Development and Evaluation of Eco-friendly Biopesticides Against Aphids and Pod borer of Food Legumes in Morocco

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Rational

Temperate food legumes, such as lentils and chickpeas, play a crucial role in human nutrition across West Asia, North Africa, and East Africa. However, climate change and variability are significantly impacting the productivity of these crops, primarily due to increased outbreaks of green pea aphids (*Acyrthosiphon pisum*) on lentils and pod borers (*Helicoverpa armigera*) on chickpeas (Damte and Ojiewo, 2017; Boulamtat et al., 2021; El Fakhouri et al., 2022). In addition to their direct effects on legume crops, aphids also serve as vectors for viruses such as Pea seed-borne mosaic virus, further threatening lentil and chickpea production in Ethiopia (Ademe et al., 2023). Currently, there are no known sources of resistance to lentil aphids and chickpea pod borers in landraces, which forces farmers to rely on broad-spectrum insecticides to protect their crops. Considering this, the objective of this study is to identify and validate safe biopesticides as alternative control methods to manage these key pests, while ensuring environmental safety and promoting sustainable pest management strategies for food legumes

Methodology

Development and validation of biopesticides against green pea aphids (*Acyrthosiphon pisum*) and chickpea pod borer (*Helicoverpa armigera*) were conducted in the laboratory and on-station trials at ICARDA Research Platform for North Africa in Morocco in the 2023/24 cropping season.

1. *Effects of essential oils on green pea aphids*

1.1. ***Laboratory bioassay***: Essential oils (EOs) from *Ocimum basilicum* and *Origanum vulgare* were tested under laboratory assays and greenhouse conditions. The EOs were formulated using Chitosan+Tween (0.01%) nanoparticles according to the protocol adopted by the Bioformulation Laboratory of AgBS (AgroBioSciences) at UM6P (Mohammed VI Polytechnic University), Morocco using the ionic gelation method (*Unpublished*). The formulated EOs were tested in four concentrations (500, 1000, 3000, and 5000 ppm) on green pea aphids in the laboratory. A completely randomized design with five replications was used with five adult aphids per replication. Chitosan+Tween and water sprayed aphids were used as controls.

1.2. ***Greenhouse tests***: Based on the laboratory bioassay results, EOs were further tested under greenhouse conditions using seedlings of the lentil variety *Bakria* (ILL-4605). The plants were grown in pots under greenhouse conditions (25 ± 2 °C, 75% RH, 14/10 h) and infested with two aphids/plant after 10 days of seedling emergence. The most effective concentration from the laboratory bioassay (5000 ppm) and the standard insecticide Pirimor (a.i. Pirimicarb) was sprayed on the infested seedlings. Controlled seedlings were sprayed with water.

1.3. ***On-station field trials***: The improved lentil variety *Bakria* (ILL-4605) was used in the field trial with a plot size of 3.6 m^2 and each plot has 6 rows in the 2023/24 cropping season. The treatments were *O. basilicum* and *O. vulgare* EOs at 5000 ppm and the standard insecticide Pirimor (a.i. Pirimicarb) and non-treated plots were arranged in randomized complete block design with three replications. The treatments were applied one time before flowering stage of the lentil crop. The treatments were applied when the aphid population reached 20 to 66 aphids per sweep (Zhou et al, 2023). Aphid counts were made 1, 2, 3, 4, and 5 days after treatment applications.

2. Effects of entomopathogenic fungi on chickpea pod borer

2.1. Laboratory bioassay of entomopathogenic fungi: Larvae of *H. armigera* were collected from infested chickpea crops and reared on an artificial diet under laboratory conditions. Two strains of entomopathogenic fungi species (*Beauveria bassiana-* strain- *SGSK and Verticillium lecanii-* Strain-*SPT-2-271)* were obtained from the Fungal Culture Collections of ICARDA Terbol, Lebanon, and were evaluated using second instar larvae. The larvae were treated with 10^6, 10^7, and 10^8 conidia mL^{-1} in five replications (5 larvae/Petri dish) and the insecticide Proclaim® 05 SG (a.i. Emamectin benzoate). Water treated larvae were used as control.

2.2. On-station field trials: The two entomopathogenic fungi and the insecticide Proclaim® 05 SG at 250 g/ha used under laboratory bioassay were also evaluated under field conditions at ICARDA Merchouch Research Station, Morocco in 2023/24 cropping season. The trial was planted in randomized complete block design with three replications. The Plot size was 3.6 m^2 with four rows of chickpea (cv. *Farihane)*. Spores of the two entomopathogenic fungi (10^8 per ml) and the insecticide Proclaim® 05 SG were applied two times at seven days intervals at the podding stage of chickpea. Larval mortality was recorded three times after each application.

