

AgroSym

BOOK OF PROCEEDINGS



IX International Scientific Agriculture Symposium
"Agrosym 2018"
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PREFACE

A Word from the Editor-in-Chief

Dear colleagues,

In your hands are the Proceedings of the 9th International Scientific Agricultural Symposium “AGROSYM 2018” held on 4-7 October 2018 in Jahorina, Bosnia and Herzegovina. The Symposium gathers about 1200 participants from 85 different countries and organizers received over 1200 abstracts/full papers. Symposium themes covered all branches of agriculture and were divided into seven sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry 6) Forestry and Agro-forestry, and 7) Rural Development and Agro-economy.

In the plenary lectures was presented the importance of new information and communication technologies for agriculture in the 21st century and biological protection in plant production. Furthermore, a particular attention was devoted to avoiding knowledge waste through networking and partnership.

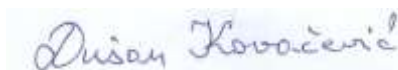
Agriculture has a complex relationship with natural resources and the environment, thus attributing specific environmental effects to agriculture is difficult and not fully understood. Today, it is obvious that conventional methods of agricultural production, in addition to providing sufficient food and other products, have led to a number of negative impacts, including direct or indirect effects on human health. Excessive use of agrochemicals can cause various disorders in the biological equilibrium of agroecosystems and beyond. These negative impacts raise serious questions about long-term sustainability of high-input agriculture. Measures to protect soil and water in agriculture include comprehensive and complex undertakings and pre-planned measures. These problems are a constant reason for ‘popularisation’ of all ecological trends in agriculture (e.g. organic agriculture, permaculture, biodynamic agriculture, conservation agriculture, regenerative agriculture, integrated farming, agroecology, etc.). Meanwhile, there are also calls for a genuine, deep transformation of agro-food systems that goes beyond ‘ecologisation’ of agricultural production. All these developments in agricultural research field, as well their implications on farmers’ fields, were discussed during the 4 days of AGROSYM 2018.

All papers included in the Proceedings were peer-reviewed. Full texts of the accepted contributions are available in electronic form on AGROSYM website (<http://agrosym.unssa.rs.ba>).

I hope that the Proceedings will be useful to many agriculturalists and to those engaged in related fields and enable better collaboration of scientists, researchers and producers.

Many thanks to all the authors, reviewers, session moderators and colleagues for their help in editing the Proceedings “AGROSYM 2018”. Special thanks go to all co-organizers for their unselfish collaboration and comprehensive support.

East Sarajevo, 07th October 2018



Prof. Dušan Kovačević, Editor-in-Chief

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MORPHOLOGICAL DIFFERENCES AMONG STRAINS OF OYSTER MUSHROOM GROWN ON DIFFERENT SUBSTRATES

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Abstract

Distribution of oyster mushroom production worldwide is based on its cultivation on available and low-priced substrates prepared using residues of cellulose materials from agricultural production. It is also based on its nutritive value and content of medically active matters that favourably affect the human organism, as well as the specific taste, which offers wide possibilities in cooking. Forms of oyster mushroom differ by colour, size and shape of the cap, length and width of stems, taste, as well as the physiological requirements during cultivation on different substrates in various climate conditions. Three isolated and determined strains of oyster mushroom (*Pleurotus ostreatus*) were studied - NS 77, NS 355 and NS 244. They were grown on residues of agricultural production most frequent on the territory of Vojvodina, such as wheat and soybean stems, and stalks of maize and sunflower, as individual substrates and in combinations with wheat straw. Variability was recorded among strains in most important morphological traits. The study monitored the ratios of cap weight (CW) and stem weight (SW), cap length (CL), cap width (CW_i), stem length (SL) and stem width (SW_i), as well as the number of mushrooms per bunch (NM). The monitored morphological traits have been expressed differently most of the time, depending on the strain, while in some cases the substrate was the determining factor. The number of fruiting bodies in strain NS 244 therefore varied in range 4.2-5.5, depending on the substrate. Strain NS 77 ranged from 7.4 to 11.2, while strain NS 355 ranged from 8.6 to 10.7. However, the highest values of cap weight of all three strains were recorded on maize stalk substrate.

