Effect of fluopyram and liquid chicken manure preparation on root-knot nematodes (*Meloidogyne* spp.) in carrot crop

Utjecaj pripravka na bazi fluopirama i tekućega pilećeg stajnjaka na nematode korijenovih kvržica (*Meloidogyne* spp.) u nasadu mrkve

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EFFECT OF FLUOPYRAM AND LIQUID CHICKEN MANURE PREPARATION ON ROOT-KNOT NEMATODES (*Meloidogyne* spp.) IN CARROT CROP

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SUMMARY

The aim of this study was to investigate the nematocidal effect of the preparations based on fluopyram (pesticide) and liquid chicken manure (natural amendment) on the population density of the root-knot nematodes,* Meloidogyne *spp. in carrot crops. The field experiment was set up in four treatments: control (C), fluopyram (FLU), fluopyram and liquid chicken manure (FLU+LCM) and liquid chicken manure (LCM) by a random block design in four replicates. Population density of* Meloidogyne *spp. juveniles varied between the treatments, and the number of* Meloidogyne *spp. was significantly decreased in all treatments with fluopyram (FLU and FLU+LCM). All amended treatments (FLU, FLU+LCM, LCM) decreased galling of the roots and had a positive effect on carrot yield. The population of* Meloidogyne *spp. juveniles increased with the plant growth, regardless of the treatments applied. Fluopyram negatively affected the biodiversity indicating greater disturbance for the nematode community structure in the soil. It can be concluded that fluopyram and liquid chicken manure have nematicidal potential, while liquid chicken manure maintained or enhanced nematode biodiversity.

Keywords: root-knot nematodes, fluopyram, liquid chicken manure, root gall index, carrot

INTRODUCTION

Carrots (*Daucus carota* L.) are the most widespread root vegetable in Europe. According to Agriculture, forestry and fishery statistics (2018), around 5.8 million tons of carrots were produced in Europe in 2016. The United Kingdom was the greatest carrot producer in the EU with 0.9 million tons (15.3 %), followed by Poland (14.3%), Germany (12.7%) and the Netherlands (10.8%). In Croatia, the total carrot production in 2017 (13 676 t) declined compared to 2016 (18 225 t), as a result of a reduced production area (from 696 ha in 2016 to 600 ha in 2017) and reduced yield per ha (from 24.7 t/ha in 2016 to 20.5 t/ha in 2017).

Many abiotic and biotic factors affect carrot production, which is reflected in the loss of yield. One of the important groups of pests is the phytoparasitic nematodes. Nematodes can cause great economic damage by feeding on carrots, the most common nematodes being the root-knot nematodes (*Meloidogyne* spp.) and pin nematodes (*Paratylenchus* spp.).

Root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites that can cause great damage on the roots of a host plant by feeding on the cytoplasm of host plant cells. When cortical cells are attacked, they hypertrophy and the affected root becomes galled (Whitehead, 1997). Their presence in the soil makes the crop rotation more difficult because they can feed on 2000 different plant species (Sasser & Carter, 1985).

Species like *M. javanica*, *M. incognita* and *M. arenaria* are the most active at soil temperatures ranging 24-32°C, causing the most damage in the tropical and subtropical climate, but also in the temperate climate from April to October, with the highest activity in the middle of the summer (Perry et al., 2009). In the temperate climate of Croatia, these nematodes cause great problems for Croatian vegetable producers (Grubišić, 2016; Ivezić, 2014). On a global scale, *Meloidogyne* spp. cause annual losses of more than 5% of the yield, therefore they repre-

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sent one of the most damaging plant pathogens (Kaplan and Noe, 1993).

Chemical pesticides, harmful to humans and the environment, are still the most commonly used method for nematode control (Kankam et al., 2015). Due to this fact, it is recommended to use pesticides with the least harmful effect on human health and the environment. One of these pesticides, whose effect on the phytoparasitic nematodes was observed in this research, is fluopyram. Fluopyram was registered in 2014 in Germany as a succinate dehydrogenase inhibitor (SHDI) (Faske and Hurd, 2015) and it was used for the control of nematodes and sudden death syndrome (fungal pathogen *Fusarium virguliforme*) in soybean.

