Aspects of Applied Biology ***147****, 2022*
International Advances in Pesticide Application

New method to increase pesticide deposition: Copper microencapsulation

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Summary

Copper plant protection products have been severely restricted in the EU according to soil and ground water contamination due to the traditional use as a fungicide, especially in vineyards. This limitation, together with the dependence to copper use for mildew control, places winegrowers in a great disadvantage, especially in organic production. Therefore, EURECAT together with the UPC, have developed a new copper product, more efficient in terms of deposition, in order to reduce the amount of active ingredient necessary for good disease control. Preliminary trials have been carried out by the UPC, in order to select the best formulation and to compare it with a conventional copper-based product in terms of deposition using filter paper as a collector in an artificial vineyard. The obtained results show that deposition of different developed products is statistically different from the control product, even doubling the amount of copper deposited in the collectors, which would be a promising solution to solve the problem outlined above.

Key words: Copper, microencapsulation, mildew, deposition, vineyards

Introduction

The traditional use of copper plant protection products as fungicides, and in particular in viticulture to control mildew, has generated a challenge with the Cu^{2+} accumulation in soils and the potential ground water contamination it causes (Jacobson *et al*., 2005; Komárek *et al*., 2010). This has pushed the authorities to severely restrict these formulates in the EU (COMMISSION IMPLEMENTING REGULATION (EU) 2018/1981 of 13 December 2018), resulting in a maximum total application of 28 kg copper per hectare over a period of 7 years.

The widespread use of copper in the vineyard and the large vineyard area in Europe (OIV, 2014), is an indicator of the high impact that this new restriction will lead on European agriculture. Moreover, this is an even bigger challenge for organic farmers as directly affects the most important available solution they have against mildew. This translates into an urgent need for farmers to dispose of alternative methods to combat these fungi diseases.

The encapsulation of bioactive agents has been developed in recent years as a new significant and breakthrough tool for ecological and sustainable plant production. Encapsulation in biopolymer matrices has been recognized as an effective method for controlled release of a bioactive agent used for plant protection. Following the new trends, scientists from Eurecat Centro Tecnológico de Cataluña and from Universitat Politècnica de Catalunya have developed and recently protected by

European Patent Application a new aqueous formulation for the delivery of copper to agricultural crops. This formulation comprises non-toxic and non-contaminant polymeric materials, and copper cations which are present both in solution and forming part of polymeric microcapsules. The liquid formulation contains adjuvants that increase the deposition on the crop leaves

The main aim of this investigation is to develop a novel formulation containing microcapsules that could be a better alternative to the traditional applications of copper, thus reducing the use of this heavy metal in plant protection.

Materials and Methods

Semi-field assays were carried out at UPC research facilities, of Agropolis Research Campus at Viladecans (Barcelona, Spain) (41°17′18.44″N/2°2′43.39″W) in February 2021.

Tested products

In order to obtain the formulation containing microcapsules formed by copper-anionic polymer network and suspended in copper-cationic solution, a protocol described in European Patent Application with the deposition number EP21382965.8 was used. Anionic polymers (named NA4012 and NA7580) were purchased from C.E. Roeper GmbH, Germany, while cation polymer was purchased from Sigma-Aldrich, Spain. $CuSO_4$ $5H_2O$ was obtained from Alfa Aesar, Spain. Commercially available aqueous solution containing 1 g·L^{-1} of Cu^{2+} was prepared using Ossirame 50wp (MANICA COBRE S.L.) following instructions provided by Manica Cobre, Spain and it was used as a control reference. Table 1 shows the composition of the investigated formulations.

Table 1. *Tested products*

Formulation	Copper Cu^{2+}content [g·L^{-1}]	Anionic polymer [g·L^{-1}]	Cationic polymer [g·L^{-1}]
Control	1.0	-	-
F1	1.0	1.5 (NA4012)	1.25
F2	1.0	1.5 (NA7507)	1.25
F3	1.0	1.5 (NA4012)	2.5
F4	1.0	1.5 (NA7507)	2.5

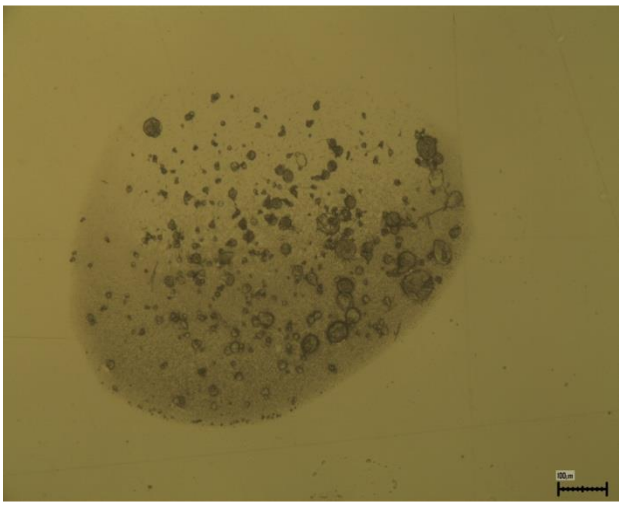

Fig. 1. Optical micrograph of dried droplet (formulation F3) containing copper microcapsules.

