



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2024; 12(1): 38-43

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Received: 08-12-2023

Accepted: 09-01-2024

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Study of the effects of biological extracts of «heals everything» *Gymnanthemum amygdalinum* and garlic *Allium sativum* on fruit flies *Bactrocera dorsalis* and their stage L3 larvae

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DOI: <https://doi.org/10.22271/j.ento.2024.v12.i1a.9277>

Abstract

The attacks suffered by crops have led to the use of various control methods, especially against fruit flies. The aim of this work is to study the actions of biological extracts of *Gymnanthemum amygdalinum* and *Allium sativum* on *Bactrocera dorsalis* and their larvae of stages L3. Methodology and results: fruits infested with mango and citrus have been incubated. Mass breeding of lots of 30 individuals was made in 7 boxes in a bottle cut and covered by a mosquito net to allow asparagus. The different dosages are: dosage 1 (25 g of leaves per 250 ml of water), dosage 2 (50 g of leaves per 100 ml of water, dosage 3 (25 g for 150 ml of water), garlic dosage (three garlic cloves and their peels macerated in 150 ml of warm water), mixture of dosage 2 with garlic solution and pure water is sprayed on a sixth batch. The seventh batch is taken as a control and does not undergo any spraying. Every day a contact spray is made on the flies. The spraying of dosage 2 + garlic dosage on *Bactrocera dorsalis* and their larvae allowed us to record higher mortalities than those had with other dosages. Dosage 2 also recorded significant mortalities compared to other less concentrated dosages but its results are lower than those obtained with cocktail dosage 2 + garlic. The flies from mango and fed with it are more resistant compared to those from citrus fruits and fed with them. Citrus fruit flies are more sensitive to spraying with water than mango flies. Similarly, citrus larvae are also more sensitive to water.

Keywords: *Gymnanthemum amygdalinum*, *Allium sativum*, *Bactrocera dorsalis*

Introduction

The economic difficulties currently known to the Sahelian population require the exploration of all possible ways to obtain effective protection of food stocks at the lowest cost ^[1]. Synthetic pesticides are generally harmful to natural enemies (Predators and parasitoids) of pests. They also have a negative effect on human health ^[2] especially among producers and on the environment ^[3, 4]. Even more, the resistance of bioagglers is one of the main limits of synthetic pesticides ^[3, 2]. To this end, biological control has seen its importance and scale increase over the years to meet this demand. Indeed, scientific literature shows that many plants of the West African flora have an enormous biocidal potential on a wide range of bioagglers ^[2]. Under certain conditions, plant extracts can be as effective as conventional insecticides. However, they can help keep the pest population below the pest threshold and reduce the use of synthetic pesticides used on vegetables ^[2]. In Senegal, several plants are used in biological control ^[5]. Bio pesticide of lemongrass oil (Concentration 5 ml/l), lemongrass extract (Concentration 25 ml/l) and lemongrass ash (concentration 25 g/l) were effective against fruit fly on pepper plants ^[6]. *Gymnanthemum amygdalinum* extracts have inhibitory activity on tested bacteria (*Klebsiella pneumonia*, *Salmonella enterica*, *Klebsiella pneumonia* and *Streptococcus aureus*) ^[7]. Hanh and Hang ^[8] demonstrated that extracts of *A. indica* (neem seeds), *W. trilobata* (Wedelia), *Allium cepa* (onion), *Artemisia absinthium* (mugwort) and *Allium sativum* (garlic) effectively prevented the laying of fruit flies at 82.22%, 71.50%, 67.86%, 63.28% and 42.82% respectively. *Gymnanthemum amygdalinum* has various bio-activities with little or no side effects ^[9].

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It is in this sense that the objective of this work is to study the actions of biological extracts of the «heals all» (*Gymnanthemum amygdalinum*) and garlic (*Allium sativum*) on the fruit fly *Bactrocera dorsalis* and their larvae of stages L3.

Materials and Methods

Sampling: The biological materials consisted of infested fruits and young adults of *Bactrocera dorsalis*. The *Bactrocera dorsalis* samples in this study were collected in the Niayes Zone locality at Sangalkam (14° 47'58"N and 17° 14'17"W) on mangoes, tangerines and cashew apples in shepherds to be incubated in the laboratory at room temperature.

