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EFFICACY OF REDOXIL AND RIDOMEX GR 5% (METALAXYL) AND MANCOLAXYL WP 72% (METALAXYL + MANCOZEB) FUNGICIDES IN CONTROLLING CUCUMBER SEEDLING DAMPING-OFF DISEASE CAUSED BY *PYTHIUM APHANIDERMATUM*

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Abstract

Kitchen garden crops, especially cucumbers, play a substantial role in people's life both nutritionally and economically. In recent years, mass production of cucumbers in greenhouse cultures has become an important factor in farmers' earnings at vegetable markets. Therefore, it is particularly important to pay attention to this plant in the country. Due to favorable greenhouse conditions for the development of various microorganisms, this plant is constantly exposed to pathogens including the fungus causing the seedling damping-off disease *Pythium aphanidermatum*. The disease is prevalent in hot regions with heavy soils, and is very widespread in the fields and greenhouses of Tehran province (Varamin) and Isfahan. Chemical control by the use of fungicides has been effective in reducing the disease, but it is also necessary to examine the efficacy of new fungicides in controlling the disease due to the resistance of disease agents to conventional fungicides. This study was conducted in a randomized complete block design with nine treatments and four replications in three different areas of Tehran, Varamin, and Isfahan. Treatments were healthy control, infected control, redoxil (2g/L), ridomex (2g/L), mancolaxil (1.5g/L), mancolaxil (1.75g/L), manocloxil fungicide(2g/L), previcur energy (3ml/L), and Downy-G (2g/L). The disease was evaluated by determining the percentage of disease incidence through calculating the number of healthy seedlings and the efficacy of fungicides used in disease control at the four-six leaf stage. Data obtained from the three different regions were statistically analyzed based on composite analysis through the MS TAT C software. If the effect of treatment × place was significant, analysis of variance of results was done separately for each region and mean values of studied traits (seedling damping-off or efficacy percentage) were grouped based on Duncan's multiple range test. In case of non-significant effect of treatment × place, mean values of traits were grouped using Duncan's multiple range test based on composite analysis. According to the composite analysis, the effect of treatment × place was significant for the percentage of seedling damping-off incidence. In the three studied regions, percentage of seedling damping-off incidence decreased significantly in all the fungicide treatments compared to infected control. The composite analysis also revealed that the effect of treatment × place was not significant for the percentage level of efficacy. Besides, no significant differences were observed between the different fungicidal treatments in terms of grouping mean percentages of efficacy based on composite analysis. The efficacy ranged from the lowest (75%) for mancolaxil with the lowest dose used (1.5g/L) to the highest level (88%) for ridomex and previcur energy fungicides.

Key words: *Pythium aphanidermatum*, Cucumber, Chemical fungicide, Seedling damping-off disease.

Introduction

Cucumis (*Cucumis sativus*) is a plant in the Cucurbitaceae family and an important commercial crop in Iran and worldwide. The crop is a tropical vegetable, is compatible with the climate of Iran, is rich in fiber and vitamin, and therefore of nutritional value. In recent years, cucumbers have been exported in various forms and are important in earning revenue for the country (Kanaskie

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et al., 2008). Studies indicate that cucumber diseases, particularly those caused by edaphic factors, not only cause significant economic damage to crop yield, but also reduce the quality and marketability of produced crop. One of the most important and destructive edaphic diseases of cucumber is seedling damping-off and seed/root rot caused by *Pythium aphanidermatum*. In Iran, seedling damping-off in plot farming has been estimated to be up to 100% of total field plants. The causative agent of this fungal disease is cosmopolitan with worldwide distribution (Postma *et al.,* 2009) and is particularly prevalent in tropical and temperate regions (Van der Plaats Niterink, 1981). The hosts of this pathogen vary widely among different plants, in particular the Cucurbitaceae (Waterhouse *et al.,* 1983) and cucumber is one of its main hosts (Zheng *et al.,* 2000).