Results

1. Effects of essential oils against green pea aphids under laboratory and greenhouse bioassays

The formulation increased the effectiveness of the essential oils (EOs) in controlling aphid mortality under laboratory bioassay (Fig. 1). The *O. basilicum* EOs at concentrations of 5000 ppm and 3000 ppm achieved 100% mortality 1 and 48 h after application, respectively.

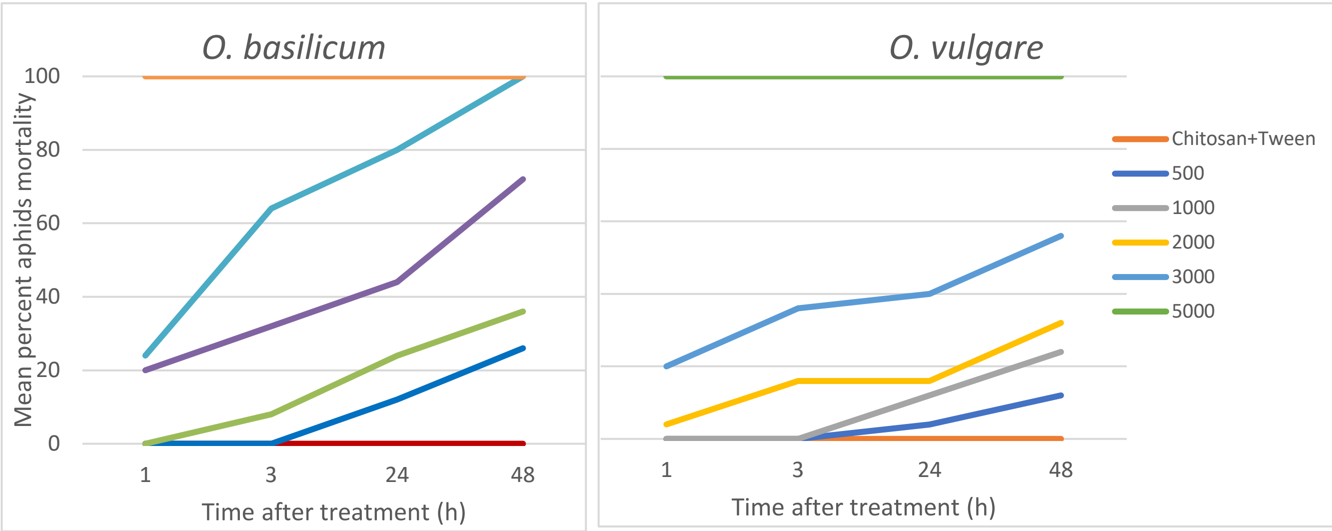

Figure 1. Effects of formulated essential oils with chitosan + tween on mean percent aphid mortality under laboratory bioassay,

Under greenhouse conditions the insecticidal activity of EOs was increased over time, leading to aphid mortality rates ranging from 60% to 80% (Fig. 2). The standard insecticide showed immediate mortality. The Chitosan+Tween control treated plots did not cause high mortality, and the former can be used in EOs formulation.

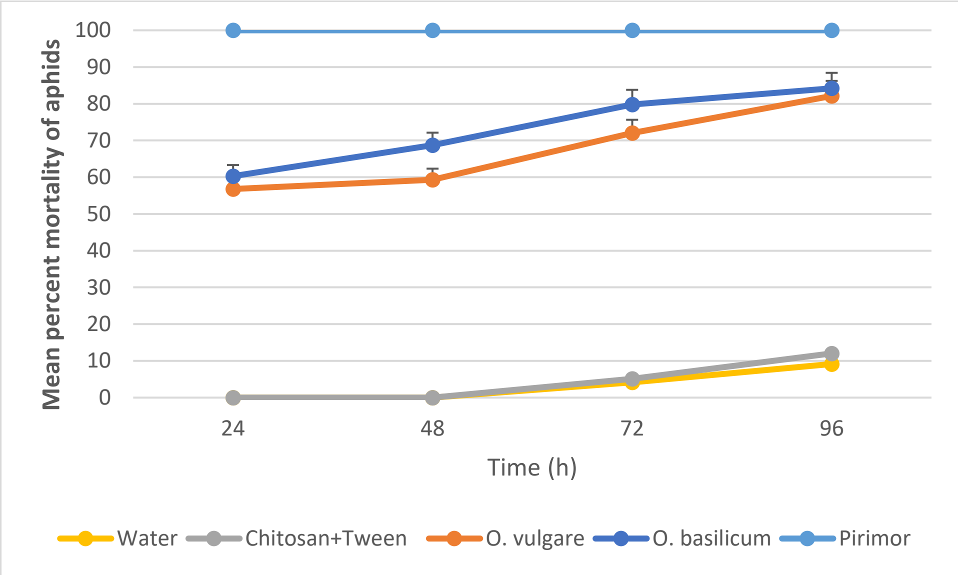

Figure 2. Effects of essential oils on mean percent aphid mortality under glasshouse tests at 5000 ppm concentration

