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REVIEW

Control of plant diseases by natural products: Allicin from garlic as a case study

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Abstract This review aims to increase awareness of the potential for developing plant protection strategies based on natural products. Selected examples of commercial successes are given and recent data from our own laboratory using allicin from garlic are presented. The volatile antimicrobial substance allicin (diallylthiosulphinate) is produced in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine sulphoxide) mixes with the enzyme alliin-lyase (E.C.4.4.1.4). Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. It is thought that these properties are the basis of its antimicrobial action. We tested the effectiveness of garlic juice against a range of plant pathogenic bacteria, fungi and oomycetes *in vitro*. Allicin effectively controlled seed-borne *Alternaria* spp. in carrot, *Phytophthora* leaf blight of tomato and tuber blight of potato as well as *Magnaporthe* on rice and downy mildew of *Arabidopsis*. In *Arabidopsis* the reduction in disease was apparently due to a direct action against the pathogen since no accumulation of salicylic acid (a marker for systemic acquired resistance, SAR) was observed after treatment with garlic extract. We see a potential for developing preparations from garlic for use in organic farming, e.g. for reducing the pathogen inoculum potential in planting material such as seeds and tubers. We have tested various encapsulation formulations in comparison to direct treatment.

Keywords Alginate · Encapsulation · Formulation · Plant protection

Introduction

In the course of evolution, plants have developed chemical defence mechanisms against potential pathogens and pests. Society's dependence on intensive agriculture and horticulture for food production has accentuated the need to reduce crop losses. Environmental considerations have highlighted the requirement for sustainable solutions in agriculture and consumer pressures for green alternatives have accompanied a boom in the organic farming sector. This has awakened new interest in natural products as a source for novel industrial plant protection strategies. The purpose of this review is to give an overview of the use of some natural products in plant protection

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Note added in proof: The complete chemical synthesis of azadirachtin has now been achieved. Nature (2007) 448: 630–631

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and to highlight the potential of allicin from garlic, which we believe holds much promise for future development in at least some specialised areas of agriculture and horticulture.

What is a natural substance?

A scientist and a lay-person perhaps have a different understanding of what the term ‘natural’ conveys. Similarly, the motivations for investigating the use of natural products in plant protection might also be different. By definition, a natural or biogenic substance is either synthetized directly by a living organism or is derived from substances of biogenic origin by chemical reactions occurring without human intervention; for example by decomposition of biological materials. Thus, humus, or in the wider sense coal, oil and limestone, are examples of natural or biogenic substances. In the context relevant to this review, a natural product is viewed as a physiologically active chemical which is synthetized by plants, animals or microbes. In contrast, a synthetic chemical is one which does not occur naturally and must be synthetized from other substances by human intervention. Of course, many naturally occurring substances can also be synthetized in the laboratory, and indeed the use of a pure, chemically synthetized molecule in laboratory tests is usually a pre-requisite for the acceptance of biological activity attributed to a particular substance in a complex mixture from a natural source.

Why consider natural substances for plant protection?

In the public perception ‘natural’ is often equated directly with ‘benign’ and ‘environmentally friendly’ and for any given purpose natural products are a priori assumed to be a more desirable alternative to synthetic chemicals. Obviously, this is per se not correct and many natural products are very toxic. For example botulinum toxin, a bacterially produced peptide with an LD_{50} of 1 ng kg^{-1} body weight is perhaps the most acutely toxic substance known (Fleming and Hunt 2000). In contrast, highly toxic inorganic arsenic has an LD_{50} (oral) of 763 mg kg^{-1} (http://ptcl.chem.ox.ac.uk/MSDS/AR/arsenic.html) making it nearly 8×10^8 times less toxic than botulinum on a weight for weight basis. Nevertheless, living organisms, particularly plants, are brilliant synthetic chemists and produce a huge variety of physiologically active substances, thus providing an alternative to the combinatorial chemistry approach in the search for useful chemicals. To quote from a recent *Science* article: “Around half of the drugs currently in clinical use are of natural product origin.” The authors go on to state that “Despite this statistic pharmaceutical companies have embraced the era of combinatorial chemistry, neglecting the development of natural products as potential drug candidates in favour of high-throughput synthesis of large compound libraries” (Peterson and Anderson 2005). This perhaps highlights a common perception among scientists that natural substances, per se are probably less effective than synthetic alternatives, or in a greater extreme that natural products are almost in the realm of esoterics and folk-lore. This can lead to a sceptical approach to each other’s perspectives by both scientists and lay people and emphasizes the need for strict objectivity.

