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SOME ECOLOGICAL ASPECT OF MUSTARD APHID *Lipaphis erysimi*, INFESTATION ON BROCCOLI (*Brassica oleracea*) AND EFFICACY OF BIO-INSECTICIDES FOR MANAGEMENT OF MUSTARD APHID IN BANGLADESH

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Abstract

Brassica oleracea (broccoli) is a major, economically important, and commercially produced winter vegetable in Bangladesh. A study was conducted on the ecology of the mustard aphid, *Lipaphis erysimi* and the bio-efficacy of bio-insecticides (Biotrin and Ecomac) against *L. erysimi* using leaf dipping methods at different doses. *L. erysimi* was recorded as a destructive and major pest of broccoli. This aphid species infested broccoli as a secondary host plant, invading the leaves and shoots during the early harvesting season (December to January). The infestation continued and infested the entire vegetative parts during the peak and late cropping seasons. Aphid incidence was significantly higher (1171.62 ± 11.14) in April but declined drastically (42.25 ± 3.2) from May when seasonal temperature increased. The results demonstrated that compared with the control (untreated) aphids, the Biotrin and Ecomac treated doses induced significant mortality ($F = 3.76, df = 2, P < 0.05$) of the aphids. Greater concentrations (1.4 ml/1000) of both bio-insecticides significantly reduced fitness traits (reproduction and survival rate) of *L. erysimi's* ($F = 18.57, df = 2, P < 0.001$). The mixed bio-insecticides (Biotrin 1: Ecomac 1) were most effective for 24 h. It caused the highest rate of mortality (81.31 ± 14.75) followed by Ecomac (65.62±25.83) and Biotrin (52.71±15.07). However, the mortality rate of aphids was declined at 73.85±15.84 after 48 h, indicating that the synergistic effects of mixed insecticides gradually decreased. After 48 h of treatment, the mortality rate of Ecomac and Biotrin treated aphids was significantly higher ($F = 22.74, df = 2, P < 0.001$). This study will provide important information on seasonal infestation intensity, interactions with *L. erysimi* and bio-efficacy of bio-insecticides. The use of bio-insecticides may be an effective control measure for aphid management in Bangladesh.

Key words: *Lipaphis erysimi*; Aphid ecology; *Brassica oleracea*; Fitness traits; Bio-insecticides; Bio-efficacy.

INTRODUCTION

Broccoli (*Brassica oleracea*) is a winter vegetable that has several nutritional benefits and is high in fiber (Jibrin and Anton 2019). Broccoli's antioxidants, vitamins, minerals, and anti-carcinogenic properties have been scientifically linked to benefits in several kinds of medical illnesses (Singh *et al.* 2014, Ola *et al.* 2019). Aphids are the most destructive and economically important insect pests of many vegetables and crops worldwide (Hemming *et al.* 2022). The mustard aphid *Lipaphis erysimi* (Apididae: Homoptera), is a severe pest that heavily infests broccoli (Sadeghi *et al.* 2009, Bakhetia 1986, Rohilla *et al.* 1987 and Kumar 2004). *Lipaphis erysimi* infests broccoli throughout the growing season, peaking in early March (Boopathi and Pathak 2012). Mustard aphid is usually associated with cruciferous vegetables and cause significant damage in the winter season (Raju and Tayde 2022). Wintertime temperatures are ideal for aphid development, growth, and reproduction (Maurya *et al.* 2018).

