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Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis*

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Abstract The volatile antimicrobial substance allicin (diallylthiosulphinate) is produced in garlic when the tissues are damaged and the substrate allicin (*S*-allyl-L-cysteine sulphoxide) mixes with the enzyme alliin-lyase (E.C.4.4.1.4). Allicin undergoes thiol-disulphide exchange reactions with free thiol groups in proteins and it is thought that this is the basis of its antimicrobial action. At 50 μg ml^{-1}, allicin in garlic juice inhibited the germination of sporangia and cysts and subsequent germ tube growth by *Phytophthora infestans* both in vitro and in vivo on the leaf surface. Disease severity in *P. infestans*-infected tomato seedlings was also reduced by spraying leaves with garlic juice containing allicin over the range tested (55–110 μg ml^{-1}) with an effectiveness ranging from approximately 45–100%. Similarly, in growth room experiments at concentrations from 50–1,000 μg ml^{-1}, allicin in garlic juice reduced the severity of cucumber downy mildew caused by *Pseudoperonospora cubensis* by approximately 50–100%. These results suggest a potential for developing preparations from garlic for use in specialised aspects of organic farming, e.g. for reducing pathogen inoculum potential and perhaps for use under glass in horticulture.

Keywords Natural fungicides · Tomato leaf blight · Plant antibiotic · Antimicrobial · Phytoanticipin

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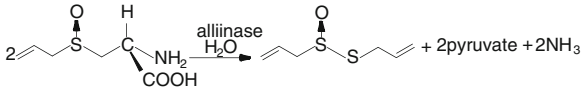
Introduction

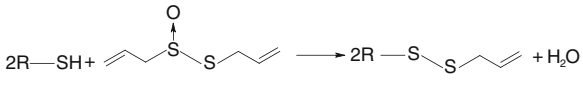
Downy mildews and diseases caused by oomycetes in general are among the most destructive and economically important agricultural problems world-wide. According to Gisi (2002) almost 17% of the world fungicides market in 1996 was for agents used in downy mildew control. Effective control by planting resistant varieties is in many cases not possible and disease management problems have been compounded by the emergence of fungicide-resistant/tolerant variants of several oomycete pathogens (Gisi 2002; Urban and Lebeda 2006, 2007; Urban et al. 2007). Furthermore, the increasing public demand for organically-grown produce, and the intended phasing out by the EU of the use of copper-containing formulations, has precipitated an urgent need for alternative control methods. In this regard resistance-inducing treatments and substances conditioning systemic acquired resis-

tance (SAR) are considered an alternative (Mauch-Mani 2002; Körösi et al. 2007) and there is increased interest in developing treatment strategies based on natural plant defence products (Konstantinidou-Doltsinis and Schmitt 1998; Konstantinidou-Doltsinis et al. 2006; Slusarenko et al. 2008).

We have reported previously that the natural antimicrobial substance allicin, which is a volatile phytoanticipin produced in garlic (*Allium sativum*) upon wounding, is active against a broad range of phytopathogenic organisms in vitro and in planta (Curtis et al. 2004) and indeed there are several reports of garlic preparations containing allicin being used to treat plant disease (e.g. Ark and Thompson 1959; Russell and Mussa 1977). Allicin (diallylthiosulphinate) is produced in garlic when the substrate alliin (*S*-allyl-L-cysteine sulphoxide) mixes with the enzyme alliinase (alliin-lyase, E.C.4.4.1.4; see diagram below). The antimicrobial



activity of garlic juice had long been known and Cavallito and Bailey (1944) showed that this activity was due to allicin, which they reported to be as active against test bacteria as penicillin. Allicin crosses the cell membrane easily and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins (see diagram below). It is thought that these properties are the basis of its antimicrobial action (Miron et al. 2000; Rabinikov et al. 1998). Allicin thus has several



targets in the cell and this makes it difficult for organisms to develop resistance to it.

The use of natural products in plant protection, either directly or as starting points for targeted enhancement of desirable qualities by industry, has been reviewed recently (Slusarenko et al. 2008) and the current paper presents results using garlic juice containing allicin to combat diseases caused by the important plant pathogenic oomycetes *Phytophthora infestans* and *Pseudoperonospoa cubensis*. The effect of allicin in garlic juice was tested on the germination rate and subsequent germ tube growth of sporangia and cysts of *P. infestans* in vitro and in vivo on the surface of tomato leaves. The effectivity of allicin in garlic juice was tested in reducing leaf infection of tomato seedlings by *P. infestans*, and cucumber seedlings by *Pseudoperonospora cubensis* was also tested under growth room conditions.

