Are bryophyte extracts inhibiting or promoting seed growth ?

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Abstract: Germinating tests with bryophytes were undertaken with three moss species and two hepatic species. For the first time these tests were performed with aquaceous extracts of bryophytes in contrast to previous studies in which seeds were directly placed on moist bryophytes. First tests showed an inhibition of the germination rate in 3 species but an increase of the germination in one species. A second study revealed that aquaceous as well as alcoholic extracts in high concentrations inhibited the growth of cress, highly diluted extracts promoted the growth. Other authors found either only germination promoting effects or both, promoting and inhibiting effects. Experiments with compounds extracted from bryophytes cited in the literature had only growth inhibiting effects. Brachythecium rutabulum revealed growth inhibiting effects in a previous but growth promoting effects in this study. The conflicting results are hard to explain and should stimulate further more detailed studies. They maybe due to various combinations of different concentrations of bryophyte extracts, different bryophyte species, different pH and different seeds but also contamination by dust, soil, bacteria and fungi .

Introduction

Bryophytes are known to have a variety of biological activities such as fungicide, bacterizide, molluscicide, activities or antifeedant acitivity (Asakawa 1982, Huneck 1983, Ando & Matsuo 1984). In contrast to most flowering plants, these biological activities are not confined to a single species, species of a genus or a family but concern the whole plant division. Reason is, that flowering plants possess components with biological activities just by chance. They are producing secondary metabolites of which the one or other has a side effect. In contrast, the biological activity of all bryophytes is part of their life strategy. They would not have survived if they would not have a protection against bacteria and fungi, with which they live together on he forest floor without being attacked, or herbivores, which would haven eaten them up without antifeedant activity.

Huneck & Schreiber (1972) were the first describing growth regulatory acitivities, Lunularic acid as well as drimenol, gymnocolin, longiborneol, longifolene and scapanin inhibit cress root growth, oat seedling and coleoptile growth. Lunularic acid inhibits the elongation of Avena coleoptiles. Longifolene promoted the growth of Avena coleoptiles at lower concentrations.

Asakawa (1982) and Huneck (1983) gave a survey of plant growth regulatory acticities of hepatics viz. bryophytes. Polygodial (from Porella arboris-vitae) completely inhibited the germination of rice at a concentration of 100 ppm but promoted root elongation at concentrations < 25 ppm (Asakawa et al. 1980). Plagiochiline inhibited germination of rice at 200 ppm (Matsuo et al. 1981) as well as Epoxyfrullanolide (Askawa et al. 1980).

Matsuo et al. (1981, 1984a, 1984b) analyzed the structures of substances from liverworts having plant growth inhibitory acitivities.
In summary, the biochemical compounds of hepatics usually had a growth inhibiting effect, although the opposite was found at low concentrations.
Other studies revealed effects of seeds in direct contact with moist bryophytes. Huneck & Meinunger (1990) tested 52 species of mosses and 29 species of liverworts on growth regulation acitivity. They added seeds of *Lepidium sativum* to fresh, moistened bryophytes and measured the length of the *Lepidium* roots after 5 days at room temperature. According to the results, they divided the bryophyte species into three groups (1) bryophytes that promote growth of the shoot, (2) bryophytes that promote growth of the root, and (3) bryophytes which retard growth of roots and shoots.
Zhou et al. (1998) described regulating activities of extracts of *Mnium* spp., *Anomodon* spp., *Entodon* spp. and *Bryum* spp. on seeds of corn, sunflower, hot pepper, wheat and tomato. They showed that the germination rate of the seeds increased by 20 to 70 percents. Furthermore, the length of the seedlings germinated in moss extracts were much higher than those germinated in water. The activity of species of *Mnium* was higher than that of *Anomodon.*
The fact that bryophytes have partly growth inhibiting, partly growth promoting activities stimulated additional tests with additional species and different concentrations of the extract.

