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A Novel Biopesticide Formulation for Organic Management of *Aphis gossypii* in Cucumber Greenhouses †

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Abstract: Intensive chemical pesticide usage in crop protection for pest control causes major pollution of the environment. Replacing chemical pesticides with biopesticide is an essential agro-ecological principle that should be considered in agro-ecosystems. In this study, a novel biopesticide formulation based on plant extract was prepared, and then aphid mortality in cucumber greenhouses was evaluated in comparison to common chemical insecticide. Our eco-friendly insecticide consists of methanolic extract of *Rosmarinus officinalis* (4.38 g in 1 L water) and succinic acid (0.5 g in 1 L water) in combination with Triton® X-100 (10 mL in 1 L water), canola seeds oil (10 mL in 1 L water), and potassium nitrate (5 g in 1 L water). Additionally, the common insecticide against *Aphis gossypii* in cucumber greenhouses is dichlorvos (Dichlorvos® 48% EC) which was applied at the recommended dose (0.6 g a.i./L). Twenty-four hours after sprayings, aphids that survived through each treatment were recorded, and mortality percentages were calculated. Results showed that in biopesticide treatment, mortality (65.6 ± 2.8%) has no significant difference compared to dichlorvos (71.1 ± 2.9%) (p = 0.0629). Finally, plant performance including numbers of leaves (p = 0.0951), flowers (p = 0.0842), fruits (p = 0.0730), and branches (p = 0.0698) were not influenced by the biopesticide application. Our results propose that the mentioned biopesticide can be used in cucumber greenhouses for aphid control with no adverse effect on plant growth and development, leading to zero-pollution tactics in crop protection, which is necessary for sustainable agriculture.

Keywords: *Aphis gossypii*; biopesticide; *Cucumis sativus*; plant growth; rosemary extract

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1. Introduction

Excessive usage of synthetic pesticides in agroecosystems has led to adverse effects on crop protection including resistance, resurgence, and residual impacts on agricultural products [1]. New tactics in pest management programs investigate alternative substances like plant extracts, which have bioactive components against pests that replace chemical pesticides [2]. Biopesticides based on plant extracts have been introduced as unique equipment in crop protection due to their inexpensive, low residual effects, and eco-friendly characteristics [3]. Additionally, the presence of various secondary metabolites containing flavonoids, terpenoids, alkaloids, phenolics, and anthocyanins make them toxic against major pests in greenhouses such as thrips, aphids, whiteflies, and mites [4].

In contrast to suitable properties to use in pest management programs, plant extracts have some problems like a need for high concentration, low stability on sprayed surfaces, and susceptibility to photolysis and oxidation that caused limited application in agricultural ecosystems [2]. Thus, the addition of auxiliary compounds based on anionic adjuvants, oils, organic acids, and mineral salts to their formulation can greatly increase the stability of plant extracts and their insecticidal actions as well.

The cotton aphid, *Aphis gossypii*, is a cosmopolitan pest with a wide host range. The intensive use of insecticides against this pest has led to them developing resistance [5]. Consequently, this study aimed to evaluate the insecticidal activity of a novel formulation based on Rosemary methanolic extract in combination with auxiliary compounds including Triton® X-100, canola seed oil, succinic acid, and potassium nitrate. Meanwhile, the mortality percent of *A. gossypii* in response to the biopesticide was compared to dichlorvos as a recommended insecticide against the aphid. Furthermore, morphological parameters of cucumber plants' growth and development were assessed to shed light on the side effects of the biopesticide formulation on non-target organisms.

2. Materials and Methods

Cucumber seeds (*Cucumis sativus*, cultivar super N_3, HED Seed Productions Company, USA) were planted and grown in 15 cm diameter plastic pots filled with sterilized planting mix consisting of coco peat: perlite: peat moss (1:1:1). Plants were grown in a greenhouse under climate-controlled conditions of 26 ± 2 °C, 50 ± 5% RH, and 16L: 8D photoperiod. A colony of *A. gossypii* was established from aphids collected from cucumber greenhouses. Aphids were reared for five generations on cucumber seedlings in growth chambers set to 26 ± 2 °C, 50 ± 5% RH, and L16:D8 photoperiod.