Key words: *oyster mushroom, morphological traits, different substrates.*

Introduction

Oyster mushroom is a cosmopolitan plant which grows on all continents. It has long been adapted to different growing environments (Chang and Miles, 2004), and it can be cultivated on any surface which contains residues of cellulose materials from agricultural production - cellulose, hemicellulose, and nitrogen (Diana et al. 2012). It appears in different forms, varied in colour, size, shape and taste. Material included in the substrates prepared for oyster mushroom cultivation depends on the growing area (Bugarski *et al.*, 2012). Agricultural waste area, as it is physically more accessible and therefore more cost-effective, is usually the substrate chosen for mushroom cultivation. For the purpose of increased yields and quality, mushroom cultivation substrates have lately been enriched with different supplements. In Spain, Pardo-Giménez et al. (2015) enriched wheat straw with 5, 10 and 15% degreased pistachios. In China, Yang et al. (2013) added cotton seed hulls and bran to wheat straw and rice straw. Mushrooms grown on substrates with a proportional content of cotton seed hulls developed larger caps, with greater cap length and shorter stems. As the quantity of cotton seed hulls in the substrates decreased 40-30-20, cap diameter decreased proportionally, ranging from 9.4 cm, 8.8 cm, to 7.8 cm. Cap weight also decreased from 22.93 g, 19.19 g, to 17.77 g, while stem length increased from 2.6 cm, 1.9 cm, to 3.2 cm.

In Taiwan, Hoa et al. (2015) showed significant differences between the morphological parameters of different species of *P. ostreatus* and *P. cystidiosus* grown on various substrates

(combination of sawdust, sugarcane and corncob). The highest cap length in both mushrooms was obtained if they were grown on corncob 100%, and lowest on sawdust 100%. Besides the effect of the substrate, type of mushroom also has a significant role; the number of obtained fruiting bodies in *P. cystidiosus* therefore ranged from 2.32 on sawdust 100% to 1.85 on substrate formula 50% sawdust +50% sugarcane bagasse, while it ranged from 10.32 on sawdust 100%, to 7.93 on corncob 100% in *P. ostreatus*. Stem length and stem width of oyster mushroom *P. ostreatus* (35.28 -39.21 mm) were lower on all substrates compared to *P. cystidiosus* (46.06 -57.84 mm).

In India, Mondal et al. (2013) enriched rice straw with different quantities of banana leaves, and the number of obtained fruiting bodies varied from 8.5 on banana leaves to 37.25 on sawdust. Stem length of oyster mushrooms grown on substrate formula banana leaves + rice straw 1:1 (2.47 cm) was significantly reduced compared to those grown on sawdust (3.80 cm), while cap diameter was significantly smaller on substrates banana leaves + rice straw 1:3 (4.13 cm), banana leaves + rice straw 3:1 (3.70 cm), and banana leaves + rice straw 1:3 (3.11 cm) compared to sawdust (7.79 cm). Contrary to the above, cap thickness was lower on sawdust in 2. (0.49 cm), and larger on banana leaves + rice straw mixture 3:1 (0.66 cm) and rice straw (0.64 cm).

Kitamoto et al (1995) explained the emergence of more fruiting bodies on some substrates by the higher content of carbohydrates (glucose, fructose and trehalose) in the substrate, while Shah et al. (2004) explained variations in cap weight by the relatively better availability of nitrogen, carbon and minerals from the substrates.

In Vojvodina, wheat straw is mainly used as the substrate for oyster mushroom growing. Wheat straw can be baled automatically after combining and thus made accessible for removal from the growing plots and transport. Since it is used in livestock breeding, it can be obtained throughout the year, so planning its purchase in advance is not necessary. Although soybean straw, corn stalks and sunflower stalks have to be collected and stored, previous studies have shown that they are better substrates than pure wheat straw. We have therefore focused our further studies on examining their effect on mushroom quality.