A potential advantage offered by natural products is that their effectiveness has been optimised by evolution for their particular task. In terms of plant protection this might be an antimicrobial, insecticidal or anti-feedant activity. Several microorganisms produce antibiotics, and many preformed and induced antimicrobial substances are known from plants (Mansfield 2000). These are obvious candidates to be considered for use in plant-protection strategies. Furthermore, many substances of natural origin which do not show direct antimicrobial activity might act as resistance inducers to prime systemic acquired resistance (SAR) relying on the plant’s own defences (Goellner and Conrath 2007).

Although some structural components which are natural in origin, e.g. lignin or $CaCO_3$- or silica-containing shells, are very stable, natural products are generally easily biodegradable and after they have served their purpose do not tend to persist in the environment. This can also be a disadvantage, however, because the plant protectant has to be around for long enough to do its job before it is degraded and taking a natural product out of its cellular environment is usually de-stabilising.

Increasing interest in environmentally sustainable agriculture and horticulture, and organic farming, has opened up a niche on the market for plant protection products compatible with regulations for labelling food as ‘organic produce’ and has forced the need to consider new alternatives. Thus, the use of copper-containing compounds, for example in combating potato blight, has traditionally been allowed in organic farming. Acknowledgement that the release of large amounts of this toxic heavy metal into the environment are not compatible with the ethos behind the organic farming movement has led to its phasing out as an allowed substance in the latest EU directives (Council Regulation 2092/91 on Organic Farming); however, an effective practical alternative has yet to be found.

Problems with natural substance development for plant protection

Substances honed by evolution for their function within the natural plant-pathogen context are not generally optimised for industrial production or external application. Thus, as mentioned above they may not have optimal stability for field applications and there may be a much cheaper synthetic alternative available which does the same job. Thus, early successes in the laboratory do not always transfer to the field situation. Furthermore, to be attractive to industry a product must be patentable and the status of many natural substances is unclear in this regard (see the example of neem products below). Nevertheless, as a starting point for derivatisation and formulation to enhance desirable and reduce undesirable properties, natural product structures can be an important starting point.

Examples

Some natural-product-inspired, natural-product-derived, or natural-product-similar plant protection chemicals have been important commercial successes:

Example (1) The systemic benzimidazole fungicide Benomyl (Fig. 1a), which was released on to the market by the DuPont Company in 1968, has a heterocyclic ring structure reminiscent of benzoxazinones/benzoxazolinones which are weakly antifungal substances accumulating in some grasses (e.g. wheat, rye, maize) (Fig. 1b). It seems that the biological activity of these compounds was not the inspiration that led to the development of Benomyl (Harvey Loux, personal communication); however, the structural similarity of Benomyl to these natural antifungals is clear. Benomyl interferes with microtubules and affects cell division and intracellular transport processes. Fungal microtubules seem particularly sensitive to benomyl which is probably the basis of its selective action. Although benomyl has such a low acute toxicity in mammals that it has not been possible to establish an LD_{50} for it (http://www.inchem.org/documents/ehc/ehc/ehc148.htm); concern about the effects of chronic exposure led to its phasing out and withdrawal from the market in 2001/2002.

Example (2) Strobilurins are produced as natural antibiotics by the wood-rotting Basidiomycete fungus *Strobilurus tenacellus* whose fruiting bodies emerge from between pine-cone scales (Fig. 2). The fungus produces antibiotics in a ‘chemical warfare strategy’ to reduce competition for its habitat from other species. Strobilurins act at the outer ubiquinol binding site in the electron transport chain in aerobic respiration (cytochrome bc_1 complex, complex III) and are classed as Q_o inhibitors or ‘Q_oI’ (Grasso et al. 2006). Complex III is an integral component in mitochondrial electron transport in all eukaryotes and why strobilurins are selectively toxic to fungi is not understood. The basic strobilurin structure has been modified in the laboratory to improve characteristics for field application, such as UV-stability, and several analogues are marketed as successful fungicides. Interestingly, some novel strobilurin derivatives have been reported to have a resistance-inducing or ‘priming’ effect in

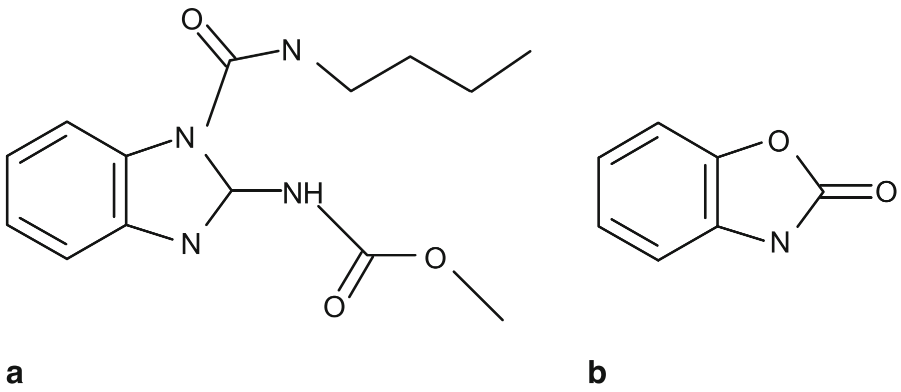

Fig. 1 The chemical structures of (**a**) the imidazole Benomyl, (**b**) 2-benzoxazolinone