Mass breeding: The infested fruits were incubated in large containers, all deposited in basins to have a large number of individuals. After emergence, images are collected in cages made from transparent 10-litre bottles with the top removed. Then both sides face to face were removed with a knife to leave about 3 cm up and down and create windows for spraying flies. Finally, the whole was covered with a mosquito net with opening above the bottle to bring in and out flies and food. The latter consisted of mango slices for those from mangoes, citrus slices and bananas for those from citrus. Thus, they can be licked and cotton soaked with water to drink preventing the drowning of flies. This food was renewed every 48 hours or 36 hours as needed.

Effects of *Gymnanthemum amygdalinum* extract and fermented *Allium sativum* on fruit fly: These experiments were done during the periods of July-August 2022 for individuals from mango, and November, December and January 2022 for individuals from citrus. This difference in sampling periods is due to the maturity of mangoes coinciding with the rainy season ^[10, 11] which is between June and September. While the ripening of citrus fruits occurs most often in the month of October-February ^[12]. For this experiment, seven cages were used for the first experiment, each of which has 30 individuals of *Bactrocera dorsalis*.

One hundred (100) g of *Gymnanthemum amygdalinum* «heals everything» leaves were soaked for 30 min in bleach to sanitize. They are then rinsed in filtered water and mixed with warm water in a blender and left for maceration for 24 hours. The decoctions were then sifted or filtered cleanly and then put into bottles and kept in the refrigerator. A small amount is taken daily from bottled pumps for spraying. This maceration process is renewed as needed.

A first dosage (Dosage 1) 25 g of leaves per 250 ml of water was used. Then higher concentrations than the previous one. Fifty grams (50 g) of leaves were used with 100 ml of water for dosage 2. A third dose of 25 g for 150 ml of water (Dosage 3) was also used. Three garlic cloves and their peels were also macerated in 150 ml of warm water (Garlic dosage) to be used on a fourth batch. A mixture of dosage 2 + garlic dosage was used on the fifth batch. Pure water is sprayed on a

sixth batch. Every day a contact spray is made on the flies. A seventh batch is taken as a control and does not undergo any spraying. Mortality is counted in each cage per day.

Effects of *Gymnanthemum amygdalinum* extract and *Allium sativum* fermented on L3 fruit fly larvae: For the second experiment, the same sprays were done on batches of 30 stage L3 larvae in Petri dishes containing sand to see if there will be hatching or emergence. The sand is moderately wet to avoid death by drowning or choking. A control lot was reserved with dry sand. After five days, the sand was removed from the petri dishes and the pupae were left for incubation. After 20 days, the number of emergence and abortion in each Petri dish is counted.

Statistical analyses: The results obtained from all the experiments were recorded in tables in Excel. Each table has been translated into a grouped histogram in order to compare the different parameters. The significance of these results on mortality according to the different sprays was tested on R version 3.6.1 (R Core Team, 2019)

Results

Effects of *Gymnanthemum amygdalinum* extract and fermented *Allium sativum* on fruit fly

Spraying *Bactrocera dorsalis* from mango gave 6.67% mortalities in the controls, 10% deaths with dosage 2, 3.33% deaths with garlic dosage and 20% deaths with garlic dosage + dosage 2 at day 5. At day 10, the 1st batch control, the 2nd sprayed with dosage 2 and the 3rd sprayed with the mixture garlic dosage + dosage 2 gave more mortality than the other lots. The same observation was made on the 15th day with respectively 53%, 56.67% and 73.33%. On the 20th day, we observed 96.67% mortality for the garlic dosage + dosage 2 and 80% for dosage 2 while the mortalities noted in the control, water batch, dosage 1, dosage 3 and garlic dosage were respectively 56.67%, 56.67%, 63.33%, 50% and 60%. The garlic dosage + dosage 2 had a significantly more pronounced action on the 20 days than the other dosages. Dosage 1 and dosage 3 had no visible actions during the first 5 days (Figure 1).