Materials and methods

Cucumber/*Pseudoperonospora cubensis*

Plant cultivation

Plants were cultivated in 8×8 cm plastic pots filled with a 1:2 parts mixture of sand: commercial potting substrate (Fruhstorfer® Erde Typ T; Industrie-Erdenwerk Archut, Lauterbach). Twelve seeds of *Cucumis sativus* cv. Chinesische Schlange were sown in each pot. The pots were watered carefully and kept in a growth room at 20°C (cycle of 16/8 h light/dark). After 1 week the seedlings were transplanted to fresh pots (one plant per pot).

Inoculation

P. cubensis was maintained on plants grown as outlined above. Fresh inoculum was prepared from plants 10 days after previously being inoculated with *P. cubensis*. Plants were incubated overnight in a moist chamber to encourage sporulation and sporangia were harvested by washing the lower leaf surface with water. The resulting suspension was adjusted to 5×10^3 sporangia ml^{-1} using a haemocytometer.

Plants were harvested approximately 3 weeks after transplanting when the second true leaf was expanded. The upper, non-expanded leaves were excised and the first and second leaves sprayed with the treatment solution on both sides using a chromatography sprayer. Control plants were sprayed with water or with 0.2% Cuprozin Flüssig ™ (460.6 g l^{-1} copper hydroxide) (Spiess-Urania, Hamburg). After 24 h the first and second leaves were inoculated using a chromatography sprayer on both sides with a suspension of *P. cubensis* sporangia (5×10^3 ml^{-1}). The pots were then incubated overnight at 15°C in a moist chamber and the following day returned to the growth room. Disease was rated 2 weeks after inoculation by

estimating the percentage of the affected leaf area. The effectivity of the treatment was calculated according to Abbott (1925):

$$\%\ \text{Effectivity} = \frac{\text{affected leaf area (control)} - \text{affected leaf area (treatment)}}{\text{affected leaf area (control)}} \times 100$$

Tomato/*Phytophthora infestans*

Plant cultivation

Tomato seeds (cv. Hoffmanns Rentita®, Schmitz & Laux GmbH, Hilden, Germany) were sown in seedling trays for germination in moist potting compost covered with fine moistened sand and incubated at 22°C in a light/dark cycle of 16/8 h. After germination, 1 week-old seedlings were transferred to individual 7×7 cm pots and grown on for a further 2 weeks.

Inoculation

The *P. infestans* isolate used in this work was kindly donated by Bayer CropScience AG, Monheim. The virulence of the isolate was ensured by regular passaging through potato tuber discs. *Phytophthora infestans* was cultivated under sterile conditions on tomato juice agar (TJA) at 18°C in the dark (TJA=3 g $CaCO_3$, 12 g PDB (Difco™), 20 g agar (AppliChem GmbH), 200 ml tomato juice (Fa. Krings Fruchtsaft GmbH, Mönchengladbach) made up to a volume of 1 l and autoclaved at 121°C for 15 min). *Phytophthora infestans* inoculum was prepared by washing the surface of 8 day-old Petri plate cultures with cold (10°C), sterile deionized water and sieving through a plastic kitchen sieve. Sporangia were adjusted to a concentration of $4–5\times10^4$ ml^{-1} with a haemocytometer. Zoospores were released from sporangia after approximately 2 h at 10–12°C. After spray-inoculation, plants were placed in a seedling tray and covered with a transparent plastic lid in the growth chamber at 20°C with a light/dark cycle of 16/8 h.

Treatment with garlic juice

Unless otherwise stated, 3 week-old tomato plants were sprayed with diluted garlic juice and the leaves allowed to dry (approx. 2 h) before being spray-inoculated. As a soil drench a single application of 5 ml of the appropriate dilution of garlic juice was applied per 7×7 cm pot containing a single plant. Five to seven intact tomato seedlings were inoculated per experiment and each experiment was repeated at least three times. A representative set of results for each experiment is shown.

Preparation of garlic juice

Garlic bulbs were purchased from the supermarket and stored at 4°C in the dark until required. Axillary buds from the composite garlic bulb were peeled, weighed and a domestic juicer (Turmix Fabr. Nr. 1068, Turmix AG, 8645 Jona, Switzerland) was used to extract the juice. The juice was poured into a sterile 50 ml Falcon tube and centrifuged at 5,000 rpm (3,000×*g*) for 10 min in order to separate the majority of the pulp from the liquid (Megafuge 1.0R, Heraeus Instruments, Osterode, Germany). Floating debris was removed from the top of the liquid with a spatula and discarded. Filtering under pressure separated the remaining pulp from the pure extract (Diaphragm Vacuum Pump, Vacuubrand GmbH + Co., Wertheim, Germany). The filtrate was transferred into a second sterile 50 ml Falcon tube and sealed. The average yield was approximately 1 ml of extract from 3 g fresh weight of garlic tissue and typically contained approximately 5 mg ml^{-1} allicin (determined by HPLC). The garlic extract was used either immediately after appropriate dilution or stored undiluted at 10°C for a maximum of 2 weeks. Dilutions were carried out with de-ionized water. Appropriate amounts of stock solution to give the required end dilution in Petri plates were incorporated into agar medium kept just molten at 45°C. Plates were poured immediately after adding and mixing the stock.