the plant and also to stimulate plant growth and drought tolerance in addition to their direct antifungal activities and are thus beneficial for the plant even in the absence of any infection (Goellner and Conrath 2007).

Example (3) Neem oil/neem cake are products made from the seeds of the Neem tree (*Azadirachta indica*) a native of India and a member of the mahogany family (Meliaceae). Neem products have a long history of nutritional and medicinal uses by indigenous peoples and were the subject of an international dispute about patenting natural resources (Wolfgang 1995). Neem products are perhaps best known for their pesticidal and antifeedant activities but broad-range anti-mycotic properties have also been reported (Carpinella et al. 2003). The major insecticidal-active substance in Neem preparations is the triterpenoid azadirachtin (Butterworth and Morgan 1968). The structure is complex (Fig. 3) and despite early synthesis of the two sub-fragments of the molecule (decalin and a hydroxy furan), each of which shows independent insecticidal effects, total synthesis has remained elusive (Aldhous 1992; Ley 1994; Nicolaou et al. 2003). The exact mechanism of action is not well understood but azadirachtin acts as both a feeding deterrent and an insect-growth regulator. The molecule is acid and base-unstable and, because of the large number of double bonds, extremely UV-labile. More stable variants of the parent molecule have been developed but work is hampered because of the lack of an easy, cheap synthetic strategy (Aldhous 1992). Thus, the majority of uses employ preparations of or from neem seeds themselves. Azadirachtin is specifically listed as an acceptable plant protection substance for organic farming in EU directive 2092/91.

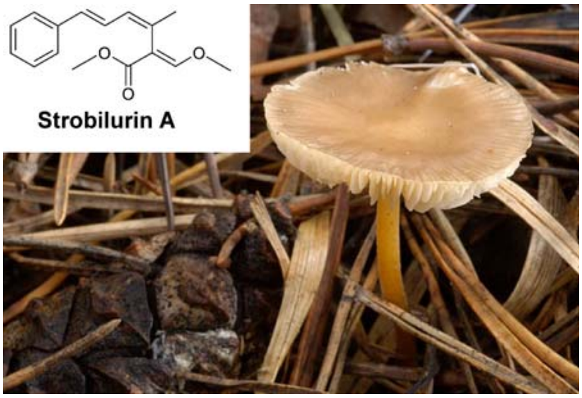

Fig. 2 A fruiting body of *Strobilurus tenacellus* (reproduced with the kind permission of Darek Karasinski, (http://grzyby.strefa.pl.). The inset shows the structure of strobilurin A

Perspectives

Certainly natural products are being considered in the search for plant-protection chemicals, as illustrated by the last two examples above. However, while many companies advertise their services for 'natural drug

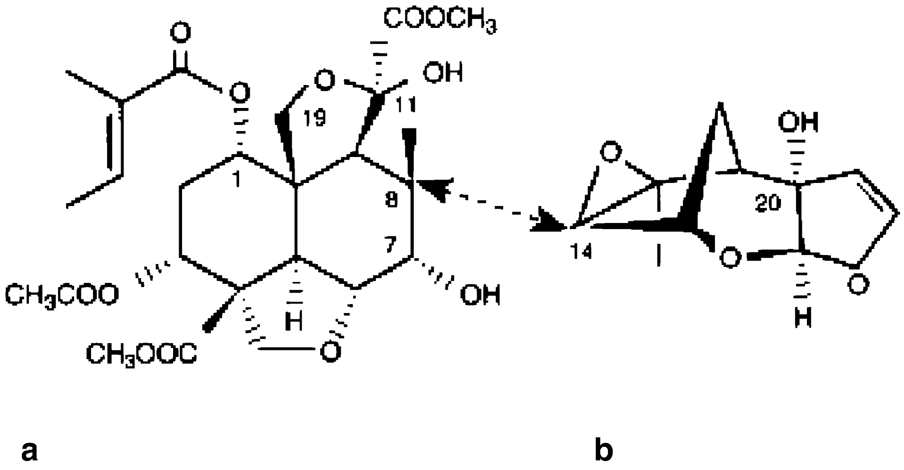

Fig. 3 The two component fragments of azadirachtin. (**a**) the decalin fragment and (**b**) the hydroxy furan fragment. From Aldhous (1992) reprinted by permission of AAAS

discovery,' the question remains as to whether plant protection will attract similar financial investment as for applications in human medicine. Since the use of raw extracts from plants is in many cases not economical for industrial scale applications due to the bulk of material needed, the future may well see the development of single molecules or mixtures that can serve as indicator structures or 'lead compounds' for derivatisation. Nevertheless, systematic scientific improvement of 'low-tech' solutions, where subsistence farmers might 'grow their own' plant protection, may be of real social value.

