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Fungal Diseases of Persian Walnut in Uzbekistan and their Control

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ABSTRACT

Surveys have been made in Persian walnut plantations and groves in 2018 and 2019 in three regions of Uzbekistan. It has been revealed that anthracnose caused by Gnomonia leptostyla had the widest distribution among all diseases registered. Walnut varieties have varied significantly on resistance to the disease. Occurrence of downy mildew (Microstroma juglandis) was much lesser. Shaggy bracket fungus (Inonotus hispidus), the cause of white heart rot has been more or less common on walnut trees.

Two-years field trials have been carried out to determine biologic efficacy of several fungicides against anthracnose on leaves, twigs and fruits of walnut. Difen Super 55% WP, Syllit 40% SC, Falkon 46% EC and Score 25% EC have revealed the highest efficacy (90% to 100%). Efficacies of lower rates of Syllit 40% SC and Falcon 46% EC, and Curzate WP, Coritus 50% WDG and Cresoxin 50% WDG were between 74.7% and 88.8%, while that of Myseb M-45 WP was under 65%; biological fungicides Phytolavin Water Soluble Concentrate and Sporagin WSC have shown efficacies between 20.9% and 45.0%.

Keywords: *Persian walnut, Disease, Anthracnose, Fungicide, Biologic efficacy.*

INTRODUCTION

Persian, or English walnut (*Juglans regia* L., literally "royal walnut") is a gift of the nature to humans. Walnuts are an excellent source of protein, dietary fiber, dietary minerals, B vitamins, and polyunsaturated α-linoleic, linoleic acids, and monounsaturated oleic acid. These substances have cardio protective effects, induce and regulate sleeps, reduce cancer, and delay neurodegenerative diseases of aging (Hassan & Ahmad, 2017).

Walnut is grown in 54 countries of the world. In 2017, the largest walnut producing countries were China, USA, Iran, Turkey, Mexico, Ukraine and Chile, whereas Uzbekistan stood eighth, and third in the world on fruit productivity per hectare (FAOSTAT, 2019).

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During years of independence production of walnut fruits in Uzbekistan has increased year-by-year. So, if 4 to 5 tonnes of fruits (with shells) had been annually harvested in 1992-1996, then 50,000 tonnes were harvested each year since 2015 (FAOSTAT, 2019).

Forest stands of walnut are widespread in Uzbekistan up to the area of about 4,000 ha on slopes of Western Tien-Shan and Pamir-Alay at heights 800 to 2,300 m above sea level (Mapelli & Vildanova, 2009). Uzbekistan takes steps for subsequent development of walnut growing. According to the decree of the President of the country of June 1, 2017, the "Association of walnut producers and exporters" has been established. It was planned to import seedlings of walnut and to expand areas of plantations up to 10,000 hectares. Realization of this program is in progress, and it requires to ground it scientifically, and to secure protection of walnut trees from diseases in particular.

According to the scientific literature more than 140 diseases occur on Persian and other walnut species. Reportedly about 60 diseases are registered on walnut trees in Uzbekistan. However, these data were generated basing mostly on mycological studies, and purposeful surveys for diseases are few and outdated (Kleyner, 1963). Recommendations on controlling walnut diseases included almost only cultural methods, but using chemical control has not been investigated properly. Purpose of our studies was to determine occurrence and severity of the main fungal diseases of Persian walnut, and to evaluate an efficacy of modern fungicides against walnut anthracnose.

MATERIALS AND METHODS

Plantations and groves of walnut trees have been surveyed in 2018 and 2019 in 11 sites of the following 7 districts located in Tashkent, Samarkand and Fergana regions.

TP – Tashkent region, Piskent district, M. Khaydarov farm, plantation of Gold Dried Fruit Co. Ltd., 3rd year seedlings.

TK – Tashkent region, Kibray district, Yusuf Kadyr Ziyo farm, 3rd year seedlings**.**

FK – Fergana region, Kushtepa district, Feruzbek nuri farm, Ideal var. – 15 year-old trees, and Jubilee var. – 12 year-old trees.