Symptoms of disease caused by *P. aphanidermatum* in cucumber appear as seedling damping-off before or after bud emergence and plant damping-off. The disease agent also causes seed rot (Hendrix & Campbell, 1973) and (Van der Plaats Niterink, 1981). In addition to young seedlings, the pathogen may also infect adult cucumber plants and lead to abrupt plant wilt and reduced fruit production (Moulin *et al.,* 1994); however, seeds and seedlings are more susceptible (Hendrix & Campbell, 1973). Since this pathogen is one of the main causes of crop decline and considerable economic damage to greenhouse and field systems under favorable conditions of disease development (Van der Plaats Niterink, 1981), it is essential to control this disease. However, the wide host range, edaphic nature of the disease, long-term survival in the soil at resting modes (Hendrix & Campbell, 1973), and the high rate of disease spread by zoospores have made the disease management very difficult and the available controlling methods have not effectively controlled the disease. For example, regulation of temperature and irrigation can only be used in limited areas, and soil sun exposure is also used in hot climates and impractical in winter (Whipps & Lumsden, 1991). In addition, no cucumber cultivars have so far been identified with resistance to this disease (Paulitz & Belanger, 2001).

Chemical control of the disease by seed and soil treatment using fungicides (propamocarb and metallaxil) is a common approach in the disease management. However, it is essential to apply various fungicides with effective control of the disease due to the loss of susceptibility of disease agents to these fungicides (Whipps & Lumsden, 1991).

Literature Review

Studies have shown that the use of single methods has not been successful in the disease control and the use of combined methods is necessary with emphasis on prevention of disease entry into uninfected farms (Babadoost, 2004). Timely use of effective fungicides has always been considered as one of the most effective methods of integrated management (Davidse *et al.,* 1984). The use of Mefogsam (Apron®XL LS) and metalaxil (Allegiance®FL) protects cucumber seedlings from disease by 5 weeks after planting (Mc Grath, 2001) and (Babadoost, 2004). The use of dimetomorph (Acrobat® WP 50%) at 448 g/ha andcopper sulfate (Coprofix® Disperss) at 25.2 kg/ha at seven-day intervalswas reported to provide good protection against host infection with the pathogen (Davidse *et al.,* 1984).

Field damage can be minimized using Apron®XL LS for seed disinfection and Acrobat® WP 50% for spraying aerial parts (Babadoost, 2004). The effect of commercial fungicides (Copper, Acrobat, Bravo, and Aliette) on disease control has been confirmed with sufficient controlling effect on the disease (Mc Grath, 2001). In Iran, studies have been carried out on crown rot disease, its etiology, pathogenicity (Alavi & Strange, 1979), host range, and chemical and non-chemical control methods (Sharifi Tehrani & Nazari, 1995), as well as on the feasibility of using the defense potential of the host (phytoaloxins) (Strange *et al.,* 1981).

Metalaxyl is a systemic fungicide of the phenylamide group (acylalanines) with protective and therapeutic effects, which is absorbed through the root, shoot, and leaf, and inhibits the growth of pathogenic fungi by preventing protein synthesis in the fungal cell. This fungicide is miscible with soil and must be mixed with the substrate soil before planting or sprayed around the plant after planting (Kumar *et al.,* 2017).

For the control of cucumber seedling damping-off, there have been recent reports on the use of new registered fungicides, including previcur energy, rosalaxil, and propellant (Azimi & Shahriyari, 2016). Rprevicu (2ml/L), rosalaxil (3g/L), and propellant (1ml/L) were used in two stages after initial cultivation and irrigation, and at two-four true leaves through the irrigation system (Kumar *et al.,* 2017).

Materials and Methods

Preparation of cucumber seedling damping-off causative agent

To conduct the study, the pathogenic isolate of *Pythium aphanidermatum*, as one of the most important causes of seedling and plant damping-off disease in cucumber was obtained from the Agricultural and Natural

Resources Research Center (Varamin, Tehran province).

Macroscopic and microscopic examination of *P. aphanidermatum* isolates

For macroscopic and microscopic examinations, the *P. aphanidermatum* isolate was identified using the identification keys of Dick (Dick, 1990) and (Van der Plaats Niterink, 1981). To this end, the colonyform and production of aerial hyphae were examined on the media Corn Meal Agar (CMA) [the extract of shredded corn grain (40g), agar (15g) and distilled water (1L)] and Potato Carrot Agar (PCA) [extracts of chopped carrot (20g) and chopped potatoes (20g), agar (15g) and distilled water (1L)] at 25°C (Tuite, 1969).