Although this review is focused on plant products it is pertinent at this point to mention micro-organisms as sources of natural products for plant defence. The control of fireblight caused by the phytopathogenic bacterium *Erwinia amylovora* with the antibiotic streptomycin is a well known, if controversial, example. However, many groups of bacteria and fungi have not been studied as a source of plant protection chemicals per se although there has been much progress in their direct use as antagonists.

Many phytoanticipins exist as precursors that need to be modified by enzymatic activity to achieve their anti-microbial potential. The future development of 'two-component' enzyme-substrate strategies for plant protection might therefore be productive. Work in this direction with the alliin-alliinase combination has already been published (Fry et al. 2005).

Case study: Allicin in garlic (*Allium sativum*)

When garlic is sliced or crushed it develops its characteristic odour because cellular damage leads to mixing of the vacuolar enzyme alliin lyase (E.C.4.4.1.4) and its cytosolic substrate alliin (S-allyl-L-cysteine

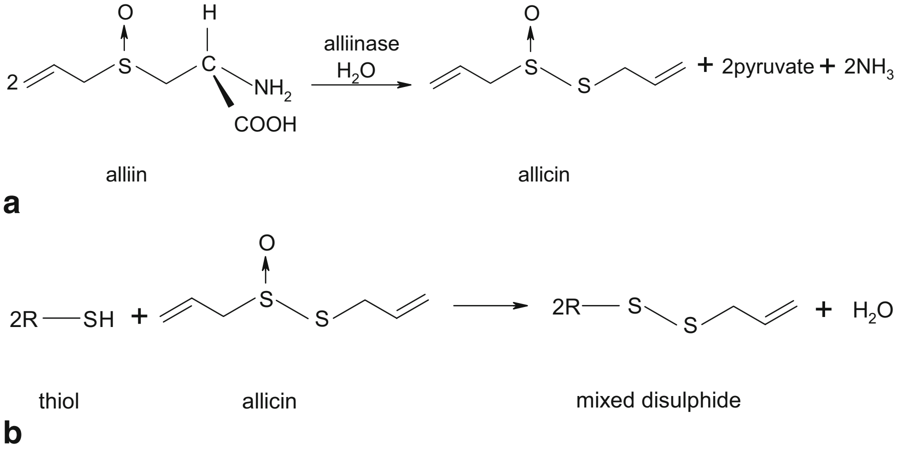

Fig. 4 (**a**) The production of allicin from alliin by alliin lyase, and (**b**) the thiol-disulphide exchange reaction with SH-compounds including amino acids in proteins thought to be the basis for allicin's biocidal activity

Fig. 5 Clear zones of inhibited growth of a wild-type *E. coli* K12 isolate around filter discs spotted with 20 µl of garlic juice containing a total of 90 µg allicin

sulphoxide). The immediate product is thiosulphenic acid which undergoes spontaneous dimerization to diallylthiosulphinate (allicin) (Fig. 4a). It is allicin that gives garlic its characteristic odour. Garlic has a long history of use in folk medicine. For instance the Codex Ebers, an Egyptian papyrus from 1800 BC, describes more than 800 medicinal preparations including 22 containing garlic (Block 1985). Allicin was shown to be the major antimicrobial substance in garlic by Cavallito and Bailey (1944) and the allicin metabolite ajoene shows potent anti-coagulent activity by inhibiting platelet aggregation (Jain and Apitz-Castro 1987). Allicin undergoes thiol-disulphide exchange reactions with free thiol groups in proteins (Fig. 4b) and it is thought that this, together with its ready membrane permeability (average LogP octanol:water=1.52±0.80, Tetko et al. 2005; http://www.vcclab.org), is the basis of its antimicrobial action (Miron et al. 2000; Rabinikow et al. 1998). Because of these attributes allicin has several potential targets within the cell and it is difficult for organisms to mutate to resistance. Allicin has been reported to be active against a broad-spectrum of taxonomically diverse organisms (Curtis et al. 2004; Portz et al. 2005 and references therein). Nevertheless, resistance to allicin is known and garlic is susceptible to *Puccinia porri*, which is presumeably insensitive to allicin or can colonise garlic without causing allicin production. Similarly we have isolated a non-pathogenic allicin-resistant *Pseudomonas* isolate from fresh garlic cloves. However, the basis of the allicin resistance of this isolate is unclear.

