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Homeopathy for Treating Contaminated Cucumber Seedlings With the Herbicide Residues

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Abstract

The aim of this study was to evaluate the effect of homeopathic medicines in the neutralization of waste herbicide 2,4-D+picloram in cucumber seeds, in the municipality of Alta Floresta-MT process. The experiment was carried out to evaluate two herbicide concentrations and five *Nux vomica* dynamizations (6CH, 12CH, 18CH, 24CH and 30CH), in addition to two controls, one with distilled water and the other contaminated with herbicide alone. The variables assessed were germination percentage, germination speed index, fresh weight, dry weight, shoot length, root length and stem thickness, where we observed a significant difference in most of the variables analyzed. From the results, it can be concluded that the homeopathic preparations in five dynamics of Nux vomica (6CH, 12CH, 18CH, 24CH and 30CH), act positively on vigor and development of cucumber seeds.

Keywords: dynamizations, 2,4-D+picloram, *Nux vomica*, family farming

1. Introduction

The use of increasingly simplified cultivation techniques coupled with continuous growth with large areas of monoculture resulted in negative impacts for several sectors. This simplification leads to ecological imbalance, the disruption of food chains, the artificialization of production areas, and a permanent and growing need for external subsidies, such as agrochemicals and fertilizers (Andrade & Casali, 2011).

In this way, the indiscriminate use of herbicides comes to draw attention. These products, whose purpose is to kill or paralyze the growth of other plants, have been widely used both in large areas of cultivation and in small areas, with practice of family agriculture and peasant.

Among the most used herbicides is 2,4-D (2,4-dichlorophenoxyacetic acid). It is a chemical compound synthesized with a growth regulator characteristic due to its structural similarity to the natural auxin of plants (Santos et al., 2013). It is widely used in the control of spontaneous plants in plantations, interfering in the growth of annual broadleaf plants and some perennials (Felito et al., 2019). This product is characterized by causing various metabolic disturbances in sensitive plants, such as abnormal growth, root tissue death, epine- sis and phloem obstruction (Rodrigues & Almeida, 2018).

2,4-D alone or in combination with picloram, another compound of the same chemical group, can cause serious environmental problems, such as soil, water and soil microbial contamination (Oliveira Jr., 2011). Although several studies (Bjorling-Poulsen et al., 2008; Belo et al., 2011) show the serious consequences that these can

imply, there are still several obstacles in Brazil that prevent the development of agriculture that is less aggressive to people and the environment (Andrade & Casali, 2011).

In this context, the need arises to search for new production methods to reduce the impacts caused by these products. The use of agroecological practices is an indispensable tool for family farmers, because they allow their autonomy in the field, leading to sustainable development.

Among agroecological practices, homeopathy has been expanding among producers in the most varied regions of the world. Its beneficial effects on various agricultural crops are reported by several authors (Roscoe et al., 2006; Edvan & Carneiro, 2011). Homeopathy, inserted in agriculture as a general practice, provides health to the rural environment, resulting in the abandonment of agrochemicals and other inputs, providing independence for the family farmer (Andrade & Casali, 2011).

Homeopathy is recognized as a field of knowledge of great potential within the modern view of food quality and biosafety, because it leaves no residues in the environment, as well as in food (Carneiro et al., 2011). Based on the experimental results, homeopathy was officialized as an agricultural input in several countries (Felito, 2016).

Some homeopathic preparations are chosen by analogy with pathogenesis, such as the homeopathic preparations of *Nux vomica*, *Arnica montana*, among others (Andrade et al., 2010; Felito et al., 2019). The homeopathic preparations of *Nux vomica* have been used with great potential in the decontamination of plants intoxicated by pesticides and soluble synthetic chemical fertilizers, acting on plant and soil tissues, promoting homeostasis (Cupertino, 2008).

The cucumber (*Cucumis sativus* L.) is a very popular crop in the world, being very popular because of the possibility of cultivation in small areas and limited technical knowledge for its production. In addition, it is nutritious and intensely marketed in fairs, small markets and even in large wholesalers. Despite its nutritional value, this culture has also been used and indicated for several medicinal purposes (Santos et al., 2013).