After sporangium production, vegetative organs of the isolates were examined by placing pieces (5mm) of *Poa annua* on PCA medium for 24h, which was then transferred to sterile distilled water and placed at 20cm from light source (Van der Plaats Niterink, 1981). The reproductive organs were evaluated on Hemp-Seed Agar (HSA) medium [cannabis seed (60g) extract, agar (15g), and distilled water (1L)] (Dhingra & Sinclair, 1985).

Preparation of pathogenic inoculum of the disease agent

At this stage, the inoculum was prepared using the seedbed peat moss + cannabis + carrot at a ratio of 80 + 10 + 10, respectively, based on the modified method of (Ramoorthy *et al.,* 2002). For artificial infection, the inoculum was mixed with greenhouse combined soil (clay + perlite + humus + garden soil including sand and clay at amounts of 15 + 10 + 40 + 40, respectively). The obtained mixture was covered by a nylon bag on to maintain moisture and better growth of the disease causative fungus. The required moisture content was considered at 2% by the use of 10L of water per 500L of soil. The mixture was sprayed and aerated every second day for 7-10 days to mix the soil mixture with the causative agent. After 7-10 days and with observation of the fungal mycelial spread on the soil mixture, the soil was prepared to be used for treatments containing the disease agent in the greenhouse.

Evaluation of the efficacy of mancolaxil, ridoxyl, and ridomax fungicides in controlling greenhouse cucumber seedling damping-off and comparison their efficacy with Downy-G and previcur energy

The study was carried out in three separate regions namely Tehran, Varamin and Isfahan in a completely randomized block design. In Tehran area, the experiment was conducted in a randomized complete block design with nine treatments and four replications in transplant trays (340×530 cm)each with 70 holes, 50ml volume and a dimension of 7×10cm Fig. 1. Treatments were healthy control, infected control, redoxil (2g/L), ridomex (2g/L), mancolaxil (1.5g/L), mancolaxil (1.75g/L), mancolaxil fungicide (2g/L), previcur energy (3ml/L) and Downy-G (2g/L). Each treatment contained two planting trays (140 holes), with half of each planting tray (35 holes) assigned to each replicate.

In each area of Varamin and Isfahan, pot experiments were conducted in randomized complete block designs with nine treatments and four replications similar to those in the greenhouse of the Iranian Institute of Medical Research. In Varamin area, each replicate consisted of 101-liter pots with three seedlings per pot, and in Isfahan region, each replicate contained ten 1-liter pots with four seedlings per pot Fig. 2 and Fig. 3.

The six fungicides were used by the soil drench method in two times, after the cotyledon stage with the emergence of the first true leaf and the two-leaf time, so that the surface and the whole soil volume were completely impregnated with the fungicide solution. The fungicides of redoxil GR5% (metallaxil), ridomex GR5% (metallaxil) and Downy-G WP72% (metalaxil+ mancozeb) were used at 2g/L dose. Previcur energy SL84% (fosetyl aluminum + promocarp hydrochloride) was applied at 3ml/L dose. Mancolaxyl was used at 1.5, 1.75 and 2 g/L doses. The application level of fungicides at each turn was adjusted to 1 liter for each planting tray, and for each pot based on the calibration and volume of

Fig. 1: A view of greenhouse survey using transplanting trays in Tehran.

Fig. 3: A view of greenhouse survey using pot planting in Isfahan region.

Fig. 2: A view of greenhouse survey using pot planting in Varamin region.

water used to cover the surface and the total soil volume of the pot. In each region, the study was carried out using the seed Sultan variety in January 2018.

Evaluation of treatments in terms of efficacy in the control of seedling damping-off disease

Treatments were evaluated for disease incidence at four-leaf and six-leaf stages in order to determine the percentage of seedling damping-off based on the difference between the number of healthy seedlings in the healthy control and individual treatments. The following relationship was used to determine the percentage of seedling damping-off:

Percentage of seedling = (number of healthy seedlings in healthy control) / (number of healthy seedlings per treatment - number of healthy seedlings in healthy control) × 100

The efficacy of treatments in reduction of the disease incidence compared to control was calculated using the following formula:

$Ef = 100 - (xt/xc \times 100)$, where Ef stands for efficacy, xt denotes mean incidence of disease per treatment and xc indicates mean incidence of disease in infected control.

