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(54) Title: USE OF LIGNOSULPHONATES FOR PLANT PROTECTION

(57) Abstract: The present invention relates to the use of lignosulphonates in the protection of plants. The lignosulphonates have a variety of applications, such as forming a top layer on the soil in which plants are growing to inhibit emergence of weeds or reduce evaporation of water from the soil. Lignosulphonates also find application in the induction of plant defense and in the control of plant pathogenic nematodes.

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USE OF LIGNOSULPHONATES FOR PLANT PROTECTION

The present invention relates to plant protection methods, such as weed control, inhibition of water evaporation, control of plant pathogens, such as nematodes, and to compositions and substrates for use in these methods.

Control of weeds is an important aspect of agriculture. In common agricultural practice a great variety of herbicides are used to reduce adverse effects of weeds on crop production. The world pesticides sales in 1997 amounted to US\$ 37 billion. More than half of the costs farmers spend on crop protection are linked to reach sufficient control of weeds. These costs have an effect on the price of agricultural products.

Moreover, herbicide application has negative effects on the environment and therefore governmental policies aim at a reduction of the use of herbicides. In several European countries policy documents have been drafted in which farmers are being asked to achieve substantial reductions in the use of herbicides.

Organic farming is of growing importance in Europe. An organic farmer is not allowed to use any herbicides. Since mechanic methods like hoeing often do not result in sufficient weed control, very expensive hand-weeding is required to solve weed problems on organic farms. Agro-economic studies have shown that the presently required amount of hand-weeding is one of the major constraints to a further increase of organic farming in countries like the Netherlands.

An important factor to achieve reductions in the use of herbicides is the development of weed preventive or alternative methods for weed control. Possibilities to prevent weed germination and establishment are essential components of modern strategies for weed control or what is called "integrated weed management". Moreover, insights in the competitive relation between crop and weeds have led to the

conclusion that a complete elimination of the weeds is often not necessary. Negative effects occur only above a minimum weed development. Also the crop itself can play a role in weed control, provided that the development of the crop is ahead of the growth of weeds. As soon as the crop canopy is closed, that means that no open spots are present, the development of new weed plants is strongly suppressed.

It is thus a first object of the present invention to provide a new method for weed control that is environmentally friendly and relatively cost-effective.

Another problem encountered in the agricultural industry is evaporation of water from in particular sandy soils. Sandy soils have difficulty in retaining water and easily become too dry for crop production. Also, the vegetation already existing on dry sandy soils may die because of water evaporation. Because of this problem, frequent irrigation is necessary. Pumping up of large amounts of water from the soil may result in lower levels of groundwater thus leading to a vicious circle.

A further problem related to water evaporation is drying out of the soil in pots during dry or hot weather. Such problems may be encountered in the horticultural industry, in the production of pot plants for in-house or garden use and at a consumer level.

The second object of the invention is therefore to provide a method for preventing or inhibiting evaporation of water from soils and other substrates for plant growth.

Another threat to plants are various pathogenic microorganisms. It is known that plants can be immunized against microorganisms (Lyon et al. 1995. "Novel disease control compounds: the potential to "immunize" plants against infection". In: Plant Pathology 44: pp 407-427).

The plant uses certain toxins for its defense against microorganisms. These toxins, the so called phytoalexins, inhibit or hamper growth and development of the pathogens

in the plant tissue. Each plant species forms several (different types of) phytoalexins. The signal for the plant to start with the synthesis of the phytoalexins are the so called elicitors produced upon attack of
5 infection. It is desirable to be able to dispose of further environmentally friendly elicitors for immunization of plants.

Therefore, it is a further object of the invention to provide a new elicitor.

10 Plant-parasitic nematodes are a further major threat to agriculture world wide. Almost all crops are attacked by plant-parasitic nematodes. Damage estimates range from 5-25 % yield reduction, but 100 % losses occur locally, due to plant death or quality loss. Nematodes
15 are conventionally managed by crop rotation and chemical control. For many nematode problems crop rotation is not very efficient, due to the large host range of the nematodes and the lack of suitable resistant crops or plant varieties.

20 Chemical control often uses compounds that are dangerous to the environment and the user. Many effective nematicides have been banned due to these risks. A widely used and effective nematicide, methyl bromide, will be banned in the USA and the EU shortly, leaving the farmers
25 with very little management options for nematode problems. Biological control could be an alternative to chemical control, but at the moment no commercial nematode biocontrol products are available on the European or American market.

30 According to a further aspect thereof, it is thus an object of the present invention to provide a means for the control of plant pathogens, in particular nematodes.