2. Performance of Essential Oils in the Control of Green Pea Aphids under Field Conditions: Green aphid population was higher than the economic threshold (20 to 66 aphids per 180-degree sweep) during the cropping season. The mean percent aphid mortality increased over time after the application of treatments (Fig. 3). The insecticide treatment showed 100% mortality one day after spraying and the two EOs showed over 60% mortality three days after application. Chitosan used for Eos formulation showed low levels of effects on the aphids and most mortality was caused by EOs.

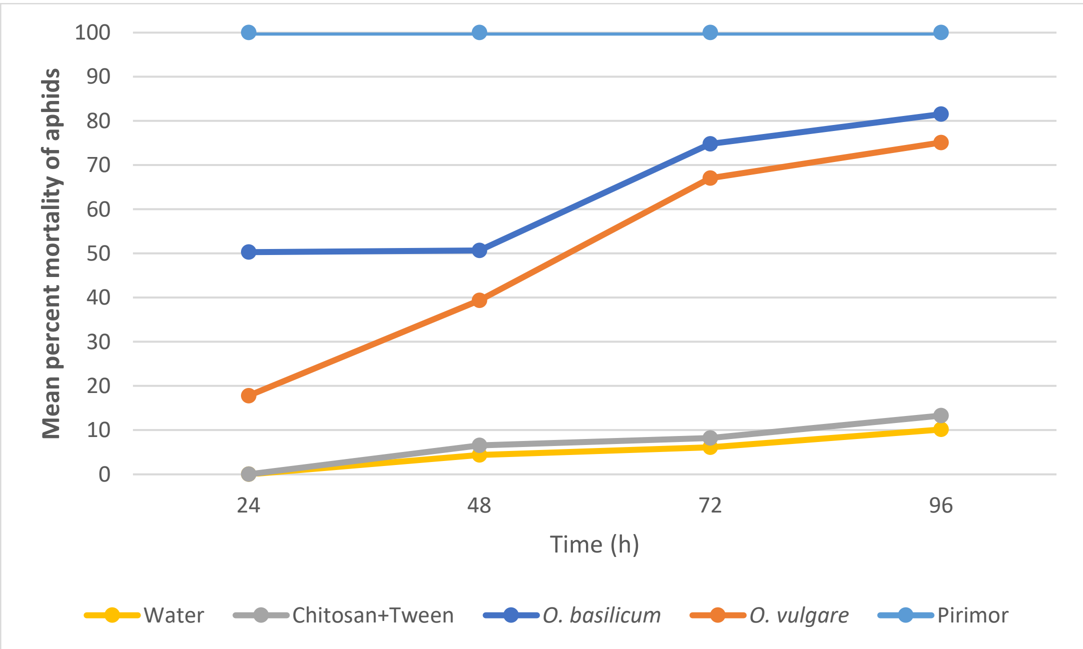

Figure 3. Effects of formulated essential oil on mean aphid mortality in 2023/24 cropping season, Merchouch, Morocco.

2. ***Performance of Entomopathogenic fungi under laboratory bioassay and field trials:*** Under laboratory condition, the two entomopathogen fungi at 10^8 conidia mL^{-1} (100%) followed by 10^7 mL (84%) concentration gave high mean larval mortality 15 days after application (Fig. 4).

