Evaluation of soursop (*Annona muricata linn)* leaf extract *as a* preventative measure against flies contamination in salted fish

Rinto Rinto[1,*], *Gama* Dian Nugroho[1], *Yulia* Sari[2], and *Aulia* Arisviani[1]

[1]Fisheries Product Technology Study Program, Faculty of Agriculture, Sriwijaya University, 30662, Indralaya, South Sumatera Indonesia
[2]Agribusiness Study Program, Faculty of Agriculture, Sriwijaya University, 30662, Indralaya, South Sumatera Indonesia

Abstract. This study aimed to determine the effectiveness of soursop (*Annona muricata linn*) leaf extract as a natural flies repellent for salted fish. The concentrations used in salted fish consist of 0%, 10%, and 20% soursop leaf extract. The results showed that based on data analysis the larvae left the container and died, the 10% concentration of soursop leaf extract effectively caused flies larvae to vacate the observation vessel and significantly reduced their movement. In contrast, a 20% concentration of soursop leaf extract could lead to the death of the larvae. The impact of soursop leaf extract on the number of flies that landed on salted fish during the drying process was significant. The effect was observed by both the concentration of extract and variations in drying time related to temperature and humidity. Specifically, about 10% concentration of soursop leaf extract could reduce the number of flies by 62.1%, while about 20% concentration achieved about 91.4% reduction. The sensory analysis showed that soursop leaf extract treatment did not significantly affect the appearance, taste, texture, and the presence of fungus on salted fish. However, it did have a noticeable effect on the smell and color of fish.

1 Introduction

Salted fish is a preserved fish product that is regularly consumed by Indonesians. Salted fish is made by carrying out two preservation processes, consisting of salting and drying. There are three fundamental methods used for salting fish, including wet, dry, and combination of both wet and dry salting. The main objective of these methods is to increase fish's shelf life and durability, which is similar to other processing and preservation processes [1].

During the drying process, salted fish is normally exposed to sunlight while laid out on bamboo racks covered with nets, mats, or similar materials, and sometimes left uncovered, making it susceptible to contamination by flies and other parasites. House flies (*Musca domestica*), which commonly settle on food, contaminate fish by laying eggs and carrying bacteria and feces. This condition also occurs in the manufacture of other salted fish products such as bekasam and peda [2]. The eggs develop into larvae, or maggots, that will decrease the quality and damage the finished product. As disease vectors, flies could transmit a range

* Corresponding author: rinto@fp.unsri.ac.id

of pathogens, including worm eggs, viruses, bacteria, protozoa, and parasites [3]. Therefore, there is a need for safer alternative methods, such as using natural pesticides, to minimize flies contamination during salted fish production.

The drying procedure is one of the most important steps in the production of salted fish, as flies infestation increases the risk of microbiological growth. This is because fish is dried outdoors in direct sunlight, which makes it more vulnerable to contamination. Flies, with their bodies covered in small hairs, can easily spread disease and serve as carriers of pathogenic bacteria and chemicals, including *Acinobacter*, *Vibrionaceae*, and *Staphylococcus*. Previous investigations Abdullah & Wahyudi [4] have shown that in the dry season, each fly could carry 10^2–10^3 CFU/g bacteria, and in the rainy season, they may carry 10^8–10^9 CFU/g bacteria. To address the risk posed by flies during the drying process, salted fish producers often use synthetic insecticides directly on fish. However, consumers may face long-term health risks from these insecticides, including cancer, neurological conditions, reproductive problems, Parkinson's disease, and immune system damage.

Based on the explanation above, a more environmentally friendly method is needed to prevent flies from contaminating salted fish. One of the methods is using insecticides derived from natural plant substances that are biodegradable, break down quickly, do not pollute the environment, and are generally safe for human use [5]. Soursop leaf (*Annona muricata linn*) has been identified as a potential source of natural insecticides due to its high content of acetogenins, saponins, and flavonoids. These compounds have been shown to function effectively as natural insecticides, with high concentrations of acetogenins having anti-feedant effects that stop pests from feeding. Acetogenins at low temperatures can also be hazardous to pests, potentially leading to their death [6]. Therefore, the chemical compounds found in soursop leaf extract can be used as insecticides, contact poisons, anti-feedants, and repellents for various harmful organisms.

