

# Rice bran as non-chemical soil fumigant for organic strawberry cultivation

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## Abstract

*The interest in eco-friendly alternatives to suppress soilborne pathogens is currently growing, especially in strawberry crop. Within this framework, a trial was carried out in the strawberry specialized valley of South Tyrol, the Martell Valley (1,312 m a.s.l.).*

*This experiment was conducted in a field where strawberry has been cultivated for many years consecutively and the symptoms of soilborne diseases were clearly visible in the year before beginning the test. Four weeks before transplanting the plants, rows were randomly assigned to each of the following treatments: untreated control, Dazomet (Basamid®) as positive control and ASD (anaerobic soil disinfestation) with rice bran (RB). Vegetative biomass, yield and plant mortality were evaluated during the first and second cropping years (2022 and 2023).*

*Promising results came from ASD-RB which had similar effects to the chemical fumigant, in terms of reducing plant mortality during the double cropping cycle (-12% as compared to untreated plants). In addition, RB treatment significantly increased the plant biomass (+65%), probably due to induced changes in the soil microbiome and soil nutrient dynamics (e.g., leaf nutrient content was significantly increased in plants subjected to treatment with ASD-RB). Nevertheless, the plant yield did not vary significantly among treatments.*

*Our data suggest that strawberry cultivation in Martell Valley needs strategies for maintaining and improving soil health. The ASD method offers a worthwhile solution to counteract soilborne diseases, even under organic management conditions.*

**Keywords:** *Fragaria × ananassa*, soilborne diseases, nonchemical alternative, organic amendment, plant growth.

## Introduction

Repeated cultivation cycles over several years on the same field lead to soilborne disease complexes, considered one of the main limitations of strawberry crop cultivated in soil condition (Harris, 1990). The loss of fertility resulting from a decrease in organic matter content, the increased activity of telluric pathogens (fungi, bacteria, nematodes and viruses), the presence of toxic substances and nutritional imbalances are common triggers of crop decline phenomena, resulting in compromised regular growth of the plant root systems (Sneh et al., 1983; Benlioğlu et al., 2004; Abd-Elgawad, 2019). Non-cultivation of strawberry crop in succession to itself is advisable and therefore a good practice in soil health management would be to leave to fallow (Katan, 2000). Alternatively, chemical means could be used (fumigants with high efficacy but with a large environmental impact), or physical (e.g., solarization), or agronomic (e.g., crop rotation with crops of the *Brassicaceae* family) and biological solutions (Katan, 2000; Clarkson et al., 2015; Mihajlovic et al., 2017; Panth et al., 2020).

Anaerobic soil disinfestation (ASD), developed in the Netherlands and Japan, is considered an attractive biological alternative to chemical fumigants for the management of soilborne

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diseases (Blok et al., 2000; Muramoto et al., 2014; Muramoto et al., 2016). Three fundamental steps must be carried out: -add an organic carbon source to the soil, -irrigate to field capacity, -cover with impermeable film. The success of the technique consists in generating anaerobic soil conditions, i.e. the achievement of oxygen concentration around 1%, the soil redox potential between -300 and 200 mV and soil temperature of 30°C for at least 300 h (Strauss and Kluepfel, 2015; Song et al., 2023; Hernández-Muñiz et al., 2023). According to several studies conducted on herbaceous and woody crops, the ASD method showed to efficacy suppress the soilborne disease symptoms (Muramoto et al., 2016; Browne et al., 2018; Song et al., 2023).

The scientific literature on rice bran applied as ASD treatment is relatively well provided. However, our present study aimed to evaluate the effects of rice bran in comparison to a chemical fumigant in a unique alpine mountain environment.

## Material and Methods

### I. Field Management and Experimental Design

The experiment was conducted over two growing seasons (years 2022 and 2023) in a commercial strawberry field located in the municipality of Martell (46°33'30.618" N; 10°46'53.649" E; 1.312 m a.s.l.) in South Tyrol, Italy. The field has been cultivated with strawberry for many years consecutively, and the symptoms of soilborne diseases were clearly visible in the year before beginning the test. In addition, microbiological analyses were also conducted to confirm those stress conditions (as reported in Soppelsa et al., 2021). The treatments tested in this study included a chemically sanitized soil product based on Dazomet as positive control (99%, Basamid® Granulat, Certis Europe, Saronno, Italy; at an application dosage of 255 kg ha<sup>-1</sup> of effective treated surface). An organic amendment based on rice bran (RB) (Table 1), a by-product of rice production during milling, was tested as soil fumigant product. As reported in previous studies, the RB application dosage considered in our experiment was 20 t ha<sup>-1</sup>. Actually, our treated area referred only to the raised beds, which corresponds to 36.4% of one hectare and thus we used about 7.3 t ha<sup>-1</sup>. As is well known, Italy is an important producer of rice and therefore this by-product can easily be sourced locally. RB was purchased from Riseria, a mill in the Verona area (Isola della Scala, Verona, Italy).