Fig. 4 shows unhealthy seedlings (infected with seedling damping-off disease caused by *P. aphanidermatum*).

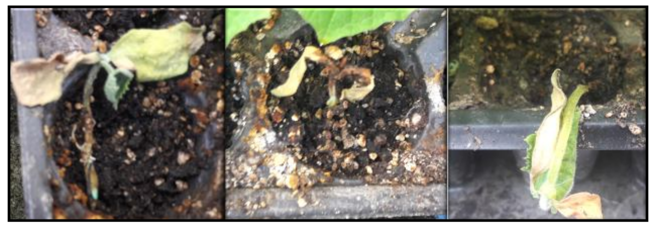

Fig. 4: A view of unhealthy seedlings (infected with seedling damping-off disease caused by *P. aphanidermatum*.

Statistical analysis of the results from the three regions was performed using composite analysis. If the effect of treatment × place was significant, analysis of variance of results was done separately for each region and mean values of studied traits were grouped based on Duncan's multiple range test. In case of non-significant effect of treatment × place, mean values of traits were grouped using Duncan's multiple range test based on composite analysis. Statistical analysis of the results was performed using the MS TAT C software through Duncan's multiple range test.

Results and Discussion

Microscopic and macroscopic examination of *P. aphanidermatum* isolate

Colonies of *P. aphanidermatum* isolates formed cottony aerial hyphae on CMA media Fig. 5., but not on HSA and PCA media. The isolate had shape less colony on the three different media. The swollen finger like sporangia formed in large numbers on solid and liquid media Fig. 6.

Spherical oogonia with a smooth wall formed at the end. The antheridia were calviform. Spherical oospores had a smooth wall without ornaments and the volume of oogonia was not fully filled with oospores aplerotic Fig. 7.

Evaluation of different fungicidal treatments in four-

Fig. 5: Colony of *Pythium aphanidermatum* on Corn Meal Agar medium.

Fig. 6: A finger likes porangium of *Pythium aphanidermatum*.

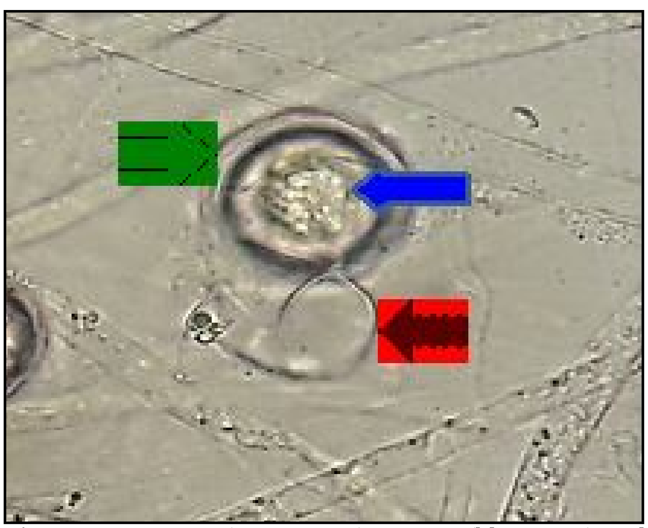

Fig. 7: An oogonium (green arrow), oospore (blue arrow), and antridium (red arrow) in *Pythium aphanidermatum*.

six leaf stage in Tehran, Varamin, and Isfahan in terms of percentages of seedling damping-off incidence and efficacy based on composite analysis

Composite analysis for the incidence rate of greenhouse cucumber seedling damping-off

The effect of treatment × place on percentage of seedling damping-off was significant for different fungicide treatments at 1% probability level in Tehran, Varamin, and Isfahan. Therefore, ANOVA was performed separately for the data obtained from each region and mean percentages of the disease incidence were grouped based on the ANOVA tables for each region tables 1-3.