The spraying of *Bactrocera dorsalis* from citrus fruits gave the results of figure 2. Mortality exceeded 50% on the 5th day for all flies. It was 53.33% for the control batch, 63.33% for the water batch, 56.67% for dosing 1, 66.67% for dosing 2, 63% for dosing 3, 60% for dosing garlic and 60% for dosing garlic + dosing 2. The 100% mortality was reached on: 11th day for the control batch, 10th day for the lot water as well as with the dosage 1 and the dosage garlic, 9th day with the dosage 2, the dosage 3 and the garlic dosage + dosage 2. In all lots of citrus flies, the mortality rate exceeded 50% in 5 days. The 100% mortality was obtained on the 9th day by spraying with dosage 2, dosage 3 and garlic dosage + dosage 2. On the 10th day, we recorded 100% mortality for treatment done with water, with dosage 2 and that with garlic dosage.

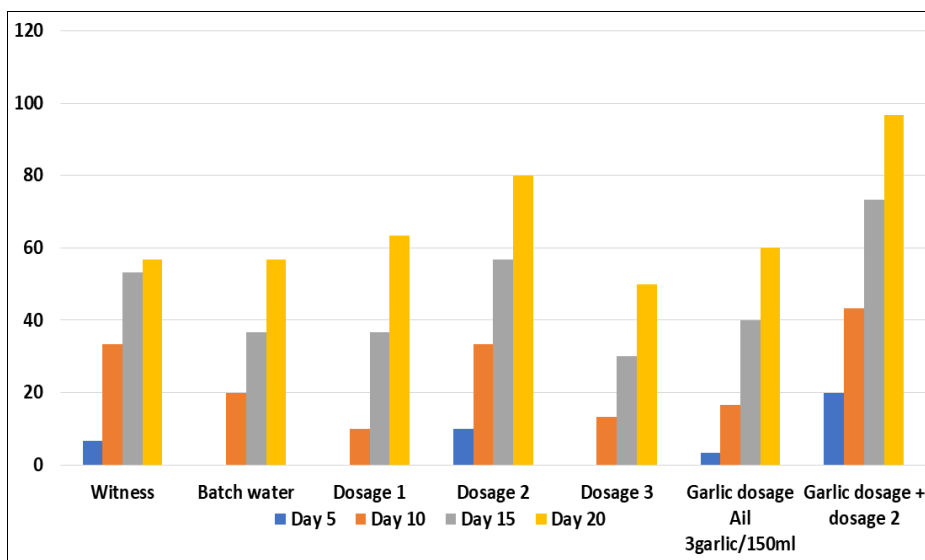


Fig 1: Mortality (%) of *Bactrocera dorsalis* flies from mango as a function of days and different sprays

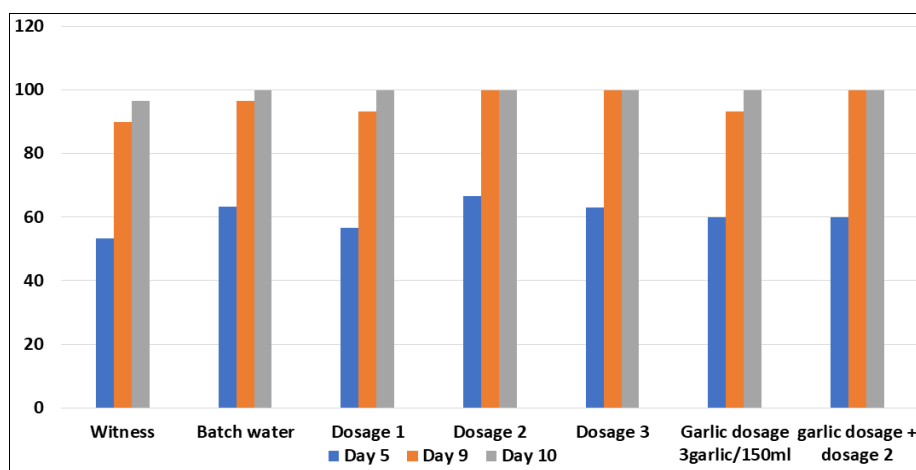


Fig 2: Mortality (%) of *Bactrocera dorsalis* flies from citrus fruits according to days and dosages

Effects of *Gymnanthemum amygdalinum* extract and fermented *Allium sativum* on L3 fruit fly larvae: From mango larvae, the control batch, the water batch and the batch sprayed with dosage 1 did not record mortality during the first 5 days. However, abortions that were respectively 3, 4 and 3

were noted for these lots. Larval and abortive mortalities of 4 and 2 for dosing 2; 5 and 3 for dosing 3; 4 and 4 for dosing garlic and 14 and 6 for dosing garlic + dosing 2 were also recorded (Figure 3).

