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Investigation note

Protection against frosts by antifreeze, amino acids, and vermicompost in phenological stages of the bean

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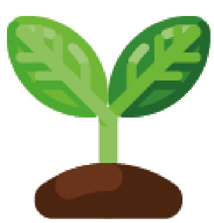
Abstract

Bean cultivation is one of the main activities of the peasant economy in Mexico; however, it is subjected to different types of stress, where frosts significantly affect its cultivation, for this reason, it is necessary to look for alternatives that provide greater tolerance to plants. Thus, the objective was to evaluate the effect of different products against frosts on the stem, in the vegetative (V) phenological stages of the bean. The research was conducted in the greenhouse of the Puebla Campus of the College of Postgraduates in 2018. The Negrito CP variety was used, which was sown in expanded polystyrene cups. The treatments used were antifreeze (10 ml L^{-1} of water), amino acids (1.5 g L^{-1} of water), and the mixture of both in the same dose used individually; these were applied with an interval of 48 h and 96 h and exposed to a simulated frost in a freezer of magnitude equal to 0 °C for 1.5 h. The frosts presented different effects on the vegetative stages of the bean, the most tolerant were V1 and V2. The leaves were the most damaged, followed by the petioles and stem. The treatments that provided the greatest protection to the bean seedlings were An (antifreeze) and the combination Ver+An (vermicompost+antifreeze).

Keywords:

Phaseolus vulgaris L., phenology, stress.



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Bean is considered an important staple food for its significant contribution of protein, carbohydrates, and fiber to consumers, who also receive numerous nutrients and phytochemicals that protect against multiple diseases (Cámara *et al*., 2013). Nevertheless, the species does not escape the climatic changes that are experienced in the present, a phenomenon considered the main cause of the decline in crop production worldwide (Bhat *et al*., 2022). Particularly in the country, the bean is exposed to adverse factors, including frosts.

This climatic factor has been a recurrent phenomenon occurring in places where it did not occur, attributable to climate changes, with the presence of catastrophic cases that cause irrecoverable losses of agricultural crops, according to Pearce (2001), frosts negatively affect the growth and development of plants, limit their geographical distribution, and decrease yield worldwide. In addition, they are becoming increasingly intense and frequent, causing direct damage to the production not only of beans but of different crops (García *et al*., 2017).

Partial damage or total losses due to frosts are of economic importance, especially in susceptible plants such as bean which has an optimal growth temperature of 16-21 °C, with the maximum at 27 °C and the minimum at 10 °C (Nadal *et al*., 2004). Its normal physiology is maintained under ideal environmental conditions; however, when the temperature drops below the minimum degrees it withstands, it is affected considerably.

Similarly, the bean has different degrees of tolerance to cold stress, according to the phenological phase, where the reproductive phase is more sensitive than the vegetative phase, this fact has been detected evidently in the broad bean crop, where sufficient tolerance has been reported in the vegetative phase but not in the reproductive phase (Alharbi and Adhikari, 2020). In particular, in the vegetative phase, low temperatures affect the growth of plants, and in the reproductive phase, mainly the reproductive organs. Thus, frosts cause damage at all stages of growth and the magnitude depends on the severity of the frosts, exposure time, frost frequency, crop sensitivity, and plant growth stage (Hawthorne, 2007).

In relation to the mechanisms that prevent irreversible damage to plants, there is avoidance and tolerance to freezing. The former delays the formation of intercellular ice and prevents the spread of ice that has already formed, while the latter allows tolerance of ice formation in the apoplast (Wisniewski *et al*., 2014). The freeze tolerance mechanism allows plants to survive at freezing temperatures, in this case, the plant can withstand ice formation in its extracellular spaces, while intracellular water migrates to extracellular ice to dehydrate cells without causing irreversible cell damage (Hoermiller *et al*., 2018).

Among the alternatives for protecting crops susceptible to cold stress is the search for genetic resistance, agronomic management, and the use of products with antifreeze potential. The latter is the most practical and easy to adopt, according to their mechanism of action, there are products to prevent freezing, products that provide tolerance to freezing, and products that act with both mechanisms (Román-Figueroa *et al*., 2021).

The products that are currently on the market contain different formulations, showing the search for greater effectiveness in this way. According to Román-Figueroa *et al*. (2021), more scientific support is needed in their preparation and in the studies of their effects directly on crops. In order to find products with antifreeze potential in beans, research was conducted on the effect of frosts in the different vegetative stages of development of the bean, on the stem, as well as to identify the product or combination of products with greater protection against frosts.

