Effectiveness of different fungicides in controlling botrytis grey mould of tomato

M S Mamiev¹, A A Khakimov¹*, M A Zuparov¹, and U N Rakhmonov²

¹Department Agrobiotechnology, Tashkent State Agrarian University, 100140 Tashkent, Uzbekistan
²Department Plant Pathology, Tashkent State Agrarian University, 100140 Tashkent, Uzbekistan

*Email: alberthakimov@mail.ru

Abstract. Tomato is the most common and widespread vegetable among other vegetable crops in Uzbekistan. It ranks the first place by its planting area and gross yield. Tomato, like other farming crops, is affected by a number of infectious diseases. Among them, grey mould causes considerable damage to the crops. This article contains the data on testing fungicides with active ingredients Difenoconazole and Cyprodinil against grey mould disease of tomato in greenhouse condition. In fungicides sprayed variants, the inhibitory effect of fungicides on the development of diseases was considerable, that is, disease development index constituted 3.4-6.4%. When 0.08% concentration of fungicide Difen Super, 55% WP was applied, then development of disease was 3.9% at the first spray; and 4.5% at the second spray; 5% at the third and 5.8% at the forth spray. Among the fungicides, 0.05% concentration of Skor, 250 g/l EC fungicide applied variant performed much less development of grey mould disease compared to other variants, disease development index indicated 3.4-4.7%. According to experiment results, it has been recommended to conduct treatment with Difen Super, 55% WP (0.08%) and Skor, 250 g/l EC (0.05%) fungicides against tomato’s grey mould disease three times with 10 days interval.

1. Introduction
Tomato plant is grown in 4.76 mln ha area in the world nowadays, while in 2018 gross yield of 180.9 million tons were produced. The most part of this amount accounts for the share of China, India, and USA. As well as, in Uzbekistan the tomato is the most common and widely grown vegetable among other vegetable crops, and ranks the first by growing area and gross yield. According to FAO statistics, total yield in 2018 throughout the republic made 2,284 mln tons. In 2018, tomato sale in the world grew 6.5% and profited 190.4 billion dollars compared to that in 2017. Growing in protected conditions or growing in greenhouses is the most modern approach to qualitative and quantitative cultivation of mainly horticultural crops, which has spread widely throughout the world over the past few decades. Protected cultivation, also known as Controlled Environment Agriculture (CEA), is highly productive, conserves water and land, and protects the environment [1]. About 115 countries in the world are engaged in the commercial cultivation of greenhouse vegetables. The world area of protected cultivation about 623,302 hectares, while the total estimated area of global production of greenhouse vegetables is 402,981 hectares. Of the total area of greenhouse vegetables in
the world, hydroponic cultivation systems account for 95,000 hectares. Tomato makes the main part of the crops cultivated in greenhouse conditions [2, 3]. Like other farming crops, tomato is infected by a number of viral, bacterial and fungal diseases during its growth and storage stages, which cause to decreasing of not only its productivity and yield, but also product quality. Tomato diseases damage all organs of the plant in growth period, including sprout diseases, fusarium, verticillium wilt, phytophthora, browning and stippled leaves (cladosporium), alternaria, grey mould, viral diseases, nematode causing diseases and non-infectious diseases. Among these diseases, damaging level of Botrytis cinerea Pers. ex. Fr. fungus causing grey mould disease is considered high enough in greenhouses [4].

Botrytis cinerea Pers.; Fr. is the main pathogen of grapes and greenhouse crops causing gray mould. In greenhouses, B. cinerea is a ubiquitous pathogen that causes serious loss of many fruit, vegetable and ornamental crops and can damage or even kill plants and affect product quality. The pathogen infects the leaves, stems, flowers and fruits of greenhouse plants [5, 6]. B. cinerea is a widespread fungus. It causes gray mold on almost all major greenhouse crops, initially entering greenhouses, probably through open windows, cracks, on people and on young cuttings and seedlings. In later stages of production, infected plants or infected and decaying plant parts in the greenhouse form an inoculum source. Younger plant parts are generally not very susceptible, while older plants or plant parts are more likely to become infected. The emergence of B. cinerea is expressed in different cultures in different phases. Tomatoes, cucumbers and bell peppers are the most common vegetable crops. B. cinerea can cause serious problems in these and many other vegetable, flower, pot and herb crops [6].

