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Rapid communication

Basic substances under EC 1107/2009 phytochemical regulation: experience with non-biocide and food products as biorationals**

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Abstract: Basic Substances are a newly effective category of Plant Protection Product under EC Regulation No 1107/2009. The first approved application of *Equisetum arvense* L. opened Part C of Implementing Regulation (EU) No 540/2011, which lists the basic substance approved. Although *E. arvense* was described as a fungicide extract, subsequent applications like chitosan were related to non-biocide molecules. Consequently, plant protection product data were collected from research on alternative or traditional crop protection methods. They are notably issued or derived from foodstuffs (plants, plant by-products, plant derived products, substances and derived substances from animal origin). Applications are currently submitted by our Institute, under evaluation at different stages of the approval process or already approved. Remarkably, this Basic Substance category under pesticide EU Regulation was surprisingly designed for these non-biocidal plant protection products. In fact, components described as the "active substance" of most of the actual applications are food products like sugars and lecithin. Basic Substance applications for these foodstuffs are therefore a straightforward way of easily gaining approval for them. Here we describe the approval context and detail the agricultural uses of theses food products as Biological Control Agents (BCAs) or biorationals for crop protection. From all deposited or approved Basic Substance Application (BSA), a proof has been provided that non-biocide and food products via physical barrier or lure effects may be effective plant protection products with an acceptable low profile of concern for public and agricultural safety.

Key words: basic substance, Biological Control Agent (BCA), biorationals, food products, Regulation (EC) No 1107/2009_article23

Introduction

The opportunity of the approval of biorationals in agriculture as Basic Substances at EU level under the Plant Protection Product (PPP) Regulation (EC) No 1107/2009 (EC 2009) is now fully operative (EU 2016a). Pilot projects were constituted with a wide range of natural products or substances included in the Biological Control Agent (BCA) area from mineral, plant and animal sources (EU 2014a; EU 2014b). These approvals under article 23 of Regulation (EC) No 1107/2009 with inscription in Part C of Implementing Regulation (EU) No 540/2011 (EU 2011) are described in various papers (Villaverde *et al*. 2014; Marchand 2015a).

Prospective applications were opened using an organic farming regulation, which provides many examples of the uses of natural products (Felsot and Racke 2007), listed in Annex II of organic farming regulation (EC 2008). Identified products in accordance with traditional biological insecticide activity such as the bark of *Quassia amara* are typically of interest, but other types of mode of action (MOA) were to be investigated as well. Looking for non-biocide compounds or substances, we reviewed our previous research programs on field trials and literature in order to complete the applications as basic substance. In this respect, food products are comparably unproblematic as substance to generate Basic Substance Application (BSA) because of their intrinsic basic substance status as satisfactory to article 2 of EC Regulation No 178/2002 (EC 2002). These products or candidate substances are able to meet all the requirement of food security (Popp *et al*. 2013), ethic of organic farming (OF) and regulatory requirements organic production (OP) (EC 2008).

This paper is focused on the practical development of these natural substances under new phytopharmaceutical regulation for traditional or newly developed uses, together with current field efficacy trials. These applications are at the evaluation stage under EU regulations. We describe here "core" data from the end-user agricultural point of view. In fact, these BSA can be reduced for the farmers to the recipe and the Good Agricultural Practices (GAP) table.

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Collection of candidate substances and methods

Substances collection

Plant protection product data were collected from research programs on alternative or traditional crop protection methodologies. They are notably issued or derived from foodstuffs (plants, plant by-products, raw, composted or converted botanical substances and animal products). This collection is instructive regarding the MOA of these products, showing some non-biocidal way of "efficacy" largely in contrast to conventional chemical crop protection products. Alternative expedient for crop protection like plant strengtheners, plant defences stimulators and lure compounds were evaluated in fields and correlated with existing results in the literature. Earlier non-approved pesticide list of low concern substances (EC 2007) was also an inspiration or further validation for considered substances.