Material and Methods

Determination of morphological traits of oyster mushroom (*Pleurotus ostreatus*), produced in different substrates during three-year long studies, was conducted under the auspices of Institute of Field and Vegetable Crops Novi Sad, using the strains of *Pleurotus ostreatus* NS 77, *Pleurotus ostreatus* NS 355 and *Pleurotus ostreatus* NS 244 maintained in the collection of the Vegetable Crops Department (Bugarski, et al., 2012).

The substrates were prepared individually or with wheat straw (the substrate most often used in oyster mushroom cultivation in Serbia), in the following combinations: 1. wheat straw (S1), 2. wheat straw 50% + soybean straw 50% (S2), 3. wheat straw 50% + corn stalks 50% (S3), 4. wheat straw 50% + sunflower stalks 50% (S4), 5. soybean straw (S5), 6. corn stalks (S6), and 7. sunflower stalks (S7).

In order to obtain morphological traits of strains from each individual sample, three first bunches were taken and total fruiting bodies were counted and weighed individually. Cap weight and stem weight was measured, and cap-stalk ratio was determined. Cap diameters were measured using nonius (vernier) lines from stem root to tip and cross the widest part of the stem, at an angle of 90° to the previous diameter. Stem diameter was measured in the central part, while stem length was measured from the root, along the cap to the point of separation from the substrate.

For the analysis of morphological traits of *P. ostreatus*, principal components (PCA) were visualized so as to reduce the multi-dimensional nature of experimental data. Principal components are orthogonal and synthetic variables obtained as a linear combination of the

optimally-weighted observed variables, whose significance is defined by the regression coefficient (or weight) for observed variable as used in creating a principal component. The total number of components equals the total number of original variables. Since most of the obtained principal components are weighted with non-systematic components or noise to graphically display the results of PCA method, the first two artificial components are used to compute the scores on the principal component. Interpretation of a graphical display of data follows the geometry of a biplot (Kroonenberg, 1995).

Analysis and visualization of data was carried out in the R environment for statistical computing (R Core Team, 2015).

Results and Discussion

In strain NS 77, cap weight (CW) was uniform across all substrates, ranging from 10.08 to 11.24 g (Table 1.), while strain NS 355 on substrate Corn (S6) weighed 17.96 g, which was significantly higher than cap weight (CW) 14.90 g found on substrate wheat+soybean (S2). In strain NS 244, cap weight (CW) 26.17 g on substrate Corn (S6) was significantly higher compared to other substrates. Cap weight (CW) variations across substrates were the highest in strain NS 244 21.32%, compared to strain NS 355 17.03%, and strain NS 77 weighing only 10.32%. Cap weight (CW) was lowest in strain NS 77, and highest in strain NS 244, i.e. double the weight of strain NS 77.

Cap weight (CW) in all three NS strains was significantly greater than cap weight obtained by Mondal et al. (2010) for oyster mushrooms grown on six different substrates, ranging from 1.77 g on sawdust to 6.41 g on rice straw, and equal to cap weight obtained by Yang et al., (2013) in their analysis of oyster mushroom growth on nine substrates (16.66-25.13 g).