Flies can be eliminated within 20 minutes using a 50% soursop leaf extract solution. Acetogenin, a bioactive compound found in leaf, acts as an antifeedant by producing a smell that reduces flies' appetite and is cytotoxic, causing cell death [7]. No previous reviews have examined the use of soursop leaf extract as organic flies repellent for salted fish. Considering the above explanation, further investigations and testing are needed to evaluate its potential application in preventing flies infestation during the drying process of salted fish.

This study aims to determine the effectiveness different concentration of soursop leaf extract as a method to protect salted fish from flies contamination. It will thoroughly examine the potential of extract as organic flies repellent during the processing of salted fish. Additionally, the analysis explores the possibility of using soursop leaf extract as a substitute for conventional organic anti-fly products in the production of salted fish and other fishery products.

2 Material and methods

2.1. Material

The materials used in this study included three-spot gourami (*Trichogaster trichopterus*), soursop leaf, salt and ethanol.

2.2. Methods

2.2.1. Preparation of soursop leaf extract

The preparation of soursop leaf extract followed the method described in [8]. Soursop leaf was washed under running water to remove any dirt before being chopped into pieces of approximately 1cm in length to ensure a consistent drying process. The chopped leaf was subsequently dried in an oven at 60°C for two hours. After drying, leaf was ground into a finer powder using a blender for 20 minutes and then sifted. The resulting soursop leaf powder was weighed, with 20 g set aside for extraction. The powder was macerated with 200 mL of 95% ethanol for 24 hours, and extract was filtered using filter paper to obtain filtrate I and a residue. The residue was macerated twice, each time using 200 mL of 95% ethanol for 24 hours. Filtrates II and III were obtained through further filtration, and their combination was concentrated using freeze-drying after the ethanol had evaporated.

2.2.2. Making salted fish by spraying soursop leaf extract

The preparation of salted fish followed the method described in [9]. Initially, the entrails of fish were removed before being washed thoroughly under running water. Each salted fish was then weighed, and a salt solution was prepared by dissolving 25% salt in 100 mL of purified water. Fish were soaked in this salt solution for 24 hours in a cool environment in the refrigerator (0-4°C) before being drained and dried in the sun (3 days). Once dried, salted fish were sprayed with whether 10% or 20% soursop leaf extract on the entire surface of salted fish, with a ratio of salted fish and extract of 1g/1mL. Throughout the drying process, the presence of flies and their eggs was monitored periodically.

2.3. Analysis and statistical

The study's observation parameters included the yield of soursop leaf extract, the effect of extract on flies larvae [10], the count of flies that landed, and the measurement of temperature and humidity during the count. Additionally, the quantity of the eggs of flies on salted fish and a sensory analysis were recorded.

The collected data were analyzed using analysis of variance (ANOVA). When a significant difference was found, the Honestly Significant Difference (HSD) test was used for further evaluation. Furthermore, the sensory data was examined through the use of the Kruskal-Wallis test.

3 Results and discussion

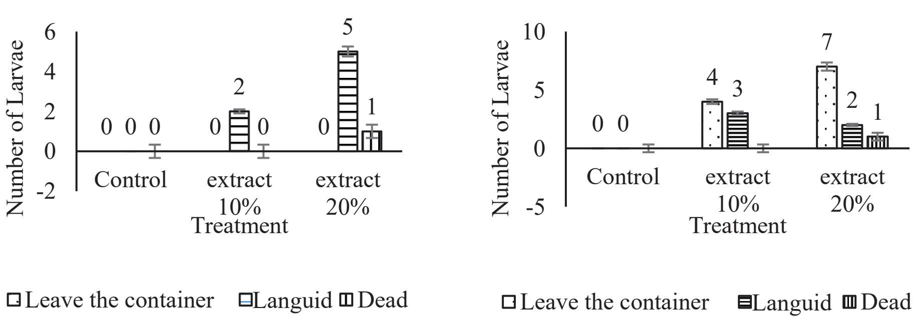
3.1. Extract yield

The yield of soursop leaf extract (Fig. 1), which was 3.004±0.023%, was calculated by comparing the weight of the extract with the initial weight of the unextracted simplisia, as shown in Table 1. Several factors could influence extract yield, including the solvent's polarity, the particle size of leaf, the concentration of the solvent, and the soaking period. Acetogenin, a compound in soursop leaf functioning as a natural insecticide, was effectively extracted with ethanol and has a similar polarity to acetogenin. This was consistent with extraction principle of "like dissolve like," where substances dissolved best in solvents with similar properties. The study conducted by Sukaryo [11] showed that the yield of soursop leaf extract using 95% ethanol was consistent with these results.