Field experiments

Field trials were conducted in France between 2009 and 2015, on organic and non-organic farms. Plants tested in the "4P" Casdar program (Marchand *et al*. 2014) for their antifungal properties are horsetail (*Equisetum arvense* L.), and white willow bark (*Salix* cortex). Vinegar was studied against *Tilletia caries*, the common blunt in the "Carie" program (Fontaine 2012). "Usage" program (Arnault 2015; Arnault *et al*. 2015) was dealing with sugars (sucrose, fructose, glucose) as an elicitor of the crop's natural defence mechanisms against insects and fungistatic in field investigations on grapevine and fruit tree protection; and currently the "Sweet" Casdar program is amplifying these protection effects on more crop pathosystems. Lecithin was tested for its antifungal properties against grape downy and powdery mildews (*Plasmopara viticola, Erysiphe necator*) in vineyards during in the "HE" Casdar program (Vidal 2016). Talc was tested for its properties in arboriculture (Compo Expert 2015). Whey was tested by Chambre d'Agriculture of Aude during a trial program in 2011 (CA 2011). Di-ammonium phosphate (DAP) was studied by Afidol in mass trapping of the olive fly program "Piegeage massif de la mouche de l'olive" (Afidol 2016). Calcium hydroxide was mainly studied by FÖKO, an organic fruit tree association in Germany (Montag *et al*. 2006).

Ecotoxicological assessments

In order to assess ecotoxicological impact of submitted substances compared with organic and chemical insecticide references, assessments were conducted with some of our plant extracts or decoctions on bees measuring contact toxicity (Giffard and Mammet 2012).

Recipes

All uses of plant extract and natural product as decoctions, herbal teas or solutions in water were tested and defined during field trials (above) or identified from the literature and checked or cross-checked with the producer surveys. Whenever water is mentioned in these tests clearly natural spring or cold rain water is used. All products recipes described in Point 2.5 (EU 2012) of the applications (BSA) are stated below.

- Plant extracts

Horsetail (*Equisetum arvense*)

The decoction is processed as follows: 200 g of the aerial part of *E. arvense* dry plant tissues are macerated in 10 l of water for 30 min (soaking) and then boiled for 45 min. After cooling down, the decoction is filtrated with a fine sieve or more generally with a stocking and then further diluted by 10 with water. The solvent for extraction and preparation was water (spring water or rainwater) and the pH was 6.5. Decoction is described in the implementing regulation (EU 2014a) and a further "Report Review" is available in EU pesticide database (EU 2016b).

White willow bark (*Salix* cortex)

Infuse 200 g of the aerial part of *Salix* cortex dry bark plant for 2 h in 30 l of natural spring or rain water brought to a simmer at 80°C in a covered stainless steel tank. After cooling down, and filtration with stainless steel sieve, adjust the pH to 6.2 and dilute with 3 with parts water for agricultural uses.

- Substances used as water dilutions or suspensions

Sugars (sucrose, fructose)

Typical solutions of sugars at concentration range from 0.1 to 100 ppm in water are used in fields as an insect lure through plant defence metabolism at 2 to 10 g · ha^{-1} rate per application.

Vinegar

Distilled vinegar, cider vinegar or wine vinegar at 5–10% acetic acid: 1 l vinegar + 1 l water; 2 l preparation (50 : 50 vol : vol) mix with 1 ql (100 kg) of seeds.

Lecithin

Typical used concentrations of lecithin in water are from 75 to 200 g per hl and amounts from 0.075 to 2 kg · ha^{-1} for fungicide uses.

Whey

Whey is used with spray rate ranging from 6 to 30 l · ha^{-1} diluted in water.

Di-ammonium phosphate (DAP)

Used concentrations of DAP from 30 to 50 g · l^{-1} in water for trapping olive flies in bottles with 3 mm holes. Regulatory note: field mass trapping uses require plant protection product regulation approval of the corresponding active substances in opposition to occurrence monitoring.