On the other hand, some authors pointed out the possibility and the effectiveness of using many natural resources for biological control of nematodes, such as garlic essential oil (Anastasiadis et al., 2011), leaves of dried weeds, residues of some plants and chicken or cattle manure (Whitehead, 1997; López-Pérez, et al., 2005). In Australia, poultry manure or sawdust at 24, 36 or 48 t/ha, incorporated in infested soil with urea at 1.8 t N/ha, controlled *M. incognita* on ginger more effectively and increased yield similarly or more than the best nematicide applied in the treatments, while the urea alone did not show the positive effect on the nematode suppression (Stirling, 1989 cited by Whitehead, 1997).

Many scientists have studied the influence of the liquid chicken manure on the nematodes of *Meloidogyne* spp. among the other parameters. According to the research of Kankam et al. (2015), application of different amounts of liquid chicken manure per ha showed that the higher dosages of the applied liquid chicken manure reduced the population of *Meloidogyne* spp. and consequently lowered the values of the root gall index. Also, the application of the liquid chicken manure has a significant effect on the yield, plant growth, chemical properties of the soil and the nematode biodiversity in the soil (Brmež et al. 2018). In the Philippines, 10 t/ha of chicken manure applied in the planting furrows controlled *M. incognita* on tomatoes until harvest and were more effective than 10 kg carbofuran/ha (Duhaylongsod, 1988 cited by Whitehead, 1997).

The aim of this study was to investigate the nematicidal effect of the preparation based on fluopyram and the preparation based on liquid chicken manure on the root-knot nematodes of the genus *Meloidogyne*, as well as the effect of the preparations combined together.

MATERIAL AND METHODS

Setup of the field experiment

The field experiment was setup on March 18, 2017, in Detkovac area, Croatia (43°52'53" N; 17°37'40" E). Four treatments were performed: 1st - application of pure water without nematicides - control treatment (C); 2nd - application of the preparation based on fluopyram (FLU); 3rd - application of the preparation based on fluopyram together with the preparations based on liquid chicken manure (FLU+LCM); 4th - application of the preparations based on liquid chicken manure (LCM). The field experiment was set by a random block design in four replicates. The total size of the experimental plot was 14,400 m^2, and the size of one treatment parcel was 900 m^2. Every treatment parcel consisted of seven raised beds, and on April 9, 2017, the carrot seeds were sown in two rows in each raised bed. Carrot cultivar used in this field experiment was Soprano F1, mid-early Nantes type. In 2017, the Croatian Ministry of Agriculture issued a certificate for the application of fluopyram in carrots for the purpose of this field experiment.

The preparations based on liquid chicken manure were sprayed onto the soil surface on March 18, 2017. The preparations were applied on the plots with LCM and FLU+LCM treatments in a dose of 2 l/ha. At the same time, pure water was applied on the plots of the remaining treatments (C and FLU). Preparations based on liquid chicken manure consisted of: 21% organic matter, 10% of nitrogen (N), 1% of phosphorus (P) and 4% of potassium (K); and 2.8% organic matter, 14% of nitrogen (N), 2% of phosphorus (P) and 5% of potassium (K).

The preparation based on fluopyram was sprayed onto the soil surface in a dose of 0.625 l/ha on April 8, 2017. The soil was turned over with a cultivator for making beds in order to incorporate the preparation into the soil depth of 10 cm. The preparation was applied with the addition of 240 l of pure water. On the same day, pure water was also applied on the remaining treatments, C and LCM.

Soil sampling and nematode assessment

The soil samples for nematode analysis were taken on three occasions. First sampling (before the application of any preparation) was carried out on March 18, 2017, and 20 soil samples were taken per treatment (five samples per replicate), a total of 80 samples. The second sampling was carried out on the June 6, 2017, a total of 80 samples. The third sampling was carried out on July 18, 2017, with an additional 16 soil samples for the nematode community structure analysis, 96 samples altogether.

Samples were taken from the soil in a zig-zag pattern with a probe (diameter of 2 cm) in the arable layer on a depth of 0-30 cm. After that, samples were collected in plastic bags, labelled, stored in a portable cooler and delivered for analysis to the Faculty of Agrobiotechnical Sciences Osijek, Croatia. The extraction of nematodes from the soil, counting and determination were carried out at the Department for Phytomedicine, Faculty of Agrobiotechnical Sciences Osijek.