It was surprisingly found that all these
35 objects can be achieved by the use of lignosulphonates. Lignosulphonates (LS) are products resulting from the pulping process of trees. They are separated from the cellulose fibers of the wood where they acts as a binder

between the fibers. LS are isolated as a mixture of molecules that are typical repeating monomers of the formula $[\text{CH}_2\text{OHCHCH}(\text{OH})_{0.5}(\text{SO}_3)_{0.5}\text{C}_6\text{H}_3\text{O}(\text{CH}_3\text{O})]_n$.

Therefore, the invention according to a first aspect thereof relates to a method for controlling weed growth in crops, which method comprises the formation of a top layer on the soil in which the crop is growing, which top layer has a mechanical resistance that exceeds the germinal force of the germinating weed, which top layer is formed by application of lignosulphonates on or in the soil. Thus, the emergence of the weeds is hampered or inhibited at least during some time to allow the crop plants to grow sufficiently to be able to compete with the weeds.

The top layer can be either formed after emergence of the crop plants from the soil or before sowing. In the latter case the layer has a mechanical strength that prevents or inhibits the emergence of weed plants but allows the emergence of crop plants.

The amount of lignosulphonates applied to the soil after emergence of the plants is 60 to 2000, preferably 300 to 1200, more preferably 300 to 900, most preferably 480 g/m².

When the top layer is formed before sowing the amount of lignosulphonates applied to the soil is 60 to 2000, preferably 300 to 1200, more preferably 300 to 900, most preferably 600 g/m².

A further aspect of the invention relates to a method for reducing the evaporation of water from soils, in particular sandy soils, which method comprises the formation of a top layer on the soil in which the crop is growing by application of lignosulphonates on or in the soil. For this application, the amount of lignosulphonates applied to the soil is 60 to 2400, preferably 300 to 2200, more preferably 800 to 2000, most preferably 1800 g/m². The top layer thus formed closes off the soil and inhibits or prevents the evaporation of water thereunder.

The invention further provides a method for immunizing plants against microorganisms, which method comprises application to the plant of a solution of lignosulphonates. The lignosulphonates may be combined
5 with one or more fungicides to obtain an enhanced or synergistic effect.

In addition, a method is provided for the protection of plants against nematodes, comprising the application of lignosulphonates in the substrate in which
10 the plant is growing. Lignosulphonates will usually be applied against nematodes in soil or in artificial substrates. The most efficient application will be in the situation where plant establishment and the first period of growth is important for final yield. Specific
15 situations in which lignosulphonates can be used are described hereinbelow. These examples are however not intended as limitations.

Protection of sugar beet seedlings against nematode attack (for instance against Heterodera
20 schachtii and Paratrichodorus teres) can be obtained by in-furrow application of a lignosulphonate slurry at seeding.

For potatoes, it is possible to coat seed potatoes with lignosulphonates or apply a lignosulphonate
25 slurry at planting of the tubers. This will protect the potato plant against early attack by Pratylenchus, Paratrichodorus, Globodera, Meloidygyne and other nematodes, that are most harmful during the first period of potato growth.

30 Protection of tulips against tobacco rattle virus, which is transmitted by Paratrichodorus and Trichodorus nematodes, can be achieved by coating of tulips or application of lignosulphonate slurry at planting. Tulip is sensitive to tobacco rattle virus
35 transmission only for a limited period after planting and the strong effect of lignosulphonates on the vector nematode Paratrichodorus teres shows that virus transmission will be inhibited.

A further application of lignosulphonates is for amelioration of replant problems. Replant problems are often caused by nematodes, that attack young trees or shrubs that are replanted at a site where the same species was growing before. Addition of lignosulphonate slurry in the plant hole will inhibit nematode (Pratylenchus, Meloidogyne and others) attack and give the young plant a better chance of establishing a vital root system. Replanting can take place in soil but also in other substrates, such as potting soil, vermiculite, coconut fibers etc. These substrates can be pre-treated with the lignosulphonates. Such pre-treated substrates for plant growth are also part of this invention.

The invention furthermore relates to the use of lignosulphonates in the applications described above and to compositions that contain lignosulphonates and are intended for the above identified use.

Such compositions comprise lignosulphonates in an amount to meet the required goal as described above. An optimal amount lies usually around 1 kg/m². The compositions take usually the form of a solution that can be sprayed. Such solution contains about 10-55%, preferably around 30% LS. LS does usually not dissolve above an amount of 55% without additional measures.

In this application the term "lignosulphonates" is used to indicate either a mixture or isolated lignosulphonate molecules. Usually a crude mixture is used, but the invention may in some applications, such as immunization, benefit from the use of pure LS. Crude mixtures still contain 5 to 10% reducing sugars that may lead to stickiness of plant parts, such as leaves, when a solution thereof is sprayed onto or otherwise applied to the plant. Crude mixtures are however more cost-effective as they do not require a further fractionation to remove the sugars. "Lignosulphonates" as used in this application are usually Ca-lignosulphonates unless otherwise indicated, such as NH₄-lignosulphonates.

