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Application of Fungal Complexes to Improve Flax Dew-Retting

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The effect of fungal complexes on the flax dew-retting accelerating under the field conditions was examined. Active strains of fungi – *Chrysosporium merdarium, Sarcopodium tortuosum, Geomyces pannorum, Oidiodendron griseum, Alternaria alternata, Cladosporium tenuissimum, Cladosporium cladosporioides, Cladosporium herbarum* – were selected and four complexes for a field treatment were formed. The fungal spore suspensions were used for the additional contamination of laid flax at the moment of harvesting or swath returning. The amount of fungi on flax stems, species composition and density of *Cladosporium* population as well as meteorological conditions were estimated periodically during the flax dew-retting. The analysis showed that the largest amount of fungi persisted on the flax treated with fungal complex N 3 containing 6 fungal strains. Totally 160 fungal species were isolated from the retted flax, but only 29–35 of them were found more frequently, and *Alternaria alternata, Cladosporium cladosporioides, C. herbarum* prevailed among them. The best results of fibre separation were observed in the variants where the population density of species *Cladosporium* was comparatively high (25–29%) at the end of retting. The additional contamination of laid flax had a positive effect on the fiber separation index and the quality of flax fiber.

Fungi, flax, dew-retting, fiber separation index.

Introduction

Flax (*Linum usitatissimum* L.) is a traditional fiber crop in Lithuania. Dew-retting of flax is used commonly for a fiber production. The pulled stems are laid on the ground in swaths and left to dew-ret for several weeks. Indigenous fungi colonize the laying flax stems, and hydrolytic enzymes produced by fungi decompose fiber-bundle matrix releasing the bast fibers from each other and from the woody core. Polysaccharide degrading enzymes such as pectinases, xylanases, hemicellulases, produced by the fungi, are primarily responsible for fiber separation (Sharma, 1986 a, b; Henriksson et al., 1997; Evans et al., 2003; Zhang et al., 2005).

The fungi participating in dew-retting depends on geographical region –the fungi important in Europe may be different from those in the USA (Henriksson et al., 2000). Fungi *Cladosporium herbarum, Epicoccum nigrum, Fusarium culmorum, Alternaria alternata* and species of genera *Mucor, Rhizopus* were isolated from the retting flax stems in Northern Ireland (Sharma, 1986 a). The best retting abilities were obtained among species of *Aspergillus* and *Penicillium* genera in southern Europe (Fila et al., 2001). According to Russian scientists the main role in separating of flax fiber belongs to dematiaceous fungi such as *Alternaria alternata* and *Cladosporium herbarum* (Белова и др., 2000). Fungi from genera *Alternaria, Cladosporium, Fusarium* as well as *Aureobasidium pullulans, Chrysosporium merdarium*, *Embellisia chlamydospora, Oidiodendron griseum* were frequently isolated from laid flax rather in Lithuania (Jankauskienė ir kt., 2005).

The number of fungi and species diversity on retted flax depend on meteorological conditions, therefore, the influence of weather humidity and temperature on dew-retting process is extremely high (Mercer et al, 1986; Белова и др., 2000; Fila et al., 2001; Foulk et al., 2002). The lack of humidity or warmth is the main cause of insufficient dew-retting in Lithuania.

Long-term dew-retting and some other disadvantages motivate research of new retting methods. Various new chemical, enzymatic and microbial methods are used to improve retting of flax. Application of some chemical desiccators such as glyphosate, Reglon improves fiber separation (Mercer et al, 1986; Sharma, 1986 a; Jankauskienė, 2006). The extensive research of enzyme-retting has been developed since 1980s. Polygalacturonase plays a key role in the enzymatic retting of flax. An extracellular polygalacturonase has been purified from zygomycete *Rhizopus oryzae,* a potential retting organism (Zhang et al., 2000; 2005). The commercial preparations Novozym, Pectinol, Ultrazym and other pectinase-rich enzymes are used for retting in tanks. Results suggest that enzymatic-retting could produce fibers with particular properties, thus providing diversity in fiber characteristics for various applications (Sharma, 1987; Akin, et al., 1997; Foulk et al., 2002).

The additional contamination of retted flax with selected indigenous active fungal strains for the acceleration of flax dew-retting may be successfully used. Similar experiments with bacteria producing pectin-lyase and xylanase were carried out (Sharma, 1986 b). Some experiments were performed *in vitro* with fungi. Flax stems were artificially inoculated with single fungal strains and their ability to decompose pectin was estimated (Henriksson et al., 1997; Fila et al., 2001). Some attempts to accelerate flax dew-retting using microorganisms were made in Russia. Suspensions of *Alternaria alternata*, *Cladosporium herbarum, Alternaria alternata* + *Cladosporium herbarum* + *Fusarium avenaceum* were spread on the flax straw in the field. The best results of flax fiber separation were obtained after application of *Alternaria alternata* (Боярченкова и др., 1989).

The aim of the investigation was to examine the ability of selected fungal strains to improve the flax dew-retting process under field conditions.

Methods and conditions

Trial design and details in the field. The field trial was established and carried out in 2005 at the Upyte Research Station of the Lithuanian Institute of Agriculture. The trial was conducted on an Endocalcari-Endohypogleyic Cambi-

sol (Buivydaitė ir kt., 2001). The content of available P_2O_5 in the soil plough layer was 164 mg kg^{-1}, content of K_2O – 136 mg kg^{-1} (determined in A-L extraction), pH_{KCl} level – 7.3 (potenciometrically), humus content – 1.53 % (by Thyurin method).