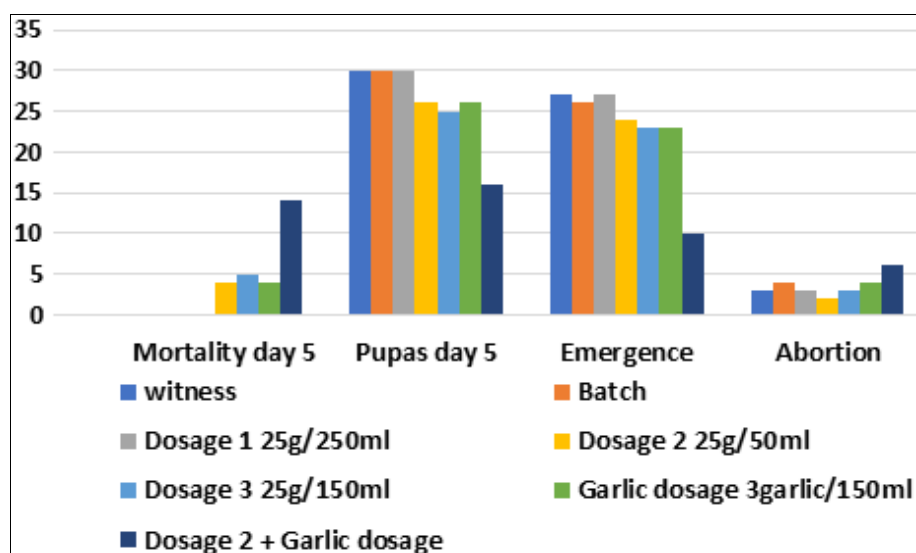


Fig 3: Mortality, emergence and abortion of *Bactrocera dorsalis* larvae from mangoes according to days and dosages

The larvae of *Bactrocera dorsalis* from citrus fruits present the results. Only dosage 3 did not give mortality on larvae or pupal abortion. Larval mortality was 2 for the control group, dosage 2 and garlic dosage and their abortion are respectively

2, 4 and 3. The mortality was 3 for the water batch and dosage 1 and their abortion was respectively 3 and 4. Dosage 2 gave a dead larva and 5 abortions (Figure 4).