The use of garlic preparations or allicin against plant pathogens has already been documented (Arya et al. 1995; Bianchi et al. 1997; Cao and vanBruggen 2001; Russell and Mussa 1977) and there are several preparations based on garlic compounds available commercially, although the latter are primarily aimed at controlling pests rather than pathogens.

Fig. 6 Control of leaf blight of tomato by spraying tomato plants with garlic juice 2 h before inoculation. Inoculation was done by spraying whole plants with a sporangial suspension of *P. infestans* (4–5×10^4 sporangia ml^{-1}). *Top panel*, inoculated plants; *middle panel*, inoculated and sprayed with diluted garlic juice containing 110 µg ml^{-1} allicin or, *bottom panel*, 85 µg ml^{-1} allicin

Fig. 7 An alginate formulation of garlic juice deposited on the soil surface in a pot test with *Phytophthora*-inoculated tomato seedlings

How potent an antibiotic is allicin?

Perhaps the best way to illustrate the potency of allicin is to make a comparison with a 'household' antibiotic like kanamycin which is used routinely in the laboratory in selective media. Spotting garlic juice or pure allicin to a Petri plate containing growth medium seeded with bacteria gives rise to clear halos where bacterial growth has been inhibited (Fig. 5). When the concentration of bacteria suspended in the agar and the depth of agar in the plate are standardized, the diameter of the inhibition zone is highly reproducible between replicates. On this basis a Petri-plate bioassay to quantify the amount of allicin in crude garlic extracts was developed and originally calibrated by determining allicin using an approximate spectrophotometric assay (Curtis et al. 2004). The accuracy of this bioassay was subsequently improved by using an HPLC method to quantify a pure allicin standard (Krest and Keusgen 2002; Portz and Slusarenko unpublished).

Using a wild-type *E. coli* K12 isolate as an indicator strain, the diameter of the inhibition zone caused by 50 µg kanamycin was matched by 55 µg of allicin. On a molar basis this makes allicin approximately a quarter as potent as kanamycin. The quantity of the substrate alliin in garlic cloves varies but in our hands garlic purchased in the supermarket routinely yields approx. 2 mg allicin g^{-1} fresh weight. A single clove of garlic weighs around 5 g and a composite bulb around 50 g; this means 2 g allicin can be obtained from a kg (approximately 20 bulbs) of garlic. Thus, the antibiotic potential present in fresh garlic is considerable.

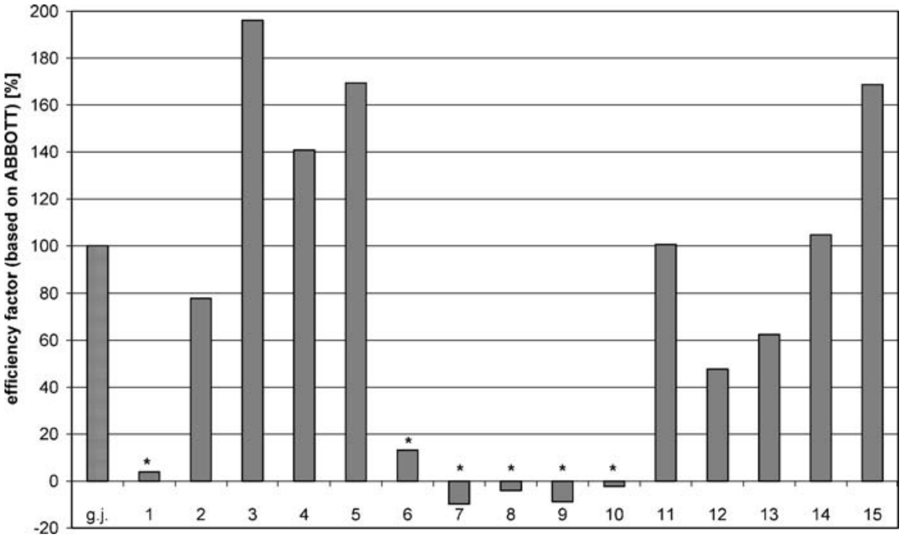

Fig. 8 The relative effectivity against *P. infestans* on tomato of applying 1.5 g of various garlic encapsulations onto the soil compared with a direct spray of 100 µg ml^{-1} allicin in garlic juice onto leaves. Both applications were done 2 h before whole plants were sprayed with sporangial suspensions of *P. infestans* ($4–5\times10^4$ sporangia ml^{-1}). The data were treated according to the method of Abbott (1925). The asterisks indicate a significant difference to the treatment with diluted garlic juice ($\alpha=0.05$, Dunn's Test). (g.j.=diluted garlic juice (100 µg ml^{-1} allicin), 1–15=different capsules)