Flax (cv. Hermes) was sown on May 6th with a sowing machine NODET at a seed rate of 25 million seed per ha, at 10 cm interrow spacing. Treatment plot was 13.0 (6.5 × 2.0) m^2, 4 replications, randomised plot design was used. Flax full germination was recorded on May 20th. The weather was warm and dry, thus the flax grew up short, and flowering began on June 27th. The flax was pulled at the stage of early yellow ripeness (on August 5th) with DEHONDT puller and laid on the soil into swathes for dew-retting. During the dew-retting process the swathes were returned twice.

The fungal spore suspensions were sprayed once on flax straw just after pulling (on August 5th; treatments 2–5) or just after first turning over the swathes (on August 15th; treatments 6–9) according to trial design (Table 1).

Table 1. Design of field trial
1 lentelė. *Bandymo schema*

Treatment No. *Varianto Nr.*	Fungal complex *Grybų kompleksas*	Fungal strains used for flax stem contamination *Grybų padermės, naudotos linų stiebelių užkrėtimui*	Time of treatment *Panaudojimo laikas*
1.	Control (water) *Kontrolė (vanduo)*	–	after flax harvesting *nurovus linus*
2.	N1	*Alternaria alternata* (Fr.) Keissl. 1-4U, *Oidiodendron rhodogenum* Robak 7-2U-20, *Cladosporium herbarum* (Pers.) Link ex Gray 8-5U-10	after flax harvesting *nurovus linus*
3.	N2	*Chrysosporium merdarium* (Link ex Grev.) J. W. Carmich. 11-6U-30, *Geomyces pannorum* (Link) Sigler ex J. W. Carmich. 6-III, *Sarcopodium tortuosum* (Wallroth) Hughes 7-20	after flax harvesting *nurovus linus*
4.	N3	all strains of fungi from complexes N1 and N2 *visos padermės iš kompleksų N1 ir N2*	after flax harvesting *nurovus linus*
5.	N4	*Cladosporium tenuissimum* Cooke 2-1U-20, *Cladosporium herbarum* (Pers.) Link ex Gray 8-5U-10, *Cladosporium cladosporioides* (Fresen.) G. A. de Vries 9-7U-50	after flax harvesting *nurovus linus*
6.	N1	*Alternaria alternata* (Fr.) Keissl. 1-4U, *Oidiodendron rhodogenum* Robak 7-2U-20, *Cladosporium herbarum* (Pers.) Link ex Gray 8-5U-10	after first swath turning *po pirmojo juostos apvertimo*
7.	N2	*Chrysosporium merdarium* (Link ex Grev.) J. W. Carmich. 11-6U-30, *Geomyces pannorum* (Link) Sigler ex J. W. Carmich. 6-III, *Sarcopodium tortuosum* (Wallroth) Hughes 7-20	after first swath turning/ *po pirmojo juostos apvertimo*
8.	N3	all strains of fungi from complexes N1 and N2 *visos padermės iš kompleksų N1 ir N2*	after first swath turning *po pirmojo juostos apvertimo*
9.	N4	*Cladosporium tenuissimum* Cooke 2-1U-20, *Cladosporium herbarum* (Pers.) Link ex Gray 8-5U-10, *Cladosporium cladosporioides* (Fresen.) G. A. de Vries 9-7U-50.	after first swath turning *po pirmojo juostos apvertimo*

The strains of fungi for the investigation of flax dew-retting acceleration were chosen in accordance to the data of fungal growth intensity on flax stems and their ability to assimilate pectin under laboratory conditions (Repečkienė et al., 2007). Fungi from genus *Cladosporium* (3 strains) as well as *Chrysosporium merdarium* were also chosen taking into account their abundant sporulation and/or wide distribution on flax under field conditions in Lithuania (Jankauskienė ir kt., 2005). Consequently, 8 fungal strains were selected and 4 complexes for additional contamination of retting flax were formed: fungal strains containing melanin in their mycelium were included in the complex N1, fungi containing carotene pigments – in the complex N2, all 6 strains from complexes N1 and N2 composed complex N3, and strains only from genus *Cladosporium* – complex N4.

Fungi were grown on malt extract agar for 7 days and suspensions ($1x10^6$ conidia ml^{-1}) were made. Suspensions were filtrated and sprayed on laid flax with knapsack sprayer Hardy RY-2 100 ml m^{-2} for good moistening.

The mycological analysis of dew-retted flax was carried out at the Institute of Botany. Flax stems (100–150 g from each variant) for the mycological analysis were taken at harvesting time before spraying with fungal suspension, at the first swath returning and every 10 days when dew-retting run (8 times totally from 8 August till October 14th). Fungi were isolated using dilution plating technique. A gram of flax stems cut to 2–3 cm pieces was shaken with 100 ml of sterile water for 10 minutes, dilutions (1:100, 1:1000, 1:10000) were made and suspension was sown on malt agar in three replications. Colony forming units (cfu) of fungi per 1 gram of flax stems, detection frequency of prevailing species (as relation of number of samples where species was found to total number of examined samples, expressed in %) and population density of *Cladosporium* genus fungi (as re-

lation of total number of strains of genus to total number of all isolated strains, expressed in %) were calculated (Мирчинк, 1988). Isolated species of fungi were identified according to the manuals (Ellis, 1971; 1976; Domsch et al., 1980; Oorschot, 1980; Watanabe, 2002).

