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Influence of *Fusarium oxysporum*-resistant pumpkin rootstock on the productivity of the F_1 Asylum cucumber hybrid

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ABSTRACT

We examined five pumpkin cultivars (including three selected by the Kazakh Institute of Fruit and Vegetable Growing) for resistance to *Fusarium*. Among resistant rootstocks to the *Fusarium* pathogen, we distinguished Mozoleevskaya 10, Stofuntovaya, and Karina cultivars for further work. The results showed that when grafting cucumber on pumpkin, the best rootstock options compared to the root-related standard of the cucumber hybrid Asylum were the pumpkin cultivars Karina and Stofuntovaya. Their early yield indicators were in the range of 3.46 and 3.39 kg m^{-2}. It exceeded the standard by 36.7% and 33.9%, respectively. In terms of total productivity, the grafting varieties for the pumpkin cultivars, i.e., Karina and Stofuntovaya also showed high yields: 27.56 kg m^{-2} and 26.65 kg m^{-2} respectively, which significantly exceeded the rootstock standard by 42.38% and 36.68%, respectively.

Keywords: Pumpkin, Plant, *Fusarium*, Cucumber, Cultivar.

Article type: Research Article.

INTRODUCTION

The protected ground vegetable growing is one of the most intensive branches of crop production, which uses modern, technological cultivation methods. Cucumber, *Cucumis sativus* L. is one of the main vegetable crops of protected soil. However, its yield remained at a low level. One of the reasons for the decline in yield in protected soil is the influence of pathogenic microflora, including fungi of the genus *Fusarium*. The harmfulness of the disease depends on the period of infection of the plant, the degree of development of the disease, the susceptibility of the variety or hybrid, and climatic conditions (Feoktistova 2000, EL-Saman *et al.* 2023; Al-Enezi & Jamil 2023). Wilts caused by fungi of the genus *Fusarium* link are widespread. Breeding work to identify resistant samples is essential in vegetable crops. Fusarium wilt, caused by the pathogen *Fusarium oxus* (Schlecht.) f. sp. *lycopersici* (Sacc.) et Hansen, is one of the most common and harmful diseases. This type of lesion leads to significant yield losses, both in the open and protected ground. With the development of protected soil, Fusarium has become one of the most common diseases in greenhouses. According to V. D. Poliksenova, Fusarium wilt causes great damage in greenhouses, especially during the winter-spring crop rotation. When the pathogen is in a greenhouse, it can survive in the soil for a long time, colonizing plant residues or forming chlamydospores. Sometimes, the source of infection is superficially infected seeds (Fletcher 1987). Using a resistant or tolerant varietal material to many critical fungal diseases (such as pumpkin varieties resistant to Fusarium wilt), one can achieve high productivity of grafted plants. Varieties with complex resistance (multiple resistance) are of great importance. Increased resistance of cucumber and pumpkin varieties and hybrids to pathogens, their correct selection, and management of their cultivation according to local conditions are essential elements of integrated plant protection (Zorina *et al.* 1981). Selection in the finding of disease resistance is not fundamentally different from the selection for other properties. However, according to the technique of execution, the selection for resistance to harmful organisms has its differences. The study includes the following main stages: (i) the creation of an infectious (invasive) background, (ii) the development or selection of methods for assessing resistance to

