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The use of bio-based liquid formulations in pest control of citrus groves

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ABSTRACT

Over the last decade, many pest control techniques have seen substantial changes mainly due to the EU directive 2009/128/EC which relates to the registration process for new products and their toxicity. Thus, the list of pesticides is continuously revised and modified to include new plant protection products which have to be safer for human health, food quality and less polluting for the environment. These decisions increase the importance of and consequently the interest in non-chemical alternatives, including physical systems and bio-based products and the Directive clearly reports the need to define new non-chemical control systems in crop management and defense, as a partial or total alternative to conventional pesticides.

At the same time, several crops particularly adapted to the pedoclimatic conditions of southern Italy, such as trees belonging to the genus *Citrus*, are suffering due to presence of several pests and pathogens that can lead to considerable reductions of yield quality and quantity, and that are often resistant to the most common pesticides. This paper reports and discusses the results of trials carried out in 2011 on the efficacy of natural formulations based on products deriving from *Brassica carinata* (Ethiopian mustard) on *Citrus* groves in southern Italy against aphids (*Aphis gossypi*), mites (*Tetranychus urticae, Panonychus citri*), scales (*Protopulvinaria pyriformis*, *Icerya purchasi, Unaspis yanonensis*) and whiteflies (*Aleurothrixus floccosus,*). In general, the results were positive, suggesting that the experimental liquid formulations can represent a total or partial alternative to agrochemical pesticides and also an interesting option for organic farming.

1. Introduction

Citrus (*Citrus* spp.) is one of the most widespread fruit-tree genus cultivated in the world and particularly suited to Mediterranean regions. Italy is the second producer in Europe with almost 150,000 hectares harvested (FAOSTAT, 2012), entirely located in the south, where there is a wide pedoclimatic area suitable for different types of production, such as citrus for fresh consumption and for industrial processing.

Such a wide diffusion of citrus has facilitated the spread of pests and diseases that have to be controlled to avoid considerable plant damage with significant economic losses of yield quality and quantity (Benfatto, 2006). In the meantime, treatments that are frequently necessary can cause the development of resistance phenomena to some widely applied pesticides (Nauen et al., 2001). In addition, over the last decade many pest control techniques have seen substantial changes due to the EU directive 2009/128/EC which introduced restrictive limits for the registration of new pesticides, the definition of the phase-out of some conventional products characterized by high environmental impact (Rasmussen and MacLellan, 2001). Thus, the list of pesticides is continuously revised and modified in order to include new plant protection products that are safer for human health and better for food quality (Carroll, 2000). New pesticides must have the lowest possible environmental impact and, where necessary, must contemplate the growing consumer demand for safer and healthier food even

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from organic farming. In 2010, in fact, 8.7% of cultivated areas in Italy, corresponding to more than 4000 firms (Bioreport, 2012), were involved in organic farming production. This market has been growing rapidly in Europe and in the USA, where it increased from 1.0 in 1990 to 26.7 billion dollars in 2010 (Organic Trade Association, 2011).

According to the EU Directive 2009/128/EC, a fundamental strategy in reaching a more sustainable use of pesticides derives from: (i) new non-chemical options that range from agronomic techniques to physical and biological approaches with a low impact on the agro-ecosystem (Gilardi et al., 2011), and (ii) treatments with short or no withholding periods (Liu et al., 2010). the application of oil/water emulsions using oil and defatted oilseed meal (DSM) of the *Brassica carinata* A. Braun (Ethiopian mustard) was one of several alternatives that showed interesting perspectives. These formulations have proved to be effective in containment of *Aonidiella aurantii* Maskell (Rongai et al., 2008) and are also potentially applicable against some citrus grove pests. They combine the protective action of oil with the action of the bioactive compounds released from the DSM. In fact, the addition of water to the mixture containing oil and formulated *B. carinata* DSM causes a clearly defined release of glucosinolate degradation products, mainly allyl-isothiocyanate (AITC), a compound that is well-known for its biological activity on several pathogenic fungi (Manici et al., 1997), nematodes (Lazzeri et al., 2004), and pests (Furlan et al., 2010).

The aim of this paper is to evaluate, in greenhouse and at open field level, the efficacy of liquid formulations based on *B. carinata* oil and DSM in controlling some Citrus pests such as aphids, mites, scales and whiteflies.