Materials and methods
Specimens of the hepatic *Porella platyphylla* and the mosses *Eurhynchium striatum*, *Dicranodontium denudatum* and *Brachythecium rutabulum* were collected in the Vosges Mountains, France, in June 1997, and air dried. In contrast to the previous authors, who added seeds to moistened bryophytes, aquaceous extracts were prepared from bryophytes, in which the seeds were germinating. 10,2 g viz. 5,1 g of the bryophytes were soaked 12 hrs in tap water. In addition, 10,2g of bryophyte material was soaked in distilled water. Two ml of the extract were poored on filter paper in petri dishes. Distilled water was used as control ("Nullwert" of the graphs). Five seeds of *Lepidium sativum*, or *Lactuca sativa* were put in every petri dish. The petri dishes were kept in a climate chamber at 16°C and 1500 Lux with 12 hrs dark phase. The length of the germinating roots were measured every day and the results illustrated in a graph.
Stimulated by the conflicting results of the first study, a second study was initiated using aquaceous and alcoholic extracts in differenr concentrations of the liverwort Bazzania trilobata and seed of cress, wheat and radish. Each 6 seeds were places in a petri dish on filter paper moistened with 3 ml extract. The liverwort was extracted in water as well as in ethanol (70%). Each 4 g of the liverwort were homogenized in a blender in 100 ml liquid. The extracts were used in dilutions of 1:100, 1:200 and 1:1000. Bazzania trilobata extracts have been studied for antimicrobial effects (Merkuria). Since the effects varied according to the season in which the plants were collected and the geographical origin, plant material from the United States and France were tested.

Results
In the first study, the effect of the extract of *Brachythecium rutabulum* resulted in an increase of the length of the germinating roots by 30% in *Lactuca sativa* and 10% in *Lepidium sativum* (figs. 1,2). The differences in the concentrations of the extracts did not much effect the results. In contrast, the extracts of *Porella platyphylla* (figs. 3,4) resulted in an inhibition of the germination of cress seeds by 30% and lettuce seeds by 40%. *Eurhynchium striatum* (figs. 5,6) reduces the length of the roots of cress seedlings by 40%, whilst differences in the concentration of the moss extract had almost no difference. The length of lettuce roots was slightly longer after two days but was 20% shorter after 5 days. The extract of *Dicranodontium denudatum* (figs. 7,8) showed no differences compared with the control in both lettuce and cress. The extract in distilled water (10,2g AD in the graphs) gave the highest effects, presumably because of a higher dissolving

capacity. However, since distilled water is unrealistic as compared with the conditions in the nature, tap water was used, too.
In the second study the following results were obtained:
Cress
Undiluted aquaceous liverwort extract showed a distinct inhibition of the growth of the radicula (fig. 9). Diluted aquaceous extracts (1:100, 1:1000) showed no difference as compared with the control.
Alcoholic extracts revealed a slightly promoting effect (ca. 10%) in a dilution of 1:1000 but showed an inhibiting effect (ca. 20%) at a dilution of 1:100 .

Radish
The germination was slightly inhibited by aquaceous extracts but slightly more by alcoholic extracts (fig. 10).

Wheat
Aquaceous extracts (1:100, 1:1000) showed promoting effects, the alcoholic extract (1:1000) a slightly reduced growth (fig. 11).

There were small differences in the results with extracts from different geographic origin but these were to small to be statitistically significant.

Discussion
Most of the biological acitivities of bryophytes are biologically understandable. Thus the direct contact with fungi and bacteria e.g. on the litter of the forest floor requires a protection against these destruents due to the fact that bryophytes have no mechanical protection such as bark or a cuticle. Their soft leaves would be an ideal food for any herbivores, but bryophytes are not eaten by beetles, snails or slugs. Insofar, a seed inhibiting activity would make sense, as it would prevent that a bryophyte would be overgrown by a much larger competitor. The effect of bryophytes to promote germination of seeds of seed plants is, however, ecologically difficult to interpret. However, not every promotion of seedlings by bryophytes or bryophyte extracts are a true promotion. Huneck & Meinunger stated that part of the bryophytes promote growth of the shoots. This could mean that the seedling is using up the nutrients provided by the endosperm without getting adequate roots to continue its growth. So this “promotion” of growth could be interpreted as special tricky method to kill the seedling by exhaustion. It seems to make no sense that bryophytes do not even allow seed plants to germinate in their cushions and do not only supply a favourate, moist habitat but even promote the germination of the seeds of their strongest competitors. The extinction of the bryophyte by nutrient competition and especially in a later stage by shade is programmed by this way. Insofar it is interesting to see that not all bryophyte species have this stimulating effect but according to our results at least some bryophyte species try to inhibit growth of their competitors.
According to Huneck & Meinunger (1990), who tested 80 species of bryophytes, species of Sphagnum showed no promoting effect on seed germination, but this could be due to the low pH in Sphagnum cushions.
The divergent results of the Chinese colleagues (Zhou et al. 1998) stating only germination promoting effects, Huneck & Meinunger (1990) and this study, which showed both promoting and inhibiting effects, is difficult to explain. Of course the problem is very complex, it includes seeds of different flowering plants with different ecological requirements, different bryophyte species growing under different ecological conditions (Sphagnum!) and different treatments (direct contact of seed and wet bryophytes, extracts of different concentration).