FD – Fergana region, Dangara district, Hamid G'ulom farm, Ideal var. – 17 year-old trees.

SU – Samarkan region, Urgut district, Amankutan farmers' society, plantation of the forest farm, 21 year-old trees**.**

SD – Samarkand region, Jambay district, Ko'lbasty farmers' society, plantation of the forest farm, 3rd year seedlings**.**

ТБ – Tashkent region, Bostanlyk district, BMRS-RIOG, 39 to 40-year old trees, and 3rd year seedlings in the nursery.

Table 1: Walnut plantations and groves surveyed in 2018 and 2019

No.	Survey location and date	Area, ha		Height above sea level, m	Variety
		total	surveyed		
1.	**TP** – 26.04.2018	230	2	478	Chandler, Franquette
2.	**TK** –31.05.2018	14	1	486	Chandler
3.	**FK** – 29.06.2018	1	1	440	Ideal
4.	**FK** – 10.07.2019	1	1	440	Ideal
5.	**FK** – 10.07.2019	0,5	0,5	440	Jubilee
6.	**FD** – 29.06.2018	0,3	0,3	387	Ideal
7.	**FD** – 10.07.2019	0,3	0,3	387	Ideal
8.	**SU** –11.07.2018	2	2	925	Bostanlyk
9.	**SU** –10.-7.2019	2	2	925	Bostanlyk
10.	**SD** –10.07.2019	545	2	708	Chandler
11.	**TB** – 12.07.2018	0,4	0,4	1050	Ideal
12.	**TB** – 16.06.2019	0,4	0,4	1050	Ideal
13.	**TB** – 16.06.2019	0,11	0,11	1050	Ideal*
14.	**TB** – 12.07.2018	0,11	0,11	1050	Jubilee
15.	**TB** – 16.06.2019	0,11	0,11	1050	Jubilee

* – Nursery of the BMRS-RIOG, 3rd year seedlings.

For assessing diseases in areas up to 1 hectare diagonally each third tree was examined on four sides, and 100 leaves, 50 twigs and 50 fruits (when available) were checked for disease symptoms. Seedlings were examined in 10 sites distributed evenly in plantations, 100 plants per site.

Table 2: Fungicides tested for efficacy against walnut anthracnose in 2018-2019

Trial year, treatments (fungicides used, active ingredients, producing company, and FRAC-codes)	Rate, kg, L/ha
1. 2019. Score 25% EC (difenoconazole 250 g/L), Syngenta Crop Protection AG, Switzerland (FRAC G1: 3)	0.2
2. 2018, 2019. Difen Super 55% WP (difenoconazole 200 g/L + thiametoxam 300 g/kg), Euro Team Ltd., Uzbekistan-Germany (FRAC G1: 3 + IRAC 4 A)	0.15
3. 2018, 2019. Difen Super 55% WP	0.25
4. 2018. Syllit 40% SC (dodine 40 g/kg), Euro Team Ltd., Uzbekistan-Germany (FRAC U 12)	1.0
5. 2018. Syllit 40% SC	1.5
6. 2018. Phytolavin WSC, 120,000 AU/ml (complex of streptotricin antibiotics, produced by Streptomyces lavendulae, 32 g/L), Biophytopharm Ltd., Uzbekistan (FRAC D4: 25?)	1.5
7. 2018. Phytolavin WSC	2.0
8. 2018. Sporagin WSC, 1,500 AU/ml (Bacillus subtilis), AnGuzal Agroservice Private Venture, Uzbekistan (FRAC F6: 44)	0.75
9. 2018. Sporagin WSC	1.0
10. 2018. Curzate WP (copper oxychloride 450 g/kg + cymoxanil 42 g/kg), Du Pont International Operations Sarl, Switzerland (FRAC M 1 + 27)	3.0
11. 2019. Falcon 46% EC (tebuconazole 167 g/L + spiroxamine 250 g/L + triadimenol 43 g/L), Bayer CropScience, Germany (FRAC G1: 3 + G2: 5 + G1: 3)	0.3
12. 2019. Falcon 46% EC	0.5
13. 2019. Coritus 50% WG (cyprodynil 500 g/kg), Agrobest Group, Turkey (FRAC D1: 9)	0.3
14. 2019. Coritus 50% WG	0.4
15. 2019. Cresoxin 50% WDG (cresoxim-methyl 500 g/kg), Ifoda Agro Kimyo Himoya Ltd., Uzbekistan; (FRAC C3: 11)	0.2
16. 2019. Cresoxin 50% WDG	0.3
17. 2019. Myseb M-45 80% WP (mancozeb 800 g/kg), Agrobest Group, Turkey; (FRAC M 3)	2.0
18. 2019. Myseb M-45 80% WP	2.5
19. 2018, 2019. Check untreated	–