From each sample, 50 g of soil were separated and nematodes were extracted from the soil by Baermann's funnel method (Van Bezooijen, 2006). Biodiversity assessment of nematodes genera was carried out after the third soil sampling and the nematodes were examined from 16 samples in order to analyze the effect of FLU and LCM on nematode community structure. Nematodes were determined to the genera level according to Eisenback (1985), Andrassy (2005, 2007, 2009), Bongers (1994), May and Lyon (1975). It is important to take into account that the preculture in these plots was also carrot, which could influence the number of *Meloidogyne* spp. juveniles.

Root galling and yield estimation

On July 18, 2017, the samples of carrot roots were taken in order to evaluate the root galls and estimate the yield. The root gall index was estimated according to Barker (1985) on the scale 0-10, where 0 represents a totally healthy root without the galls and 10 represents a completely infected and galled root. The root gall index was evaluated on 80 samples of carrot roots. The yield was calculated by weighing carrots and presented as mean value (from 4 repetitions per treatment) in kilograms per square meter.

Chemical analyses of soil properties

Before setting up the experiment, chemical analyses of soil properties were carried out in September 2016, at the Department for Agroecology, Faculty of Agrobiotechnical Sciences Osijek. Chemical analysis consisted of determining pH reaction of the supernatant soil suspension in water (1:5 volume fraction) for pH (H_2O) and 1 M KCl solution in water for pH (KCl) (ISO, 1994). The organic matter in the soil was determined by bicarbonate method (ISO, 1998), while P and K were extracted by the ammonia-lactate method (Egner et al., 1960). Soil samples taken at arable layer on the depth of 0-30 cm contained 1.55% organic matter, 5.04 % $CaCO_3$, 28.86 mg100g^{-1} P_2O_5, 10.86 mg 100g $^{-1}$ K_2O. The pH (H_2O) was 8.20 (moderately alkaline), while pH (KCL) was 7.89 (ISO, 1994).

The results of the research were statistically processed and the analysis of variance and LSD test were established by using SAS 9.1. The correlation between population density of *Meloidogyne* spp. juveniles and the root gall index was established using Excel descriptive statistics tool.

RESULTS AND DISCUSSION

The nematicidal effect of preparations based on fluopyram and liquid chicken manure was investigated on the root-knot nematodes, *Meloidogyne* spp.

The determined number of *Meloidogyne* spp. juveniles in samples significantly differed in investigated treatments. Treatments FLU and FLU+LCM had the statistically highest nematicidal effect on *Meloidogyne* spp. juveniles (Table 1). Riegel and Noe (2000) reported that the population densities of *M. incognita* decreased with higher rates of chicken litter incorporated into the soil because of the bacterial and fungal activity in the amended soil.

Faske and Hurd (2000) isolated *M. incognita* and *Rotylenchulus reniformis* from cotton and treated nematodes with water solution of 10, 1 and 0.1 μg/ml of fluopyram. After two hours of continuous exposure, 78% of *M. incognita* (48% of *R. reniformis*) were immotile and 91% (87% of *R. reniformis*) after 24 hours at a dose of 10.0 μg/ml fluopyram. The nematicidal effect of fluopyram on other economically important phytoparasitic nematodes can vary among nematode genera depending on the concentration of fluopyram needed to paralyse nematodes.

Table 1. The average number of *Meloidogyne* spp. juveniles in 50 g of soil

Tablica 1. Prosječna brojnost ličinki Meloidogyne spp. u 50 g tla

Treatments *Tretmani*	C	FLU	FLU+LCM	LCM
The average number of *Meloidogyne* spp. juvenile *Prosječna brojnost ličinki Meloidogyne spp.*	31.9^{A}	13.850^{C}	22.817^{B}	24.417^{AB}

Treatments: control (C), fluopyram (FLU), liquid chicken manure+fluopyram (FLU+LCM), liquid chicken manure (LCM)
Tretmani: kontrola (C), fluopiram (FLU), tekući pileći stajnjak+fluopiram (FLU+LCM), tekući pileći stajnjak (LCM)