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the pathogen, (iii) the evaluation of breeding material against an infectious background to identify resistant forms, and (iv) the inclusion of donors of resistance to the pathogen in the selection process (Amini & Monakhos 2005) Grafting can overcome many of these problems. It is a routine technique in continuous cropping systems in many parts of the world (Wang *et al.* 2020; Liu & Zhang 2020). It was first used in Japan in the late 1920s by grafting watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai on the pumpkin (*Cucurbita moschata Duchesneex.* Poir) rootstocks (Oda *et al.* 1993). Shortly after that, the watermelons were grafted onto the rootstocks of the bottle gourd (*Lagenaria siceraria* (Molina) Standl.). This practice helped controlling the decline in yield due to soil diseases. China produces more than half of the world's watermelons and cucumbers, *Cucumis sativus* L. About 20% of them are grafted (Lee 1994). The grafting of vegetable crops on stable rootstocks is widely used in Europe and Asia (Oda 2002). In South Korea and Japan, almost all cucumbers in the protected ground are grafted on different resistant rootstocks depending on the season and cultivation methods. In open ground, the plantings of grafted cucumber plants reach 30% (Ahn *et al.* 1999; Alan *et al.* 2007). When used as a pumpkin rootstock, cucumber plants receive enhanced nutrition (Shoibekova *et al.* 2021). Grafted cucumber plants grow faster, develop better, and are more resistant to diseases (Karataev & Borak Siham 1987). More powerful, resistant to many weather factors, the root system of pumpkins provides high yield, allows to tolerate stresses more easily, and increases plants' resistance to diseases and pests (Gruzdov 1954). In addition, grafting can affect vegetative growth, flowering, ripening time, and fruit quality and provide higher yields, especially in low-temperature conditions. Rootstock-graft combinations affect pH, taste, sugar, colour, and carotenoid content (Shoibekova & Dzhantassov 2021). The main motive for inoculating pumpkin crops is to avoid the damage caused by soil pathogens when genetic or chemical approaches to disease control are not available (Alexopoulos 2007). There are various ways of grafting vegetable crops. O. V. Yurin describes six methods of grafting pumpkins. The most common ways of grafting pumpkin crops in greenhouse production are (i) inside cleft grafting, (ii) in a cleft-green grafting, and (iii) by approaching the shoots. Three methods produce the grafting of cucumber on pumpkin. The first method is to make a cleft right into the mesocotyl. The second method is when the shoots are brought closer together by cutting on the crusts. The third method is the convergence between the tongues in the side cleft grafting of the stems themselves. The simplest and most optimal grafting options are the last two of them (Alan *et al.* 2007).

MATERIALS AND METHODS

We conducted the study in 2018-2020 in the Immunity Laboratory and Breeding Greenhouse of the Kazakh Research Institute of Fruit and Vegetable Growing LLP in the Karasai district of the Almaty region. The study object was five varieties of pumpkin: Aphrodite, Karina, Mindalnaya, Mozoleevskaya 10, Stofuntovaya, and the cucumber hybrid Asylum (Shoibekova *et al.* 2021).

Table 1. Classification of varieties and species of pumpkin and cucumber used in the experiment.

Type, variety	Fruit shape	Fruit color	Yield ton ha^{-1}	Average fruit weight (kg)
Large-fruited (Karina)	round	green, white, pink, red	7.50	7.50
Muscat Aphrodite	rounded cylindrical with an interception	brown-yellow	25.5-38.3	4.35
Solid pumpkin Mozoleevskaya 10	round	bright yellow with a pattern	25.0-30.0	5.15
Solid pumpkin Mindalnaya	round	yellow with an orange tinge	30.0-35.0	5.00
Solid pumpkin Stofuntovaya	round	orange, white, gray, pink	25.0-30.0	4.50
Asylum	elongated cylindrical	green	29-30	0.09-0.1

Table 1 shows that the highest yield is characterized by the variety Karina from the studied pumpkin cultivars. The weight of the fruit reaches up to 10 kg. It has denser flesh, better quality, and higher yield of marketable products. It stores longer than other types of pumpkin. In addition, Karina cultivar has good friability. Karina is a large-fruited pumpkin of the selection of the Kazakh Research Institute of Potato and Vegetable Growing. It is long-leaved, its fruits are round-flattened, medium-sized, grey and green, and less often variegated, pinkish when stored. The average weight of the fruit is 2 to 10 kg. The cultivar is medium-ripe, yielding. Keeping quality and transportability are high. It has high taste qualities of fruits: the content of dry matter: 17%, sugars: 11.5%, vitamin

