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Preparation of local protein hydrolyzate from liquid wastes of breweries: a protein bait for the Mediterranean fruit fly *Ceratitis capitata*

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Abstract

Protein hydrolyzates were prepared locally from breweries liquid wastes. The liquid wastes were cooked for 48 hours, a papain enzyme was added and mixed for 24 hours. Field experiments were carried out to test the attractiveness of the Mediterranean fruit fly (Med fly) *Ceratitis capitata* to the resulting product. Two orchards (1 hectare/orchard), were selected containing (each) several varieties of citrus fruit trees. Delta traps were used to monitor Med fly populations. The objective of this study is to convert the liquid waste resulting from breweries into substances of economic value (Protein hydrolyzates). Field experiments have shown that locally prepared hydrolyzates are successful, and outperform imported ones. This product will be used as attractive bait for an insect that is considered one of the most important agricultural pests (Med fly) in Syria. The local product will enable us to use it as an alternative to the imported one.This will reflect positively on the sustainable economic development plans in our country.

Key words: *Ceratitis capitata*, Protein hydrolyzates, liquid wastes of breweries.

1. Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824), is one of the most damaging insect pest of fruit trees in the world [1]. The larvae of this insect attack more than 200 wild and cultivated hosts [2]. As a result of the seriousness of the damage caused by this pest, agricultural quarantine laws were strictly applied to limit its spread to other parts of the world [3, 4] and trade barriers were imposed against agricultural products coming from infested countries. The appropriate temperatures and the continued presence of fruits of different host species throughout the year have made this insect a serious economic pest on a wide range of fruit trees in Syria. Protein baits (protein hydrolyzates) are widely used in an integrated pest management (IPM) approach to control the Mediterranean fruit fly. These techniques are applied, either by their use in traps for monitoring, or as a procedure of control by spraying the material after mixing it with a pesticide (toxic baits) on trees and agricultural crops [5]. The use of toxic protein baits in the controlling the Mediterranean fruit fly began at the beginning of the twentieth century [6]. In 1952, a mixture of protein baits, brown sugar, and parathion was used to control fruit flies in the Hawaiian Islands [7]. In Brazil, the technique of spraying protein baits has been widely applied after the addition of phosphorus or parathyroid insecticides in the control of fruit flies since the sixties of the last century [8]. Some studies have observed that adding the pulp or juice of some kind of fruits like mango, banana, orange and pineapple increases the ability of protein baits to attract fruit flies [9], but the rate of attraction varies according to the kind of juice or pulp added.

Animal and agricultural wastes are also widely used as raw materials for the production of protein hydrolyzates. Microbial enzymes, such as pepsin, trypsin, alpha-chymotrypsin and amino peptidase in addition to proteins and

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papain, play an essential role in converting proteins into hydrolyzates, where this conversion is moderate or low [10]. Liquid wastes of breweries are considered one of the most widely used materials in preparing protein baits for fruit flies due to its richness in proteins, sugars and mineral elements [11].

Research conducted on fruit fly attractants has indicated that most fermentation products from breweries were effective in attracting the flies in proportions related to the quality of the ingredients or species of flies. Mangan and Lloyd [12] and Drew [13] reported that protein baits contain all essential amino acids, and rich source of B vitamins, in addition to containing most of the important mineral elements. Bateman and Morton [14] pointed out that ammonia released from protein baits are the main reason why flies are attracted to them. However, Ekesi and Tanga [15] indicated that the reason for the attraction of fruit flies to protein baits is not limited to the rise of the ammonia compound alone, but also to a mixture of four other compounds: Ammonium bicarbonate, linolenic acid, putrescence and pyrolidine.

