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*Scientific Paper*

# Use of fish residue as a substrate in lettuce germination

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## Abstract

The productive chain of the fish generates solid residues that are discarded in an inadequate way compromising the environment. Thus, the objective of this work was to evaluate the use of composting and silage elaborated with fish residue as substrate in the germination of lettuce. The fish residues obtained in the municipal market of the city of Bragança - PA were decomposed in composting and in the manufacture of the biological silage, being later used as substrates in the germination of lettuce cv. Monica. The seed was performed using three seeds per cell in trays of 200 cells using as substrates: S1 (control), S2 (fish composting), S3 (fish composting + fish silage), S4 (control + fish composting) e S5 (control + fish silage). The experimental design was a randomized complete block (DBC) with five treatments and four replicates of 50 seeds. During the 25 days, the germination speed index and germination rate were evaluated, and at the end of this period seedling vigor, shoot and root length, and fresh and dry shoot and root mass were evaluated. The substrate S2 (fish composting) higher germination percentage (93.2%), speed of germination (9.1), seedling vigor (91,3%), fresh shoot mass (0.43 g) and roots (0.18 g) differing significantly ($p<0.05$) from the other substrates. The addition of compost and biological silage gave better results on germination and vigor of seedlings when compared to substrate S1 (control). Thus, fish residue is a viable alternative to be used as a substrate in the germination and formation of lettuce seedlings.

**Keywords:** *Lactuca sativa L.*, fish, composting, silage.

## Utilização do resíduo de peixe como substrato na germinação de alface

## Resumo

A cadeia produtiva do pescado gera resíduos sólidos que são descartados de forma inadequada comprometendo o meio ambiente, assim, objetivou-se neste trabalho avaliar a utilização da compostagem e silagem elaborada com resíduo de peixe como substrato na germinação de alface. Os resíduos de peixe obtidos no mercado municipal do município de Bragança – PA foram decompostos em compostagem e na fabricação da silagem biológica sendo, posteriormente, utilizados como substratos na germinação de alface cv. Mônica. O semeio foi realizado utilizando três sementes por célula em bandejas de 200 células utilizando como substratos: S1 (controle), S2 (compostagem de peixe), S3 (compostagem de peixe + silagem de peixe), S4 (controle + compostagem de peixe), S5 (controle + silagem de peixe). O delineamento experimental adotado foi em blocos casualizados (DBC) com cinco tratamentos e quatro repetições de 50 sementes. Ao longo de 25 dias avaliou-se porcentagem e o índice de velocidade de germinação e, ao final deste período o vigor das plântulas, comprimento da parte aérea e da raíz e massa fresca e seca da parte aérea e raíz. O substrato S2 (compostagem de peixe) conferiu maior porcentagem de germinação (93,2%), velocidade de germinação (9,1), vigor das plântulas (91,3%), na massa fresca da parte aérea (0,43 g) e da raíz (0,18 g) diferindo significativamente ($p<0,05$) dos demais substratos. A adição da compostagem e da silagem biológica conferiu melhores resultados sobre a germinação e vigor das plântulas quando comparados ao substrato S1 (controle). Deste modo, o resíduo de peixe é uma alternativa viável para ser utilizada como substrato na germinação e na formação de mudas de alface.

**Palavras-chave**: *Lactuca sativa L.,* peixe, compostagem, silagem.

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## Uso de residuos de peces como sustratp en la germinación de lechuga


## Resumen

La cadena de producción de pescado genera residuos sólidos que se desechan de manera inadecuada, lo que compromete el medio ambiente. El objetivo de este trabajo fue evaluar el uso de compostaje y ensilado preparado con residuos de pescado como sustrato en la germinación de lechuga. Los residuos de pescado obtenidos en el mercado municipal de Bragança - PA fueron descompuestos en compostaje y fabricación de ensilajfe biológico y posteriormente se usados como sustratos para la germinación de lechuga cv. Monica. La siembra se realizó utilizando tres semillas por celda en bandejas de 200 celdas utilizando como sustratos: S1 (control), S2 (compuesto de pescado), S3 (compuesto de pescado + ensilaje de pescado), S4 (control + compuesto de pescado), S5 (control + ensilaje de pescado). El diseño experimental fue un diseño de bloques al azar con cinco tratamientos y cuatro repeticiones de 50 semillas. Durante 25 días, se evaluó el porcentaje y la velocidad de germinación y, al final de este período, el vigor de las plántulas, la longitud del brote y l raíz, y la masa fresca y seca del brote y de la raíz. El sustrato S2 (compostaje de pescado) resultó en un mayor porcentaje de germinación (93,2%), velocidad de germinación (9,1), vigor de plántulas (91,3%), masa de brotes frescos (0,43 g ) y raíz (0,18 g), que difieren significativamente (p <0,05) de otros sustratos. La adición de compostaje y ensilaje biológico resultó en mejor germinación y vigor de las plántulas en comparación con el sustrato S1 (control). Por lo tanto, el residuo de pescado es una alternativa viable para usar como sustrato para la germinación y la formaón de plántulas de lechuga.