The work was carried out in the greenhouse of the Puebla Campus, of the College of Postgraduates in 2018, using the Negrito CP variety. The sowing was carried out in expanded polystyrene cups with a capacity of one liter, placing the bean seed 2.5 cm deep. Fertilization was carried out on the same day of sowing with the dose 40-40-00, using ammonium nitrate and calcium triple superphosphate as sources for nitrogen and phosphorus, respectively. The identification of each of the vegetative (V) phenological stages studied was based on the characteristics mentioned by Escalante and Kohashi (1993), which were from V1 to V4.

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In V4, a sampling was obtained at the beginning and end. The solutions used were Antifreeze (thermal protector) of the Ibarquím© group applied in doses of 10 ml L^{-1} of water, aminocel 500 amino acids of Cosmocel® in the amount of 1.5 g L^{-1} of water, and the mixture of both in the same dose used individually. The following treatments were generated: vermicompost 50% (Ver), amino acids (Am), vermicompost 50% + amino acids (V+Am), amino acids + antifreeze (Am+An), vermicompost 50% + amino acids + antifreeze (V+Am+An), antifreeze (An), vermicompost 50% + antifreeze (V+An) and agricultural soil (As).

The applications of the products were made at the time that the plants reached the desired vegetative stage (V1, V2, V3, and V4), there were two applications at an interval of 48 h, with the exception of stage V1 which only had one application. Frosts were simulated in a Torrey CV16B 1276 freezer. From each treatment, three plants per stage were exposed to 0 °C for 1.5 h, before putting them in the freezer they were sprayed with drinking water using a manual spray. The plants were randomly distributed inside the freezer.

Plants in stages V2, V3, and V4 were introduced to the freezer 96 h after the application of the products, and plants in stage V1 after 48 h. After 24 h of the simulated frost, the damage produced in the stem of the plants was evaluated by means of a scale of 0 (no damage) to 100 (totally damaged or dead), with the possibility of taking any percentage within the scale depending on the damage produced by the frost.

We performed an analysis of variance to detect statistical differences between treatments and the Tukey mean separation test (#= 0.05) in order to detect the best treatments for frost damage. In both analyses, the statistical package SAS ver. 9 for Windows was used.

The analysis of variance showed a highly significant difference for frost damage caused in bean seedlings between stages, treatments, and the interaction between the two. The stage of development of the seedling influenced the susceptibility to frost damage and at least one treatment protected the seedlings to a greater degree, which led to a positive effect when both factors interacted. In the leaves of each plant, the leaf blades received the direct impact of frost, while the petioles presented less damage because the leaf blades provided protection against frost, which coincides with what was mentioned by Restrepo *et al*. (2013), they pointed out that in corn plants, the cell membranes of the leaves are the main structural component affected during frost seasons.

It was observed that the damage during the vegetative development of the plants increased in magnitude as it progressed to the reproductive phase (Figure 1), the lowest percentage of damage was in the plants of stage V1, and the greatest damage was in stage V4. Elías *et al*. (2001) and Augspurger (2013) have also reported that frost damage depends on factors such as phenological status, tissue maturity, and fertilization.

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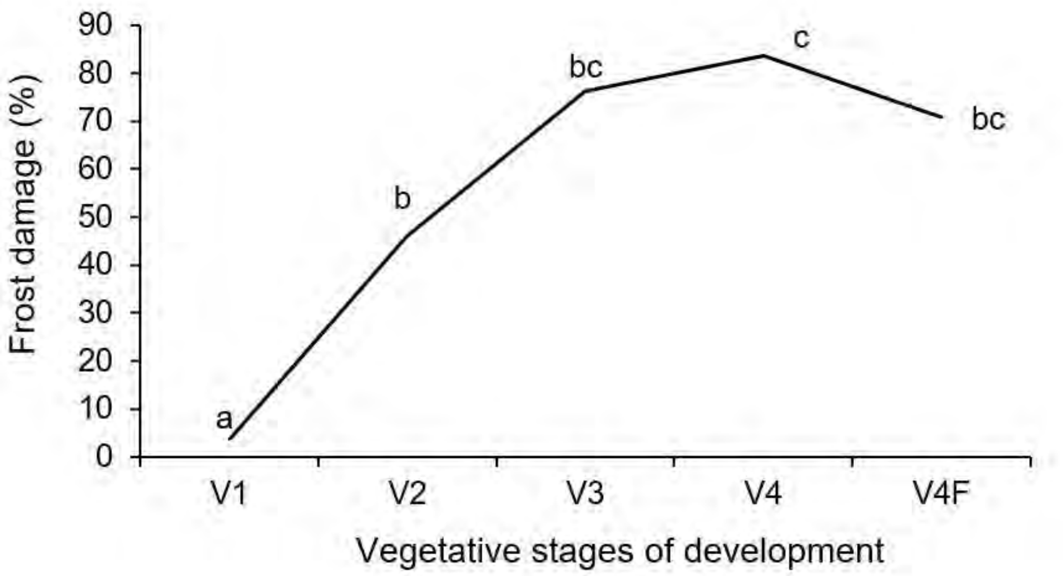
Figure 1. Percentage of damage in plants 48 h after frost in the different vegetative stages ($p \leq 0.05$). Identical letters are not significantly different. V1= emergence; V2= primary leaves; V3= first trifoliate leaf; V4= third trifoliate leaf; V4F= end of stage V4.