The McPhail trap was the first trap used protein baits to attract fruit flies, followed by the Steiner trap in 1952 and Jackson trap in 1971 [16]. These traps are currently used in various countries to conduct fruit fly surveys and to support control activities and eradication programs [16]. Toxic baits are prepared by mixing protein hydrolyzates with effective chemical pesticides against the insect, such as malathion, spinozad, and others [17, 18]. Some studies showed that hydrolyzate mixed with pesticides remains effective in killing fruit flies for up to 14 days [19]. This technique has become widespread for controlling many agricultural pests, including the Mediterranean fruit fly [20]. Protein baits are often used in insect traps in order to monitor the emergence of their generations in the fields, and thus apply control at the right time. Increasing the number of traps per unit area may play a role in reducing the insect's numbers. Adding pesticides with attractive baits and spraying them on trees within an area of 1 m^2 (partial spraying) leads to attracting the insects and getting rid of them at high rates. This, in turn, will lead to rationalizing the employ of chemical pesticides and reducing their use in the environment [21, 22]. For this reason, preparing such materials locally, and reducing the amount of pesticides used to control the pest, will assist in decreasing importing agricultural pesticides and to reduce the harmful effects resulting from the extreme use of pesticides in the environment. Protein hydrolyzates had never been prepared in Syria from local sources before. Therefore, preparing such a compound locally as an alternative to similar ones imported from abroad, will constitute a new step in controlling fruit flies.

So, preparing this product locally will reflect positively on the sustainable economic development plans in our country. The objective of this study is to convert the liquid waste resulting from breweries into substances of economic value (Protein hydrolyzates). This product will be used as attractive bait for an insect that is considered one of the most important agricultural pests in Syria, which is the Mediterranean fruit fly.

2. Material and methods

2.1. Experimental site

This experiment was carried out in the citrus orchards of Al-Bassa village which is 6 km south of the city of Latakia. Citrus orchards occupy the largest percentage of agricultural land, while the rest of area land is cultivated with vegetables and tobacco. The average annual precipitation is about 770 mm and the average daily temperature is about 23°C. August is the hottest month of the year with an average temperature of 32°C. The orchards were planted with citrus varieties whose maturity ranges from the end of September to February. Ripening begins first with Clementine, followed by Tangerine, Satsuma varieties, and then followed in succession by Shamouti, Navel, Pomelo, Grapefruit and Mandolin. However, Valencia variety matures late until February. This diversity of varieties extends the presence of fruits on trees for about 6 months, allowing fruit flies to reproduce for several generations during this period. This helped in maintaining the Med fly population in the orchards for a long period of time and facilitated the successful testing of the studied material for two consecutive years.

2.2. Fields of experiment

The experiment was carried out in two citrus orchards, each with an area of about 1 hectare, and the distance between them is about 1 km. Both orchards contain early, medium and late maturing varieties. The trees were planted in

both orchards on lines, the distance between the lines is 3.5 m, and between the trees on the same line is 3 m. The traps were hanged on the trees at an altitude of 160 cm from the southeastern side of trees. The traps were checked weekly and the number of captured flies was counted and removed. The traps were placed in the first week of October 2021, while they were placed in mid-September in 2022.

2.3. Preparing the local protein hydrolyzates

The liquid waste was brought from Afamya-Beer Factory located in the industrial city of Adra near Damascus. The waste was subjected to the process of preparing protein hydrolyzates by the following steps: first, liquid waste was cooked in an electric kettle with a mixer (capacity 60 liters), at a temperature of 95C° for 48 hours. The cooked waste was left until the temperature dropped to 65°C. Second, a papain enzyme was added at rate of 2 grams per liter and mixed by an electric shaker at a temperature of 65°C for 24 hours. Third, ammonium acetate was added at a rate of 20 g/L after the temperature dropped to 35°C and mixed well. At this final step, the compound (locally hydrolyzates) (LH) becomes ready for testing.

2.4. Insect traps

Delta type traps were used to test the studied protein hydrolyzates and compared with the control which is the imported protein hydrolyzate. Each trap was provided with sticky tape (Figure 1) and a cylindrical plastic cup (3 × 3.5 cm) with a mesh base and cover (Figure 2) that allowed air to pass through was hung to it from the top using a metal wire. Each cup is provided with 1.5 grams of cotton.

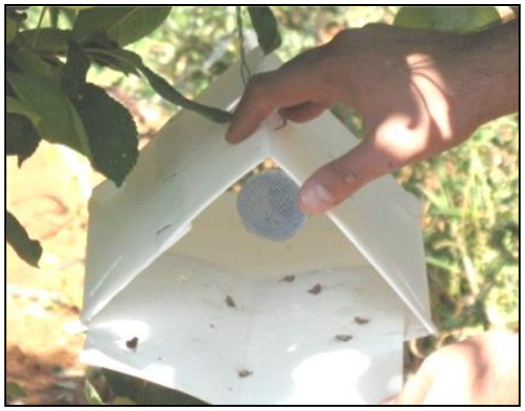


Fig.1. Delta trap with sticky tape

Fig.2. Cylindrical plastic cup with a mesh

The tested hydrolyzate and control were added into the cup by injecting them with plastic syringe at a rate of 20 ml/cup. The cotton and hydrolyzates were replaced every two weeks. The traps were checked out weekly and the sticky tape was replaced when necessary.