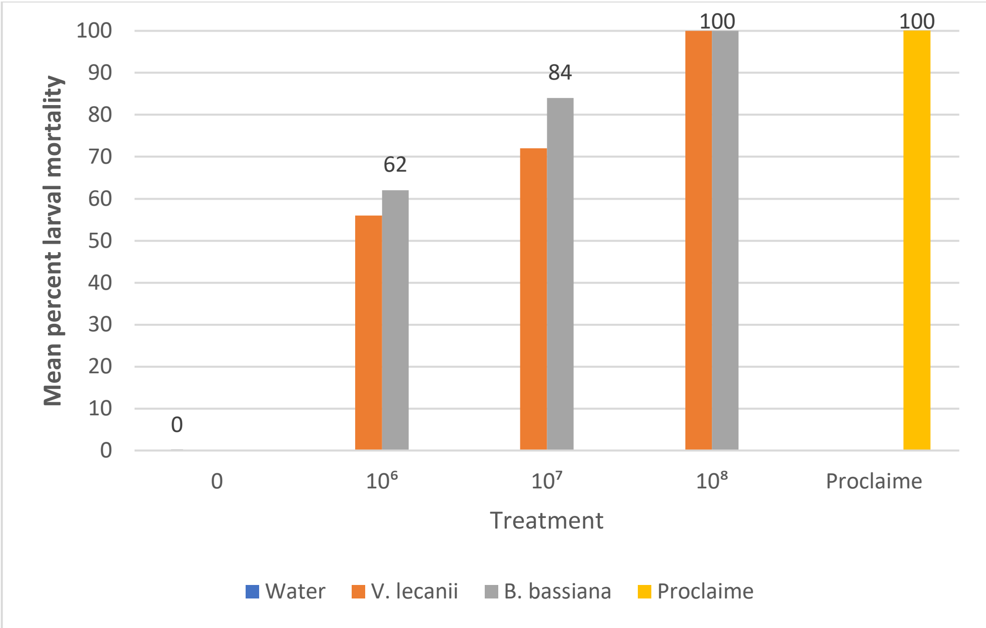

Figure 4. Effects of entomopathogenic fungi and insecticide application on mean percent larval mortality 15 days after treatment

Under field conditions, the application of entomopathogenic fungal strains resulted in high larval mortality at one- and two-times applications (Fig. 5 A&B). Following the second application, plots treated with entomopathogenic fungi strains reduced the pod borer below five larvae per plot, compared to 17 larvae per plot in the control plots. The pesticide (Proclaim® 05 SG) demonstrated high efficacy in both applications but the entomopathogenic fungi did not keep the population below its economic threshold levels like the insecticide. So, the second application of the biopesticide supplemented with minimum spray of insecticides can be used as IPM of pod borer on chickpea.

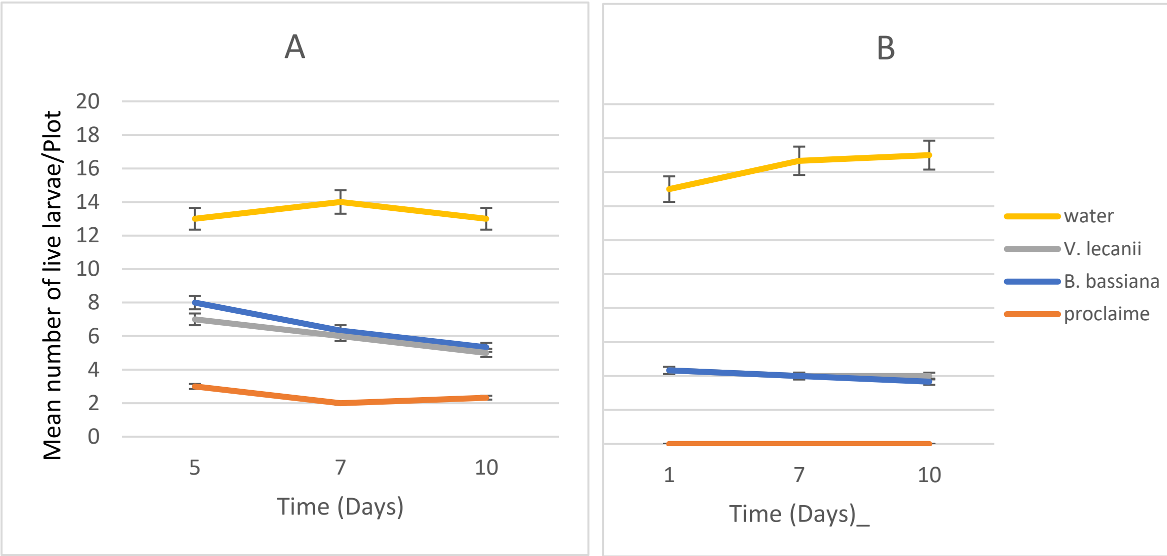

Figure 5. Mean number of live larvae of chickpea pod borer with one-time (A) and two times (B) applications of entomopathogenic fungi, 2023/24 cropping season, Merchouch, Morocco.

Conclusion and recommendations

We found that *O. basilicum* and *O. vulgare* essential oils significantly reduced green pea aphid populations, although it was less effective than conventional insecticides. Additionally, the entomopathogenic fungi *V. lecanii* and *B. bassiana* reduced chickpea pod borer larvae. Formulations of EOs Chitosan+tween showed encouraging results so that farmers can easily apply biopesticides against key legume pests. Our research showed the potential of essential oils and entomopathogenic fungi as components of managing food legume aphids and pod borers with a wide host range. Hence the innovation should be tested on large plots on farmers' fields in 2024/25 cropping season.

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FUNDING

This activity was supported by CGIAR Plant Health and Rapid Response to Protect Food Security and Livelihoods Initiative (Plant Health Initiative) through CGIAR Trust Fund.