Talc

Quantities of talc, suspended in water, ranged from 25 to 100 kg · ha^{-1} and are sprayed for fungistatic uses.

Calcium hydroxide

Lime water [$Ca(OH)_2$] was used as a suspension in water, ranging from 15 to 350 kg · ha^{-1}.

Results

Previously, field research investigations in order to validate the traditional uses of naturally occurring substances was coordinated by our institute or conducted by partners. Numerous solutions from different sources are now characterized and reasonably efficient for plant protection. Distinct from active chemical substances in terms of efficacy, but in the same proportion not of concern for their toxicological effects and residues, some natural substances are nevertheless promising as crop protectant. Clearly, these substances are fully within the scope of the Recital (Whereas) 18 of Regulation (EC) No 1107/2009 regarding food status and utility in crop protection. As the French Institute is in charge of research/experimentation coordination and transfer of knowledge in organic agriculture, we continuously develop regulatory expertise on natural substances for plant protection, which are one part of the widely used alternative means for crop protection, including physical barriers. An interesting issue of these products is an appreciation or understanding of these non-biocidal modes of action. These aspects are of importance when considering the need to reduce the toxicity of pesticides while still meeting the expectations of organic producers.

Innovative substance characteristics

History of dossier submission

The first approved basic substance as of *E. arvense* stems extract is definitively not considered as an intrinsic biocide mixture, but more like a plant strengthener with fungistatic effects. Later applications had even less to do with any biocide considerations. The next approved basic substance, chitosan (EU 2014b) is clearly not a biocide at all. Subsequently, limewater used as an agent in cooking and diarrheal treatment in baby medicine was the fourth basic substance approved (EU 2015a). Later, *Salix* cortex (EU 2015b) voted for approval at the Standing Committee on Plant, Animal, Food and Feed (PAFF Committee) is once more clearly not biocidal; as it is a plant extract used for headache treatments, willow bark extract (glycoside conjugates), cannot be considered as biocide.

From non-biocidal mode of action to food products

As foodstuffs (EC 2002) are intrinsically entitled as basic substance (EC 2009) the registry of food products was investigated. Plant products and by-products have been known for a while in agriculture; simple food products such as sugar molecules, together with vegetable oils are therefore automatically entitled to be approved at general EU pesticide regulation level for plant protection product (PPP) (EC 2009), although some of them are already allowed at Organic Farming regulation level (EC 2008).

Again, there was no requirement to reclaim food status for approved basic substances such as sucrose (EU 2014c), lecithin (EU 2015d) fructose (EU 2015e) and vinegar (EU 2015c). Focusing on these products for their non-biocidal MOA has generated many applications. Talc as a non-soluble mineral and physical barrier was one pilot project. As a food grade compound at EU level (E553b) and not of environmental concern (ANA 2010) during operations in the quarry (Imerys, Luzenac, France), basic substance status was again automatic. Again, sodium bicarbonate (EU 2015f) for which approval at Standing Comitee on Plants, Animals, Food and Feed (PAFF) was examined in October 2015 is also treated as a foodstuff. Latest approved (EU 2016c) DAP against olive flies is used as food grade phosphorus provider in oenology during wine fermentation. Finally, whey is the latest approved food based substance in this category (EU 2016d).

Uses

Horsetail (*Equisetum arvense*)

The aqueous extract of horsetail as decoction is intended to be used in fields for plant protection on grapevines and apple trees (Fauteux 2006; Garcia *et al*. 2011) and vegetable gardening to control diseases such as mildew, downy mildew and others caused by foliar fungi such as *Pythium* and *Alternaria* spp. Horsetail has long been known in the botanical tradition, organic and biodynamic agriculture as having a preventive effect on fungal diseases of plants. The effect is based on the high percentage of silica in the plant, which helps to reduce the impact of moisture. Silica would reduce the effects of excess water on plants that lead to the growth of fungi. *Equisetum arvense* decoction shows anti sporulation activity (Marchand *et al*. 2014). It would also be an activator of the defence mechanisms of these plants. Field typical suitable concentration is 200 g of active ingredient (a.i.) · hl^{-1}.

