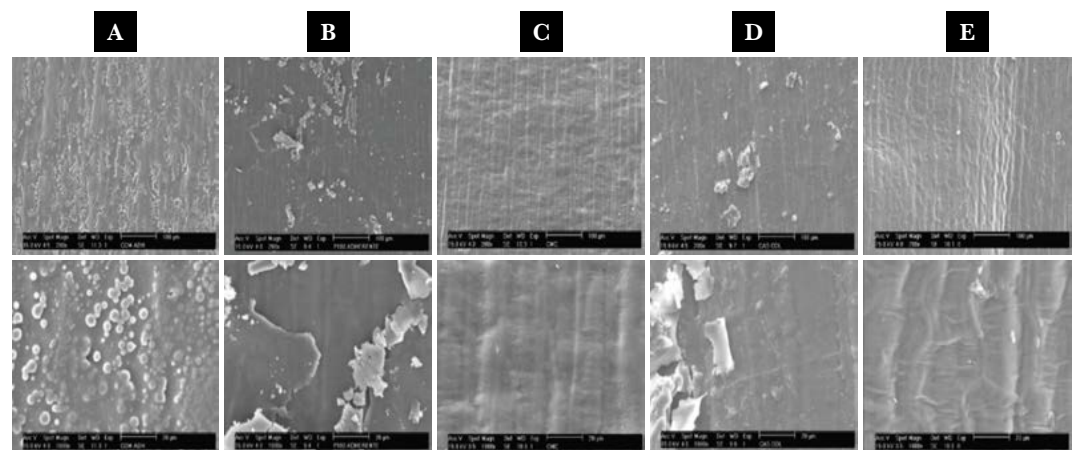


Figure 4. Views through scanning electron microscopy (SEM) of the coatings, with lenses 200X (top images) and 1000X (bottom images), on the surface of the seeds, with the different adherents. A: commercial adherent; B: polyterpene; C: carboxymethyl; D: maize starch; E: casein.

The SEM visualizations of the seed conditioned with the commercial adherent and the vegetable flour are presented (Figure 5). We can observe a homogeneous coating on the surface of the seed (Figure 5, A1), and when making a cross-section of the seed, it is observed how the HV forms volumes of different sizes on the surface (Figure 5, A2). The same is true for seeds conditioned with 100% Polyterpene (Figure 5, B1 and B2). An adequate coating with the biostimulant facilitates to handle the seed and allows an adequate release in the immediate environment to germination. The choice of adherent depends on the ability to keep these products attached to the surface of the seed (Sohail *et al.*, 2022).

Physiological and biochemical indicators

There were non-significant differences in the germination percentage between seed lots treated with HV or without HV (Figure 6), which indicates that germination was not influenced by the application of HV. Regarding only the adherent factors, there were statistical differences ($p \leq 0.05$) in the treatment with maize starch without HV ($86\% \pm 15$) which was the lowest and similar to the commercial adherent ($92\% \pm 5$), compared to the others, polyterpene ($93\% \pm 9$) and casein ($94\% \pm 5$) with HV. In regard to the treatments with adherents with HV (control, carboxymethyl and casein) and without HV (control, polyterpene, carboxymethyl and casein), the germination percentages were higher than 99%, with non-significant difference among them.

In their study Gorim & Asch (2012) determined that there is a strong influence of seed coat thickness and composition on the germination rate and mobilization efficiency of reserves stored in the endosperm. Those authors used hydrophilic adherents with humic acid and biplanto (a commercial biostimulant); with those the coating significantly reduced