**Palabras clave**: *Lactuca sativa* L., pescado, compostaje, ensilaje.

## Introduction

The world fishery production reached a range of around 171 million tons in the year 2016 (FAO, 2018). In this way, the fish productive chain has the potential to occupy a prominent place among the main economic activities in the world, thus generating employment and income. However, the rise of this activity as a result of increased fish consumption generates solid waste that can be disposed of inappropriately in the environment (GUIDONI et al., 2013).

Organic solid wastes are those classified as of animal or vegetable origin and in their decomposition process generate nutrients that are extremely important to the soil in a process called composting (FURTADO et al., 2016). In soil, organic waste improves water retention capacity, maintains temperature and acidity levels, hinders or prevents the germination of seeds from possible invasive plants, as well as increasing organic matter and soil biological activity, improving your fertility (OELOFSE et al., 2010; SANTOS e MACHADO, 2011).

Lettuce (*Lactuca sativa* L.) is a herbaceous plant whose germination varies from 4 to 6 days depending on the cultivar and the sowing season (FILGUEIRA, 2008). In this context, the substrate is the main factor in the germination and formation of seedlings with quality, since it plays a fundamental role in the development of the root system, serving as support and source of nutrients (MOREIRA et al., 2011).

Several researches have been developed with the application of organic residues in the germination and development of lettuce (SILVA et al., 2013; FREITAS et al., 2013; TERRA et al., 2014; SOUSA et al., 2017; BOHM et al., 2017). Thus, it was assumed that the use of fish waste in the formulation of a substrate could lead to lower cost and dependence on industrialized compounds. Thus, the objective of this work was to evaluate the fish residue as substrate in lettuce germination.

## Material and methods

### Obtaining materials

The fish residue used for composting and silage formulation was obtained in the municipal fish market, in the municipality of Bragança-PA, and was sent to the Probiotics Laboratory of UFPA - Bragança Campus. The sawdust was obtained from the city sawmills, lettuce seeds cv. Monica was acquired in the local commerce and the substrate used for the formation of seedlings (control) with local horticulturists of the municipality of Bragança – PA.

### Elaboration of composting

The preparation of composting was based

on the methodology of Pilotto (2014), being done anaerobically. Fish waste and sawdust and plastic containers with 20L lids were used. The fish residue was taken to the Probiotics Laboratory where it was ground in a grinding machine. The containers were then filled with sawdust and shredded residue so that the waste portions were arranged over the sawdust layers, respecting the distance of 10 cm between the bottom and top walls of each container. The containers were filled with 1.2 kg of sawdust and 400 g of residue. At the end, the containers were capped to avoid oxygen entry and left in this way for 90 days, mixing the sawdust and observing the physical (color and moisture) and biological (odor) characteristics every 15 days.

**Elaboration of biological silage**

The process for the preparation of biological silage from fish processing residues was based on the methodology of Machado (2010). The ingredients used for the elaboration of biological silage were: fish residue (including viscera), yogurt, sucrose, sugar cane molasses and vitamin C to avoid rancidification.

First, the residues were selected: head, viscera, pimples and fish out of the commercial standard, later this residue was submitted to cooking for 20 min at 100 ºC aiming to paralyze the enzymatic action and destroy pathogenic and putrefactive bacteria present in the raw residues. The cooking water was drained, and the residue, after cooling to milled ambient temperature and mixed with sucrose, molasses and inoculum of yogurt and vitamin C. After this process, the ready-made silage was added in a pack, and sealed, the In order to avoid the entry of light and oxygen, the compound remained in this condition for a period of 7 days for biological fermentation by lactic acid bacteria to occur.

**Analysis of germination**

The experiment was carried out in a protected area, located in the municipality of Bragança-PA (01º 03 '13' 'S, 46º 45' 56 " W and altitude: 19 m). The climate of the city is warm and temperate the winter has much more rainfall than the summer. In the city the average temperature arrives at 11.6 ºC and the average annual rainfall is of 744 mm.