Trials for evaluation of fungicide efficacy against anthracnose have been carried out in 2018 and 2019 in walnut groves (variety Ideal) of the Bostanlyk mountain research station of the Scientific research institute of orchard, grape growing and wine-making (BMRS-RIOG) after acad. M. Mirzaev, located in Bostanlyk district of Tashkent region.

Fungicides were applied three times with a first spray in spring when walnut leaves were fully unfolded, second spray at the end of flowering, and the last one in 14 to 30 days after the previous application. Rate of working volume was 1,000 liters/ha. Treatments and a list of 8 chemical and 2 biological fungicides used are shown in the Table 2.

Trials were carried out in 3 replications, each replication was one tree. Four assessments were made during a season: first assessment – before 1st application of fungicides, 2nd, 3rd and 4th assessments, respectively, in 25, 50 and 80 days after 1st assessment. This has been done by examining all leaves, twigs and fruits (when available) on four labelled shoots on four sides of each tree.

Disease severity has been assessed on the following two empiric scales: 0-5 points scale for assessing a disease on leaves and fruits, where 0 – no disease, 0.1 – 1-5 barely seen flecks; 1 to 5 – 10%, 11-25%, 26-50% and more than 50% of surface of leaves and fruits are covered by spots/lesions, respectively; 0-3 points scale for assessing a disease on green twigs, where 0 – no disease, 0.1 – 1-5 barely seen flecks; 1 to 3 – 5%, 25%, and more than 25% of surface of twigs are covered by lesions, respectively

Incidence and intensity of anthracnose was assessed and calculated using known formula of All-Russian Institute of Plant Protection (Chumakov et al., 1974). Biologic efficacy of fungicides was determined using empiric formula $B_e = I_c - I_t \bullet 100/I_c$, where B_e is a biologic efficacy, I_c is disease index in check treatment, and I_t is a disease index in trial treatment. Disease index has been calculated also empirically by multiplying parameters (%) of incidence and intensity of the disease (Chumakov et al., 1974), and dividing resultant sum by 100.

RESULTS AND DISCUSSION

During surveys anthracnose, downy spot and infection caused by the bracket fungi have been registered on walnut trees (Table 3).

Anthracnose. Causal agent of anthracnose is an ascomycete fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not. It has occurred only in the conidial stage (anamorph) called *Marssonina juglandis* (Lib.) Magn. This disease has been identified based on distinctive symptoms on green leaves and twigs, and microscopic characteristics of the causal fungus (Belisario et al., 2008, Hassan et al., 2017). Conidia were two-celled, hyaline, crescent shaped and measured 16-22 x 2.5-4 μm. Anthracnose was the most frequently spread disease of walnut trees. Our findings confirm reports of local (Mapelli & Vildanova, (2009); Ozolin, G.P. (1990) and foreign scientists (Hassan & Ahmad, K. 2017, Belisario et al., 2008, Hassan et al., 2017, Saremi & Amiri, 2010) about prevalence of this disease on walnut plantations and groves.