Investigation of fiber separation. Dew-retting degree of flax straw was evaluated visually after scutching of dew-retted straw samples (150 g) by scutching tool SMT-200 M. When flax straw became grey (because of dew-retting process) fibre separation analyses were started with OOV tool. The samples were analysed with interval of 1 week starting from the 27th retting day. The sample of 100 dew-retted straws was tested in 4 replications from each treatment. Fibre separation was measured in three places of the flax stem (top, middle and foot) cutting out 10 cm length segments and averaged data of fiber separation index were calculated (Andrišiūnas, 1975). The obtained results were processed using statistical programme ANOVA (Tarakanovas ir kt., 2003).

Meteorological conditions. Average weather temperature and amount of precipitation were recorded during flax dew-retting period (Table 2). After the flax harvesting the weather was warm, it was raining already on the next day after harvesting (heavy rain) and also some days later (some showers). Thus the conditions for flax straw dew-retting were suitable, and in 10 days the colour of flax straw from yellow turned to grayish. But later dry period started (in the second ten-day period 12.5 mm and in the third ten-day period of August only 2.5 mm of precipitation fell down) and dew-retting was suspended. It was warm but dry in September – nary precipitation in the first ten-day period, and only 5.2 mm - in the second ten-day period of September. Some precipitation (19 mm) fell down at the end of September, but the weather become cool, the first frost at night came already on September 17th. At the beginning of October great mists at night and until the midday were dominating, but there was lack of precipitation, thus the dew-retting run in un-favourable conditions.

Table 2. Average air temperature, precipitation and relative humidity during flax dew-retting period, Upytė, 2005
***2 lentelė**. Vidutinė oro temperatūra, kritulių kiekis bei santykinis oro drėgnumas linų klojėjimosi metu periodu, Upytė, 2005 m.*

August / *Rugpjūtis*			
10-day period *Dešimtadienis*	Precipitation, mm *Krituliai, mm*	t, °C	Relative weather humidity, % *Santykinis oro drėgnumas, %*
I*	85.0	17.2	–
II	12.5	16.1	84.9
III	2.5	16.8	83.5
Month average *Mėnesio vidurkis*	100.0	16.7	87.3
September / *Rugsėjis*			
10-day period *Dešimtadienis*	Precipitation, mm *Krituliai, mm*	t, °C	Relative weather humidity, % *Santykinis oro drėgnumas, %*
I	0	15.8	85.3
II	5.2	13.1	86.6
III	19.0	14.4	91.1
Month average *Mėnesio vidurkis*	24.2	14.4	87.7
October / *Spalis*			
10-day period *Dešimtadienis*	Precipitation, mm *Krituliai, mm*	t, °C	Relative weather humidity, % *Santykinis oro drėgnumas, %*
I	0	11.5	94.3
II**	0	8.6	88.2

* – beginning at September 8th / *pradžia rugsėjo 8 d.;* ** – end at October 17th / *pabaiga spalio 17 d.*

Results

The amount of fungi on dew-retted flax. During dew-retting period 72 samples of flax stems were taken for mycological analysis. At flax harvesting time 0.6×10^5 cfu of fungi per gram of straw were found. The amount of fungi increased to $11–39 \times 10^5$ cfu g^{-1} in separate variants of the experiment after 10 days at swath turning time (Fig. 1). Particular increase of fungi ($17.7–39.3 \times 10^5$ cfu g^{-1}) was evaluated in the variants after application of suspensions of fungi at harvesting, whereas $11–15.3 \times 10^5$ cfu g^{-1} were found on un-treated flax.

After the additional contamination of retted flax with fungal complex N1 the number of fungi fluctuated from 14 to 42×10^5 cfu g^{-1} of stems during the experiment. Some decrease in their number was noticed after 40 and 50 days of harvesting due to drought. The greatest amount of fungi was found at the end of dew-retting.

The number of fungi was $12.7–43.3 \times 10^5$ cfu g^{-1} of stems in variants of treatment where fungal suspensions of complex N2 were applied. The total amount of fungi increased due to abundant development of fungi from genus *Penicillium* after 50 days of dew-retting.

The most stable number of fungi on laid flax was found in the variants after the application of complex N3, consisting of 6 fungal strains. After contamination at harvesting time the number of fungi gradually decreased until the 50th day of retting (from 33.0 to 19.0×10^5 cfu g^{-1}) and again increased (to 48.0×10^5 cfu g-1) at the end of dew-retting. The total amount of fungi stabilized at rather high level ($25.0–31.3 \times 10^5$ cfu g^{-1}) since the 30th day of retting after spraying of fungal suspensions at swath turning.

