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Dual role of milk on aphid and powdery mildew control in kale

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ABSTRACT

Powdery mildew (PM) is the most important kale disease and it is more difficult to manage when plants are simultaneously infested with aphids. In this work, we aimed at evaluating the contribution of a sulphur-based fungicide, water, or cow's milk to control PM as well as the aphids *Brevicoryne brassicae* and *Myzus persicae* on kale under greenhouse conditions. We also assessed *in vitro* the effect of the products mentioned on their selective action on growth of the entomopathogenic fungus and the aphids. Four week-old plants naturally infested with aphids and PM were evaluated for disease severity and aphid counts. Plants were sprayed weekly with milk (10% v/v), sulphur (2 g/L), or water. From 7 to 28 days after the experiment onset (DAEO), plants were evaluated weekly for the disease severity, number of each aphid species, and number of fungal-colonized dead aphids. Milk and fungicide sprayed on plants reduced disease (30% and 10%, respectively) compared to the water control ($P \leq 0.001$). In addition, *B. brassicae* population was reduced for the milk but not for the fungicide treatment at 21 DAEO. Surprisingly, the dead aphids were parasitized by a fungus identified as *Cladosporium cladosporioides*. Moreover, milk treatment did not interfere with *C. cladosporioides in vitro* growth. On the contrary, sulphur, copper oxychloride, and azoxystrobin reduced fungal growth. Additionally, *C. cladosporioides*, milk, and milk + *C. cladosporioides* reduced *B. brassicae* and *M. persicae* populations. A dual role of milk on both powdery mildew and aphid control reinforces the usefulness of this product on glasshouse kale production.

1. Introduction

Among the diseases of kale (*Brassica oleracea* L. var. *acephala* D.C.), powdery mildew (*Erysiphe polygoni* DC.) is the most important under greenhouse conditions. The disease is widespread and is a major concern for many commercially important crop hosts such as Vigna radiata (L.) Wilczek, *Pisum sativum* L., *Beta vulgaris*, and *Lycopersicon esculentum* (Chankaew et al., 2013; Azmata et al., 2012; Hanson and McGrath, 2011; Segarra et al., 2009). Besides the disease, kale plants can also be simultaneously infested with aphids, such as *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) and *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae). These aphid species can cause significant damage to cruciferous plants (Liu and Sparks, 2013). Through continuous sap suction, *B. brassicae* leads to leaves curling which harbour the insects and affect plant development. Current control of powdery mildew and *B. brassicae* relies on application of sulphur fungicides and neonicotinoid insecticides, respectively (Agrofit, 2015; Stein and Teixeira, 2010). For either insect or plant diseases, eco-friendly approaches have proven to be efficient, such as entomopathogenic fungi for the biocontrol of aphids (Rashki and Shirvani, 2013; Anwar et al., 2007) and alternative plant extracts or milk for powdery mildew management in horticultural crops (Bettiol et al., 1999; Medeiros et al., 2012a). These are pest management strategies that respond to the negative public perception of pesticide effects on the environment and human health as well as to the growing legislative pressure to reduce their use in agriculture (Medeiros et al., 2012b; Segarra et al., 2009). However, the consequences of adopting one biocontrol strategy to manage disease on the ecological balance of a pest has not been addressed.

In overall, plant diseases and insects losses are respectively responsible for an average of 17% and a range of 26–80% annual losses of important crops produced worldwide (Oerke, 2006). To reduce such losses, an application of one single product to manage both pest and disease of kale would be environmentally safe

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and based on a product easily accessible to growers represent a plausible strategy to be researched.

The aim of this work was to evaluate the contribution of milk application compared to sulphur fungicide and water (control) on kale under greenhouse conditions. We examined powdery mildew control and its effect on the population dynamics of *B. brassicae* and *M. persicae* aphids, on fungal colonized dead aphids as well as on the growth of the putative entomopathogenic fungus.

2. Materials and methods

The experiment was performed under greenhouse conditions at the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil (915 m altitude, 21°22′67″s and 44°97′53″W). Four week-old plants naturally infested with the aphids *B. brassicae* and *M. persicae* as well as powdery mildew were evaluated for the disease severity, aphid count, and for number of aphids naturally colonized by fungi. During 28 days plants were sprayed weekly until runoff with raw cow's milk (10% v/v), sulphur (2 g/L), or water (control). Four replicates and two plants per treatment were used. The experiment was performed twice in order to confirm the results.

2.1. Assessment of the analyzed variables

From the 7th to the 28th days after the experiment onset (DAEO), plants simultaneously infested by aphids and infected by powdery mildew were evaluated weekly for the disease severity, number of each aphid species (only adults), as well as number of fungal-infected pests. For the disease severity, the percentage of leaves affected by powdery mildew was determined by counting two infected leaves per plant. The disease ratings were based on a 0–4 scale, adapted from Matsumoto et al. (2011), where: 0 = leaves free of disease symptoms, 1 = leaves with 1–25% covered with fungus hyphae, 2 = leaves with 26–50% covered with fungus hyphae, 3 = leaves with 51–75% covered with fungal matt, and 4 = leaves with 76–100% covered with fungal matt.