Formulate selection

Deposition measurements were made using an artificial vineyard, of 1.2 m long and 0.6 m high. For each trial 12 filter papers (FANOIA quantitative filter paper, cod. HK1238/30-80, 24 cm^2 area) were placed on the artificial leaves, distributed four times along the vegetation at three different heights (low, medium and high) to cover all the target surface, each one as a sample repetition. It means that four papers were located at 0.5 m high, other four papers at 0.8 m high, and finally four papers at 1.1 m. The distance between the collector papers in the same row was approximately 20 cm.

The spray application was performed using a Matabi Evolution 15 L (Goizper-Matabi, Antuzuola, Spain) with a manual boom attached provided with four ISO 025 flat fan nozzles model XR (Teejet Technologies, Glendale Heights, USA) separated each one by 0.5 m, set at three bars of pressure, and 50 cm from the vegetation to achieve the optimum spray distribution. The forward speed for all tests was 1 $m \cdot s^{-1}$.

Field validation

Once the formulation was selected according a higher deposition, spray application was performed in real vineyards at BBCH55 stage. The field was divided in two areas of 150 m^2 to test the two hypotheses. Deposition experiments were performed using vegetal collectors. Blank leaves samples were collected before spray products to determine possible copper remnant from the current production system management.

Each one of the treatments, control and the selected microcapsules formulate, were applied under the same conditions, using a Hardi Arrow Ilemo sprayer (Hardi International A/S, Norre Alslev, Denmark) (Fig. 2) at 80$L \cdot ha^{-1}$ (4.9 bar, TR01 Lechler nozzles, 5 $km \cdot h^{-1}$). Thirty vegetal samples composed each one by five leaves were collected randomly in each one of the two tested hypotheses.

Fifty leaves were taken in order to calculate the relation between weight and surface and be able to estimate the collector's area.

Fig. 2. Hardi Arrow Ilemo sprayer. Spray application of the Formulate 4 in field tests.

Petri dishes were placed among the vegetation (attached to the support poles) to visually check if the spray affected the structure of the microcapsules.

Sample analysis

The copper content of the samples was measured by pouring the collectors using 25 ml of 0.05 N of a solution HNO_3 during 5 mins. Then, the remained acid solution containing the copper cations was analyzed by means of atomic absorption spectrometer (SpectrAA-110, Varian, Inc. USA) according to Salyani & Whitney (1988). From the copper concentration ($mg \cdot L^{-1}$) the copper deposition (µg·cm^{-2}) was calculated as a function of the collector area. The area for the artificial collectors was fixed (24 cm^2) and for the vegetal ones, was estimated according to the weight-surface ration (Gil *et al.*, 2011).

The collected data was analysed using R studio software (PBC, Boston, USA). An ANOVA test was carried out, and in the case, it was significant, a *post hoc* Tukey HSD test to observe the significant differences between groups.

Obtained microcapsules were observed by means of Microscope "Axiovert" 40C for transmitted-light brightfield and phase contrast with condenser 0.4, inclusive object traverser M, while the micrographs were captured by DeltaPix Invenio 3S digital camera connected to the microscope.

Results

Once the results have been analyzed, the deposition observed of the microencapsulated copper formulations was greater than the one of conventional copper (Fig. 3) by a percentage between 38% (Formulate 3) and 85% (Formulate 1). However, only Formulate 1, Formulate 2 and Formulate 4 are significantly higher than control because significant differences were not found between Formulate 3 and the conventional copper product.

Although F1 presented the highest deposition (0.063 ± 0.0194 µg·cm^{-2}), the high data dispersion was a reason for choosing formulation 4, with a 79.4% higher deposition than conventional copper and a lower standard deviation data than formulation 1 (0.061 ± 0.0129 µg·cm^{-2}) (Fig. 3).