C: 20 mg/% and carotene: 13 mg/%. The marketability of fruits is high at 97%. The variety is relatively resistant to powdery mildew and peronosporosis. Approved for use: Since 2003 in Aktobe, Almaty, Atyrau, East Kazakhstan, Zhambyl, Kyzylorda, Pavlodar, South Kazakhstan regions. Mozoleyevskaya 10 is a solid pumpkin selected by the Kazakh Research Institute of Potato and Vegetable Growing. The plants are long-branched; the fruits are cylindrical, ribbed at the peduncle, light orange at full maturity. The pattern is broad spotty bands, initially dark green and dark orange at full maturity. The flesh is yellow or creamy, 3-5 cm thick, medium-bodied, slightly sweet. The seed nest is large. The seeds are medium oval, yellowish-cream with a rim. The average mass of the fruit is 4.5-7.0 kg. Intermediate variety (102-117 days), productive, with high commodity qualities and good taste. Maintaining quality and transportability is high. Approved for use: Since 1955 in Akmola, East Kazakhstan, Karaganda, Kyzylorda, Kostanay, Pavlodar, North Kazakhstan regions. Aphrodite (Muscat) is a selection of the Kazakh Research Institute of Potato and Vegetable Growing, a sample obtained from the originator. The fruits are elongated, with a swollen end, of the "intercept" type. The tail part of the fruit occupies up to 2/3 of its entire length and has no voids. The colour of the fruit is orange with a pattern in the form of brown indistinct torn stripes. When fully matured, the pattern disappears. The flesh is thick orange, sweet and dense. The surface of the fruit is smooth, with a powdery-bluish waxy coating, and the peduncle is ribbed. The average weight of the fruit is 5-8 kg. The variety is medium-ripe for table consumption. The cultivar has been approved for use since 2004 in Almaty and East Kazakhstan regions. Mindalnaya is a large-fruited, long-fruited pumpkin. It is a medium maturing long-fruited variety with orange-red flattened fruits that have broad longitudinally broken stripes. The average weight of the fruit is 4-7 kg. The yellow flesh has an orange hue. The flesh is dense, crisp and sweet. This cultivar ripens in 115-130 days. Quality yielding, stored well. Approved for use: Since 2005 in Aktobe, Almaty, Zhambyl, Kyzylorda, Pavlodar, South Kazakhstan regions. Stofuntovaya is a large-fruited, versatile pumpkin. It is a late-maturing cultivar with long stalks. The oval fruits are smooth, slightly segmented. They are yellow, orange, white, grey, or pink. The fruit weighs 6-10 kg. The flesh is white-yellow or orange, thick, little sweet and sugar content is 4%-5%. The cultivar has been approved for use since 2003 in Almaty, Kyzylorda, Pavlodar, and South Kazakhstan regions. Asylum greenhouse hybrid is a medium maturing cultivar. The total yield is 29-30 kg m^{-2}. The marketability of fruits is 80%-85%. The pumpkin is resistant to powdery mildew and feather dew. The shape is elongated-cylindrical. The fruit is green. The average length of the fruit amounts to18-20 cm. The average diameter is 3-3.5 cm. The pumpkin weighs 90-100 g. The skin is thin, soft, crisp. The cultivar has been approved for use since 2016 in all regions of the Republic of Kazakhstan. We evaluated pumpkin cultivars to the *Fusarium oxysporum* pathogen with the isolation of resistant forms in the Immunity Laboratory. We did experiments to study the effects of *F. oxysporum*-resistant pumpkin rootstocks on the productivity of the F_1 Asylum cucumber hybrid in the spring-summer in a breeding greenhouse. Phenological observations and biometric measurements were carried out according to the methods recommended in vegetable growing. We used the generally accepted methods approved by many years of practice: field experiment methodology (Dospekhov 1985) and methodological recommendations for conducting experiments with vegetable crops in protected ground structures (Vashchenko *et al*. 1976). The nutritional regime of plants is generally accepted for protected soil. Soil moisture during the growing season was maintained within the optimal parameters.