Table 1. Cap weight (CW) and stem weight (SW) in NS strains of *P. ostreatus*

| Substrate | Cap weight (CW) (g) | | | Stem weight (SW) (g) | | |
|-----------------------|---------------------|---------|---------|----------------------|--------|--------|
| | NS 77 | NS 355 | NS 244 | NS 77 | NS 355 | NS 244 |
| Wheat (S1) | 10.82a | 15.38ab | 21.44ab | 3.53ab | 4.80ab | 5.43ab |
| Wheat+ soybean (S2) | 11.24a | 14.90a | 21.65a | 4.47ab | 4.47a | 6.24a |
| Wheat +corn (S3) | 11.02a | 16.18ab | 20.75a | 3.33b | 5.35ab | 6.44a |
| Wheat +sunflower (S4) | 10.29a | 16.61ab | 20.83ab | 4.19ab | 4.71ab | 3.67c |
| Soybean (S5) | 10.76a | 15.74ab | 25.16bc | 4.52a | 4.37a | 5.86a |
| Corn (S6) | 11.13a | 17.96b | 26.17c | 3.79ab | 5.80b | 5.79a |
| Sunflower (S7) | 10.08a | 16.58ab | 20.59a | 3.85ab | 5.46ab | 4.00cd |

Note: a, b, c, d, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Stem weight (SW) in strain NS 77 on the substrate soybean (S5) was significantly greater than stem weight on the substrate wheat+corn (S3) (Table 1). Stem weight (SW) of strain NS 355 on substrate Corn (S6) was significantly greater than stem weight on the substrate soybean (S5) and wheat+soybean (S2), while significantly lower in strain NS 244 on wheat+sunflower (S4) and sunflower (S7), compared to other substrates. Stem weight variations according to substrates were the highest in strain NS 244 43.01%, compared to only 24.65% in strain NS 355, and 26.33% in strain NS 77. Stem weight (SW) was the lowest in strain NS 77, while the highest in strain NS 244.

Cap length (CL) (Table 2), in strain NS 77 was significantly lower on wheat+sunflower mixture (S4) compared to soybean (S5) and wheat (S1). In strain NS 355, it was significantly lower on wheat (S1) compared to wheat+corn (S3), whereas in strain NS 244 it was significantly higher on wheat+soybean (S2) compared to soybean (S5) and corn (S6). The

observed cap length (CL) variations, depending on the substrate, were the highest in strain NS 244 7.28%, compared to 7.12% in strain NS 355, and 6.27% in strain NS 77.

Depending on the substrate, cap width (CWi) (Table 2.) in strains NS 77 and NS 244 was uniform, but significantly lower in strain NS 355 on substrate soybean (S5) compared wheat+corn (S3). Cap width (CWi) variations were insignificant across substrates; the highest CWi was observed in strains NS 355, NS 244, and NS 77: 7.79%, 5.45%, and 4.07%, respectively.

Cap length (CL) and cap width (CWi) determined the shape of the cap, which was uniform across all the substrates, but varied depending on the strain; the cap of strain NS 355, especially strain NS 77 was almost round shaped, but an elongated cap was observed in strain NS 244 .

Table 2. Cap length (CL) and cap width (CWi) in NS strains of *P. ostreatus*

| Substrate | Cap length (CL) (mm) | | | Cap width (CWi) (mm) | | |
|-----------------------|----------------------|---------|---------|----------------------|---------|--------|
| | NS 77 | NS 355 | NS 244 | NS 77 | NS 355 | NS 244 |
| Wheat (S1) | 65.83a | 77.03a | 93.01ab | 59.85a | 70.05ab | 70.15a |
| Wheat+ soybean (S2) | 64.99ab | 78.01ab | 99.05b | 58.95a | 69.56ab | 73.35a |
| Wheat +corn (S3) | 62.55ab | 83.79b | 97.33ab | 60.41a | 74.03b | 74.29a |
| Wheat +sunflower (S4) | 61.70b | 78.75ab | 97.26ab | 59.05a | 71.27ab | 74.02a |
| Soybean (S5) | 66.17a | 79.46ab | 91.93a | 59.46a | 68.26a | 70.57a |
| Corn (S6) | 65.25ab | 79.98ab | 91.84a | 59.80a | 71.25ab | 70.82a |
| Sunflower (S7) | 63.65ab | 78.53ab | 93.06ab | 57.95a | 69.69ab | 71.05a |

Note: a, b, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Mondal et al. (2010) obtained the results of cap diameters, ranging from 41.3 mm on banana leaves+rice straw (1:1) to 77.9 mm on sawdust, while Tupatkar & Jadhao (2006) obtained cap diameters of 28.38 cm² (equal to diameter of 60 mm) on wheat+soybean, 27.80 cm² on soybean straw, 27.05 cm² on corn stalk and leaves, and 24.50 cm² on wheat straw, which is at the threshold of obtained NS values. Cap diameters of 63 mm on wheat straw 80%+wheat bran 20% and 104 mm on cotton seed hulls 80%+ wheat bran 20%, obtained by Yang et al. (2013), were the closest to CL and CWi values of NS strains.