2. Materials and methods

In 2011, eight trials were conducted on orange, lemon, mandarin and cedar trees in representative areas of southern Italy citricultures, heavily infested by citrus pests. After some preliminary trials, specific protocols were defined to verify the efficacy of liquid formulations based on *B. carinata* oil and DSM in the containment of the infestation in greenhouse and at open field level of the following key-pests considered as endemic in Sicily and Calabria regions (Benfatto, 2006): the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae); the twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae); the citrus red mite *Panonycus citri* McGregor (Acari: Tetranychidae); the pyriform scale *Protopulvinaria pyriformis* Cockerell (Hemiptera: Coccidea); the japanese citrus scale *Unaspis yanonensis* Kuwana (Hemiptera: Diaspididae); the cottony cushion scale *Icerya purchasi* Maskell (Homoptera: Margarodidae); and the woolly whitefly *Aleurothrixus floccosus* Maskell (Homoptera: Aleyrodidae). These reported species, largely present in Italy for many many years, were classified by the author, Benfatto (2006); the classification of *Protopulvinaria pyriformis* Cockerell (1894) and *Unaspis yanonensis* Kuwana (1923), which appeared on Italian citrus in more recent years, wasdone by John Martin, the Natural History Museum of London – Identification & Advisory Service (personal communication). The parameter used to measure the efficacy was the evaluation of the dead insect on an average of 250 specimens for each repetition. Considering that the repetitions were 4 it means that around one thousand specimens were evaluated for each thesis. The effect of the bio-based products was compared with that of chemical pesticides, using untreated plants as reference. In all the trials, both in open field and in greenhouse, the plants were naturally infested. Protocol required that some plants remain unsprayed to highlight the different effects of treatments on the infesting pests. A buffer zone was allowed between treatments to avoid any influence from neighboring treatments.

The phytotoxic effect was tested, assessing the presence of necrosis, stains or other tissue damage to leaves or fruits. Adult plants of mandarin cv. Nova and orange cv. Tarocco were sprayed until drop, every two weeks for 8 weeks from August to October. The treatments were applied with a sprayer powered by a 2-cycle engine. A spray pressure of 5 bars was used. Spraying was stopped when the sprayed mixture began to drip off the plants. Spraying operative conditions were those of good agricultural practice.

To verify the impact on natural enemies of pests, in-field collateral observations were performed on beneficial Arthropods spp., at the beginning and the end of the experimental trials, recording the number of living and dead individuals on the sprayed plants and on the untreated ones.

2.1. Description of chemicals and bio-based liquid formulations

The products used for these experiments are widely available in commerce: Afidane 200 SL (Chimiberg, active ingredient *N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl}nitramide, 200 gL^{-1}), Teppeki ISK (ISK Biosciences Europe, active ingredient *N*-cyanomethyl-4-(trifluoromethyl) nicotinamide, 50 g/g), Paraffinic oil (SIPCAM, Biolid E®, 20 mL L^{-1}), Clorpirifos (Dow Agrisciences, active ingredient *O*,*O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate 210 mL L^{-1}).

In the trials, three-phase patented formulations (Rongai et al., 2006) based on *B. carinata* oil and formulated DSM, purchased from Agrium Italia S.p.A. (Livorno, Italy) were used. The DSM was obtained with an endless screw pressing device. The pressure procedure applied for oil extraction was preferred to extraction by solvent. In this way, thanks to the absence of any chemical treatment, DSM application can also be admitted in organic farming. After extraction, the oil was filtered and partially refined (degummed).

2.1.1. Biomass characterization

The oil extracted was characterized for its fatty acid composition by the ISO 5508 method (1990); the DSM was analyzed for its main components using the following methods:

- Moisture content was determined by oven-drying DSM at 105 °C for 12 h and evaluating, by precision balance, weight differences before and after drying.
- Nitrogen content was determined by the Kjeldahl method (Standard UNI 22604, 1992), using a Tecator digestion system 20 and an automatic Büchi distillation unit (B 324).
- Glucosinolate content was determined following the ISO 9167-1 method (1992), with some minor modifications (Lazzeri et al., 2011).

2.1.2. The formulations

The DSM was formulated following a patented procedure (Lazzeri et al., 2010) aimed at optimizing the enzymatic system that catalyzes glucosinolate hydrolysis, thus releasing bio-active compounds. The details of this procedure have to be considered as confidential and will not be extensively reported.

The three tested formulations were essentially based on fertilizers Duolif and Duofruit distributed by Agrium S.p.A.. The formulations were prepared by mixing the DSM with the oil and then adding the corresponding amount of water.

After some preliminary evaluations (data not shown) the following products were defined and used in the trials:

a) formulation 1 (F1) that contained 1% (v/v) of *B. carinata* oil supplemented by a natural emulsifier in water, 0.2% (w/v) of formulated DSM;