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Drug effectiveness in laboratory conditions

To develop a laboratory test for the horn manure preparation

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The effect of tiny amounts of bioactive substances is not only a fundamental question for biodynamic agriculture, but also for other research areas such as medicine, physiology or toxicology. What is striking is how the knowledge gained about these disciplines over the last few decades leads to new insights and even paradigm shifts. Particularly in toxicology, the paradigm of limit values1 is wavering today , at least with regard to chemical substances that cause endocrine disorders. Vandenberg et al. (2012) have shown, based on hundreds of scientific studies, that the effect of these substances at low concentrations cannot be predicted based on the effect at higher concentrations because the relationship between dose and effect is not linear. This scientific finding can have far-reaching legal consequences because it calls current limit values into question. In everyday life, this particularly affects the consumption of numerous objects made of polycarbonate plastic (food packaging, beverage bottles, etc.) that contain the plasticizer bisphenol A.

Test sensitivity: ability to provide results for the determination of a substance being tested.

Test stability: Stability of the method as a function of time

Test specificity: ability to determine a specific substance without interference from other components.

Impact research on bioactive substances

Other significant examples can be found in other areas. In plant physiology, the detection of plant signaling substances led to new areas of research such as plant communication. In agriculture, biostimulants, such as algae extract or humic substances, are increasingly being considered to stimulate plant growth and reduce the effects of stress. In this respect, these scientific advances of the last few decades have brought the question of the effectiveness of biodynamic preparations closer to the scientific discourse than 90 years ago. The small amounts used are no longer strange for today's science.

But how were these scientific advances achieved?

This is particularly a question of methodology. The effect of small amounts of bioactive substances is sensitive to a variety of factors, making their repeatability a challenge. Accordingly, laboratory tests became more important. In particular, the last few decades have seen a systematic development of standardized laboratory biotests in toxicology, medicine and physiology. The targeted reactions of test organisms for bioactive substances are tracked and reproduced under controlled conditions. These laboratory tests are to be understood as measuring instruments for the bioactivity of substances.

However, a different approach was taken when researching biodynamic preparations. Since the justification of the trial or Research in 1924, the investigations were mostly carried out in field trials.
One reason for this was probably that biodynamic farming is based on a holistic approach. This research has brought valuable fruit, as the effectiveness of biodynamic preparations on yield, quality and soil properties has been successfully demonstrated several times statistically. However, the repeatability of the results remains insufficient and a basic scientific understanding of how biodynamic preparations work is missing.

Therefore, the potential of laboratory studies should be investigated in order to better follow the complex plant reactions to the use of the preparations (Scheper et al. 2007). With this background, a research project ran from 2011 to 2017 at the Dottenfelder-hof Agricultural School (Bad Vilbel) in cooperation with the biodynamic coordination office at the University of Kassel. The aim was to develop a standardized biotest for the horn manure prep clink. A cress test was chosen that was developed at the Hiscia Institute for pre-clinical testing for the cancer cure Iscador® (Baumgartner et al. 2014). An overview of the investigations carried out is presented below.2

method

Cress seeds are placed on chromatography paper and placed in a plastic bag filled with water (16 seeds per bag). As a treatment, a single drop was placed on the chromatography paper with a microliter syringe when setting up the experiments (Fig. 1). Three dosage variants were examined: (1) 1 ÿl drop of water as a control variant; (2) 1ÿl drop of a freshly stirred horn manure solution (variant D1ÿl); (3) 0.1ÿl drops of the horn manure solution (D0.1ÿl). The dosages examined were based on the dilution of the horn manure preparation suspension in the soil water under biodynamic practical conditions, based on estimates by Giannattasio et al. (2013).

20 bags (repetitions) were prepared for each variant. The bags were hung in the dark in the temperature cabinet in a randomized complete block system. With the exception of the application of the drop, all steps of the test procedure were carried out on coded bags.

This is marked every day Growth development of shoot and root of the seedlings (Fig. 2). At the end of the experiment (after about a week), the bags are photographed, the growth development markings are quantified using an image analysis program and the growth progression of each seedling is reconstructed. The tests were carried out at the Dottenfelderhof (Bad Vilbel). In the experiments described below, the local farm preparation was examined.

Results 2011-2013:
Test sensitivity and stability

A long-term series of 76 individual experiments was carried out between 2011 and 2013. The experiments were scheduled weekly with the three dosage variants. It was found that the root growth of the cress plants was significantly influenced by the horn manure treatments in 28 of 76 experiments.
The effect of the fluctuated between the individual experiments Horn manure preparation was strong (from -32.7% to +17.7% compared to the control).