The damage that the plants presented after the frost was of a different magnitude, directly proportional to the advance of vegetative development in stages V1, V2, V3, and V4. Within each stage, the treatments tested had a different degree of protection against frost damage. In stage V1, no significant differences were found in the protective effect of the treatments (Figure 2). In stage V2, significant statistical differences were detected, the outstanding treatments were Ver + An, As, and An, with less than 20% damage in the plants.

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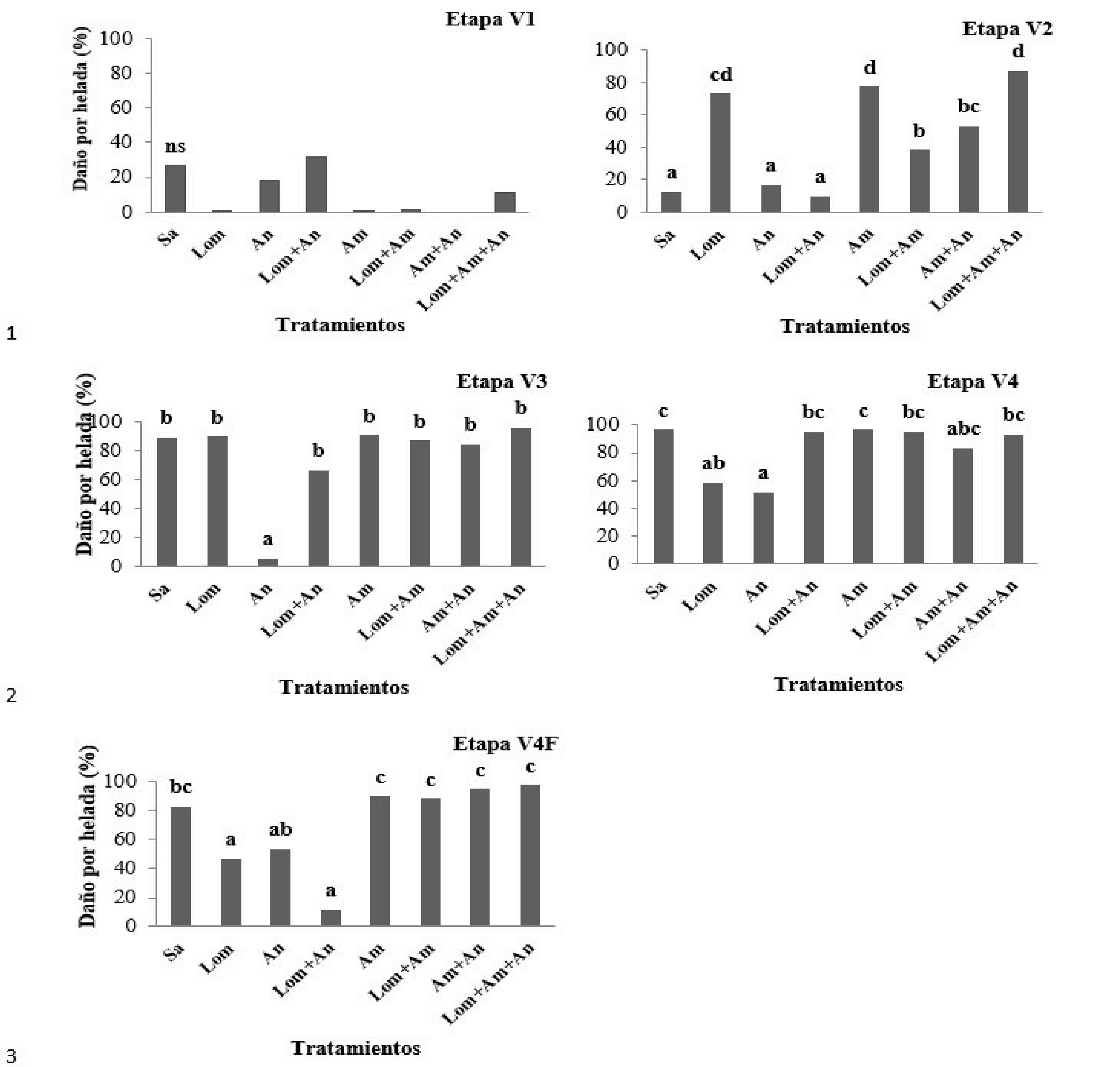
Figure 2. Damage caused by frost in bean plants after the application of treatments (p≤ 0.05). Identical letters are not significantly different. Sa= agricultural soil (As); Lom= vermicompost (Ver); An= antifreeze; Lom+An= vermicompost+antifreeze (Ver+An); Am= amino acid; Lom+Am= vermicompost+amino acids (Ver+Am); Am+An= amino acids+antifreeze; Lom+Am+An= vermicompost+amino acids+antifreeze.