The statistically significant differences ($P \leq 0.05$) are marked with different letters in a row (LSD 8.7479)
Statistički značajne razlike ($P \leq 0,05$) označene su različitim slovima unutar reda (LSD 8.7479)

The population density of *Meloidogyne* spp. juveniles increased during the vegetation, and the highest population number was established in July. With statistical significance, more *Meloidogyne* spp. juveniles were found in July than in March and June (Table 2), which was expected as *Meloidogyne* spp. favour high temperatures and high soil moisture (Whitehead, 1997; Perry et al., 2009; Ivezić, 2014). Also, Faske and Hurd (2000) investigated the recovery in nematode motility for *M. incognita* when removed from fluopyram after 1 hour treatment. They showed that *M. incognita* has an ability to recover motility (for 58%) 24 hours after being rinsed and removed from the fluopyram-water solution. On the other hand, Vrain (1982) concluded that the tolerance levels increased with the age of plants, but in our research, carrots were sown in an already infested soil, as the preculture was also carrot.

Table 2. The average number of *Meloidogyne* spp. juveniles in 50 g of soil for three sampling occasions

Tablica 2. Prosječna brojnost ličinki Meloidogyne spp. u 50 g tla za sva tri vremena uzorkovanja

Soil sampling *Uzorkovanje tla*	1st sampling (March 18, 2017) *1. uzorkovanje (18. ožujka 2017.)*	2nd sampling (June 6, 2017) *2. uzorkovanje (6. lipnja 2017.)*	3rd sampling (July 18, 2017) *3. uzorkovanje (18. srpnja 2017.)*
The average number of *Meloidogyne* spp. juveniles *Prosječna brojnost ličinki Meloidogyne* spp.	16.68^{B}	18.338^{B}	34.763^{A}

The statistically significant differences ($P \leq 0.05$) are marked with different letters in a row
Statistički značajne razlike ($P \leq 0,05$) označene su različitim slovima unutar reda

Carrots were harvested during the third sampling, in July 2017, and the root gall index was evaluated on a scale of 0-10. The treatments with fluopyram (FLU and FLU+LCM) had the lowest value of the root gall index (4), followed by the treatment LCM (6). The control treatment had the highest average value of the index (9) indicating the greatest damage caused by nematodes in this research (Table 3). All treatments based on soil amendments were significantly better in the evaluation of the carrot root gall index than the control treatment. Oka and Yermiyahu (2002) explained that high nitrogen concentrations and high EC values in natural amendments contribute to the nematode suppression. Higher population density of *Meloidogyne* spp. juveniles was noted in FLU+LCM compared to LCM, although value of the root gall index was the same in FLU and FLU+LCM, which is probably due to better suppression of plant parasitic nematodes in plant treated with natural amendment. Many different factors can impact the root galling, such as *Meloidogyne* species present in the soil, cultivated plant species, cultivar, defensive mechanisms of the plants etc. According to Raspudić et al. (2006) and Ivezic et al. (2001), the average population density of *Meloidogyne* spp. in pepper was 601 per 100 g of soil and 3570 nematodes in 1 g of pepper root without visible symptoms on the roots, although above ground symptoms were observed.

Table 3. The average root gall index of carrot root per treatments (July, 2017)

Tablica 3. Prosječan indeks gukavosti korijena mrkve po tretmanima (srpanj, 2017.)

Treatments *Tretmani*	**C**	**FLU**	**FLU+LCM**	**LCM**
The root gall index *Indeks gukavosti*	9^{A}	4^{C}	4^{C}	6^{B}

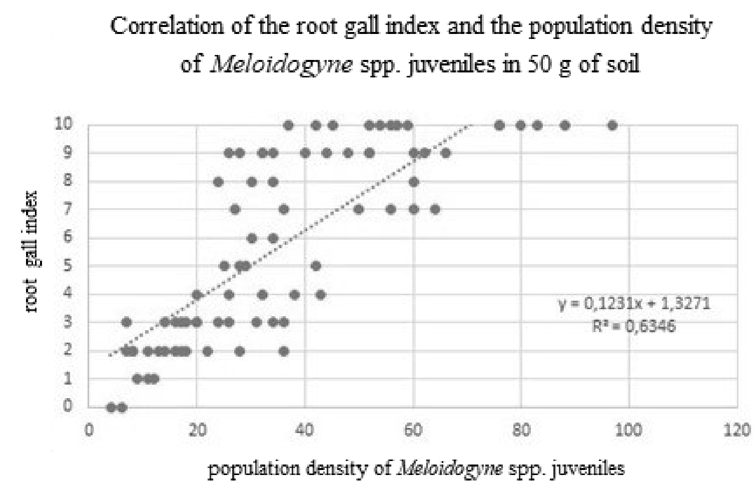
Treatments: control (C), fluopyram (FLU), liquid chicken manure+fluopyram (FLU+LCM), liquid chicken manure (LCM)
Tretmani: kontrola (C), fluopiram (FLU), tekući pileći stajnjak+fluopiram (FLU+LCM), tekući pileći stajnjak (LCM)