RESULTS AND DISCUSSION

During the growing season of vegetable crops in the greenhouse, we observed the development of the disease. The symptoms of the disease were described, and plants with signs of infection with *Fusarium* fungi were selected for phytopathological analysis (VASKhNIL 1981). We isolated *Fusarium* pathogens according to the generally accepted method (Chumakov1974; VASKhNIL 1986) and cultivated crops under the optimal temperature (24-26 °C) and light conditions to develop pathogens (Alpatiev *et al*. 1976; Podkina & Kotlyarova 1989). We also studied the morphological and cultural characteristics of the isolated isolates on a nutrient medium (potato-sucrose agar). The identification of the *Fusarium* pathogen was carried out using the determinants (VASKhNIL 1986). To determine the harmfulness of *Fusarium* on cucumber in the phase of flowering and fruit formation in greenhouse conditions, we simultaneously identified and tested 20 plants (in three-fold repetition) with various degrees of *Fusarium* damage. The degree of the lesion was determined visually according to a 4-point scale. Infection with a pathogen and assessment for resistance to *Fusarium* were carried out according to the guidelines for conducting stationary experiments to study measures to combat cucumber diseases in film greenhouses [20]. A visual record was made of the infestation of the plants by *Fusarium*. A 4-point scale was applied 40-50 days after planting to

determine the extent to which the aboveground parts of the plants are affected by *Fusarium* wilt (point 0: healthy plants; point 1: grafting of lower leaves during hot hours of the day; point 2: binding of middle leaves, yellowing of lower leaf tips; point 3: wilting of all leaves, death of lower leaves; point 4: the plant turns yellow and withers). During the statistical processing of the experimental data, the sample averages and their standard deviations were calculated. During 2018, we visually identified cucumber plants affected by *Fusarium* in the greenhouse during the growing season. Then, leaves with petioles and stems were selected from the affected plants to isolate *Fusarium* isolates from the affected plants. Parts of the plants were thoroughly washed under running water, then disinfected by immersion in a sodium hypochlorite solution (the preparation "Belizna" bleach diluted with distilled water 1:3). We placed the disks of filter paper moistened with distilled water in the cups. The discs were kept for up to 48 h at a temperature of 15-20 °C. The mycelium of the pathogen on the slices was placed on a nutrient medium in Petri dishes on potato agar. Isolates were isolated from the affected cucumber plants into a pure culture by transferring to a nutrient medium, then maintained *in vitro*. When the colony grew, a pathogen passage was performed.

Fig. 1. Sporulation of the *Fusarium* pathogen in a moisture chamber.

We prepared the nutrient medium (potato-sucrose agar), then potato broth. We grated 250 g of potatoes, supplemented with three times the amount of distilled water, filtered and sterilized for 1 h at 75 kPa. We composed the nutrient media: added 230 mL of potato broth to 770 mL distilled water, 20 g agar, 20 g sucrose, filtered, supplemented with distilled water to 1000 mL, and sterilized in an autoclave for 20 min at 120 °C. The next step was to identify the pathogen, i.e., to identify it. We identified the pathogen using a microscope. Fig. 3 shows *Fusarium oxysporum* spores obtained from the affected plants. The pathogen was grown on potato-sucrose agar before spore formation. When assessing pathogenicity, one should prepare a sufficient volume of infectious material. Spores were collected by removing the air mycelium with a spatula. The concentration of spore suspension used for infection was determined. There should be at least 20 conidia of the *Fusarium* pathogen in the field of view in the eyepiece of a microscope at a magnification of 8 × 15. We prepared a collection of five cultivars of pumpkin with subsequent planting of plants on an infectious background. *Fusarium* infection of pumpkin plants was carried out by dipping the pruned roots in a suspension of spores, then planting in pots. The plants were grown under optimal conditions for the pathogen (light, temperature, and air humidity). The registration was started after the appearance of symptoms. The duration of the incubation period under optimal environmental conditions was 2-3 weeks. We evaluated five pumpkin cultivars from the institute's gene pool collection for resistance to fusariosis on an artificial infection background. The varieties were sown. The varieties Mozoleyevskaya 10, Mindalnaya, Stofuntovaya, and Karina showed no signs of *Fusarium* infestation on the first date of counting, while the variety Aphrodite showed minor symptoms in 5 % of plants. By the second count, three varieties showed signs of *Fusarium* infestation: Mindalnaya and Aphrodite 10% each, and Stofuntovaya 5%. At the very beginning of infection, the Aphrodite variety showed a slight susceptibility to *Fusarium* at an early stage of development. However, by the third count, the percentage of damage was at the level of the other affected varieties. At the third census, no plants with signs of *Fusarium* were noted in the variety Mozoleyevskaya 10. We detected the disease in 4 varieties (10% of plants in the varieties Stofuntovaya and Karina, 20% in the varieties Almond and Aphrodite). We can identify Mozoleyevskaya 10, Stofuntovaya, and Karina cultivars as *Fusarium*-