Sugars

Sugars (sucrose, fructose, glucose) are known to be efficient against insect pest species in a quite different way than more conventional insecticides. Typically, MOA of sugars at 1 g · hl^{-1} is a lure effect, by changing acids and amino acids leaf surface composition. Sugars were found to carry activity "against" insects in orchards. In fact, insect lure effects are managed at plant surface (leaves and stems) by change in acids/amino acids composition and ratio (Derridj 2013).

White willow bark (*Salix* cortex)

Willow bark extract is used to control foliar fungal diseases caused by *Taphrina deformans*, *Venturia inaequalis*, *Plasmopara viticola*, *Erysiphe necator* and *Podosphaera leucotricha*. Regarding the fruit trees (peach-tree, *Prunus persica* L. and apple fruit, *Malus pumila* Mill.) and the grapevine (*Vitis vinifera*), trials were carried out in France. A small fungicidal effect was observed with treatments of willow extract (typical concentration 220 g · hl^{-1}). The effect of this aqueous extract is due to the high percentage of salicylic glycosides or salicylate in the plant that works to reduce the impact of plant stress and also activates certain plant defence mechanisms. However, salicylic acid alone, in compared to the willow extract, did not provide the same level of effect. *Salix* cortex decoction shows anti sporulation activity (Marchand 2015b).

Vinegar

Diluted vinegar is intended to be used in fields for plant protection as fungicide/bactericide on wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.). Experiments with vinegar dressings have been performed on organic farms and have proven to be effective against common bunt (*Tilletia tritici, T. caries and T. foetida*) and barley leaf stripe (*Pyrenophora graminea*). *In vitro* trials, vinegar, cider vinegar, red wine vinegar and white wine vinegar have shown inhibitory effect against bacteria and fungi. Vegetables seed; carrots (*Daucus carota* L.), tomatoes (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L.) and bell pepper (*Capsicum* spp.) can also be protected from fungal and bacterial diseases including (*Clavibacter* spp., *Alternaria* spp., *Botrytis* spp., *Pseudomonas* spp. and *Xanthomonas* spp.).

The trials have shown that different tested vinegar types do not affect seed viability if the concentration of acetic acid is low (0.5–2%) but higher concentrations (more than 5%) drastically reduce the viability. Practically, acetic acid concentration should not be higher than 2% to avoid germination problems. However, even at 0.5% red- and white wine vinegar have positive effect on germination capacity (Borgen and Bent 2001; Tobias *et al*. 2007; Bruyère 2013). Regarding technical uses, typical on farm process 1 l of vinegar and 1 l of water are added to 1 ql of seeds during mixing. Industrial seed treatments units may avoid large liquid volumes and supress water addition since only amount of vinegar per quintal is compulsory.

Lecithin

Lecithin solution is intended for field use as a fungicide on vineyards, fruit trees, vegetable gardening and ornamentals (Misato *et al*. 1977; Trdan *et al*. 2008). Field trial concentrations of lecithin between 0.01 to 0.1% provided 25 to 30% protection against *Plasmopara viticola*. Surprisingly, low concentration (0.05%) was even more efficient than higher (0.5%) in contact *in vitro* trials (Aveline *et al*. 2013). Statistical difference with control (no treatment or water control) is positive for all concentrations (0.01% to 0.2%) with foliar disks. An additional field efficacy trial demonstrated a reduction in powdery mildew in vineyards, which supports the existing registration of lecithin in Switzerland and used at 75–200 g · hl^{-1} concentration.

Sodium bicarbonate

Sodium bicarbonate, a food additive known as baking powder, was tested in orchards in 2006 and 2007 (Kelderer *et al*. 2008) to overcome technical problems and/or phytotoxicity observed with formulated products. No phytotoxicity was observed at high temperatures (> 35°C). Field typical suitable concentrations are from 0.33 to 2 kg of active ingredient (a.i.) · hl^{-1}.

