EVIDENCE OF CAFFEINE INSECTICIDE'S NEGATIVE IMPACT ON BUCKWHEAT SEEDS' GROWTH

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Abstract

Pesticides are overused all around the world, in agricultural fields, gardens, parks and homes. France is the first European pesticide consumer and fourth consumer worldwide, which is a considerable proportion regarding its agricultural area (approximately thirty millions of hectares). Six thousand products containing authorized chemicals are approved in France, and two thousand five hundred are used regularly [0].

Furthermore, it has been proved that pesticides have a serious impact on the environment [1] *and on human health* [2]*: even low levels of exposure can harm human health, and children are particularly vulnerable.*

Insecticides are generally the most toxic pesticides. Many are designed to attack insects' brain and nervous system, which can mean they can also have neurotoxic effects on humans. That is why natural and less harmfull alternatives to pesticides are actively searched.

Caffeine seems to be an efficient natural insecticide [3]*, but its impact on plants is not documented yet.*

Here we show that adding caffeine to buckwheat seeds' soil, slows or blocks their growth. The concentration of caffeine is therefore correlated with the alteration of seeds' growth.

As caffeine slows buckwheat's growth, it may also have a negative impact on many other plants. Therefore, caffeine does not seem to be an appropriate natural alternative to chemical pesticides.

Introduction

The use of insecticides is a major environmental problem as it is one of the causes of water pollution and soil contamination [4]. Indeed, more than 98% of sprayed insecticides reach water, air and soil instead of reaching insects [5]. Therefore, the organisms living in the soils are often damaged by this frequent use of insecticides [6].

However, some inseticides are less toxic for plants than others. Indeed, the contact insecticides are less dangerous for plants than the systemic ones which circulate in their vascular system, penetrating their tissues and thus kill the plants [7].

Caffeine is one of the well-known natural insecticides [3] and, as the majority of insecticides, it targets insect's nervous system. Also, *"the side effects of caffeine-like compounds appear to be relatively minor in humans and animals"* Dr. Nathansan said [8]. However for plants, caffeine's impact remains still unkown. It is why we were interested in studying plants' growth in different caffeine concentrations, having in mind the caffeine median lethal dose for insects lays around 2mg/mL [9].

As we had only one week to answer this question, we chose to work on buckwheat seeds, which grow fast, are easy to access and cheap. Moreover, buckwheat is more and more consumed by humans and mainly in the form of flour which makes the caffeine impact (especially if it's a negative one) even more important [10].

Buckwheat seeding in different caffeine concentrated media

Five solutions with 500mL of water and five different caffeine concentrations (0mg/mL, 0.2mg/mL, 0.6mg/mL, 1mg/mL and 5mg/mL) were prepared using 500mL beakers, a cruet and a precise balance.

Caffeine, in each solutions, was totaly disolved thanks to a thermal shaker.

Once the five solutions back to a room temperature, more than a hundred buckwheat seeds were poured in each of them for thirty minutes. In the meantime, twenty five Petri dishes were labelled: five Petri dishes named 0mg/mL, five Petri dishes named 0.2mg/mL, five Petri dishes named

0.6mg/mL, five Petri dishes named 1mg/mL and five Petri dishes named 5mg/mL. Each of these Petri dishes were then filled with a thin layer of cotton and 15mL of the corresponding solutions, with 20mL pipettes.

Thirty minutes later, a hundred buckwheat seeds were taken out of each solutions, with a clean fork, and disposed in their respective Petri dishes (twenty seeds in each Petri dish).

The twenty five Petri dishes were then placed randomly in a transparent greenhouse, previously built with plexiglass and a laser cutter, for two days. This greenhouse was pierced with two holes, on two extremities, in order to insert two DHT11 humidity and temperature sensor through it. This sensor allowed monitoring of humidity and temperature in the greenhouse and checking the biological noise (see **Figure 1**).