resistant rootstocks for further work. In the subsequent stages of the work, the Asylum, a regionalized cucumber hybrid was grafted on the previously evaluated *Fusarium*-resistant pumpkin rootstocks. Seedlings of five pumpkin varieties were sown to evaluate the best rootstocks, as well as a cucumber hybrid Asylum as a rootstock. The standard was a rootstock hybrid Asylum. By an identical date of sowing, grafting, and planting in a permanent place of all samples, the beginning of flowering, early laying of the ovaries, and earlier arrival of the crop on the standard were noted earlier, these indicators in the grafted plants were affected by the time of accretion of the grafting site.

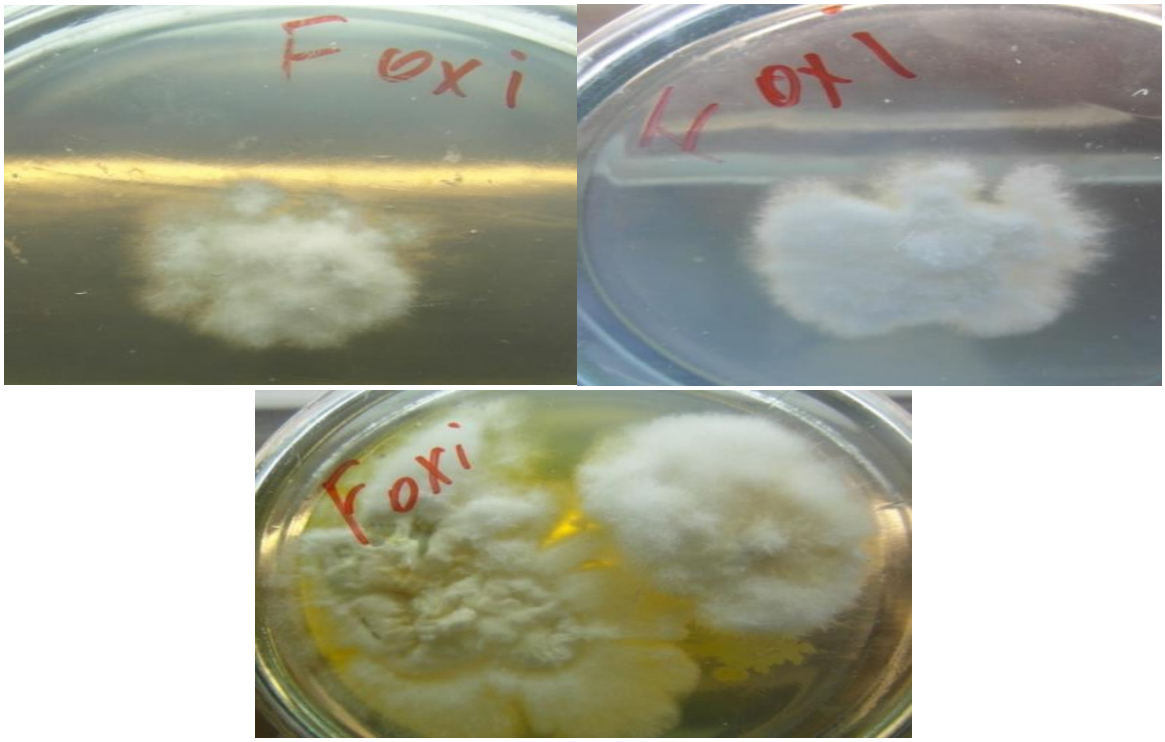

Fig. 2. Development of the Fusarium pathogen on nutrient media.