A comparison of our results with those of Huneck & Meinunger revealed that aquaceous extract of *Brachythecium rutabulum* promoted the growth of the roots by 150%, whereas the germination in direct contact as reported by Huneck & Meinunger reduced the growth of the roots by about 50% as compared with the control treated with water.

Conclusions

1. Different concentrations of the bryophyte extracts vary the amount of promotion viz. inhibition. Thus growth regulation activity depends on the concentrations, is inhibiting growth at higher and promoting growth at lower concentrations, as suggested by the results of Matsuo et al (1981) and Asakawa (1982).
2. A weak point in all experiments is the fact that bryophytes are affected with dust, humus, soil particles, bacteria, fungi etc., which could have an influence on the results. These components are active when seeds are placed directly on moist bryophytes or unwashed plant material is extracted. The biochemical studies reveal that certain compounds of bryophytes such as lunularic acid inhibit growth of seeds. If this is generalized, all bryophytes should have a growth inhibiting effect. If this is not the case or the opposite, other factors as the compounds of bryophytes mentioned above could have an effect. Therefore studies with washed bryophyte material is suggested.
3. The solution of the problem, under which conditions bryophytes have a germination promoting effect, has a potential commercial aspect: bryophytes (also extracts of bryophytes) have antimicrobial effects. If such an extract would have a promoting effect on seed germination, it would be an ideal stain to treat seeds before sowing.

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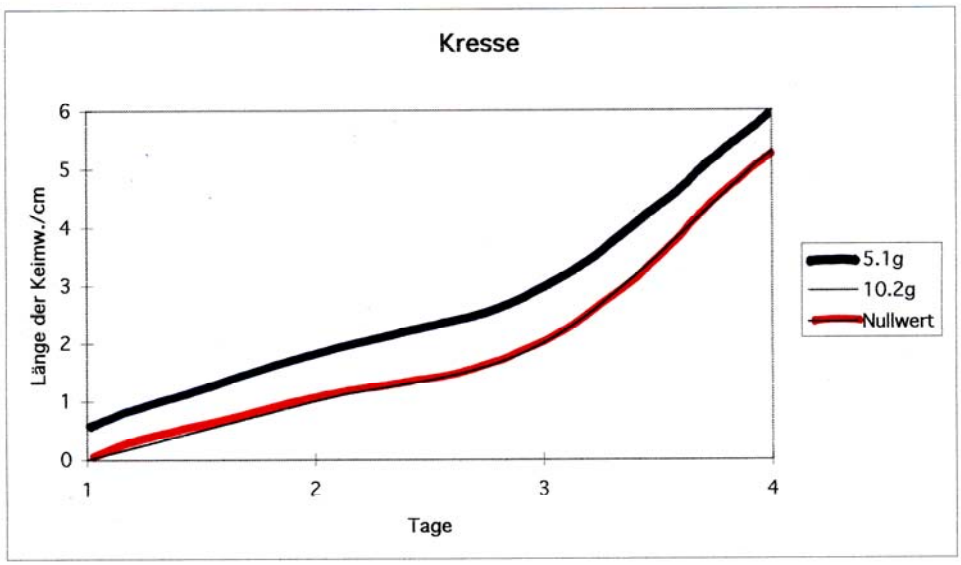

Fig. 1: Effect of an aquaceous extract of Brachythecium rutabulum on the germination of Lepidium sativum. Blue = extract, red = control.