The invention will be further illustrated in the examples that follow and are not given to limit the invention. In the examples reference is made to the following figures:

5 **Figure 1:** Effect of LS on germination of wheat and weed.

Figure 2: Effect of LS and Eupareen™ on infection of Botrytis cinera spores on tomato leaves. Values are averages $\pm$ SD.

10 **Figure 3:** Experiments on the effect of Ca-lignosulphonates on the attack of plants by various phytoparasitic nematodes.

Figure 4: Attractiveness of Ca-lignosulphonates and NH_4 -lignosulphonates to Pratylenchus penetrans.

15 Presented is percentage nematodes preferring the test solution to the control. Control was demineralised water. The experiment had 5 replications.

EXAMPLES

20 EXAMPLE 1

In an experiment plant pots with a diameter of 14 cm were filled with sandy soil contaminated with seeds of both dicotyledonous and monocotyledonous weeds. In these plant pots 15 maize and wheat seeds were planted. Hereafter, the surfaces of the pots were sprayed with different amounts of a 30% solution of lignosulphonates (LS) (30 g LS in 100 ml water). The total amount of LS applied to the pots were 0, 10, 20, or 30 g. Fourteen and eighteen days after sowing 20 ml of water was sprayed on top of the soil in the pots. After 8, 12 and 19 days seed germination of weeds and of the wheat and pea seeds was determined (table 1).

Table 1

	Amount of LS sprayed on soil in pot	Number of germinated weed (w), wheat (t) or pea (p) seeds after 8 days	Number of germinated weed (w), wheat (t) or pea (p) seeds after 12 days	Number of germinated weed (w), wheat (t) or pea (p) seeds after 12 days
5	0	5(w)	7(t), 2(p), 5(w)	7(t), 3(p), 9(w)
	10	(2t)	10(t), 1(p)	10(t), 1(p), 1(w)
	20	-	5(t)	6(t), 2(p), 2(w)
	30	-	-	1(p)

10 Table 2

	Amount of LS sprayed on soil in pot	Number of germinated weed (w), wheat (t) or pea (p) seeds after 8 days	Number of germinated weed (w), wheat (t) or pea (p) seeds after 12 days	Number of germinated weed (w), wheat (t) or pea (p) seeds after 12 days
15	0	1(t), 6(w)	10(t)	7(t), 3(p), 9(w)
	10	1(t)	10(t), 1(p)	10(t), 1(p), 1(w)
	20	-	5(t)	6(t), 2(p), 2(w)
	30	-	-	1(p)

The experiment presented in table 1 was repeated with 20 only wheat and weed seeds (table 2).

The results from table 1 and 2 show that the weed seeds rarely germinate in the soil after application of 10 g of LS or more. In contrast, the germination force of the wheat seeds is enough to break through the soil 25 treated with LS. Application of LS concentrations lower than 10 g per pot also significantly reduced weed germination. So, LS can be used to control weed formation. If crop seeds are planted after the LS application, the large crop seeds can easily germinate 30 while the small weed seeds can not.

EXAMPLE 2Reduction of evaporation by lignosulphonates

In a pot experiment without seeds evaporation related to LS application was measured (table 3). The experimental set-up was as described for the experiments described in Example 1 (tables 1 and 2).

Table 3

Amount of LS sprayed on soil in pot	Evaporation (ml) from the surface area in 4 days	Evaporation (ml) from the surface area after 7 days
0	19.8	30.1
10	18.5	27.2
20	17.8	28.2
30	12.8	20.8

Application of 30 g LS per pot led to a reduction of 34% after 4 days and 31 % after 7 days. This shows that LS can be used to overcome water losses due to evaporation.

EXAMPLE 3Induction of plant defense by LS

In an experiment it was tested if the large LS molecules were suited to immunize plants against attack by microorganisms.

Six weeks old tomato plants were sprayed with 20 ml of:

- the fungicide Eupareen™ (recommended concentration of the manufacturer)
- Eupareen™ 20% of the recommended concentration
- 1 mM LS (the average molecular weight was 10.000 g/mol)
- 1 mM LS + 20 % Eupareen™

The control plants were sprayed with water.

One day after this treatment the plants were infected by applying 1 μ l Botrytis cinera spores (12000 spores per μ l) in Gamborg B5 medium (Gamborg 3.16 g/l;

Sodium phosphate 10 mM pH = 6.5; 10 mM sucrose) on the third and fourth leaf counted from the basis of the plant. The spores were treated before application as described by Benito et al. (1998) "Fungal and plant gene
5 expression during synchronized infection of tomato leaves by Botrytis cinera". In: European Journal of Plant Pathology 104: pp 207-220. The spores were applied (pipet) in duplicate on five leaf segments of two leaves per plant. Eight plants were used per treatment.