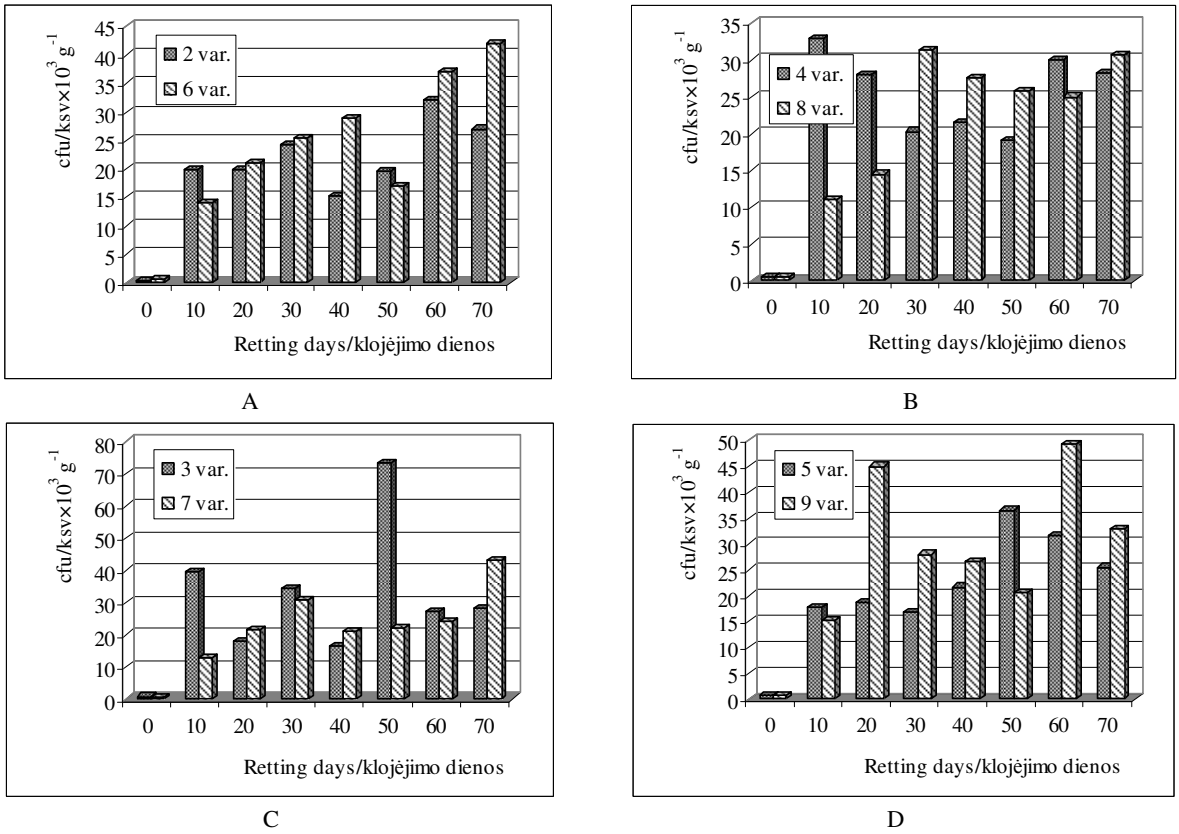

Fig. 1. The number of fungi on laid flax stems during dew-retting after the application of spore suspensions of fungi at harvesting and at swath turning (treatments 1–9 according to trial design): A – complex N1, B – complex N2, C – complex N3, D – complex N4

1 pav. *Grybų skaičius ant paklotų linų stiebelių klojėjimosi metu apkrėtus juos grybų sporų suspensijomis linų rovimo arba paklotos stiebelių juostos vartymo metu (variantai nuo 1 iki 9 pagal bandymo schemą): A – kompleksas N1, B – kompleksas N2, C – kompleksas N3, D – kompleksas N4*

The greatest amount of fungi (to 36.5×10^5 cfu g^{-1}) was estimated after the application of suspensions of *Cladosporium* (complex N4) maybe because of their abundant sporulation.

Fungal species, dominating on dew-retted flax. Before harvesting 44 fungal species were isolated from flax stems but only 13 (29.5%) of them were more abundant. *Alternaria alternata, Cladosporium cladosporioides, C. herbarum* (detection frequency 100%), yeasts *Rhodotorula rubra* (Demme) Lodder (66.7%), *Gilmaniella humicola* (55.5%), *Alternaria pluriseptata, Scytalidium lignicola, Ulocladium chartarum* (44.4%) were isolated more frequently.

From the dew-retted flax 160 species of fungi were isolated during the experiment. The main part (61.25%) of them was found only on one flax sample and may be termed as sporadic. More than once 62 (38.75%) species were isolated. More rich species composition was evaluated in the variants where complexes N1 and N 2 had been used for contamination (Table 3). Having applied the complex of *Cladosporium* fungi (N4), the amount of colony forming units increased but the number of species was poorer.

Plenty of fungi, known as the ones participating in a separation of flax fibre, were found from the beginning on dew-retting in all variants of treatment. Fungal species containing melanin from genera *Alternaria, Aureobasidium, Cladosporium, Humicola, Oidiodendron* were distributed more widely. The detection frequency of 23 species was 12.5–79.2% (Table 4).

