Garlic – from Nature's ancient food to nematicide

With the imperative to protect crops from pest damage while protecting the environment there is a clear need for 'green' alternatives to synthetic pesticides. UK-based company, ECOspray, has steered a course through the EU and UK pesticide regulatory frameworks to finally bring a garlic-based product to market. Its story is one of dogged determination in the face of numerous set-backs. ***Dr. Awais Anwar, Dr. Murree Groom*** *and* ***David Sadler-Bridge*** *report.*

Garlic has been cultivated for around 4,000 years and has not only been used in food preparation but also as a medicine and crop protection product. This long history gave garlic a head start in recent efforts to gain EU regulatory approval for its use in agriculture. A vast body of literature was available to drawn upon when compiling the required data, for example data on toxicity, residues, ecotoxicity and fate and behaviour in the environment. As the regulatory data package matured, more effort was focused on establishing the physical chemistry and efficacy of garlic. Within the current framework for regulating pest control products, these two areas are the most technically challenging to satisfy when registering botanical extracts.

The key difficulty in registering botanical products is the intrinsic complexity of extracts or concentrates produced from a plant. The regulatory framework, now broadly similar in all countries, has been developed to register single molecules that have been produced synthetically with the rigorous quality control possible in large industrial facilities. This makes experimentation on toxicity, residue retention, breakdown in the environment and metabolism a relatively straightforward, although expensive, undertaking.

By contrast a plant extract may contain many biologically active molecules, the quantities and composition of which may vary with the cultivar used, environmental conditions during growth and conditions used through processing. Such complexity is not easily addressed within the present regulatory framework.

Despite these difficulties, many plant extracts have shown consistent and potent biological activity. Garlic is a good example.

Chemistry of garlic

Garlic, onions and related plants of the genus *Allium* have been part of folk medicine for many centuries all over the world. Although their precise health benefits are a matter for debate, recent research has provided a number of new insights which deepen our understanding of *Allium* plants and derived products, such as garlic oils and powders[1,2]. The name 'allium' itself is derived from the Celtic word 'all', which means pungent, referring to the characteristic odour of these plants. Numerous biologically active ingredients of garlic, including allicin and polysulfides, have been identified and their interactions with biomolecules, cells and organisms described. In many cases, there seems to be a broad-range of antimicrobial activity associated with the sulfur-containing compounds derived from *Allium* species, which manifests itself in diverse antibacterial, antifungal and pesticidal activities. These activities seem to be the result of an intrinsic cytotoxicity of various sulfur compounds present in these plants. The highly toxic sulfur compounds often known as polysulfides or organosulfur compounds are generated in the clove under certain conditions, such as when injured (by chopping or crushing) or attacked by microbes[3]. Under these conditions the sulfur containing amino acids are hydrolysed by an enzymatic process to yield an unstable compound called allicin.

Allicin, once formed, decomposes upon 'ageing' or heating. The decomposition process is complex and results in a wide range of sulfur compounds, including diallylsulfide (DAS), diallyldisulfide (DADS), diallyltrisulfide (DATS), diallyltetrasulfide (DATTS), as well as a range of 'higher' sulfides, such as diallylpentasulfide and diallylhexasulfide, vinyl diithins, ajoene and others. The allyl sulfides show high levels of activity. In particular, various biological tests have confirmed DATS and DATTS as the most active compounds[4,5].

Regulatory developments

Post-war changes

War time endeavours in the field of chemistry produced a huge resource of biologically active synthetic chemicals, some with an insidious history. In the immediate post war period, the chemical industry sought new applications for these chemicals. Some entered agriculture as pesticides. In the three decades that followed WW2, food production increased significantly, heralding the era of cheap food for developed economies.

The regulatory framework for introduction of new active chemicals to agriculture evolved during this time. It became increasingly complex as methods for detection and analysis of these chemicals advanced.

Ecosystem effects

In the mid 1960's broader biological understanding of host/pest interactions and ecology helped develop the concept of an agricultural ecosystem. This led to even greater complexity in the regulatory framework, fueled by increasing unease over the emerging evidence of the ecotoxicity of products such as the organochlorines.

The evolving regulatory process forced up costs of registration to the point that a major new active currently costs at least £100 million to take from the point of discovery to broad market entry. The ever increasing costs of agricultural crop protection products locked the industry and regulators into a waltz from which neither party could retire. With increasingly complex data requirements come increased costs; with increased costs comes the need for bigger companies with sufficient capital for product development; with bigger companies comes more regulatory interest and so on. The high cost of product development can now only be justified on six major world crops including maize, rice, cereals and soya, leaving minor crops, including potatoes, at risk of having fewer protective products.