Fig. 3. Conidia of the pathogen Fusarium oxysporum in the microscope eyepiece.

a) b) c) d) e)

Fig. 4. Preparation of pumpkin varieties for infection with a suspension of Fusarium spores: a) - Mozoleevskaya 10; b) – Mindalnaya; c) – Stofuntovaya; d) - Aphrodite; e) - Karina.

Table 2. Evaluation of pumpkin varieties for resistance to Fusarium on an artificial infectious background.

No.	Cultivars	22.12*		10.01		30.01	
		%	Score	%	Score	%	Score
1	Mozoleevskaya 10	0	0	0	0	0	0
2	Mindalnaya	0	0	10	1	20	1
3	Stofuntovaya	0	0	5	1	10	1
4	Afrodita	5	1	10	1	20	1
5	Karina	0	0	0	0	10	1

* The first assessment for infestation was carried out on the fourteenth day after infection.

Table 3. Dates of pssing of phenological phases by pumpkin and cucumber plants.

Samples	Seeding	Sowing (75%)	Grafting	Vegetation	The start of blooming	Beginning of fetal maturation	Sowing to (days)		
							seedling mass	flowers of the main axis,	1 fruit collection
Afrodita	February 28	March 6	March 12	April 5	April 19	May 3	7	50	64
Karina	February 28	March 6	March 12	April 5	April 20	May 1	7	51	62
Mindalnaya	February 28	March 6	March 12	April 5	April 21	May 5	7	52	66
Stofuntovaya	February 28	March 6	March 12	April 5	April 19	May 5	7	50	66
Mozoleevskaya 10	February 28	March 6	March 12	April 5	April 20	May 1	7	51	62
Asylym, st. (rootstock)	February 27	March 5	-	April 5	April 17	April 29	7	47	59

Table 4. Biometric indicators of grafted cucumber plants.

No.	Variety, Hybrid	Height (cm)		Quantity (pieces)		Leaf diameter (cm)
		plants	internodes	leaves	flowers	
1	Afrodita	131.3	15.7	21.7	3.9	9.0
2	Karina	134.6	20.0	23.3	8.3	9.7
3	Mindalnaya	122.0	17.7	21.3	2.7	1.3
4	Stofuntovaya	111.7	16.7	22.7	4.7	9.0
5	Mozoleevskaya 10	135.3	21.7	24.3	4.3	9.7
6	Asylym, st. (rootstock)	128.4	18.4	24.6	4.8	8.9

Given the height of plants, we noted that on the rootstock of Mozoleyevskaya 10, Karina, and Aphrodite developed better, and the height of plants exceeded the rootstock standard by 5.3%, 4.8%, and 2.2%, respectively. Regarding the number of flowers on the plant, the root-related standard exceeded the variant grafted on the Karina pumpkin by 72.9%. In other variants, this index was within the standard. We noticed an increase in the leaf blade in three variants grafted onto the pumpkin varieties i.e., Mindalnaya, Karina, and Mosoleyevskaya 10 (15.7%, 8.9%, and 8.9%, respectively). This fact shows a well-developed root system of rootstock on these variants and good fusion with the scion. When evaluating the options of grafting on different pumpkin varieties, the variants stood out with respect to rootstock hybrid - standard in both early yield arrival of the crop and total productivity. For early yield (for the first month) stood out the variants with grafting on Karina and Stofuntovaya: indices were in the range of 3.46 and 3.39 kg m^{-2} that exceeded the standard by 36.7% and 33.9%, respectively. In terms of total productivity, the variants grafted on Karina and Stofuntovaya exhibited 27.56 kg m^{-2} and 26.65 kg m^{-2}, which significantly exceeded the rootstock standard by 42.38% and 36.68%, respectively. The other variants were within the margin of error of the experiment or did not exceed the standard. In terms of index, the average fruit weight exceeded the variant grafted on Mozoleevskaya 10: 115 g; the other variants were within the standard. No mineral fertilizers and growth regulators were used in the cultivation of cucumbers. An increase in cucumber fruit yield was obtained by applying a new cultivation technology: grafting cucumber plants onto pumpkin varieties. When evaluating grafting options for different varieties of pumpkin, the variants of the root-related hybrid-the standard for both early harvest and overall productivity were distinguished. Thus, according to the early yield (for the first month), the variants with grafting on the varieties of pumpkin, i.e., Karina and Hundred-pound were distinguished. The indices were in the range of 3.46 and 3.39 kg m^{-2}, which exceeded the standard by 36.7% and 33.9%, respectively.