For germination or initial development of seedlings of crisp lettuce cv. Monica, 5 substrates were used, where: substrate (S1) was only the control, being the standard substrate used by local horticulturists constituted by black soil, poultry manure and sawdust; S2 (fish composting), S3 (fish composting + fish silage 1:1), S4 (control + fish composting 1:1) e S5 (control + fish silage 1:1).

After homogenization of the substrates these were arranged in 200 cell polystyrene styrofoam trays of dimensions 34 x 68 x 5.5 cm in width, length and depth, respectively, moistened and allowed to stand for 24 hours until sowing. For the seeds, the purity and germination data were taken into account, and the values should be as close as possible so that the effect of the substrates did not suffer influence of these characteristics.

Seeding was carried out using about three seeds per cell, arranged about 3 cm deep. The experimental design was a randomized complete block (DBC) with five treatments and four replicates of 50 seeds.

The variables analyzed were: Percentage of germination (% G): Determined daily by direct counting of germinated seeds until the 25th day and calculated by the following formula:

$$G = (N/A).100$$

Where: G = germination; N = total number of germinated seeds; A = total number of seeds placed to germinate.

Index of germination speed (IVG): Determined by registering the number of seeds germinated daily until the 12th day and considering as emerged, the seedlings that presented the cotyledons totally free and normal. The germination speed was calculated according to Edmond e Drapala (1958) by expression:

$$VG = (N1\,G1) + (N2\,G2) + ... + (Nn\,Gn)\ (G1 + G2 + ... + Gn)$$

Where: VG = Germination speed N1 = number of days for the first count; G2 = number of germinated seeds computed in the first count; N2 = number of days for the second count; G2 = number of seeds germinated in the second count; Nn = number of days for the last count; Gn = number of seeds germinated at the last count.

Classification of seedling vigor (CVP): Determined at the end of 25 days by direct counting of normal seedlings (well developed, morphologically perfect, without lesions) being computed as strong and vigorous and of seedlings considered not normal (that is, poorly developed) (NAKAGAWA, 1994). The results were expressed as mean percentage of strong seedlings.

Root length (CR): Determined using a millimeter ruler and results expressed in centimeters (cm).

Fresh aerial and root mass (MFPA and MFR):

Determined by weighing (balança Bioprecisa®) and the results expressed in grams (g).

Dry shoot and root dry mass (MSPA e MSR): The roots and leaves of each treatment / replicate were placed in paper bags (Kraft) and kept in an oven at 60 ºC for 72 hours until complete drying. The results were expressed in grams of dry root and shoot mass.

The data were submitted to analysis of variance (ANAVA) and the means were compared by the Tukey test at the 5% probability level. The analyzes were performed by SISVAR software version 5.3 (FERREIRA, 2011).

## Results and discussion

There was a significant effect ($p < 0.05$) of the substrate on the variables: germination percentage and germination speed index (Table 1).

**Table 1.** Mean and standard deviation on germination percentage and germination speed index of lettuces cv. Monica.

| Substrates | Variables | |
|---|---|---|
| | Germination percentage (%) | Germination speed index |
| S1 | 61.8 ± 2.6 c | 4.3 ± 0.36 c |
| S2 | 93.2 ± 1.4 a | 9.1 ± 0.16 a |
| S3 | 82.5 ± 3.3 b | 6.7 ± 0.34 b |
| S4 | 76.3 ± 2.9 b | 6.3 ± 0.31 b |
| S5 | 72.9 ± 3.6 b | 5.2 ± 0.29 b |
| CV= | 3.38 | 1.87 |

Means followed by the same letter do not differ by Tukey test at the 5% probability level. S1 (control), S2 (fish composting), S3 (fish composting + fish silage), S4 (control + fish composting) e S5 (control + fish silage).

The percentage of germination (93.2%) and germination rate index (9.1) were higher in the substrate S2 (fish composting), differing significantly ($p<0.05$) from the other combinations. In general, the use of fish waste (composting and silage) favored an increase in the germination parameters, when compared to the control (Table 1).

Second Santos et al. (2010) the use of organic residues in the substrate composition contributes to the aeration, water retention capacity and the formation of a physical structure adequate to the development of radicle and epicotyl, corroborating the effects of fish silage composting observed in this study.

On the other hand, Aragão et al. (2010) did not observe effect of the amino acid of fish on the germination percentage and the rate of germination speed in seeds of watermelon. The use of agroindustrial residues as substrates undergoes low aeration space, making it necessary to mix with other materials such as coconut fiber, pine bark or vermiculite (LORIN, 2009). In this study, both preparation and mixing of compost and fish silage may have contributed to better aeration of the substrate favoring germination.