In tomatoes in unheated greenhouses, the fungus attacks flowers, fruits and leaves and can grow through the petiole into the stem [7, 8]. In heated greenhouses, Botrytis infestation is almost entirely confined to stem infection. Recently, tomato is mostly grown in greenhouses to the High Wire method [9], with plants reaching lengths of 20-30 m with bundles of stems running along the floors and grown for up to 50 weeks. The leaves around the ripening fruits are removed thus causing pruning wounds. In heated greenhouses, B. cinerea mainly causes stem lesions by infecting such pruning wounds. Damage to the stem can encircle the plant, resulting in plant death. Stem infections may remain quiescent for up to 12 weeks [10].

The chemical control of Botrytis diseases is impeded by the development of resistance to many fungicides and the negative public perception regarding the safety of pesticides. As a consequence, in many countries, the regulatory authorities have restricted the use of new and established pesticides [11].

A number of research work and investigations were conducted on the study of fungicide resistance of B.cinerea isolates. In Israel the fungicide resistance of B.cinerea isolates extracted from sweet basil, grapes and tomato was studied [12, 13, 14]. Occurrence of resistant strains to fungicides is not the same in different countries. The emergence of isolates of B.cinerea fungus resistant to dicarboximide was observed after three years of application of these fungicides in England [15], while in Japan the strains resistant to benomyl were determined in the fields where the treatment with this fungicide though was not performed [16]. The emergence of B.cinerea fungus isolates resistant to benzimidazol occurred a bit slowly in Hungary than in France, while in the fields where dicarboximide was applied, this case was not observed [17]. In Ontario province of Canada the occurrence of fungicide resistant isolates was lower in vineyards where benzimidazol was applied against disease causing B.cinerea fungus compared to Europe [18].

In Germany since 1978 Botryticide fungicide with dicarboximide was used against this disease in vineyards, where in 1979 fungicide resistant isolates were observed. The occurrence of fungicide resistant isolates varied depending on regions [19, 20]. Use efficiency of ronylan, rovral, procimidin fungicides in the fields began decreasing constantly from 1981 [21]. In Italy incubation of resistant isolates of B.cinerea fungus occurred slowly. It was recorded that pathogen isolates resistant to preparations with dicarboximide and benzimidazole active ingredients were also observed in greenhouses and vineyards.
From different provinces of England isolated *B. cinerea* fungus isolates performed varied levels of resistance to different concentrations of iprodione and benomyl. The strains of *B. cinerea* isolated from different greenhouses of Netherlands were found to have resistant forms of 0 to 100% to benomyl. It was recorded that isolates of *B. cinerea* resistant to benomyl were contrarily non-resistant to the fungicides with active ingredient benzimidazole. Most systematic fungicides are inactivated by fungi enzymes [22]. Under mutation impact, fungicides lose their inhibitory effect. Therefore, the resistance of pathogens to systematic fungicides increase due to the changes of metabolites in fungus cell. Taking into account aforementioned scientific references, it can be known that Botrytis types perform different resistance to different fungicides. However, in Uzbekistan special research work haven’t been conducted yet on the study of efficacy of fungicides against grey mould and the occurrence resistance with it. Considering this case, several fungicides were tested against grey mould disease of tomato.

2. Material and methods

On the study of grey mould disease of tomato plant, the research work in the form of experiments were conducted during the years 2018-2019 in “Innovative work and counseling center in agriculture” State unitary enterprise at Tashkent state agrarian university and in greenhouses of “Fresh rose” LLC in Urtachirchik district of Tashkent region. While on the study of efficacy of fungicides at in vitro condition and the disease, the experiments were carried out in the laboratory of Agro-biotechnology at Tashkent state agrarian university.

2.1. Incidence of grey mould disease on tomato plant.

Infected amount of tomato plant in a certain area of greenhouse in a percentage rate is called disease spread and it is found with the help of the following formulae:

\[
P = \frac{100 \times n}{N},
\]

\(P\) – spread of the disease, %;

\(N\) – a total number of recorded plants;

\(n\) – a number of infected plants in the pattern [23, 24, 25].

An average value of disease spread over greenhouse is identified with this formulae:

\[
P_y = \frac{\sum(S\times P)}{S},
\]

\(P_y\) – an average value of disease spread over greenhouse, %;

\(\sum S\times P\) – a sum of multiplication of disease spread in percentage in the sections of each greenhouse where observations were conducted;

\(S\) – a total area of the observed greenhouse [23, 24, 26, 27].