Table 5. Productivity indicators of grafted cucumber plants.

No.	Variety, Hybrid	Yield				Average fruit weight, g
		For 1 month	for the growing season		in %	
			in total	marketability		
1	Afrodita	2.73	21.18	21.0	+9.89	110.8
2	Karina	3.46	27.56	27.21	+42.38	104.6
3	Mindalnaya	2.1	17.76	17.54	-	107.8
4	Stofuntovaya	3.39	26.65	26.12	+36.68	102.0
5	Mozoleevskaya 10	2.59	20.93	20.77	+8.68	115.2
6	Asylym, st. (rootstock)	2.53	19.45	19.11	-	111.0
	$HCP_{(05)}$		1.46			

According to the indices such as total productivity, grafting options for Karina and Stofuntovaya showed yields of 27.56 kg m^{-2} and 26.65 kg m^{-2}, which significantly exceeded the root standard by 42.38% and 36.68%, respectively. The rest of the options were in the case of experience errors or did not exceed the standard. According to the indices, the average weight of the fruit exceeded the vaccination option for the pumpkin variety Mozoleevskaya: 10-115 g. The other options were within the standard. When growing cucumbers, no mineral fertilizers and growth regulators were used. The increase in the yield of cucumber fruits was obtained with new growing technology: grafting cucumber plants on pumpkin varieties. When evaluating the options of grafting on different pumpkin varieties, the options stood out relative to the rootstock hybrid-standard in terms of both early yield and overall productivity. Thus, for the early yield (for the first month) stood out options with grafting on pumpkin varieties, Karina and Stofuntova - indices were within 3.46 and 3.39 kg m^{-2} that exceeded the standard by 36.7% and 33.9%, respectively. In terms of total productivity, the variants grafted on Karina and Stofuntovaya exhibited yields of 27.56 kg m^{-2} and 26.65 kg m^{-2}, which significantly exceeded the rootstock standard by 42.38% and 36.68%, respectively. The other variants were within the margin of error of the experiment or did not exceed the standard. The variant grafted on Mozoleevskaya 10 (115 g) exceeded the standard in terms of average fruit weight. Other cultivars were within the limits of the standard. No mineral fertilizers and growth regulators were used when growing cucumbers. An increase in cucumber fruit yield was obtained by applying a new cultivation technology: grafting cucumber plants onto pumpkin varieties.

CONCLUSION

We evaluated five pumpkin cultivars against the *Fusarium ochysporum* pathogen. The varieties Mozoleyevskaya 10, Stofuntovaya, and Karina were selected for further work as resistant rootstocks to fusariosis. The results showed that when grafting cucumber on the pumpkin, the best rootstock options compared to the root-related standard of the cucumber hybrid Asylum were the pumpkin varieties, i.e., Karina and Stofuntovaya, whose early yield indices were in the range of 3.46 and 3.39 kg m^{-2}, exceeding the standard by 36.7% and 33,9%, respectively. In terms of total production, the grafting options for Karina and Stofuntovaya also displayed high yields: 27.56 kg m^{-2} and 26.65 kg m^{-2}, which significantly exceeded the rootstock standard by 42.38% and 36.68%, respectively.

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