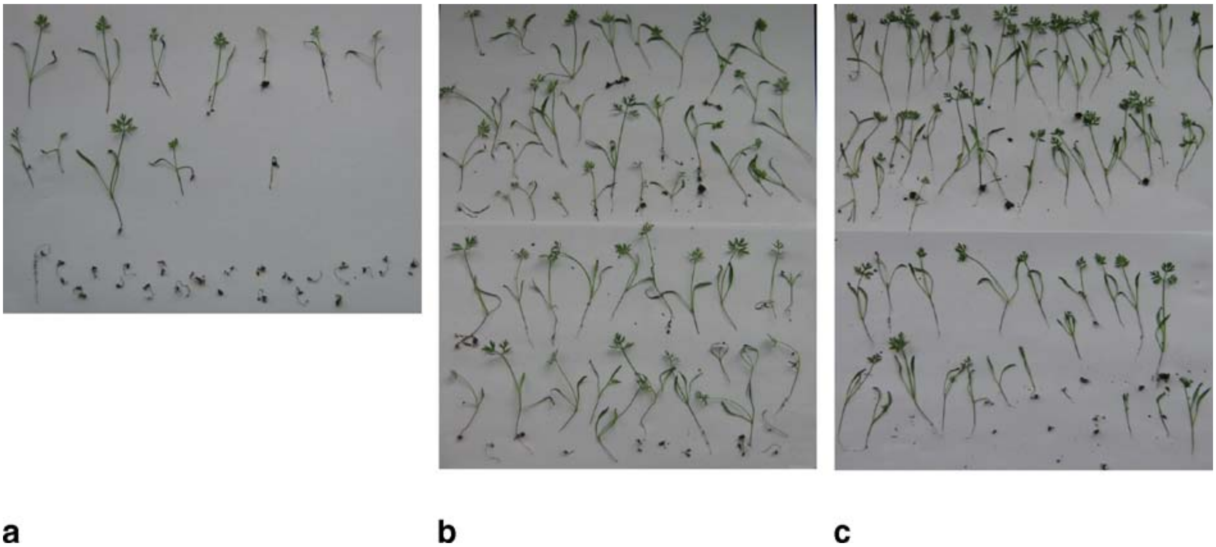

Fig. 9 Disinfection of *Alternaria*-infested carrot seed by imbibing with garlic juice. (**a**) Control seed without treatment, 12/100 seeds germinated, (**b**) Aatiram®-treated seed, 48/100 seeds germinated, (**c**) garlic juice treatment, 47/100 seeds germinated

Activity of allicin in garlic juice against plant pathogens in vitro and in planta

In vitro antibacterial, antifungal and anti-oomycete activity from garlic have been reported in many instances in the literature (see Curtis et al. 2004 and references therein). There are also reports of disease being reduced by treatment of infected plants and in the laboratory. We showed that garlic juice was able to reduce disease severity in several test pathosystems such as rice/*Magnaporthe oryzae*, *Arabidopsis*/*Hyaloperonospora parasitica*, and potato/*Phytophthora infestans* (*ibid*). In the latter case, tuber infection was investigated and control was achieved by applying allicin directly to the inoculation site as well as via the vapour phase in an enclosed space. This raises the possibility for developing fumigation protocols in special circumstances and relies on the volatile nature of the active principle allicin. Control of leaf blight of tomato after *P. infestans* inoculation was also achieved by spraying leaves with dilutions of garlic juice (Fig. 6).

The possibility that allicin might not only be acting directly against the pathogen but also by conditioning SAR was investigated in the *Arabidopsis*/*Hyaloperonospora* interaction by looking for accumulation of the SAR marker salicylic acid (SA) in local (treated) and systemic (untreated) leaves. No significant induction of either free or glycosylated SA was observed, leading us to conclude that garlic juice at the concentrations tested did not induce SAR directly (*ibid*).

Can allicin be transferred from the laboratory to the field?

The antibiotic potential and success in the laboratory of allicin/garlic treatments are clear. In a plant protection context, the big question is: can the small-scale effects in controlled environments be transferred at manageable cost to applications in the field? For this to happen several aspects must be considered.