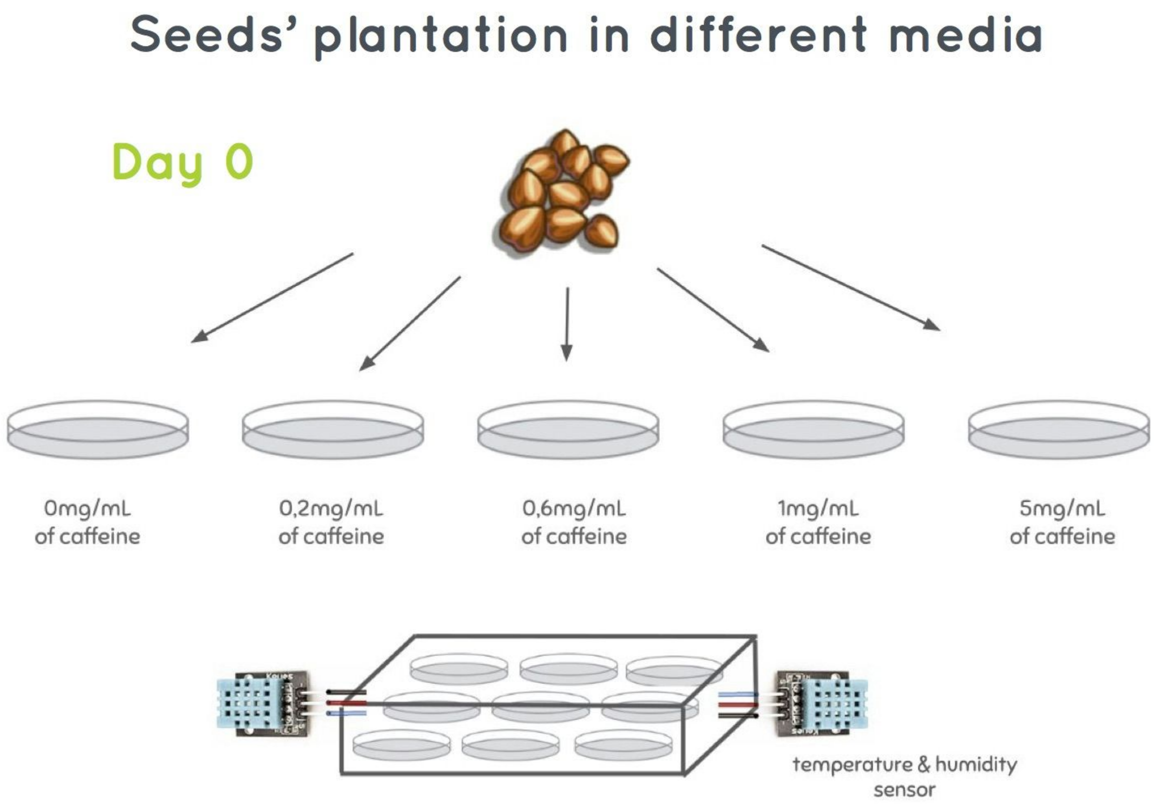

Figure 1: Visual protocol of what was done on day 0. Preparation of the Petri dishes with cotton and five different caffeine concentrations, and buckwheat seeding on them. Five Petri dishes replicates were prepared for each caffeine concentration.

Measuring the impact of different caffeine concentrated media on buckwheat seeds' weight, opening and buckwheat germs' height

The next day, five more solutions with 200mL of water and the same five different caffeine concentrations (0mg/mL, 0.2mg/mL, 0.6mg/mL, 1mg/mL and 5mg/mL) were prepared in other clean 200mL beakers.
Caffeine, in each solution, was also totaly dissolved thanks to a thermal shaker.
Once all solutions at room temperature, all Petri dishes were taken out of the greenhouse and 15mL of the five solutions were spread again on the corresponding Petri dishes.
After that, each buckwheat seeds from all Petri dishes were weighted on a very precise balance, wich was tared between each measurement (see **Figure 2**).
Weight data was collected on a google sheet, exported as a tsv file and plotted thanks to ipython (see **Figure 4**).

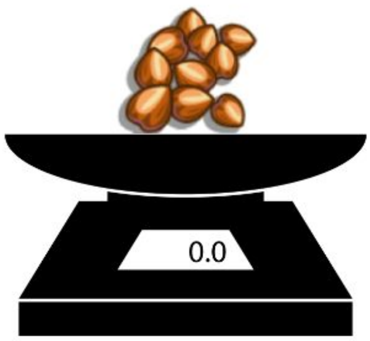

Figure 2: Visual protocol of what was done on day 1. Weighing of all buckwheat seeds with a precise balance.