Consumer power

The rapid empowerment of consumers in the early 1980's coupled with broader definitions of food quality, changed the situation again. Consumer concern about the safety of pesticides and the sustainability of production methods started to influence the retailers. This opened up another market influence, that of consumer selectivity.

Increasing consumer concern within Europe and the need to harmonise regulations within the expanding European Union prompted a review of the pesticide regulatory process in Europe. This has been taking place under EU directive 91/414 since the early 1990s.

ECOspray enters the scene

Identifying a change in the market, ECOspray, a UK-based business, came into existence in 1997 focusing on the chemistry of garlic as a potential source of new pesticides from a benign botanical

origin.

Developing country agricultural systems have limited financial resources and generally operate on low input models. They have a long history of using plant juices and extracts, such as neem, for the protection of crops from pest and disease. However, after 10 years of intense work to register garlic in the EU, the reaction to the use of a plant extract as a pesticide is still very variable.

In the UK in the late 1990's, natural products were of fringe interest with their primary entry point being the organic sector or as general plant stimulants. To avoid the stringent requirements of a regulatory system designed to regulate synthetic chemicals these natural products were marketed with no assertive claims about pesticidal function on the product label. This tended to keep these products off the regulatory radar but as a consequence they suffered from low commercial credibility.

When ECOspray sought to bring garlic to market as a pesticidal product it had no choice but to adopt the guidelines applied to the chemistry of a new synthetic active under the Control of Pesticides Regulations (COPR, 1986). It had to take full account of the biochemical complexity of garlic in order to plot a course through the chemical and manufacturing regulatory data requirements, which had been evolving around single molecule activities for more than fifty years. This involved characterizing and developing quality control for a complex plant extract, developing new analytical technology and proving batch-to-batch consistency. This was not an easy task but was completed around 2004.

Initially several poor quality garlic based materials such as Garlic Barrier (distributed at that time by ECOspray) entered the market. But results using these products were very inconsistent. On one occasion, two sequential deliveries of Garlic Barrier were subjected to a quality control bioassay. One batch exhibited 100% mortality on the test organism while the other batch exhibited 0% mortality. With this scale of variability, scepticism about the potential of these products persisted in both the market and regulatory bodies. Critical mass for greater market penetration of natural products had still not been achieved by the early 2000s.

However, there were some initial signs that within the complex mixtures of plant extracts, viable biological activity as crop protection agents could be identified. Garlic and neem based products started to attract growing interest around this time. And ECOspray identified a much more consistent source of garlic extract.

Garlic's biological activity

The extensively studied sulphur compounds derived from garlic[6] represent excellent candidates for green nematicides or insecticides, with approvals being given to ECOspray for both these uses. However, the nematicide function is particularly important due to the toxicity of the conventional nematicides currently on the market.

Recent work carried out by ECOspray and their collaborators: Professor Claus Jacob (University of Saarland, Germany), Dr. Colin Fleming (Agri-Food and Biosciences Institute, Northern Ireland), and Professor Eric Block (University at Albany, New York) proved that the polysulfides present in garlic are potential nematicides. Further evidence came from field trials where the garlic-based products were effective in preventing nematode

Garlic's nematicidal activity

The nematicidal activity of garlic has been effectively demonstrated in a series of experiments.

Figure 1 shows the toxicity of individual garlic polysulphides (at 200 µM) against a benign nematode *Phasmarhabditis hermaphrodita*. Nematode mortality increases in the presence of the polysulfides. The polysulfides are similarly toxic to another nematode *Steinernema feliate* (not shown).

Figure 1

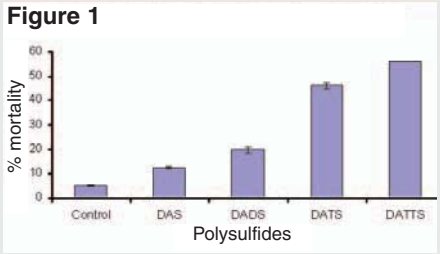
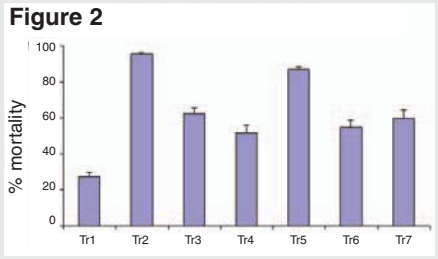


Figure 2 shows the nematicidal effects of ECOspray's NEMguard formulations (garlic polysulfides) compared with Temik (aldicarb) *in vivo* against *Longidorus elongates*, a known plant parasitic nematode. Tr1 - Control, Trt 2 - Temik, Tr3-7 various NEMguard formulations. The direct nematicidal effect of both Temik (aldicarb) and NEMguard is clear.