Determination of allicin by HPLC

The method used was based on that of Krest and Keusgen (2002). Garlic juice was diluted 1:10 with HPLC-grade water and 1.5 ml of a 0.05 mg ml^{-1} solution (in methanol) of butyl-4-hydroxybenzoate (internal standard). To protect the column, this mixture was first filtered through a polyethersulfon-membrane (0.2 μm pore size, Steriflip, Millipore) before 20 μl were injected into the HPLC (Kontron

system with diode array detector, Kontron Instruments GmbH, Neufahrn). Using the HPLC software Geminyx (version 1.91) a mixed gradient elution (solvent A, 30% (*v*/*v*) HPLC grade methanol adjusted to pH 2.0 with 85% (*v*/*v*) orthophosphoric acid; solvent B, 100% HPLC grade methanol) was performed. Spectra were recorded between 200–600 nm during elution with detection at 254 nm for the chromatogram.

Effect of garlic juice on *P. infestans* sporangium and cyst germination in vitro

Droplets (20 µl) of inoculum suspension, prepared as described above and containing sporangia and zoospores, were pipetted onto the surface of 1% agar containing 50 µg ml^{-1} allicin. Control plates contained no allicin. Plates were sealed with Micropore™-tape and incubated in a plastic container with moistened tissue paper at 18°C in the dark for 4 h. Germination rate and germ tube length were measured using a microscope (Leica DM R) at 50- to 200-fold magnification. At least 50 sporangia or encysted zoospores were scored for germination per plate and photographed using a JVC digital camera (KY-F75U) and Discus software (Version 32, Hilgers Co., Königswinter, Germany). Germ tube lengths of at least 15 germinated sporangia or cysts were measured per plate.

Effect of garlic juice on *P. infestans* sporangium and cyst germination in vivo on tomato leaves

After spraying 3-week-old tomato plants with diluted garlic juice containing 50 µg ml^{-1} allicin and allowing them to dry, leaves were excised and placed in plastic boxes (12×12 cm) on moistened tissue paper. Droplets (20 µl) of sporangial or cyst suspensions were then pipetted onto the leaves and the lids placed on the boxes for incubation for 4 h in the dark at 20°C. The leaf lamina under the droplets was then excised and stained with acid fuchsin (modified after Carmichael 1955). Excised leaf segments were fixed and decolourised for 48 h at 60°C in aqueous chloral hydrate (2.5 g ml^{-1}). Leaf segments were then stained for 1–2 h in 0.01% acid fuchsin-lactophenol solution and de-stained in 50% (*v*/*v*) glycerol before viewing using a confocal laser-scanning microscope (Leica TCS SP, using Leica software TCS NT) at 630- to 1000-fold magnification (excitation 543 nm; emission filter 575–640 nm, 63× PL APO w, and 100× PL FLUOTAR oil objective lenses).

Statistical treatments

Raw data were first tested for normal distribution and variance homogeneity using Sigmastat® 3.1 (SYSTAT software 2004) to a limit of $P \leq 0.05$. If the data showed normal distribution and variance homogeneity they were subjected to parametric statistic tests to show significant differences (*t*-test or one-way ANOVA) to a probability of $P \leq 0.05$. Non-normal data were analysed with either the Mann–Whitney Rank Sum Test for two groups or the Kruskal–Wallis ANOVA on Ranks for more than two groups. If these treatments pointed to a significant difference between groups, a *post hoc* test (Dunn's or Tukey's) was used to determine which groups differed significantly at the $P \leq 0.05$ level.

Results

Pseudoperonospora cubensis/*Cucumis sativus* pathosystem

Cucumber plants were sprayed with either dilutions of garlic juice, water (untreated controls) or Cuprozin™, and spray-inoculated the next day with a suspension of sporangia of *P. cubensis* (Fig. 1A). Two weeks after inoculation infected leaf areas were estimated (Fig. 1B, Table 1). Dilutions of garlic juice over a wide range of allicin concentrations (50–1,000 µg ml^{-1}) led to a reduction in disease severity which compared favourably with the degree of disease control achieved with a copper-containing commercial fungicide (Cuprozin™).