Allicin has the reputation of being rather unstable. However, in a study on the degradation kinetics of allicin in different solvents Canizares et al. (2004) reported that allicin kept at 6°C retained its bacteriostatic activity against *Helicobacter pylori*, which causes stomach ulcers, for at least 10 months. In our hands the antimicrobial activity of garlic juice was destroyed after 10 min at 80°C but showed no loss of activity after 10 days at 4°C. Storage at room temperature led to a 50% reduction in the diameter of the inhibition zone against *E. coli in vitro* over the same period (Curtis et al. 2004). On a hobby gardener scale stability is not a problem because garlic juice can be freshly prepared and used immediately. The onus is on the plant protection industry to acknowledge the potency of this natural product and find ways to develop a suitable commercial product from it.

One approach to formulation of plant extracts is encapsulation which has been used successfully to stabilize and establish bio-pesticides in soil (Patel et al. 2004). For this reason we have carried out experiments using alginate and other formulations to encapsulate garlic juice and applying the capsules at various dosages to the soil around *Phytophthora*-inoculated tomato seedlings (Fig. 7). The results were promising in comparison to direct spraying of the plants. Thus, some formulations enhanced activity whereas others were clearly less effective (Fig. 8). Probably such factors as the rate of release and stabilisation of the garlic preparation were important. It seems there is scope for further optimisation in this area.

Whether allicin can be derivatized to improve its field qualities and still retain antimicrobial activity is unclear. A systematic investigation by synthetic chemists is needed to determine whether other related molecules or modification of allicin structure could lead to desirable plant protection chemicals.

Special applications of allicin: seed disinfection

In the EU-wide regulations governing organic farming (Council Regulation 2092/91 on Organic Farming) it is laid down that organic produce must be derived from the sowing of organically-produced seeds. Seed-borne diseases and seed hygiene are increasing problems in this sector and acceptable seed treatment procedures are urgently needed.

Commercial seed companies often employ a procedure called ‘priming’ to improve the germination rate and uniformity of germination of their seeds. Basically, seeds are allowed to imbibe for a period and are then dried down again to let them remain dormant (Bradford 1999; Gao et al. 1999). Priming procedures are generally empirically determined for particular seeds and each company has its own ‘secret’ protocol. By allowing seeds to imbibe allicin-containing preparations, followed by subsequent drying down, we have achieved improvements in the germination rate of *Alternaria*-infested carrot seed that are comparable to results obtained with the industrial seed dressing Aatiram® (active ingredient: 670 g kg^{-1} thiram) (Fig. 9). Whether these laboratory successes will transfer to commercial applications is yet unknown.

Concluding remarks

Whether the use the natural substance allicin, or garlic juice, for seed-disinfection or other plant protection strategies, conforms with organic farming practice must still be determined by the relevant regulatory body. Nevertheless, allicin seems to offer a promising alternative to the use of synthetic compounds and the analogy to the accepted use of azadirachtin and neem seed products is clear. Hopefully the future will see the increased development of successful plant protection strategies based on natural products.

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References

Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Econonomic Entomology*, *18*, 265–267.

Aldhous, P. (1992). Neem chemical: The pieces fall in place. *Science*, *258*, 893.

Arya, A., Chauhan, R., & Arya, C. (1995). Effect of allicin and extracts of garlic and bignonia on two fungi. *Indian Journal of Mycology and Plant Pathology*, *25*, 316–318.

Bianchi, A., Zambonelli, A., Zechini D’Aulerio, A., & Bellesia, F. (1997). Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi *in vitro*. *Plant Disease*, *81*, 1241–1246.

Block, E. (1985). The chemistry of garlic and onions. *Scientific American*, *252*, 94–99.

Bradford, K. J. (1999). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *HortScience*, *21*, 1105–1112.

Butterworth, J. H., & Morgan, E. D. (1968). Isolation of a substance that suppresses feeding in locusts. *Chemical Communications (London)*, 23–24.

Cao, K.-Q., & vanBruggen, A. H. C. (2001). Inhibitory efficacy of several plant extracts and plant products on *Phytophthora infestans*. *Journal of Agricultural University of Hebei*, *24*, http://www.cipotato.org/gilb/Pubs/proceedings_easa/caokeqiang%2821%29.pdf.

Canizares, P., Gracia, I., Gomez, L. A., Garcia, A., de Argila, C. M., & Boixeda, D., et al. (2004). Thermal degradation of allicin in garlic extracts and its implication on the inhibition of the *in-vitro* growth of *Helicobacter pylori*. *Biotechnology Progress*, *20*, 32–37.

Carpinella, M. C., Ferrayoli, C. G., & Palacios, M. S. (2003). Antimycotic Activity of the Members of Meliaceae. In M. Rai, & D. Mares (Eds.) *Plant-derived antimycotics* (pp. 81–115). New York: Food Products.