Figure 2



The effects illustrated in figures 1 and 2 have been transferred to the field and in independent certified trials. NEMguard has delivered comparable levels of efficacy to the synthetic nematicides on the market.

Figure 3 shows an independent trial on the effects on a carrot crop carried out by AgriSearch (trial 2, 2004) for the British Carrot Growers Association. Nematodes cause carrot roots to fork and NEMguard applied at 20 kg/ha delivered the greatest reduction in damage.

Figure 3

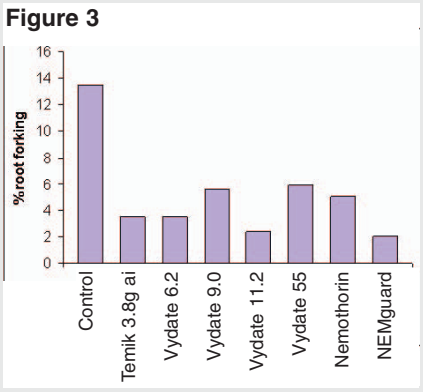


Figure 4 shows the effect of NEMguard in a parsnip crop. The trial was carried out by the Processors and Growers Research Organisation (2006, trial 1) and clearly showed that NEMguard had comparable levels of efficacy with Temik (aldicarb). The 15 and 20 kg/ha rates of NEMguard are statistically equivalent to Temik used at its recommended rate.

Figure 4

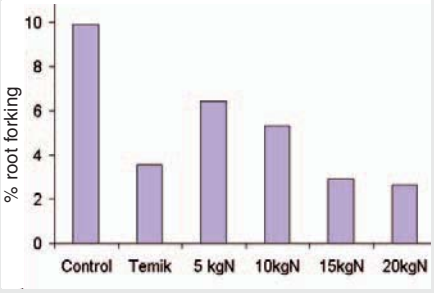
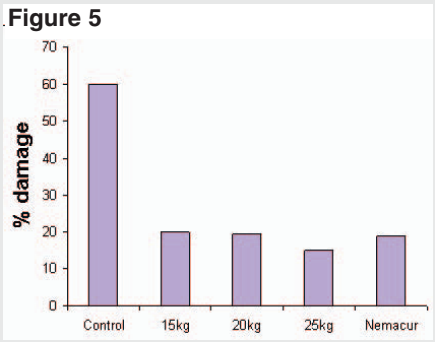


Figure 5 shows a 2008 trial by IntraChem in Italy comparing the effect of NEMguard on carrots with Nemacur (a Bayer nematicide). Nemacur applied at the recommended dose of 300 kg/ha compares to the 25 kg/ha maximum use rate for NEMguard.

Figure 5



attack. The work focused on various free living nematodes in the UK that damage carrot and parsnip crops, and root knot nematodes which damage carrot and tomato crops in Italy. The ECOspray product was as effective against both types of nematode as the synthetic nematicides (Temik, Nemacur and Basimid) (see Box).

ECOspray developed a highly refined garlic extract, manufactured in a food processing plant, using extracts similar to those used for food flavours. The combination of this new very consistent food grade garlic juice concentrate with a granulation technology, based on mixing the garlic extract with wood flour and biodegradable binders in a granulator produced NEMguard®, a new nematicidal product capable of delivering efficacy comparable to synthetic products such as Temik (aldicarb) and Vydate (oxamyl) and Nemacur (see Box), all of which are under intense international scrutiny.

NEMguard was recommended for full 'on label' approval as a nematicide by the Pesticide Safety Directorate (PSD) in the UK in November 2007. It had taken ECOspray seven years to work the formulation through the COPR regulatory process as no alternative regulatory framework for natural products was in place during this period.

The full range of ECOspray's garlic active ingredients was voted through the EU Commission for inclusion into Annex 1 of 91/414 in October of 2008. Finally there was some light at the end of a very long tunnel.

ECOspray is now able to start developing commercial opportunities whilst still working hard on technical issues as approvals are being sought in many countries both within and outside the European Union including the USA, Australia and South Africa. It has already received a registration for ECOguard®, a garlic-based insecticide registered for cabbage root fly control in Denmark in February 2006 (it received organic registration for the same product in september of the same year in Denmark). And in early 2008 it received a registration for ECOguard® also for cabbage root fly control in Norway.