Table 3. Stem length (SL) and stem width (SWi) in NS strains of *P. ostreatus*

| Substrate | Stem length (SL) (mm) | | | Stem width (SWi) (mm) | | |
|-----------------------|-----------------------|---------|---------|-----------------------|--------|---------|
| | NS 77 | NS 355 | NS 244 | NS 77 | NS 355 | NS 244 |
| Wheat (S1) | 52.18a | 38.72ab | 30.76ab | 11.34abc | 12.23a | 18.00a |
| Wheat+ soybean (S2) | 54.32a | 38.99ab | 30.32ab | 12.25ab | 12.07a | 21.55b |
| Wheat +corn (S3) | 49.98a | 41.88ab | 34.82b | 9.82c | 12.73a | 20.35ab |
| Wheat +sunflower (S4) | 54.73a | 37.98b | 46.01c | 13.95a | 12.62a | 46.99c |
| Soybean (S5) | 54.53a | 40.38ab | 31.56ab | 12.22ab | 13.38a | 20.54ab |
| Corn (S6) | 56.38a | 44.71a | 33.52ab | 10.59ac | 12.68a | 19.06ab |
| Sunflower (S7) | 52.97a | 40.28ab | 27.91a | 11.70abc | 14.17a | 18.40ab |

Note: a, b, c, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

Stem length (SL) (Table 3) in strain NS 77 was uniform on all substrates. It was significantly lower in strain NS 355 on wheat+sunflower (S4) compared to corn (S6), and significantly lower in strain NS 244 on sunflower (S7) compared to wheat+sunflower (S4), although higher in wheat+sunflower (S4) compared to other substrates. Stem length (SL) in strains NS 355

and NS 244 equaled the range of SL from 35.20 mm on substrate 50% sawdust+50% corn straw to 39.21mm on substrate 80% sawdust+20% sugarcane, as obtained by Hoa et al. (2015), while it was significantly higher in strain NS 77. Mondal et al. (2010) reported lower SL values than the values found in NS strains, ranging from 24.7 mm on banana leaves+rice straw mixture (1:1) to 38.0 mm on sawdust. Tupatkar & Jadhao (2006) obtained the SL values of 35.6 mm on wheat+soybean, 32.9 mm on soybean straw, 25.4 mm on corn stalk and leaves, and 22.6 mm on wheat straw. Jonathan et al. (2013) reported SL values within the range of 53-71 mm on sawdust, the closest to the SL value of strain NS 355.

Stem width (SWi) (Table 3) was uniform on all substrates for strain NS 355 (12.07-14.17 mm). Strain NS 77 had significantly lower SWi on wheat+corn mixture (S3) compared to wheat+sunflower (S4), wheat+soybean (S2) and soybean (S5), while significantly higher values were observed on wheat+sunflower mixture (S4) compared to wheat+corn (S3). The obtained SWi values were close to SWi of 8.52 mm on sawdust and 11.06 mm on corn straw, as reported by Hoa et al. (2015). The value of SWi in strain NS 244 was significantly lower on wheat (S1) compared to wheat+soybean (S2) and wheat+sunflower (S4), but significantly higher on wheat+sunflower (S4) compared to other substrates.