Table 1: Physicochemical amendment properties.

Carbon source	pH (in CaCl <sub>2</sub> )	Organic matter (%)	Humidity (%)	C:N ratio	Total N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Rice Bran (RB)	6.1	85.3	7.5	21	2.4	3.6	1.8	<0.1	1.2

An untreated variant was also included. Field preparation took place in Juni-July 2021 with tillage followed by the formation of raised beds. A furrow over each raised bed was made and Dazomet or RB were incorporated at a depth of 20-30 cm. Following, furrows were closed and raised beds were covered with black plastic mulch film. During a 4-week period, the ASD technique was implemented. The soil temperature was measured with sensors and reached maximum values of 26°C (similar values among treated and untreated parcels).

One month after soil treatments, fresh plug plants (cv. Elsanta) from the Società Agricola Salvi Vivai s.s. (Ferrara, Italy) were transplanted in a double row, with 15 cm intra-row and intra-plant spacing. As the cultivar Elsanta is highly susceptible to soilborne pathogens, it was chosen in this experiment.

Plants were managed in the same way in terms of watering, fertilization, and pest control.

The experiment setup was organized as a randomized block design with 3 replicates composed of 320 plants per experimental unit (approximately 1,000 plants per treatment).

## II. Evaluated Parameters

Plant mortality was monitored at the beginning of June each year by counting the number of dead plants in the entire row of each parcel. Plant biomass (as g FW plant<sup>-1</sup>) was measured at the end of first and second production cycle on four plants per replicate. Nutrient leaf content (as % DW) were analysed with microwave-assisted acid digestion using the ICP-OES.

Ripe strawberry fruits (uniformly red) were harvested every four days during the month of July. From each experimental unit and at each picking time, the commercial production (healthy fruit with a diameter  $\geq 22$  mm; expressed as g plant<sup>-1</sup>) and the waste, represented by small fruit, deformed and with the presence of rot, were weighed with a digital scale (Valor™ 2000, OHAUS Europe GmbH, Nänikon, Switzerland).

## III. Statistical Analysis

Data normality was examined with the Shapiro–Wilk test, and homogeneity of variance was confirmed using Flinger–Killeen’s test. A two-way ANOVA was performed on data collected from both years and mean separation of the dependent variables obtained with the LSD Fisher’s test ( $p < 0.05$ ). All analyses were carried out in R v. 3.3.1. (R Development Core Team 2022). Values were expressed as mean  $\pm$  standard deviation (SD).

## Results

Compared with the Control, the ASD-RB and the chemical fumigant significantly helped the survival of the plants by 10% in growing year 2022 and without significant differences in 2023 (Figure 1). If we consider the total percentage of strawberry plant mortality at the end of the two growing years, a reduction of 12% was achieved in the rows subjected to anaerobic conditions induced by treatment with rice bran (Figure 1).