10 Five days after applying the Botrytis spores, the diameter of the infections were measured (Figure 2). The average infection size was significantly reduced by all treatments compared to the control treatment with water. The effectiveness of LS 1 mM and Eupareen™ were
15 comparable and significantly better than treatment with 20% Eupareen™. Treatment of the plants with 20 % Eupareen™ + 1 mM LS was the most optimal treatment and significantly better than all other treatments.

These results show that LS, which is
20 environmentally acceptable is equally effective as the fungicide. Combination of LS with low doses of fungicide leads to even further reduction in the susceptibility of plant leaves for fungi (Botrytis).

25 **EXAMPLE 4**

Protection against nematodes by LS

The effect of Ca-lignosulphonates was tested on two plant parasitic nematodes: Pratylenchus penetrans and Paratrichodorus teres. Pratylenchus penetrans is an
30 endoparasitic nematode, feeding inside the plant roots, whereas Paratrichodorus teres is ectoparasitic, feeding on the outside of the plant roots. Three experiments were performed:

Experiment A:

Effect of Ca-lignosulphonates on survival and feeding of Paratrichodorus teres.

5 Experimental set-up

Two-week old petunia plans were transferred to 150-ml pots filled with fine dune sand. The pots were placed in a growth cabinet at 15°C, 80 % relative humidity and provided with water through a watering blanket. The water potential was 1733 Pa. See **Figure 3**. After four days a Ca-lignosulphonate solution was added in a dose of 10 g (dry content) per Kg soil. The Ca-lignosulphonates were suspended in water (25 g per 100 ml) and pipetted into two 4-cm deep holes next to the stem of the plant.

Three days after addition of the Ca-lignosulphonates 100 individuals (adults and juveniles mixed) of Paratrichodorus teres were added, suspended in 5 ml of water. The nematodes were pipetted into two holes of 4 cm deep, next to the plant stem.

One week after the inoculation the nematodes were recovered from the sand by decanting and filter extraction (according to J.J. 's Jacob and J. van Bezooijen, "A Manual for Practical Work in Nematology", 77pp, Wageningen Agricultural University (1986)).

The number of Paratrichodorus teres individuals were counted and the feeding state was assessed by estimation of the percentage of the gut filled with food. The animals were classified as well fed (gut >50 % full) and not well fed (gut < 50 % full). The control of the experiment had the same set-up as the treatment with Ca-lignosulphonates, except that instead of the lignosulphonates a corresponding volume of water was added to the pots. The experiment had 4 replicates. The results of the experiment were tested for statistical significance with analysis of variance, using the Genstat V statistical software.

Results of experiment A:

Ca-lignosulphonates addition resulted in a strong reduction in recovery of Paratrichodorus teres. (Table 4). This effect was highly significant ($p < 0.002$). Furthermore, the percentage of well-fed individuals was significantly lower ($p < 0.007$) in the pots treated with Ca-lignosulphonates.

Table 4

Effect of Ca-lignosulphonates on survival and feeding of Paratrichodorus teres. Represented are means ($n = 4$) $\pm$ standard deviation.

Treatment	Number of <u>Paratrichodorus teres</u> recovered	% of <u>Paratrichodorus teres</u> well fed
control (water)	75.0 $\pm$ 16.4	71.1 $\pm$ 12.0
Ca-lignosulphonates	18.8 $\pm$ 15.4	40.1 $\pm$ 9.9

Experiment B

Effect of Ca-lignosulphonates on root penetration by Pratylenchus penetrans.

Experimental set-up

Two-week old leek plants were transferred into 150-ml pots filled with a mixture of coarse sand and potting soil (1 : 4 v/v). There were seven plants per pot. After four days a Ca-lignosulphonate solution was added in a dose of 10 g (dry content) per Kg soil. The Ca-lignosulphonates were suspended in water (25 g per 100 ml) and pipetted into two 4-cm deep holes next to the stem of the plant.

Three days after addition of the Ca-lignosulphonates 500 individuals (adults and juveniles mixed) of Pratylenchus penetrans were added, suspended in 5 ml of water. The nematodes were pipetted into two holes of 4 cm deep, next to the plants.

One week after inoculation the plants were removed from the pots. The roots were rinsed free of

adhering soil and mixed in a Waring blender for 20 seconds, at the low speed setting. The root suspension was filtered over a cotton wool filter (J.J. 's Jacob and J. van Bezooijen, 1986, supra). Active nematodes were 5 recovered after 48 hrs of extraction and counted.

The control of the experiment had the same set-up as the treatment with Ca-lignosulphonates, except that instead of the lignosulphonates a corresponding volume of water was added to the pots. The experiment had 5 10 replicates. The results of the experiment were tested for statistical significance with analysis of variance, using the Genstat V statistical software.