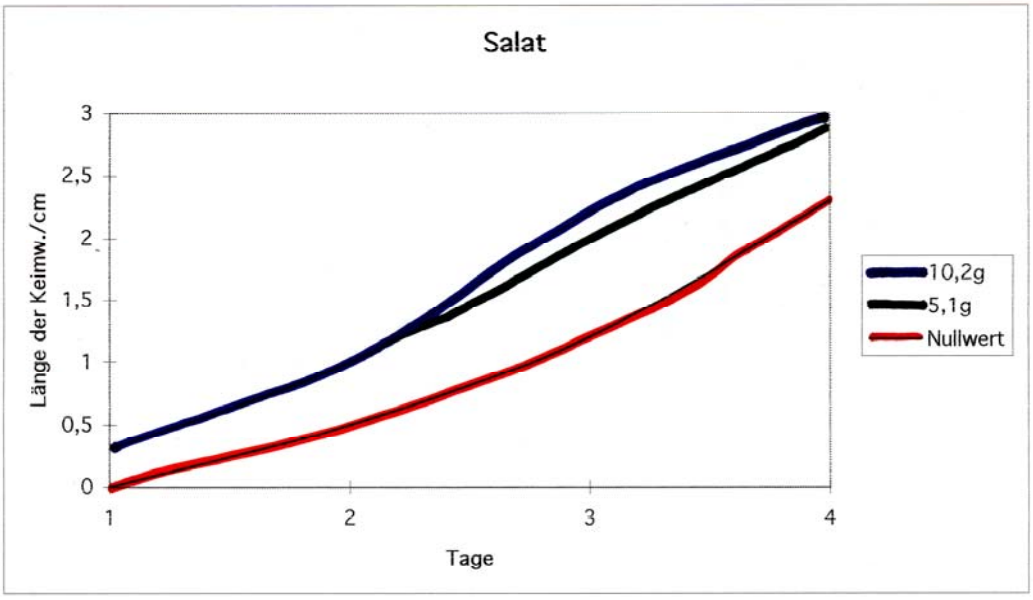

Fig. 2: Effect of an aquaceous extract of Brachythecium rutabulum on the germination of Lactuca sativa. Blue = extract, red = control.

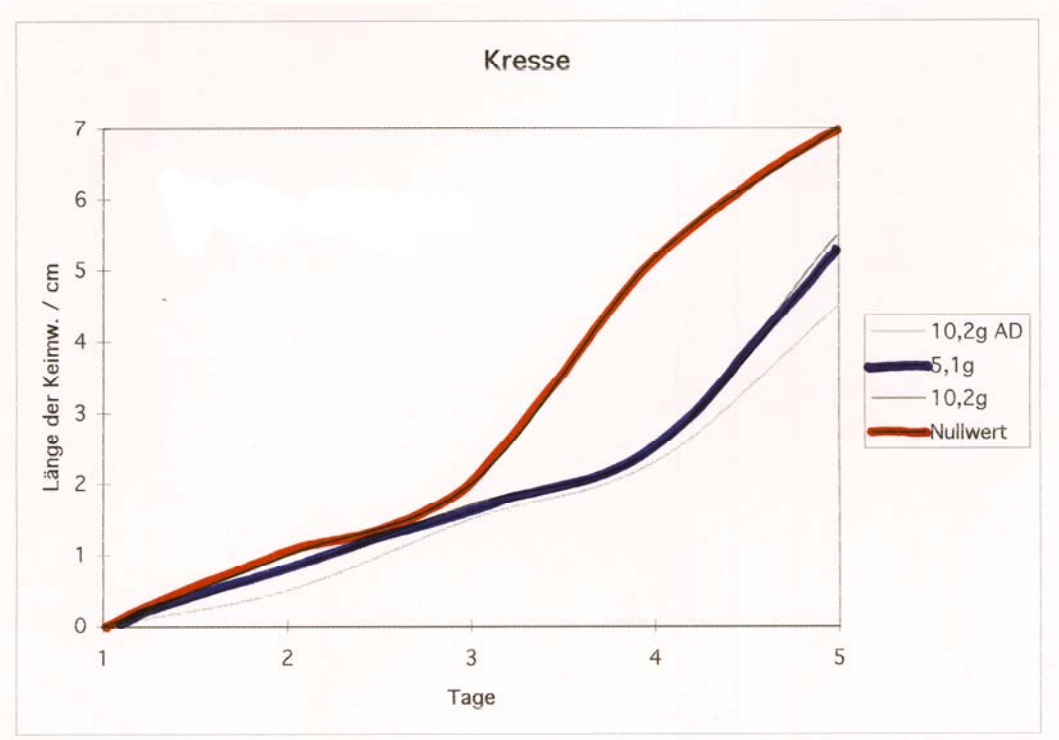

Fig. 3: Effect of an aquaceous extract of Porella platyphylla on the germination of Lepidium sativum. Blue = extract, red = control.