Table 3: Occurrence and intensity of development of diseases in plantations and groves of the Persian walnut (2018 and 2019)

No.	Survey date	Survey site and walnut variety	Anthracnose		Downy spot		Bracket fungi
			O*	In*	O	In	O
1.	26.04.2018	**TP** (C, F**)	0	0	0	0	0
2.	31.05.2018	**TK** (C)	0	0	0	0	0
3.	29.06.2018	**FK** (I)	70.0	32.5	5,5	>0,1	10.0
4.	10.07.2019	**FK** (I)	73.0	33.5	8,0	>0,1	5.5
5.	10.07.2019	**FK** (J)	45.2	14.3	8,0	>0,1	16.0
6.	29.06.2018	**FD** (I)	40.0	18.0	3,5	1.3	8.0
7.	10.07.2019	**FD** (I)	46.0	19.0	6,0	1.2	9.0
8.	11.07.2018	**SU** (B)	40.0	18.7	0	0	>0.1
9.	10.07.2019	**SU** (B)	85.0	48.2	0	0	>0.1
10.	10.07.2019	**SD** (C)	0	0	0	0	0
11.	12.07.2018	**TB** (I)	74.0	31.7	10,0	3.2	5.0
12.	16.06.2019	**TB** (I)	80.0	35.2	7,0	>0.1	6.0
13.	16.06.2019	**TB** (I)	23.0	11.2	21,0	9.4	0
14.	12.07.2018	**TB** (J)	45.0	10.6	4,5	>0.1	0.5
15.	16.06.2019	**TB** (J)	48.0	11.6	9,0	2.8	0

Notes: * O – occurrence, %; In – intensity of the disease, % (Chumakov et al., 1974).

** *Varieties*: B – Bostanlyk; C – Chandler; F – Franquette; I- Ideal; J – Jubilee.

We have noted that walnut varieties varied on their resistance to the disease. Recently it has been reported that Ideal variety belonged to the group of susceptible cultivars while Jubilee and Bostanlyk varieties were in the group of relatively less susceptible genotypes

(Khasanov & Safarov, 2018). Earlier it has been reported that among 20 tested varieties Chandler was the most resistant (Arnaudov & Gandev, 2009, Arnaudov et al., 2014), while Franquette and several other varieties have had moderate resistance to the anthracnose of walnut (Salahi & Jamshidi, 2009, Cerović et al., 2017). Besides Persian walnut, *Gnomonia leptostyla* infects many other *Juglans* species. So, it has been found, that black walnut species *J. hindsii* (Jeps.) R.E. Smit, *J. major* (Torrey) Heller and *J. nigra* L. were highly susceptible to anthracnose (Black & Neely, 1978, Berry, 1981).

Downy spot. Causal agent of this disease is basidiomycete fungus *Microstroma juglandis* (Berenger) Sacc. Incidence of the downy spot, generally, was low and varied between >0,1% and 3,2%. Its notable development has been registered only on leaves of young seedlings of Ideal variety at the nursery of the BMRS-RIOG (Table 3). This disease has been easily recognized by its very distinctive symptoms, namely snowy-white, powdery growth confined by leaf veins on the undersurface of leaves, and yellow or light green spots on upper surface opposite to white growth (Garcia-Jímenéz et al., 1995, Juhásová et al., 2005). Microscopy of white growth has revealed basidiospores of the fungus that were oviform, hyaline and measured 16-22x3-4 μm. Except the Persian walnut, *M. juglandis* infects more than 6 species of the *Juglans* genus and more than 8 species of hickory (Hickory, 2015).

Bracket fungi. Reportedly more than 20 species of the bracket fungi occur on the Persian walnut trees, including 17 species that cause infection of the wood and produce characteristic fruiting bodies (conks) of different forms and sizes on stem and large branches of trees. In Uzbekistan and neighbor countries more than 10 species of the bracket fungi were reported as parasites of walnut trees (Kleyner, 1963, Mapelli & Vildanova, 2009, Akhmedova, 1960, Prutenskaya, 1968). Each of these fungi can cause some definite harm to trees, but the most widely distributed and harmful species is the shaggy bracket fungus *Inonotus hispidus* (Bull.) P. Karst. (syns. *I. hirsutus* (Scop.) Murrill, *Polyporus hispidus* (Bull.) Fr. a. o.). It causes destruction of walnut trees' wood (heart rot), produces cavities on stems, decreases life time and yields of trees (Akhmedova, 1960, Prutenskaya, 1968). Data in the table refer to the shaggy bracket that had occurred on stems and larger branches of 0% to 16% of walnut trees surveyed (Table 3).