Evaluation of fungicidal treatments in Tehran area (greenhouse of Iranian Institute of Medical Research) in terms of percentages of seedling damping-off incidence at 4-6 leaf stage

Efficacy of the studied chemical fungicides in reducing the incidence of seedling damping-off at the six-leaf stage in Tehran was significant at 1% probability level. Results of statistical grouping for mean values of seedling damping-off incidence showed that all treatments were placed in three statistical groups. The disease incidence decreased significantly in all treatments with chemical fungicides compared with the infected control, with the lowest disease incidence rate belonging to ridomex and previcur energy treatments. Also, the above treatments were not significantly different from healthy controls in terms of disease incidence table 1.

Evaluation of fungicidal treatments in Varamin (a greenhouse at medical plant research laboratory) in terms of seedling damping-off percentage at four-six leaf stage

Efficacy of the studied chemical fungicides in reducing the incidence of seedling damping-off at the four-six leaf stage in Varamin was significant at 1% probability level. Results of statistical grouping for mean values of seedling damping-off incidence showed that all treatments were placed in three statistical groups. The disease incidence decreased significantly in all treatments with chemical fungicides compared with the infected control, with the highest disease incidence rate belonging to mancolaxil treatment with the lowest dose (1.5/1000). Also, all the treatments were not significantly different from healthy controls in terms of disease incidence table 2.

Table 1: Grouping of mean incidence rates of greenhouse cucumber seedling damping-off among different treatments of chemical fungicides (ridomex, redoxyl, and mancolaxil) at four-six leaf stage in Tehran.

Treatment	Mean seedling damping -off incidence (%)
Redoxil® 5% G (metalaxil)	b*22.31
Ridomex® 5% G (metallaxil)	bc16.42
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.5g/L)	b32.13
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.75g/L)	b30.71
Mancolaxil® 72% WP (metalaxil + mancozeb)(2g/L)	b32.13
Downy-G® 72% WP (metalaxil + mancozeb)	b22.24
Precvicur energy ®840 SL (promocarp + hydrochloride)	bc17.85
Infected control	a62.85
Healthy control	c0.71

* There is no statistically significant difference among the averages with similar letters at a probability level of 1%.

Table 2: Grouping of mean incidence rates of greenhouse cucumber seedling damping-off among different treatments of chemical fungicides (ridomex, redoxyl, and mancolaxil) at four-six leaf stage in Varamin.

Treatment	Mean seedling damping -off incidence (%)
Redoxil® 5% G (metalaxil)	bc*1.67
Ridomex® 5% G (metalaxil)	bc4.16
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.5g/L)	b11.67
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.75g/L)	bc3.33
Mancolaxil® 72% WP (metalaxil + mancozeb)(2g/L)	bc7.50
Downy-G® 72% WP (metalaxil + mancozeb)	bc4.16
Precviur energy ®840 SL (promocarp + hydrochloride)	bc2.50
Infected control	a85.500
Healthy control	c0

* There is no statistically significant difference among the averages with similar letters at a probability level of 1%.

Table 3: Grouping of mean incidence rates of greenhouse cucumber seedling damping-off among different treatments of chemical fungicides (ridomex, redoxyl, and mancolaxil) at four-six leaf stage in Isfahan.

Treatment	Mean seedling damping -off incidence (%)
Redoxil® 5% G (metalaxil)	b*3.75
Ridomex® 5% G (metalaxil)	b1.88
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.5g/L)	b5.00
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.75g/L)	b3.13
Mancolaxil® 72% WP (metalaxil + mancozeb)(2g/L)	b6.25
Downy-G® 72% WP (metalaxil + mancozeb)	b3.13
Previcur energy840 SL (promocarp + hydrochloride)	b1.25
Infected control	a61.25
Healthy control	b0

* There is no statistically significant difference among the averages with similar letters at a probability level of 1%.

Evaluation of fungicidal treatments in Varamin (a greenhouse at the Agricultural and Natural resources Research Center) in terms of seedling damping-off percentage at four-six leaf stage

Efficacy of the studied chemical fungicides in reducing the incidence of seedling damping-off at the four-six leaf stage in Varamin was significant at 1% probability level. Results of statistical grouping for mean values of seedling damping-off incidence showed that all treatments were placed in two statistical groups. All treatments with chemical fungicides showed significant decreases in disease incidence compared to the infected control. There were no significant differences between fungicide treatments, which were not significantly different from the healthy control in terms of the disease incidence table 3.