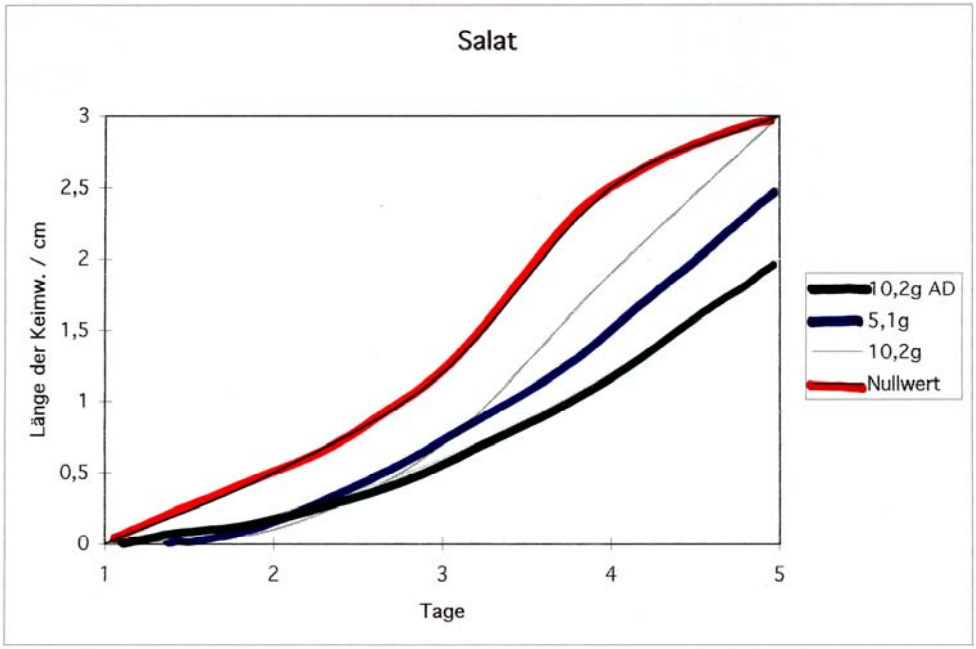

Fig. 2: Effect of an aquaceous extract of Porella platyphylla on the germination of Lactuca sativa. Blue, black = extracts of different concentration, red = control.

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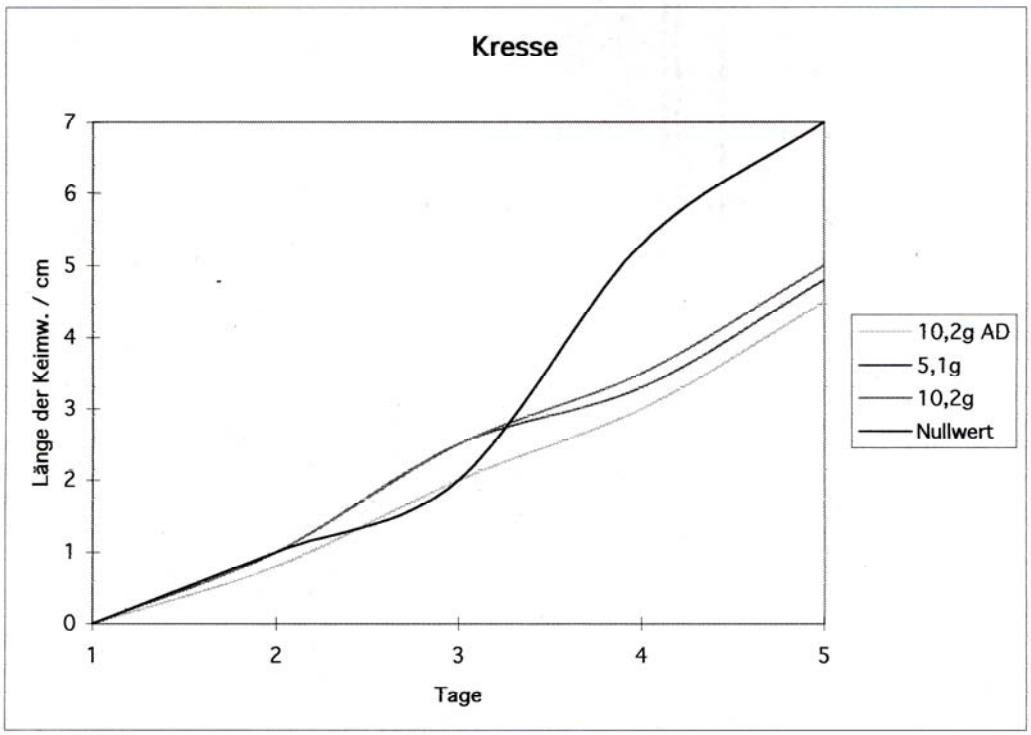