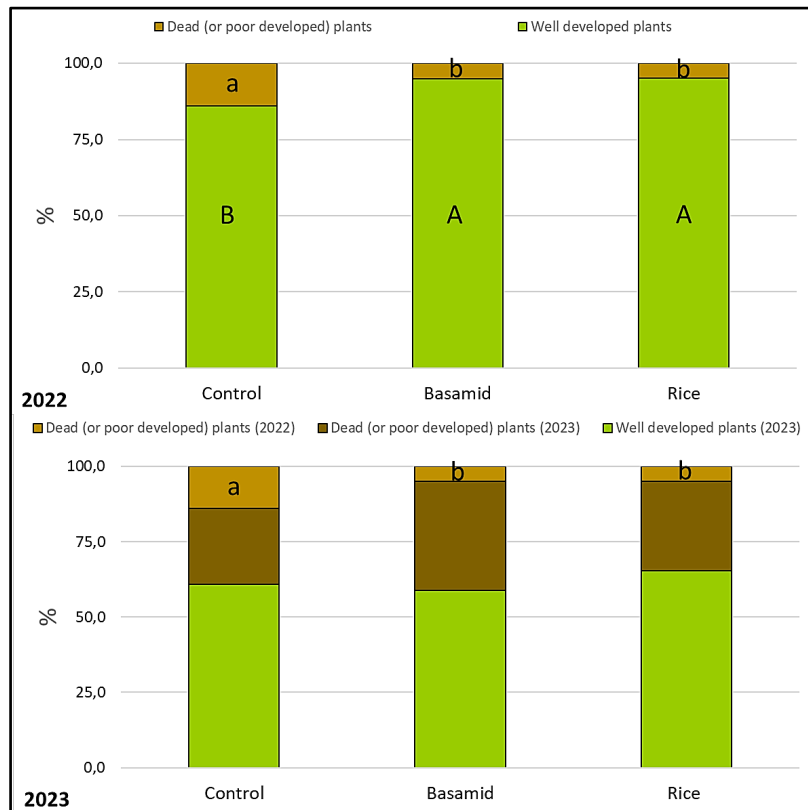


Figure 1: Percentage of well-developed or dead plants as affected by treatments and growth season. Means  $\pm$  SD ( $n = 3$ ). Different upper- and lowercase letters indicate significant differences across treatments for years 2022 and 2023, according to LSD Fisher's test;  $p < 0.05$ .

In addition, we observed an improvement of individual plant biomass (especially related to the aerial part) by pre-treating soil against soilborne diseases (Table 2). RB treatment showed plants with significantly increased plant biomass (around +65%) compared to untreated plants. The chemical fumigant (Basamid) increased plant biomass, but not differently than the Control.

Table 2: Total biomass (fresh weight – FW) composed of aboveground and root biomass, as affected by treatments and growth season.

		Plant biomass (g FW plant <sup>-1</sup> )			
<b>Treatment (T)</b>	Control	115.58	$\pm$	34.78 <sup>1</sup>	b
	Basamid	148.50	$\pm$	34.66	ab
	Rice Bran (RB)	191.67	$\pm$	33.88	a
<i>Significance</i>				**	
<b>Year (Y)</b>	2022	139.83	$\pm$	38.65	
	2023	164.00	$\pm$	50.85	
	<i>Significance</i>				<b>ns</b>
<b>T x Y</b>					<b>ns</b>

<sup>1</sup>: Means  $\pm$  S.D. ( $n = 3$ ) followed by the same letter do not significantly differ according to LSD Fisher's test;  $p < 0.05$ . Two-way ANOVA significant differences: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; ns: not significant

Leaf analysis can be considered a highly effective approach for monitoring the nutritional status of crop cultures and thus identifying potential stress situations. Concerning the macro-nutrient leaf content analysed, phosphorus and magnesium were found to be significantly higher in leaves treated with RB as compared to Control (+40% and +20%, respectively) (Table 3).

Table 3: Macro-nutrient content of strawberry leaves as affected by treatments and growth season.

		P (% DW)				Mg (% DW)			
<b>Treatment (T)</b>									
	Control	0.24	±	0.03 <sup>1</sup>	b	0.41	±	0.02	b
	Basamid	0.23	±	0.03	b	0.41	±	0.03	b
	Rice Bran (RB)	0.32	±	0.04	a	0.48	±	0.03	a
<i>Significance</i>				<b>***</b>				<b>***</b>	
<b>Year (Y)</b>									
	2022	0.28	±	0.05		0.45	±	0.04	
	2023	0.24	±	0.05		0.41	±	0.04	
<i>Significance</i>				<b>ns</b>				<b>ns</b>	
<b>T x Y</b>				<b>ns</b>				<b>ns</b>	

<sup>1</sup>: Means ± S.D. ( $n = 3$ ) followed by the same letter do not significantly differ according to LSD Fisher's test;  $p < 0.05$ . Two-way ANOVA significant differences: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; ns: not significant

Although the vegetative parameters were increased as a result of the treatments, they had no effect on plant yield in both years (Table 4). Treated plants showed only a tendency to increase marketable and total yield.

Table 4: Yield (g per plant), as affected by treatments and growth season.