Phytophthora infestans/Lycopersicon esculentum pathosystem

Effects of garlic juice on P. infestans *germination and growth in vitro*

The effect of garlic juice on *P. infestans* in vitro was assessed by investigating the effects on sporangial and cyst germination and on germ tube growth. Garlic juice (50 µg ml^{-1} allicin) caused a clear reduction in

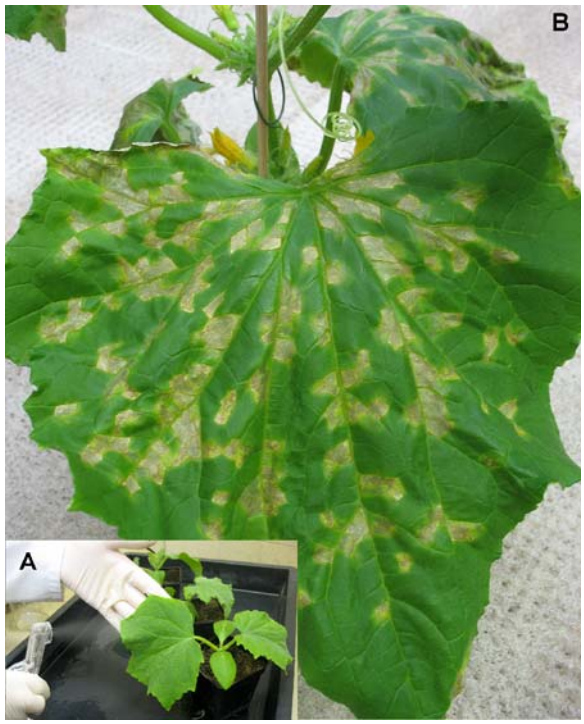


Fig. 1 Leaf of cucumber showing *A*, the spray inoculation procedure and *B*, symptoms 14 days after inoculation with *P. cubensis* (5×10^3 sporangia ml^{-1})

the germination of encysted zoospores and of sporangia under conditions where they germinate directly with a germ tube (i.e. behave like conidia) (Fig. 2). Hyphal growth from germinated sporangia or cysts was also reduced by the presence of garlic juice in the medium (50 μg ml^{-1} allicin) (Fig. 3).

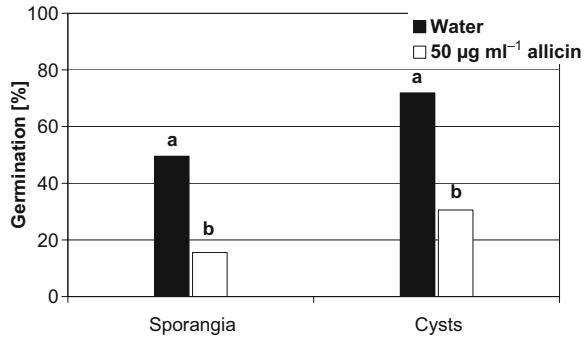


Fig. 2 Influence of garlic juice in agar (50 μg ml^{-1} allicin) on the germination of sporangia and encysted zoospores of *P. infestans* (in vitro). Means of nine replicate Petri plates of sporangia and cyst preparations. Columns which differ significantly from one another are marked with a different letter (*t*-test, $P\leq0.05$)

Effects of garlic juice on P. infestans *germination and growth* in vivo

The behaviour of sporangia and cysts on the tomato leaf surface after treatment with garlic juice is shown in Fig. 4. It can be seen that the inhibitory in vitro effects of garlic juice are mirrored in the in vivo behaviour of sporangia and cysts on the tomato leaf surface.

Effects of garlic juice on disease severity in tomato leaves inoculated with P. infestans

To assess whether the inhibitory effects of garlic juice on *P. infestans* observed in vitro and in vivo on the leaf surface translated into an effect on disease development, a systematic investigation on tomato

Table 1 Effect on disease severity of spraying garlic juice containing allicin at the concentrations shown onto leaves of 40-day-old cucumber plants 24 h prior to spray inoculation with 5×10^3 conidia ml^{-1} of *P. cubensis*

Treatment	Infected leaf area (%) ±SD			Average effectivity[a] (%)
	Experiment 1	Experiment 2	Experiment 3	
Water control	73.3±17.1	33.8±8.9	81.8±9.9	
Allicin 1000 μg ml^{-1}	N.T.[b]	0.2	0.4	>99
Allicin 500 μg ml^{-1}	N.T.	1.0	1.0	96–98
Allicin 200 μg ml^{-1}	3.7	2.8	2.0	84–94
Allicin 100 μg ml^{-1}	19.0	N.T. ±	N.T.	55
Allicin 50 μg ml^{-1}	8.2	N.T. ±	N.T.	52
Cuprozin™ (0.2%)[c]	N.T.	20.0±9.8	20.0±11.9	41–76

Plants (four per experiment, eight leaves in total) were scored 2 weeks after inoculation.