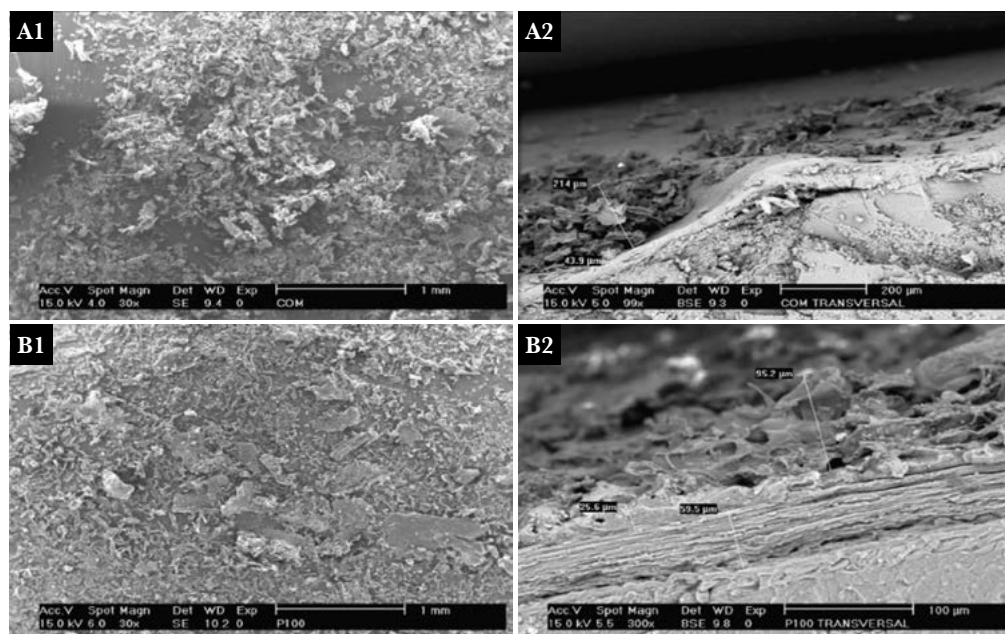


Figure 5. Surface area of maize seeds viewed in a scanning electron microscope (SEM). A1: commercial adherent with HV; B1: 100% polyterpene with HV [both, 30X objective lens]. Cross-sectional views of seeds with A2: commercial adherent plus HV and B2: 100% polyterpene plus HV [99X and 300X objective lens].

the total germination of barley, rye and wheat, compared to uncoated seeds. Germination is a useful parameter to measure the effect of a product with biostimulant action, since this latter either can or cannot improve the metabolic processes in the initial stages of the seedling (Kola & Carvalho, 2023).

In this study, the application of HV as biostimulant extract did not have an effect on germination. However, when evaluated together with the different adherents, it was observed that the adherents with the highest percentage of coverage (Figure 2) were statistically equal to the lowest value of germination without HV of the maize starch (Figure 6). The reduction in the percentage of germination with this latter treatment may be due to its physical properties, since it forms a thick layer that can act as a physical barrier that hinders the absorption of water and gas exchange, which delays or prevents imbibition in seeds.

The combined effect of maize seed coating with the different adherents and the application or not of HV on plumule length and radicle length is presented in Figure 7. Statistically significant differences ($p \leq 0.05$) were observed, between applying or not applying HV, in the plumule length, but not in radicle length (Figure 7). Plumule growth was reduced by 7% in HV treatments compared to non-HV treatments. These results suggest that the biostimulant activity of HV was not positive on the initial development of the seedling. On the other hand, type of adherent did have a variable behavior with or without HV (Figure 7).

In the variable plumule length, the zero control with HV presented the lowest values compared to most treatments with or without HV. Although it was statistically equal ($p > 0.05$) to the maize starch adherent (with or without HV), and to the commercial adherent with HV. Regarding the radicle length variable, most of the adherent treatments

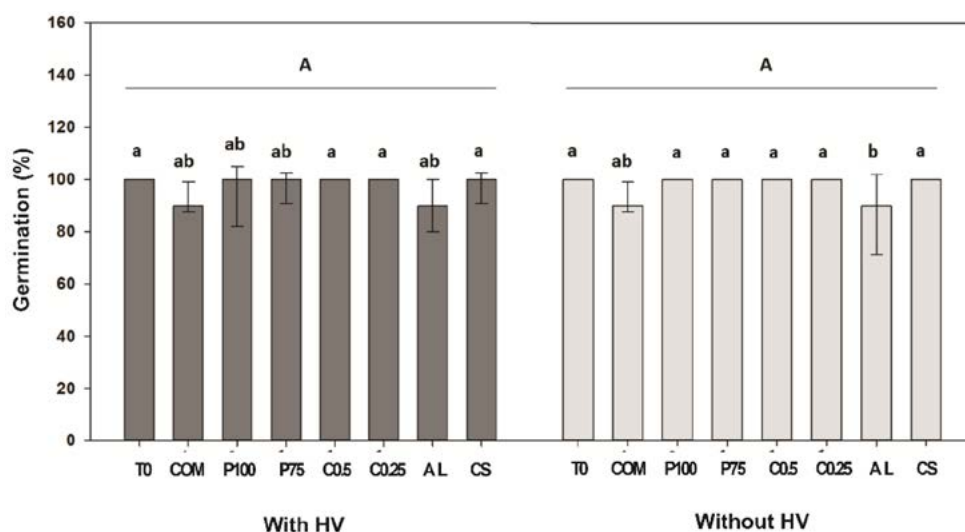


Figure 6. Germination percentage in maize seeds conditioned with different adherents and vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