Seed vigor, shoot length and root length were significantly affected ($p<0.05$) by the substrate used (Table 2).

The seedlings were more vigorous in the substrate S2, mean of 91.3% when compared to the other substrate combinations ($p<0.05$), especially in substrate S1 (control), whose average corresponded to 66.4% of seedlings with good vigor (Table 2).

The aerial and root lengths were higher on substrates S2, S3 and S4, respectively, differing significantly ($p<0.05$) from substrates S5 and S1 (Table 2). It is observed that fish waste is an excellent source of

**Table 2.** Mean and standard deviation on seedling vigor, shoot length and root length on lettuce seedlings cv. Monica.

| Substrates | Variables | | |
|---|---|---|---|
| | Seedling vigor (%) | Shoot lenght (cm) | Root lenght (cm) |
| S1 | 66.4 ± 2.4 c | 4.42 ± 0.19 b | 3.82 ± 0.16 b |
| S2 | 91.3 ± 2.6 a | 6.81 ± 0.15 a | 5.63 ± 0.13 a |
| S3 | 81.5 ± 3.7 b | 6.56 ± 0.21 a | 5.41 ± 0.14 a |
| S4 | 83.2 ± 3.4 b | 6.44 ± 0.22 a | 5.52 ± 0.11 a |
| S5 | 69.3 ± 2.6 c | 4.67 ± 0.11 a | 4.21 ± 0.26 b |
| CV= | 2.91 | 1.05 | 3.92 |

Means followed by the same letter do not differ by Tukey test at the 5% probability level. S1 (control), S2 (fish composting), S3 (fish composting + fish silage), S4 (control + fish composting) e S5 (control + fish silage).

nutrients, especially when associated with composting.

The use of substrates based on organic residues in lettuce (SIMÕES et al., 2015; BOHM et al., 2017), cabbage and beet (COSTA et al., 2014), rucula (LUIZ et al., 2017) and coriander (CUNHA et al., 2017) also favored an increase in the aerial and root art length, reflecting the better vigor and development of the seedlings.

There was a significant effect ($p<0.05$) of the substrate on the fresh and dry mass of shoot and root (Table 3).

The substrate S2 gave a higher fresh mass (0.43 g) and dry (0.0024 g) of the aerial part differing significantly ($p<0,05$) from the other substrate combinations whose average was around 30 g for fresh mass and 0.0016 g for the dry mass.

For the fresh and dry root mass, the highest averages were verified in the seedlings of the substrates S2, S3 and S4, respectively, differing ($p<0.05$) from those from the substrates S5 and S1, respectively. Similarly, the gain in fresh and dry shoot and shoot mass in lettuce seedlings were higher when grown on substrate with alternative residues (TEIXEIRA et al., 2014; BRITO et al., 2017; FONTANA et al., 2018).

**Table 3.** Average and standard deviation on shoot and root fresh mass and shoot dry matter on lettuce seedlings cv. Monica.

| Substrates | Variables | | | |
|---|---|---|---|---|
| | Fresh shoot mass (g) | Fresh shoot roots (g) | Mass aerial part (g) | Root dry mass (g) |
| S1 | 0.26 ± 0.008 c | 0.10 ± 0.06 b | 0.0018 ± 0.0021 c | 0.0013 ± 0.0016 b |
| S2 | 0.43 ± 0.011 a | 0.18 ±0.053 a | 0.0036 ± 0.0001 a | 0.0024 ± 0.0003 a |
| S3 | 0.31 ± 0.017 b | 0.15 ±0.006 a | 0.0025 ± 0.0015 b | 0.0021 ± 0.0004 a |
| S4 | 0.33 ± 0.009 b | 0.16 ± 0.054 a | 0.0027 ± 0.0012 b | 0.0022 ± 0.0005 a |
| S5 | 0.28 ± 0.006 c | 0.11 ± 0.021 b | 0.0019 ± 0.0017 c | 0.0014 ± 0.0012 b |
| CV= | 2.21 | 0.93 | 0.29 | 1.16 |

Means followed by the same letter do not differ by Tukey test at the 5% probability level. S1 (control), S2 (fish composting), S3 (fish composting + fish silage), S4 (control + fish composting) e S5 (control + fish silage).

The increase in these characteristics is of fundamental importance as they reflect the gain of water and nutrients by the seedlings reflecting in the vigor, thus, the substrate elaborated with fish residue is an excellent alternative, since it favored increase in the fresh mass of the aerial part and root, especially.

## Conclusion

Composting with fish residue is an excellent alternative for substrate use in germination and vigor of lettuce seedlings.

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