Efficacy of fungicides against walnut anthracnose. Various fungicides have been tested and recommended for controlling an anthracnose in various countries, e.g. copper-based substances (Bordeaux mixture, copper oxychloride, copper hydroxide), dithiocarbamates (zineb, maneb, mancozeb), thyram, captan, vinclozoline, benomyl, thiophanate-methyl, dodine, chlotothalonil, dithianon, flusilazole, cymoxanil, fenarimol, hexaconazole a. o. These fungicides were recommended to apply on walnut trees 2-3 to 5-6 times a season (Hassan & Ahmad, 2017, Berry, 1977, Zamani et al., 2011). It has been reported that injection of benomyl into soil reduced incidence and intensity of the anthracnose of black walnut for two years (Neely, 1977).

In the USA, except copper containing compounds, more than 10 fungicides with one or more active ingredients are currently recommended for control of anthracnose on walnut trees (Table 4).

Taking into consideration the above information we have chosen for testing some of those fungicides that were reported as an effective in the literature, namely formulations of difenoconazole, dodine, cymoxanil, copper oxychloride, mancozeb and tebuconazole (Table 2).

In our field trials the highest biologic efficacy against anthracnose on leaves, twigs and fruits has been provided by Difen Super 55% WP at rates 0.15 kg/ha (80.4-96.1%, 90.3-96.3% and 87.5-94.9%, respectively), and 0.25 kg/ha (99.6-99.3%, 99.1-100% and 98.7-100%), Syllit 40% SC at rate 1.5 L/ha (92.3%, 90.3% and 91.1%), Falcon 46% EC at rates 0.3 L/ha (on leaves – 91.8%), and 0.5 L/ha (96.8%, 92.5% and 92.4%), and Score 25% EC at rate 0.2 L/ha (93.6%, 94.4% and 93.7%, respectively) (Table 5).

Table 4: Fungicides recommended against anthracnose of walnut trees in the USA (except copper containing products) (Pscheidt & Ocamb, 2019)

Fungicides, active ingredients, and producers	FRAC code
Gem 500 SC (trifloxystrobin), Adama	11
Inspire Super 33,5 SC (difenoconazole 86 g/L + cyprodinil 249 g/L), Syngenta	3 + 9
Luna Experience 400 SC (fluopyram 200 g/L + tebuconazole 200 g/L), Bayer	7 + 3
Luna Sensation 50 SC (fluopyram 250 g/L + trifloxystrobin 250 g/L), Bayer	7 + 11
Merivon 50 SC (fluxapyroxad 250 g/L + trifloxystrobin 250 g/L), BASF	7 + 11
Pristine 38 WG (boscalid 25,2% + pyraclostrobin 12,8%), BASF	7 + 11
Bumper 41.8 EC, Propi-Max EC, Tilt 25 EC a. o. (propiconazole), various producers	3
Quadris Top 29.6 SC (azoxystrobin 18,2% + difenoconazole 11.4%), Syngenta	11 + 3
Quash 50 WDG (metconazole), Valent, USA	3
Quilt Xcel 26.38 suspo-emulsion (azoxystrobin 141.4 g/L + propiconazole 122.4 g/L), Syngenta	11 + 3
Syllit FL (dodine, 39,6%), Arysta LifeScience North America	U12
Topguard EQ 43,93 SC (flutriafol 18,63% + azoxystrobin 25,3%), FMC	3 + 11

Table 5: Disease indices and biologic efficacy of tested fungicides against walnut anthracnose (2018-2019)