Fig. 5: Effect of an aquaceous extract of Eurhynchium striatum on the germination of Lepidium sativum.

Fig. 5: Effect of an aquaceous extract of Eurhynchium striatum on the germination of Lactuca sativa.

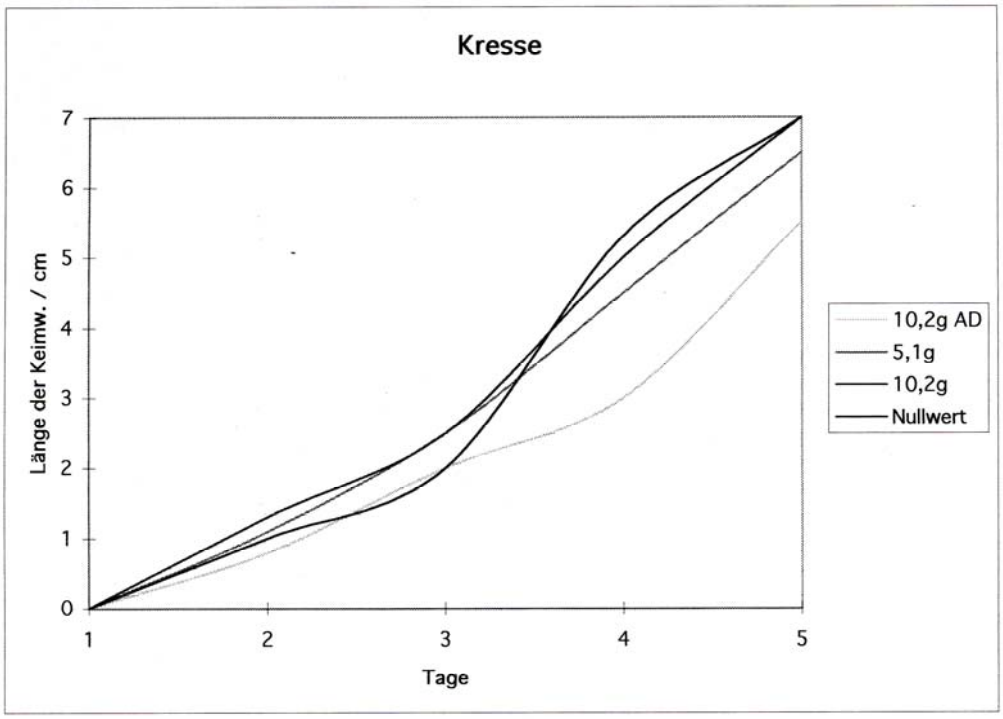

Fig. 7: Effect of an aquaceous extract of Dicranodontium denudatum on the germination of Lepidium sativum.