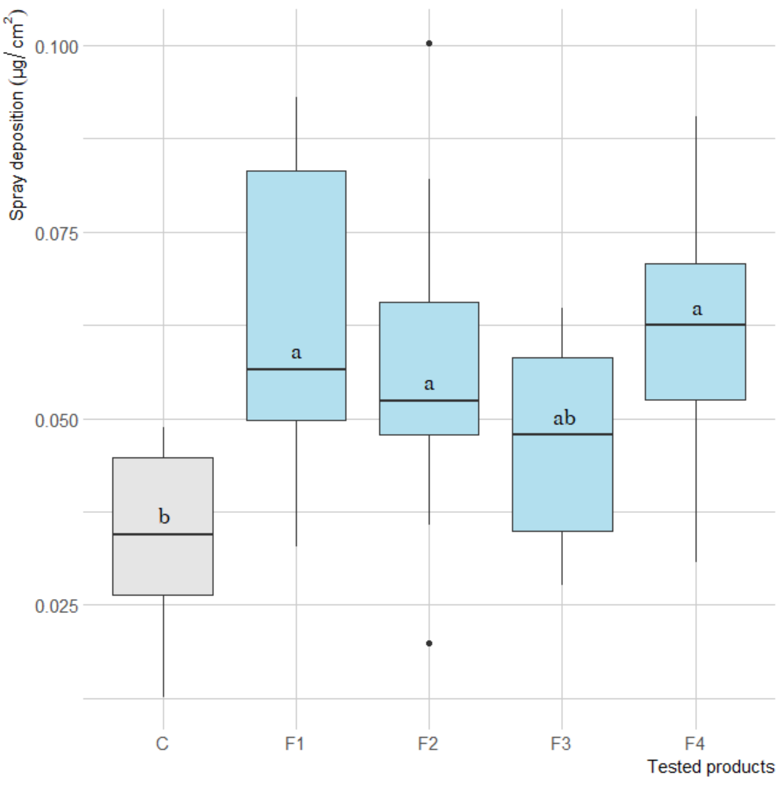

Fig. 3. Boxplot of spray deposition collected (µg·cm^{-2}) comparison for the different formulates. HSD Tukey ($P < 0.05$) differences expressed with a central letter on the boxplot. C: control; F1: Formulate1; F2: Formulate2; F3: Formulate3; F4: Formulate4.

This higher deposition of Formulation 4 compared to a conventional copper application is also observed in the copper concentration found in leaves during field validation. Formulation 4 deposited a copper concentration of 2.072 ± 0.981 µg·cm^{-2} on the leaves, while conventional copper deposited 0.802 ± 0.389 μg·cm^{-2}, which is approximately 2.5 times less than the microcapsulated product (Fig. 4).

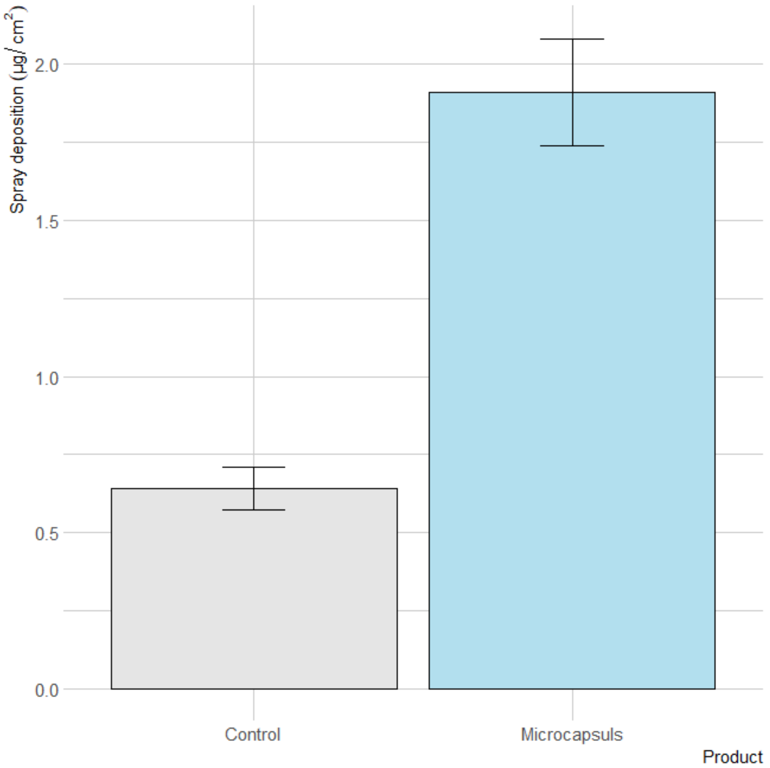

Fig. 4. Average level of spray deposition collected in real vegetation (μg·cm^{-2}) ± SEM.

The visual check of the microcapsules structure concluded that they did not lose their structural shape after being applied with the sprayer.

Discussion

The higher copper deposition found in all the microcapsule formulations than the conventional product (Fig. 3), clearly indicates that microencapsulation technique improves deposition in filter paper even with different types of anionic polymer or different concentrations of cationic polymer (Table 1). However, Formulation 4 was selected as it had a higher deposition and a lower standard deviation data than formulation 1.

This increased deposition of the microencapsulated formulation is clearly observed in the field validation (Fig. 4). The higher deposition on leaves, which is 2.5 times higher, would be due to the interaction between the cationic and anionic polymers and the composition of the leaf surface. New studies are underway to further investigate this interaction.

Focusing on field application, spraying pressure (3 and 4.9 bar) and size of the nozzles does not affect the constitution of the microcapsulated.

These results lead us to the conclusion that the use of copper microcapsules would allow us to greatly reduce the use of copper in the vineyard and to comply with the new European regulations on the use of this product (COMMISSION IMPLEMENTING REGULATION (EU) 2018/1981 of 13 December 2018). In turn, as the formulations only contain organic components, copper microencapsulated will also be useful for organic farming, where available plant protection products are restricted.

Although the copper concentration in the microcapsules is adequate for crop protection against fungal diseases (1 g·L^{-1}), further trials are needed in order to ensure the functionality of the microencapsulated. For that reason, experiments are currently underway at UPC for testing in real to test the efficiency.

Acknowledgements

The presented research is part of the COPPEREPLACE project (INTERREG SUDOE, Ref. SOE4/P1/E100) in the frame of the activity 3.3 in the GT3.

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