Fig. 1. The extraction of soursop leaf.

3.2. Test of the effect of soursop leaf extract on flies larvae

Fig. 2 presented the effect of soursop leaf extract on flies larvae in sealed container and open container. The purpose of the difference in the use of 2 types of containers is to determine the durability of the larvae/effectiveness of the extract against the larvae. In open containers, the larvae can escape from the extract, whereas in closed containers the larvae cannot get out of the container that is sprayed with the extract. Extract contained acetogenin compounds that had a toxic effect on insects. According to Piffer [12], acetogenin compounds enter through natural holes or insect skin, disrupting the nervous system. This disruption inhibited respiration at the NADH bond which caused a decrease in ATP (energy) levels and impaired electron transport in mitochondria respiration, thereby making the test larvae become lethargic and eventually die.



(a) Sealed container (b) Open container

Fig. 2. Effect of spraying soursop leaf extract on flies larvae (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$)).

According to Kolo [13] and Kewa 14], soursop leaf extract was effective in killing Aedes agephty mosquito larvae with a concentration of 5.000 ppm within 90 minutes. The study conducted by Agustin [15] also showed that extract was effective in killing *Cilex* sp mosquito larvae with a concentration of 1.25% within 8 hours. These results showed that soursop leaf extract could serve as a natural larvicide.

This study showed that higher concentrations of soursop leaf extract led to greater changes in the physical condition and mortality of flies larvae. The results were in line with the observation of showing that both the concentration and exposure duration of chemical substances impacted the number and speed of larval deaths.

3.3. The calculation of the number of flies, humidity, and temperature

Houseflies were attracted to salted fish during the drying process due to its high protein content. Fig. 3 provided the differences in the number of flies landing on salted fish treated with 10% and 20% soursop leaf extract compared to the control category.

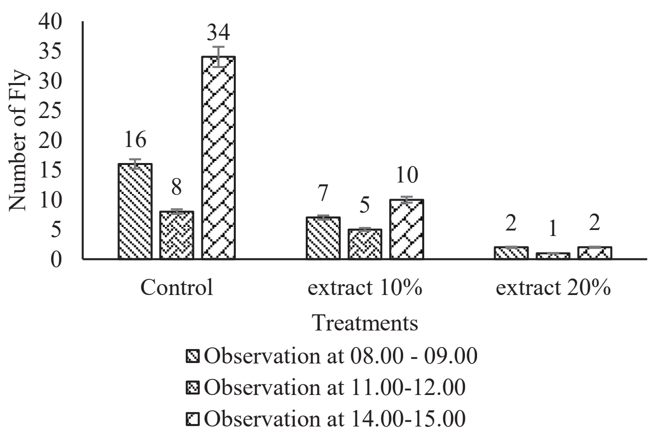


Fig. 3. Observation of flies on salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

Based on the observations in Fig. 3, some flies landed on all treatments. The data showed that spraying 10% soursop leaf extract reduced the number of landed flies by 62.1%, and using 20% soursop leaf extract reduced the number by 91.4%.

Table 1. Analysis of the effect of soursop leaf extract treatment on salted fish

No.	Treatment	Average* (number of flies)
1.	F2 (20%)	0.56^{a}
2.	F1 (10%)	2.67^{a}
3.	F0 (0%)	6.44^{b}

*)Values with different superscripts are statistically significantly difference ($p < 0.05$).

Based on the ANOVA test results, spraying soursop leaf extract on salted fish significantly affected the number of flies that landed. The HSD test further showed that fish treated with 10% and 20% soursop leaf extract differed significantly from the control treatment. Soursop leaf extract effectively repelled flies from salted fish due to the presence of acetogenin chemicals. The chemicals acted as repellents and antifeedants (appetite inhibitors) to prevent flies from feeding on fish. The antifeedant effect functioned by activating chemoreceptors, which were chemical receptors in flies' mouths. These receptors interacted with other receptors to potentially inhibit the perception of feeding impulses [16]. According to Romansyah [17], the evaporation of extract's scent produced gas droplets that combine with surrounding air, forming a repelling agent. The gas molecules were detected by flies' olfactory sensilia (chemoreceptors), prompting flies to move away from extract and towards other preferred materials. Therefore, spraying soursop leaf extract on salted fish caused flies to avoid the treated fish and move toward untreated samples.