The treatments with the least protection were Am and Ver+Am+An with about 80% damage. In stage V3, the minor damage in the plants was recorded with the application of An (5%), with the other products damages greater than 60% were quantified. In stage V4, the best treatment was An (51.6%) followed by Ver (58.3%). The rest of the treatments showed damage greater than 80%. In stage V4F, the treatments Ver+An (11.6%), Ver (46.6%), and An (53.3%) stood out. The other treatments allowed damage above 80%.

The slight damage detected in stage V1 is attributed to the fact that products such as An were not applied to the entire seedling, because only the plumule hook was observed, so, when put into the freezer at 48 h, the first leaves were opening and were exposed with little protection of

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the products. In stage V2, variation was detected in the effect of the treatments, in the case of As, the damages were similar to An and Ver+An, probably because at this stage the seedlings have greater resistance to frost, as observed in (Figure 1), a fact that did not occur in the other stages.

The combinations of amino acids with antifreeze did not have a positive effect, it is likely that the mixture of these two products will be antagonistic; in this regard, Arjona *et al*. (2004) detected adverse effects on onion growth when applying combinations of urea with amino acids and molasses with amino acids to foliage and soil, which causes complex relationships between products that affect the physiological response of plants. In combined treatments, the accumulation of nutrients is greater so that excess nitrogen produces a deficiency, however, plants acquire great development, dark green leaves, and delay in their maturation, Fuentes (2002) mentions that these characteristics reflect less resistance to frost, drought, and diseases.

The best protection of plants against frosts caused by An is due to the fact that the product is made to cushion the harmful effects of the phenomenon, which prevents the exit of water because it is formulated to form a thin biodegradable waxy layer that acts as a physical barrier between the plant surface and sudden changes in temperature (Grupo Ibarquim, 2023). In general, plants with An and Ver were favored in frost tolerance because the stem was protected by An and the root by Ver by keeping more moisture, this kept the tissues with water and prevented the total freezing of the plant.

The water content in the tissues has been reported as a frost tolerance trait in wheat (Limin and Fowler, 1994), in general, the plants suffer dehydration, and after a while, they wither (Sánchez-Díaz and Aguirre-Olea, 2000), an important reason to keep the plants hydrated before exposure to frost.

The damage caused to the leaves is evident, which is related to the work carried out by Barrales *et al*. (2002), in which, during the cooling or frost phenomenon, the corn genotypes used did not present a difference in cold tolerance, since the foliage died with light frosts, while the death of the stem occurred due to more intense frosts. In the bean, it is affected by low temperatures (without reaching the level of frost, 0 °C).

Nevertheless, sensitivity to damage is a function of the intensity of the phenomenon and the stage of development of plants, because plants maintain various physiological functions at each stage of development that go according to temperature changes, as mentioned by Rodríguez *et al*. (2018), who report that during the winter, tree leaves use a high proportion of carbohydrates to resist low temperatures, while in spring, this ratio decreases because they are destined to processes related to growth.

Conclusions

Frosts had a different effect on the different vegetative stages of bean development, the most tolerant were V1 and V2. The effect was directly observed in the stem structures, where the leaf blades were the most damaged, followed by the petioles and stem.

With the application of the products, the plants had different degrees of protection in each of the stages, thus, the application of foliar An provided a greater degree of cushioning against frost from stage V2, the same happened with the combination Ver-An. Future studies should work with reproductive stages, test other products with different doses. Take experimentation to regions with frost problems with the participation of bean producers and install the work on their own land.

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