Many previous studies reported that the use of chemical insecticides is the only quick and effective method for controlling this aphid species for sustainable production of cruciferous vegetables (Devonshire *et al.*1998, Kerns and Stewart 2000, Kumar *et al.* 2001, Ameta and Sharma 2005, Sule *et al.* 2008, Ahmed *et al.* 2007, Edwards *et al.* 2008, Maurya *et al.* 2018, Khan *et al.* 2020). Understanding how aphids interact with secondary host plants is crucial for maintaining consistent seasonal broccoli production with the lowest possible aphid infestations (Umina *et al.* 2022). However, it has been observed that aphid populations quickly acquire a variety of chemical resistances after insecticides application (Mamun *et al.* 2010, Ragsdale *et al.* 2011). In addition, chemical insecticides are seriously threatened for beneficial insects and natural predators in agricultural fields (Amad *et al.* 2003, Chandler *et al.* 2011, Bass and Nauen 2023, Ulusoy 2024, Delnat *et al.* 2019). Furthermore, the long-term impact of chemical pesticides on the environment has disrupted the balance of ecosystems and biodiversity (Paul *et al.* 2017, David *et al.* 2022). Therefore, to reduce insecticide residues, environmental risks, and need-based treatments using less harmful bio-insecticides with the right dosages are necessary for managing aphids on seasonal vegetables. Additionally, plant extract chemicals with insecticidal properties are being explored as alternative chemical insecticides for aphid control (Singh 2007, Singh *et al.* 2012, Agale and Wawdhane 2019). However, the susceptibility and effectiveness of bio-insecticides for *L. erysimi* may differ in different regions. To determine the effectiveness of bio-insecticides on local *L. erysimi* aphid populations, management actions necessitate frequent information gathering and monitoring.

Thus, this study was designed to evaluate the impacts of local bio-insecticides against *L. erysimi* and explore potential alternative management strategies. This study elucidated the bio-efficacy of Biotrin and Ecomac against *L. erysimi* at different concentrations on fitness traits at 24-hour and 48-hour intervals. Hence, the first objective of this study was to clarify the host plant interactions, seasonal incidence, and nature of injury by *L. erysimi* in broccoli and to evaluate and compare the susceptibility of Biotrin and Ecomac against *L. erysimi*. The study was also extended to investigate the effectiveness of the tasted formulations and doses.

MATERIAL AND METHODS

Study area

The infested broccoli plants *B. oleracea* and mustard aphid *L. erysimi* were randomly selected and regularly collected from the agricultural field of the Sher-e-Bangla Nagar Agricultural University (latitude 23º46'37.66" N and longitude 90º 23'58.02" E) and vegetable garden of the Curzon Hall, Dhaka University, Dhaka (latitude 23º72'68.99" N and longitude 90º40'15.42" E).

Sample collection and preparation

The ecological study was conducted from December 2022 to May 2023 and December 2023 to February 2024. During the study period, the infested broccoli leaves and vegetative parts were visually identified with the help of a magnified glass and collected weekly as samples. The collected samples were placed in a beaker containing a wad of cotton soaked with water to prevent dryness. The samples were immediately transported to the Laboratory of

Entomology, Department of Zoology in a protective plastic case being covered with mosquito nets. The infested leaves and vegetative parts were observed under a portable binocular stereo microscope (Euromex Edu Blue series), and images were captured using a digital camera. The collected adult aphids were preserved for identification at 40x magnification. The morphometric characteristics of aphids (body shape, color, cornicle size and cauda) were recorded regularly according to the methods of Dixon (1977), Dixon (1987) and Doris *et al.* (2017).

Studies on seasonal incidence, infestation intensity and nature of damage by Lipaphis erysimi

The ecological study, seasonal abundance and infestation intensity were recorded during the study period (December 2022 to May 2023). The aphids from the infested leaves and vegetative parts were removed using a fine camel hair brush and collected on the leaves using 75% ethanol. The aphids from one shoot or vegetative part were considered replicates. The total number of aphids was counted using a Finger Ring Watch with the help of a magnified glass. The association between *L. erysimi* and broccoli, and the nature of injury were observed under a stereo microscope and investigated according to the methods of Maurya *et al.* (2018) and Chaudhary *et al.* (2020). The field temperature and humidity were recorded with the aid of a clock and a Hygro-thermometer (Brannan, England) during the field survey and sample collection.