Cavallito, C. J., & Bailey, H. J. (1944). Allicin, the antibacterial principle of *Allium sativum* L. Isolation, physical properties and antibacterial action. *Journal of the American Chemical Society*, *66*, 1950–1951.

Curtis, H., Noll, U., Störmann, J., & Slusarenko, A. J. (2004). Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and Molecular Plant Pathology*, *65*, 79–89.

Fleming, D. O., & Hunt, D. L. (Eds.) (2000). Biological safety: Principles and practices. (ASM Press) p. 267.

Fry, F. H., Okarter, N., Baynton-Smith, C., Kershaw, M. J., Talbot, N. J., & Jacob, C. (2005). Use of a substrate/alliinase combination to generate antifungal activity in situ. *Journal of Agricultural and Food Chemistry*, *53*, 574–580.

Gao, Y.-P., Young, L., Bonham-Smith, P., & Gusta, L. V. (1999). Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. *Plant Molecular Biology*, *40*, 635–644.

Goellner, K., & Conrath, U. (2007). Priming: It's all the world to induced disease resistance EJPP this issue.

Grasso, V., Palermo, S., Sierotzki, H., Garibaldi, A., & Gisi, U. (2006). Cytochrome *b* gene structure and consequences for resistance to Q_o inhibitor fungicides in plant pathogens. *Pest Management Science*, *62*, 465–472.

Jain, M. K., & Apitz-Castro, R. (1987). Garlic: molecular basis of the putative 'vampire-repellant' action and other matters related to heart and blood. *Trends in Biochemical Science*, *12*, 252–254.

Krest, I., & Keusgen, M. (2002). Biosensoric flow-through method for the determination of cysteine sulfoxides. *Analytica Chimica Acta*, *469*, 155–164.

Ley, S. V. (1994). Synthesis and chemistry of the insect antifeedant azadirachtin. *Pure & Applied Chemistry*, *66*, 2099–2102.

Mansfield, J. (2000). Antimicrobial compounds and resistance: The role of phytoalexins and phytoanticipins. In A. J. Slusarenko, R. S. S. Fraser, & L. C. van Loon (Eds.) *Mechanisms of resistance to plant diseases* (pp. 325–370). Dordrecht: Kluwer.

Miron, T., Rabinikov, A., Mirelman, D., Wilchek, M., & Weiner, L. (2000). The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochimica et Biophysica Acta*, *1463*, 20–30.

Nicolaou, K. C., Roecker, A. J., Monenschein, H., Guntupalli, P., & Follmann, M. (2003). Studies towards the synthesis of azadirachtin: Enantioselective entry into the azadirachtin framework through cascade reactions. *Angewandte Chemie International Edition*, *42*, 3637–3642.

Patel, A. V., Slaats, B., Hallmann, J., Tilcher, R., Beitzen-Heineke, W., & Vorlop, K. D. (2004). Encapsulation and application of bacterial antagonists and a nematophagous fungus for biological pest control. In J. L. Pedraz, G. Orive, & D. Poncelet (Eds.), *Proceedings of the 12th International Workshop on Bioencapsulation, Vitoria, Spain, Servicio Editorial de la Universidad del Pais Vasco* (pp. 137–140). ISBN: 84-8373-649-7.

Peterson, I., & Anderson, E. A. (2005). The renaissance of natural products as drug candidates. *Science*, *310*, 451–453.

Portz, D., Noll, U., & Slusarenko, A. J. (2005). Allicin from garlic (*Allium sativum* L.): A new look at an old story. In H.-W. Dehne, U. Gisi, K. H. Kuck, P. E. Russell, & H. Lyr (Eds.) *Proceedings of the 14th International Reinhardsbrunn Symposium, Modern Fungicides and Antifungal Compounds IV* (pp. 227–234). Alton, U.K.: British Crop Production Council.

Rabinikov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D., & Weiner, L. (1998). The mode of action of allicin: Trapping of radicals and interaction with thiol containing proteins. *Biochimica et Biophysica Acta*, *1379*, 233–244.

Russell, P. E., & Mussa, A. E. A. (1977). The use of garlic (*Allium sativum*) extracts to control foot rot of *Phaseolus vulgaris* caused by *Fusarium solani* f.sp. *phaseoli*. *Annals of Applied Biology*, *86*, 369–372.

Tetko, I. V., Gasteiger, J., Todeschini, R., Mauri, A., Livingstone, D., & Ertl, P., et al. (2005). Virtual computational chemistry laboratory—Design and description. *Journal of Computer-Aided Molecular Design*, *19*, 453–463.

Wolfgang, L. (1995). Patents on native technology challenged. *Science*, *269*, 1506.