[a] According to Abbott (1925), see "Materials and methods" section

[b] *N.T.* Not tested

[c] Equivalent to 0.92 g $Cu(OH)_2$ l^{-1}

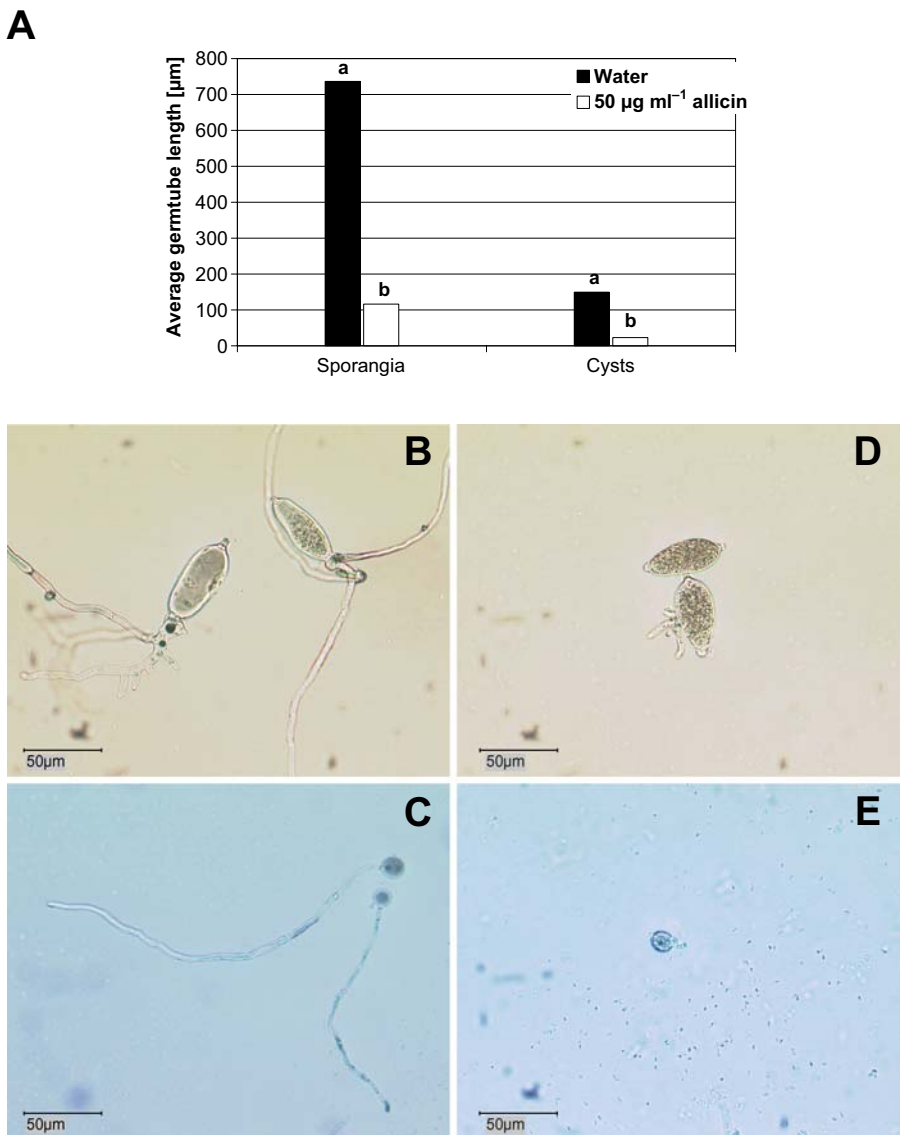


Fig. 3 Influence of garlic juice on germ tube growth from germinating sporangia and encysted zoospores of *P. infestans* (in vitro). **A** Means of ~75 measurements (sporangia) and ~135 measurements (cysts). Columns which differ significantly from one another are marked with a different letter (Mann–Whitney Test, $P \leq 0.05$). **B** Untreated sporangia. **C** Untreated cysts. **D** Sporangia on agar incorporating garlic juice to give a final concentration of 50 µg ml^{-1} allicin. **E** Cysts on agar incorporating garlic juice to give a final concentration of 50 µg ml^{-1} allicin. *Bar*= 50 µm

leaf infections was carried out. Firstly, potential phytotoxic effects of garlic juice on leaves were monitored. As shown in Table 2, spraying tomato leaves of 3-week-old plants with dilutions of garlic juice containing 200–800 µg ml^{-1} allicin led to leaf damage in category 2 (<2.5% of the leaf area showing chlorosis or necrosis), the least severe, and only at the highest concentration tested.

The effect on disease development of spraying tomato leaves with a single application of garlic juice containing a range of allicin concentrations 2 h before inoculation with *P. infestans* is shown in Fig. 5 (for a photograph showing the appearance of control and allicin-treated leaves see Fig. 6 in Slusarenko et al. 2008). In the experiment shown in Fig. 5, control tomato plants had lesions covering 77% of the leaf area 4 days after inoculation (dai). As can be seen, spraying with garlic juice very effectively reduced disease development, with a 1:50 dilution (110 µg ml^{-1} allicin) suppressing lesion development completely (Fig. 5).