Treatment, dose selection and preparation

The bio-assay and susceptibility of bio-insecticides were recorded during the study period from December 2022 to May 2023 and from December 2023 to February 2024. The Biotrin contains 0.5% Matrine; Ecomac contains 0.25% Abamectin. The chemicals were analyzed in High liquid chromatography at the Drug Analysis and Research Laboratory, Centre for Advanced Research in Sciences (CARS), University of Dhaka. The treatment doses used in the present study were prepared following the manufacturer's instructions as an applied dose of 1 ml with 1000 ml deionized water (DO). The higher dose (1.4 ml with 1000 ml DO) was prepared based on pre-treatment mortality results (less than 30% for 1 hour). The equal ratio and quantities of Biotrin and Ecomac were combined with DO to generate mixed doses (1/1000 ml and 1.4/1000 ml) by following the methods of Khan *et al.* (2012). The doses of the mixed pesticide treatments were T_1 (Biotrin 1 ml mixed with 1000 ml DO); T_2 (Biotrin 1.4 ml mixed with 1000 ml DO); T_3 (Ecomac 1 ml mixed with 1000 ml DO); T_4 (Ecomac 1.4 ml mixed with 1000 ml DO); T_5 (1ml of Biotrin and 1 ml of Ecomac mixed with 2000 ml DO); T_6 (1.4 ml of Biotrin and 1.4 ml of Ecomac mixed with 2000 ml DO); and T_7 (Control with water (1000 ml DO) dipping).

Used insecticides for dose preparation

The amounts of insecticides for treatments were calculated by using the formula given below and following manufacture instructions.

$$Required\ amount\ of\ bio-insecticides = \frac{Volume\ of\ deionized\ water\ (lit.\ per\ hectare) \times\ desired\ concentration}{\%\ \text{Strength of formulated insecticides}}$$

Aphid-dip bio-insecticidal bioassay treatments

The initial numbers of aphids (total adults and nymphs) on each leaf and vegetative body were recorded under a portable binocular stereo microscope (Euromex Edu Blue series) before being treated with the insecticides. The collected samples (infested broccoli) with adult aphids and nymphs of different instars were randomly allocated to one of the six treatments: the Biotrin (T_1, n = 16 and T_2, n = 15), the Ecomac (T_3, n = 16; T_4, n= 16) and the mixed doses (T_5, n = 15 and T_6, n= 15). To assess the effectiveness of bio-insecticides on aphid fitness traits, the control samples were always taken. Along with the treatments, the control treatments (CT, n = 15) were also prepared. Replicates of the treatments were used on the same day, and aphids were subjected to the dipping of insecticide treatments simultaneously. The untreated samples (leaves or vegetative parts) were dipped in the test solution with a metal mesh tea strainer for laboratory bioassay. The replicate numbers of all treatments were gradually increased. For each treatment, young leaves and vegetative bodies of the same maturity were selected. Separated and fresh leaves for each treatment were used to evaluate the bio-efficacy according to the method of Ulusoy (2024) and Shannag *et al.* (2014). The samples were dipped in insecticide solutions for 20 seconds and dried in air being placed on a piece of clean tissue paper to remove excess moisture by following the methods of Chandersena *et al.* (2011), Shannag *et al.* (2014), Sadeghi *et al.* (2009), Gokulakrishnaa and Trirunavukkarasu (2023). Each leaf disc placed in a 200 ml. beaker with a layer of cotton and filter paper saturated with deionized water and placed on the bottom by following the methods of Calvin *et al.* (2020).

Post-treatment observation

All the samples in treatments and controls were transferred at 25°C, 16 hours of light, 8 hours dark cycle and 65% RH in the climate chamber (BIOBASE). The responses (mortality) of aphids were recorded after 24 hours and 48 hours of post-exposure period according to Bhati and Sharma (2014) and Seni and Naik (2017). The treated aphids were observed under the microscope. An aphid showing no perceptible movement after being prodded for 2-3 seconds with a fine brush was considered dead according to the methods of Calvin *et al.* (2020). The total dead aphids in all replicates were calculated individually. The number of newly born nymphs was counted and compared to the initial numbers of nymphs in order to determine the impact of pesticides on the reproduction rate of adults.