One hypothesis to explain the fluctuations was the obsolescence of the horn dung preparation that was excavated in 2010. A second horn manure preparation from 2012 was therefore examined in another series. This series of 38 experiments was carried out in parallel to the first one in 2012-2013. In 17 individual experiments, an effect of the horn manure preparation was significant, which also fluctuated greatly (between -16.9% and +16.3% compared to the control).
The results of the two series were coherent with each other.

To understand the considerable fluctuations, it was checked whether the horn manure preparation had a balancing effect on high or low growth levels. With a suitable statistical test, the hypothesis of a stabilizing effect was confirmed

Figure 1: Adding a drop of the treatment (including horn manure preparation) to the seedlings using the microliter syringe

Footnotes

1) In toxicology, Paracelsus' words "The dose makes the poison" is interpreted as meaning "that for each substance there is an individual dose or concentration, below which the risk of poisoning is zero or at least negligibly small." (German Society for Toxicology eV http://www.toxicologie.de/toxikologie.html)

2) Scientific publications on these results are in preparation, so they cannot yet be presented in detail here.

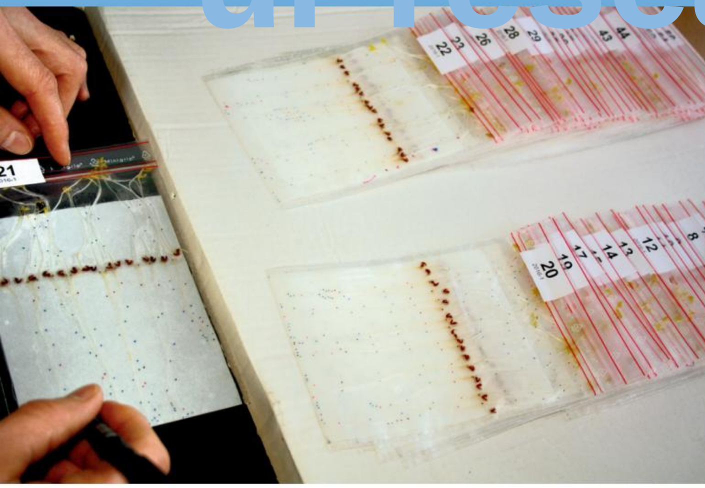

Figure 2: Daily Length measurement of the Rootlets of the treated Seedlings

of horn manure on seedling growth has been confirmed.

2013–2014: the "dry spell"

The first series of tests was continued in 2013–2014, but hardly any significant effects were demonstrated. The reason for this was only understood at the beginning of 2015, when the importance of one parameter was recognized: namely the disruption of the geotropism of the cress seedlings during the daily horizontal positioning of the bags during the length measurements (Fig. 2).

2015–2017: systematic Determination of influencing factors

From 2015 to 2017, influential factors were systematically identified (Fritz and Morau 2017). In 2015, the influence of the disruption of geotropism was examined. In a series of 8 experiments were 2 factors examined; the dosages (control, D1µl and D0.1µl) and the duration of the bags lying horizontally (1, 20, 40, and 60 min). There were highly significant ($p < 0.001$) interactions between the two factors. In the variants with pronounced disturbance (20, 40 and 60 min), root growth was significantly inhibited compared to the disturbance-free variant (1 min) (on average: -14.7%, $p<0.001$). In addition, the treatments with horn manure preparation had a promoting effect on root growth in cases of severe disturbance (compensating effect, Fig. 3).

These effects were highly significant at 40 min (D1µl: + 9.5%, $p<0.001$; D0.1µl: + 8.1%, $p=0.001$), and tended towards 20 min and 60 min. The lying time of 40 minutes was therefore optimal for the test.

The influence of the water volume in the bags was also examined. The results were similar: (1) with high doses of water, the root growth of the cress seedlings was inhibited and (2) only under these stress conditions did the horn manure preparation have a growth-promoting effect.

This resulted in two building blocks for understanding the compensatory effect of the horn manure preparation based on these two stress factors. Furthermore, the influence of light exposure was determined under these stressful conditions. During the daily length measurement, the cress seedlings were illuminated with different light sources for a short time (1 minute): uncontrolled room light or neon light at different strengths (100, 500, 1000, 1500 lux). There were interactions again. The results confirmed the promoting effect of the horn manure preparation, which was present in all light variants.