The effectivity of a single pre-inoculation spray with garlic juice containing a low concentration of allicin (60 µg ml^{-1}), which did not completely suppress disease development, decreased with time but was still apparent 10 dai (data not shown). Thus, in plants treated with 60 µg ml^{-1} allicin the affected leaf area increased from 16% at 4 days to 37% at 10

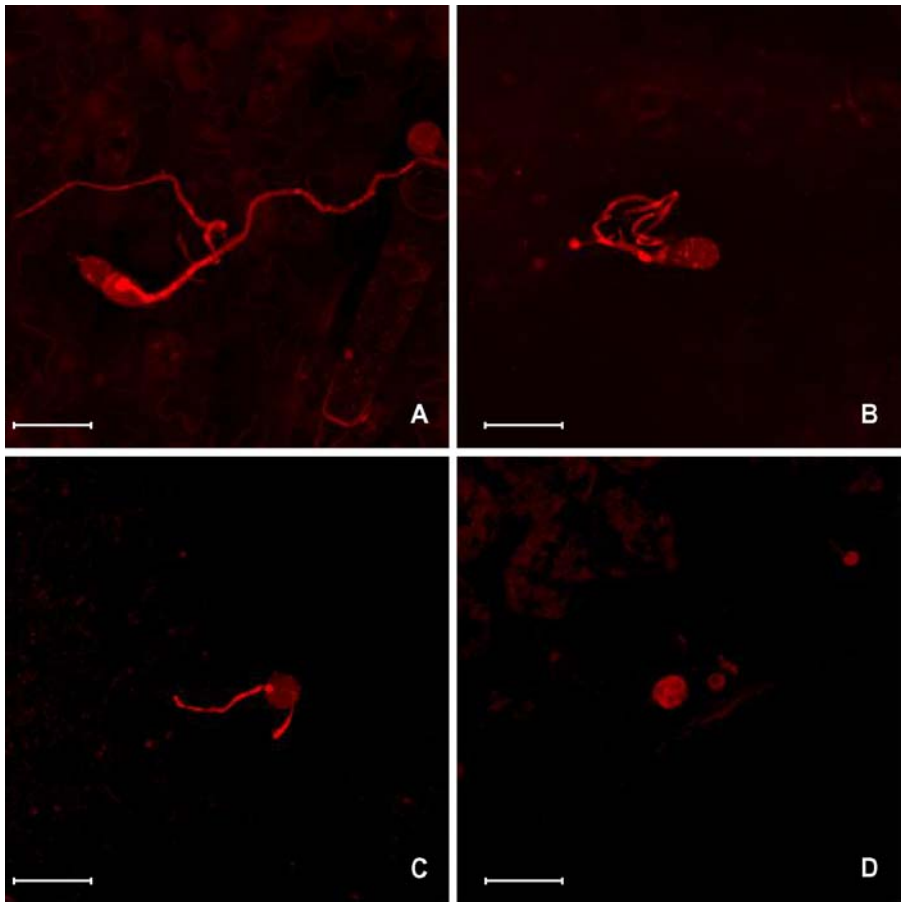


Fig. 4 Influence of garlic juice on germ tube growth from germinating sporangia and encysted zoospores of *P. infestans* on the tomato leaf surface (in vivo) shown after acid fuchsin staining under a confocal laser scanning microscope excitation, 543 nm, emission, 575–640 nm; *Scale bars* = 50 μm (A & B), 25 μm (C & D). **A** Untreated sporangia showing germination and healthy germ tube growth. **B** Sporangia on a leaf sprayed with garlic juice (50 μg ml^{-1} allicin) approximately 2 h prior to inoculation, germinated at a lower rate and has formed abnormal germ tubes with reduced growth. **C** Untreated cyst showing normal germ tube growth. **D** Ungerminated cysts on a leaf sprayed with garlic juice (50 μg ml^{-1} allicin)

dai. In the untreated controls, however, the infected leaf area was 60% after 4 days and increased to 63% by 10 dai.

The effect of various garlic juice application times in relation to the time of inoculation with *P. infestans* was investigated and it was found that the nearer to the inoculation time that allicin was sprayed, the more effective a given dosage was in suppressing disease development (Fig. 6). In contrast, spraying leaves with garlic juice 24 h after inoculation had little effect. Direct spraying onto leaves was also compared with a single application as a soil drench. As can be seen in Fig. 7, allicin was more effective when sprayed on the leaves than when applied to the soil.

Discussion

Curtis et al. (2004) previously reported that dilutions of garlic juice containing allicin were effective in reducing the production of conidiophores and

Table 2 Phytotoxicity scores for individually potted 3-week-old tomato seedlings sprayed to run-off with dilutions of garlic juice containing various concentrations of allicin

Concentration of allicin in garlic juice (μg ml^{-1})	Phytotoxicity category[a]
0	1
200	1
400	1
800	2

[a] Phytotoxicity categories (Gorog née Privitzer et al. 1988): 1= no damage, 2=<2.5% leaf area damaged (showing chlorosis or necrosis), 3=<5% leaf area damaged. The scale progresses to 9=100% leaf damage. Scores<2 are considered acceptable in screens of potential candidates for plant protection substances.