Table 3. The amount of fungal species isolated from flax during dew-retting period

3 lentelė*. Mikromicetų rūšių, išskirtų nuo linų stiebelių klojėjimosi metu, skaičius*

Complexes of fungi *Mikromicetų kompleksai*	Number of isolated species / *Išskirtų rūšių skaičius*		
	Contamination after harvesting *Užkrėsta nurovus*	Contamination at swath turning *Užkrėsta verčiant juostą*	Totally *Iš viso*
Control (water) *Kontrolė (vanduo)*	–	–	37
Complex N1 *Kompleksas N1*	51	57	83
Complex N2 *Kompleksas N2*	45	58	78
Complex N3 *Kompleksas N3*	52	48	77
Complex N4 *Kompleksas N4*	43	48	70

Table 4. Species of fungi frequently isolated from the flax treated with spore suspensions
4 lentelė*. Mikromicetų rūšys, dažniausiai išskirtos iš linų, užkrėstų sporų suspensijomis*

Fungi *Mikromicetai*	Isolation frequency, % *Išskyrimo dažnis, %*
Acremonium charticola (J. Lindau) W. Gams	23.6
Acremonium strictum W. Gams	13.9
Alternaria alternata (Fr.) Keissl.	75.0
Alternaria pluriseptata (P. Karst et Har. Ex Peck) JØrst	20.8
Alternaria radicina Meier, Drechsler et E. D. Eddy	25.0
Arthrinium phaeospermum (Corda) M. B. Ellis	12.5
Aureobasidium pullulans (de Bary) G. Arnaud	13.9
Candida albicans (C. P. Robin) Berkhout	16.7
Chrysosporium merdarium (Ehrenb.) J. W. Carmich.	12.5
Cladosporium cladosporioides (Fresen.) G. A. de Vries	79.2
Cladosporium herbarum (Pers.) Link	72.2
Exophiala jeanselmei (Langeron) McGinnis et A. A. Padhye	16.7
Fusarium avenaceum (Fr.) Sacc.	27.8
Fusarium graminearum Schwabe	27.8
Fusarium heterosporum Nees et T. Nees	19.4
Fusarium oxysporum Schltdl.	13.9
Fusarium poae (Peck) Wollenw.	19.4
Gilmaniella humicola G.L. Barron	30.6
Penicillium palitans Westling	13.9
Sclerotinium sclerotiorum (Lib.) de Bary	15.3
Scytalidium lignicola Pesante	12.5
Ulocladium chartarum (Preuss) E. G. Simmons	20.8

The differences in number of species on flax stems from various variants of treatment were noticed. In control variant 20 species were detected more than once, in the variants treated with fungal complexes N1, N2 and N4 – 35 and in the variants treated with complex N3 – 29 fungal species.

Not all species, used for the additional contamination, developed on retted flax equally. *Sarcopodium tortuosum* was isolated more frequently from the flax contaminated with fungal complex N1, *Alternaria alternata* and *Cladosporium herbarum* – with complex N2, *Alternaria alternata, Chrysosporium merdarium, Cladosporium cladosporioides* and *C. herbarum* – with complex N3*, Cladosporium cladosporioides* and *C. herbarum* – with complex N4. *Oidiodendron echinulatum* and *Geomyces pannorum* were found rarely.

The most of isolated species belonged to genera *Acremonium, Alternaria, Cladosporium, Fusarium* and *Penicillium*. The other genera accounted for 35–49% of more often detected species. Such fungi were isolated more frequently from the variants contaminated with fungi of complexes N3 and N4.

Species diversity changed during dew-retting. At 10 days after harvesting fungi of genus *Cladosporium* prevailed in most variants of treatment. At 20 days after beginning of dew-retting a number of *Fusarium* increased significantly. At the same time *Rhodotorula rubra* yeasts were isolated more frequently. Separate species of fungi survived and developed on the flax contaminated with different complexes of fungi at the end of dew-retting. For example, from the flax in variant 2 fungi from genus *Acremonium* as well as melanin containing species used for additional contamination were isolated frequently, but species containing carotene pigments in their mycelium were detected rarely.

The main fungal species participating in dew-retting were isolated from the soil were flax grew, therefore, they were found on the flax additionally contaminated with fungal complexes as well as on those in control variant. Fungi from genus *Alternaria* were isolated more frequently from the flax contaminated with spore complexes N2, N3 and N4 comparing with the control variant and the variant treated with complex N1 (17 and 11%, respectively). Species of genus *Cladosporium* formed 8–10% of all isolated species, except in the variants additionally treated with spores of complex N4. *C. cladosporioides* and *C. herbarum* prevailed in these variants. It was noticed that their prevalence inhibited development of fungi from genus *Fusarium* – their part in mentioned variants was 14% compare with 21–26% in other variants.

Distribution of Cladosporium fungi on dew-retted flax. Special attention was given to fungi of genus *Cladosporium*. Four species – *Cladosporium cladosporioides, C. herbarum, C. sphaerospermum* and *C. tenuissimum* – were isolated from flax stems. The number of *Cladosporium* was the greatest till the 30th day of retting (11.3×10^5 cfu g^{-1}), and reduced twice in the following ten-day periods ($4.0–6.3\times10^5$ cfu g^{-1}) in control. The same tendency was observed in the variants treated with fungal complex N1. The complexes N2 and N3 included strain *Cladosporium herbarum;* consequently, the total number of *Cladosporium* was greater. For example, a number of these fungi fluctuated within 15.7–25.0×10^5 cfu g^{-1} till the 30^{th} day of retting and within 5–6.7 $\times 10^5$ cfu g^{-1} till the end of process in the variants treated with complex N2 at harvesting, or within 8.0–14.7×10^5 cfu g^{-1} and 3.0–14.7×10^5 cfu g^{-1}, respectively, when spores were sprayed at swath turning. No significant increase of *Cladosporium* was determined in the variants treated with complex N4 consisting only of strains of this genus. There-

fore, their abundance was more stable during dew-retting compared with other variants and fluctuated within 7–16.3 × 10^5 cfu g^{-1} after additional contamination at harvesting and within 3.0–10.3×10^5 cfu g^{-1} – at swath turning.