Although it is a relatively easy-to-grow crop, there is concern that herbicides applied to other crops in previous crops affect its development and consequently its production. In addition, this species is considered as one of the indicative cultures of substrates contaminated by auxinic herbicides, due to their sensitivity to these chemical compounds (Carvalho et al., 2012).

Based on analogies made with homeopathic medicine, the objective of this research was to evaluate the homeopathic potential of *Nux vomica* in the process of neutralizing the residual effect of the 2,4-D+picloram herbicide on cucumber seeds.

2. Material and Methods

The experiment was developed at the Laboratory of Seed Technology and Weed Science (LaSeM) of the Mato Grosso State University (UNEMAT), Campus of Alta Floresta, Brazil. The experiment consisted of the study of the germination of organic cucumber seeds on substrate moistened with ultra-dilute doses with herbicide 2,4-D+picloram, belonging to the chemical group of syntetic auxin.

The organic cucumber seeds, free of any chemical treatment, were acquired in the local trade, being previously tested the germination of these, to verify their viability. The herbicide was also purchased locally and used within the validity specified on the label.

The homeopathic preparations of *Nux vomica* were chosen respecting the first principle of Homeopathy, "*similius similibus curanter*" (similar cure similar). For the production of homeopathic preparations, the standard methodology proposed by Hahnemann (Barthel, 1993) was followed in his experiments. Firstly a vial or glass containing the mother tincture (MT). Subsequently, a portion of the MT was used, which was placed in another glass containing 99 parts of 70% alcohol (the so-called dilution procedure). Subsequently it was shaken 100 times with rhythmic movements (sucussion). From there, the first dinamization (dilution and sucussion) was produced which is called 1CH (Centesimal Hahnemanianna). To obtain 2CH, 1 part of 1CH was used, which was placed in another glass containing 99 parts of 70% alcohol or water and sucussionando by 100 times, obtaining at the end 2CH. Same procedure adopted to get 3CH, 4CH and so on.

The experiment was arranged in a completely randomized design in a 2 × 5 factorial scheme, where the treatments consisted of two concentrations of herbicide 2,4-D+picloram (0.125 and 0.0625 mL to 100 mL of distilled water) and five dynamizations of homeopathic preparations of *Nux vomica* (6CH, 12CH, 18CH, 24CH and 30CH), plus two controls, consisting of seeds contaminated by herbicide without homeopathic treatments and seeds without contamination soaked only with distilled water.

In order to determine the doses of the herbicide, preliminary tests with different product concentrations (2,4-D+picloram) were carried out, and two concentrations were selected that showed potential influence on the development of the crop.

As recommended, the homeopathic preparation filled only 2/3 of the volume of the vial in which it was stored. The capacity of the flask used was 30 mL. Therefore, only 20 mL were filled. The ratio of one drop of the homeopathic drug per 99 drops of the carrier was respected, in which case the vehicle used was 70% alcohol. The succussion process was performed manually prior to the assembly of the experiment, being dynamized from 3CH to 30CH.

The treatments for each concentration of herbicide consisted of: 0-seeds soaked with distilled water (control 1); 1-seeds contaminated by herbicide without homeopathic treatment (control 2); 2-*Nux vomica* 6CH; 3-*Nux vomica* 12CH; 4-*Nux vomica* 18CH; 5-*Nux vomica* 24CH; 6-*Nux vomica* 30CH.

The seeds were divided into groups of 100 and placed in disposable plastic cups containing the weeds of the herbicide at concentrations of 0, 125 and 0.0625 mL for 100 mL of distilled water for a period of 60 minutes. Afterwards the seeds were removed and dried on paper towel. Then the seeds corresponding to the homeopathic treatments were placed in disposable plastic cups, containing the homeopathic treatments in the proportion of 1.0 mL of the homeopathic solution to 1.0 liter of distilled water, remaining for a period of 30 minutes, and later placed to germinate.

For the germination, transparent acrylic boxes (11.0 × 11.0 × 3.5 cm) were used as experimental unit, submitted to previous aseptic treatment by washing with neutral soap and subsequent cleaning with sodium hypochlorite (10%) and alcohol (70%). Each treatment consisted of four replicates with 25 seeds per box.