		Marketable yield (g plant <sup>-1</sup> )				Total yield (g plant <sup>-1</sup> )			
<b>Treatment (T)</b>									
	Control	135.68	±	21.29 <sup>1</sup>		177.06	±	28.80	
	Basamid	136.10	±	32.41		180.87	±	34.00	
	Rice Bran (RB)	144.65	±	37.26		183.62	±	35.05	
<i>Significance</i>				<b>ns</b>				<b>ns</b>	
<b>Year (Y)</b>									
	2022	152.30	±	6.95		194.72	±	11.29	
	2023	125.32	±	37.26		166.32	±	37.99	
<i>Significance</i>				<b>ns</b>				<b>ns</b>	
<b>T x Y</b>				<b>ns</b>				<b>ns</b>	

<sup>1</sup>: Means ± S.D. ( $n = 3$ ) followed by the same letter do not significantly differ according to LSD Fisher's test;  $p < 0.05$ . Two-way ANOVA significant differences: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; ns: not significant

## Discussion

Soilborne diseases are considered some important limitation factors in the cultivation of many crops, especially those belonging to certain botanical families such as *Solanaceae* (tomato), *Cucurbitaceae* (cucumber), *Liliaceae* (garlic), *Asteraceae* (lettuce), *Apiaceae* (carrot) and *Rosaceae* (strawberry) (Koike et al., 2003; Cesarano et al., 2017).

Although the most efficient techniques to suppress soilborne plant pathogens are: - to fumigate the soil with chemicals (highly harmful to the environment), - to plant in soilless culture (not allowed in organic farming), - to let a field lie fallow (economic loss), there are some eco-friendly techniques that have been recently gaining increasing interest (López-Aranda et al., 2016; Panth et al., 2020; Braun and Supkoff,). These techniques include: - biological control with organisms; - resistant varieties and grafting; - cropping system such

as mixed cropping, intercropping and crop rotation; - biofumigants (e.g., isothiocyanates – ITC) released by *Brassica* crops; and - anaerobic soil disinfestation – ASD (Bonanomi et al., 2007; Panth et al., 2020; Chadfield et al., 2022).

More in detail, the ASD has been proposed as a non-fumigant alternative against pathogens in several crops, especially for strawberry crop (Muramoto et al., 2016). Existing literature has assessed different residual wastes (e.g., rice bran, sugarcane molasses, fishmeal, soybean flour, mustard seed meal) as amendments for ASD (Hernández-Muñiz et al., 2023). ASD-treated soil with rice bran (application dosage 20 t ha<sup>-1</sup>) was observed to generate the appropriate anaerobic conditions to suppress pathogens. The reduction in *Verticillium dahliae* microsclerotia was not significantly different to treatment with chloropicrin fumigant (Shennan et al., 2018). According to study conducted by Hernández-Muñiz et al. (2023), the ASD-treated soil with rice bran was able to significantly reduce the disease severity induced by *Fusarium oxysporum* f. sp. *fragariae*. Similarly, Muramoto et al. (2016) and Márquez-Caro et al. (2022) observed a reduction in plant mortality by *Macrophomina phaseolina* (charcoal rot) after treating with ASD-RB.

Our study confirmed the increased survival and development of the ASD-treated strawberry plants, probably due to induced changes in the soil properties and microbiome (Figure 1 and Table 2, Table 3). ASD induced modification on soil pH, EC and nutrient dynamics. The anaerobic conditions in the soil created by the decomposition process of C-source amendments allow to accumulate organic acids (e.g., acetic acid, butyric acid) with consequent soil pH reduction (Muramoto et al., 2016). Though some studies showed an increase in soil pH (Song et al., 2023; Hernández-Muñiz et al., 2023). Carbon input used for ASD treatment leads to increase the organic matter, soil nutrient content and plant nutritional status (Butler et al., 2014; Muramoto et al., 2016; Shrestha et al., 2021). Indeed, leaf nutrient content (P and Mg) was significantly increased in our plants subjected to ASD-RB.

The reduced plant mortality resulted in a benefit in terms of marketable yield and net returns (Song et al., 2023; Hernández-Muñiz et al., 2023). However, no improvement in yield emerged in our experiment (Table 4).

In summary, our results suggest that ASD-treated soil with rice bran as a C-source amendment can be a promising solution to counteract the soilborne diseases with outcomes similar to those of a chemical fumigant, thus offering an interesting opportunity for organic growers.

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