Whey

Whey has been studied by many researchers (Crisp *et al*. 2006) but the final recommended dried whey powder concentrations (i.e. 45 g · l^{-1}) are much higher than quantities of liquid whey (30 l · ha^{-1} max) normally used. Calculations ended with a factor of x38 since liquid whey is at 6% solid residue (94% water).

Di-ammonium phosphate (*DAP*)

Di-ammonium phosphate is a non-lethal attractant for both the male and female Mediterranean fruit fly [*Ceratitis capitate* (Wiedeman)] placed at 30 g · l^{-1} rate in physical traps (Caleca *et al*. 2007; Braham M. 2013; Gil-Ortiz 2015).

Talc

Talc usages were tested in arboriculture against pear psylla [(*Psylla pyri* (Linnaeus)] and olive fruit fly [(*Bactrocera oleae* (Rossi)] and show good efficacy as physical barrier (La Pugère 2011; GRAB 2013).

Calcium hydroxide

The post-infection activity of hydrated lime against conidia of *Venturia inaequalis* was evaluated using an *in vitro* test system based on isolated apple leaf cuticles. Experiments were conducted at 20°C and treatments were applied 24 or 48 h after inoculation. Experiments were assessed by counting living primary stromata 72 h after inoculation using fluorescence microscopy and fluorescein diacetate as a vital stain. At the conditions of the *in vitro* test system, hydrated lime had some post-infection activity. Suspensions of 5 g · l^{-1} applied 24 h after inoculation (16 h after infection) killed all primary stromata and stopped their further development. Treatments 48 h after inoculation reduced the number of vital primary stromata to 60% of control (Montag *et al*. 2006).

Discussion

The selection process for the identification of promising candidate substances was successful to gather plant protection substances derived from food products; used by farmers or determined by agronomic research. Investigating the new category of basic substances, introduced by EC Regulation No 1107/2009 and later better described by guidance document (EU 2012), the work of collecting usages was effective to produce many application dossiers.

Following the Pilot project *Equisetum*, a few food products were submitted from 2013 to 2015 and some approved at PAFF Committee. Up to now we submitted more than 20 new dossiers carried by Institut Technique de l'Agriculture Biologique (ITAB) alone or in collaboration with other institutes or small and medium-sized enterprices (SMEs). Some were approved and the remaining dossiers are following ongoing EU evaluation. Next, after this first successful approach, we selected more compounds issued from existing field results or trials from our research projects to be able to complete GAP tables. Accurate bibliographic data were also collected to complete all the chapters, especially toxicological ones. Although approved as active substances, they unmistakably meet the initial requirements and criteria of this new pesticide category and especially "Utility" cited in Whereas 18 (EC 2009). Proof then was afforded that regularization of these popular crop protection knowledge and practices could be performed in accordance with EU pesticide regulation. By means of the BSA procedure this requirement can be achieved with a minimum of investment and cost, for all Europe. This mandatory step also allows distinguishing efficient circumscribed uses from tales.

Conclusions

Substances without known biocidal activity were selected as candidate substances. For these substances evidence was provided that the putative MOA was not linked to biocidal activity. It has been proved that non-biocide and food products may be effective and approved as crop protection products with a low concern profile via physical barrier or lure effects.

More than 10 dossiers of various candidates were prepared and submitted. The approval rate was very high, demonstrating that the procedure for approval of safe and useful basic substances is appropriate and feasible. Most of them are now allowed in OF following 2016 OP regulation changes (EU 2016e).

Many foodstuff applications are deposited or engaged in ongoing evaluation (i.e. talc, capsicum spice, citrus pulp, sunflower oil, mustard powder and potassium sorbate) considering the demand from the organic and non-organic sectors coupled with societal expectations.

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