The greatest part of *Cladosporium* strains among total amount of fungi was estimated at the first 10 days of experiment and reached 49.2–56.5% (Fig. 2). Actually, the part of these fungi was significant in control (56.5%) and on the flax left for spraying at swath turning. At 40 days after beginning of dew-retting the part of *Cladosporium* became similar in all variants of treatment (27.5–32.3%), except on the flax treated with spore complex N3 at harvesting (44.2%). At the end of dew-retting the density of *Cladosporium* population decreased but it remained the greatest on the flax treated with complexes N3 and N4 at harvesting (25–29.4%) or with complexes N2 or N3 – at swath turning (about 20%). Their population density was significantly lower in control and in the variants after treatment with complex N1 without *Cladosporium* strains (11.1–13.3%) at the same time.

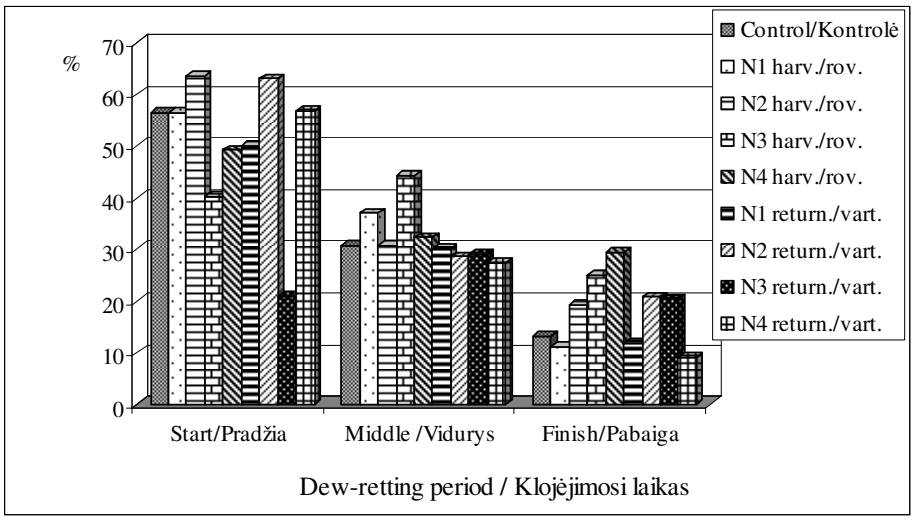

Fig. 2. Population density (%) of *Cladosporium* genus fungi on flax stems at different time of dew-retting
2 pav. *Cladosporium genties grybų populiacijos tankis (%) ant linų stiebelių įvairiu klojėjimosi laiku*

Fibre separation and quality. Fibre separation, colour of fibre and its homogeneity were estimated during flax dew-retting in field. When scutched fibre was well separated from the wooden part of the stem, it contained no impurities and was dew-retted enough. As mentioned above (Table 1), just after harvesting the meteorological conditions were favourable for dew-retting, and flax straw colour changed from yellow to yellow-greyish in just 10 days. But later the weather become dry again and the straw did not change the colour for a long time. After 27 days after flax harvesting straw was not dew-retted enough, fibre contained impurities, and colour was variegated, not homogenous. No significant differences among treatments were observed. Colour of fibre was darker in the variants where fungal strains containing black pigment had been applied for the additional contamination. The top of stems was already dew-retted, but the foot of the stem remained un-retted for a long time. Sharp changes in colour of straw and fibre were noticed after some rains (62 days after harvesting). Dew-retting was sufficient in 70 days after harvesting (fibre separation index reached 8–9 at this time).

The evaluation of fibre dew-retting degree showed that generally the best fibre was obtained in the treatment 4, where complex N3 was applied after harvesting, and fibre was coarser in the treatment 5, where the complex N4 was applied after harvesting. The last evaluation (on October 13th) of the fibre dew-retting degree showed that fibre in the treatments 1–3 was not homogenous. The fibre had the best image in the treatments 4, 5 and 6 (it was clean, homogenous, soft), while the fibre in the treatments 7, 8, 9 was well scutched, clean, but the colour was not homogenous.

The determination of fibre separation index (FSI) was started on September 1st (27 days after harvesting). The data of the investigation show that application of fungal spore complexes had a positive effect on the fibre separation index – FSI for treated variants was higher than that for untreated straw (Fig. 3).