Petri dishes were again disposed in the greenhouse with both sensors.
On the day after (two days after the seeds were planted in the Petri dishes), all Petri dishes were taken out of the greenhouse again.
All open seeds and non-opened seeds were counted and data was collected on a google sheet and plotted with ipython (see **Figure 5**).
With a sharp scalpel, the visible stems were then cut from the buckwheat seeds of each Petri dishes and gently placed on labnotebooks (specific spaces for each groups of germs were correctly labelled on papers sticked on the labnotebooks). A 1cm scale was also drawn on a piece of paper and placed on the notebooks. With a smartphone, photos of each group of germs (one group

corresponds to germs from the same Petri dish) with the scale, were taken. Precise measurments of the size of the germs could then be done with the software ImageJ (see **Figure 3**) using the 1cm drawn line as a scale, and size data was collected on a google sheet, exported as a tsv file and plotted with ipython (see **Figure 6**).

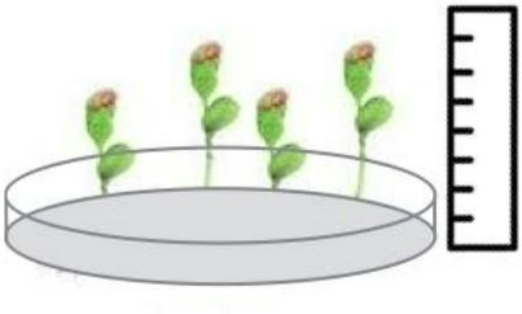

Figure 3: Visual protocol of what was done on day 2. Size measurement of all visible buckwheat germs.

Observing the impact of different caffeine concentrated media on buckwheat seeds' weight

Buckwheat seeds' weight and caffeine concentration in the seeds' media show no correlation (see **Figure 4**).

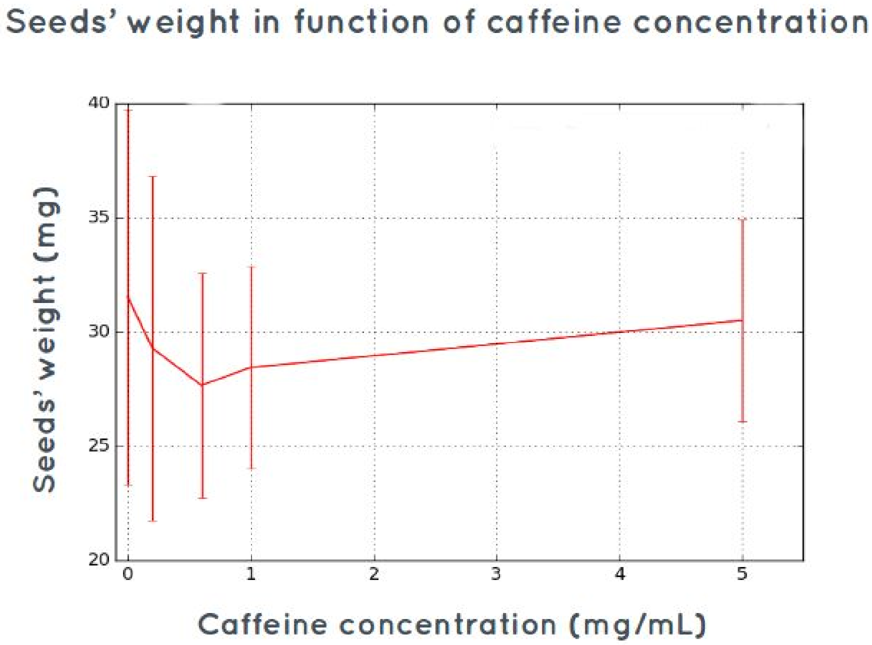

Figure 4: Visual results of all buckwheat seeds' weighing. Graph showing the seeds' weight at day 1, in function of the different caffeine concentrations in the media.

In fact, the weight starts to decrease from 0mg/mL to 0.6mg/mL and then increases up to 5mg/mL. Besides, the standards deviations of weight measures at each caffeine concentration are very important. Thus, these weight results are not revelant and conclusions can not be drawn.
Considering the time to weight each buckwheats seeds and the time left for our experiment, weighing the seeds was not performed on the second day.

Observing the impact of different caffeine concentrated media on buckwheat seeds' opening

Buckwheat seeds' opening and caffeine concentration in the seeds' media show a real correlation (see **Figure 5**). Seeds were classified in three categories: opened, not opened (did not grow) and dead.