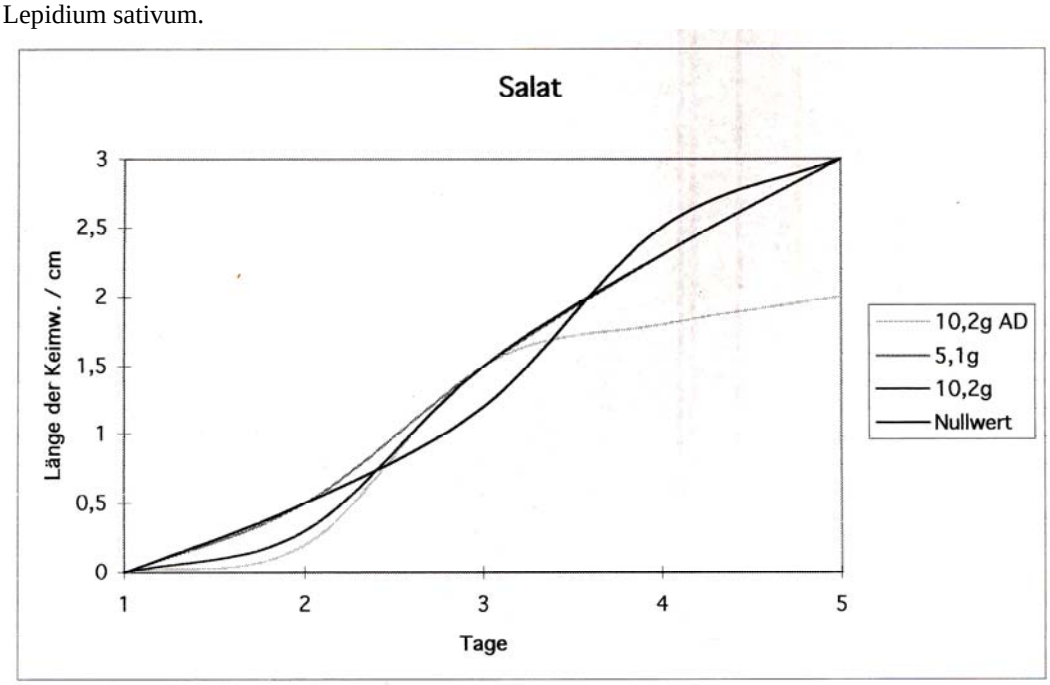

Fig. 8: Effect of an aquaceous extract of Dicranodontium denudatum on the germination of Lactuca sativa.

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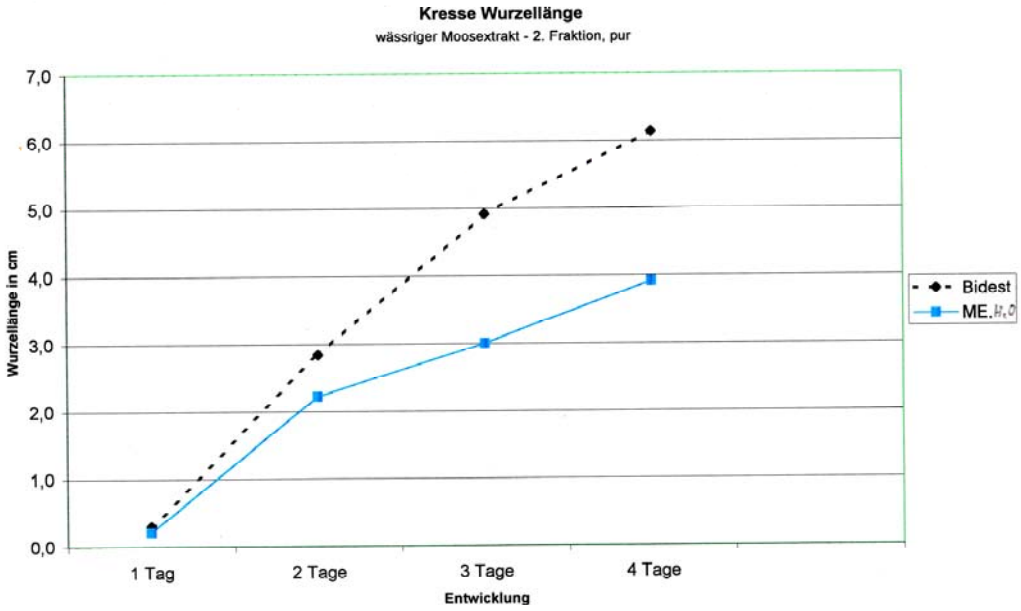

Fig. 9: Effect of undiluted aquaceous Bazzania-extract on the germination of cress.