The seeds were placed to germinate in germitest boxes on two sheets of germitest paper moistened with each homeopathic solution (according to each treatment), in the proportion of 2.5 times the mass of the dry substrate (Brazil, 2009), and later packed in germination chambers type BOD with light regime of 12 h, under white fluorescent lamps of 40w for ten days, being considered germinated the seed whose radicle reached 2.0 mm in length.

After the incubation time, 10 seedlings were randomly withdrawn per replicate. The variables evaluated were percentage of germination (%), germination speed index (GSI), shoot length (mm), root length (mm), stem thickness (mm) and fresh matter and dry matter (g).

The shoot length, main root length and stem thickness were measured using a digital caliper. Fresh matter and dry matter of the seedlings were obtained by analytical weighing, where the dry matter was dried in a forced circulation oven at 70 ºC for 72 hours (until constant weight). The germination speed index was determined according to Maguire (1962), in which the number of germinated seeds was counted daily.

The results obtained, after meeting the assumptions of homogeneity of variance, were submitted to analysis of variance and the means were compared by Tukey's test at 5% of probability, using Sisvar® software (Ferreira, 2011).

3. Results and Discussion

When evaluating the response to the neutralizing potential of the residual effect of 2,4-D+picloram herbicides on cucumber seeds using *Nux vomica* homeopathic preparations, it was verified that the treatments promoted significant differences in most of the evaluated variables (Table 1). Too, it was verified that only for the germination percentage variable, there was no difference between treatments.

Table 1. Medium squares of germination percentage (G%), germination speed index (GSI), fresh mass (FM) dry mass (DM), plant height (PH), root length (RL) and stem thickness (ST) in a study of *Nux vomica* homeopathic preparations on herbicide residues 2,4-D+picloram in cucumber seeds

Variation Font	Medium Squares						
	G%	GSI	FM	DM	PH	RL	ST
Dose (D)	0.07143ns	0.05786ns	0.0105*	1.6071ns	2442.370*	34.1422ns	0.044409*
Treatment(T)	15.6012ns	9.36059*	0.0050*	0.0006*	915.0057*	5006.291*	0.134856*
D × T	8.7797ns	3.58202ns	0.0062ns	2.85714ns	43.9503ns	8.78512ns	0.0083ns
Error	6.869048	2.419405	0.000275	6.964285	25.307913	30.377176	0.005864
CV (%)	3.21	4.24	8.19	6.59	13.09	23.93	3.64

Note. ns = Not significant; * Significant at 5% probability.

Despite the contamination of the substrate, the germination of the cucumber seeds was close to 80%, demonstrating their high vigor, regardless of the treatment (Table 2). The process of imbibing the seeds with the solution that moistened the substrate was not impaired and, for the species in question, was not enough to inhibit the germination process, even in the higher concentration of the herbicide.

The fact that there is no difference in germination between homeopathic treatments is related to the considerations presented by E. Kolisko and L. Kolisko (1978). The authors considered that among homeopathic medicinal products, depending on their similarity to the plant, it is possible to observe a stimulating, inhibitory or even no effect on the metabolism of living beings, among them, in the germination process of the seeds, as occurred in the present job.

Among the treatments, it was verified difference for GSI, fresh mass and dry mass (Table 2). Treatments 1 and 2 were the ones with the lowest mean values, differing from the treatment that used distilled water. The averages obtained in these two treatments were almost 8% lower than the 0 treatment, although they did not differ from the other treatments. The other homeopathic treatments were approximately 3% lower than the means obtained in the control treatment (distilled water). Thus, it was observed that the treatments with dynamizations 12CH, 18CH, 24CH and 30CH were similar to the control, with no difference in germination speed of cucumber seeds.

Table 2. Mean values of germination percentage (G%), germination speed index (GSI), fresh mass and dry mass of cucumber seedlings contaminated by 2,4-D+picloram herbicides submitted to *Nux vomica* homeopathic treatment

Treatment	G (%)	GSI	Fresh mass (g)	Dry mass (g)
0	83.57 a	38.162 a	0.230 a	0.014 a
1	80.57 a	35.238 b	0.150 c	0.011 c
2	80.42 a	35.275 b	0.198 b	0.012 bc
3	82.43 a	36.963 ab	0.203 b	0.012 bc
4	82.43 a	37.350 ab	0.214 ab	0.013 b
5	82.85 a	37.138 ab	0.214 ab	0.012 bc
6	79.57 a	36.875 ab	0.206 ab	0.012 bc
CV (%)	3.21	4.24	8.19	6.59

Note. The averages followed by at least one letter in the column do not differ significantly from each other by the Tukey test at 5% probability.