Experiment B was performed under the same conditions as experiment A (see above) and at the same 15 time.

Results of experiment B

Addition of Ca-lignosulphonates reduced the number of individuals of Pratylenchus penetrans recovered 20 from the plant roots significantly ($p < 0.003$) (Table 2).

Table 5
Effect of Ca-lignosulphonates root penetration by Pratylenchus penetrans. Represented are means ($n = 5$) $\pm$ 25 standard deviation

Treatment	Number of <u>Pratylenchus penetrans</u> recovered
control (water)	12.2 $\pm$ 5.2
Ca-lignosulphonates	2.0 $\pm$ 1.8

Experiment C

Effect of NH_4 - and Ca-lignosulphonates on the migration of Pratylenchus penetrans on agar

5 Experimental set-up

The experiment was carried out in 5-cm plastic Petri dishes, without notches. The agar used was technical agar no. 3, Oxoid, at a concentration of 15 g/l. The agar is dispersed in demineralised water and autoclaved for 20 minutes at 120°C. Two ml of agar are dispensed per Petri dish. After cooling of the agar, the nematodes are placed in the middle of the agar dish, while the substances tested are spotted on 2 cm distance from the center. The nematodes (about 100 individuals per sample, juvenile and adult stages mixed) are dispensed in a droplet of 50 μl tap water. The amount of test solution added was 25 μl of Ca- or NH_4 -lignosulphonates at a concentration of 5 g/l. After application of the nematodes and the test solutions, the agar dishes are placed in a flow hood, to let the droplets dry.

Since the nematodes can move freely over the agar only when the droplet in which they are applied has dried, the moment of drying of the droplets is considered the starting time of the experiment. After the droplets have dried, the Petri dishes were placed in a plastic box, lined with wet filter paper to ensure 100 % relative air humidity and to prevent drying out of the agar. The boxes with Petri dishes were stored in an incubator at 20°C. Nematode activity was recorded by counting the number of nematodes in a radius of 1 cm from the spot where the test solutions were applied. Counts were made at 2, 8 and 23 hr after start of the experiment.

Attractiveness of the test solution was expressed as percentage of the nematodes preferring the test solution to the control. Only nematodes inside the test sections of the agar dish are accounted for in this measure. Non-mobile nematodes at the point of nematode application or active nematodes outside the test sections

are not taken into account. The following formula to calculate attractiveness was used:

Attractiveness = $(\# \text{ nema in section 1} / (\# \text{ nema in section 2} + \# \text{ nema in section 1})) \times 100$,

- 5 In which section 1 is the treated section and section 2 is the control section. Attractiveness is expressed as percentage: a value of 50 % means no effect (test solution and control equally attractive). For statistical testing analysis of variance was performed on log-
10 transformed counts. The experiment had 5 replicates.

Results experiment C

- Ca-lignosulphonates were repulsive to Pratylenchus penetrans, during the whole observation
15 period. NH_4 -lignosulphonates were less repulsive than Ca-lignosulphonates. Only at the first observation time there was a significant repulsion effect (Figure 4).

Conclusions of experiment A, B and C

- 20 Addition of Ca-lignosulphonates caused a reduction of survival and feeding of Paratrichodorus teres and of root penetration by Pratylenchus penetrans. This shows that Ca-lignosulphonates are capable of inhibiting and/or killing both ectoparasitic and
25 endoparasitic nematodes. The effect sizes were considerable, at a dose that is not unrealistic in field application at seeding or planting, when Ca-lignosulphonates are used in an in-furrow application, like most conventional nematicides are used.
30 Ca-lignosulphonates can protect plants form attack by both ecto- and endoparasitic nematodes, through direct nematicidal action and possibly through increase of the plant defense reaction and disturbance of nematode orientation.

CLAIMS

1. Use of lignosulphonates in plant protection.
2. Use as claimed in claim 1, wherein the plant
5 protection resides in the control of weeds.
3. Use as claimed in claim 1, wherein the plant
protection resides in the reduction of water loss.
4. Use as claimed in claim 1, wherein the plant
protection resides in the induction of plant defense.
- 10 5. Use as claimed in claim 1, wherein the plant
protection is against nematodes.
6. Method for controlling weed growth in crops,
which method comprises the formation of a top layer on
the soil in which the crop is growing, having a
15 mechanical resistance that exceeds the germinal force of
the germinating weed, which top layer is formed by
application of lignosulphonates on or in the soil.
7. Method as claimed in claim 6, wherein the
top layer is formed after emergence of the crop plants
20 from the soil.
8. Method as claimed in claim 7, wherein the
amount of lignosulphonates applied to the soil is 60 to
2000, preferably 300 to 1200, more preferably 300 to 900,
most preferably 480 g/m².
- 25 9. Method as claimed in claim 6, wherein the
top layer is formed before sowing and has a mechanical
strength that prevents or inhibits the emergence of weed
plants but allows the emergence of crop plants.
10. Method as claimed in claim 9, wherein the
30 amount of lignosulphonates applied to the soil 60 to
2000, preferably 300 to 1200, more preferably 300 to 900,
most preferably 600 g/m².
11. Method for reducing the evaporation of
water from soils, in particular sandy soils, which method
35 comprises the formation of a top layer on the soil in
which the crop is growing by application of
lignosulphonates on or in the soil.