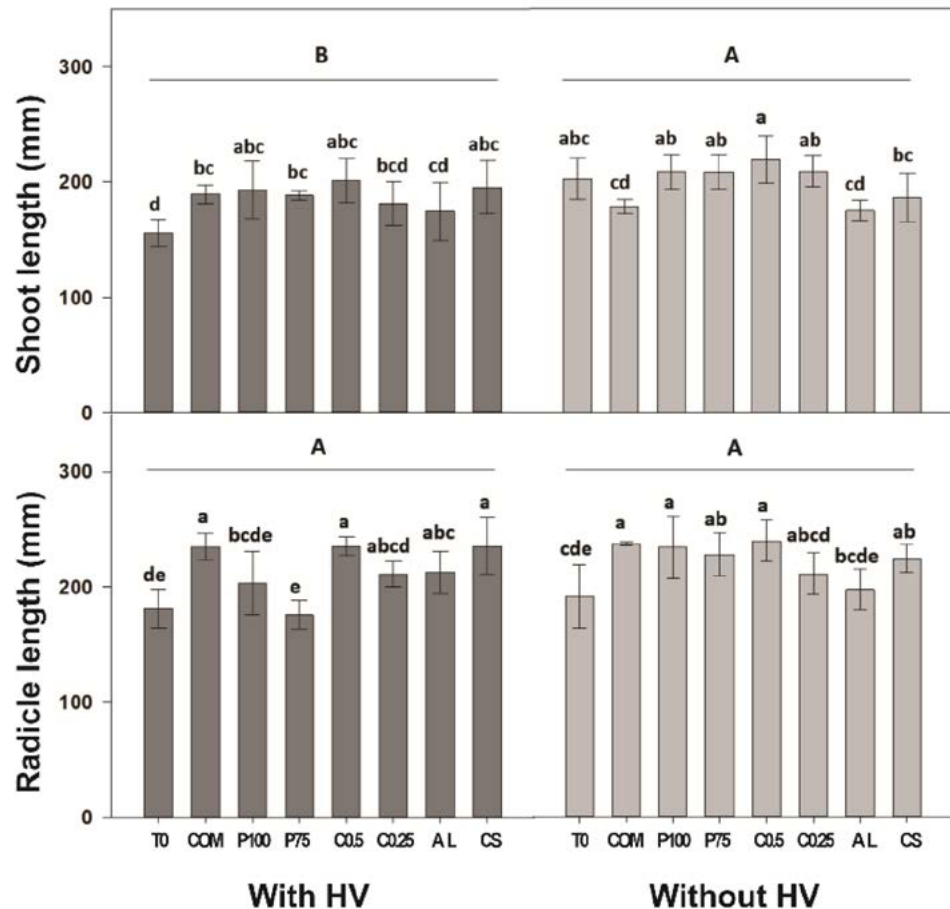


Figure 7. Plumule length and radicle length of maize seedlings with different adherent treatments with or without vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

(with or without HV) were statistically equal, except the zero control and polyterpene in its two concentrations with HV. This is of great importance since the greatest coverage with HV was with commercial adherent and polyterpene (Figure 2). These same treatments without HV showed the highest values in radicle length.

Results indicated that seeds with greater coverage percentage and with HV had a similar development in plumule length, compared to seeds without HV. In contrast, regarding radicle length, the lowest values were present in seeds treated with HV, compared to treatments without HV. This, although there were non-significant differences ($p > 0.05$), indicates there could be an effect between the adherent and the HV. Our results coincide with those obtained by Qiu *et al.* (2020), who evaluated the seed coating with a mixture of soybean flour, diatomaceous earth, and vermicompost extracts, obtaining positive results in germination rate and growth of red clover plants, but a reduction on these same variables in seeds of perennial grass (Ray grass).