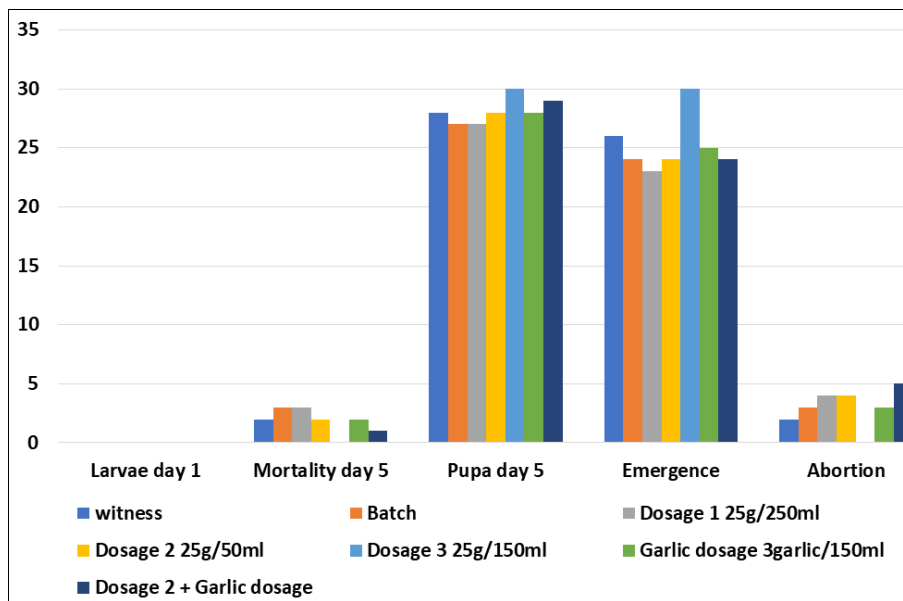


Fig 4: Mortality, emergence and abortion of *Bactrocera dorsalis* larvae from citrus fruits as a function of spraying

Discussion

Effects of *Gymnanthemum amygdalinum* extract and fermented *Allium sativum* on fruit fly: The application of dosage 2 in mango flies as those from citrus allowed us to record mortalities higher than other dosages but lower than those obtained with cocktail garlic dosage + dosage 2. This could be due to the biocidal actions that could have the healer all on *Bactrocera dorsalis*. Indeed, *Gymnanthemum amygdalina* has been shown to inhibit certain bacteria such as *Klebsiella pneumonia*, *Salmonella enterica* and *Streptococcus aureus* [7]. So, everything could act in the same way on the symbiont bacteria residing in the digestive tract of *Bactrocera dorsalis* (*Gluconobacter cerinus*) [13]. The mortality rate obtained with the application of garlic dosage + dosage 2 is higher than the others at the level of all intervals (5, 10, 15 and 20 days). Indeed, in mango, this difference is significant (With any adjustment) except for that obtained between the cocktail (Garlic dosage + dosage 2) and dosage 2. The combined actions of the healer all and garlic on *Bactrocera dorsalis* are more effective on the latter. Indeed, the insecticidal and/or larvicidal effects of plants are linked to active compounds such as alkaloids, flavonoids, tannins anthracene derivatives, sterols, terpenes saponins soluble in their various extracts, extract fractions and essential oil [3]. It also contains terpenoids, alkaloids [14], flavonoids, polyphenol tannins [15]. Similarly, alliinase, accounts for more than 82% of the total sulphur content of garlic. In the presence of alliinase, it produces allicin that acts on the pathogenic microorganism [16]. Cold macerated garlic inhibits the development of strains of *Listeria innocua*, *Bacillus thuringiensis*, *Escherichia coli*, *Saccharomyces cerevisiae* [17], *Pseudomonas syringae* pv. *syringae* [18] as well as a mixture of unidentified bacterial strains and yeasts that were taken from carrot [17]. From these different results we can say that garlic acts on the bacteria of the adults of *Bactrocera dorsalis* by passing through their cuticle and their pores causing the rupture of the symbiotic relations between these living beings.

The flies obtained from mangoes had a longer lifespan than those obtained from citrus fruits. This remark could be explained by the organoleptic compounds that are different between the two fruits. Indeed, sugar is part of the diet of flies. The high level of sugar in mango, a major component and a fast source of energy, gives it a fairly good energy value of around 74 kCal/100 g [19, 21]. It is a very nutritious fruit and has a carbohydrate content of 14 g/100 g [22] while citrus fruits have an energy intake of 32 to 45 kcal/100 g and a carbohydrate content of 8.63 g/100 g [21]. These values probably led *Bactrocera dorsalis* to choose mango as the main fruit hence the name mango fly. These results can also be explained by those of Sembène *et al.* [23] who studied the fertility of female insects that differ from one host plant to another and can be explained by the existence of a barrier. This finding could also have an impact on the development time of larvae and flies, hence the difference in lifespan of *Bactrocera dorsalis* in this study. Indeed, climatic conditions can impact the development cycle of *Bactrocera dorsalis* [10, 11]. Similarly, the population peak most often coincides with the maturity of mangoes [24]. The lifespan could be explained by a water sensitivity of individuals from citrus fruits compared to those from mango. Indeed, the ripening of citrus fruits that occurs most often in the month of November-December does not fall on the optimal temperatures for the proper development of the fruit fly described by Mouly *et al.* [25]. The latter also stated that the population increase of *Bactrocera dorsalis* is noted with precipitation in the range 100-200 mm per month, however, it decreases with precipitation below 50 and above 25 mm per month. Similarly [26], found that fruit infestation was positively correlated with the level of *Bactrocera dorsalis* populations in orchards under the influence of abiotic factors including temperature, rainfall and relative humidity.