2.5. Statistical analysis

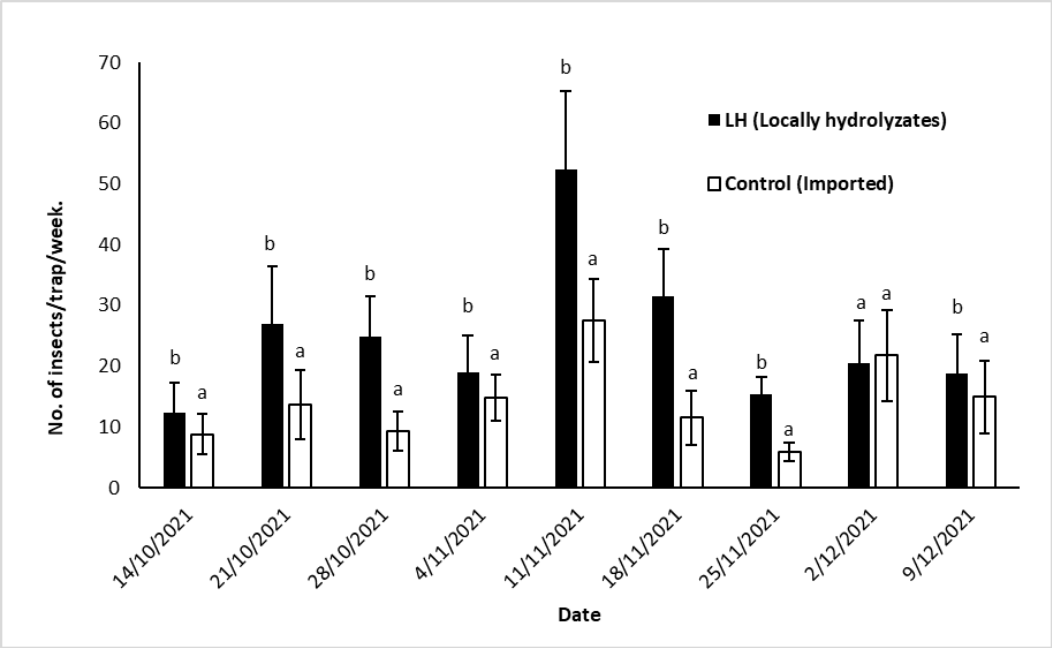
Data from these experiments were subjected to analysis of variance (ANOVA). The means were separated by Fisher's protected least significant difference (LSD) test (StatView, 4.57® Abacus Concepts, Berkley, Calif) to compare the average values at a significance level of $P<0.05$.

3. Results and discussion

3.1. Fruiting season 2021

Figure 3 shows that the number of captured flies in the traps containing locally protein hydrolyzates (LH) was significantly higher than the control (imported hydrolyzates). This superiority remained whether the population of insects decreased or increased during all stages of the experiment that lasted for 9 continuous weeks.

The same (Figure 3) shows that when the number of flies in the control traps in the first week was 8.8 insect/trap, the number increased significantly ($P<0.05$) to 12.4 insect/trap in LH traps in the same week. The figure also indicates that when the density of fly population increased during the fifth week, to reach 27.5 in control traps, the number of insects also increased significantly in the LH traps to reach 52.3 insects/trap. In return, the number of insects decreased at the end of the fruiting season in control traps to 14.9 insects/trap.