Data collection and analysis

The seasonal incidence was calculated by the mean abundance of aphids with the standard error value of a month's samples (broccoli leaves and vegetative parts) by using Microsoft Excel 2007. The calculated living and dead aphids of all treatments were separately considered as a response or effect of bio-insecticides on aphids. The mean survival and mortality rates were calculated as the relative percentage of survival and mortality individually for all treatments of Biotrin and Ecomac, compared with controls (non-treated). Differences among treatments were tested using the analysis of variance (ANOVA). The critical differences at 5% level of probability were compared by the F value (treatment means). Where 'F' was significant, critical difference = SE ± x V2 x t (at error degree of freedom). For comparing the effects of doses and treatments on the fitness traits of aphids, two-way ANOVA (analysis of variance) was used at 95% significant level. The effects of bio-insecticides on the reproduction of aphids were compared with control aphids after 24 hours and 48 hours separately by ANOVA and followed the post-hoc Tukey Kramer method to separate the means (a = 0.05). All analyses were performed by Minitab Statistical Software (Version 21.1. 0).

RESULTS AND DISCUSSION

Seasonal incidence and nature of injury

The infestation of mustard aphid (*Lipaphis erysimi*) started in December 2022 (172.62 ± 2.20); the numbers of aphids gradually increased by reproduction. The *L. erysimi* associated with mustard plants infestation then spread to broccoli when quality of primary host plants was declined. The notable higher aphid's incidence (1171.62 ±11.14) and infestation was recorded in April 2023 and the lowest was in May 2023 (42.25 ± 3.2). The infestation intensity was related with aphid abundance (Fig. 1).

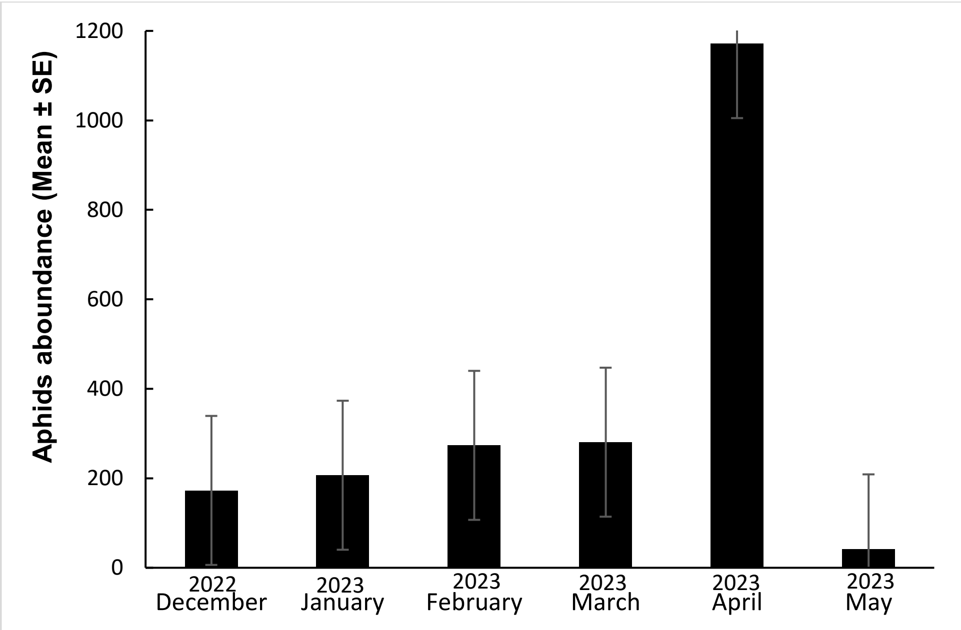


Fig. 1. The seasonal aphid incidence and infestation intensity by *Lipaphis erysimi* on broccoli.