The statistically significant differences ($P \leq 0.05$) are marked with different letters in a row
Statistički značajne razlike ($P \leq 0,05$) označene su različitim slovima unutar reda

Graph 1 shows the correlations between the root gall index and the population density of the *Meloidogyne* spp. juveniles in the soil for all treatments. The coefficient of correlation (R=0,84*) showed statistically significant influence of the number of *Meloidogyne* spp. juveniles in the soil on the root gall index.

In treatment C, the coefficient of correlation (R=0.58) showed no statistical significance between the number of *Meloidogyne* spp. juveniles and the root gall index, because a large number of samples had the same root gall index (10). The coefficient of correlation in treatment LCM (R=0.81*) and treatment FLU (R=0.84*) indicates a significant influence of liquid chicken manure preparation and fluopyram on the number of *Meloidogyne* spp. juveniles on the root gall index. The coefficient of correlation (R=0.91**) in combined treatment (FLU + LCM) showed that the population density of *Meloidogyne* spp. juveniles in the soil had very significant effect on the root gall index.

In the paper of Vrain (1982), the relationship between *Meloidogyne hapla* density and damage to carrots in organic soils was researched and showed significant negative correlation between weight of the roots and initial nematode density in field plots. Nematodes caused malformations, galling, and hairiness of carrot roots and affected weight of roots and foliage.



Graph 1. Correlations of the root gall index and the number of *Meloidogyne* spp. juveniles in the soil (all treatments)

Grafikon 1. Korelacija indeksa gukavosti korijena i broja ličinki **Meloidogyne** *spp. u tlu (svi tretmani)*

Yield (Table 4) was statistically significantly different between treatment C and treatment FLU, but FLU treatment does not differ significantly from FLU+LCM and LCM, indicating that all amended treatments had a positive influence on the carrot root yield. In the research of Riegel and Noe (2000), plant growth increased as chicken litter rates increased, regardless of the presence or absence of *M. incognita*.

Table 4. Average yield (kg/m²) of carrot root by treatments, July 2017

Table 4. Prosječan prinos korijena mrkve (kg/ m²) po tretmanima, srpanj 2017.

Treatments *Tretmani*	C	FLU	FLU+LCM	LCM
Average yield of carrot root (kg/ m²) *Prosječan prinos korijena mrkve (kg/ m²)*	$4,050^{A}$	$5,475^{B}$	$4,55^{AB}$	$4,425^{AB}$

Treatments: control (C), fluopyram (FLU), liquid chicken manure+fluopyram (FLU+LCM), liquid chicken manure (LCM) / *Tretmani: kontrola (C), fluopiram (FLU), tekući pileći stajnjak+fluopiram (FLU+LCM), tekući pileći stajnjak (LCM)*

The statistically significant differences (P≤0.05) are marked with different letters in a row / *Statistički značajne razlike (P≤0,05) označene su različitim slovima unutar reda*