The highest SL with the variable SWi was obtained in strain NS 77, where SL was 5.3 times higher than SWi (56.38 x 10.59 mm) on substrate corn (S6), and 3.9 times higher than SWi (54.73 x 13.95 mm) on wheat+sunflower (S4) (Table 3). The lowest SL and highest SWi were observed in strain NS 244, often giving away the impression that the cap is attached to the substrate. On wheat+sunflower mixture (S4), SL was lower than SW (46.01 mm x 46.99 mm), while the stem was uniform in strain NS 355 where SL/SWi ratio ranged from 2.84 (40.28 mm x 14.17 mm) on sunflower (S7) to 3.61 (44.71 mm x 12.68 mm) on corn (S6).

Table 4. The number of fruiting bodies (NM) in NS strains of *P. ostreatus*

| Substrate | NS 77 | NS 355 | NS 244 |
|----------------------|---------|--------|--------|
| Wheat (S1) | 7.44a | 8.96ab | 4.93a |
| Wheat+soybean (S2) | 9.44ab | 10.67a | 4.22a |
| Wheat+corn (S3) | 8.19ab | 8.63b | 4.70a |
| Wheat+sunflower (S4) | 11.22b | 10.56a | 5.52a |
| Soybean (S5) | 10.74ab | 10.59a | 5.04a |
| Corn (S6) | 10.48ab | 9.93ab | 5.22a |
| Sunflower (S7) | 10.52ab | 9.04ab | 5.52a |

Note: a, b, - Means followed by the same letters within the columns are not significant (Tukey's multiple comparison test)

The number of fruiting bodies was uniform in strain NS 244 (4.22-5.52) (Table 4). In strain NS 77, it was significantly lower on substrate wheat (S1) compared to wheat+sunflower (S4), and significantly lower on wheat+corn mixture (S3) in strain NS 355 compared to wheat+sunflower (S4), wheat+soybean (S2), and soybean (S5). Kitamoto et al. (1995) explained the variation of fruiting bodies on certain substrates by the higher content of glucose, fructose and trehalose in the substrates.

The biplot revealed 72.9% of sample variability in the first dimension, and 14.8% in the second dimension (Fig. 1.). The first dimension separated the substrates which established morphological traits of strain NS 244, from the substrates defining morphological traits of strain NS 77, whereas substrates establishing morphological traits in strain NS 355, as the strain with the lowest variability, were displayed along the first axis located in the coordinate origin of the biplot. Strain NS 244 connected to stem width (SWi) was isolated in the second dimension, while other strains along the axis were located in the coordinate origin of the biplot as the substrates and strains with the lowest variation coefficient.

Cap weight (CW), stem weight (SW), cap length (CL) and cap width (CWi), as well as stem width (SWi) were positively correlated variables, with high values observed in strain NS 244. High values for the mushroom number (NM) were observed in strains NS 77 and NS 355. Positively correlated group of traits - cap weight (CW), stem weight (SW), cap length (CL), cap width (CWi), and stem width (SWi) - were negatively correlated with stem length (SL) and mushroom number (NM).