Plants were allowed to dry, and then pots were placed under plastic hoods for 4 days before the hoods were removed. Plants were incubated in a growth chamber (22°C, cycles of 18 h light 6 h dark) and scored 6 days after spraying.

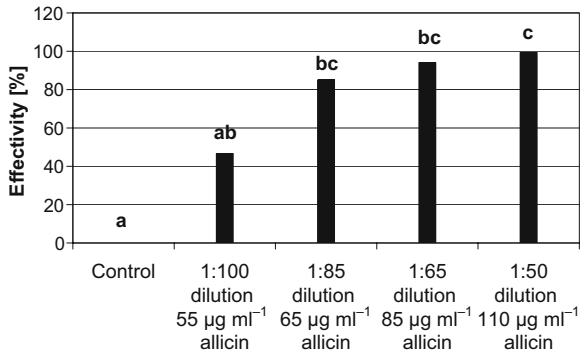


Fig. 5 Dose-dependency of disease control by allicin in garlic juice in the *P. infestans*/tomato leaf pathosystem. Three-week-old plants (cv. Hoffmans Rentita) were sprayed with garlic juice, the leaves allowed to dry (approx. 2 h) and then spray-inoculated with $4–5\times10^4$ sporangia ml^{-1}. The effectivity of treatment (Abbot 1925) is shown at 4 dai. Columns which differ significantly from one another are marked with a different letter (Dunn's Test, $P\leq0.05$)

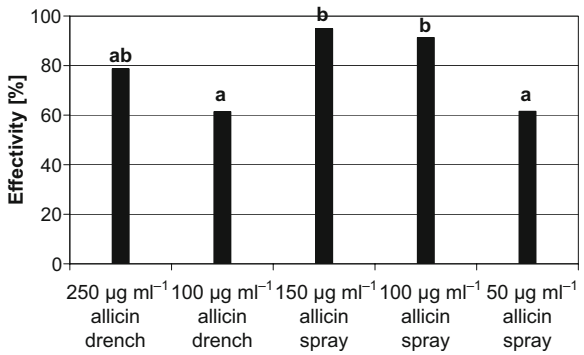


Fig. 7 Comparison of the effectivity of garlic juice containing allicin as a soil drench or a foliar spray in the *P. infestans*/tomato pathosystem at 4 dai. Columns which differ significantly from one another are marked with a different letter (Tukey's Test, $P\leq0.05$)

oospores in downy mildew of *Arabidopsis* caused by *Hyaloperonospora parasitica*. In the present study these observations are extended to show that macroscopic disease symptoms of cucumber downy mildew can be markedly reduced by spraying the leaves with garlic juice containing a range of allicin concentrations 24 h prior to inoculation. The disease reduction compared very favourably with a commercial copper-fungicide treatment and suggests that development of garlic products for at least small-scale application such as in glasshouse situations might be feasible and desirable as an alternative to standard treatments (Fig. 1, Table 1). Resistance of *P. cubensis* against conventional fungicide treatments is increasing (Urban and Lebeda 2006, 2007; Urban et al. 2007) and because allicin appears to have a multi-site mode of action (Portz et al. 2005; Slusarenko et al. 2008) it will presumably be difficult for pathogens to mutate to resistance against it, thus conferring a strong advantage on allicin-based disease treatments.

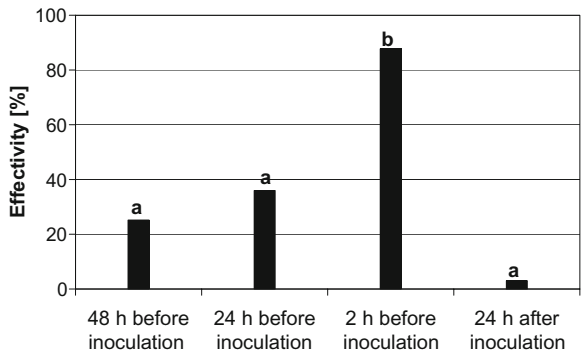


Fig. 6 Influence of time between treatment with garlic juice (70 µg ml^{-1} allicin) and time of inoculation on effectivity in the *P. infestans*/tomato pathosystem at 4 dai. Columns which differ significantly from one another are marked with a different letter (Dunn's Test, $P\leq0.05$)