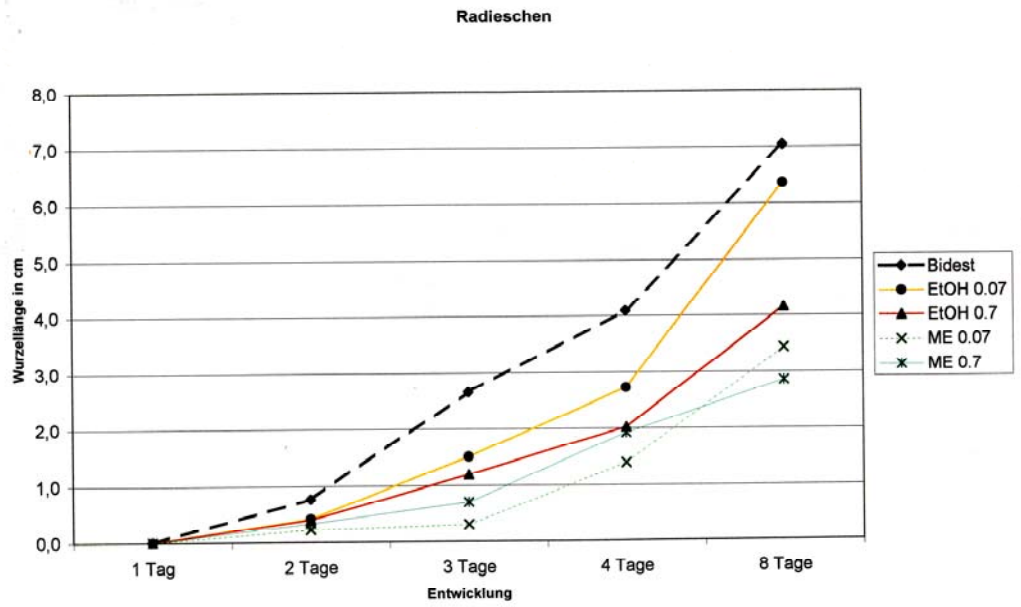

Fig. 10: Effect of various aquaceous (ME) and alcoholic (EtOH) Bazzania-extracts on the germination of radish.

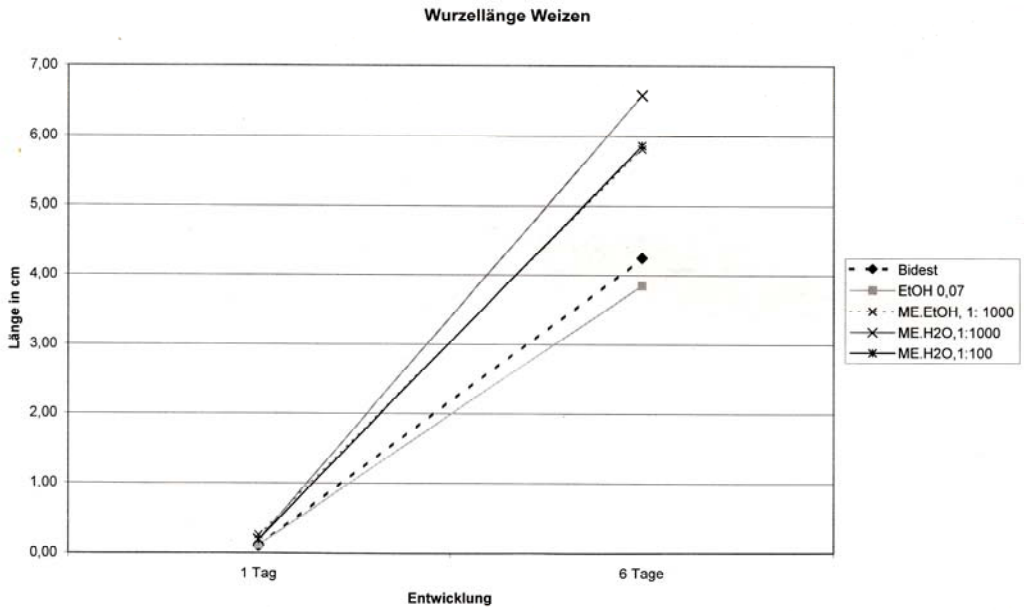

Fig. 11: The effects of aquaceous (ME)and alcoholic (EtOH) extracts on the length of the radicula of wheat.

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