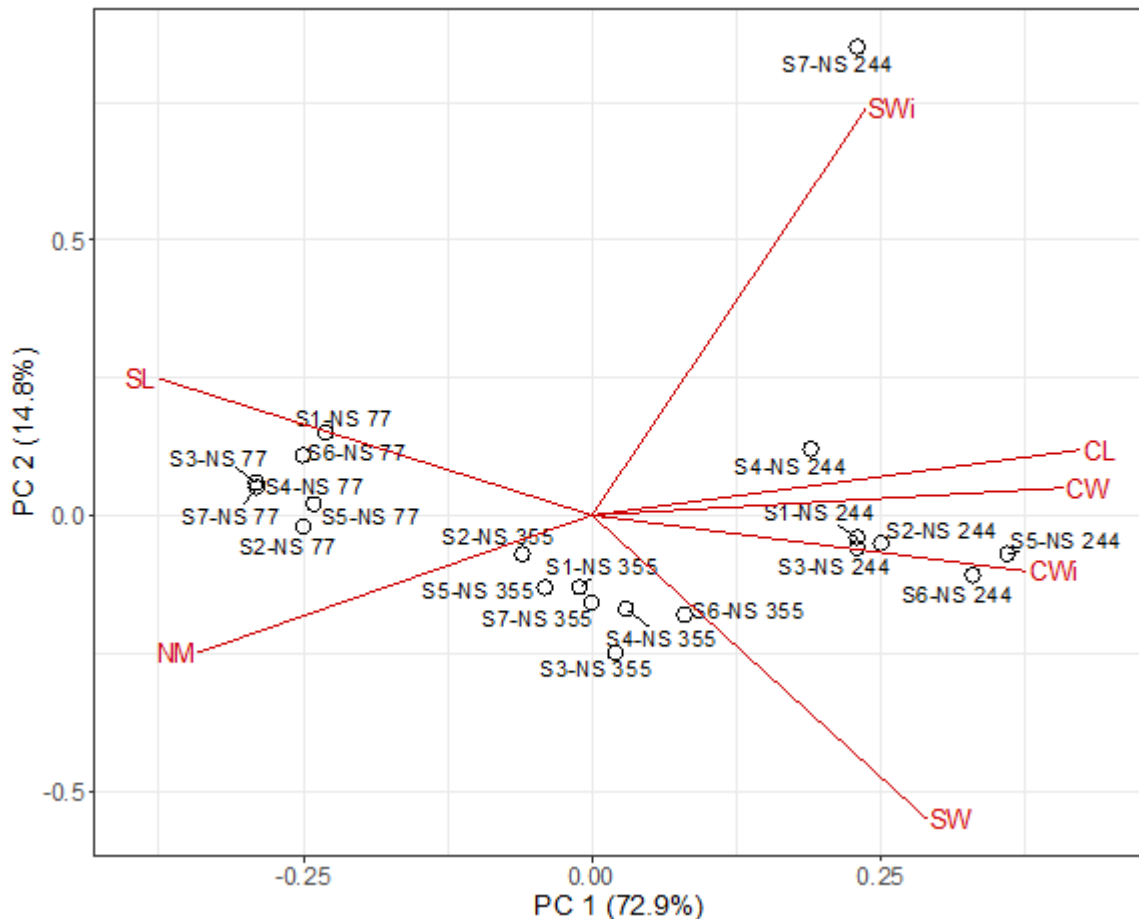


Fig. 1. Biplot display of morphological traits of NS strains of *P. ostreatus*

Conclusions

Comparison of morphological traits of the caps (cap weight CW, cap length CL and cap width CWi) revealed high variability between strains *P. ostreatus* (cap weight CW was doubled in strain NS 244 compared to strain NS 77); across substrates they were strain-specific and not significant.

The obtained results of stem weight (SW) and stem length (SL) revealed high variability between strains (NS 77 > NS 355 > NS 244) as well as the results of stem width (SWi) (NS 244 > NS 355 > NS 77), depending on the substrate. Soybean substrate (S5) had the highest effect on stem length (SL) in all three strains (NS 77, NS 355, and NS 244); a significant effect of substrate wheat+soybean (S2) was observed in strains NS 77 and NS 244; wheat+corn mixture (S3) significantly affected strains NS 355 and NS 244.

The number of fruiting bodies was uniform in strain NS 244, depending on the substrate, while strains NS 77 and NS 355 were significantly higher in wheat+sunflower (S4).

The biplot indicated a positive correlation between the morphological traits of stems - stem width (SWi) and stem weight (SW), a positive correlation between cap morphological traits - cap length (CL), cap width (CWi) and cap weight (CW), but a negative correlation with stem

length (SL) and the number of fruiting bodies in a bunch (NM). Depending on the composition of the tested substrates, high values of trait correlations - stem length (SL) and cap width (CWi) - were found on corn (S6); the highest values of cap width (CWi) and stem width (SWi) were observed on wheat+corn mixture (S3) as a group of positive variables, while the number of fruiting bodies (NM) had high values on sunflower stem substrates (S4 and S7).

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