After investigating the effectiveness of preparations based on fluopyram and liquid chicken manure on nematodes of the genus *Meloidogyne* in carrot crop, their impact on total biodiversity of nematode community in the soil was also determined. Lowest nematode biodiversity was found in samples treated with FLU (18 different genera) and FLU+LCM (15). The largest biodiversity of the whole nematode community in the soil was established in the treatment C (22) and treatment LCM (23). Genera that were found in all 4 treatments are *Acrobeloides, Acrolobus, Aphelenchoides, Aphelenchus, Ditylenchus, Eucephalobus, Gracilacus, Meloidogyne, Paratylenchus, Plectus, Rhabditis* and *Teratocephalus*. In addition to these genera, *Acrobeles, Acrobelophis, Drilocephalobus, Eudorylaimus, Mesodorylaimus, Panagrolaimus, Pratylenchus, Tyelencholaimellus, Tylenchorhynchus* and *Tylenchus* were determined in C, *Eudorylaimus, Filenchus, Panagrolaimus, Pratylenchoides, Pratylenchus* and *Tylenchorhynchus* in FLU, *Metateratocephalus, Tylenchorhynchus* and *Tylenchus* in FLU+LCM and *Acrobeles, Alaimus, Eudorylaimus, Filenchus, Mesodorylaimus, Metateratocephalus, Panagrolaimus, Pratylenchus, Psilenchus, Turbatrix* and *Tylenchus* were determined in LCM. Riegel and Noe (2000) state that the microbivorous nematode densities increased as chicken litter rates increased. Liquid chicken manure contributed to enhanced nematode activity through increased microbe activity in the soil.

CONCLUSION

Population density of *Meloidogyne* spp. juveniles varied between the treatments, and in all treatments with fluopyram (FLU and FLU + LCM) the number of *Meloidogyne* spp. was significantly decreased indicating the nematicidal efficacy. The value of the root gall index was the lowest in all treatments with fluopyram preparation, but the liquid chicken manure preparation also reduced the galling of the roots. The coefficient of correlation showed statistically significant influence of the population density of *Meloidogyne* spp. juveniles in the soil on the root gall index in the amended treatments (FLU, FLU + LCM, LCM). Amended treatments had a positive effect on the carrot yield, although only FLU significantly differed from the control treatment, but it did not significantly differ from the other treatments. Based on the soil sampling periods through March, June and July, it was evident that the population of *Meloidogyne* spp. juveniles increased with the plant growth, regardless of the treatments. The highest biodiversity of the nematode community in the soil was established in the treatments LCM and C, while fluopyram negatively affected the biodiversity indicating greater disturbance for the nematode community structure.

It can be concluded that fluopyram and liquid chicken manure preparation have nematicidal potential and because of the positive effect of liquid chicken manure on nematode biodiversity and, according to literature data, great potential in *Meloidogyne* spp. control, higher doses of this treatment should be tested in further research.

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UTJECAJ PRIPRAVKA NA BAZI FLUOPIRAMA I TEKUĆEGA PILEĆEG STAJNJAKA NA NEMATODE KORIJENOVIH KVRŽICA (*Meloidogyne* spp.) U NASADU MRKVE

SAŽETAK

Cilj ovoga istraživanja bio je ispitati nematocidni učinak pripravaka na bazi fluopirama (pesticid) i tekućega pilećeg stajnjaka (prirodni poboljšivač tla) na gustoću populacije ličinki nematoda korijenovih kvržica, **Meloidogyne** ***spp. Poljski pokus postavljen je u četiri tretmana: kontrola (C), fluopiram (FLU), fluopiram i tekući pileći stajnjak (FLU + LCM) te tekući pileći stajnjak (LCM) slučajnim blok-rasporedom u četiri ponavljanja. Gustoća populacije ličinki*** **Meloidogyne** ***spp. varirala je između tretmana, a broj ličinki*** **Meloidogyne** ***spp. značajno je smanjen u svim tretmanima s fluopiramom (FLU i FLU + LCM). Svi pripravci (FLU, FLU + LCM, LCM) utjecali su na smanjenje gukavosti korijena. Navedeni tretmani pozitivno su utjecali na prinos mrkve. Populacija ličinki*** **Meloidogyne** ***spp. porasla je razvojem biljaka, bez obzira na primijenjeni tretman. Fluopiram je negativno utjecao na bioraznolikost, koja ukazuje na veća uznemirenja u strukturi zajednice nematoda u tlu. Može se zaključiti kako pripravci na bazi fluopirama i tekućega pilećeg stajnjaka imaju nematocidni potencijal, a tekući pileći stajnjak održava ili povećava bioraznolikost nematoda.***

Ključne riječi: nematode korijenovih kvržica, fluopiram, tekući pileći stajnjak, indeks gukavosti korijena, mrkva

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