Outlook

There has been considerable interest in the development of pesticides based on organosulphur compounds derived from garlic, onions and other *Allium* species since such food-based compounds should be environmentally benign, potentially widely available and low in cost. It is known that *Allium* species are resistant to insects and fungi and that extracts and distillates of these plants can function as nematicides and repellents against a range of pests. Not surprisingly, *Allium* species are used in inter-cropping to protect crops such as carrots from pests[7]. In addition, certain compounds from *Allium* species have been found to function as growth stimulators.

Recent initiatives by the pesticide regulatory departments of European and North American governments have stimulated renewed interest in biopesticide technologies to replace toxic synthetic pesticides with more benign natural products. Although researchers have looked for microorganisms with biopesticidal activity, very few have considered formulating microorganisms with commercial applications in mind. A concerted effort in formulation development for biopesticides by multi-disciplinary teams is required to optimize biopesticide yield, efficacy, storage stability and delivery for this technology to evolve and meet today's agricultural and societal demands.

Most remarkable is the evidence that a food grade extract of garlic can kill nematodes with the same efficacy as a material that started off as a chemical developed as a nerve gas during the war. This story is one of dogged determination from a very small company, pushing against closed doors for several years. The doors are now opening.

ECOspray

ECOspray is committed to the advancement of ethical and sustainable food production through the scientific development of naturally based products and decision support systems. It was set up in 1997 with Dr Murree Groom as sole employee and was privately funded by two Norfolk-based farming families. David Sadler Bridge joined in 1999 as Managing Director, Stephen Silvester in 2002 as sales manager and Awais Anwar in 2008 as a biochemist.

ECOspray have been successful in obtaining various grants including Framework 5B, SMART, EEDA. They have now obtained support at EU level through Red Cat.

Their first approval was in 2005 for a garlic-based biocide for control of European red mite in poultry. ECOspray's interests now extend to sports turf nematicides and the therapeutic effects of polysulfides against cancer and blood feeding pests such as mosquitoes, mites and ticks.

In existence for twelve years, ECOspray has evolved and survived through major changes in the regulatory process associated with the introduction of alternative crop protection products to the market. From the mid-1990s there has been an intent to broaden the current regulatory framework. But despite this crop protection product regulation is still based heavily around synthetic chemistry. Botanical extracts, although widely used in many parts of the world to protect crops, have not made much headway in the EU regulatory framework. ECOspray is an exception.

Save the Date!

'Birds and pesticides – Is the threat of a Silent Spring really behind us?'

In the 2009 Rachel Carson Memorial Lecture, **Pierre Mineau** will describe the highs and lows of the ongoing struggle to protect our feathered friends from the ravages of modern pest control. Dr. Mineau conducts research on pesticides for Canada's federal department of the environment at the National Wildlife Research Centre in Ottawa.

The **2009 Rachel Carson Memorial Lecture** will take place on the evening of **Wednesday 2 December** in London. More details in the next issue of Pesticides News.

References

1. A Anwar, Natural polysulfides- Reactive sulfur species from Allium with applications in medicine and agriculture, PhD dissertation 2009, University of Saarland Germany.

2. A Anwar, T Burkholz, C Scherer, M Abbas, C-M. Lehr, M Diederich, and C Jacob, Naturally occurring reactive sulfur species, their activity against Caco-2 cells, and possible modes of biochemical action, Journal of Sulfur Chemistry 2008, 29(3), 251-268.

3. C Jacob and A Anwar, The chemistry behind redox regulation with a focus on sulphur redox systems, Physiol Plant. 2008, 133(3), 469-80.

4. E Block, The Organosulfur Chemistry of the Genus Allium - Implications for the Organic Chemistry of Sulfur, Angew. Chem. Int. Ed. Engl. 1992, 31(9), 1135-1178.

5. U Muenchberg, A Anwar, S Mecklenburg and C Jacob, Polysulfides as biologically active ingredients of garlic, Org. Biomol. Chem 2007, 5(10), 1505-1518.

6. LD Hjeresen and R Gonzales, Can green chemistry promote sustainable agriculture?, Environ Sci Technol. 2002, 36 (5), 102-107

7. Op cit 6

Dr. Murree Groom, Technical Director, ECOspray, Grange Farm, Hillborough, Norfolk, IP26 5BT, UK (01760 756 100), jgroom@btinternet.com; David Sadler-Bridge, Chief Executive, ECOspray, Sadler-Bridge@ecospray.com; Dr. Awais Anwar, Biochemist, ECOspray, a.anwar@mx.uni-saarland.de