Soursop leaf contained flavonoids, alkaloids, and saponins, which were toxic to flies. Flavonoids were known for their harsh taste and strong smell, which could prevent insects from feeding [18]. The compounds might also function as a toxin that entered the insect's body through its respiratory system and disrupted metabolic and respiratory systems, ultimately causing death [19]. Saponins entered the insect's body through its exoskeleton and were toxic, while alkaloids affected flies' muscles, leading to oxygen depletion, paralysis, and death [20].

Table 2. Analysis of the effect of observation time on salted fish

No.	Treatment	Average* (number of flies)
1.	11.00-12.00	1.89[a]
2.	08.00-09.00	2.78[ab]
3.	14.00-15.00	5.00[b]

*)Values with different superscripts are statistically significantly difference ($p < 0.05$).

The ANOVA test showed that the observation time had a significant impact on the number of flies landing on salted fish. The HSD test found no significant difference between morning (08.00-09.00), early afternoon (11.00-12.00), and late afternoon (14.00-15.00) observations. However, there was a significant difference between the early afternoon and late afternoon observations. The variation in the number of landing flies at different times was related to temperature fluctuations, which were detailed in Fig. 4.

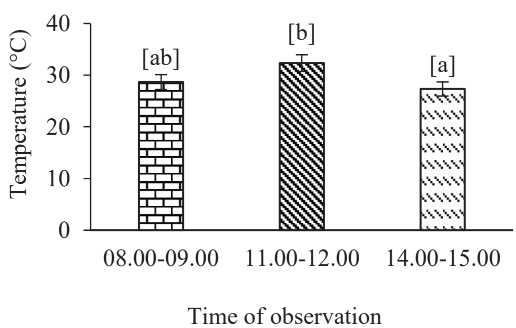


Fig. 4. Observation of temperature of flies landing on salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

As the temperature increased, it moved away from the ideal range for flies, leading to a reduction in flies activity. The observation results showed that flies activity became denser when the temperature dropped between 20°C and 34°C. When the temperature fell between 15°C and 20°C or rose between 35°C and 40°C, flies activity reduced. Extremely low temperatures (10°C) and high temperatures (40°C) could cause flies to die or cease to exist [21]. Therefore, both extremely high and low temperatures had the ability to reduce flies activity and even result in death.

The ANOVA showed that temperature significantly affected flies activity. The HSD test showed no significant difference in flies activity between the morning (08.00-09.00), early afternoon (11.00-12.00), and late afternoon (14.00-15.00) observations. However, flies activity in the early afternoon (11.00-12.00) differed significantly from that in the afternoon (14.00–15.00). The results were in line with the observation of [22], which showed a large number of flies were trapped in the morning and afternoon when temperatures were between 20°C and 28°C. Similarly, the investigation conducted by [23] at the Chicken Slaughterhouse in Baraya Village found high flies density levels during morning and evening measurements. Temperature conditions during the observation were related and inversely proportional to humidity levels. When the temperature was low, humidity would be high, and when the temperature was high, humidity tended to decrease, as shown in Fig. 5.

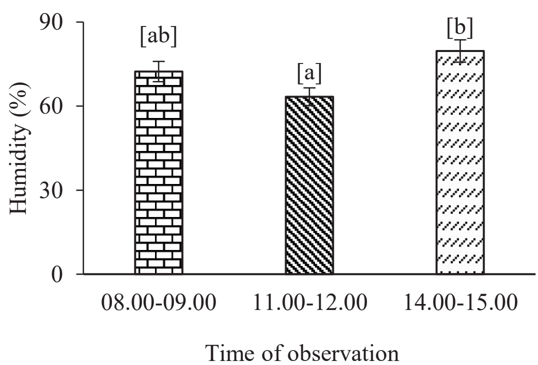


Fig. 5. Observing the humidity of flies landing on salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

The results showed that the average observed humidity fell within the optimal range for flies activity. According to [21], flies were most active when the humidity was between 45% and 90%. During the afternoon observations, humidity levels were high, and the ANOVA results showed that air humidity significantly affected flies activity. The HSD test found no significant difference in flies activity between morning (08.00-09.00), early afternoon (11.00-12.00), and late afternoon (14.00-15.00) humidity levels. However, flies activity in the early afternoon (11.00-12.00) differed significantly from that in the late afternoon (14.00-15.00). Air humidity was influenced by environmental and weather conditions, which included wind, clouds, rain, temperature, humidity, and air pressure.