Composite analysis for the efficacy percentage of chemical fungicides in the control of Pythium seedling damping-off in greenhouse cucumber

The results for the efficacy percentage of chemical fungicides in controlling Pythium seedling damping-off in greenhouse cucumber in three regions of Tehran, Varamin, and Isfahan showed that the effect of treatment × place was not significant at 1% probability level. According to the composite analysis table, therefore, the mean percentages of efficacy were grouped in different fungicide treatments table 4. The results of grouping mean percentages of efficacy in different fungicide treatments revealed that mean values of all fungicide treatments were in one statistical group and there were no significant differences between different fungicide treatments at 1% probability level table 4.

Overall, the results demonstrated that the fungicides tested in the present study, including ridomex and redoxil with metalaxil active ingredient and mancolaxil with mancozeb + metallaxil active ingredients reduced significantly the incidence of *P. aphanidermatum* seedling damping-offdisease. According to the results, no significant differences were observed between the efficacy percentages of these fungicides in the disease control.

Over the past decade, the rapid growth of cucumber production in Iran has made it one of the

Table 4: Grouping mean percentages of efficacy among different treatments of the tested chemical fungicides (ridomex, redoxil, and mancolaxil) at four-six leaf stage in three regions of Tehran, Varamin, and Isfahan based on composite analysis.

Treatment	Mean efficacy (%)
Redoxil®5% G (metalaxil)	a*86.39
Ridomex® 5% G (metalaxil)	a88.63
Mancolaxil®72% WP (metalaxil + mancozeb)(1.5g/L)	a75.65
Mancolaxil® 72% WP (metalaxil + mancozeb)(1.75g/L)	a80.70
Mancolaxil® 72% WP (metalaxil + mancozeb)(2g/L)	a76.60
Downy-G® 72% WP (metalaxil + mancozeb)	a84.92
Precviur energy®840 SL (promocarp + hydrochloride)	a88.86

* There is no statistically significant difference among the averages with similar letters at a probability level of 1%.

most important vegetables produced in the country (Zamanizadeh *et al.,* 2011). Various species of oomycete fungi are the causative agent of cucumber seedling and plant damping-off disease that incur great damage to the crop under favorable environmental conditions. The use of effective chemical fungicides is one of the successful methods of controlling these diseases in the field and greenhouse cultures (Zamanizadeh *et al.,* 2011). Therefore, it is of great importance for manufacturers to access to diverse and effective fungicides from different groups and to use them in appropriate methods in disease management (Azimi & Shahriyari, 2016).

Conclusion

Our results indicated that the use of fungicides Redoxil® and Ridomax® Granules 5% (metallaxil) and Menacolaxil® Powder and 72% (metallaxil + mancozeb) was effective to reduce significantly the incidence of cucumber plant damping-off disease caused by the fungus *P. aphanidermatum*. In this study, no statistically significant differences were observed in the disease incidence rate and the percentage of efficacy in the disease control among fungicides with different active ingredients including metallaxil (ridomex and redoxyl), metallaxil + mancozeb (Downy G and Mancolaxil), and promocarp hydrochloride + foztyl aluminum (precviur energy).

Results of previous research reveal the effects of Precviur energy ® 840SL (promocarp hydrochloride + foztyl aluminum) and Ridomil® 5% G (metallaxil) on cucumber plant damping-off induced by *Phytophthora drechsleri*, indicating the superiority of precviur energy to ridomil in terms of effective control of the disease (Azimi & Shahriyari, 2016).

Moreover, the present results showed that there were no statistically significant differences in the disease control efficacy among the fungicides with two different active ingredients, including metalaxil + mancozeb (mancolaxil and Downy G) and metalaxil (ridomex and redoxyl). There was no disease. In this regard, (Azimi, 2014) also presented evidence that both rosalaxil (metallaxil + mancozeb) and ridomil (metalaxil) were in a single statistical group in terms of reducing the incidence of cucumber plant damping-off disease caused by *Phytophthora drechsleri* (Azimi, 2014).

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