Plant crude extract was prepared based on the methanolic method [2]. According to this method, 50 gr of dried powder of *R. officinalis* leaves were dissolved in 250 mL of methanol (Emsure®, Merck, Germany). After 24 h incubation in dark conditions and room temperature, solutions were filtered through a Buchner funnel to be ready for extraction with a rotary evaporator at 40 °C for 20 min. Then, plant crude extract (4.3 gr) was mixed with succinic acid (0.5 g, ACS reagent, Sigma-Aldrich, USA) and Triton® X-100 (10 mL, GR for analysis, Merck, Germany) in 1 L sterile distilled water. This solution was stirred thoroughly to achieve a homogenous mixture. Then, 10 mL of canola seed oil (10 mL) and potassium nitrate (10 g, Emsure®, Merck, Germany) were added to the mentioned mixtures.

The toxicity evaluation of *A. gossypii* in response to biopesticide and dichlorvos (Dichlorvos® 48% EC, Ariashimi Company, Iran) was accomplished based on the leaf dip method [5]. Leaf disks (2.5 cm diameter) of cucumber leaves were dipped in either biopesticide (1% *v*/*v*) or dichlorvos (0.6 g a.i./L, as a recommended dose against cotton aphids in greenhouses) for 5 s and each placed upside down on wet cotton in a Petri dish (3 cm diameter). Then, twenty apterous adults of *A. gossypii* were transferred on cucumber leaf discs and moved to a growth chamber at the same conditions as above. After 24 h, the mortality rate was recorded. Aphids failing to respond after being probed with a fine brush were considered dead. Percentage mortalities were corrected using Abbott's formula [6].

Cucumber growth and development in response to biopesticide application were assessed using the account of numbers of leaves, flowers, fruits, and branches at the end of harvest. All experiments were carried out in three independent biological replications.

3. Results and Discussion

Bioassay results revealed the significant effect of biopesticide on *A. gossypii* survival with 65.6 ± 2.8% mortality. On the other hand, the survival rate in chemical insecticide treatment showed 71.1 ± 2.9% mortality in *A. gossypii* that was not a significantly different (t = 0.453, p = 0.0629) with biopesticide. Additionally, plant performance in response to biopesticide usage showed that numbers of leaves (t = 0.259, p = 0.0951), flowers (t = 0.367, p = 0.0842), fruits (t = 0.169, p = 0.0730), and branches (t = 0.508, p = 0.0698) had no significant difference with the control.

Results showed that the biopesticide based on methanolic extract of *R. officinalis* had a satisfactory efficiency on the control of cotton aphids without adverse effects on the plant's health. In this study, an attempt was made to select various components to achieve a homogenous solution in addition to high insecticidal activity. This issue explains the logic behind the selection of properties used in investigation, where there is Rosemary crude extract with insecticidal properties [2] in combination with Triton® X-100 as a non-ionic

adjuvant [7], canola seed oil as a facilitator to insect cuticle penetration [8], succinic acid as a plant defense inducer against herbivores [9], and potassium nitrate as a stabilizer agent prepared [10]. According to observed results, the biopesticide formulation can lead to lower plant extracts usage because other components in the formulation act as auxiliary compounds. Additionally, the presented eco-friendly pesticide had high stability on the sprayed surface. This finding may solve one of the main limitations in using pesticide formulation based on plant extracts.

4. Conclusions

It seems that using a combination of plant extracts with adjuvant, oil, organic acid, and a mineral salt is an acceptable approach towards controlling cotton aphids. Comparing mortality percentages between dichlorvos and biopesticide showed no significant differences. Authors conclude that the application of biopesticide formulation based on Rosemary methanolic extract can be recommended to control *A. gossypii* in cucumber greenhouses with no adverse effects on feeding plants. This recommendation contributes to the sustainable agriculture through decreasing synthetic insecticide usage and thus reduction in insecticide resistance, pest resurgence, and residual impacts on agricultural products.

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