2.2. Entomopathogenic fungus in vitro assay

To assess the effect of milk or chemical fungicides on the development of the putative entomopathogenic fungus, an *in vitro* experiment was set up. The fungus was isolated from *B. brassicae* and then one 5 mm mycelium disk was put on the surface of PDA media amended with cow's milk (10% v/v), sulphur (2 g/L), azoxystrobin (8 g/L), copper oxychloride (2 g/L), or water (control). Petri dishes were then transferred to a growth chamber at 21 °C. Three days after plating (DAP), radial mycelium growth (mm) was measured daily until the 8th DAP.

2.3. Rearing aphid populations

M. persicae and *B. brassicae* were collected from *Brassica oleraceae* var. *acephala* plants in the region of Lavras, Minas Gerais, Brazil. Aphids were reared on *Brassica oleracea* var. *acephala* in 500 mL pots in wooden framed cages (45 × 50 × 70 cm) covered by cheesecloth and were maintained in a greenhouse until enough aphids reached adulthood to provide a significant population to perform the experiments.

2.4. Effect of milk on aphid populations

In order to assess the direct and indirect effects of milk to reduce *B. brassicae* and *M. persicae* populations, two *in vitro* assays were set up using the following treatments: milk (10%), *Cladosporium cladosporioides* (10^4 conidia/mL), *C. cladosporioides* (10^4 conidia/mL) + milk (10%), the sulphur fungicide (2 g/L), the sulphur fungicide + milk (10%) and water (control). A volume of 0.5 mL of each product was sprayed on an initial population of *B. brassicae* or *M. persicae* composed by 50 adult aphids with 5 repetitions per treatment each experiment. 9cm-diameter leaf disks of kale were infested with 50 adult aphids each and incubated at 25 °C ± 2 during 7 days, the time period chosen according to approximately *C. cladosporioides* life cycle. After that, the number of each aphid population (only adults) was counted to assess the effect of each treatment. To confirm the presence of *C. cladosporioides* on dead aphids, after the experiment five aphids were plated on potato-dextrose agar and checked for the presence *C. cladosporioides* after five days of incubation at 25 °C.

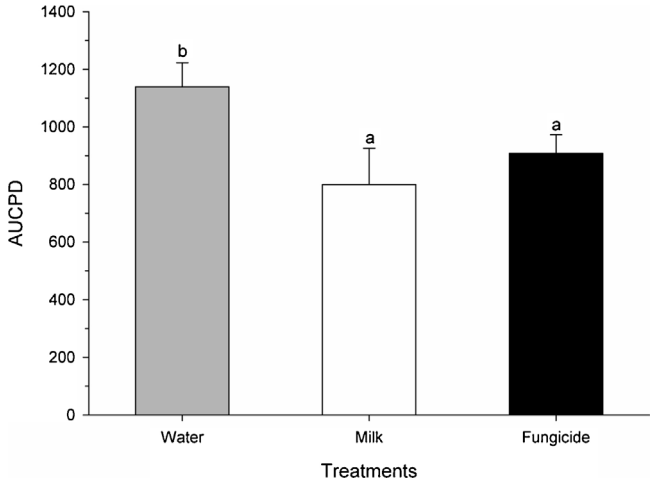

Fig. 1. Effect of spraying with Kumulus® (fungicide), milk (10%), or water (control) on the area under the disease progress curve (AUDPC) in kale plants. Bars headed with the same letter are similar at the 5% level according to Tukey's test. The line on each bar represents ±SE. (Means of two experiments of four replicates of two plants each).

2.5. Experimental design and statistical analysis

The experimental design was in randomized blocks for the greenhouse experiment with a total of four replicates for each treatment. For the *in vitro* assays, a completely randomized design was used with a total of five replicates for each treatment in each assay. Data from both experiments were submitted to variance analysis (ANOVA; $P = 0.05$), and to separate treatment means, Tukey's multiple range tests were applied if necessary. For all analyses, the assumption of normality was checked by Shapiro-Wilk test prior to analysis and no transformation was necessary, except for the radial mycelium growth last assessment where log(×/1000) were applied in order to meet the ANOVA assumptions. SigmaPlot® version 11 was used to create the artworks and Sisvar (Build 72) was used for statistical analyses (Ferreira, 2011).

3. Results

There was no difference between experiments ($P = 0.8532$) whilst there was a significant effect for treatments ($P \leq 0.001$) with disease reduction for milk (30%) and fungicide (10%) treated plants compared to the water control (Fig. 1).

Considering the population of *B. brassicae*, a reduction in 56% of the pest population was observed on milk treated plants at 21 DAEO (Fig. 2). For fungicide treatment, an increase in the aphid population was observed compared to the water control. However, there was no statistical difference regarding *M. persicae* population for the period considered ($P = 0.4525$; $P = 0.0832$; $P = 0.0946$; $P = 0.7475$, respectively for 7, 14, 21, 28 DAEO).