20°C temperature and 70-76% relative humidity were suitable for mustard aphid reproduction. Initially, the aphids damaged the leaves of growing broccoli. Later on, gradually their population increased and vegetative parts congregated. The adults were capable of producing 5-8 aphids within 24 hours. Within the couple of weeks, the aphid population grew significantly. The *L. erysimi* was considered a key pest of broccoli due to its severe injury and hampered production. The nymphs and adults sucked cell sap from leaves, shoots, flower buds, and pods. The infested leaves turned yellow and the vegetative parts developed slowly over time (Fig. 2. a, b, c, d). This aphid began infesting on rapeseed mustard in November and had continued until March. According to Pradhan *et al.* (2020), the population peaked in January and February. The mustard aphids began infestation on broccoli in December 2022 at the study areas and a severe infestation was recorded in late winter (April 2023). Aphids covered most of the broccoli leaves and vegetative body during a severe infestation. The aphids were largely concentrated underside of the leaves and stems, and vegetative parts were failed to develop properly. The aphid infestation indirectly acts as a vector of plant viruses (Mamun *et al.* 2010 and Maurya *et al.* 2018).

Fig. 2. Infestation by mustard aphid *L. erysimi* on broccoli: **a**. Initial infestation and newly emerged adult and 1st instar nymph; **b**. The severe aphid infestation on the stem of broccoli; **c**. Aphids and nymphs are congregated on the ventral surface of leaves; and **d**. Nature of injury by feeding the sap from leaves.

Efficacy of Biotrin on the survival of Lipaphis erysimi

The results demonstrated that all of the treatments were effective and considerably increased the mortality of *L. erysimi* than the aphids in controls. The highest survival rate was found among control aphids. The mortality rate was considerably increased during the second day of treatment. The results indicated that Biotrin had significant impacts on mustard aphids. The aphids' fitness was significantly decreased by both of the doses of treatment ($F = 18.57, df = 1, P < 0.0005$). Aphids treated with 1 ml/1000 of Biotrin had 48.27 ± 15.54 survival rate. The survival rate was 46.31 ± 14.61 when treated with 1.4 ml/1000 (Fig. 3. a). The development of nymphs was declined and affected by feeding duration on plants and concentrations of insecticides (Ahmed *et al.* 2007).

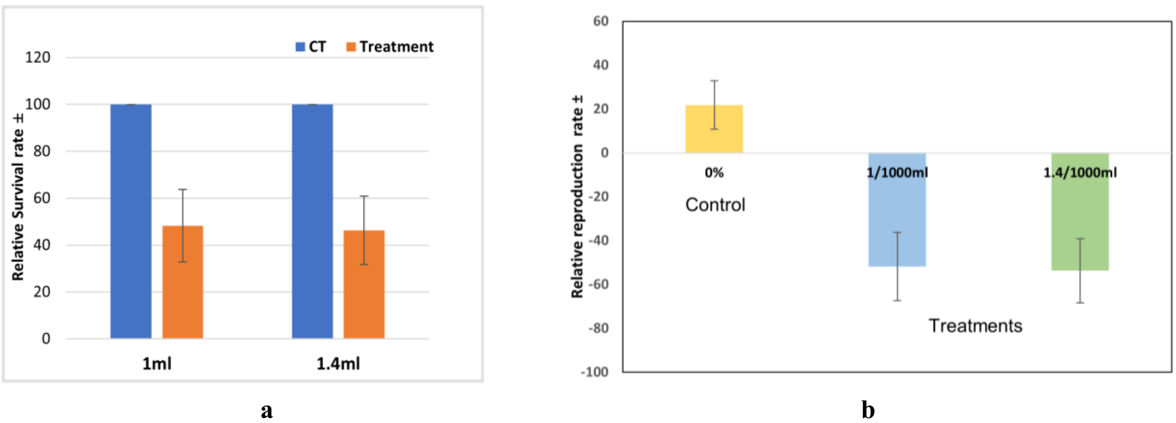


Fig. 3. **a**. Bio-efficacy of Biotrin on the survival rate of *Lipaphis erysimi* on broccoli after 24 hours in comparison with control aphids; and **b**. Bio-efficacy of Biotrin on the reproduction of *Lipaphis erysimi* after 24 hours of application.