The inhibitory effect of allicin on the vegetative mycelial growth of *P. infestans* and the reduction of potato tuber colonization by allicin in the gas phase have been reported previously (Curtis et al. 2004). Now, the inhibitory effects of garlic juice containing allicin on the germination of sporangia and encysted zoospores and subsequent reduction in germ tube growth, both in vitro and on the tomato leaf surface (Figs. 2, 3, 4) are reported. These effects presumably contribute to the reduction in infection seen in inoculated tomato seedlings (Fig. 5). The tomato leaf/*P. infestans* pathosystem was used in preference to potato/*P. infestans* because it is easier to work with in the laboratory. Nevertheless, since it appears that the effect of allicin is directly against the pathogen, rather than via an induced resistance mechanism (Curtis et al. 2004), it seems likely that a similar degree of control might be expected in the potato/*P. infestans* pathosystem, particularly in view of the effects of allicin in reducing tuber colonisation at least under controlled conditions (Curtis et al. 2004). The effectivity of garlic juice in reducing disease in tomato leaves was very high and approached 100%

at an allicin concentration of 110 μg ml^{-1} (Fig. 5). In fungicide screening, substances are usually only considered for further development if they do not cause leaf damage above category 2 (<2.5% leaf area affected) on a scale of 1–9 (Gorog née Privitzer et al. 1988) (see Table 2). Garlic juice was assessed at various dilutions for phytotoxicity, and disease control was achieved at allicin concentrations well below those where phytotoxicity was observed (Table 2, Fig. 5). Thus, allicin in garlic juice would not be excluded in a conventional screening programme based on this criterion.

The effectivity of the allicin treatment in reducing disease on tomato seedlings is more pronounced in the early stages after treatment. If allicin is working mainly via a reduction of successful infections by killing a certain proportion of the spores and subsequently by suppressing germ tube growth from surviving propagules, then a time-lag in disease development would be expected until inoculum levels had reached those present before the sanitation treatment. However, the dynamics of disease development in fungicide-treated plants are difficult to model and disease development often deviates from the ideal mathematical description (Jeger 1987). In control plants not treated with allicin, the disease level 4 dai was already high and this increased only marginally in subsequent days. In the allicin-treated plants the affected leaf area increased from 16% at 4 days to 37% by 10 dai. Thus, even a single treatment with allicin at a dose (60 μg ml^{-1}) below that necessary to completely eradicate disease (~110 μg ml^{-1}, see Fig. 5) is already effective at reducing the rate of disease progress over a substantial time period.

The data presented in Fig. 6 show the effectivity of a single allicin treatment in relation to the time of inoculation and support a low-persistence, contact-fungicide type of effect for allicin. Thus, the effectivity of the treatment increases with decreasing time before inoculation (from 48 to 24 h), is maximal when inoculation takes place approximately 2 h after treatment with garlic juice, and is least effective at later times after inoculation (e.g. 24 h) when the pathogen has already penetrated the leaf and is perhaps less easily accessed by allicin. In this regard the kinetics of allicin behaviour on the leaf surface, and its uptake by the leaf, are aspects which need further investigation.

In downy mildew of *Arabidopsis* it was shown that treatment of the plant with garlic juice did not lead to the accumulation of SAR markers (Curtis et al. 2004) and the authors suggested that garlic juice was exerting its antimicrobial effect directly on the pathogen rather than via inducing SAR in the plant. The data presented in Fig. 6 for tomato support this conclusion and extend it to a further pathosystem.

Interestingly, in the tomato/*P. infestans* pathosystem, applying garlic juice as a soil drench was also effective at reducing disease levels, although a better degree of control was achieved with lower concentrations of allicin as a direct spray on the leaves (Fig. 7). As stated earlier, allicin appears to act directly against the pathogen and it is unclear whether the disease reduction after applying garlic juice as a soil drench is due to the action of allicin against the pathogen via the gas phase, or whether allicin is also taken up via the roots and transported systemically within the plant. Allicin is readily membrane-permeable (Miron et al. 2000; Rabinikov et al. 1998; Slusarenko et al. 2008) and could therefore enter the symplast in the roots, but whether it is transported within the plant is unknown at present. In this regard, it is perhaps important to mention that it is difficult to quantify allicin in the gas phase because the temperature of the injection port in the GC is too high and leads to modifications producing other polysulphides (Block 1992).

The potential for allicin in garlic juice to be used as an effective control agent against diseases caused by oomycetes is clear, although there is scope for optimisation of treatment regimes, and field testing is certainly necessary. Very clearly, transfer from the laboratory to the field/glasshouse is a stumbling block which many otherwise promising compounds fail to negotiate successfully (Slusarenko et al. 2008). This may also prove true for allicin in garlic juice. Also, it will be necessary to carry out organoleptic assessment of harvested plant parts to ensure the absence of undesireable flavour notes in any development of garlic products for plant protection. Neither garlic juice nor allicin are named presently as plant protection substances specifically permitted for organic farming in the EU (Directive 2092/91). However, it is not likely that these substances, which are a common foodstuff or a component thereof, have properties that would not allow them to be added to the list in the future. Furthermore, chemical modifi-

cation of allicin, which has an activity comparable to several conventional antibiotics (Cavallito and Bailey 1944; Curtis et al. 2004; Slusarenko et al. 2008), to enhance its desirable properties and reduce its undesirable ones, might even lead to a new multi-target plant protection compound useable in conventional agriculture and horticulture.

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