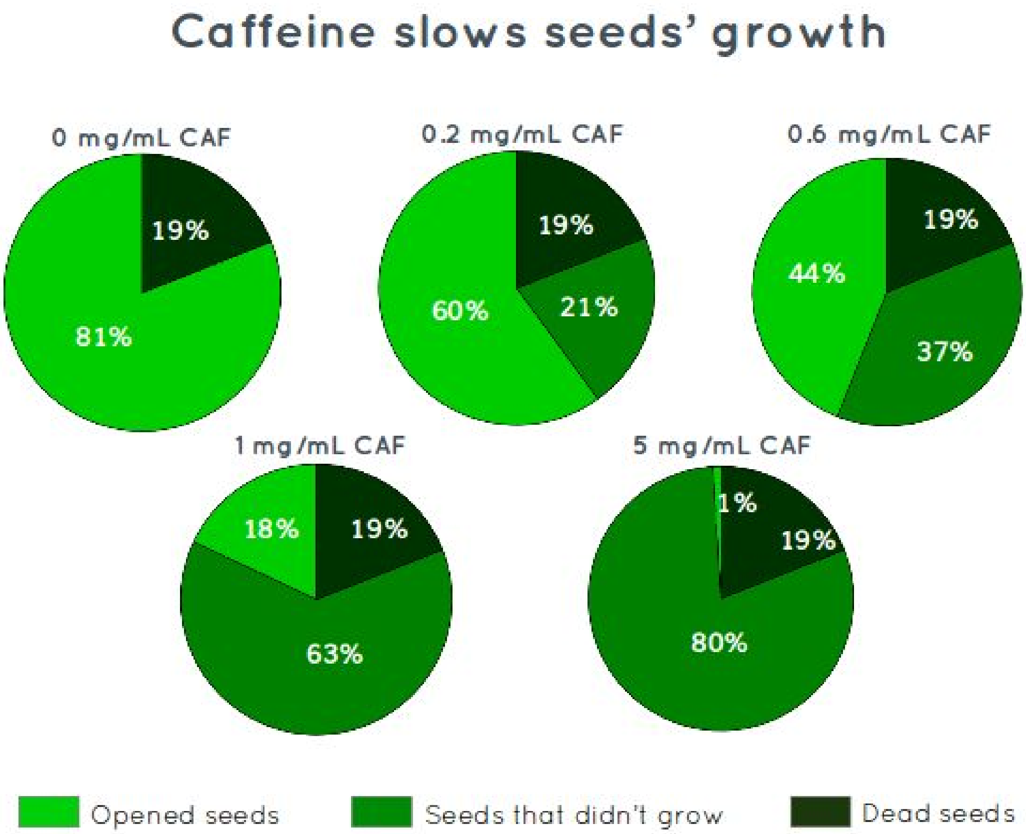

Figure 5: Visual results of all buckwheat seeds' opening. Pie charts showing the buckwheats seeds opening, at day 2, in function of caffeine concentration in the media. Three categories are here represented in three different greens: opened seeds, seeds that did not grow and dead seeds.

With 0mg/mL of caffeine in their media, so in the most natural media, 81% of the buckwheat seeds opened and germed. So we assumed that the 19% other seeds were dead. This percentage was then used as the average of dead seeds for all the other media with different caffeine concentrations (we assumed that 19% of the seeds that did not open, in each Petri dish, were dead).
Results of buckwheat seeds' opening show that when the caffeine concentration in the seeds' media increases, the amount of opened seeds decreases from 81%, with 0mg/mL of caffeine, to 1%, with 5mg/mL of caffeine, and the amount of seeds that did not grow increases from 0%, with 0mg/mL of caffeine, to 80%, with 5mg/mL of caffeine.

Observing the impact of different caffeine concentrated media on buckwheat germs' height

Buckwheat germs' height and caffeine concentration in the seeds' media show also a real correlation (see **Figure 6**).