The efficacy of Biotrin on the reproduction rate of Lipaphis erysimi

The results indicated a higher rate of adult's deaths and a declining trend of reproduction with the application of Biotrin. Aphids were incapable of reproducing, and their population growth rate was negative at -51.73± 15.55 following at 1 ml/1000 treatment dose and -53.695± 14.62 following at 1.4 ml/1000 treatment dose (Fig. 3. b). Reproduction increased the mean number of aphids (13.12 ± 6.59) in control conditions. The significant reduction of aphid reproduction was also observed as an effect of neem-treated plant feeding. The reproduction of *Aphis febae* was also limited when the host plant was treated with neem products (Ahmed *et al.* 2007, Dimetry and Schmidt 1992). Similar effects of plant saponins on mustard aphid reproduction were reported by Singh and Kaur (2018). Compared to untreated aphids in controls, treated aphid populations on plants were lower. Aphids experienced developmental delay, a drop in the rate of reproduction, and a decrease in fitness due to an increase in aphid mortality.

Efficacy of Ecomac on survival of Lipaphis erysimi

Aphids administered Ecomac with 1/1000 ml had a lower survival rate of 45.65 ± 13.61 than control aphids. Similar impacts on survival were observed when treated with 1.4/1000 ml, of Ecomac. The survival rate was gradually decreased to 25.93 ± 13.43 (Fig. 4. a). The relative mean mortality rate was also increased along with a higher concentration of Ecomac. Magalhaes *et al.* (2008) found that the chemical component of imidacloprid derived from plant seed had toxic effects on the survivorship of soyabean aphids. The impacts were increased along with the concentration of imidacloprid.