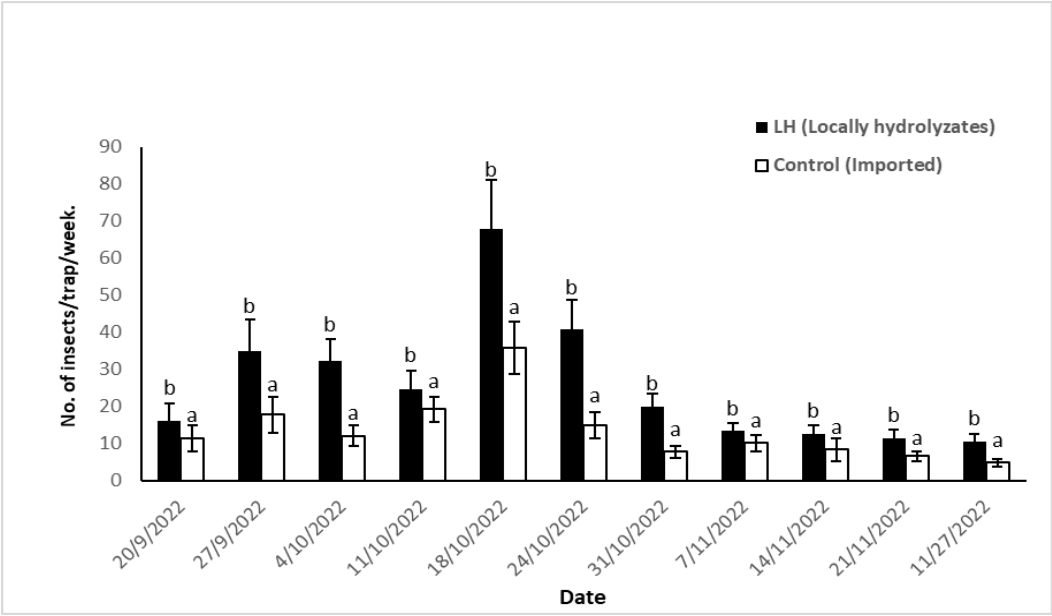
- *Values for bars sharing different letters are significantly different (ANOVA, P<0.05)*
- *Values for bars sharing the same letter are not significantly different (one-way ANOVA, P<0.05)*

Fig.3. Number of Med flies captured in the control and locally hydrolyzates (LH) at fruiting season 2021

This decrease was accompanied by a decrease in the LH traps as well recording 18.8 insects/ trap in control. So, the significant difference between the two treatments continued to favor the superiority of the LH compound during fruiting season 2021.

3.2 Fruiting season 2022

The results presented in Figure 4 show that the ability of protein hydrolyzates to attract the flies, whether in control or LH traps, took a similar trend to that in 2021.



- *Values for bars sharing different letters are significantly different (ANOVA, P<0.05)*
- *Values for bars sharing the same letter are not significantly different (one-way ANOVA, P<0.05)*

Fig.4. Number of Med flies captured in the control and locally hydrolyzates (LH) at fruiting season 2022

However, the traps in both treatments gave results indicating a relative increase in the density of the insect population for this year, compared to what it was in the previous year. The peak of that density appeared in the second half of October, while the peak of its density during the year 2021 was during the first half of November. Nevertheless, it is noticeable that despite the variation in the number of captured insects from one week to another, whether up or down, the significant differences ($P<0.05$) between the both indicated the superiority of the LH compound in comparison with the control during all weeks of the study. This confirms for the two consecutive fruiting seasons of the study. The variation in the number of captured insects using protein baits may vary from time to time due to the appearance of generations, in addition to environmental conditions and the availability of the host. These results are consistent with what was mentioned by [23, 24].

The results of this study show that the protein hydrolyzates attractant made from local sources of beer factory liquid wastes was more effective than the imported hydrolysates. That may be due to the time that the imported attractant takes for transporting and storing, comparing with fresh prepared local hydrolyzates [25, 18].

4. Conclusion

Based on the results of this study, the following conclusions can be reached:

- This study showed that the number of insects caught under the influence of locally prepared hydrolyzates was higher than their number under imported hydrolyzates (control).
- Converting liquid waste, resulting from beer factories into substances of economic value (protein hydrolyzates) is something that can be achieved in Syria.
- This research is important in the field because it generates new insight in expanding the possibility to add value to waste from the food industry. In particular, it is worth mentions that by using the obtained protein hydrolyzates it will help to monitor *Ceratitis capitata* in the field, the time of its appearance and the number of its generations and to apply the control of the insect at the right time
- local protein baits based on waste brewer's yeast and production and localization of such materials make them more available and cheaper than imported ones
- This, in turn, will reduce the harmful effects resulting from the intensive use of pesticides in the environment and decrease the import of such materials from foreign sources.

Protein hydrolyzates are made from liquid waste of beer factories in many countries around the world, but it has never been prepared in Syria before.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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