Effects of *Gymnanthemum amygdalinum* extract and fermented *Allium sativum* on L3 fruit fly larvae: In larvae

from mango like citrus fruits, the abortion rate with the application of garlic dosage + dosage 2 is respectively 6/30 and 5/30 individuals. This corresponds to rates of 20% and 16.67% respectively. This cocktail records the highest number of deaths compared to other dosages. This could be due to the combined action of garlic and treats everything. The chemical compounds of these two plants (Alkaloids, FL avoids, tannins, terpenes, allicin, etc.) add to give higher concentrations or new compounds that could have a greater impact on larval development. Larvae have enter bacteria in their abdomen that help with nutrition [27]. Thus, treating everything could have an impact on the development of larvae through *Bactrocera dorsalis* bacteria [28]. When the garlic bulb is damaged (Crushed, chopped, etc.) alliin is released from its compartment and interacts with the alliinase enzyme present in the adjacent vacuoles to form allicin [16]. The latter is responsible for the recognized actions of garlic (biocides). Indeed, according to Bourgoin *et al.* [17], it is possible to obtain an extract of garlic whose active ingredient inhibits, more effectively than bleach at 0.04 mol/L, the *in vitro* development of strains of *Listeria innocua*, *Bacillus thuringiensis*, *Escherichia coli*, *Saccharomyces cerevisiae* and a mixture of bacterial strains and unidentified yeasts taken from carrot.

The mortality rate with this same dosage is 14 or 46.67% for mango larvae and 1 or 3.33% for citrus larvae. The biocidal action of this cocktail is more pronounced in larvae from mango than in those from citrus. This could be explained by the fact that mango larvae are more sensitive than citrus larvae for this cocktail. The nutritional values of mango [22] are higher than those of citrus fruits [21] which would have allowed the larvae to acquire more bacteria for their digestion. Thus, biocidal plants would have a wider spectrum of action on these larvae by killing most symbiotic bacteria which would weaken these larvae from mango.

The application of dosage 3 on citrus larvae does not record larval mortality or pupal abortion. It could be due to the fact that the concentration of care everything is adequate to the development of the latter. The cure all presents not only biocidal qualities it is also endowed with virtues used for the treatment of certain diseases or used for the improvement of nutritional quality [7]. It contains nutrients (carbohydrates, proteins and fiber), vitamins (A, B, C) and minerals (Magnesium, potassium, sodium, phosphorus, calcium, zinc, copper, iron) [29]. Spraying with water results in mortality and abortion of citrus larvae while only abortions are noted with mango larvae. This confirms the observations made on citrus fruit flies in relation to their sensitivity to water. So, the larvae from citrus fruits are more sensitive to water compared to those from mangoes.

Conclusion

The spraying of extract *Gymnanthemum amygdalina* combined with that of *Allium sativum* (Dosage 2 + garlic dosage) on fruit flies *Bactrocera dorsalis* and their larvae allowed us to record higher mortalities than those had with other dosages. Dosage 2 also recorded significant mortalities compared to other less concentrated dosages but its results are lower than those obtained with cocktail dosage 2 + garlic. The flies from mango and fed with it are more resistant compared to those from citrus fruits and fed with them. Citrus fruit flies are more sensitive to spraying with water than mango flies. Similarly, citrus larvae are also more sensitive to water.

Acknowledgements

I thank my coach Professor Mbacké Sembène, Dr. Toffène Diome head of the Entomology speciality, Dr. Adiouma Georges Robert Jacque Sarr, the team of Genetics for Population Management (GenGesPop), for their availability, their support and guidance. I also thank Dr. Bathie Ka, Miss Ndèye Amy Diémé Department of Vegetal Biology of the UCAD for their help in identifying the *Gymnanthemum* plant species. Thanks to Mrs Maryam Keita for their help in writing the document. Any conflict of interest related to this work

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