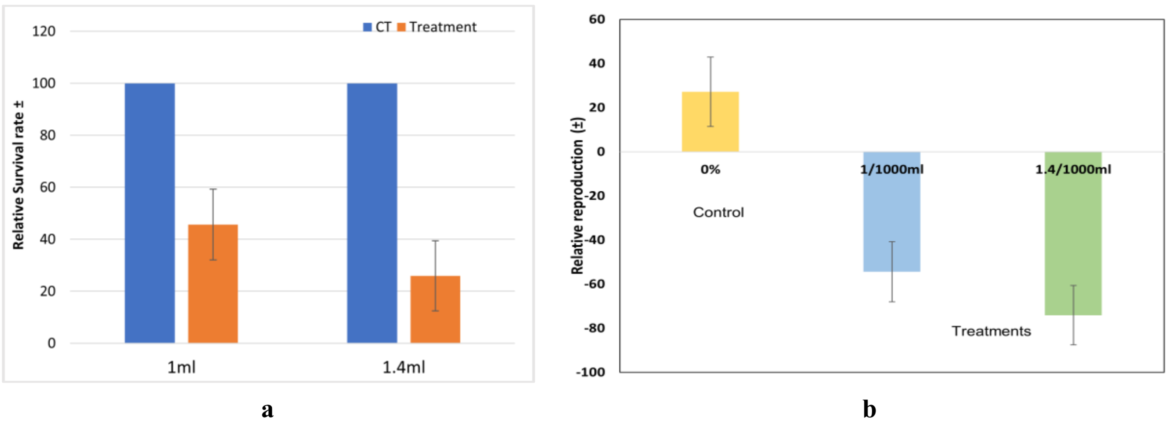


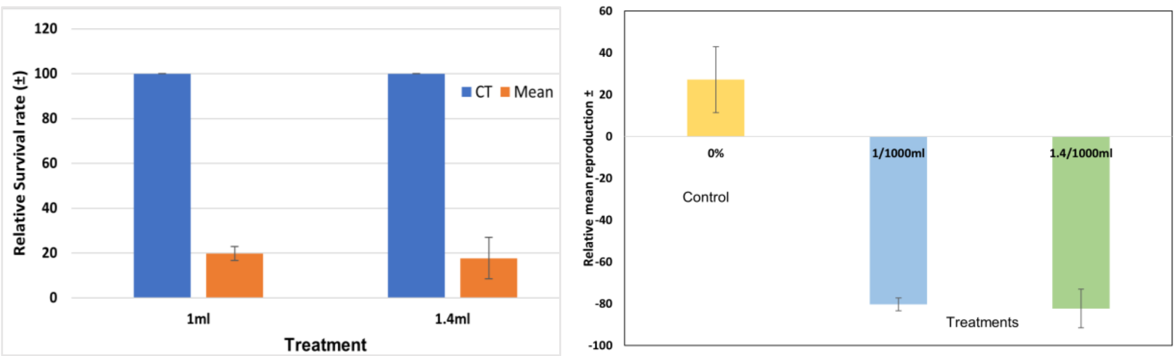
Fig. 4. **a.** Bio-efficacy of Ecomac on the survival of *Lipaphis erysimi* in comparison with control aphids after 24 hours; and **b**. Bio-efficacy of Ecomac on the reproduction of *Lipaphis erysimi* in compared with non-treated aphids after 24 hours of application.

Effect of Ecomac on the reproduction of Lipaphis erysimi

Aphids treated with Ecomac (Dose-1 1/1000 ml and Dose-2 1.4/1000 ml) were unable to reproduce and their numbers decreased at - 54.36 ± 13.61 and - 74.07±13.43, respectively. The untreated aphids were able to reproduce and the numbers increased at 27.14 ± 15.74 after 24 hours. (Fig. 4. b). According to Mandal *et al.* (2012), imidacloprid and diethoate plant-origin pesticides dramatically decreased the ability of aphids to reproduce after seven days of spraying. According to Debnath *et al.* (2018), *Melia azedarach* plants reduced aphid populations at 50.5-61.9% by imidacloprid and chlorpyrife toxic chemicals. Tang *et al.* (2002) found that commercial formulation of neem plants significantly reduced the fecundity of citrus aphid. Pyriproxyfen, a plant-based pesticide, reduced aphid longevity by about 50% and resulted in infertility in adults (Kerns and Stewart 2000). Aphid reproduction and survival are significantly impacted by bio-insecticides (Satoh and Plapp 1993). Aphids, when treated with sulprofos and dicrotophos died more and changed into dark-colored morphs than aphids that were not treated (Kerns and Gaylor 1993). According to Devee *et al.* (2011) the plant-origin products contained the imidacloprid, bifenthrin, and lambda-cyhalothrin were all found to be highly effective for controlling aphids.

Efficacy of mixed bioinsecticide on the survival of Lipaphis erysimi

Aphids treated with an equal combination of Biotrin and Ecomac insecticides had a much lower survival rate. The survival rates were 19.72 ± 3.09 and 17.66 ± 9.24 by mixed 1/1000 ml and 1.4/1000 ml dosages of bio-insecticides, respectively. Moreover, the mixed bio-insecticides decreased the ability to survive more than untreated aphids (Fig. 5. a). The bio-insecticide neem oil when mixed with entomophathogenic fungus were found compatible and the synergistic activities caused mortality of water lily aphid (Halder *et al.* 2020).



a **b**

Fig. 5. **a**. Efficacy of mixed bioinsecticide (Biotrin 1: Ecomac 1) on the survival rate of *Lipaphis erysimi* after 24 hours of application; and **b**. Efficacy of mixed bio-insecticide on reproduction rate of *Lipaphis erysimi* in comparison with control after 24 hours of application.