Treatment 0 = Witness 1 (distilled water); Treatment 1 = Witness 2 (herbicide); Treatment 2 = *Nux vomica* 6CH; Treatment 3 = *Nux vomica* 12CH; Treatment 4 = *Nux vomica* 18CH; Treatment 5 = *Nux vomica* 24CH; Treatment 6 = *Nux vomica* 30CH.

The variability of the responses as a function of the dynamics used has already been reported by several authors (Carneiro et al., 2010; Damin et al., 2014). In studies with homeopathy, it is not uncommon to report that the same drug was able to cause different responses in the body undergoing treatment in accordance with the dynamization (Muller et al., 2009), in the same way that was verified in this study. Since the first research with homeopathy, it was common to observe the same solution causing different effects, due to the dynamizations, sometimes increasing, or inhibiting a certain variable (Castro, 2013; Rissato et al., 2016). Like Castro (2002), when evaluating the effect of homeopathic solutions on the growth of carrot and beet plants, the effect of different homeopathic energies was differentiated.

As for the fresh mass, the highest mean was observed in control 1 (distilled water), with a value of 0.23 g and less in control 2 (herbicide 0.0625 mL/100 mL distilled water), with a mean of 0.15 g. Thus, the presence of the herbicide in the substrate promoted the reduction of the accumulation of plant material in the seedlings, significantly impairing its development.

Similar results were also observed in the dry mass. The lowest value or weight occurred in the control treated as herbicide (0.011) and the highest mean corresponded to the control with distilled water (0.014) followed by the homeopathic treatment in 18CH (0.013) dynamization reaching values close to a normal seedling. These values indicated a possible beneficial effect of *Nux vomica* dynamizations on the accumulation of dry mass in plants poisoned by auxinic herbicides. This result is consistent with the *Law of Similitude*, where the substance that in toxic dose generates several symptoms in the healthy living being, when given to the patient with the same

symptoms, causes the state of equilibrium (Moreno, 2000). That is, when receiving similar information, the environment is stimulated to the reaction (Casali et al., 2006).

It was observed in the variables plant height and root length, that the averages of control 1 (distilled water) showed higher growth than the others (Table 3). However, it was found that homeopathic treatments provided the greatest aerial development when compared to control 2 (with herbicide).

Lisboa (2010), when evaluating the effect of homeopathic preparations of *Nux vomica* and *Carbo vegetabilis* on 7CH dynamization in different water sources, observed a beneficial effect of these on their physicochemical properties. According to the same author, the significance is due to the fact that the water used would probably have some contaminant, thus manifesting the reaction. In the same way, it can be said that there was a reaction of the homeopathic preparations by the presence of the toxic compound, providing aerial growth of the cucumber seedlings. When the homeopathic preparation that would be able to produce the same symptoms in the plant is applied, the result will be the minimization of the harmful effects caused by the biotic and abiotic factors, these being the same considerations in this work (Bonato & Peres, 2007).

The results obtained in aerial development were not observed in the reestablishment of root growth, presenting a mean value of 23 mm, showing a higher sensitivity to the applied herbicide (Table 3). Differences in the reaction of two plant species when submitted to treatment with the same substance at high dilutions have been reported (Rossi et al., 2005), and need to be better understood.

These results suggest that substances in high dilutions and homeopathic medicines that produce an effect in one plant species may not produce the same effect in another species, which prevents the generalization of treatment for many plant species (Carneiro et al., 2011). The effects of homeopathy observed in this study, as a function of the analyzed variables, can also be explained by the stress conditions caused by the dilutions of the herbicide and subsequent treatment of the seeds during the assembly of the present experiment. Plants have the capacity for self-regulation, and homeopathy acts in the disorder of the living being, and stimulates the vital force for the organism to enter again in homeostasis, where the level of stress in which the plants are inserted can influence the process of regulating the (Casali, 2004; Lisboa et al., 2005).