3.4. The number of flies egg on salted fish

During sun-drying, no flies eggs were found on any of salted fish samples, including untreated fish, fish treated with 10% soursop leaf extract, and fish treated with 20% soursop leaf extract. Flies preferred moist organic materials, such as food scraps left in public spaces, animal excrement, or areas not directly exposed to sunlight, for settling and laying their eggs [24]. Before landing on a surface to feed or lay eggs, flies were subjected to an adaptation process to ensure they did not contain any hazardous materials. Flies had highly sensitive chemoreceptors, or porous olfactory sensilia, located on the head and thorax, which could detect unpleasant smells, allowing them to avoid unsuitable surfaces.

3.5. Sensory analysis

Sensory analysis was a method used to measure food acceptance based on human senses (hedonic analysis). This study adopted organoleptic tests to evaluate factors such as appearance, smell, taste, texture, color, and mold. The sensory values were determined according to the standards of SNI 8273: 2016, with data collected from 25 panel members.

3.5.1. Appearance

Appearance was the first attribute that people notice, evaluate, and consider when selecting or using a product. The results of the appearance test of salted fish samples were presented in Fig. 6, with the hedonic quality sensory testing yielding values ranging from 7 to 8.

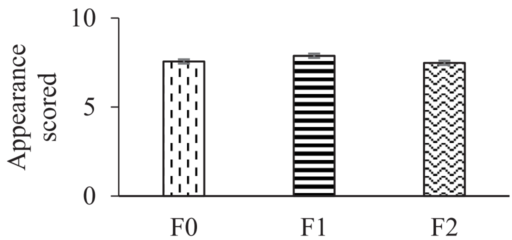


Fig. 6. Appearance of salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

Based on the results of the Kruskal-Wallis test ($p<0.05$), spraying soursop leaf extract did not have a significant effect on the appearance of salted fish. The observed changes in appearance were primarily due to the salt concentration used. According to Mardalisa [25], copper (Cu) and iron (Fe) compounds in salt could cause the product to appear duller or take on a brownish-yellow hue, making it less attractive to panelists.

3.5.2. Smell

The smell was one of the sensory qualities of food that could be evaluated using the sense of smell. The components used in food preparation had a significant impact on the final product's smell. The smell analysis carried out on samples of salted fish was shown in Fig. **7**, with the hedonic quality scores ranging from 7 to 9. Salted fish treated with a 20% soursop leaf extract (F2) received the lowest hedonic quality score, while the control treatment without soursop leaf extract (F0) received the highest score.

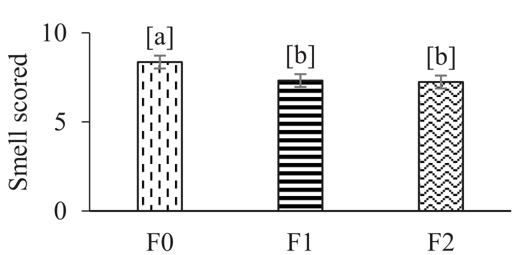


Fig. 7. The smell of salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

Based on the Kruskal-Wallis test (p<0.05), the use of soursop leaf extract significantly affected the smell of salted fish produced. The HSD test further showed that the control treatment (F0) was significantly different from the treatment using 10% (F1) and 20% (F2) soursop leaf extract. This difference was attributed to the distinctive aroma of soursop leaf extract, which contained acetogenin content that imparted an additional smell to salted fish. The results were in line with the observation of [26], that the addition of lemongrass and green tea to salted catfish products could disguise and reduce fishy smell, respectively.

3.5.3. Taste

Taste was one of the factors that determine food quality. The taste test results for salted fish samples were detailed in Fig. 8, with the hedonic quality scores ranging from 7 to 8.

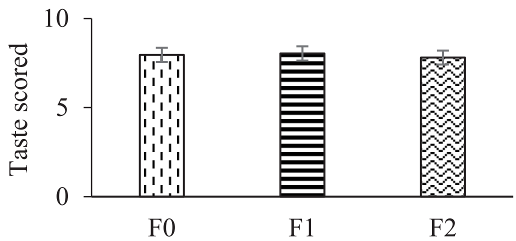


Fig. 8. Taste of salted fish (each treatment was repeated 3 times

The Kruskal-Wallis test (p<0.05) showed that spraying soursop leaf extract on salted fish did not significantly affect its taste. The results might be attributed to the specific characteristics of soursop leaf used in extract, which were not too mature. This was in line with the observation of [27], that the amount of active saponin in soursop leaf extract varied depending on the age of leaf. It was found that younger soursop leaf contained less saponin, a compound imparting an unpleasant taste.