Efficacy of mixed biopesticide on the reproduction rate of Lipaphis erysimi

The control aphids reproduced at 27.13 ± 15.74. After treated with a 1/1000 ml mix and 1.4 ml/1000 ml doses of bio-insecticides, the mean aphid reproduction rate decreased to - 80.28 ± 3.09 and - 82.34 ± 9.24, respectively, due to high mortality. The reproduction and population growth rate were gradually declining, and the mortality rate was dramatically rising (Fig. 5. b). The mixed bio-insecticides were more effective and had a higher mortality rate at 81.31 ± 4.27 than Biotrin (65.61± 5.82) and Ecomac (52.71 ± 5.60). The mixed bio-insecticides 1 ml/1000 ml treatment increased mortality after 24 hours of application at 93.52 ± 4.46 along with a higher concentration (1.4 ml/1000) due to the synergetic actions. The synergetic actions gradually decreased after 48 hours of application. No significant differences on aphid's survival were observed among doses 4 (Fig. 6).

The plant origin insecticides were highly effective as a control measure of mustard aphids on cruciferous vegetables. The bio-insecticides significantly reduced survival and caused substantial mortality, resulting in various behavioral and physiological impacts (Kaadeh *et al.* 2001, Mariappan and Saxena 1983, Ahmed *et al.* 2007). The effects of carbosulfan, phosphamidon, malathion, fenvalerate, dimethoate and oxydemeton-methyl applied as foliar sprays against *L. erysimi* in mustard in Bangladesh (Islam *et al.* 1990). The oxydemeton-methyl, dimethoate, endosulfan, imidacloprid, and thiamethoxam were also effective as control measures against *L. erysimi* (Bapari *et al.* 2007).

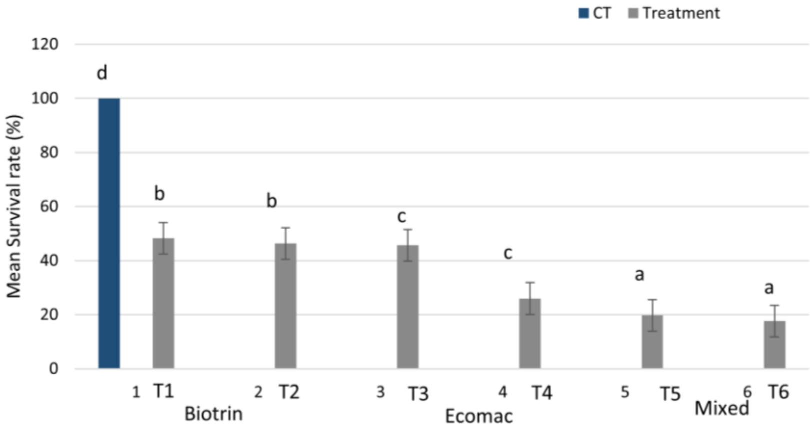


Fig. 6. Efficacy of Biotrin, Ecomac and mixed bio-insecticides on the survival of *L. erysimi* compared with non-treated aphids (control) after 48 hours**.**

The results of the present study revealed that biological insecticides, including Biotrin and Ecomac and the mix of Biotrin and Ecomac might control the *L. erysimi* infestation in broccoli. These bio-insecticides have significant impacts on the survival and reproduction of *L. erysimi*. The current research indicates a clear correlation between the bio-efficacy and the concentrations of bio-pesticides. Therefore, further research is necessary and should address application methods, environmental conditions and rates conducive to the optimal efficacy of these insecticides in the field. Information will enhance our comprehension of how plant-origin insecticides will perform as control measures of aphids. The use of these insecticides would also support organic cruciferous vegetable production and insecticide resistance mitigation.

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