No differences were established in fibre separation index between treated and un-treated straw at 27 days after harvesting. On September 15th (41st day after harvesting) the mean of FSI changed un-significantly (because of dry weather); the highest FSI mean was determined for the treatments where contamination with complexes N3 and N4 was used. Significant differences in FSI among different treatments were established on the flax samples taken on the 48th day after harvesting (on September 22nd), although the mean of FSI in a week changed slightly (dry weather). Significant differences of FSI (comparing to the untreated straw) were established for the variants 4, 5, 7 and 8 of the experiment. On September 29th (at 55 days after harvesting) the differences of FSI were again slight, but higher for all treated than for the untreated straw. Significant changes of the FSI and fibre colour were noticed on the flax samples taken one week later – on October 6th (at 62 days after harvesting). The mean of FSI became higher than 7.0. Significantly higher FSI (than that of untreated straw) was determined for the variants 4, 8 and 9 (complexes N3 and N4 were used). At the end of experiment (on October 13th, at 69 days after harvesting) the mean of FSI mainly was higher than 8.0.

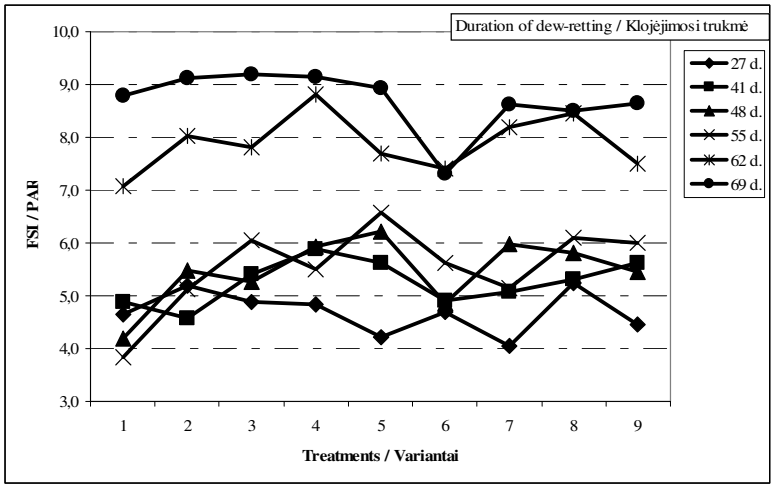

Fig. 3. Change of fibre separation index (FSI) of the flax treated with fungal complexes (treatments 1–9 according to trial design) (LSD_{05} after 27 days of retting – 1.15; LSD_{05} after 41 day – 0.99; LSD_{05} after 48 days – 1.12; LSD_{05} after 55 days– 0.80; LSD_{05} after 62 days– 0.82; LSD_{05} after 69 days – 0.90)

3 pav. *Pluošto atsiskiriamumo rodiklis (PAR) paveikus linus skirtingais mikromicetų kompleksais (variantai nuo 1 iki 9 pagal bandymo schemą) (R_{05} po 27 klojėjimo dienų – 1,15; R_{05} po 41 dienos – 0,99; R_{05} po 48 dienų – 1.12; R_{05} po 55 dienų – 0,80; R_{05} po 62 dienų – 0,82; R_{05} po 69 dienų – 0,90)*

Discussion

The eight fungal strains were selected and 4 complexes were formed for the additional contamination of retting flax with intention to improve flax retting process under field conditions. The amount of fungi on dew-retting flax increased just after 10 days when additional contamination with fungal complexes was used. Some decrease in their number was noticed at 40 and 50 days after harvesting due to drought. The greatest amount of fungi was found at the end of dew-retting (at 60–70 days after harvesting). More fungi were isolated when additional contamination was used at swath turning. The abundance of fungi fluctuated from 13 to 43×10^5 cfu g^{-1} of stems during the experiment and correlated with amount of precipitation. It is known that abundance of microorganisms on retted flax depends on meteorological conditions and may fluctuate within $3 \times 10^5 – 1 \times 10^9$ cfu per gram of air dry stems (Белова и др., 2000).

Fungi *Alternaria alternata, A. solani, Cladosporium cladosporioides, C. herbarum, Fusarium avenaceum, F. graminearum* and *Gilmaniella humicola* may have determinant influence on the duration of flax retting because they were detected the most frequently (30–80%).

Additional contamination of flax with active fungal strains had positive effect on the diversity of species of laid flax and at the same time their activity in conjunction improved retting process. The data confirms proposition of other researches that fungi may act synergistically during the retting process. A consortium of enzymes consisting pectinases, xylanases and hemicellulazes produced by retting fungi take part in depolymerisation of noncellulosic materials in dew-retted flax (Henriksson et al., 1997).

The richest species diversity in the variants with additional contamination may show that introduction of fungi stimulated development of various fungal species, what had a positive effect on the index of fibre separation in these variants.

Fungi belonging to genus *Cladosporium* are rather important in dew-retting (Mercer et al., 1986; Белова и др., 2000). The additional contamination with *Cladosporium* spores had a positive effect on development of mentioned fungi on retted flax stems and simultaneously on the index of fibre separation index. Furthermore, the prevalence of *Cladosporium* spp. inhibited development of fungi from genus *Fusarium* – their part was 14% among all isolated species in the treated variants, compare with 21–26% in other variants. *Fusarium* fungi are known as agents of plant diseases, item, their cellulolytic activity may have negative effect on fibre quality (Evans et al., 2003).