However, the neon light increased the growth-promoting effect of the horn manure preparation (up to +11.6% at 1000 lux, $p<0.0001$) more than the room light (+3.7%, not significant). This time the interaction between the light and the horn manure preparation was synergistic.

In 2016–2017, the focus was on the composition of cultured water. In the experiments presented above, local well water was used, which turned out to be an undesirable factor that could not be controlled. In order to find a standardizable alternative, various waters (distilled water and mineral water) were examined. The results indicated an influence of the mineral content of the culture water on the effectiveness of the horn manure preparation. Significant effects of horn manure preparation treatments occurred mainly on spring water with a medium mineral content (100–600 mg per liter). Based on these results, a special mineral water was further investigated as water for the cultures (Morau and Fritz 2017). However, the preliminary results showed that the effect of the horn manure preparation in this mineral water was less reproducible than in the local well.

water.

discussion

The investigations from 2011 to 2017 went through several phases. From 2011 to 2013, the test sensitivity and stability were examined over a long period of time. The period 2013–2014 was a "dry spell" in which hardly any significant effects could be demonstrated. In 2015–2017, influencing factors were identified and the stability of the test was thereby better understood. From the

thanksgiving

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Three main conclusions emerge from the results written:

• The repeatability of the effectiveness of the horn manure solution could be guaranteed over several months. This is an important result because the effectiveness of biodynamic preparations was statistically shown in previous studies, but the results were not repeatable. However, this repeatability in the cress test remains unstable over a longer period of time.

• The regulating effect of the horn manure preparation has been statistically proven. This mode of action corresponds to the evidence from previous studies (Dewes and Ahrens 1990, Raupp and König 1996, Goldstein and Barber 2005). Since these studies mostly took place under natural conditions, this correspondence suggests that the test results can be transferred to practical conditions.

• The mode of action of the horn manure preparation was specified by describing a compensatory mode of action against two stress factors (disruption of geotropism, water overdose). interaction Effects with light and water composition have also been described. The sensitivity of the effectiveness of the horn manure preparation in interaction with such different factors indicates that the horn manure preparation increases the adaptability and resilience of plants to their environment. However, with regard to successful test development, other influencing factors must be better understood, standardized and controlled in order to ensure test stability.

The question also arises to what extent the biodynamic production process of the horn manure preparation underlies the effectiveness shown in this cress test (question of test specificity). In fact, substances chemically comparable to the horn manure preparation, such as humic substances, can also show effectiveness in laboratory studies at very low doses. It therefore remains unclear to what extent the horn manure preparation differs in its effectiveness from other substances.

conclusion

The results of this research have proven the test sensitivity and thereby the power viability of the project has been demonstrated. However, the repeatability of the results over long periods of time remains difficult to achieve. It was shown how complex the mode of action of the horn manure preparation is due to the interactions with several factors. The development of such a biotest is a challenge, but it also leads to significant gains in knowledge regarding the effectiveness of the horn manure preparation. In this respect, this research project showed the potential of laboratory studies to determine biodynamic preparations and should be continued. I

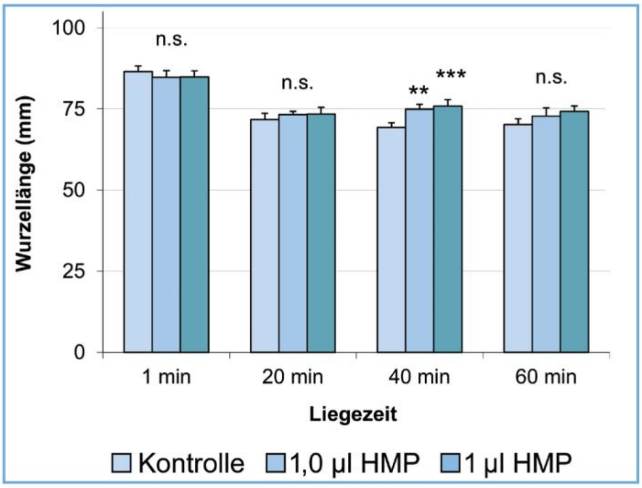

Figure 3: Root length depending on lying time and dosage. Mean values of 8 experiments (bars: standard error). Asterisks indicate significant differences in HMP Treatment for control with the same length of stay: ns not significant; * $0.01<P<0.05$; ** $0.01<P<0.001$: *** $0.001<P$ (Wald statistics, Tukey-Kramer test).

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