3.5.4. Texture

Texture referred to the tactile properties of food, such as wetness, dryness, hardness, smoothness, roughness, and others. The results of the texture test for salted fish samples were presented in Fig. 9, with the hedonic quality scores ranging from 8 to 9.

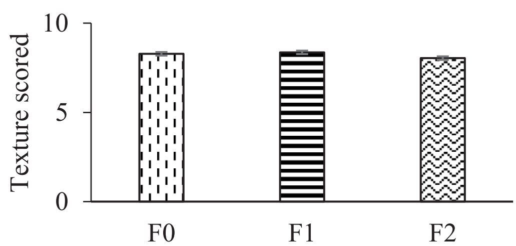


Fig. 9. The texture of salted fish (each treatment was repeated 3 times)

According to the Kruskal-Wallis test (p<0.05), the use of soursop leaf extract did not significantly affect the texture of salted fish. Salted fish was dried directly in the sun, which reduced its water content to an optimal level, resulting in a dry texture. According to [28], soaking materials in a salt solution facilitated the autolysis process in the food tissue.

Observably, proteolytic enzymes produced by bacteria played a significant role in the autolysis process.

3.5.5. Color

Color was a sensory attribute that could be observed directly by panelists. Generally, the quality of food was determined by its color. The results of the color test for salted fish samples were detailed in Fig. 10, with the hedonic quality scores ranging from 7 to 9.

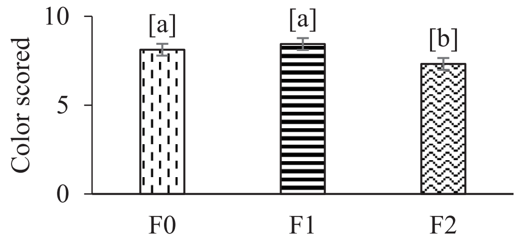


Fig. 10. Color hedonic of salted fish (each treatment was repeated 3 times; values with different superscripts are statistically significantly difference ($p < 0.05$))

Fig. 10 presented that the organoleptic test results for the color of salted fish showed the highest average value for the treatment without soursop leaf extract spraying (F0). Conversely, the lowest average value was found in the treatment with 20% soursop leaf extract (F2). The Kruskal-Wallis test ($p < 0.05$) showed that soursop leaf extract significantly affected the color of salted fish. The HSD test further showed that the color of salted fish treated with F0 and F1 was significantly different from the treatment of F2. The lower color quality in F2 was attributed to the higher concentration of soursop leaf extract, which imparted a yellowish-brown color due to pigments and phenol components in extract.

3.5.6. Molds

Mold presence could damage food ingredients, and the result of the organoleptic assessment was detailed in Fig. 11. The assessment showed that salted fish samples, whether untreated (F0), treated with 10% soursop leaf extract (F1), or 20% soursop leaf extract (F2), all scored 9, showing no mold presence.

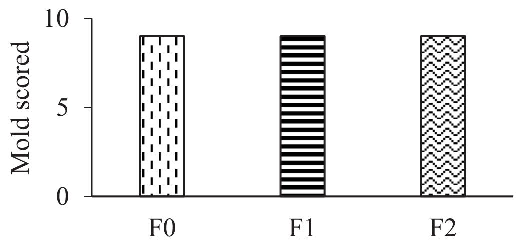


Fig. 11. Molds assessment on salted fish (each treatment was repeated 3 times)

Based on the results of the Kruskal-Wallis test ($p<0.05$), the use of soursop leaf extract did not significantly affect the presence of mold in salted fish. This was attributed to the salting process that reduced the water content in fish. The ability of salt to draw moisture from fish inhibited the growth of rot-causing microorganisms [29]. Additionally, the absence of mold was supported by immediate drying under direct sunlight, which prevented mold development. The results were in line with the observation of [30], that fresh raw materials and immediate processing contributed to the absence of mold.

4 Conclusions

In conclusion, the effectiveness of soursop leaf extract as a natural flies repellent increased with its concentration. At 10% and 20% concentrations, extract showed 62.1% and 91.4% effectiveness, respectively in repelling flies from salted fish. At a 20% concentration, soursop leaf extract effectively acted as a larvicide, killing flies larvae. Additionally, the use of extract had a significant impact on the smell and color of fish.

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