In a study developed by Qiu *et al.* (2020), those authors proposed a method for the application of bio-based biostimulants as seed coatings. Those authors used different sources of liquid and powder forms of vermicompost and soybean flour, and the formulations did not decrease germination parameters and were non-significantly different from those of the control. The efficiency of biostimulants, applied as a coating on seeds, may be influenced by the adherent material. These may or may not favor the solubility and release of the bioactive compounds present in biostimulants, moreover, adherents can even interfere with the biostimulant activity (Ayed *et al.*, 2022). As an example, adherents with hydrophilic properties can promote the release of the compounds during imbibition, whereas more compact or hydrophobic adherents can delay that process (Tweddell *et al.*, 2000).

On the other hand, the use of biostimulant extracts can modulate different physiological and metabolic processes since they contain biocompounds such as phenols and flavonoids. Therefore, it is important to consider the application doses of those biocompounds, because in high doses they can generate toxicity (Van Duijn, 2024).

Figure 8 shows fresh weight and dry weight of the maize seedlings treated with seed coatings. It can be observed that there were significant differences ($p \leq 0.05$) in fresh weight, but there were non-significant differences in dry weight with or without HV (Figure 8). The application of HV reduced fresh weight by 12.02% in relation to treatment without HV. As for adherents, the highest values were found in fresh weight with adherents without HV, although statistically equal with only the maize starch adherent with HV (Figure 8). The opposite case was observed in dry weight; the highest value (maize starch $102.34 \text{ mg} \pm 4.34$) and the lowest value (casein $56.83 \text{ mg} \pm 9.43$) were found with adherents plus HV.

Fresh weight and dry weight in seedlings are important indicators of water processes and physiological development, because they are related to structural biomass and to the yield as growth of functional structures. The application of biostimulants has a positive effect, in a generalized way, on fresh and dry biomass, this effect is closely related to the application dose. In a study where the application of a plant-based biostimulant in forest seeds was evaluated, positive results were obtained in some variables, such as biomass and photosynthetic capacity (Huang *et al.*, 2019). Overall, these results highlight the importance of considering carefully the product to be used, the application dose, and the interaction with the adherent selected to apply to seeds.

Regarding the results obtained in the biochemical variables, those only highlighted the seedlings from seeds treated with the adherents that had the highest coverage percentage (Figure 2) and coated with HV. Table 3 presents the results obtained, where we can observe statistically significant differences ($p \leq 0.05$) in the content of phenols, total proteins and pigments, but not in glutathione or flavonoids contents. In the case of phenols, the treatment with commercial adherent and 100% polyterpene had a higher content (46.87% and 54.41%), compared to the control with HV; also 20.25% and 26.42% more compared to the control without HV. For total proteins, the commercial adherent had the highest values, and was statistically equal to the control without HV; indicating that the increase cannot be attributed to HV application. In pigments (chlorophylls and carotenoids) the control without HV had the lowest contents in relation to HV treatments.