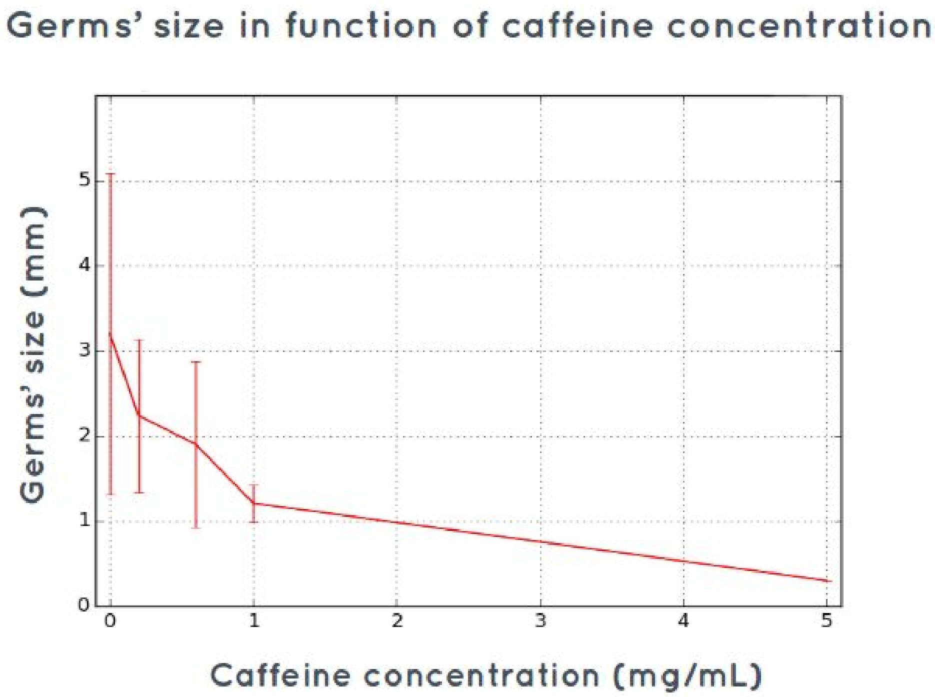

Figure 6: Visual results of visible buckwheat germs' size measuring. Graph showing the buckwheat germ's size that were visible (not dead and non-opened seeds), at day 2, in function of the different caffeine concentrations in the media.

Results of buckwheat germs' size show that as caffeine concentration in the seeds' media increases, the germs are smaller. The size of the germs goes from 3.2mm at 0mg/mL of caffeine to 0.3mm at 5mg/mL of caffeine.
Also, the standards deviations for each caffeine concentration decrease. This is due to the amount of germs (and so the amount of height data) that decreases when the caffeine concentration in the seeds' media increases (81% of opened seeds with 0mg/mL of caffeine, to 1% opened seeds with 5mg/mL of caffeine, see **Figure 5**).

Caffeine alters buckwheat seeds' growth

Through this experiment, it is clear that buckwheat seed's planted in soils containing different caffeine concentrations have an altered growth. What can be noticed, is the correlation between caffeine concentration and buckwheat seeds' growth alteration. In fact, 81% of buckwheat seeds grew in a soil without caffeine, while only 1% of them grew in a soil containing 5mg/mL caffeine. Looking at the germs' size in function of the caffeine concentration, the exact same phenomenon is observed: the more caffeine is added in the buckwheat seeds' soils, the less seeds grow. These results show that caffeine blocks buckwheat seeds' growth or slows it. As our experiment was conducted on a weekly basis, we were not able to understand if caffeine killed the seeds or just slowed their growth. What can though be concluded, is that caffeine has a negative impact on buckwheat seeds growth.

Monitoring buckwheat seeds germination for more relevance

During this project , we showed that caffeine slows buckwheat seeds' growth so we can suppose that it may also have a negative impact on many other plants. Therefore, caffeine does not seem to be an appropriate natural alternative to chemical pesticides. Unfortunately, we were not able to draw any solid conclusion from the results concerning the germs' weight. In fact, in order to get reliable results, we should have weighed the seeds before planting them, and weighed them again everyday for at least one month. In that case, we would have been able to study seeds' weight evolution over their whole germination time. In a more general way, the experiment would have been wiser if it had been designed in order to study buckwheat seeds' germination evolution. A longer lasting experiment would enable us to deeply follow buckwheat seed's growth by analyzing numerous parameters such as weight, height and photosynthesis. It seems to be fundamental to analyze more parameters than just weight in order to understand not only if caffeine affects buckwheat seeds' growth but also how it does. Just as for germs' weight, height could be studied overtime, by repeating the measurements everyday for one month. Concerning photosynthesis, as it is known that it is an essential process for plants [11], it would be interesting to understand if caffeine affects buckwheat's photosynthesis. To do so, a chlorophyll extraction could have been performed on buckwheat's leaves.

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