According to the investigations of Russian scientist (Mukhin, 1992), flax straw is fully dew-retted when fibre separation index (FSI) for the straw is within the limits of 4.0–7.0. However, in the trials of this experiment the fibre was not clean enough, contained wooden impurities even when the fibre separation index was close to 6.0–7.0. Thus, the dew-retting in the field was carried on until the fibre visually looked clean and well dew-retted. Significantly higher FSI was determined for the variants 4, 8 and 9 where fungal complexes N3 and N4 were used. The similar results were obtained by Russian researches. No significant differences in dew-retting period, fibre separation index and quality parameters were found among treatments, but it was established that the dew-retting ran more intensive after the treatment with *Alternaria alternata* (Боярченкова и др., 1989).

Conclusions

160 fungal species were isolated from dew-retted flax straw during the dew-retting period in 2005. *Alternaria alternata, A. solani, Cladosporium cladosporioides, C. herbarum, Fusarium avenaceum, F. graminearum* and *Gilmaniella humicola* prevailed (detection frequency was 30–80%).

The greatest number of fungi colony forming units was detected after the application of complex N4 (consisting *Cladosporium* genus strains only), but during dew-retting period this rate was more stable after the application of complex N3 (consisting of 6 fungal strains from different genera). A greater diversity of fungal species was obtained on the flax additionally contaminated with spore suspensions of fungal complexes, comparing with untreated flax.

The density of *Cladosporium* species population on retted flax decreased at the end of dew-retting but remained high and reached 21–29% in the variants where the additional contamination with fungal complexes consisting of strains of this genus was used.

The application of fungal spore suspensions positively influenced the dew-retting process, fibre separation index and the quality of fibre. The best results were obtained after the application of complexes N3 and N4 at harvesting and at swath turning, and of complex N2 at harvesting.

Additional contamination of retted flax with indigenous active fungal strains is advisable for the improving of flax retting, especially under unfavorable weather conditions. It allows the increase in number of pectinolytic fungi at the start of dew-retting.

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Mikromicetų kompleksų panaudojimas linų klojėjimuisi pagerinti

Santrauka

Straipsnyje pateikiami duomenys apie mikromicetų kompleksų efektyvumą paspartinant linų klojėjimąsi lauko sąlygomis. Bandymams buvo atrinktos 8 mikromicetų padermės: *Chrysosporium merdarium, Sarcopodium tortuosum, Geomyces pannorum, Oidiodendron griseum, Alternaria alternata, Cladosporium tenuissimum, Cladosporium cladosporioides, Cladosporium herbarum,* iš kurių sudaryti 4 kompleksai ir jų sporų suspensijomis nupurkšti linai klojimo arba pirmojo juostų vartymo metu. Visą linų klojėjimosi laiką buvo stebimos meteorologinės sąlygos, nustatomas mikromicetų skaičius, išskiriamos ir identifikuojamos mikromicetų rūšys bei įvertinamas *Cladosporium* genties rūšių populiacijos tankis. Didžiausias mikromicetų pradų skaičius aptiktas ant linų, apdorotų komplekso Nr. 3, į kurį įėjo 6 grybų padermės, sporomis. Iš viso nuo besiklojėjančių linų išskirta 160 mikromicetų rūšių, tačiau tik 29–35 buvo dažnos kai kuriuose bandymo variantuose, o tarp jų vyravo *Alternaria alternata, Cladosporium cladosporioides* ir *C. herbarum*. Geriausi pluošto atsiskyrimo rodikliai buvo gauti apdorojus linus mikromicetų kompleksais, kuriuose *Cladosporium* genties mikromicetų populiacijos tankis klojėjimosi pabaigoje sudarė 25–29%. Papildomas klojėjamų linų užkrėtimas aktyviomis mikromicetų padermėmis turėjo teigiamą įtaką linų pluošto atsiskyrimo indeksui ir pluošto kokybei.

Linas, klojėjimasis, mikromicetai, pluošto atsiskyrimo indeksas.

Юрате Репечкене, Зофия Янкаускене

Применение комплексов микромицетов для улучшения росяной мочки льна

Резюме

В статье представлены данные об эффективности применения комплексов микромицетов для ускорения процесса росяной мочки льна в полевых условиях. Для эксперимента были отобраны 8 штаммов грибов: *Chrysosporium merdarium, Sarcopodium tortuosum, Geomyces pannorum, Oidiodendron griseum, Alternaria alternata, Cladosporium tenuissimum, Cladosporium cladosporioides, Cladosporium herbarum*, которые входили в состав 4 комплексов. Суспензиями спор грибов была опрыскана льносолома при ее расстиле или во время переворачивания разостланных лент. Во время вылежки льна фиксировались метеорологические условия, определялась численность и видовой состав микромицетов, плотность популяции грибов рода *Cladosporium.* Наибольшее количество пропагул грибов установлено на льносоломе обработанной спорами комплекса N3, в состав которого входили 6 штаммов. Всего было выделено 160 видов грибов, из которых в отдельных вариантах наиболее часто встречались 29–35 видов, а среди них доминировали *Alternaria alternata, Cladosporium cladosporioides* и *C. herbarum.* Наиболее высокий индекс отделения волокна установлен в вариантах, в которых плотность популяции грибов *Cladosporium* в конце вылежки составляла 25–29%. Дополнительная обработка льна активными штаммами микромицетов положительно влияла на ускорение процесса росяной мочки и качество волокна при неблагоприятных погодных условиях.

Лен-долгунец, микромицеты льна, росяная мочка льна, индекс отделения волокна.

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