Treatment	Year	Rate, L, kg/ha	Disease index*, point			Biologic efficacy, %		
			L**	T	F	L	T	F
1. Difen Super 55% WP	2018	0.15	2.5	0.7	0.7	89.4	90.3	875
	2019	0.15	1.1	0.4	0.4	96.1	96.3	94.9
2. Difen Super 55% WP	2018	0.25	0.1	0.0	0.0	99.6	100	100
	2019	0.25	0.2	0.1	0.1	99.3	99.1	98.7
3. Syllit 40% SC	2018	1.0	4.0	1.5	1.4	83.0	79.2	75.0
4. Syllit 40% SC	2018	1.5	1.8	0.7	0.5	92.3	90.3	91.1
5. Phytolavin WSC	2018	1.5	15.5	4.6	4.3	33.9	36.8	23.7
6. Phytolavin WSC	2018	2.0	13.7	4.0	4.1	41.6	45.0	26.3
7. Phytolavin WSC	2018	7.5	15.4	5.0	4.7	34.5	30.8	16.4
8. Phytolavin WSC	2018	10.0	14.5	4.6	4.4	38.1	35.8	20.9
9. Curzate WP	2018	3.0	4.1	1.1	1.2	82.5	84.7	78.6
10. Falcon 46% EC	2019	0.3	2.3	1.2	1.0	91.8	88.8	87.3
11. Falcon 46% EC	2019	0.5	0.9	0.8	0.6	96.8	92.5	92.4
12. Coritus 50% WG	2019	0.3	5.4	1.9	1.6	80.9	82.2	79.7
13. Coritus 50% WG	2019	0.4	4.6	1.7	1.4	83.7	84.1	82.3
14. Cresoxin 50% WDG	2019	0.2	6.3	1.8	2.0	77.7	83.2	74.7
15. Cresoxin 50% WDG	2019	0.3	5.5	2.1	1.6	80.5	80.4	79.7
16. Myseb M-45 80% WP	2019	2.0	11.0	5.9	3.6	61.0	44.9	54.4
17. Myseb M-45 80% WP	2019	2.5	10.0	5.2	3.1	64.5	51.4	60.8
18. Score 25% EC	2019	0.2	1.8	0.6	0.5	93.6	94.4	93.7
19. Check untreated	2018	–	23.5	7.2	5.6	–	–	–
20. Check untreated	2019	–	28.2	10.7	7.9	–	–	–
$LSD_{05\%}$	2018	–	1,9	3,1	3,6			
$LSD_{05\%}$	2019	–	3,3	3,7	3,9			

* – *Disease indices were calculated using data of the last assessment;* ** – **L** - *leaves*, **T** - *twigs*, **F** - *fruits.*

Biologic efficacy has been rather lower of the lesser rates of both Syllit 40% SC and Falcon 46% EC, Curzate WP, and of both rates of each of Coritus 50% WG and Cresoxin 50% WDG. Biologic efficacy of these fungicides on anthracnose varied between 77.7-83.7% on leaves, 80.4-88.8% on twigs, and 74.7-87.3% on fruits of walnut trees.

Biologic efficacy of Myseb M-45 80% WP against anthracnose was lesser than 65%, while that of biological fungicides Phytolavin WSC and Sporagin WSC varied between 33.9-41.6% on leaves, 30.8-45.0% on twigs, and 20.9-26.3% on fruits (Table 5).

CONCLUSIONS

Surveys carried out in 2018 and 2019 have revealed that anthracnose caused by ascomycete fungus *Gnomonia leptostyla* has been the most widespread disease in Persian walnut plantations and groves in three regions of Uzbekistan. Walnut varieties varied on resistance to the disease. Downy leaf spot caused by the basidiomycete fungus *Microstroma juglandis* has been registered rarely and sporadically. Among the bracket fungi only the shaggy bracket fungus *Inonotus hispidus*, a cause of white heart rot of the wood, has been registered on walnut trees more or less commonly.

In two-year field trials on testing fungicides against anthracnose on leaves, twigs and fruits of the Persian walnut trees it has been found, that Difen Super 55% WP, Syllit 40% SC, Falcon 46% EC, and Score 25% SC have provided with the highest efficacy (>90%, and in some cases up to 99-100%). Biologic efficacy of lesser rates of both Syllit 40% SC and Falcon 46% EC, Curzate WP, and of both rates of each of Coritus 50% WG and Cresoxin 50% WDG has varied between 74.7% and 88.8%. An efficacy of Myseb M-45 80% WP was lesser than 65%, and that of Phytolavin WSC and Sporagin WSC varied between 20.9% and 45.05%.

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