2.2. Severity of grey mould disease on tomato plant.

Contamination rate on infected plants was recorded by 5 scored-scale or percentage. The development of grey mould disease of tomato plant was determined by this formulae:

\[
C = \frac{\sum(a\times b)}{n},
\]

\(C\) – an average index of disease rate, %;

\((a\times b)\) – a sum (b) of multiplication of the number of plants (a) with the disease expression in scores or percentage respectively.
$n$ – a number of infected plants [23, 26, 27].

| score | Percentage (%) |
|-------|----------------|
| 0 –   | 0 healthy plant; |
| 1 –   | 10% infected plant; |
| 2 –   | 11-25% infected plant; |
| 3 –   | 26-50% infected plant; |
| 4 –   | over 50% infected plant. |

### Table 1. Contamination rate on infected plants

2.3. Testing fungicides

Experiments were carried out on tomato plants cultivated in greenhouses of “Fresh Rose” LLC in Urtachirchik district of Tashkent region. Different concentrations of Difen Super, 55% WP (200 g/kg Difenonconazole + 350 g/kg Thiamethoxam), Skor, 250 g/l EC and Chorus, 750 g/kg WDG fungicides were tested against grey mould disease of tomato (Table 2). Unfortunately, any aforementioned fungicides are not recommended for the treatment against grey mould disease of tomato in the “List of allowable pesticides to be used in the Republic of Uzbekistan”, however, they are recommended to be applied against grey mould disease of other farmland crops, and therefore, effective concentrations were used at in vitro laboratory testing.

Each experimental variant was performed in 4 repetitions, under each repetition 10 pieces of tomato plant, and total 40 tomato plant in one variant were tested and observed. Fungicide spray was performed on each 9-12 days, totally implemented 4 times. The spread and development of the disease were recorded after 7-8 days from the time of spraying each fungicide. While in control variant tomato plants were tested without treatment of fungicide.

### Table 2. Description of fungicides used in the experiment

| Active ingredient | Trade name  | Formulations* | Chemical class | Application concentration |
|-------------------|-------------|---------------|----------------|--------------------------|
| Difenonconazole   | Difen Super | WP, 200 g/kg  | Triazole       | 0.08                     |
| Difenonconazole   | Skor        | EC, 250 g/l   | Triazole       | 0.05                     |
| Cyprodinil        | Chorus      | WDG, 750 g/kg | Anilinopyrimidine fungicide | 0.13                     |

*WP - Wettable Powder; EC - Emulsifiable Concentrate; WDG - Water Dispersible Granule.

2.4. Determination of biological efficacy of preparations against the disease.

Decrease of contamination rate of infected tomato plant by pathogens in the variants treated with fungicides and biological preparations is defined in percentage comparing to control variant, and biological efficacy of preparations has been found according to the following formulae:

$$\eta = \left( \frac{P_h - P_t}{P_h} \right) \times 100,$$

$\eta$ – biological efficacy of fungicide, %;
$P_h$ – severity of diseases in control variant, %;
$P_t$ – severity of diseases in experimental variant, % [24].

3. Results and Discussions

Grey mould disease of tomato was firstly noted in October in greenhouses of “Fresh Rose” LLC in Urtachirchik district of Tashkent region. The initial spray of fungicides was implemented in the beginning of November month. The data about effect of sprayed fungicides on severity of disease has been illustrated in figure. In unsprayed control variant the dynamics of disease severity was too strong, and growth index among the records (recorded after 7-8 days from the time of applying each
fungicide) varied from 4.3% to 8.8%. In the first recording, the disease severity was 17.1%, while in the last recording, that is, in the 4th recording this index was noted to be 37.7% (Figure 1).