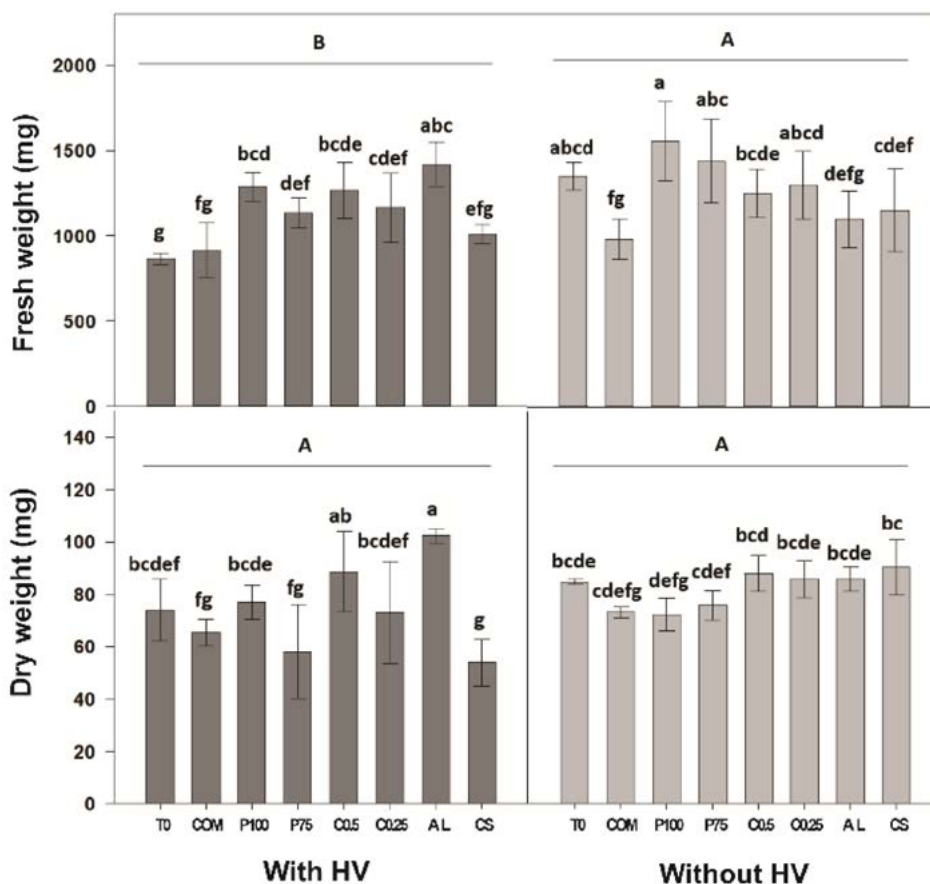


Figure 8. Fresh weight and dry weight of maize seedlings with the different adherent treatments with or without vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

Table 3. Biochemical variables of maize seedlings with different seed coating treatments done with those adherents with the highest coverage percentage.

	T0 without HV	T0 with HV	COM with HV	P75 with HV	CV
Glutathione mg 100 g ⁻¹ DW	580.07 ± 60 a	593.98 ± 80 a	632.25 ± 44 a	623.989 ± 52 a	7.03
Phenols mg GAE 100 g ⁻¹ DW	1200.23 ± 101 b	982.63 ± 148 c	1443.28 ± 74 a	1517.36 ± 108 a	8.63
Flavonoids mg CE 100 g ⁻¹ DW	5.43 ± 0.98 a	5.91 ± 0.97 a	5.98 ± 0.85 a	4.97 ± 0.83 a	17.87
Total proteins mg AE 100 g ⁻¹ DW	1055.67 ± 165 ab	686.45 ± 90 c	1184.14 ± 150 a	853.12 ± 133 bc	14.54
Total chlorophyll mg 100 g ⁻¹ DW	1810.88 ± 162 b	3133.14 ± 430 a	3412.095 ± 565 a	2513.78 ± 355 ab	18.44
Carotenoids mg 100 g ⁻¹ DW	36.45 ± 9.45 c	196.70 ± 62 b	310.90 ± 27 a	202.39 ± 37 b	20.9

Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$). CV: coefficient of variation; GAEs: gallic acid equivalents; CE: catechin equivalents; AE: albumin equivalents. DW: dry weight. T0: zero control; COM: commercial adherent; P75: 75% polyterpene.

The increase in phenol content could be associated with the activation of antioxidant and plant defense metabolic pathways, generated by stress. Stress can be identified in physiological variables such as fresh weight and plumule length, for which HV treatments had lower results compared to non-HV treatments. Biostimulants have the ability to potentiate the production of biocompounds in plants, because they regulate biochemical mechanisms and promote tolerance to oxidative stress (Mir & Khah, 2024). Other studies conclude that the application of biostimulants, in the early stages of the plant and in adequate doses, improves antioxidant production, stimulates enzymatic and non-enzymatic activity, improves cellular homeostasis and adaptation to stress (Johnson & Puthur, 2022).

CONCLUSIONS

Results obtained in this study indicated that the adherents with the best adhesion capacity were the commercial compound and the proposed polyterpene as a viable alternative. The application of vegetable flour did not have a significant impact compared to treatments without it. In most of the variables evaluated, those were statistically equal, except in plumule length and fresh weight, where they were even lower.

Seeds treated with adherents showed non-significant differences among treatments. Biochemical variables did have a positive effect with the application of adherents and vegetable flour, compared to the zero control with and without it. The information obtained in this study can serve as a model for the application of vegetable flours, as extracts in solid form with biostimulant activity in seeds or plants, towards a sustainable approach to crop conditioning and management. Additional studies are also necessary to evaluate and propose other application rates for vegetable flours and their effect on the entire plant cycle.

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