12. Method as claimed in claim 11, wherein the amount of lignosulphonates applied to the soil is 60 to 2400, preferably 300 to 2200, more preferably 800 to 2000, most preferably 1800 g/m².

5 13. Method for immunizing plants against microorganisms, which method comprises application to the plant of a solution of lignosulphonates.

14. Method as claimed in claim 13, wherein the lignosulphonates are combined with one or more
10 antimicrobial agents, such as fungicides.

15. Method for the protection of plants against nematodes, comprising the application of lignosulphonates in the substrate in which the plant is growing.

16. Method as claimed in claim 15, wherein the
15 lignosulphonates are applied to the substrate upon seeding.

17. Method as claimed in claim 16, wherein the lignosulphonates are applied in-furrow.

18. Method as claimed in claims 15-17, wherein
20 the lignosulphonates are applied to the soil via a coating of the seeds.

19. Method as claimed in claim 15, wherein the lignosulphonates are applied to the soil upon planting bulbs.

20. Method as claimed in claim 19, wherein the
25 lignosulphonates are added to the planting hole for the bulb.

21. Method as claimed in claims 15, 19 and 20, wherein the lignosulphonates are applied to the soil via
30 a coating of the bulbs.

22. Method as claimed in claim 15, wherein the lignosulphonates are applied to the soil upon planting tubers.

23. Method as claimed in claim 22, wherein the
35 lignosulphonates are added to the planting hole for the tuber.

24. Method as claimed in claims 15, 22 and 23, wherein the lignosulphonates are applied to the soil via a coating of the tubers.

25. Method as claimed in claim 15, wherein the
5 lignosulphonates are applied to the soil upon planting or replanting of plants, trees or shrubs.

26. Method as claimed in claim 25, wherein the lignosulphonates are added to the planting hole for the plant, tree or shrub.

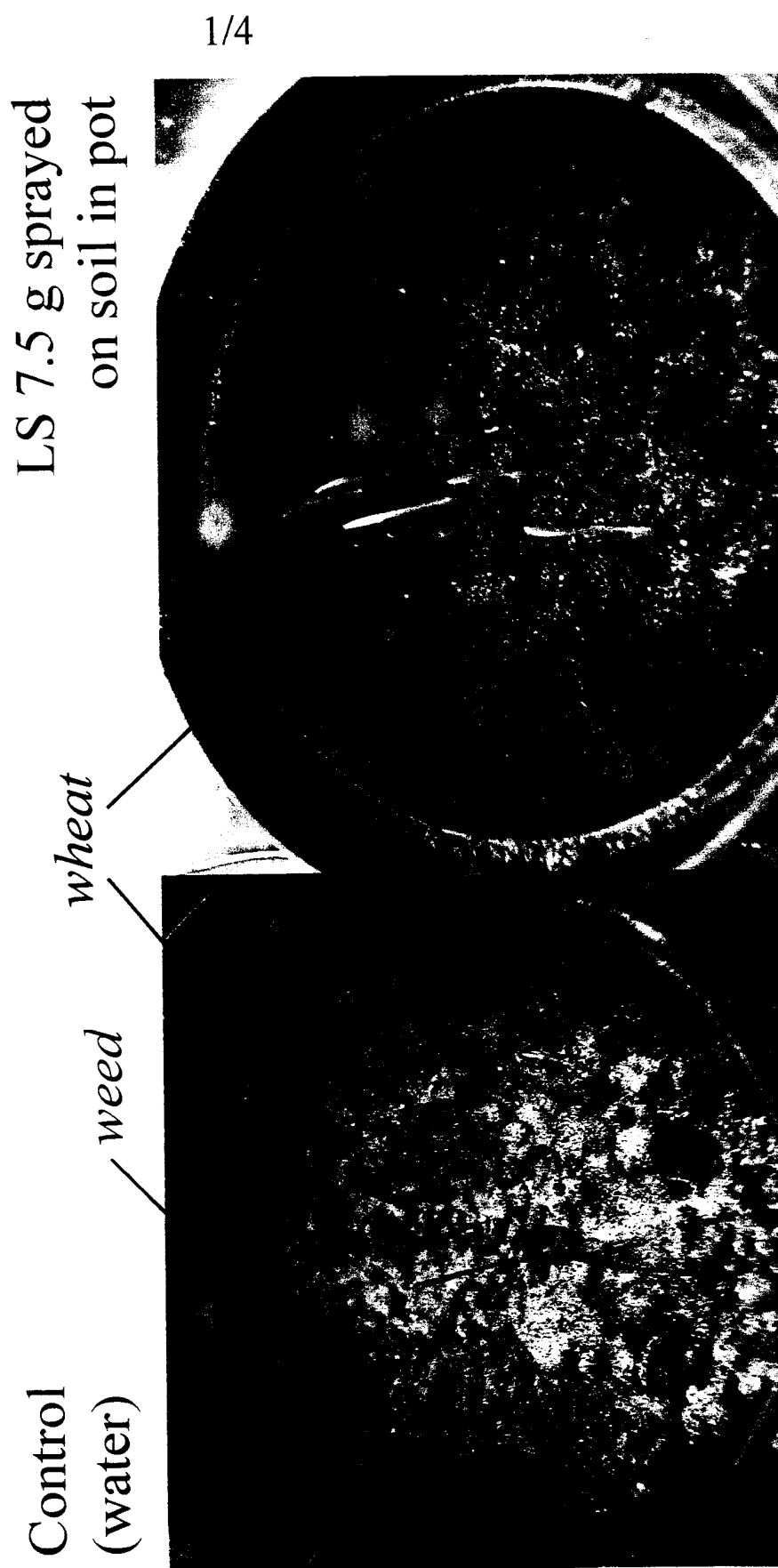
10 27. Composition for use in plant protection and development, which composition comprises a solution of lignosulphonates.