According to the conditions established for the experiment, there were observed symptoms of relatively high stress for the crop, such as the inhibition of its growth, even at low concentrations of the herbicide. Stress triggers a large amount of response variation in plants, ranging from altered gene expression and cell metabolism to altered growth rate and productivity (Bonato & Peres, 2007). Plant responses to stress depend on duration, severity, number of exposures and the combination of stressors.

For the variable stem thickness, there was a significant increase among homeopathic treatments, differing from the two controls (Table 3). Although the homeopathic preparations differ in thickness in relation to the treatment with herbicide, it is possible to observe signs of phytointoxication caused by the herbicide. In a study conducted by Nascimento and Yamashita (2009), they demonstrated the great sensitivity of seedlings such as cucumber, even at low concentrations of auxinic herbicides.

Table 3. Mean values of aerial length, root length and stem thickness of cucumber seedlings contaminated by 2,4-D+picloram herbicides submitted to *Nux vomica* homeopathic treatment

Treatment	Plant height (mm)	Root lenght (mm)	Stem (mm)
0	57.926 a	79.694 a	1.865 c
1	21.791 c	11.065 b	1.984 b
2	34.850 b	14.987 b	2.161 a
3	36.318 b	13.988 b	2.242 a
4	41.246 b	13.975 b	2.159 a
5	39.913 b	13.648 b	2.155 a
6	37.049 b	13.848 b	2.146 a
CV (%)	13.09	23.93	3.64

Note. Averages followed by at least one same letter in the column do not differ significantly from each other by the Tukey test at 5% probability.

Treatment 0 = Witness 1 (distilled water); Treatment 1 = Witness 2 (herbicide); Treatment 2 = *Nux vomica* 6CH; Treatment 3 = *Nux vomica* 12CH; Treatment 4 = *Nux vomica* 18CH; Treatment 5 = *Nux vomica* 24CH; Treatment 6 = *Nux vomica* 30CH.

During the evaluation period of the experiment, changes and callus formation were verified at the base of the plant stem, causing them to thicken. These symptoms were reported by Silva et al. (2005), as the most evident alterations caused by auxinic herbicides, together with root thickening, as well as other barely visible symptoms such as intense cell division in the vascular, endodermal, pericyclic and phloem exchange.

For all variables evaluated, it was possible to infer that there was little difference in the homeopathic treatments. These results confirm the considerations of Rossi et al. (2006), who stated that the increase in dynamizations would not necessarily have repercussions on progressive or increasing physiological responses. Casali et al. (2006) also affirm that the release of the energy of the substance by the dynamization method, does not occur in a linear way, but by jumps, presenting similarity among themselves when the jumps are expansions within the same energy level.

In relation to the applied dose (Table 4), there was a greater accumulation of fresh mass and air length there was greater accumulation of fresh mass and plant height in the concentration of 0.0625 if compared with the concentration of 0.125. From these results it can be inferred that plants poisoned by pesticides at low concentrations present a better recovery response with homeopathic treatment when compared to contamination by higher doses.

Analyzing the thickness of the stem as a function of the applied dose, it was observed that there was thickening and callosity in the highest concentration of the herbicide. This result corroborates Silva et al. (2005), who reported symptoms characteristic of those previously reported, promoted by 2,4-D+picloram herbicides.

Table 4. Influence of 2,4-D+picloram herbicide dose on fresh mass, plant height and stem thickness of cucumber seedlings

Dose	Fresh mass (g)	Plant height (mm)	Stem (mm)
0.0625	0.2164 a	45.0464 a	2.0739 b
0.125	0.1889 b	31.8383 b	2.1302 a
CV(%)	8.19	13.09	3.64

Note. Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at 5% probability.

Based on the results and the conditions under which the present work was developed, it is observed that the '*Principle of Similarity*' is applied in the choice of homeopathies for decontamination in plants caused by pesticides. However, experimental studies should be carried out under field conditions, since homeopathic preparations may present different behavior through interaction with other environmental factors.

4. Conclusions

Based on the results obtained in the present study, it can be concluded that the homeopathic preparations of Nux vomica promote partial neutralization of residues of the herbicide 2,4-D+picloram in cucumber seeds. The 18CH dynamization is promising for new studies, since it provides greater recovery of cucumber seedlings under stress.

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