![Figure 1](image-url) - The effect of the number of fungicides treatment on the severity of grey mould disease of tomato, %

| №   | Treatments          | Application concentration, % | Spread of disease, % | Severity of disease, % | Biological efficacy, % |
|-----|---------------------|-----------------------------|----------------------|------------------------|------------------------|
| **First spray**                             |                                |                        |                       |                        |
| 1   | Difen Super, 55% WP | 0.08                         | 7.8                   | 3.9                    | 77.1                   |
| 2   | Skor, 250 g/l EC    | 0.05                         | 6.5                   | 3.4                    | 80.3                   |
| 3   | Chorus, 750 g/kg WDG| 0.13                         | 9.1                   | 5.0                    | 70.8                   |
| 4   | Unsprayed Control  | –                            | 26.9                  | 17.1                   | –                      |
| **Second spray**                            |                                |                        |                       |                        |
| 1   | Difen Super, 55% WP | 0.08                         | 7.9                   | 4.5                    | 82.6                   |
| 2   | Skor, 250 g/l EC    | 0.05                         | 6.8                   | 3.8                    | 85.3                   |
| 3   | Chorus, 750 g/kg WDG| 0.13                         | 9.4                   | 5.2                    | 77.2                   |
| 4   | Unsprayed Control  | –                            | 44.0                  | 25.9                   | –                      |
| **Third spray**                             |                                |                        |                       |                        |
| 1   | Difen Super, 55% WP | 0.08                         | 8.6                   | 5.0                    | 83.4                   |
| 2   | Skor, 250 g/l EC    | 0.05                         | 7.3                   | 4.2                    | 86.8                   |
| 3   | Chorus, 750 g/kg WDG| 0.13                         | 10.2                  | 5.9                    | 80.5                   |
| 4   | Unsprayed Control  | –                            | 52.2                  | 30.2                   | –                      |
| **Fourth spray**                            |                                |                        |                       |                        |
| 1   | Difen Super, 55% WP | 0.08                         | 10.2                  | 5.8                    | 84.6                   |
| 2   | Skor, 250 g/l EC    | 0.05                         | 8.3                   | 4.7                    | 87.5                   |
| 3   | Chorus, 750 g/kg WDG| 0.13                         | 11.1                  | 6.4                    | 83.0                   |
| 4   | Unsprayed Control  | –                            | 65.9                  | 37.7                   | –                      |
| LSD₉₅ (%)                                    | 1.92                          | 1.54                  |                        |                        |

In fungicide sprayed variants their influence was considerable on the development and severity of disease that it made 3.4-6.4%. When Difen Super, 55% WP was applied in 0.08% concentration, the
disease severity constituted 3.9% at the first spray; at the second spray 4.5%; at the third spray 5% and at the fourth spray 5.8%. Among the fungicides, 0.05% concentration of Skor, 250 g/l EC fungicide decreased the severity of grey mould much more than in other variants. In this variant the index of severity made 3.4-4.7%.

Comparing to the variants with other fungicides applied, 0.13% concentration of Chorus, 750 g/kg WDG fungicide with active ingredient Cyprodinil showed less effect on the severity of grey mould (5.0-6.4%) (Figure 1).

Biological efficacy of sprayed (applied) fungicides for the control of grey mould disease of tomato and on the severity of this disease was determined by comparing unsprayed control variant with fungicide-sprayed variants. It was studied that when Difen Super, 55% WP fungicide (0.08%) was sprayed once against grey mould disease of tomato, biological efficacy made 77.1%, at the second spray it was 82.6%, while at the third 83.4% and at the fourth spray the indication was 84.6% (Table 3).

Among the sprayed (applied) fungicides, the highest biological efficacy was noted in Skor, 250 g/l EC fungicide (0.05%) and after the first spray of fungicide biological efficacy showed 80.3%, and the next sprays this indication constituted 85.3%; 86.8; 87.5% respectively.

4. Conclusions

Basing on the data obtained in the results of conducted experiments, it can be concluded that all tested norms of fungicides resulted positively, mainly, when they sprayed three and four times, their biological efficacy indicated high (80.5-87.5%) results. The fungicides with active ingredient Difenoconazole performed higher biological efficacy than the active ingredient Cyprodinil.

Though the applied fungicides Difen Super, 55% WP 0.08% and Skor, 250 g/l EC have performed the highest biological efficacy against grey mould disease of tomato, and due to less difference of biological effectiveness between the third and fourth sprays, it is recommended to apply Difen Super, 55% WP (0.08%) and Skor, 250 g/l EC (0.05%) fungicides against grey mould disease of tomato three times with 10 days interval having considered economic case, cumulativeness of fungicides and the occurrence of resistant isolates of *Botrytis cinerea* Pers. ex. Fr.

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