28. Substrate for growing plants, which substrate is mixed with lignosulphonates.

15 29. Substrate as claimed in claim 28, wherein the substrate is selected from the group consisting of soil, potting soil, vermiculite, coconut fibers.

FIG. 1

Effect of LS on germination of wheat and weed



Effect of LS in combination with eupareen on immunization against Botrytis

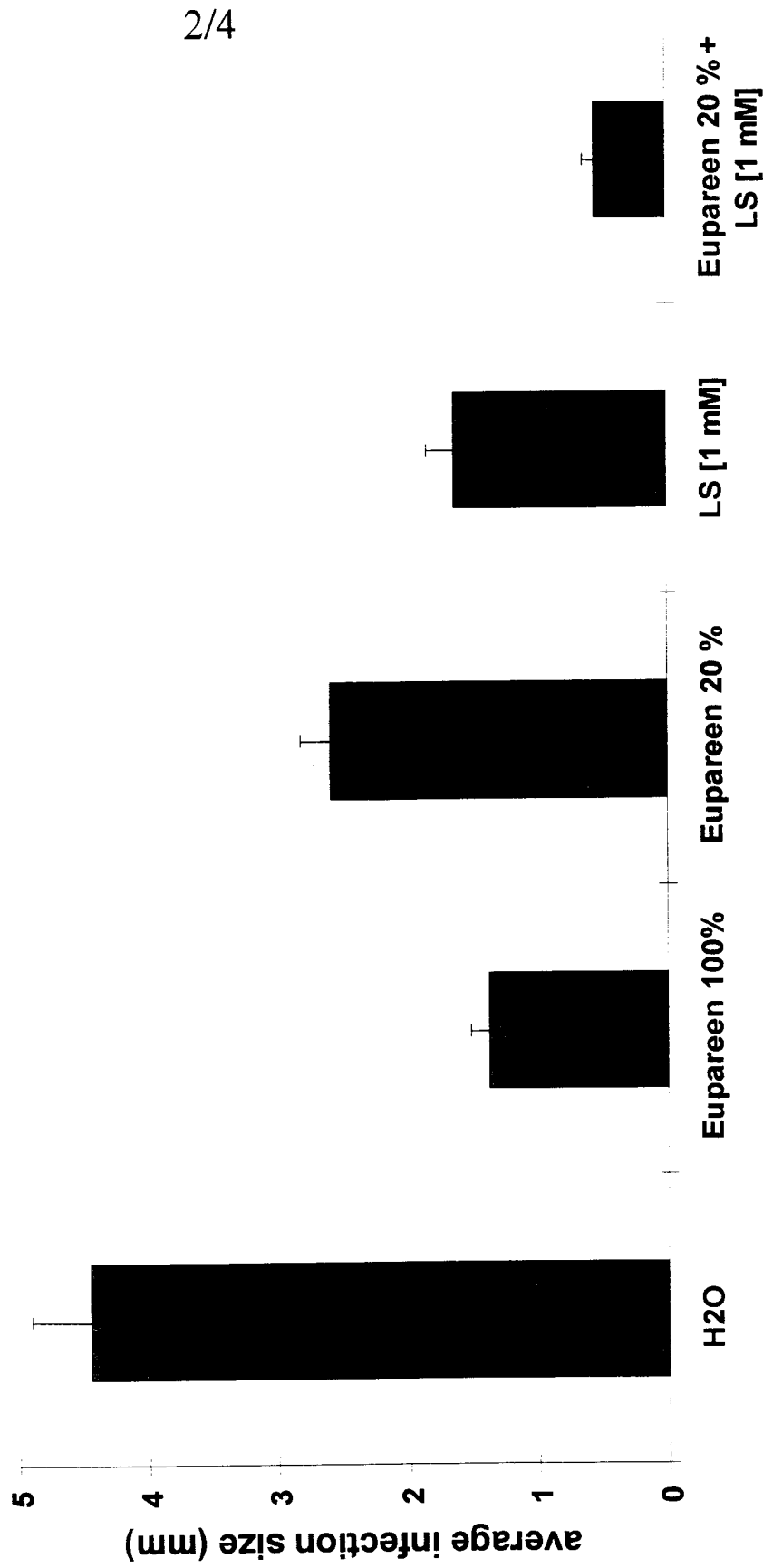


FIG. 2

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FIG. 3

Attractiveness ligno-compounds

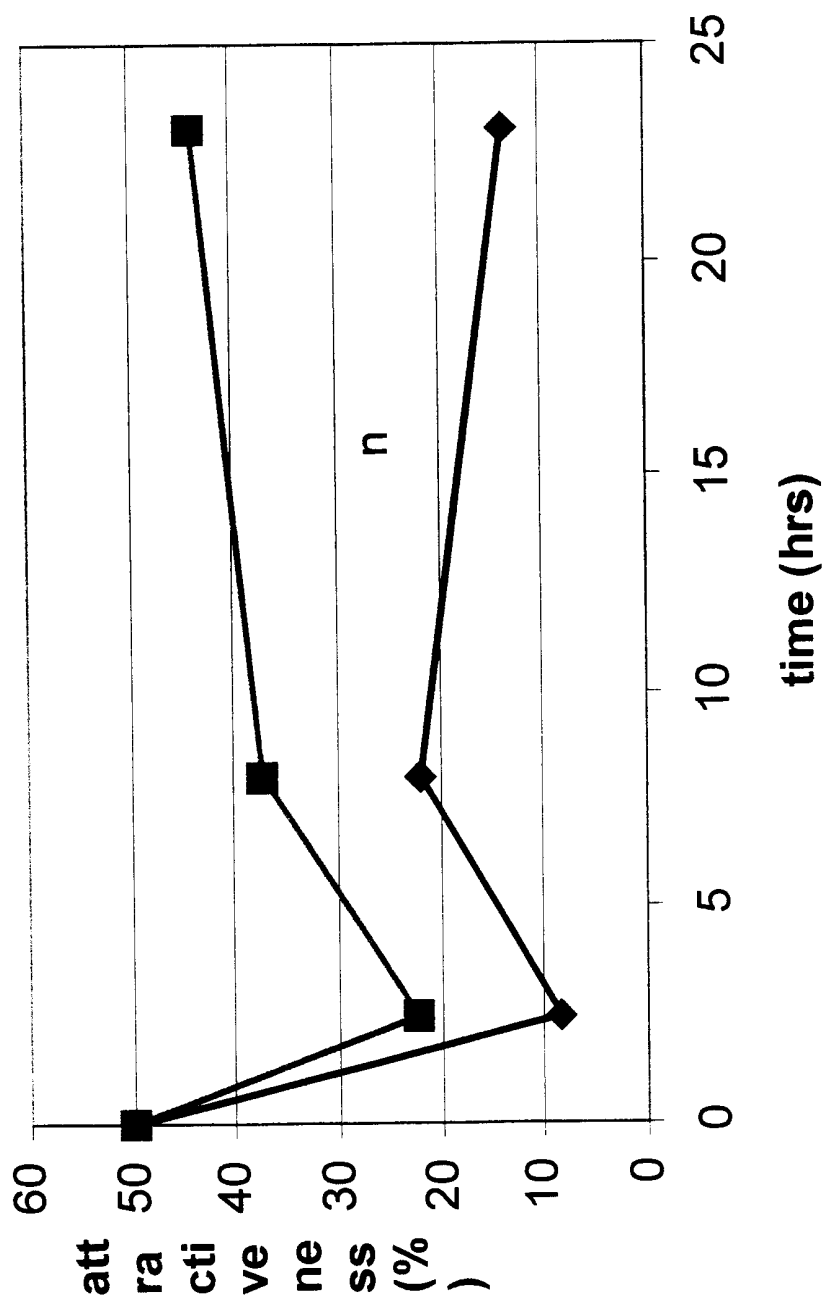


FIG. 4